MAF 2019

16TH INTERNATIONAL METHODS & APPLICATIONS OF FLUORESCENCE

UC San Diego
La Jolla, California
August 20 – 24, 2019

Diamond Sponsor

VERTEX®

Platinum Sponsor

HORIBA

Welcome to the MAF 2019

Dear Colleagues,

On behalf of the MAF PSC and local organizing committee we welcome you to the University of California San Diego in La Jolla. For the next few days, a lively group of individuals from all over the world will celebrate the science of fluorescence spectroscopy. We have an intense program filled with invited and contributed lectures as well as a keynote and award presentations. As always, poster presentations play a key role in the success of the MAF meetings. All posters will therefore be displayed throughout the meeting and be featured in two early evening sessions.

In addition to our stimulating scientific program, we have planned diverse social events for you. Your participation is essential for the success of the meeting. Engage yourself in our program and enjoy the science, your colleagues, the food, and the Southern California atmosphere. We hope you will also have the opportunity to explore our campus, La Jolla, and San Diego.

We are grateful to all involved in organizing this meeting and facilitating this gathering and the associated events. We thank our colleagues for their feedback and help. We are indebted to our sponsors and exhibitors and their support, as well as to UC San Diego's Hospitality and Conference Services and our students for their dedication and assistance.

Have a wonderful and memorable time!

Professor Yitzhak Tor
On behalf of the MAF PSC and the Local Organizing Committee

Meeting Contacts

Yitzhak Tor
Email: ytor@ucsd.edu

UC San Diego Hospitality and Conference Services Team
Anita Treviño Neubarth
Suzette Olguín
Jackie Campbell
Kristina Young
Email: meetings@ucsd.edu

Committees

Event Organizers
Professor Yitzhak Tor, Chair, *University of California, San Diego*
Dr. Michelle A. Digman, *University of California, Irvine*
Dr. Enrico Gratton, *University of California, Irvine*

Members of the MAF PSC
Professor Yves Mely, Chair, *University of Strasbourg*
Professor Manfred Auer, *The University of Edinburgh*
Professor Mario Berberan Santos, *University of London*
Professor David Birch, *Universtiy of Strathclyde*
Professor Fred M Brouwer, *Universteit van Amsterdam*
Professor Alexander P Demchenko, *Palladin Institute of Biochemistry*
Professor Alberto Diaspro, *University of Genoa*
Professor Michelle Digman, *University of California, Irvine*
Professor Katharina Gaus, *University of New South Wales, Australia*
Professor Hans C Gerritsen, *Universiteit Utrecht*
Professor Zygmunt Gryczynski, *Texas Christian University*
Professor Stefan W Hell, *Max Planck Institute for Biophysical Chemistry*
Professor Martin Hof, *J. Heyrovsky Institute of Physical Chemistry of the ASCR*
Professor Johan Hofkens, *KU Leuven*
Professor David M Jameson, *University of Hawaii*
Professor Anita C Jones, *University of Edinburgh*
Professor Clemens Kaminski, *University of Cambridge*
Professor Guruswamy Krishnamoorthy, *Tata Institute of Fundamental Research*
Professor Joseph Lakowicz, *University of Maryland*
Professor Bin Liu, *National University of Singapore*
Professor Maria Garcia Parajo, *The Institute of Photonic Sciences*
Professor Seung Bum Park, *Seoul National University*
Professor Julia Perez-Prieto, *University of Valencia*
Professor Jicun Ren, *Shanghai Jiao Tong University*
Professor Ute Resch-Genger, *Bundesanstalt fur Materialforschung und prufung*
Professor Catherine Royer, *Rensselaer Polytechnic Institute*
Professor Markus Sauer, *Universitat Wurzburg*
Professor Suzanne Scarlata, *University of Illinois at Urbana-Champaign*
Professor Gerhard Schutz, *TU Wien*
Professor Petra Schwille, *Max Planck Institute of Biochemistry*
Professor Claus Seidel, *Universtat Dusseldorf*
Professor Trevor Smith, *The University of Melbourne*
Professor Koji Suzuki, *Keio University*
Professor Jacek Waluk, *Polish Academy of Sciences*
Professor Jerker Widengren, *KTH, Royal Institute of Technology*
Professor Marcus Wilhelmsson, *University of Gothenburg*
Professor Paul Wiseman, *McGill University*
Professor Otto Wolfbeis, *Universitat Regensburg*

Thanks to all our sponsors! We couldn't have done it without you!

Diamond Sponsors

VERTEX®

Platinum Sponsors

HORIBA

Silver Sponsors

Bronze Sponsors

biosensors

Alnylam® PHARMACEUTICALS

TriLink® BIOTECHNOLOGIES
part of Maravai LifeSciences

UC San Diego
School of Medicine
Department of Pharmacology

ACS NANO

H2

THORLABS

A Journal of the German Chemical Society
Angewandte
GDCh
International Edition
Chemie
www.angewandte.org

IONIS™

AVELAS BIOSCIENCES

UC San Diego
Chemistry and Biochemistry

OLYMPUS®

Program at a Glance

	Tue 8/20	Wed 8/21	Thu 8/22	Fri 8/23	Sat 8/24
08:20 – 08:30					VCR Brown
08:30 – 08:40		Session 1 Chair: Michelle Digman IL J. Biteen	Session 5 Chair: Nathalie Weickgenannt IL U. Endesfelder Angew Chem Lec	Session 9 Chair: Yves Mély IL M. Wilhelmsson	Session 13 Chair: Paul Negulescu IL G. Zlokarnik
08:40 – 08:50					
08:50 – 09:00					
09:00 – 09:10		IL M. Roeffaers	IL T. Tahara	IL S. Magennis	IL C. Schultz
09:10 – 09:20					
09:20 – 09:30					
09:30 – 09:40		IL R. Evans	IL Y. Stahl	IL Y.-T. Chang	IL L. Lavis
09:40 – 09:50					
09:50 – 10:00					
10:00 – 10:10		OP B. Albinsson	OP Y. Hong	OP B. Fischer	OP N. Luedtke
10:10 – 10:20					
10:20 – 10:50		Coffee Break	Coffee Break	Coffee Break	Coffee Break
10:50 – 11:00		Session 2 Chair: Otto S. Wolfbeis Wolfbeis Award J. Hofkens	Session 6 Chair: Claus Seidel KN P. Weiss	Session 10 Chair: Trevor Smith OP B. Laursen	Session 14 Chair: Doug Magde KN WE Moerner
11:00 – 11:10					
11:10 – 11:20				OP J. Waluk	
11:20 – 11:30					
11:30 – 11:40		IL J. Schuck	IL M. Schnermann	IL E. Isacoff	IL J. Zhang
11:40 – 11:50					
11:50 – 12:00					
12:00 – 12:10		OP K. Gall	OP C. McLellan	OP D. Whelan	OP V. Lev-Ram
12:10 – 12:20					
12:20 – 13:30		Lunch break Posters	Posters Lunch break	Lunch break Posters	Lunch break
13:30 – 13:40		Session 3 Chair: Marcus Wilhelmsson IL T. Wohland	Session 7 Chair: Enrico Gratton IL C. Eggeling	Session 11 Chair: Jerker Widengren IL J. Elf	Session 15 Chair: Jin Zhang IL A. Palmer
13:40 – 13:50					
13:50 – 14:00					
14:00 – 14:10	**Registration** 14:00–19:00				
14:10 – 14:20		IL M. Hoernke	IL C. Stringari	IL B. Wu	IL K. Kikuchi
14:20 – 14:30					
14:30 – 14:40		OP V. Lemmens	OP V. Vukojevic	OP S. Krause	IL M. Ellisman
14:40 – 14:50					
14:50 – 15:00		OP G. Krishnamoorthy	OP L. Wang	OP Y. Erostyak	OP P. Wiseman
15:00 – 15:10					
15:10 – 15:40		Coffee Break	Coffee Break	Coffee Break	Coffee Break
15:40 – 15:50		Session 4 Chair: Jacek Waluk OP E. Cosco	Session 8 Chair: Paul Wiseman OP V. Glembockyté	Session 12 Chair: David Birch OP M. Heilemann	Session 16 Chair: Gregor Zlokarnik IL A. Ting
15:50 – 16:00					
16:00 – 16:10		OP D. Birch	OP R. Sachl	OP R. Cinco	
16:10 – 16:20					
16:20 – 16:30		OP M. Frei	OP H. Sanabria	OP M. Levitus	IL N. Devaraj
16:30 – 16:40					
16:40 – 16:50		OP G. Jung	OP A. Barth	OP H. Kim	
16:50 – 17:00					IL R. Campbell
17:00 – 17:10		Poster Session Reception	Poster Session Reception	OP G. Holst	
17:10 – 17:20					
17:20 – 17:30				Transportation to Birch Aquarium	IL P. Negulescu
17:30 – 17:40		Beer Garden			
17:40 – 17:50					
17:50 – 18:00				Conference Banquet Birch Aquarium Poster Awards	Closing
18:00	Opening KN P. Chen		MAF PSC Mtg		
19:00					
22:00	Reception				

ROGER Y. TSIEN MEMORIAL SYMPOSIUM

KEY (total time): **KN**=Keynote Lecture (40 min) **IL**=Invited Lecture (30 min) **OP**=Oral Presentation (20 min)

MAF 2019 — Methods & Applications of Fluorescence

August 20–24, 2019, La Jolla, California

UC San Diego

General Meeting Information

Conference Website: *http://maf2019.ucsd.edu*

Meeting Venue:
University of California San Diego
Price Center, 2nd Floor, East Ballroom
9500 Gilman Drive
La Jolla, CA 92093

Registration Desk:
Price Center, 2nd Floor, East Ballroom

Hours:	Tuesday (08/20/19)	**14:00 – 19:00**
	Wednesday (08/21/19)	**7:30 – 17:00**
	Thursday (08/22/19)	**7:30 – 17:00**
	Friday (08/23/19)	**7:30 – 17:00**
	Saturday (08/24/19)	**7:30 – 17:00**

If you are interested in local dining, feel free to ask at our desk.

On-Campus Housing Conference Desk:
The Village
2202 Scholars Drive North
La Jolla, CA 92093
+1-858-534-4165
Hours: 07:00 – 02:00 Daily

Internet:
For Wi-Fi Internet Access in the meeting areas, connect to "**UCSD-Guest**" or use "**eduroam**" if your institution is eduroam-enabled.

For Wi-Fi Internet access in on-campus housing, please note the information for access at http://resnet.ucsd.edu/conf and review the information on its security compliance tool: SafeConnect.

Parking Information:
Parking on campus is by Permit only Monday 7:00AM – Friday 22:00 (11:00PM).

Conference attendees should park in the ***Hopkins Parking Structure*** located at the corner of Hopkins Drive and Voigt Drive.

Important: Do not park in "A" (red) or "Reserved 24/7" spaces or you will get a parking ticket.

Shuttle Service:
Estancia Shuttle: Shuttle service to and from Estancia Hotel can be requested at the Hotel's Front Desk.

Gala Banquet Shuttle (Fri, 23 August): A shuttle will be provided to and from the Birch Aquarium (ample free parking is available at the Birch Aquarium) per schedule: one pickup at 5:45PM from the Price Center and go directly to the Birch Aquarium. The shuttle will then loop from The Village Apartments and the Estancia Hotel starting at 6:15PM.

Return service begins at 8:45PM.

No Smoking Policy:
Please note that the entire UC San Diego campus is a smoke-free, including all outdoor spaces and residential buildings. UC San Diego follows all federal guidelines.

Emergency and Security
In the event of an emergency, please dial **911.**
For campus emergency status and information:
https://ucsd.edu/about/emergency.html
For security walking escorts on-campus, please, contact 858.534.9255 (858.534.WALK), available sunset to 1:00AM.

The University of California does not sponsor this conference. The views expressed at the conference are not supported or endorsed by the University of California and or UC San Diego

Program

Tuesday, 20 August

14:00 Registration Opens

18:00 Opening
Yves Mély and Yitzhak Tor

18:10 Keynote Lecture (**KN1**)
Peng Chen, Cornell University, USA
Single-molecule fluorescence microscopy of nanocatalysis and beyond

19:00 RECEPTION

Kindly sponsored by HORIBA

Wednesday, 21 August

8:00 CONTINENTAL BREAKFAST

Session 1

Session Chair: Michelle Digman

8:30 Invited Lecture (**IL1**)
Julie Biteen, University of Michigan Ann Arbor, USA
Single-molecule fluorescence imaging reveals hidden dynamics inside living bacterial cells

9:00 Invited Lecture (**IL2**)
Maarten Roeffaers, KU Leuven, Belgium
Optical Micro(Spectro)scopic Characterization of Metal Halide Perovskites

9:30 Invited Lecture (**IL3**)
Rachel Evans, University of Cambridge, UK
Bottom-Up Design of Integrated Spectral Conversion Materials for Luminescent Solar Devices

10:00 Oral Presentation (**OP1**)
Bo Albinsson, Chalmers University of Technology, Sweden
Towards Diffusion-Free Triplet-Triplet Annihilation Photon Up-conversion

10:20 COFFEE BREAK

Session 2

Session Chair: Otto S. Wolfbeis

10:50 **Wolfbeis Award**
Otto S. Wolfbeis, University of Regensburg, Germany

11:00 Wolfbeis Award Lecture (**AL1**)
Johan Hofkens, KU Leuven, Belgium
Identifying microbiome species by single-molecule super-resolved DNA mapping and resampling statistics

11:30 Invited Lecture (**IL4**)
P James Schuck, Columbia University, Country
Amplifying the prospects of upconverting nanoparticles

12:00 Oral Presentation (**OP2**)
Karen Gall, HORIBA Instruments Inc., USA
Novel Method for Multidimensional Fluorescence Characterization of Protein Binding and Conformational Changes

12:20 LUNCH BREAK

Session 3

Session Chair: Marcus Wilhelmsson

13:30 Invited Lecture (**IL5**)
Thorsten Wohland, National University of Singapore, Singapore
Generalized fluorescence imaging for maximal information recovery with high spatiotemporal resolution

14:00 Invited Lecture (**IL6**)
Maria Hoernke, Institute Name, Country
Quantified Membrane Permeabilization: Statistical Analysis of Fluorescence Lifetimes

14:30 Oral Presentation (**OP3**)
Veerle Lemmens, BIOMED Hasselt University, Belgium
Raster spectral image correlation spectroscopy: principle and application to the glycine receptor

14:50 Oral Presentation (**OP4**)
Guruswamy Krishnamoorthy, Anna University, India
Fluorescence Window Reveals the Population Distribution During Collapse and Folding of a Small Protein

15:10 COFFEE BREAK

Session 4

Session Chair: Jacek Waluk

15:40 Oral Presentation (**OP5**)
Emily Cosco, UCLA, USA
Multiplexed in vivo optical imaging with shortwave infrared polymethine dyes

16:00 Oral Presentation (**OP6**)
David Birch, University of Strathclyde, UK
Pheomelanin sheet structure revealed by ThT fluorescence

16:20 Oral Presentation (**OP7**)
Michelle Frei, MPI for Medical Research and EPFL, Germany
Photoactivation of silicon rhodamines via a light-induced protonation

16:40 Oral Presentation (**OP8**)
Gregor Jung, Saarland University, Germany
Pyrene derivatives for single-molecule chemistry

17:00 POSTER SESSION (with beer garden and snacks)

Thursday, 22 August

8:00 CONTINENTAL BREAKFAST

Session 5

Session Chair: Nathalie Weickgenannt

8:30 Invited Lecture (**IL7**)
Ulrike Endesfelder, MPI Marburg, Germany
Visualizing the inner life of microbes by single-molecule localization microscopy

9:00 Invited Lecture (**IL8**)
Tahei Tahara, RIKEN, Japan
Microsecond structural dynamics of bio-macromolecules revealed by two-dimensional fluorescence lifetime correlation spectroscopy

9:30 Invited Lecture (**IL9**)
Yvonne Stahl, Heinrich-Heine University, Germany
Molecular control of stem cell regulation in plants

10:00 Oral Presentation (**OP9**)
Yuning Hong, La Trobe University, Australia
An Activity-based Fluorogen for Quantifying Intracellular Polarity of Protein Environment

10:20 COFFEE BREAK

Session 6

Session Chair: Claus Seidel

10:50 Keynote Lecture (**KN2**)
Paul Weiss, UCLA, USA
Submolecular resolution spectroscopic imaging for photoactive molecules and assemblies

11:30 Invited Lecture (**IL10**)
Martin Schnermann, National Cancer Institute, USA
Harnessing Cyanine Reactivity to Prepare Novel Fluorophores for Advanced Imaging Applications

12:00 Oral Presentation (**OP10**)
Claire McLellan, Stanford University, USA
Upconverting nanoparticle as optical transducers of biomechanical forces

12:20 LUNCH BREAK

Session 7

Session Chair: Enrico Gratton

13:30 Invited Lecture (**IL11**)
Christian Eggeling, Friedrich-Schiller University Jena, Germany
Molecular membrane organization: a super-resolution fluorescence spectroscopy study

14:00 Invited Lecture (**IL12**)
Chiara Stringari, CNRS- Ecole Polytechnique, France
Multicolor Two-Photon Microscopy of Endogenous Fluorescence by Wavelength Mixing for Multiparametric Metabolic Imaging

14:30 Oral Presentation (**OP11**)
Vladana Vukojevic, Karolinska Institute, Sweden
Functional Fluorescence Microscopy Imaging (fFMI). Quantitative scanning-free confocal fluorescence microscopy for characterization of fast dynamic processes in live cells

15:00 Oral Presentation (**OP12**)
Lei Wang, UCSF, USA
Nano-switches for optogenetic control and a fluorescent reporter for acidic vesicles

15:20 COFFEE BREAK

Session 8

Session Chair: Paul Wiseman

15:40 Oral Presentation (**OP13**)
Viktorija Glembockyté, LMU Muenchen, Germany
Self-assembled DNA Optical Nanoantennas for Fluorescence-based Diagnostic Applications

16:00 Oral Presentation (**OP14**)
Radek Šachl, J.Heyrovsky Institute of Physical Chemistry, Czech Republic
Are Lipid Nanodomains Inter-Leaflet Coupled? An MC-FRET Study.

16:20 Oral Presentation (**OP15**)
Hugo Sanabria, Clemson University, USA
Local order-disorder dynamics revealed in domain swapping pathway of the DNA-binding domain of human FoxP1

16:40 Oral Presentation (**OP16**)
Anders Barth, Heinrich-Heine-Universität Düsseldorf, Germany
Studying complex biomolecular dynamics by single-molecule FRET

17:00 POSTER SESSION (with drinks and snacks)

Friday, 23 August

8:00 CONTINENTAL BREAKFAST

Session 9

Session Chair: Yves Mély

8:30 Invited Lecture (**IL13**)
Marcus Wilhelmsson, Chalmers University of Technology, Sweden
Fluorescent nucleobase analogues and their utilization in studies of nucleic acid conformations and oligonucleotide-based therapeutics

9:00 Invited Lecture (**IL14**)
Steven Magennis, University of Glasgow, UK
Ultrasensitive detection of fluorescent nucleobase analogs via multiphoton excitation

9:30 Invited Lecture (**IL15**)
Young-Tae Chang, POSTECH Chemistry, South Korea
Development of Universal Platform for Live Cell Discrimination through Gating Mechanism

10:00 Oral Presentation (**OP17**)
Bilha Fischer, Bar-Ilan University, Israel
An Oligonucleotide Probe Incorporating the Chromophore of Green Fluorescent Protein is Useful for the Detection of HER-2 mRNA Breast Cancer Marker

10:20 COFFEE BREAK

Session 10

Session Chair: Trevor Smith

10:50 Oral Presentation (**OP18**)
Bo W. Laursen, University of Copenhagen, Denmark
A general approach to fluorescent crystals and nanoparticles based on organic dyes

11:10 Oral Presentation (**OP19**)
Jacek Waluk, Institute of Physical Chemistry, Poland
Tautomerization probed by single molecule fluorescence

11:30 Invited Lecture (**IL16**)
Ehud Isacoff, UC Berkeley, USA
Single molecule FRET and photo---switched ligand reveal novel conformational pathway of GPCR activation

12:00 Oral Presentation (**OP20**)
Donna Whelan, La Trobe University, Australia
Complementary Single Molecule Imaging and Infrared Spectroscopy to Characterise DNA Damage Response

12:20 LUNCH BREAK

Session 11

Session Chair: Jerker Widengren

13:30 Invited Lecture (**IL17**)
Johan Elf, Uppsala University, Sweden
Genome-wide Single Cell Biophysics

14:00 Invited Lecture (**IL18**)
Bin Wu, Johns Hopkins School of Medicine, USA
Single molecule imaging of repeat RNA translation in live cells

14:30 Oral Presentation (**OP21**)
Stefan Krause, University of Copenhagen, Denmark
Single Molecule Excitation-Emission & Lifetime Mapping at Ambient Conditions

14:50 Oral Presentation (**OP22**)
Janos Erostyak, University of Pecs, Hungary
Transition from solid state to molecular-like optical properties in silicon carbide nanoparticles

15:10 COFFEE BREAK

Session 12

Session Chair: David Birch

15:40 Oral Presentation (**OP23**)
Mike Heilemann, Goethe-University Frankfurt, Germany
Determining the stoichiometry of protein complexes with single-molecule localization microscopy

16:00 Oral Presentation (**OP24**)
Rachel Cinco, UC Irvine, USA
Multi-Modal Fluorescence Characterization of Cell Cycle Progression and Cytokinesis

16:20 Oral Presentation (**OP25**)
Marcia Levitus, Arizona State University, USA
Modulation of the oligomerization state of proteins by ions and small molecules: a fluorescence correlation spectroscopy study

16:40 Oral Presentation (**OP26**)
Harold Kim, Georgia Institute of Technology, USA
Sequence dependence of DNA strand displacement kinetics

17:00 Oral Presentation (**OP27**)
Gerhard Holst, PCO AG, Germany
Frequency Domain FLIM System Improvements and Applications

18:00 GALA BANQUET AT BIRCH AQUARIUM

19:30 Poster Awards

Saturday, 24 August – Roger Tsien Memorial Symposium

8:00 CONTINENTAL BREAKFAST

8:20 Opening Remarks
Vice Chancellor for Research Sandra A. Brown

Session 13
Session Chair: Paul Negulescu

8:30 Invited Lecture (**IL19**)
Gregor Zlokarnik, Vertex Pharmaceuticals, USA
A perspective on Roger Tsien's contribution to and impact on Chemical Biology

9:00 Invited Lecture (**IL20**)
Carsten Schultz, OHSU, USA
Novel fluorescent and thermogenetic tools

9:30 Invited Lecture (**IL21**)
Luke Lavis, OHSU, USA
From single-molecule imaging to the brain: A circuitous route to new neural activity indicators

10:00 Oral Presentation (**OP28**)
Nathan Luedtke, McGill University, Canada
Biopolymer folding studies inspired by the works of Roger Tsien, Yitzhak Tor and Neal Devaraj

10:20 COFFEE BREAK

Session 14
Session Chair: Doug Magde

10:50 Keynote Lecture (**KN3**)
William Moerner, Stanford University, USA
Roger Tsien and Blinking Fluorescent Proteins in the mid--1990s

11:30 Invited Lecture (**IL22**)
Jin Zhang, UC San Diego, USA
Illuminating the Biochemical Activity Architecture of the Cell

12:00 Oral Presentation (**OP29**)
Varda Lev-Ram, UC San Diego, USA
Do Perineuronal nets stabilize the engram of a synaptic circuit?

12:20 LUNCH

Session 15

Session Chair: Jin Zhang

13:30 Invited Lecture (**IL23**)
Amy Palmer, University of Colorado Boulder, USA
Riboglow: a new tool for tagging and tracking RNA in live cells

14:00 Invited Lecture (**IL24**)
Kazuya Kikuchi, Osaka University, Japan
In vivo Multicolor Imaging with Fluorescent Probes Revealed Dynamics and Function of Osteoclast Proton Pumps

14:30 Invited Lecture (**IL25**)
Mark Ellisman, UC San Diego, USA
Revealing Secrets Hiding in Plain Sight: Advances in Multi-scale Multi-modal Imaging

15:00 Oral Presentation (**OP30**)
Paul Wiseman, McGill University, Canada
Beating Nyquist limits for the measurement of fluorophore blinking rates using image correlation spectroscopy

15:20 COFFEE BREAK

Session 16

Session Chair: Gregor Zlokarnik

15:50 Invited Lecture (**IL26**)
Alice Ting, Stanford University, USA
Optogenetic and chemogenetic technologies for probing molecular and cellular interactions

16:20 Invited Lecture (**IL27**)
Neal Devaraj, UC San Diego, USA
Probing lipids in living cells

16:50 Invited Lecture (**IL28**)
Robert Campbell, University of Alberta, USA
The legacy of camgaroo: new fluorescent protein-based biosensors of neural activity and metabolism

17:20 Invited Lecture (**IL29**)
Paul Negulescu, Vertex Pharmaceuticals, USA
Roger Tsien's Impact on Drug Discovery

17:50 Closing Remarks

Poster abstracts may be found online at http://MAF2019.ucsd.edu/

Keynote Abstracts

Single-molecule fluorescence microscopy of nanocatalysis and beyond

Peng Chen

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA

This presentation will describe our efforts in developing single-molecule fluorescence imaging approaches to study nanoscale catalysis, focusing on two stories. The first will be about our imaging work on the catalytic properties of single nanoparticles at single-turnover resolution and nanometer precision. I will describe the insights we gained into the catalytic activity and dynamics of individual metal nanoparticles, and the surprising spatial and temporal activity patterns and dynamics within single nanocatalysts. The second story will be about our work in using redox-selective super-resolution reaction imaging and sub-particle photocurrent measurements to determine the relation between charge-carrier surface activity and water oxidation efficiency on a semiconductor photoanode during photoelectrochemical water oxidation. I will describe how the mapping of hole and electron activity leads to the discovery of optimal sites for oxygen evolution catalysts and a strategy for rationally engineering photoelectrodes with catalysts.

Acknowledgments: The related work was supported by grants from the Army Research Office, Chemical Sciences, Reactive Chemical Systems, and from the Department of Energy, Office of Science, Basic Energy Sciences, Catalysis program.

References: [1] Xu, W. et al., *Nature Mater.* **2008**, 7, 992. [2] Zhou, X. et al., *Nature Nanotech.* **2012**, *7*, 237. [3] Sambur, J. B. et al., *Nature* **2016**, *530*, 77. [4] Liu, C. et al., *Science* **2017**, *358*, 352. [5] Chen, G. et al., *ACS Cent. Sci.* **2017**, *3*, 1189. [6] Zou, N. et al., *Nature Chem.* **2018**, *10*, 607.

*Corresponding author: E-mail: pc252@cornell.edu

Submolecular resolution spectroscopic imaging for photoactive molecules and assemblies

Paul S. Weiss*[a]

[a]*California NanoSystems Institute and Departments of Chemistry & Biochemistry, Bioengineering, and Materials Science & Engineering, UCLA, Los Angeles, CA 90095, USA*

We have developed and applied new multimodal nanoscale analysis tools based on the scanning tunneling microscope (STM) to measure structure, function, and spectra simultaneously.[1-5] We are particularly interested in the interactions of photons with precisely assembled structures.[1-4] We use molecular design, tailored syntheses, intermolecular interactions, and selective chemistry to direct molecules into desired positions to create nanostructures with controlled environments and dimensionality, to connect functional molecules to the outside world, and to serve as test structures for measuring single or bundled molecules and assemblies.[1,2,4,6] The measured results of photoexcitation include photoconductivity, regioselective reaction, intramolecular charge redistribution, and environmental sensitivity.[1-5] We apply this method to optimize molecules and materials for energy conversion and storage. Concepts from sparsity and compressive sensing are developed and applied to guide efficient data acquisition and to accelerate data analysis and information assembly.

Acknowledgments: This work was supported by the US Department of Energy grants #DE-SC0005161 & DE-SC0005025-002.

References: [1] Kim, M. et al. *Science* **2011**, *331*, 1315. [2] Wang, S. et al. *J. Phys. Chem. Lett*. **2019**, *10*, 2175. [3] Kumar, A. S. et al. *Nano Lett*. **2008**, *8*, 1644. [4] Zheng, Y. B. *Adv. Matl*. **2013**, *25*, 302. [5] Bonnell, D. A. et al. *Rev. Mod. Phys*. **2012**, *84*, 1343. [6] Claridge, S. A. *Chem. Soc. Rev*. **2013**, *42*, 2725.

*Corresponding author: E-mail: psw@cnsi.ucla.edu

Roger Tsien and Blinking Fluorescent Proteins in the mid-1990s

W. E. Moerner*[a]

[a] *Departments of Chemistry and Applied Physics (courtesy), Stanford University, Stanford, California, U. S. A.*

I will describe some of my interactions with Roger Tsien in the mid-1990's relating to green fluorescent protein variants[1]. This will include descriptions of the first detection of single molecules at low temperature, surprises with single GFP variants at room temperature, the importance of single-molecule blinking and photocontrol, and the eventual implications and use[2] of these properties for super-resolved fluorescence microscopy.

References: [1] R. M. Dickson, A. B. Cubitt, R. Y. Tsien, and W. E. Moerner, "On/Off Blinking and Switching Behavior of Single Green Fluorescent Protein Molecules," Nature (1997) 388, 355. [2] J. S. Biteen, M. A. Thompson, N. K. Tselentis, G. R.Bowman, L. Shapiro, W. E. Moerner, "Superresolution Imaging in Live *Caulobacter Crescentus* Cells Using Photoswitchable EYFP," Nature Meth. (2008) 5, 947-949, published online 15 September 2008.

*Corresponding author: E-mail: wmoerner@stanford.edu

Award Lecture Abstracts

Identifying microbiome species by single-molecule superresolved DNA mapping and resampling statistics

Johan Hofkens,*[a], Raffaele Vitale,[d] Laurens D'Huys,[a] Vince Goyvaerts,[a] Cyril Ruckebusch,[d] Theo Lasser,[b] Aleksandra Radenovic,[b,c] and Kris P.F. Janssen[a]

[a] *Chemistry department, KU Leuven, Leuven, 3000, Belgium*
b Institute of Bioengineering, Ecole Polytechnique Federale de Lausanne, Lausanne, CH-1015, Switzerland
c School of engineering, Ecole Polytechnique Federale de Lausanne, Lausanne, CH-1015, Switzerland
d LASIR CNRS, Universite de Lille, Villeneuve d'Ascq Cedex, 59655, France

Single molecule DNA mapping has the potential to serve as a powerful complement to high throughput sequencing in metagenomic analysis. Offering longer read lengths and forgoing the need for complex library preparation and amplification, mapping stands to provide an unbiased view into the composition of complex viromes and/or microbiomes. To fully enable mapping-based metagenomics, sensitivity and specificity of DNA map analysis and identification need to be improved. Using detailed simulations and experimental data, we first demonstrate how fluorescence imaging of surface stretched, sequence specifically labeled, DNA fragments can yield highly sensitive identification of targets. Secondly, a new analysis technique is introduced to increase specificity of the analysis, allowing even closely related species to be resolved. Thirdly, we show how an increase in resolution, improves sensitivity. Finally, we demonstrate that these methods are capable of identifying species with long genomes such as bacteria with high sensitivity.

Acknowledgments: This work was supported by the Horizon 2020 Framework Programme of the European Union called ADgut [Grant No 686271]; 'Agentschap Innoveren & Ondernemen' in the framework of an innovation mandate [No HBC.2016.0246]; the European Union Research Council through the ERC-2017-PoC Metamapper [No 768826]

References: [1] Deen,J. et al Methyltransferase-Directed Labeling of Biomolecules and its Applications. Angew. Chem. Int. Ed., **2017** 56, 5182–5200. [2] Neely, R.K et al,. DNA fluorocode: A single molecule, optical map of DNA with nanometre resolution. Chem. Sci **2010**, 1, 453. [3] Neely, R.K. et al Optical mapping of DNA: single-molecule-based methods for mapping genomes. Biopolymers, **2011** 95, 298

*Corresponding author: E-mail: johan.hofkens@kuleuven.be

Invited Lecture Abstracts

Single-molecule fluorescence imaging reveals hidden dynamics inside living bacterial cells

Julie S. Biteen[a]

[a] *Departments of Chemistry and of Biophysics, University of Michigan, Ann Arbor, MI 48104 USA*

By sensitively detecting single fluorescent molecules, the resolution of fluorescence microscopy has been brought down to the nanometer scale, approaching the size of proteins inside cells. Single-molecule imaging therefore enables super-resolution microscopy, and our lab has been developing methods to locate, track, and analyze single molecules in living cells. In particular, because of the small size of bacterial cells, the mysteries of their subcellular structure, dynamics, and cooperativity are well suited to single-molecule investigations. I will discuss the example of how we have used single-molecule microscopy to measure and understand the dynamical interactions essential for DNA replication and in living *Bacillus subtilis* cells.[1] However, despite the power of super-resolution microscopy, single-molecule data processing algorithms can fail to provide accurate measurements of the brightness and position of molecules in the presence of backgrounds that fluctuate significantly over time and space. To address this limitation, I will present our new Single-Molecule Accurate LocaLization by LocAl Background Subtraction (SMALL-LABS) algorithm, which accurately locates and measures the intensity of single molecules, regardless of the shape or brightness of the background.[2]

Acknowledgments: This work was supported by NSF grant CHE-1807676 and NIH grant R21-GM128022 to JSB.

References: [1] Li, Y. et al., *Curr. Opin. Microbiol.* **2018**, *43*, 38-45. [2] Isaacoff, B.P. et al., *Biophys. J.* **2019**, *116*, 975-982.

*Corresponding author: E-mail: jsbiteen@umich.edu

Optical Micro(Spectro)scopic Characterization of Metal Halide Perovskites

Haowei Huang, Julian Steele, Haifeng Yuan, Johan Hofkens, Maarten B.J. Roeffaers*
Department of Chemistry, KU Leuven, Celestijnenlaan 200F,B-3001, Leuven Belgium

Corresponding author: maarten.roeffaers@kuleuven.be

The charge carrier dynamics in metal halide perovskites (MHP) materials have far-reaching consequences for developing high-efficiency materials for optoelectronic applications. In this presentation different optical micro(spectro)scopic tools are used to yield insight into the details of the charge carrier dynamics highlighting direct implications to their usability as electro-optical material.

The first key example is related to the double perovskite $Cs_2AgBiBr_6$.[1] Combining information from optical excitation and temperature-dependent emission spectroscopy, resonant and nonresonant Raman scattering the room-temperature charge carrier mobility and related electronic band structures and intrinsic lattice phonon scattering mechanisms are revealed in detail. Compared to the more popular lead halide perovskites, $Cs_2AgBiBr_6$ has deep conduction band energy levels and extremely large electron scattering from longitudinal optical phonons. These large Frohlich interactions, with an electron-phonon coupling constant of about 230 meV compared to 40-60 meV for traditional MHPs, dominate at room temperature and account for the relatively large Stokes shift, broad emission, and poor carrier transport.

A secondly approach explored at KU Leuven is the use of single MHP particle photoluminescence (PL) microscopy to reveal the charge carrier dynamics inside the material. For high quality single crystal $MAPbI_3$ nanorods with dimensions of several hundred nanometers even up to micrometers we recorded clear two-state blinking.[2,3] Detailed analysis of this single entity behavior in correlation with PL decay times and the strong environmental dependence we could link this information to the presence of crystal surface traps likely related to under-coordinated lead ions and methylammonium vacancies. Apply simultaneous luminescence and electron microscopy[4] on MHPs these photo-active can also be mapped for polycrystalline particles and trap densities can be quantified to be between 1.3×10^{14} and 8×10^{13} cm^{-3} for polycrystalline and for monocrystalline nanocrystals, respectively.[3] Environmental and synthetic impact on the trap density reveals their link to inherent structural defects.

The third example introduces an intrinsic approach for shifting the energetics of polymorphic $CsPbI_3$, in order to secure RT black phase formation over its yellow non-perovskite phase. We outline the role of glass substrate clamping using synchrotron-based grazing incidence wide angle X-ray scattering with extremely fast acquisition time (~0.1 s), to track the introduction of crystal distortions within black $CsPbI_3$ thin films as they are cooled from annealing temperatures. The thermal stability of black $CsPbI_3$ thin films are vastly improved by the strained interface, a response verified by ab initio thermodynamic modelling.[5]

In a last example, we widen the applicability and materials engineering options for MHPs by developing efficient photocatalysts for the selective oxidation of alcohols.[6] The best results were obtained with formamidinium lead bromide ($FAPbBr_3$) linked to electron and hole withdrawing materials to enhance photogenerated charge separation and utilization.

References

[1] J.A. Steele,…, M.B.J. Roeffaers, *ACS Nano* (**2018**) 12, 8081−8090.
[2] H. Yuan, … M.B.J. Roeffaers, *ACS Omega* (**2016**) 1, 148−159.
[3] H. Yuan, … M.B.J. Roeffaers,… E.H. Sargent, J. Hofkens, *Adv. Mater.* (**2018**) 30, 1705494.
[4] H. Yuan, … M.B.J. Roeffaers, *J. Phys. Chem. Lett.* (**2016**) 7, 561−566.
[5] J.A. Steele,…, M.B.J. Roeffaers, *Science* (**2019**) 10.1126/science.aax3878.
[6] H. Huang, … M.B.J. Roeffaers, *ACS Energy Lett.* (**2018**) 3, 755−75

Bottom-Up Design of Integrated Spectral Conversion Materials for Luminescent Solar Devices

Rachel C. Evans*

Department of Materials Science and Metallurgy, University of Cambridge, U.K.

Single junction photovoltaic devices exhibit a bottleneck in their efficiency due to incomplete or inefficient harvesting of photons in the low- or high-energy regions of the solar spectrum. This can be overcome through the retro-fitting of a spectral converter to the device, which is used to convert solar photons into energies that are more effectively captured by the solar cell through a photoluminescence process.[1] However, while a lumophore may show seemingly ideal optical characteristics for spectral conversion in solution (high emission quantum yield, strong absorption), disappointment frequently awaits on its translation to the solid-state, where aggregation and quenching effects lead to significantly reduced photoluminescence yields.

To overcome this challenge, we are investigating the bottom-up design of integrated lumophore-host systems for solar spectral converters based on π-conjugated lumophores in organic-inorganic hybrid polymer hosts known as *ureasils*.[2] Ureasils are composed of a siliceous skeleton that is chemically-grafted to poly(ethylene oxide) (PEO)/poly(propylene oxide) (PPO) chains through urea cross-linkages. Through judicious selection of the degree of branching and length of the organic backbone and the incorporation method (grafting *vs* immobilization *vs* permeation), we can control the packing, orientation and placement of the π-conjugated species in the ureasil host. Since the electronic properties depend explicitly on the arrangement and packing of the lumophore, this approach provides a means of modulating the optical properties – from enhanced photoluminescence quantum yields[3], to tunable emission colour via Förster resonance energy transfer.[4,5] These characteristics can be exploited to improve light-harvesting and trapping within the integrated material, which can be used to develop highly efficient spectral converters for luminescent solar concentrators[6] or as optical amplifiers for visible light communication.

Acknowledgments: This work was only possible due to the efforts of my fantastic research group (past and present) including Niamh Willis-Fox, Ilaria Meazzini, Adarsh Kaniyoor, Barry McKenna and Morton Lyu. Financial support from Science Foundation Ireland (12/IP/1608) and the European Research Council under the European Union's Horizon 2020 research and innovation programme (grant agreement No 818762) is also acknowledged.

References: [1] B. McKenna, R. C. Evans, *Adv. Mater*. **2017**, *29*, 1606491. [2] V. de Zea Bermudez, L. D. Carlos, L. Alcácer, *Chem. Mater.* **1999**,*11*, 569. [3] N. Willis-Fox *et al*., *Chem. Sci.* **2015**, *6* 7227. [4] I. Meazzini *et al*. *J. Mater. Chem. C.* **2016**, *4*, 4049. [5] N. Willis-Fox *et al.*, *Adv. Funct. Mater*. **2016**, *26*, 532. [6] A. Kaniyoor *et al*., *Adv. Opt. Mater*. **2016**, *4*, 444. [7] A. Bastos, B. McKenna, M. Lima, P. André, L. D. Carlos, R. C. Evans and R. A. S. Ferreira, *ACS Omega*, **2018**, 3, 13772.

*Corresponding author: rce26@cam.ac.uk

Amplifying the prospects of upconverting nanoparticles

P. James Schuck,*[a] Yung Doug Suh,[b] Bruce E. Cohen,[c] Emory M. Chan,[c]

[a] *Department of Mechanical Engineering, Columbia University, New York, New York, USA*
[b] *Research Center for Convergence NanoRaman Technology, Korea Research Institute of Chemical Technology (KRICT), DaeJeon, South Korea,*
[c] *The Molecular Foundry, LBNL, Berkeley, CA USA*

Though their unique optical properties are proving advantageous for numerous applications, an ongoing challenge facing upconverting nanoparticles (UCNPs) remains achieving satisfactory upconverted signal and quantum yield while illuminating at low fluences. Here, I will describe the pros and cons of various strategies used to improve UCNP signals and efficiencies, which are pushing UCNP brightness to unprecedented levels. Of particular note here are advances in dye-sensitized UCNPs,[1] high-lanthanide-content compositions,[2,3] exploitation of stimulated emission,[4] and the relationships between these approaches. The impact of these concepts are now being widely felt in fields spanning deep-tissue imaging, optogenetics, remote sensing, anti-counterfeiting, and solar light harvesting.

Acknowledgments: This work was supported in part by the Global Research Laboratory (GRL) Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (no. 2016911815).

References: [1] Garfield, D. J. et al., *Nature Photonics.* **2018***, 12*, 402. [2] Gargas, D. J., et al. *Nature Nanotechnol.* **2014**, *9*, 300. [3] Tian, B., et al. *Nature Commun.* **2018**, *9*, 3082. [4] Bravo, D. J., et al. *Nature Nanotechnol.* **2018**, *13*, 572.

*Corresponding author: E-mail: p.j.schuck@columbia.edu

Generalized fluorescence imaging for maximal information recovery with high spatiotemporal resolution

Thorsten Wohland,*[a,b] Jagadish Sankaran,[a] , Xue Wen Ng,[a] Harikrushnan Balasubramanian,[a] Wai Hoh Tang,[c] Adrian Roellin[c]

[a]*Department of Biological Sciences and Centre for Bioimaging Sciences, National University of Singapore, 14 Science Drive 4, 117557 Singapore, Singapore*
[b]*Department of Chemistry, National University of Singapore, 3 Science Drive 3, 117543 Singapore, Singapore*
[c]*Department of Statistics and Applied Probability, National University of Singapore, 6 Science Drive 2, 117546 Singapore, Singapore*

Traditionally, fluorescence microscopy records intensities and reports qualitative or at best semi-quantitative information about the sample under investigation. With the advent of super-resolution microscopy and single molecule techniques, fluorescence techniques made tremendous advances in the last decades. However, it is still required to optimize experiments for either super-resolution or fast temporal acquisitions, and measurements have to be done independently. If one records fluorescence images at high speed and multiple fluorescence parameters simultaneously, one could in principle obtain all quantitative evaluations in one single experiment, reducing the number of experiments and improving reliability since all evaluations stem from the exact same data. Here we demonstrate that at an acquisition rate of 1000 frames per second, we can record data from a live cell and obtain simultaneously information on diffusion coefficients, diffusion modes, oligomerization and spatial super-resolution from a single measurement. For this purpose we have combined imaging fluorescence correlation spectroscopy[1] with super-resolution radial fluctuation (SRRF) analysis[2] and number and brightness (N&B) analysis[3]. We test the combination of these techniques on CHO cells that were co-transfected with a filamentous actin marker (LifeAct GFP) and mApple labeled epidermal growth factor receptor (EGFR-mApple). The data acquisition of 50,000 frames takes less than a minute while data evaluation, using a new GPU based algorithm, takes on the order of 2 minutes. We achieve a spatial resolution of about 60 nm on actin filaments using SRRF, demonstrate diffusion with transient trapping for EGFR with diffusion coefficients of 0.2-0.3 $\mu m^2/s$, and demonstrate that ~35% of EGFR are dimerized in the absence of ligand. Generalized fluorescence imaging, in which images of multiple fluorescence parameters are extracted from a single recording, maximizes the extraction of information from experiments with improved reliability.

Acknowledgments: This work was supported by grants from the Singapore Ministry of Education (MOE2016-T3-1-005).

References: [1] Krieger, J.W., et al. *Nat Protoc.* **2015**, *10(12):*1948-74. [2] Gustafsson, N., et al. Nat. Com. **2016**, *7*:12471. [3] Unruh, J.R., et al. *Biophys. J.* **2011**, *95(11)*: 5385-98.

*Corresponding author: E-mail: twohland@nus.edu.sg

Quantified Membrane Permeabilization: Statistical Analysis of Fluorescence Lifetimes

Stefan Braun, [a] Anja Stulz, [a] Shuai Shi, [a] Ndjali Quarta, [a] Maria Hoernke*[a]

[a] *Pharmaceutical Technology and Biopharmacy, University of Freiburg, Hermann-Herder-Str.9, 79104 Freiburg i.Br., Germany*

In many therapeutic strategies like killing pathogenic microbes by antimicrobial peptides, drug delivery, endosomal escape, and in disease-related processes such as apoptosis, the membrane is permeabilized. Membrane permeabilization is often modelled by the release of an entrapped self-quenching fluorescent dye from lipid vesicles of varying lipid composition.

We employ time correlated single photon counting. Our statistical analysis of fluorescence lifetimes yields the strength of an individual membrane leakage event (Figure 1) [1] and replaces less stringent descriptions of dye leakage. The concept is applicable to many types of leakage events including thinning, defects, (toroidal) pores, or channels. Additionally, cumulative leakage kinetics can indicate certain membrane permeabilization mechanisms.[2]

Investigating antimicrobial peptide analogues with varying microbial selectivity, we could show how the leakage mechanism and strength of leakage events change with lipid composition. Thus, lipids play an important role for the selectivity of membrane permeabilization.

The quantitative description of leakage behavior and understanding of leakage mechanisms aid the design of membrane-active antimicrobials and increase the value of model studies for biological and biochemical processes at membranes.

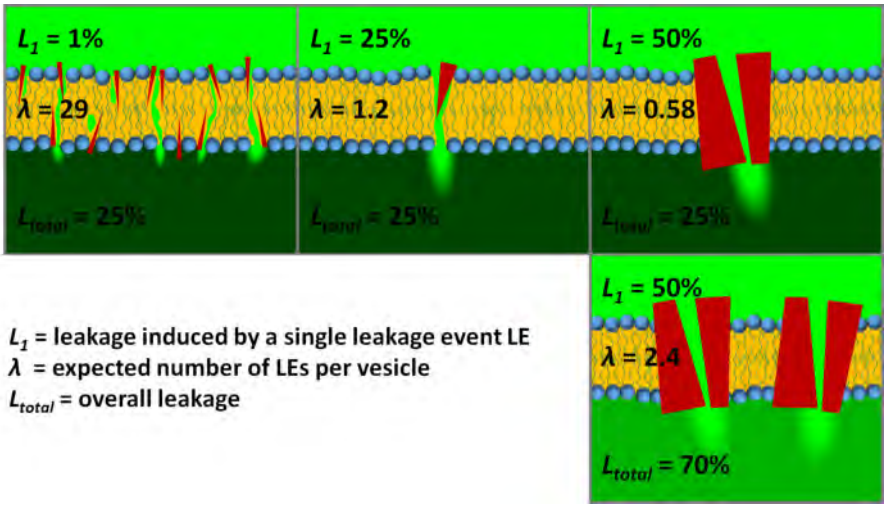


Figure. *Strength of individual leakage events L_1.*

References: [1] Braun, S. et al., *ACSnano* **2018**, *12*, 813. [2] Stulz, A., et al. Quantified membrane permeabilization indicates the lipid selectivity of membrane-active antimicrobials *submitted*

*Corresponding author: Maria.Hoernke@BIOSS.uni-freiburg.de

Visualizing the inner life of microbes by single-molecule localization microscopy

Endesfelder, Ulrike

Department of Systems and Synthetic Microbiology, Max Planck Institute for Terrestrial Microbiology and LOWE Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany

Microbes as single-cell organisms are important model systems to study cellular mechanisms and functions. In recent years and with the help of advanced fluorescence microscopy techniques, immense progress has been made in characterizing and quantifying the behavior of single bacterial cells on the basis of molecular interactions and assemblies in the complex environment of live cultures. Importantly, single-molecule imaging enables the *in vivo* determination of the stoichiometry and molecular architecture of subcellular structures, yielding detailed, quantitative, spatiotemporally resolved molecular maps and unraveling dynamic heterogeneities and subpopulations on the subcellular level. Nevertheless, open challenges remain. Here, I quickly review the past and current status of the field, discuss example applications from our own work and give insights in future trends [1, 2].

References: [1] Vojnovic, I., Winkelmeier, J. and Endesfelder, U., *Biochemical Society Transactions* **2019**, 10.1042/BST20180399 [2] Endesfelder, U., *Essays in Biochemistry* **2019**, 10.1042/EBC20190002

*ulrike.endesfelder@synmikro.mpi-marburg.mpg.de

Microsecond structural dynamics of bio-macromolecules revealed by two-dimensional fluorescence lifetime correlation spectroscopy

Tahei Tahara*[a, b]

[a] *Molecular Spectroscopy Laboratory, RIKEN, 2-1 Hirosara, Wako, Saitama 351-0198, Japan*
[b] *Ultrafast Spectroscopy Team, RIKEN Center for Advanced Photonics (RAP), 2-1 Hirosara, Wako, Saitama 351-0198, Japan*

Single-molecule spectroscopy, combined with fluorescence resonance energy transfer, is intensively utilized for studying structural heterogeneity and dynamics of protein, DNA and RNA on the sub-millisecond to second timescales. However, observation of the dynamics on the microsecond timescale is still very challenging due to the low efficiency of collecting photons from a single molecule. The microsecond timescale is important because the elementary processes of the structural change of bio-mactromolecules take place, and direct comparison between experiment and simulation is possible. To realize quantitative investigation of structural dynamics on the microsecond timescale, we developed a novel single molecule spectroscopy, i.e., two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS).[1] In 2D FLCS, we analyze the fluorescence lifetime from the donor of a FRET pair, instead of analyzing the FRET efficiency evaluated from the ratio between the donor and acceptor fluorescence photons. We first obtain the correlations of the photon pairs with respect to the excitation-emission delay times in the form of a two-dimensional (2D) map, and the 2D map is converted to the correlations between different species that have distinct fluorescence lifetimes using inverse Laplace transformation. 2D FLCS is capable of visualizing the structural fluctuation of complex molecules in equilibrium with microsecond time resolution at the single-molecule level. We have investigated the folding/unfolding dynamics of proteins, and clarified structural inhomogeneity and transitions between different structural ensembles.[2,3]

In this presentation, I report on technical extensions of 2D FLCS including implementation of the acceptor fluorescence information and the most recent study on structural dynamics of an RNA riboswitch.[4]

References: [1] Ishii, K. & Tahara, T., *J. Phys. Chem. B* **2013***, 117*, 11414 & 11423. [2] Otosu, T., et al. *Nat. Commun.* **2015**, *6*, 7685. [3] Otosu, T., et al. *J. Phys. Chem. B* **2017**, *121*, 5463. [4] Sarkar, B. et. al., in preparation.

*Corresponding author: E-mail: tahei@riken.jp

Molecular control of stem cell regulation in plants

Rebecca Corinna Burkart[a], Vivien Strotmann[a] and Yvonne Stahl*[a]

[a] *Institute for Developmental Genetics, Heinrich-Heine University, Düsseldorf, Germany*

Sessile multicellular organisms like higher plants have to adapt to a variety of biotic and abiotic challenges. Thus, plants have developed a remarkably high degree of plasticity and are able to generate new organs throughout their whole lifespan by maintaining groups of pluripotent stem cells in structures called meristems. The root system of higher plants originates from the activity of a root meristem comprising a group of highly specialized and long-lasting stem cells. Their maintenance and number is controlled by feedback signaling from differentiated cells involving peptide ligands and receptors [1,2].

Many plant transcription factors (TFs) could act as putative "stemness factors", because they are known to act as key regulators of stem cell homeostasis [3,4]. However, the exact molecular mechanisms of how the necessary tight but also dynamic regulation of the transition from stem cell fate to differentiation is conferred on a subcellular basis still remains largely unknown.

I will report on the recent advances in characterization of candidate stemness factors and their differential complexes in the regulation of root meristem maintenance by using different fluorescent techniques *in vivo*, e.g. FRET, FRAP and FCS. We propose a novel model of regulation where the dynamic differential subnuclear localization of TF complexes could act as a hub integrating external cues with stem cell regulation.

Acknowledgments: This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG).

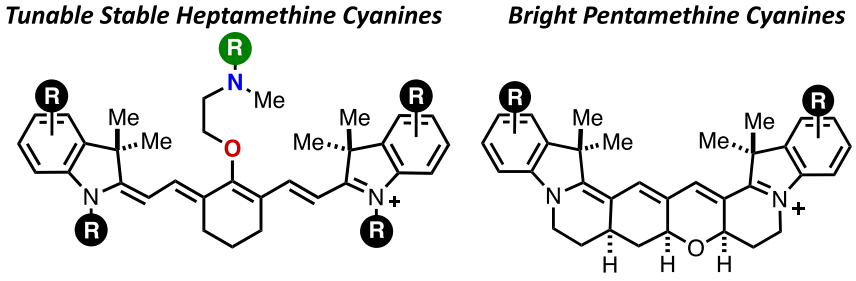
References: [1] Stahl, Y. et al., *Curr. Biol.* **2009***, 19*, 11. [2] Stahl, Y. et al., *Curr. Biol.* **2013**, *23*, 5. [3] Drisch, R.C. and Stahl, Y., *Frontiers Plant. Sci.* **2015**, *6*, 505. [4] Long, Y., Stahl, Y. et al. *Science* **2017**, 548, 7665

*Corresponding author: E-mail: yvonne.stahl@hhu.de

Harnessing Cyanine Reactivity to Prepare Novel Fluorophores for Advanced Imaging Applications

Martin J. Schnermann
Chemical Biology Laboratory, National Cancer Institute, Maryland, USA
martin.schnermann@nih.gov

Existing fluorescent probes derive from a small set of core scaffolds initially developed for various abiological dye applications, and subsequently applied for biomedical research with minimal synthetic modification. Consequently, there exists a significant opportunity to develop molecules specifically tailored for use in modern imaging applications. Cyanine fluorophores are among the most broadly used fluorescent probes, despite poor chemical stability and modest photon output. To address these limitations, we develop new synthetic transformations that modify the core polymethine chromophore unit. We have discovered a novel class of near-IR emitting heptamethine cyanines containing a C4'-O-alkyl substituent. These molecules are readily synthesized through an N- to O- transposition reaction. The new fluorophores exhibit excellent labeling properties, no covalent reactivity, and improved in vivo tumor uptake and signal compared to existing near-IR cyanines. These probes are also being using as organ-specific probes with direct application in certain abdominal surgical procedures. We have also shown that conformationally restrained pentamethine and heptamethine cyanines can be accessed through a multiple ring forming cascade reaction. The resulting far-red fluorophores exhibit improved fluorescence quantum yield (3- to 4-fold) and extended lifetime relative to typical pentamethine cyanines. Moreover, these fluorophores recover from hydride reduction with improved efficiency. These observations enable efficient single-molecule localization microscopy in oxygenated buffer without addition of conventional blinking buffers. Overall, these efforts involve a feedback loop between chemical studies focused on the design and synthesis of novel compounds and biological applications in advanced microscopy and in vivo imaging settings.



Multicolor Two-Photon Microscopy of Endogenous Fluorescence by Wavelength Mixing for Multiparametric Metabolic Imaging

C. Stringari *[a], L. Abdeladim [a], P. Mahou [a], G. Malkinson [a], S. Brizion [b], J-B. Galey [b], W. Supatto [a], R. Legouis [c], A-M Pena [b], E. Beaurepaire [a]

[a] *Ecole Polytechnique, Lab for Optics and Biosciences, CNRS, Palaiseau, France*
[b] *L'Oréal Research and Innovation, 93600 Aulnay-sous-Bois, France*
[c] *Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France.*

Multiphoton microscopy of endogenous fluorophores is emerging as an effective approach to study dynamic changes in cells and tissues metabolism in vitro and in vivo. Quantifying the metabolic coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) through optical redox ratio (FAD/(NADH+FAD)) and Fluorescence Lifetime Microscopy (FLIM) provides sensitive information on the relative balance between oxidative phosphorylation and glycolysis. However, multiphoton microscopy of endogenous fluorophores in live cells and tissues is limited by the challenge of efficient simultaneous multicolor imaging. Being able to efficiently image NADH and FAD simultaneously would make it possible to track physiological and pathological processes in dynamic systems. Endogenous fluorophores have non-overlapping two-photon absorption maxima in the range between 700 nm and 1000 nm and are present in a wide variety of concentrations in living tissues. Metabolic imaging of NADH and FAD is usually performed by sequential excitation at different wavelengths using a tunable laser, leading to difficulties in ensuring pixel-level registration between the channels in the case of a dynamic sample.
A distinct advantage of wavelength mixing for ratiometric measurements in a point-scanning multiphoton microscope is that the fluorescence images are intrinsically co-registered at the scale of the diffraction-limited excitation volume. [1] Here we present the application of wavelength mixing to the excitation of spectrally distinct endogenous fluorophores and optical redox ratio imaging in tissues and live zebrafish embryos, overcoming the difficulties associated with sequential excitation at different wavelengths such as motion artifacts. [2] Through simultaneous excitation of NADH and FAD in living tissues, reliable 2-photon ratiometric redox imaging and simultaneous FLIM of NADH and FAD is achieved with similar signal levels in both channels. We used this property to measure NADH and FAD lifetime gradients associated to cellular differentiation in living tissues such as reconstructed human skin and *c. elegans* germline. [2]

Acknowledgments: This work was supported in part by Marie Curie European Grant PIIF-GA-2013-629418 and by Agence Nationale de la Recherche under contracts ANR-10-INBS-04 France BioImaging, ANR-11-EQPX-0029 Morphoscope2 and ANR-15-CE11-0012-01 NLOMMIT.

References: [1] Mahou, P., et al. (2012). "Multicolor two-photon tissue imaging by wavelength mixing." *Nature Methods* 9 (8): 815–818 [2] C. Stringari, et al. "Multicolor two-photon imaging of endogenous fluorophores in living tissues by wavelength mixing," *Sci. Rep.* 7, 3792 (2017)

*Corresponding author: E-mail: chiara.stringari@polytechnique.edu

Multicolor Two-Photon Microscopy of Endogenous Fluorescence by Wavelength Mixing for Multiparametric Metabolic Imaging

C. Stringari *[a], L. Abdeladim [a], P. Mahou [a], G. Malkinson [a], S. Brizion [b], J-B. Galey [b], W. Supatto [a], R. Legouis [c], A-M Pena [b], E. Beaurepaire [a]

[a] *Ecole Polytechnique, Lab for Optics and Biosciences, CNRS, Palaiseau, France*
[b] *L'Oréal Research and Innovation, 93600 Aulnay-sous-Bois, France*
[c] *Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France.*

Multiphoton microscopy of endogenous fluorophores is emerging as an effective approach to study dynamic changes in cells and tissues metabolism in vitro and in vivo. Quantifying the metabolic coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) through optical redox ratio (FAD/(NADH+FAD)) and Fluorescence Lifetime Microscopy (FLIM) provides sensitive information on the relative balance between oxidative phosphorylation and glycolysis. However, multiphoton microscopy of endogenous fluorophores in live cells and tissues is limited by the challenge of efficient simultaneous multicolor imaging. Being able to efficiently image NADH and FAD simultaneously would make it possible to track physiological and pathological processes in dynamic systems. Endogenous fluorophores have non-overlapping two-photon absorption maxima in the range between 700 nm and 1000 nm and are present in a wide variety of concentrations in living tissues. Metabolic imaging of NADH and FAD is usually performed by sequential excitation at different wavelengths using a tunable laser, leading to difficulties in ensuring pixel-level registration between the channels in the case of a dynamic sample.
A distinct advantage of wavelength mixing for ratiometric measurements in a point-scanning multiphoton microscope is that the fluorescence images are intrinsically co-registered at the scale of the diffraction-limited excitation volume. [1] Here we present the application of wavelength mixing to the excitation of spectrally distinct endogenous fluorophores and optical redox ratio imaging in tissues and live zebrafish embryos, overcoming the difficulties associated with sequential excitation at different wavelengths such as motion artifacts. [2] Through simultaneous excitation of NADH and FAD in living tissues, reliable 2-photon ratiometric redox imaging and simultaneous FLIM of NADH and FAD is achieved with similar signal levels in both channels. We used this property to measure NADH and FAD lifetime gradients associated to cellular differentiation in living tissues such as reconstructed human skin and *c. elegans* germline. [2]

Acknowledgments: This work was supported in part by Marie Curie European Grant PIIF-GA-2013-629418 and by Agence Nationale de la Recherche under contracts ANR-10-INBS-04 France BioImaging, ANR-11-EQPX-0029 Morphoscope2 and ANR-15-CE11-0012-01 NLOMMIT.

References: [1] Mahou, P., et al. (2012). "Multicolor two-photon tissue imaging by wavelength mixing." *Nature Methods* 9 (8): 815–818 [2] C. Stringari, et al. "Multicolor two-photon imaging of endogenous fluorophores in living tissues by wavelength mixing," *Sci. Rep.* 7, 3792 (2017)

*Corresponding author: E-mail: chiara.stringari@polytechnique.edu

From single-molecule imaging to the brain: A circuitous route to new neural activity indicators

Luke D. Lavis*

Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, Virginia 20176, U.S.A.

Small molecules remain important tools to probe or perturb biological systems. Designing chemical reagents for modern neuroscience remains a significant challenge, however, since the brain is the most complex and least accessible organ in the body. My lab initially focused on molecular tools for neuroscience, but these efforts largely failed. Frustrated by the brain, we began developing reagents for cell biology with the goal of creating bright, cell-permeable dyes for single-molecule imaging. Inspired by computational experiments, we discovered that replacing the *N*,*N*-dimethylamino substituents in the classic dye tetramethylrhodamine with four-membered azetidine rings greatly improved brightness and photostability. The novel substitution is generalizable to fluorophores from different structural classes and enables fine-tuning of the dyes' spectral and chemical properties. This effort yielded a palette of fluorophores useful in live-cell single-molecule imaging experiments. We have since turned our focus back to the brain, learning that our dyes can be delivered to neurons *in vivo*, and tailoring new fluorophores for animal experiments. These bioavailable compounds enable the *in situ* construction of hybrid small-molecule:protein sensors with substantially higher brightness and photon yields compared to genetically encoded sensors, facilitating new functional imaging experiments to measure changes in voltage or [Ca^{2+}].

Acknowledgments: This work was supported by the Howard Hughes Medical Institute.

References: [1] Grimm, J. B. et al., *Nat. Methods* **2015***, 12*, 244. [2] Grimm, J. B. et al. *Nat. Methods* **2017**, *14*, 987. [3] Abdelfattah, A. S. et al. *bioRxiv* **2018**, 436840*,* doi: https://doi.org/10.1101/436840.

*Corresponding author: E-mail: lavisl@janelia.hhmi.org

Ultrasensitive detection of fluorescent nucleobase analogs via multiphoton excitation

Steven Magennis

School of Chemistry, University of Glasgow, University Avenue, Glasgow, G12 8QQ, UK

Fluorescence spectroscopy and imaging are powerful techniques for probing the structure, dynamics and interactions of nucleic acids. In this talk I will demonstrate the potential of new fluorescent base analogues (FBAs) for single-molecule fluorescence detection. Although FBAs have many desirable features in comparison to extrinsic labels they have not yet found application to ultrasensitive detection. Many of the disadvantages of FBAs arise from their short excitation wavelengths (often in the ultraviolet), making multiphoton excitation a potentially attractive approach.[1] It has now been shown that FBAs can have high two-photon (2P) brightness with increased photostability under 2P excitation compared with resonant absorption.[2] Using a multiphoton microscope with ultrafast excitation and pulse shaping, the limit of detection was pushed to a few molecules.[3] I will also present new results, which show that the ultimate, single-molecule level has now been reached for FBAs, opening up new possibilities to study nucleic acids.

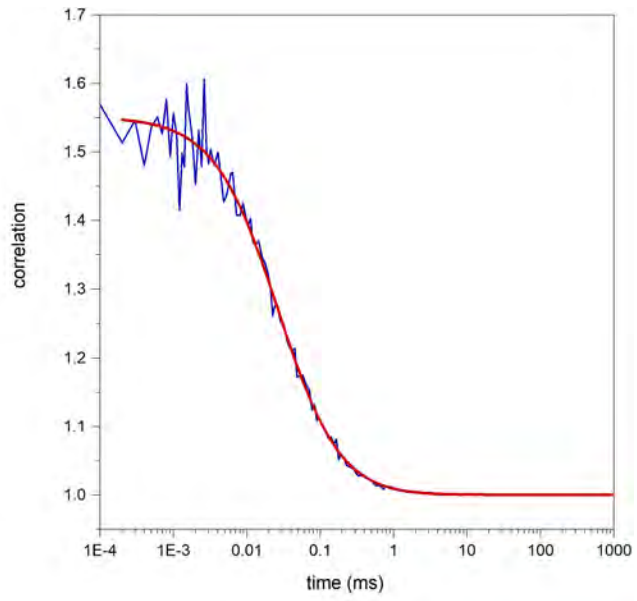


Figure 1. *Fluorescence correlation spectroscopy of a nucleobase analog (unpublished)*

Acknowledgments: This work was supported by grants from the EPSRC and BBSRC.

References: [1] Lane, R. S. K., et al. *ChemPhysChem* **2014**, *15*, 867. [2] Bood, M., et al. *Chem. Sci.* **2018**, *9*, 3494. [3] Fisher, R. S., et al. *Phys. Chem. Chem. Phys.* **2018**, *20*, 28487.

*Corresponding author: E-mail: steven.magennis@glasgow.ac.uk

Development of Universal Platform for Live Cell Discrimination through Gating Mechanism

Young-Tae Chang

POSTECH Chemistry and Center for Self-Assembly & Complexity, IBS
Email: ytchang@postech.ac.kr, Web: http://ytchang.postech.ac.kr/

Abstract: Conventional sensor development requires defining the target and design of sensor, which is so-called hypothesis driven approach. While powerful, this approach cannot be applied to unknown target or difficult to be applied to complex of analytes. To overcome the limitation, we have devised a Diversity Oriented Fluorescence Library Approach (DOFLA) where a combinatorial synthesis of fluorescent dye is combined with unbiased screening to accelerate the sensor development. More than 10,000 synthetic organic dyes were constructed as a tool box, and numerous analytes have been tested, yielding systematic platform for sensor development for almost everything. Complex or unknown target problem with biological systems were also challenged, and various cell type selective probes for live bioimaging were developed. In this presentation, especially the recently developed probe CDg16 for activated macrophages and its application to atherosclerosis will be mainly discussed with a novel gate-oriented mechanisms. The sensors and probes developed in this study will be freely available for the chemical and biological community for common usage

Single molecule FRET and photo-switched ligand reveal novel conformational pathway of GPCR activation

Ehud Isacoff

Helen Wills Neuroscience Institute and Department of MCB, University of California Berkeley, Berkeley CA 94720

Metabotropic glutamate receptors (mGluRs) are dimeric G-protein–coupled receptors (GPCRs) that operate at neural synapses. We use Foster resonance energy transfer (FRET), in live cells and on isolated protein at the single molecule level, to monitor the structural rearrangements of ligand-induced activation of the clamshell ligand binding domain (LBD) and Photoswitched Tethered Ligands (PTLs) to activate individual subunits in a timed manner. Our assays reveal unique cooperative interaction and a novel conformational pathway that tune sensitivity and efficacy and provide the receptor the ability to respond to glutamate released within a synapse and spillover from distant synapses.

Acknowledgments: This work was supported by grants from the National Institutes of Health.

E-mail: ehud@berkeley.edu

Genome-wide Single Cell Biophysics

Daniel Camsund[a], Michael J. Lawson[a,b], Jimmy Larsson[a], Daniel Jones[a], Spartak Zikrin[a], David Fange[a] & Johan Elf[a*]

[a] *Department of Cell and Molecular Biology, Uppsala University, Husarg. 3 Uppsala, Sweden*
[b] *Current Address: Division of Biology and Biological Engineering, Caltech, Pasadena, CA, USA*

Our ability to connect genotypic variation to biologically important phenotypes has been seriously limited by the gap between live cell microscopy and library-scale genomic engineering. Specifically, this has restricted studies of intracellular dynamics to one strain at a time and thus, generally, to the impact of genes with known function. Here we show how *in situ* genotyping of a library of *E. coli* strains after time-lapse imaging in a microfluidic device overcomes this problem. We determine how 235 different CRISPR interference knockdowns impact the coordination of the replication and division cycles of *E. coli* by monitoring the location of replication forks throughout hundreds of cell cycles per knockdown. The single-cell time-resolved assay allows us to determine the distribution of single-cell growth rates, cell division sizes, and replication initiation volumes. Subsequent *in situ* genotyping allows us to map each phenotype distribution to a specific genetic perturbation in order to determine which genes are important for cell cycle control. The technology enables genome-scale screens of virtually all live-cell microscopy assays and, therefore, constitutes a new approach to cellular biophysics.

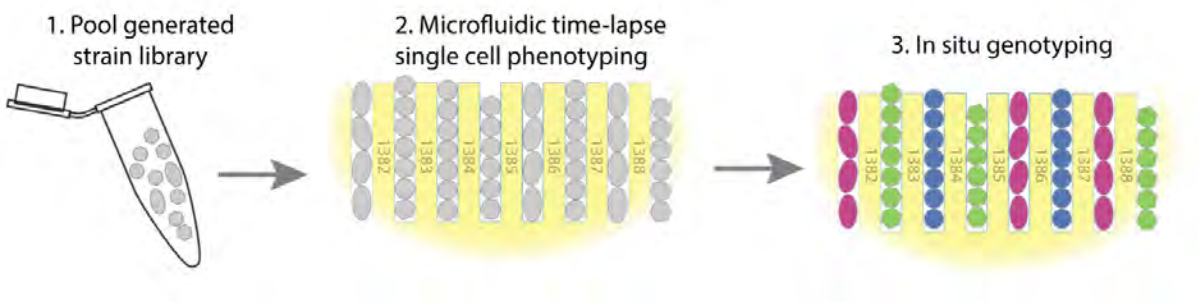


Figure. *Work flow for live cell phenotyping of a library of pool synthesized cell strains before in situ genotyping*[1]

.

Acknowledgments: This work was supported by Knut and Alice Wallenberg Foundation and the European Research Council.

Reference: [1] Lawson, M.J. et al. *Molecular Systems Biology* **2017** *13*, 947.

*Corresponding author: E-mail: johan.elf@icm.uu.se

Single molecule imaging of repeat RNA translation in live cells

Translation is the fundamental biological process that converts the genetic information in mRNAs into functional proteins. Dysregulation of translation leads to many cancer and neurodegenerative diseases. We have previously developed Single Molecule Imaging of Nascent Peptides (SINAPS) to assess translation of individual mRNAs in live cells. This approach allows direct measurement of initiation and elongation dynamics in vivo. We have applied the method to study canonical as well as aberrant translation process, which reveals rich spatial and temporal dynamics of translation process. For example, repeat expansion of GGGGCC in the intron region of C9orf72 gene is the most common cause of inheritable amyotrophic lateral sclerosis (ALS) and frontal temporal dementia (FTD). RNA containing the abnormally long GGGGCC repeats can form RNA foci, which may sequester essential RNA binding proteins. Alternatively, RNA containing repeats can be exported from nucleus and translated via repeat associated non-ATG (RAN) translation to generate different poly dipeptide repeats (DPR). The hexanucleotides are transcribed in both sense and antisense direction, which leads to two type of repetitive RNA and translation in six different reading frames. In patients the levels of DPR from each reading frame is different, which has been speculated resulting from difference in RAN translation efficiency. We used SINAPs to visualize and quantify the dynamics and heterogeneity of different dipeptides produced from C9orf72 repeat expansion. We found the nature of the intronic RNA species and the substrate of RAN translation. The molecular insights obtained through this study may help understanding the etiology of ALS and aid the therapeutic design.

A perspective on Roger Tsien's contribution to and impact on Chemical Biology

Gregor Zlokarnik

Department of Chemistry, Vertex Pharmaceuticals Inc., 3215 Merryfield Row, San Diego, CA 92121, USA

Roger Tsien was one of the founding fathers of a discipline now known as Chemical Biology. He introduced two new concepts to the field: the recording of the spatial distribution of biological messenger molecules and the progression from still-photos of tissue samples to filming messenger biology in action, resulting in dynamic movies of biological events. To achieve these advances, he taught himself chemistry, biochemistry, cell physiology, optics, mechanical engineering and informatics, and went on to invent all the components required to achieve his vision, the dynamic temporal and spatial measurement of Physiology in action.

Assembled from a comprehensive review of references that list Roger as a co-author, and subsequent papers citing them, I will present a framework to illustrate Roger's impact on Biology and Medicine.

Acknowledgments:

References: All references which list Roger Y. Tsien as co-author in SciFinder, and select references by lab members (~500 refs.).

*Corresponding author: E-mail: Gregor_Zlokarnik@vrtx.com

Novel fluorescent and thermogenetic tools

Carsten Schultz*[a,b]

[a] *Cell Biology & Biophysics Unit, European Molecular Biology Laboratory (EMBL), Meyerhofstr. 1, 69117 Heidelberg, Germany*
[b] *Department of Chemical Physiology & Biochemistry, Oregon Health and Science University, Portland, OR, USA*

The Schultz group has an interest in developing and applying novel techniques for visualization and manipulation of disease-relevant events such as protease and insulin secretion.

Cathepsin G is a serine protease secreted from activated neutrophils. In diseases with chronic lung inflammation, such as cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD), cathepsin G contributes to the well-described destruction of the extracellular matrix. Here, we introduce a set of FRET-based reporters able to monitor cathepsin G activity on the surface of neutrophils as well as on DNA (NETS) released from activated neutrophils. The reporter localization is targeted by specific molecular additions. We demonstrate that the FRET-based reporters are useful tools to measure cathepsin G activity on lung lymphocytes isolated from CF and COPD patient sputum.[1]

Failure of insulin secretion in response to elevated blood glucose levels is a hallmark of diabetes and new methods for artificially stimulating insulin secretion are in high demand. Insulin secretion is strongly regulated by intracellular calcium oscillations. Here, we report on a thermogenetic method involving snake temperature-sensitive calcium channels that translate heat or infrared (IR) light into calcium spikes in pancreatic ß-cells. The thermogenetic approach for the first time demonstrates that certain oscillatory patterns are required to efficiently stimulate insulin secretion.

Acknowledgments: This work was partly supported by the German Ministry for Education and Research (82DZL00401, 82DZL004A1) and the Molecular Medicine Partnership Unit shared by the EMBL and Heidelberg University Clinic.

References: [1] Guerra, M. et al., *ACS Cent. Sci.* **2019** *27*, 539-548.

*Corresponding author: E-mail: schulcar@ohsu.edu

From single-molecule imaging to the brain: A circuitous route to new neural activity indicators

Luke D. Lavis*

Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, Virginia 20176, U.S.A.

Small molecules remain important tools to probe or perturb biological systems. Designing chemical reagents for modern neuroscience remains a significant challenge, however, since the brain is the most complex and least accessible organ in the body. My lab initially focused on molecular tools for neuroscience, but these efforts largely failed. Frustrated by the brain, we began developing reagents for cell biology with the goal of creating bright, cell-permeable dyes for single-molecule imaging. Inspired by computational experiments, we discovered that replacing the *N*,*N*-dimethylamino substituents in the classic dye tetramethylrhodamine with four-membered azetidine rings greatly improved brightness and photostability. The novel substitution is generalizable to fluorophores from different structural classes and enables fine-tuning of the dyes' spectral and chemical properties. This effort yielded a palette of fluorophores useful in live-cell single-molecule imaging experiments. We have since turned our focus back to the brain, learning that our dyes can be delivered to neurons *in vivo*, and tailoring new fluorophores for animal experiments. These bioavailable compounds enable the *in situ* construction of hybrid small-molecule:protein sensors with substantially higher brightness and photon yields compared to genetically encoded sensors, facilitating new functional imaging experiments to measure changes in voltage or [Ca^{2+}].

Acknowledgments: This work was supported by the Howard Hughes Medical Institute.

References: [1] Grimm, J. B. et al., *Nat. Methods* **2015***, 12*, 244. [2] Grimm, J. B. et al. *Nat. Methods* **2017**, *14*, 987. [3] Abdelfattah, A. S. et al. *bioRxiv* **2018**, 436840*,* doi: https://doi.org/10.1101/436840.

*Corresponding author: E-mail: lavisl@janelia.hhmi.org

Illuminating the Biochemical Activity Architecture of the Cell

Jin Zhang, Ph.D.

University of California, San Diego

The complexity and specificity of many forms of signal transduction are widely suspected to require spatial microcompartmentation and dynamic modulation of the activities of signaling molecules, such as protein kinases, phosphatases and second messengers. We have developed a series of fluorescent biosensors to probe the compartmentalized signaling activities in living cells. In this talk, I will present several new fluorescent biosensors that we recently developed; I will then focus on cAMP/PKA and PI3K/Akt/mTORC1 signaling pathways and present studies where we combined genetically encoded fluorescent biosensors, superresolution imaging, targeted biochemical perturbations and mathematical modeling to probe the biochemical activity architecture of the cell.

Riboglow: a new tool for tagging and tracking RNA in live cells

Amy Palmer,[a] Esther Braselmann,[a] Colin Rathbun,[a] Amy Palmer*[a]

[a] *Department of Biochemistry, BioFrontiers Institute, University of Colorado, Boulder, CO, USA*

The complex spatiotemporal dynamics of messenger RNAs and non-coding RNAs affect virtually all aspects of cellular function. In addition to serving as the central intermediary between DNA and proteins, RNAs regulate gene expression at multiple levels, play roles in epigenetic regulation and genome organization, and serve as physical scaffolds to assemble and integrate macromolecular complexes. Despite the importance of RNA in biology and growing evidence of complex and dynamic localization patterns, robust tools for visualizing RNA molecules in live cells are limited. Recently, we developed an RNA imaging platform using the cobalamin riboswitch as an RNA tag and a series of molecular probes containing cobalamin as a fluorescence quencher. This highly modular 'Riboglow' platform leverages different color fluorescent dyes, linkers and riboswitch RNA tags to elicit fluorescent turn-on upon binding RNA.[1] We demonstrated the ability of two different Riboglow probes to track mRNA and small non-coding RNA in live mammalian cells. A side-by-side comparison revealed that Riboglow outperformed the dye binding aptamer Broccoli and performed on par with the gold standard RNA imaging system, the MS2-fluorescent protein system, while featuring a much smaller RNA tag. Recently, we have demonstrated that we can visualize and characterize the dynamics of single RNA molecules in living cells.[2] In this talk I will introduce the Riboglow platform and discuss our current approaches for creating orthogonal tags for simultaneous imaging of multiple RNA species, efforts to further engineer fluorescent turn on, and strategies for assessing whether RNA tags perturb underlying properties of the RNA.

Acknowledgments: This work was supported by grants from the NIH.

References: [1] Braselmann, E. et al., *Nature Chem. Bio.* **2018***, 14*, 964. [2] Braselmann, E. Y., et al. *bioRxiv* **2019**, *701649; doi: https://doi.org/10.1101/701649*.

*Corresponding author: E-mail: amy.palmer@colorado.edu

In vivo Multicolor Imaging with Fluorescent Probes Revealed Dynamics and Function of Osteoclast Proton Pumps

Masafumi Minoshima,[a] Yuta Omori,[a] Kazuya Kikuchi*[a,b]

[a] *Graduate School of Engineering, Osaka University, 2-1 Yamada-oka Suita City, Osaka 565-0871, Japan*
[b] *Immunology Frontier Research Center, Osaka University, 3-1 Yamada-oka Suita City, Osaka 565-0871, Japan*

In vivo fluorescence imaging is a powerful modality to monitor cell dynamics in biomedical studies [1],[2]. Despite considerable efforts for the development of molecular probes, present imaging methods still have certain limitations to clarify the protein function associated with cell activity. Here, a red-fluorescent small molecular probe was developed, namely Red-pHocas (Red pH-activatable fluorescent probe for osteoclast activity sensing), to reversibly detect the acidic environments and applied it to in vivo two-photon imaging to analyze the function of osteoclast proton pumps. The rational design of the probe structure successfully controlled the kinetics of the fluorescence response to acidic pH, which allowed rapid and reversible monitoring of acidic compartments upon osteoclastic bone resorption with the dynamics of osteoclast proton pumps. Multicolor two-photon imaging using Red-pHocas in fluorescent reporter mice revealed that bone acidification synchronously occurred with proton pump accumulation onto the bone surfaces, which first demonstrates the direct involvement of osteoclast proton pumps in bone resorption [3]. Our imaging system will provide a useful approach for studying protein function involved in cell activity under intravital condition.

Acknowledgments: Authors thank Professor Masaru Ishii and Professor Junichi Kikuta, Osaka University for helpful discussions.

Publications: [1] Kowada, T., et al. *J. Am. Chem. Soc.* **2011**, *133*, 17772. [2] Maeda, H., et al. *Nat. Chem. Biol.* **2016**, *12*, 579. [3] Minoshima, M. et al., *ACS Cent. Sci.* **2019**, *5*, in press. DOI: 10.1021/acscentsci.9b00220

*Corresponding author: E-mail: kkikuchi@mls.eng.osaka-u.ac.jp

Revealing Secrets Hiding in Plain Sight: Advances in Multi-scale Multi-modal Imaging:

Mark H. Ellisman, Ph.D.,
Distinguished Professor of Neurosciences; Director, the National Center for Microscopy and Imaging Research (NCMIR), UCSD
Senior Fellow, HHMI Janelia Research Campus

A grand challenge of modern biology is to better understand how molecular, cellular and tissue physiology plays out across a daunting range of spatial and temporal scales. Current imaging methods leave significant gaps in our knowledge, limiting our ability to connect information across scales. How multiple methods are now being combined to fill and help bridge critical gaps will be shared; including where recent advances to multi-tilt electron tomography (mtEMT) and development of new chemical and genetic probes for correlated light (LM), x-ray microCT (XRM), correlated multi-ion mass spectroscopy imaging (MIMS) and EM (MIMS-EM) and state-of-the-art 3D EM technologies. Examples of biological questions being addressed in ongoing projects will be described to illustrate how development and application of new contrasting methods, imaging tools and data analysis strategies are allowing the observation of otherwise complex or hidden relationships between cellular, subcellular and molecular constituents of cells.

Optogenetic and chemogenetic technologies for probing molecular and cellular interactions

Alice Y. Ting*[a]

[a] *Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA*

Spatial compartmentation underlies all cellular signaling, but existing methods to study the subcellular organization of endogenous proteins and RNA - by imaging and fractionation-mass spec for example - have important limitations. We developed an alternative approach, enzyme-catalyzed proximity labeling, for the high-resolution spatial mapping of subcellular proteomes and transcriptomes in living cells. I will describe the development of this approach, which includes enzyme directed evolution, and its application to uncover some new mitochondrial biology.

In the second part of the talk, I will describe synthetic protease-based optogenetic circuits that convert transient molecular events into stable cellular signals. I will give an example of how these tools can be used to access and study specific neuronal subpopulations that are activated during particular animal behaviors.

*Corresponding author: E-mail: ayting@stanford.edu

Probing lipids in living cells

Neal K. Devaraj*[a]

[a] *Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive La Jolla CA, USA*

Lipids remain one of the most enigmatic classes of biological molecules. Lipids were likely one of the first components necessary for life, yet our understanding of how lipid membranes could have arisen spontaneously is a mystery. Human cells produce thousands of unique lipid species, but the purpose for such diversity remains unknown. Dysregulation of lipid metabolism is a key factor in some of the most common diseases that afflict human beings. I will discuss our lab's efforts in using imaging and chemistry to understand the assembly and function lipids. I will discuss the development of chemoselective reactions that enable the manipulation and imaging of lipids within living cells during cell death and disease. Our ultimate goal is to answer fundamental questions about the origins of lipid membranes and build a functional understanding of the diverse array of lipids present in life today.

Acknowledgments: This work was supported by grants from the National Science Foundation and the National Institutes of Health.

*Corresponding author: E-mail: ndevaraj@ucsd.edu

The legacy of camgaroo: new fluorescent protein-based biosensors of neural activity and metabolism

Robert E. Campbell*[a,b]

[a] *Department of Chemistry, University of Alberta,* Edmonton, Alberta*, Canada*
[b] *Department of Chemistry, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan*

The Campbell research group is focused on the use of protein engineering for the development of fluorescent protein-based biosensors for imaging of cell signalling and metabolism. Protein engineering, using a combination of rational protein design and directed protein evolution, is the most effective and versatile approach for generating new genetically encoded fluorescent biosensors. Indeed, by exploiting iterative cycles of high-throughput fluorescence image-based screening of bacterial colonies, and lower throughput testing of promising variants in mammalian cells, the Campbell group has developed a growing selection of fluorescent protein-based biosensors with improved properties. In this seminar I will present some of our most recent efforts to engineer an improved generation of biosensors. Specifically, I will provide an update on the expanding palette of calcium ion biosensors, and describe how we are using similar engineering efforts to make bisensors for neurotransmitters, ions, and key metabolites.

Acknowledgments: This work was supported by grants from the NIH, Brain Canada, NSERC, CIHR, and JSPS.

*Corresponding author: robert.e.campbell@ualberta.ca, campbell@chem.s.u-tokyo.ac.jp

Roger Tsien's Impact on Drug Discovery

Paul Negulescu

Vertex Pharmaceuticals, Inc. 3215 Merryfield Row, San Diego CA, 92121

Roger Tsien's scientific impact includes many aspects of modern drug discovery. Most notably, optical detection of biological activity in live cells is now routinely deployed in the drug discovery process for a wide range of targets and disease indications. Attributes that made this industrialization possible include the sensitivity, reproducibility, simplicity and adaptability of the tools that Roger invented. The lecture will provide an overview of Roger's impact on drug discovery and discuss several programs at Vertex that applied fluorescence assays to the discovery of human therapeutics.

Oral Presentation Abstracts

Towards Diffusion-Free Triplet-Triplet Annihilation Photon Up-conversion

Bo Albinsson,* Victor Gray, Fredrik Edhborg, Betül Küçüköz, Kasper Moth-Poulsen, and Maria Abrahamsson

Department of Chemistry and Chemical Engineering, Chalmers University of Technology, 412 96 Göteborg, Sweden

More than 99% of all renewable energy resources near the Earth's surface are available in the form of solar radiation. A one percent efficiency increase of direct conversion of solar energy will therefore enhance the theoretical available renewable energy by as much as the potential of all other renewables, including wind, bio, hydro, ocean and geothermal energy, taken together. Our aim is to harvest the untapped parts of the solar spectrum and to transform it into useful photons or charge carriers. Down-conversion through singlet fission and up-conversion through triplet-triplet annihilation are two photophysical processes that have potential to dramatically increase the theoretical limits for *any* solar energy process. In this presentation, I will show some of our attempts towards diffusion-free triplet-triplet annihilation up-conversion (TTA-UC). By using a supramolecular approach where the sensitizers are non-covalently bound to a dendrimer annihilator we have addressed the challenge with a systematic approach in which all molecular processes could be studied and optimized separately. [1-9]

Acknowledgments: This work was supported by grants from the Swedish Research Council and the Swedish Energy Agency.

References: [1] K. Börjesson, D. Dzebo, B. Albinsson, K. Moth Poulsen, *Journal of Materials Chemistry A* 2013, *1*, 8521-8524. [2] V. Gray, K. Borjesson, D. Dzebo, M. Abrahamsson, B. Albinsson, K. Moth-Poulsen, *Journal of Physical Chemistry C* 2016, *120*, 19018-19026. [3] D. Dzebo, K. Moth-Poulsen, B. Albinsson, *Photochemical & Photobiological Sciences* 2017, *16*, 1327-1334. [4] V. Gray, A. Dreos, P. Erhart, B. Albinsson, K. Moth-Poulsen, M. Abrahamsson, *Physical Chemistry Chemical Physics* 2017. [5] V. Gray, D. Dzebo, M. Abrahamsson, B. Albinsson, K. Moth-Poulsen, *Physical Chemistry Chemical Physics* 2014, *16*, 10345-10352. [6] V. Gray, D. Dzebo, A. Lundin, J. P. Alborzpour, M. Abrahamsson, B. Albinsson, K. Moth-Poulsen, *Journal of Materials Chemistry C* 2015. [7] V. Gray, K. Moth-Poulsen, B. Albinsson, M. Abrahamsson, *Coordination Chemistry Reviews* 2018, *362*, 54-71. [8] V. Gray, B. Kucukoz, F. Edhborg, M. Abrahamsson, K. Moth-Poulsen, B. Albinsson, *Physical Chemistry Chemical Physics* 2018, *20*, 7549-7558. [9] D. Dzebo, K. Borjesson, V. Gray, K. Moth-Poulsen, B. Albinsson, *Journal of Physical Chemistry C* 2016, *120*, 23397-23406.

*Corresponding author: E-mail: balb@chalmers.se

Novel Method for Multidimensional Fluorescence Characterization of Protein Binding and Conformational Changes

Karen E. Steege Gall,*[a] Alex Siemiarczuk[a]

[a] *Fluorescence Division, HORIBA Instruments Inc., 20 Knightsbridge Rd. Piscataway, NJ 08807 USA*

We use novel fluorescence techniques to elucidate the binding mechanism and energy transfer between bovine serum albumin (BSA) protein and 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS). While this is a common experiment to demonstrate protein binding kinetics, the simultaneous use of a CCD detector and an absorbance photodiode allows for complete characterization in multiple spectral dimensions. These techniques include full fluorescence spectral kinetics measured simultaneously with absorbance. Fluorescence anisotropy is also used to measure changes in local reorientation of 1,8-ANS and BSA Trp residues and aids greatly in interpreting conformational changes upon SDS-induced unfolding. Fluorescence lifetimes are then used to further characterize the conformational changes and their effect on the Förster resonance energy transfer (FRET) between tryptophan residues in the BSA to 1,8-ANS in both native and unfolded protein. The novel instrumentation with multidimensional approach to measure full spectral kinetics allows a broader picture of the binding, changes in local environment, as well as energy transfer between 1,8-ANS and BSA.

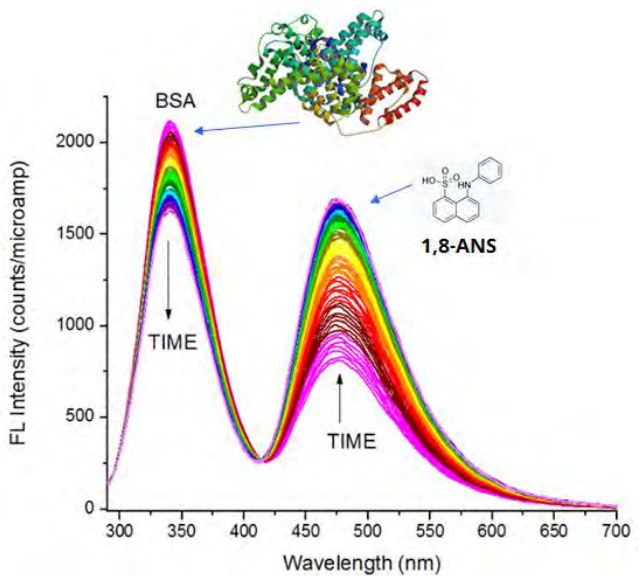


Figure. *Fluorescence Spectral Kinetics of binding an and energy transfer from BSA protein to 1,8-ANS. Structure of BSA.*[1]

Acknowledgments: This work was supported by HORIBA Scientific

References: [1] Jablonska, K., et. al., *Mol. Immuniol.* **2012**, *52*, 174.

*Corresponding author: E-mail: karen.gall@horiba.com

Raster spectral image correlation spectroscopy: principle and application to the glycine receptor

Lemmens Veerle,[ab] Waldemar Schrimpf,[c] Brône Bert, [a] Hofkens Johan,[b] Hendrix Jelle*[ab]

[a] *Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium*
[b] *Department of Chemistry, KULeuven, Leuven, Belgium*
[c] *Department of Chemistry, Ludwig-Maximilians-Universität, Munich, Germany*

In this talk I will introduce the application of spectral weighting in multicolor fluorescence data. Similar to linear unmixing it removes spectral bleedthrough, only it performs excellently even to describe dynamic processes in low-intensity live samples. We successfully applied this new method, called raster spectral image correlation spectroscopy (RSICS)[1], to resolve dyes with very similar emission spectra. Furthermore, using RSICS we were the first to do robust three-color RICS experiments in living cells allowing to simultaneously quantify molecular concentration, mobility and interaction affinity.

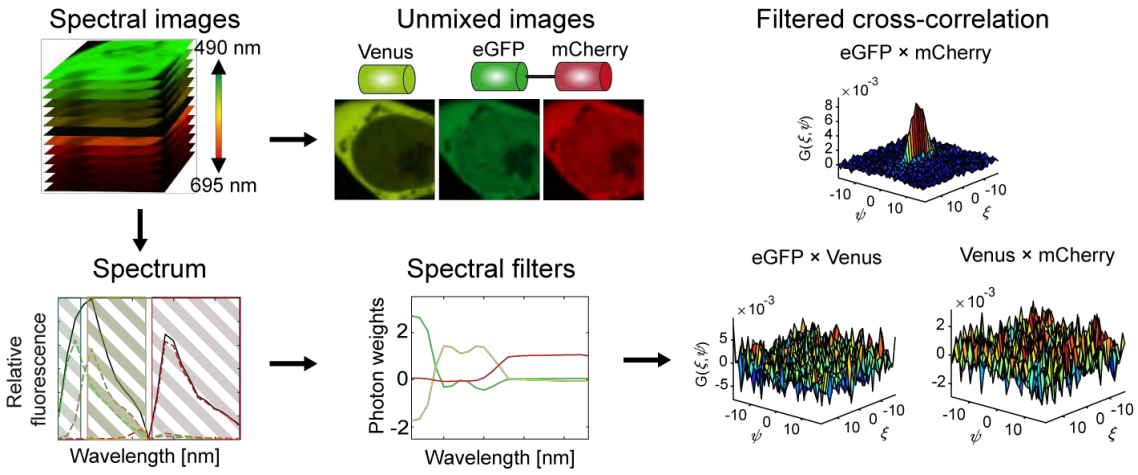


Figure. *Spectral weighting reveals true interaction in multicolor experiments*

Recently, we used RSICS to demonstate hetero-pentamerization of two splice variants of the glycine receptor (GlyR) a3 isoform involved in suppression of chronic pain and temporal lobe epilepsy. Furthermore, we complement our findings with single-molecule localization microscopy and investigate the effect on its structure and activity via combined patch clamp and fluorescence using non-natural amino acid click labeling.

Acknowledgments: This work was supported by grants from the BOF research funds of Hasselt University.

References: [1] Schrimpf, Waldemar et al., *Methods.* **2018**, *140-141*, 97-111.

*Corresponding author: E-mail: jelle.hendrix@uhasselt.be

Fluorescence Window Reveals the Population Distribution During Collapse and Folding of a Small Protein

Sandhya Bhatia[a], G. Krishnamoorthy[b]*, Deepak Dhar[c]* and Jayant B. Udgaonkar[a,c]*

[a] *National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru 560 065, India*

[b] *Department of Biotechnology, Anna University, Chennai 600 025, India*

[c] *Indian Institute of Science Education and Research, Pune 411008, India*

Revelation of the population distribution during the collapse and folding of a polypeptide chain is essential for understanding the mechanism by which structure develops during protein folding. Here, the folding process of the small protein, monellin was investigated using the time-resolved fluorescence resonance energy transfer (trFRET) technique. Decay kinetics of fluorescence intensity was used to determine the time evolution of the distribution of an intramolecular distance, using Maximum Entropy Method of analysis, when folding of monellin[1] was initiated in a stopped-flow mixer. At the earliest measurable time of folding (100 ms) in a double-kinetics set-up, a bimodal distribution of the intramolecular distances was seen, which indicated the presence of two distinct sub-populations. One of them is a molten globule intermediate (I_{MG}), which is nearly as compact as the native state, but is devoid of native-like secondary structure. The second population (named U_X) has a size slightly smaller than the unfolded state, but seems to be a metastable state that transforms to the I_{MG} state by a slow activated process. Both the populations contract continuously and slowly as structure develops. A phenomenological model of Markovian evolution of the polymeric chain undergoing folding, incorporating these features, that fits well the experimentally observed time evolution of distance distributions, has been developed.

References: [1] Bhatia, S., Krishnamoorthy, G. and Udgaonkar, J. B. *Phys. Chem. Chem. Phys.* **2018**, *20*, 3216–3232.

*Corresponding authors: E-mail: gk@tifr.res.in, deepak@iiserpune.ac.in, jayant@iiserpune.ac.in

.

Multiplexed *in vivo* optical imaging with shortwave infrared polymethine dyes

Emily D. Cosco,[a,b] Anthony L. Spearman,[a] Shyam Ramakrishnan,[b] Jakob G. P. Lingg,[b] Monica Pengshung,[a] Sarah Glasl,[b] Mara Saccomano,[b] Martin Warmer ,[b] Ryan R. McLaughlin,[a] Oliver T. Bruns,*[b] Ellen M. Sletten*[a]

[a] *Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA, 90095, USA*
[b] *Helmholtz Pioneer Campus, Helmholtz Zentrum München, D-85764 Neuherberg, Germany*

Organic fluorophores developed for the visible (400-700 nm) and NIR (700-1000 nm) regions of the electromagnetic spectrum have enabled multiplexed experiments to study live cells and simple organisms with high resolution. In more complex systems such as mammals, limitations in contrast and resolution result from diffuse light originating from background and scattering at visible and near-infrared (NIR) wavelengths.[1] By moving to detection in the shortwave infrared region (1000-2000 nm), high resolution images can be obtained non-invasively at video-rate speeds.[2] While the optical properties of tissue are ideal for SWIR imaging, non-toxic organic small molecules with bright emission > 1000 nm are necessary to obtain high signal with minimal biological perturbation. Polymethine dyes are a prime scaffold for creating SWIR probes, as they can be red-shifted with predictable structural changes. We hypothesized that a flavylium heterocycle, containing no heavy atoms and with an electron donating group, would impart red-shifted photophysical properties without diminished Φ_F.[3] With absorbance and emission in the SWIR, the 7-dimethylamino flavylium heptamethine dye is approximately 200 nm red-shifted from heptamethine cyanine dyes traditionally employed for optical imaging at NIR wavelengths. Building on this scaffold, we investigated systematic changes to the flavylium heterocycle to reveal insight into relationships between structure and photophysical properties. By accessing a library of SWIR dyes with consistent brightness, we demonstrated a method which improves the spatial and temporal resolution attainable for non-invasive multicolor imaging in mice.

Acknowledgments: This work was supported by grants to E.D.C (NSF GRFP DGE-1144087, Foote Fellowship), O.T.B. (Emmy-Noether-Programme of DRG BR 5355/2-1, Helmholtz Pioneer Campus Institute for Biomedical Engineering), E.M.S. (UCLA, NIH 1R01EB027172-01, Sloan Research Award FG-2018-10855,), and by shared instrumentation grants from the NSF (CHE-1048804) and NIH (1S10OD016387-01).

References: [1] Thimsen, E. *et al*. *Nanophotonics* **2017**, *6*, 1043. [2] Bruns, O. T. *et al. Nat. Biomed. Eng.* **2017**, *1*, 0056. https://doi.org/10.1038/s41551-017-0056. [3] Cosco, E. D. *et al. Angew. Chemie Int. Ed.* **2017**, *56*, 13126.

*Corresponding author: E-mail: sletten@chem.ucla.edu

Pheomelanin sheet structure revealed by ThT fluorescence

David J S Birch,* Alastair D Davy

Department of Physics, SUPA, University of Strathclyde, 107 Rottenrow, Glasgow G40NG, UK

Despite many decades of research important questions remain over the structure of both the brown/black pigment eumelanin and the red/yellows pigment pheomelanin, not least because of their role in melanoma, the most virulent form of skin cancer. Both melanins are thought to possess ill-defined heterogenic polymeric structures consistent with their complex absorption spectra and weak intrinsic fluorescence. The evidence for eumelanin forming a π-stacked sheet structure akin to graphite is strong, but whether or not this translates to pheomelanin has remained unresolved.

Here we report evidence from fluorescence of the rotor probe thioflavin T (ThT) that pheomelanin does indeed also form sheet structures[1]. As pheomelanin is synthesized from L-cysteine, L-DOPA and the enzyme tyrosinase the fluorescence intensity of ThT increases as a sigmoidal function akin to that for beta-amyloid aggregation[2] and previously reported for eumelanin[3]. The fluorescence decay also supports this conclusion with one of the three components needed to describe the decay increasing to ~ 5.5 ns. This component is ascribed to close coupling of ThT to pheomelanin's sheet structure as it is in excess of the 3.4 ns radiative decay of ThT[4]. Disassembly studies by raising pH[5] will also be presented. These suggest that ThT is trapped in extensive pheomelanin sheet structures not fragments of the sheets.

Melanin sheet fragmentation is a possible indicator of melanoma, and fluorescence monitoring of the extent of melanin sheet structure will be discussed with respect to finding new approaches for detecting and monitoring the progression of the disease.

Acknowledgements: The authors acknowledge the support of the National Physical Laboratories and an OPTIMA research studentship for ADD.

References:

[1] Davy, A. D. and Birch, D. J. S., *Appl. Phys. Letts*. **2018**, *113*, 263701. [2] Benseny-Cases, N. et al., *Biochem. Biophys*. *Res. Commun*. **2007**, *361*, 916. [3] Sutter, J. U., et al. *Appl. Phys. Letts*. **2013**, *100*, 113701. [4] Stsiapura V. I. et al., *J. Phys. Chem. B*. **2008**, *112*, 15893. [5] Ju, K., et al., *Biomacromolecules* **2016**, *17*, 2860.

*Corresponding author: E-mail: djs.birch@strath.ac.uk

Photoactivation of silicon rhodamines via a light-induced protonation

Michelle S. Frei,[a, b] Luc Reymond,*[c, d] Kai Johnsson*[a, b, d]

[a] *Department of Chemical Biology, Max Planck Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany*
[b] *Institute of Chemical Sciences and Engineering (ISIC), École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland*
[c] *Biomolecular Screening Facility, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland.*
[d] *National Centre of Competence in Research (NCCR) in Chemical Biology, 1015 Lausanne, Switzerland*

Photoactivatable fluorophores are important tools to investigate dynamic processes in cells. With the advent of super-resolution microscopy techniques based on single-molecule localization, these fluorophores have found even more applications. However, to make these techniques available for routine live-cell imaging, brighter, cell-permeable fluorophores are required. In our research, we develop new photoactivatable synthetic fluorophores based on the silicon rhodamine scaffold. This class of fluorophores has ideal properties for live-cell imaging: excitation and emission maxima in the far-red, high extinction coefficient, high quantum yield, photostability and cell-permeability. Instead of using bulky photolabile groups, we made use of photochemical concepts that require smaller structural modifications and generated a far-red photoactivatable fluorophore. The unusual mechanism of photoactivation and the fluorophore's outstanding spectroscopic properties make it a powerful tool for live-cell super-resolution microscopy. We showed that this fluorophore can be used not only in fixed-cells, but also for following the fast dynamics of mitochondria by single-molecule localization microscopy in live-cells. Most excitingly, we could distinguish the unlabeled interior of the mitochondria from their labeled outer membrane in several cases, showcasing the power of this probe in combination with super-resolution microscopy.

Acknowledgments: This work was supported by the Max Planck Society, the École Polytechnique Fédérale de Lausanne, a grant from the Swiss Commission for Technology and Innovation (CTI) and the NCCR Chemical Biology.

*Corresponding author: E-mail: johnsson@mr.mpg.de

Pyrene derivatives for single-molecule chemistry

Gregor Jung,*[a]

[a] *Biophysical Chemistry, Saarland University, Campus B2 2, 66123 Saarbruecken, Germany*

Hydroxy- and ammoniumpyrene derivatives are known to show an acidity increase upon electronic excitation, and excited-state acidities as strong as concentrated sulfuric acid are reachable.[1,2] The inherent dual emissivity together with the high chemical stability and the favorable fluorescence properties makes these dyes versatile substrates for enzymes with ratiometric readout. With proper design of substituents, three fluorescence colors can be obtained from one starting compound as a result of enzymatic cleavage.[3]

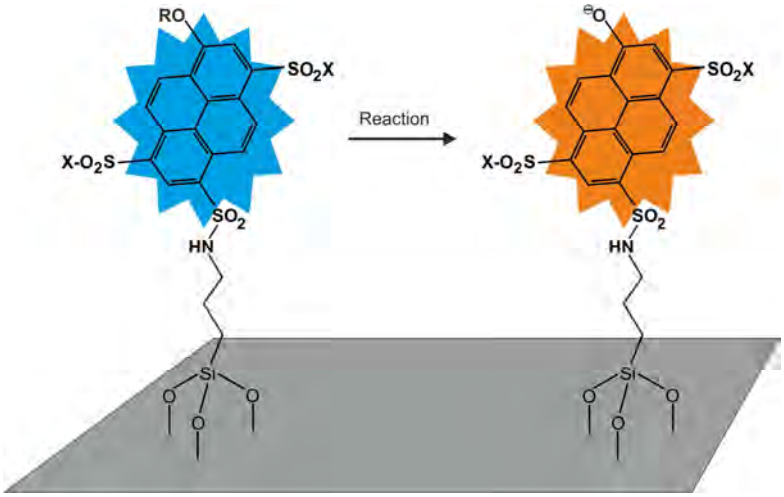


Figure: *Approach for single-molecule chemistry*

The same strategy is now transferred to substrates for metal-organic catalysis. We combined hydroxypyrene derivatives with a protection unit and observed a change in the fluorescence color upon Pd(0) deallylation.[4] Silanization leads to immobilized substrates which are then traceable by single-molecule fluorescence microscopy (figure).
In my presentation, I will report about our recent experiments with simultaneous detection of substrate and product emission and compare the outcome with the study using a fluorogenic approach.[4] Moreover, I will discuss how a single, reversible chemical reaction can be studied multiple times and what we can learn about the influence of the surrounding.

Acknowledgments: Generous support is provided by the German Science Foundation (DFG JU650/7-1 and 10-1).

References: [1] Finkler, B. et al., *Photochem. Photobiol. Sci.* **2014**, *13*, 548. [2] Maus, D. et al., *J. Phys. Chem. A* **2018**, *122*, 9025. [3] Finkler, B. et al., *Photochem. Photobiol. Sci.* **2016**, *15*, 1544. [4] Menges, J. et al., *Langmuir* **2019**, *35*, 2506.

*Corresponding author: E-mail: g.jung@mx.uni-saarland.de

An Activity-based Fluorogen for Quantifying Intracellular Polarity of Protein Environment

Tze Cin Owyong,[a,b] Wallace W. H. Wong,[b] Yuning Hong[a*]

[a] *Department of Chemistry and Physics, La Trobe Institute for Molecular Science, La Trobe University, VIC 3086, Australia.*
[b] *School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, VIC 3010, Australia.*

Maintaining proteostasis is an essential housekeeping function for cell survival. It involves chaperones and degradative pathways to ensure proteins fold correctly and to remove those that are misfolded, damaged or aggregated. In principle, proteostasis is affected in any disease that involves misfolded or mutant proteins that do not fold with normal efficiencies; and hence overdraw on the finite proteostasis resources of the cell. Tracking the proteostasis capacity of cells has the generic potential to track neurodegenerative diseases with diverse specific molecular origins. Hence, building new approaches to identify the efficiency of proteostasis is highly desired in order to track the risk of cells succumbing to damage from protein misfolding and aggregation. Previously we have demonstrated a cysteine-reactive aggregation-induced emission fluorogen is capable of reporting unfolded protein load as a measure of proteostasis capacity in cells.[1] In this project, we synthesized a new probe which can not only report on the proteostatic stress but also allow us to map the polarity changes accompanying with protein unfolding by using spectral phasor analysis method. Our results show huge change in the intracellular protein polarity when stress response is activated. We also applied this method to investigate disease-associated proteostasis collapse process.

Acknowledgments: This work was supported by grants from the Australian Research Council (DE170100058) and Rebecca L. Cooper Medical Research Foundation (PG2018043).

Reference: [1] Chen, M. Z. et al. *Nat. Commun.* **2017**, *8*, 474.

*Corresponding author: E-mail: y.hong@latrob.edu.au

Upconverting nanoparticle as optical transducers of biomechanical forces

Claire A. McLellan,*[a] Chris Siefe,[a] Stefan Fischer,[a] Alice Lay[b], Jason Casar,[a] Jennifer A. Dionne[a]

[a] *Department of Materials Science and Engineering, Stanford University, Stanford, CA*
[b] *Department of Applied Physics, Stanford University, Stanford, CA*

Mechanical forces play a critical role in biological functions, ranging from muscle contractions to immune-system intracellular communication to motor protein transport. While the improper functioning of these mechanical forces can lead to disease, it is currently difficult to detect and study mechanical forces in vivo. For example, FRET sensors provide a powerful force sensing technique but bleach after extended use; similarly, techniques such as atomic force microscopy and traction force microscopy cannot be deployed between cells. Here, I will discuss our work developing a novel optical probe of mechanical forces based on rare-earth upconverting nanoparticles (UCNPs). Our UCNPs absorb near-infrared wavelengths and emit visible wavelengths with a ratiometric color response that depends on the applied pressure. For optimal sensitivity, we are developing UCNPs with host lattices based on group II alkaline earth atoms (CaLuF, SrLuF, BaLuF) [1]. These particles are sub-15nm in diameter and are doped with 30% Yb^{3+} and 2.9% Er^{3+} to produce bright and sensitive force sensors. Using both diamond anvil cell measurements and atomic force microscopy, we quantify the force responses, showing that SrLuF particles able to detect pressures down to 37 MPa and forces down to 27 nN [2]. Finally, we discuss the cytotoxicity of these particles and their in vivo deployment, focusing on the rhythmic contractions of the digestive tract within the roundworm *C. elegans* [3].

Acknowledgments: This work is supported by grants from the National Institutes of Health and the Gordon and Betty Moore Foundation

References: [1] Fisher, S. *et al Nano Lett.* **2019** 1963878-3885 [2] McLellan, C. A. *et al In Preparation* [3] Lay, A. *et al* ACS Central Science **2019** accepted

*Corresponding author: E-mail: camcl@stanford.edu

Functional Fluorescence Microscopy Imaging (fFMI). Quantitative scanning-free confocal fluorescence microscopy for characterization of fast dynamic processes in live cells

Aleksandar J. Krmpot,[a,b] Stanko N. Nikolić,[a,b] Sho Oasa,[a] Marco Vitali,[c,d] Dimitrios K. Papadopoulos,[e] Makoto Oura,[f] Shintaro Mikuni,[f] Per Thyberg,[g] Simone Tisa,[h] Masataka Kinjo,[f] Lennart Nilsson,[i] Lars Terenius,[a] Rudolf Rigler,[a,j] Vladana Vukojević*[a]

[a] *Department of Clinical Neuroscience (CNS), Center for Molecular Medicine (CMM), Karolinska Institutet, 17176 Stockholm, Sweden*
[b] *Institute of Physics, University of Belgrade, 11080 Belgrade, Serbia*
[c] *Department of Chemistry, Berlin Institute of Technology, 10623 Berlin, Germany*
[d] *Omicron Energy Solutions GmbH, 12099 Berlin, Germany*
[e] *Max-Planck Institute for Molecular Cell Biology and Genetics, 01307 Dresden, Germany*
[f] *Laboratory of Molecular Cell Dynamics, Faculty of Advanced Life Science, Hokkaido University, Sapporo, 001-0021, Japan*
[g] *AlbaNova University Center, Royal Institute of Technology, Department of Applied Physics, 106 91 Stockholm, Sweden*
[h] *Micro Photon Devices (MPD), 39100 Bolzano, Italy*
[i] *Department of Biosciences and Nutrition, Karolinska Institutet, 14183 Huddinge, Sweden*
[j] *Department of Medical Biochemistry and Biophysics (MBB), Karolinska Institutet, 17177 Stockholm, Sweden*

Quantitative confocal fluorescence microscopy imaging without scanning is developed for characterization of fast dynamical processes. The method is based on massively parallel Fluorescence Correlation Spectroscopy (mpFCS). Simultaneous excitation of fluorescent molecules in multiple spots in the focal plane is achieved using a Diffractive Optical Element (DOE). Fluorescence from the thus generated 1024 illuminated spots is detected in a confocal arrangement by a matching matrix detector consisting of 32×32 single-photon avalanche photodiodes (SPADs), enabling quantitative imaging at a rate of 21 µs/frame. Software for data acquisition and fast auto- and cross-correlation analysis by parallel signal processing using a Graphic Processing Unit (GPU), allows temporal autocorrelation across all pixels in the image frame in 4 s and cross-correlation between first and second order neighbors in 45 s. Usefulness of this approach for the study of spatio-temporal integration of fast biochemical processes and for fluorescence lifetime imaging microscopy (FLIM) is demonstrated in live cells/tissue.

Acknowledgments: We gratefully acknowledge financial support from The Knut and Alice Wallenberg Foundation (grant KAW 2011.0218), the Swedish Research Council (grants VR 2016-01922 and 2018-05337) and the Foundation for Strategic Research (SBE13-0115). A. J. Krmpot and S. N. Nikolić gratefully acknowledge financial support from the Rajko and Maj Đermanović Fund and the Ministry of Education and Science of the Republic of Serbia (Grants No. III45016 and OI171038). D. K. Papadopoulos was supported by a Federation of European Biochemical Societies (FEBS) postdoctoral fellowship.

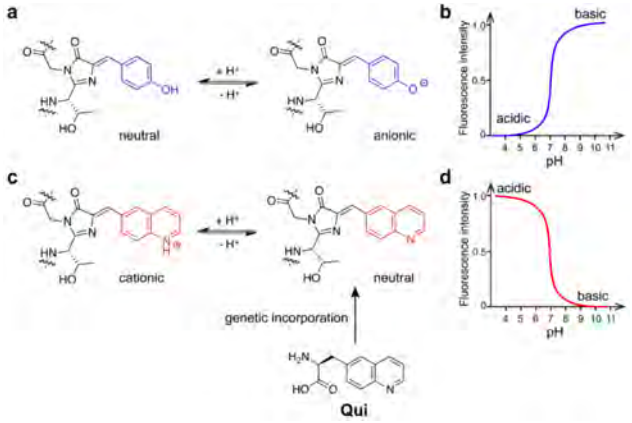
*Corresponding author: E-mail: vladana.vukojevic@ki.se

Nano-switches for optogenetic control and a fluorescent reporter for acidic vesicles

Lei Wang*[a]

[a] *Department of Pharmaceutical Chemistry and the Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA, USA*

The ability to control protein function with light provides excellent temporal and spatial resolution for precise investigation *in situ*, and thus is having significant impact on biological studies. We aim to develop a nano-switch technology for optical control of proteins in their native settings with general applicability and ultra-specificity. Through the expansion of the genetic code, we site-specifically incorporated photo-reversible unnatural amino acids (Uaas) into proteins to modulate a single site, and to build novel nano-bridges onto proteins in order to modulate secondary structures and domains, so as to photo-regulate protein activities in a reversible manner. We build such nano-bridges by developing new latent bioreactive Uaas that can react with different natural amino acid residues on proteins in situ. In another project, we unexpectedly discovered that a quinoline-bearing amino acid enabled the green fluorescent protein to fluoresces strongly at acidic pH but remain nonfluorescent at or above neutral pH, boasting a pH profile opposite to that of common fluorescent proteins. The resultant acid-brightening fluorescent protein (abFP) provides distinct fluorescent images for acidic vesicles without background fluorescence from other cellular regions. We expect that abFP will be valuable for studying acidic vesicles and organelles, which play fundamental roles in a broad range of cellular events such as endocytosis, lysosomal degradation, synaptic transmission, pathogen fate, and drug delivery.



Acknowledgments: This work was supported by grants from NIH (R01GM118384, RF1MH114079).

References: [1] Kang, J. et al., *Neuron* **2013***, 80*, 358. [2] Hoppmann, C. et al., *Angew. Chem. Int. Ed.* **2014***, 53*, 3932. [3] Hoppmann, C. et al., *J. Am. Chem. Soc.* **2015***, 137*, 11218. [4] Klippenstein, V. et al. *eLife* **2017**, *6*, e25808. [5] Fu, C., et al. *J. Am. Chem. Soc.* **2018**, *140*, 11058.

*Corresponding author: E-mail: Lei.Wang2@ucsf.edu

Self-assembled DNA Optical Nanoantennas for Fluorescence-based Diagnostic Applications

Viktorija Glembockyte,[a] Kateryna Trofymchuk,[a,b] Lennart Grabenhorst,[a] Cindy Close,[a] Martina Pfeiffer,[a] Renukka Yadaav,[a] Florian Selbach,[a] Birka Lalkens,[b] and Phillip Tinnefeld*[a]

[a]*Department of Chemistry and Center for NanoScience (CeNS), LMU München, Butenandtstr. 5 – 13, 81377 München, Germany*
[b]*Institute of Physical and Theoretical Chemistry, TU Braunschweig, Integrated Center of Systems Biology (BRICS), Rebenring 56, 38106 Braunschweig, Germany*

Signal amplification strategies are essential for improving sensitivity, speed, and robustness of sensing assays for point-of-care diagnostic applications. One strategy to achieve this relies on physical fluorescence signal amplification by plasmonic nanostructures that act as optical nanoanntenas concentrating the incident excitation light into zeptoliter volumes and enhancing the radiative decay rate of fluorescent molecules.[1-3] In this work we exploit the unique positioning precision of DNA origami to directly place the diagnostic elements in the plasmonic hotspots of silver and gold dimer nanoantennas (Figure 1).[4] Using fluorescence-quenched hairpin and sandwich binding assays we are able to detect DNA and RNA sequences specific to the Zika virus as well as antibiotic resistant bacteria with fluorescence enhancements reaching several hundred fold.

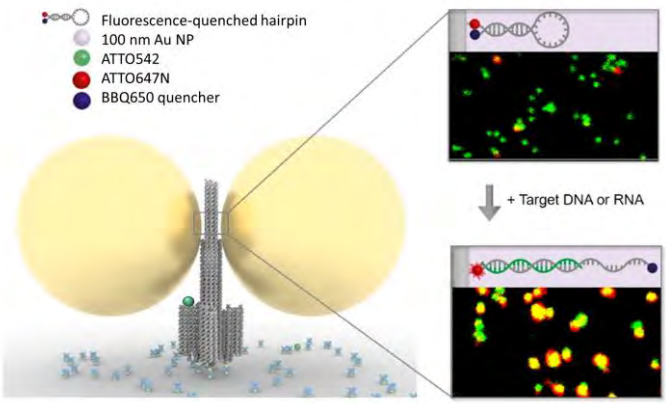


Figure 1. *Illustration of fluorescence-quenched hairpin assay incorporated in the hotspot of plasmonic gold dimer nanoantenna: upon binding of the target DNA/RNA target fluorescence enhancements of ATTO647N reaching several hundred fold can be achieved.*

Acknowledgments: This work was supported by the Federal Ministry of Education and Research (BMBF, grants: POCEMON, 13N14336, and SIBOF, 03VP03891) and the Humboldt Research Fellowship.

References: [1] Acuna, G.P. et al., *Science* **2012**, *338*, 508. [2] Puchkova, A. et al., *Nano Lett.* **2012**, *15*, 8354. [3] Vietz, V. et al., *ACS Nano* **2017**, *11*, 4969. [4] Ochmann, S. et al., *Anal. Chem.* **2017**, *89*, 13000.

*Corresponding author: E-mail: viktorija.glembockyte@cup.lmu.de

Are Lipid Nanodomains Inter-Leaflet Coupled? An MC-FRET Study.

Ivo S. Vinklárek,[a] Lukáš Vel'as,[a] Petra Riegerová,[a] Ilya Mikhalyov,[2] Natalia Gretskaya,[2] Martin Hof[a] and Radek Šachl*[a]

[a] *J. Heyrovský Institute of Physical Chemistry of the Academy of Sciences of the Czech Republic, 182 23 Prague, Czech Republic*
[b] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Science, Moscow, GSP-7, Russia*

Plasma membranes of living cells are compartmentalized into small sub-microscopic structures (nanodomains) having potentially relevant biological functions. Despite this, structural features of these nanodomains remain elusive. For instance, it is unclear whether nanodomains found in the upper bilayer leaflet are transversally registered with those found in the lower leaflet. Experiments that would prove or disprove the existence of inter-leaflet coupled nanodomains are practically missing, mainly because to resolve registered from inter-leaflet independent nanodomains requires not only high lateral resolution but also excellent resolution along bilayer's normal. We show in this work that FRET (Förster resonance energy transfer) combined with Monte-Carlo simulations (consequently termed MC-FRET) meets such high requirements on high spatial resolution in all three directions. This work provides experimental evidence that even small nanodomains of variable sizes between 10 and 160 nm are inter-leaflet coupled. Importantly, the alternative scenarios of partially registered, independent, or anti-registered nanodomains could be excluded.

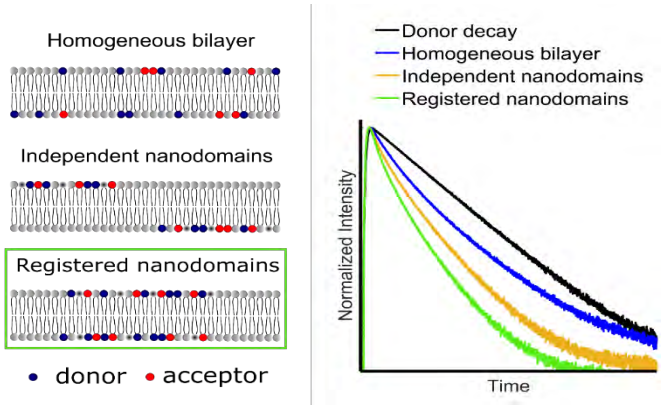


Figure. *Basic principles of FRET underlying the detection of nanodomains.*

Acknowledgments: This work was supported by the Czech Science Foundation via grant 18-04871S.

*Corresponding author: E-mail: sachl@jh-inst.cas.cz

Local order-disorder dynamics revealed in domain swapping pathway of the DNA-binding domain of human FoxP1

Hugo Sanabria,[a] Exequiel Medina,[b] Pablo Villalobos,[b] George Hamilton,[a] Elizabeth A. Komives,[c] César A. Ramírez-Sarmiento,[d] Jorge Babul,[b] Corresponding Author Hugo Sanabria*[a]

[a] *Department of Physics and Astronomy, Clemson University, Clemson SC, USA*
[b] *Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, , Chile*
[c] *Department of Chemistry & Biochemistry, University of California San Diego, La Jolla, USA*
[d] *Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile*

Forkhead box P (FoxP) proteins are unique among Fox transcription factors because they can form domain-swapped dimers through their DNA-binding -forkhead- domains, enabling the communication of distant chromosome elements by tethering two DNA molecules together. The equilibrium and kinetic properties of domain swapping suggest that a high level of structural flexibility must be a hallmark between FoxP members. However, the lack of high-resolution (spatial and temporal) models and the missing description of the local intrinsic plasticity hinders the ability to define a general mechanism that relates their domain swapping process to their function. To explore the local flexibility of FoxP proteins and their role in structural dynamics and domain swapping, we characterized the forkhead domain of human FoxP1 by single-molecule multiparameter fluorescence detection and molecular dynamics simulations. Using this hybrid approach, we present the first validation of the domain-swapped conformation of FoxP1 and further reveal a highly-populated and expanded dimeric intermediates with different degree of local disorder of the dimeric form FoxP1. These intermediates might be critical for screening distant chromosome domains and provide the path toward a stable domain-swapped structure (a.k.a. fly-casting mechanism). This unique feature of FoxP1 differentiates it from most other Fox family members, providing a glimpse of how order-disorder transitions can facilitate biologically relevant functions like protein-protein and protein-DNA interactions in order to accomplish its genetic regulatory function.

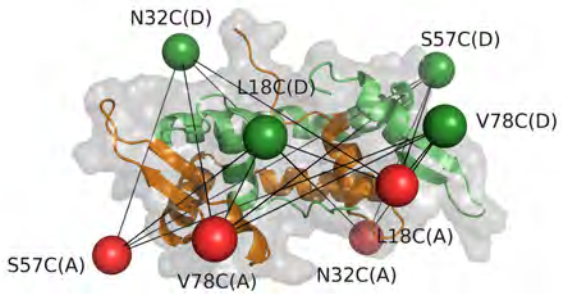


Figure. *FRET network designed used to validate domain swapped structural model.*

Acknowledgments: This work was supported by grants from the Foundation of Excited States.

*Corresponding author: E-mail: hsanabr@clemson.edu

An Oligonucleotide Probe Incorporating the Chromophore of Green Fluorescent Protein is Useful for the Detection of HER-2 mRNA Breast Cancer Marker

Bilha Fischer[a]*, Abed Saady[a], Verena Böttner[b], Melissa Meng[b], Eli Varon[c], Yaron Shav-Tal[c], and Christian Ducho[b]

[a] Department of Chemistry, Bar-Ilan University, Ramat-Gan 52900, Israel. [b] Department of Pharmacy, Pharmaceutical and Medicinal Chemistry, Saarland University, 66123 Saarbrücken, Germany [c] Faculty of Life Sciences & Institute of Nanotechnology, Bar-Ilan University, Ramat-Gan 52900, Israel.

Diagnosis and treatment of breast cancer can be greatly enhanced and personalized based on the quantitative detection of mRNA markers. Here, we targeted the development of a fluorescent oligonucleotide probe to detect specifically the HER-2 mRNA breast cancer marker. We have selected the chromophore of the Green Fluorescent Protein (GFP), 4-hydroxybenzylidene imidazolinone (HBI), as a fluorophore covalently bound to an oligonucleotide probe and potentially capable of intercalating within a probe-RNA duplex. We first synthesized the two-ring scaffold of the HBI chromophore and coupled it to 2'-deoxyuridine at C5-position via a 7-atom-spacer, to give **1**. Indeed, in the highly viscous glycerol used to mimic the reduced conformational flexibility of the intercalated HBI, chromophore **1** displayed a quantum yield of 0.29 and brightness of 20600 $M^{-1}cm^{-1}$, while no fluorescent signal was observed in methanol. Next, we synthesized a 20-mer oligonucleotide probe incorporating **1** at position 6 (5'-CCCGTUTCAACAGGAGTTTC-3'), ON^{HBI}, targeting nucleotides 1233–1253 of HER-2 mRNA. A 16-fold enhancement of ON^{HBI} emission intensity upon hybridization with the complementary RNA vs that of the oligonucleotide probe alone indicated the presence of the target oligonucleotide (quantum yield 0.52; brightness 23500 $M^{-1}cm^{-1}$). The intercalative binding mode of the HBI fluorophore was demonstrated by circular dichroism. Furthermore, an 11-fold enhancement of ON^{HBI} emission (quantum yield 0.50; brightness 23200 $M^{-1}cm^{-1}$) was observed when the probe was mixed with total RNA extract from a human cell line that has high levels of HER2 mRNA expression. Thus, we propose ON^{HBI} as a promising probe potentially useful for the sensitive and specific detection of HER2 mRNA breast cancer marker.

*Corresponding author: E-mail: bilha.fischer@biu.ac.il

An Oligonucleotide Probe Incorporating the Chromophore of Green Fluorescent Protein is Useful for the Detection of HER-2 mRNA Breast Cancer Marker

Bilha Fischer[a]*, Abed Saady[a], Verena Böttner[b], Melissa Meng[b], Eli Varon[c], Yaron Shav-Tal[c], and Christian Ducho[b]

[a] Department of Chemistry, Bar-Ilan University, Ramat-Gan 52900, Israel. [b] Department of Pharmacy, Pharmaceutical and Medicinal Chemistry, Saarland University, 66123 Saarbrücken, Germany [c] Faculty of Life Sciences & Institute of Nanotechnology, Bar-Ilan University, Ramat-Gan 52900, Israel.

Diagnosis and treatment of breast cancer can be greatly enhanced and personalized based on the quantitative detection of mRNA markers. Here, we targeted the development of a fluorescent oligonucleotide probe to detect specifically the HER-2 mRNA breast cancer marker. We have selected the chromophore of the Green Fluorescent Protein (GFP), 4-hydroxybenzylidene imidazolinone (HBI), as a fluorophore covalently bound to an oligonucleotide probe and potentially capable of intercalating within a probe-RNA duplex. We first synthesized the two-ring scaffold of the HBI chromophore and coupled it to 2'-deoxyuridine at C5-position via a 7-atom-spacer, to give **1**. Indeed, in the highly viscous glycerol used to mimic the reduced conformational flexibility of the intercalated HBI, chromophore **1** displayed a quantum yield of 0.29 and brightness of 20600 $M^{-1}cm^{-1}$, while no fluorescent signal was observed in methanol. Next, we synthesized a 20-mer oligonucleotide probe incorporating **1** at position 6 (5'-CCCGTUTCAACAGGAGTTTC-3'), ON^{HBI}, targeting nucleotides 1233–1253 of HER-2 mRNA. A 16-fold enhancement of ON^{HBI} emission intensity upon hybridization with the complementary RNA vs that of the oligonucleotide probe alone indicated the presence of the target oligonucleotide (quantum yield 0.52; brightness 23500 $M^{-1}cm^{-1}$). The intercalative binding mode of the HBI fluorophore was demonstrated by circular dichroism. Furthermore, an 11-fold enhancement of ON^{HBI} emission (quantum yield 0.50; brightness 23200 $M^{-1}cm^{-1}$) was observed when the probe was mixed with total RNA extract from a human cell line that has high levels of HER2 mRNA expression. Thus, we propose ON^{HBI} as a promising probe potentially useful for the sensitive and specific detection of HER2 mRNA breast cancer marker.

*Corresponding author: E-mail: bilha.fischer@biu.ac.il

A general approach to fluorescent crystals and nanoparticles based on organic dyes

Christopher R. Benson[b], Laura Kacenauskaite[a], Amar H. Flood[b], Bo W. Laursen[a]

[a] *Department of Chemistry, University of Copenhagen, Copenhagen, Denmark*
[b] *Department of Chemistry, Indiana University, Bloomington, IN, USA*

The bright color and fluorescence of organic dyes in solution is in general not transferable to densely packed solid state materials, where the close proximity of the dyes leads to exciton coupling and emission quenching. Here we report a new class of molecular materials called small-molecule ionic isolation lattices (SMILES) where solution-like fluorescence properties are reinstated into classical dyes, like rhodamines, styryls, oxazines, trianguleniums, and cyanines, by supramolecular control of the solid state packing. The materials are simple to make by mixing a cationic dye with an anion-binding and structure-directing cyanostar[1] macrocycle (Figure a). The macrocycle sequesters the dye's counter anion creating a wide band-gap complex that hierarchically directs alternating charge-by-charge packing for spatial and electronic isolation of the dyes in the SMILES crystals. These new supramolecular materials display very high volume-normalized brightnesses B/V≈7000 M^{-1} cm^{-1} nm^{-3} (Figure b). Formulation of SMILES materials into ligand stabilized nanoparticles provide 20 nm particles with similar high brightness that can be used for bioimaging (Figures c and d).

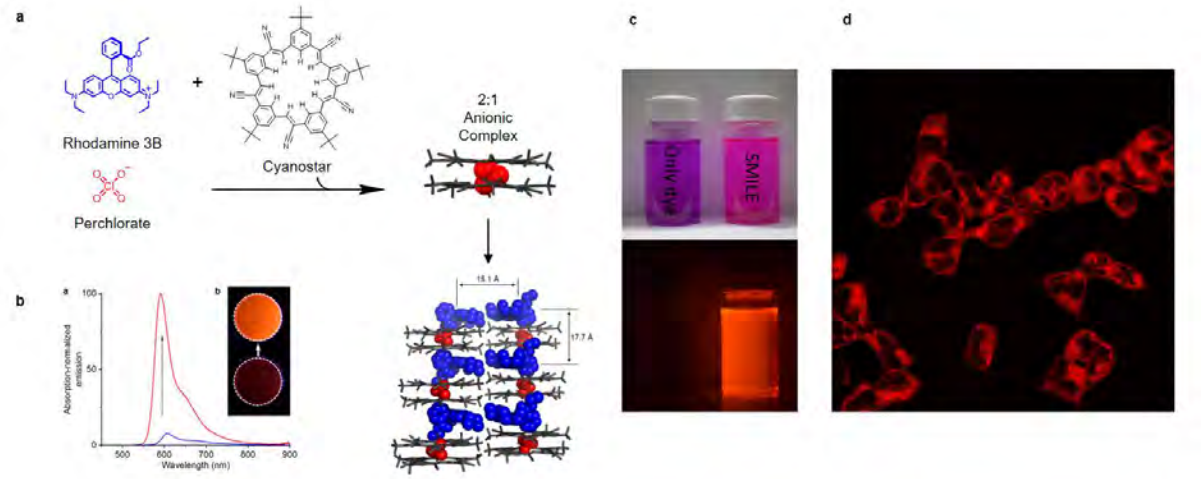


Figure. *(a) Supramolecular assembly of SMILES crystals, decoupling the dyes. (b) Fluorescence from rhodamine thin film with and without cyanostar. (c) 20 nm ligand stabilized rhodamine SMILES nanoparticles in water. (d) HeLa cells stained with SMILES nanoparticles.*

References: [1] Lee, S.; Chen, C.-H.; Flood, A. H., A, *Nat Chem* **2013,** *5*, 704-710.

*Corresponding author: E-mail: bwl@nano.ku.dk

Tautomerization probed by single molecule fluorescence

Jacek Waluk,[a,b]

[a] *Institute of Physical Chemistry, Polish Academy of Sciences, 01-224 Warsaw, Kasprzaka 44/52, Poland;* [b] *Faculty of Mathematics and Science, Cardinal Stefan Wyszyński University, Dewajtis 5, 01-815 Warsaw, Poland*

Excited state reactions that change the direction of the S_0-S_1 transition moment can be studied using fluorescence anisotropy. A special case is the self-exchange process, in which the reactant and product are formally identical (Figure). We have developed procedures for obtaining the rate constant of tautomerization in such systems from the analysis of stationary or time-resolved anisotropy measurements.[1] We also proposed a methodology based on excitation with polarized light for monitoring tautomerization in single molecules.[2] Application of this technique resulted in an unusual observation that the tautomerization rate can be slowed down by many orders of magnitude when the environment changes from a liquid to a polymer matrix. The effect has now been observed for several porphycenes[3-4] and for another porphyrin isomer, hemiporphycene.[5]

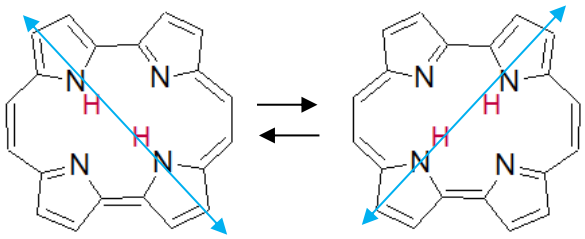


Figure. *Tautomerization in porphycene accompanied by a change in the S_0-S_1 transition moment direction.*

Acknowledgments: This work was supported by grants no. 2011/02/A/ST5/00443 and 2016/22/A/ST4/00029from the Polish National Science Centre.

References: [1] Gil, M., Waluk, J. *J. Am. Chem. Soc.* **2007***, 129,* 1335. [2] Piwoński, H., et al. *J. Am. Chem. Soc.* **2005**, *127*, 5302. [3] Piwoński, H., et al. *J. Phys. Chem. Lett.* **2013**, *4*, 3967. [4] Piatkowski, L., et al. *J. Phys. Chem. Lett.* **2018**, *9*, 1211. [5] Kim, V., et al. *Phys. Chem. Chem. Phys.* **2018**, *20*, 26591.

*Corresponding author: E-mail: jwaluk@ichf.edu.pl

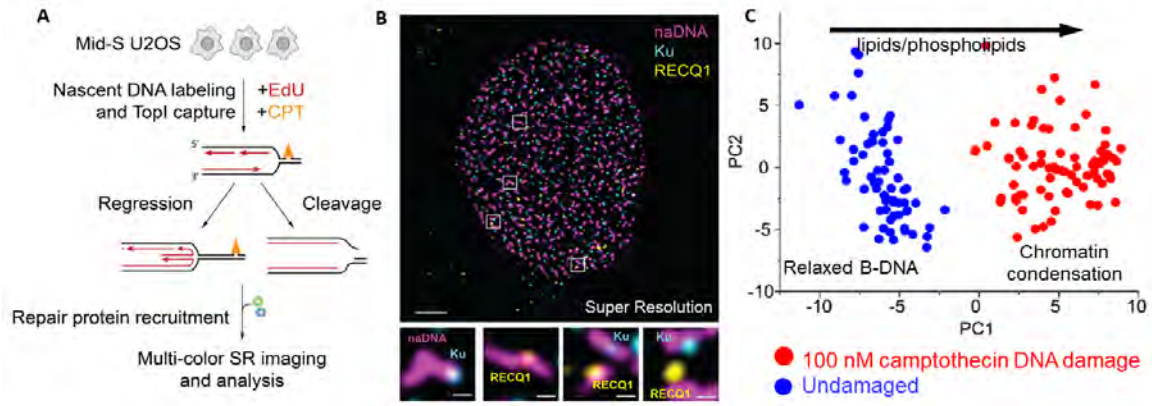
Complementary Single Molecule Imaging and Infrared Spectroscopy to Characterise DNA Damage Response

Esther Miriklis,[a] Eli Rothenberg,[b] Toby D. M. Bell[a], Donna R. Whelan*[c]

[a]*Biochemistry and Molecular Pharmacology, New York University, NY, 10016, USA*
[b]*School of Chemistry, Monash University, Clayton, 3800, Australia*
[c]*School of Pharmacy and Biomedical Sciences, La Trobe Institute for Molecular Science, Bendigo, 3555, Australia*

Single molecule localisation microscopy (SMLM) and Synchrotron Fourier transform infrared (FTIR) spectroscopy are two techniques that can elucidate unique and valuable molecular distributional and structural information about biological samples. SMLM provides images of targeted biomolecules at spatial resolutions an order of magnitude better than the diffraction limit while also achieving inherent single molecule sensitivity [1]. In contrast, FTIR objectively measures the holistic biochemistry of a sample, without any requirement for targeting or labels, to reveal variations in overall composition and molecular secondary structure [2].

We have applied a correlative approach [3] to investigate the cellular response to low levels of DNA replicative stress. In this study we analysed FTIR spectra of drugged live and fixed cells with colocalization studies of SMLM cell images which enabled visualization of the DNA damage sites [4]. This enabled detection of changes to cellular metabolism and chromatin structure and differentiation of undamaged cells from those treated with drug dosages that cannot be detected using conventional imaging and biochemical methods. These studies demonstrate the sensitivity of these combined techniques for characterizing biochemical changes and offer new insights into the mechanisms involved in DNA damage and repair.



(A) Schematic of low level DNA damage and labelling for SMLM, (B) SMLM image of a damaged call labelled for nascent DNA and repair proteins Ku and RECQ1, (C) Multivariate principal component analysis of S-FTIR spectra of damaged and undamaged cells.

References: [1] Whelan D.R. and Bell T.D.M., *J. Phys. Chem. Letts*. **2015**, *6*, 374. [2] Whelan D.R., et. al. *Analyst* **2013**, *138*, 3891. [3] Whelan D.R. and Bell T.D.M, *ACS Chem. Biol.* **2015,** *10*, 2874. [4] Whelan, D.R. et. al. *Nat. Commun*. **2018**, 9, 3882.

*Corresponding author: E-mail: d.whelan@latrobe.edu.au

Single Molecule Excitation-Emission & Lifetime Mapping at Ambient Conditions

Stefan Krause,*[a] Erling Thyrhaug,[b] Antonio Perri,[c] Martin Streiter,[d] Christian von Borczyskowski,[d] Carsten Deibel,[d] Dario Polli,[c] Giulio Cerullo,[c] Jürgen Hauer,[b] Tom Vosch[a]

[a] *Department of Chemistry, University of Copenhagen, 2100 Copenhagen, Denmark*
[b] *Department of Chemistry, Technical University of Munich, 85748 Garching, Germany*
[c] *Dipartimento di Fisica, Politecnico di Milano, 20133 Milano, Italy*
[d] *Department of Physics, Technical University of Chemnitz, 09126 Chemnitz, Germany*

Single molecule (SM) emission spectroscopy has proven to be a powerful, noninvasive tool in life science, materials science, and photophysics. Even though the first SM experiments were based on excitation spectroscopy (at low temperatures),[1] the simplicity made emission-based techniques most dominant in today's SM experiments. However, the benefit of a combination of both methods led to several successful attempts to measure the excitation spectrum of a single molecule also at ambient conditions.[2]

Here we present and compare two different modalities of SM excitation spectroscopy. The first technique relies on scanning a laser line over a large spectral range. The procedure is fast and allows for recording spectral time traces and to track the absorption and emission behavior of single bay-substituted perylene diimide molecules undergoing spectral fluctuations.[3] The second technique involves the modulation of a white light laser source by a recently developed birefringent-based common path interferometer (GEMINI Interferometer from NIREOS, www.nireos.com). The Fourier transform of the recorded interferogram provides the full two-dimensional spectral map of a single molecule with outstanding quality and enables to extract information on line-widths, Stokes-shift and absolute absorption and emission energies. We verified the technique with a standard dye (terylene diimide) and a more complex donor-acceptor dyad consisting of a linked perylene diimide-benzoperylene pair. The approach was then further extended to map single molecule lifetimes as a function of the excitation wavelength.[4]

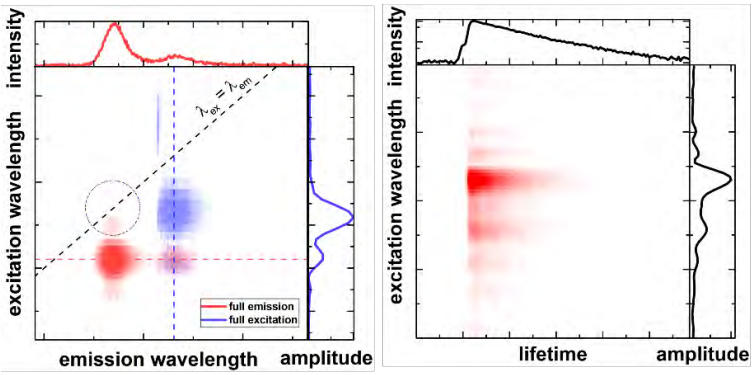


Figure. *Excitation-Emission and Excitation-Lifetime map of a single molecule.*

References: [1] Orrit, M. et al., *Phys. Rev. Lett.* **1990**, 65, 2716. [2] Stopel, M. H. et al., *J. Phys. Chem. Lett.* **2014**, 5, 3259-3264. [3] Streiter, M. et al., *J. Phys. Chem. Lett.* **2016**, 7, 4281-4284. [4] Thyrhaug, E. Et al., *PNAS* **2019**, 116, 4064-4069.

*Corresponding author: E-mail: stefan.krause@chem.ku.dk

Transition from solid state to molecular-like optical properties in silicon carbide nanoparticles

János Erostyák*[,a,b], David Beke[c], Anna Fučíková[d], Tibor Z. Jánosi[a,b], Gyula Károlyházy[c], Bálint Somogyi[c], Sándor Lenk[e], Olga Krafcsik[e], Zsolt Czigány[f], Katalin Kamarás[c], Jan Valenta[d], and Adam Gali[c,e]

* Corresponding Author and Presenter
[a] University of Pécs, Institute of Physics, Pécs, Hungary
[b] University of Pécs, Szentágothai Research Centre, Spectroscopy Research Group, Pécs, Hungary
[c] Institute for Solid State Physics and Optics, Wigner Research Centre for Physics, Hungarian Academy of Sciences, Hungary
[d] Charles University, Faculty of Mathematics and Physics, Department of Chemical Physics & Optics, Prague, Czech Republic
[e] Department of Atomic Physics, Budapest University of Technology and Economics, Budapest, Hungary
[f] Institute for Technical Physics and Materials Science, Centre for Energy Research, Hungarian Academy of Sciences, Budapest, Hungary.

Silicon carbide (SiC) is a wide band gap indirect semiconductor known for its chemical resistance and hardness. SiC nanoparticles (NPs) are promising candidates for bioimaging and sensing as NPs in size smaller than 10 nm have enhanced luminescence properties. We applied both time-dependent and steady-state photoluminescence measurements to study the size dependent optical properties of ultrasmall SiC NPs. We found that the nature of the optical transition transforms from solid state indirect gap type to molecular-like as the diameter of SiC NPs reduces from 6-4 nm to 3-1 nm with a smooth transition in between. We show, that below 3.7 nm, the influence of surface states arises and becomes significant below 2.8 nm. In line with increase of the relative number of surface atoms contributing to the surface states, the number of Si and C atoms contributing to the crystal core decreases rapidly which results in transformation from solid state to molecular-like behavior. We performed detailed time-dependent density functional theory (TDDFT) calculations in order to support the conclusions obtained from the experiments.

References: [1] Beke, D. et al. *J. Phys. Chem. C* **2016**, *120*, 685–691. [2] Beke, D. et al., *Sci. Rep.* **2017**, *7*, 10599.

*Corresponding author: E-mail: erostyak@fizika.ttk.pte.hu

Determining the stoichiometry of protein complexes with single-molecule localization microscopy

Tim N. Baldering[1], Christos Karathanasis[1], Carmen L. Krüger[1], Sebastian Malkusch[1], Marina S. Dietz[1], Gerhard Hummer[2] and Mike Heilemann[1]

[1] Institute of Physical and Theoretical Chemistry, Goethe-University Frankfurt, 60438 Frankfurt, Germany
[2] Max-Planck-Institute for Biophysics, 60438 Frankfurt, Germany

Knowledge of assembly, subunit architecture and dynamics of proteins in a cellular context is essential to infer their biological function. Optical super-resolution techniques provide the necessary spatial resolution to study protein complex nanostructures in the context of their cellular environment.[1] Single-molecule localization microscopy (SMLM)[2, 3] in addition provides access to the number of detected proteins.
A challenge for current super-resolution methods is to resolve individual protein subunits within a densely packed protein cluster. For this purpose, we apply quantitative SMLM (qSMLM), which reports on molecular numbers by analyzing the kinetics of single fluorophore blinking.[4] We developed theoretical models to describe blinking patterns,[5] and demonstrated stoichiometry imaging for synthetic and biological reference structures and membrane proteins [4, 6, 7]. Using this technology, we investigated how selected membrane receptors alter their stoichiometry in response to ligand binding and functional or chemical modification. For toll-like receptor 4 (TLR4), we found an equilibrium of monomeric and dimeric stoichiometry in the absence of a ligand, and a ligand-specific formation of monomers or dimers [8]. For tumor-necrosis factor receptor 1 (TNFR1), we found a similar equilibrium of monomeric and dimeric stoichiometry in the absence of its ligand TNFα, and the formation of trimers and higher-order oligomers upon ligand binding.

1. Stone, M. B.; Shelby, S. A.; Veatch, S. L. *Chem Rev* **2017,** 117, (11), 7457-7477.
2. Sauer, M.; Heilemann, M. *Chem Rev* **2017,** 117, (11), 7478-7509.
3. van Wijk, S. J. L.; Fricke, F.; Herhaus, L.; Gupta, J.; Hotte, K.; Pampaloni, F.; Grumati, P.; Kaulich, M.; Sou, Y. S.; Komatsu, M.; Greten, F. R.; Fulda, S.; Heilemann, M.; Dikic, I. *Nat Microbiol* **2017,** 2, 17066.
4. Fricke, F.; Beaudouin, J.; Eils, R.; Heilemann, M. *Sci Rep* **2015,** 5, 14072.
5. Hummer, G.; Fricke, F.; Heilemann, M. *Mol Biol Cell* **2016,** 27, (22), 3637-3644.
6. Baldering, T. N.; Dietz, M. S.; Gatterdam, K.; Karathanasis, C.; Wieneke, R.; Tampe, R.; Heilemann, M. *Mol Biol Cell* **2019**, mbcE18100661.
7. Karathanasis, C.; Fricke, F.; Hummer, G.; Heilemann, M. *Chemphyschem* **2017,** 18, (8), 942-948.
8. Kruger, C. L.; Zeuner, M. T.; Cottrell, G. S.; Widera, D.; Heilemann, M. *Sci Signal* **2017,** 10, (503).

Multi-Modal Fluorescence Characterization of Cell Cycle Progression and Cytokinesis

Rachel Cinco,[a] Per Niklas Hedde,[a] Leonel Malacrida,[a] Michelle A. Digman,[a] Enrico Gratton,[a]

[a] *Department of Biomedical Engineering, University of California, Irvine, Irvine CA, USA*

Fluorescence and advanced fluorescence methods such as phasor FLIM are powerful tools for examining the metabolic and biophysical characteristics of biological systems. Cell division is central to both the creation of life and the propagation of diseases such as cancer and neurodegenerative disorders. While many of the molecular mechanisms responsible for regulating cell cycle progression have been well studied, much of the cellular bioenergetics responsible for fueling cell cycle progression and biophysical properties of cell division is poorly defined. Here, we perform a multi-modal fluorescence characterization of cell cycle progression in human HeLa cells using Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI). Application of NADH phasor Fluorescence Lifetime Imaging Microscopy (FLIM) identified significant changes both in NADH concentration between several stages of the cell cycle defined by FUCCI, whereas the free/bound NADH ratio is maintained relatively consistent. We also present detailed timelapse images of human HeLa cells undergoing cytokinesis using NADH Phasor FLIM, Laurdan Phasor FLIM, and timelapse 740nm 2-Photon excitation spectral phasor to spatially characterize and quantify metabolism, membrane fluidity, and unlabeled fluorescence emission signatures during the completion of cell division.

From our characterization we identify cell cycle stage specific changes in NADH bioenergetics, spectral phasor signature, and membrane biophysics not previously identified.

Acknowledgments: This work was supported by NIH P41GM103540

*Corresponding author: E-mail: egratton@uci.edu

Modulation of the oligomerization state of proteins by ions and small molecules: a fluorescence correlation spectroscopy study

Anirban Purohit,[a] Marcia Levitus*[a]

[a] *School of Molecular Sciences and The Biodesign Institute, Arizona State University, Tempe, AZ, United States.*

It has been estimated that about 50% of all proteins are oligomeric in nature, and among these, most are homomultimers. For some proteins, the oligomerization state can be modulated by environmental factors such as pH, phosphorylation, ligand binding, etc. Different oligomeric forms of the protein may have different metabolic functions, and the modulation of the oligomerization state by environmental factors can serve as a metabolic control mechanism in the cell. The oligomerization state of a protein is often difficult to determine. Methods such as size exclusion chromatography, analytical ultracentrifugation, and chemical cross-linking can be employed, but these techniques are rarely adequate to identify transient intermediates. In addition, these traditional techniques are not always amenable to the array of buffer conditions required to investigate how environmental variables affect the oligomeric state of a protein.

Here, we present a methodology based on fluorescence correlation spectroscopy (FCS) that allows the characterization of the effects of environmental factors on the oligomeric state of a protein. We have derived models to describe the measured autocorrelation function in terms of the relative concentrations of the different oligomeric forms that exist under equilibrium conditions at a given protein concentration. In contrast to most traditional biochemical techniques, FCS allows the determination of the concentrations of different oligomers under true equilibrium conditions. Here, we will illustrate the capability of this powerful approach by discussing our recent unpublished research that unraveled previously-unknown mechanisms of modulation of the oligomeric state of the replication processivity clamp of *E. coli*. We will show that this ring-shaped protein self-assembles to form stacks of rings in the presence of glutamate, glycine betaine, and other osmoprotectants.

Acknowledgments: This work was supported by a grant from the National Science Foundation (BIO-1157765)

*Corresponding author: E-mail: mlevitus@asu.edu

Sequence dependence of DNA strand displacement kinetics

D. W. B. Broadwater, Jr. [a], Alexander W. Cook [a], Harold Kim*[a]

[a] *School of Physics, Georgia Institute of Technology, 837 State Street Atlanta GA, USA*

DNA strand displacement, where a single-stranded nucleic acid invades a duplex DNA, is pervasive in biology and DNA nanotechnology. In vitro, DNA strand displacement is initiated through hybridization of the invader strand to the exposed bases of a partial duplex. This slow bimolecular step known as toehold formation dominates the apparent kinetics in bulk and thus obfuscates the kinetic details of the actual displacement step. Here, we introduce a new single-molecule fluorescence approach to studying the unimolecular displacement kinetics of short DNA molecules (15-20 nucleotides in length). We find that despite the multistep nature of the process, the displacement time distribution exhibits a monotonic decay with little delay, and the mean displacement time varies widely in the range of 10-100 milliseconds among different DNA sequences we tested. The displacement time is also found to depend on toehold polarity and nucleic-acid type. Our study will provide novel insights into the exchange kinetics of individual bases and the sequence dependence of other genome-related processes such as R-loop formation.

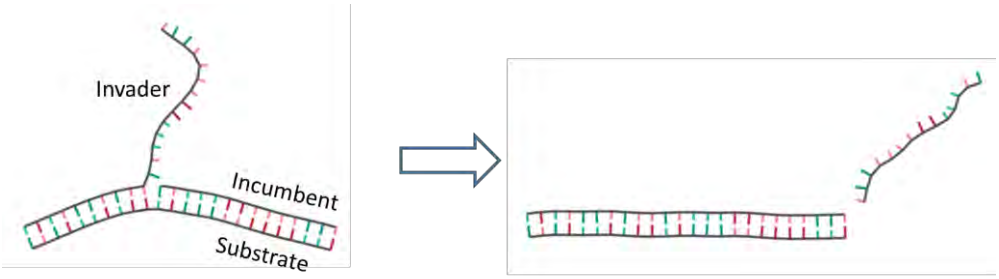


Figure. *Toehold-mediated strand displacement.*

Acknowledgments: This work was supported by grants from the National Science Foundation.

*Corresponding author: E-mail: harold.kim@physics.gatech.edu

Frequency Domain FLIM System Improvements and Applications

Gerhard Holst,[a] Robert Franke,[a] Corresponding Author Gerhard Holst*[a]

[a] *Research Department, PCO AG, Donaupark 11, D-93309 Kelheim, Germany*

Since the introduction of the first FD-FLIM pco.flim camera in 2014 the camera system has been applied to different areas[1,2,3], from FRET to endogenous fluorescence, from oxygen to pH measurement, from large technical applications in wind tunnels down to light sheet microscopy. Thanks to customer feedback and own experiences the camera system and the controlling software have been improved. Currently the full integration in the NIS Elements software v5.11 has resulted in a significant improvement concerning calibration and dark field correction. Further an optimized laser light source has been developed, which simplifies the use of the camera plus excitation light as a system. The improvements in use and applicability will be shown and discussed.

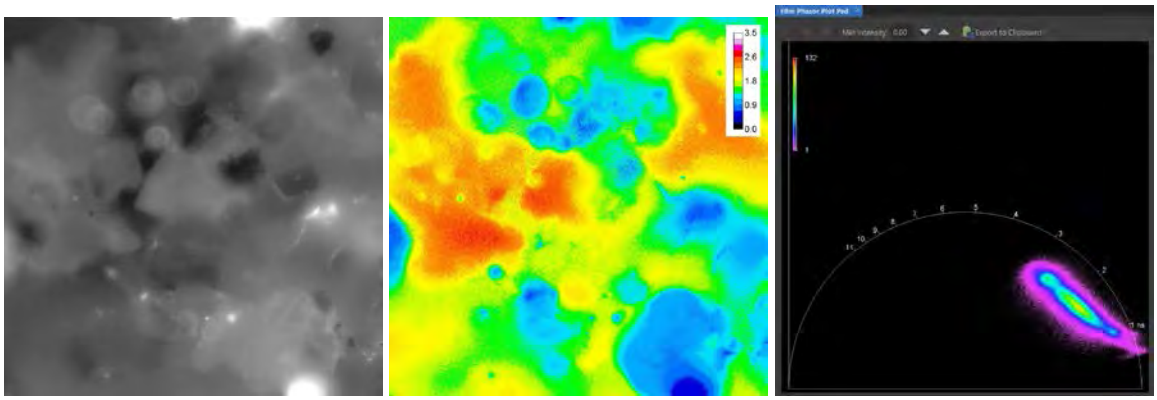


Figure 1. *From left to right: fluorescence image of a filter paper with a variety of dust and microplastic particles, phase angle based fluorescence lifetime distribution in the range of 0.6 ns – 2.9 ns, the corresponding phasor plot.*

Further new results including investigation of micro-plastics on lab filters (see above), pH measurements, oxygen measurements in marine environment, assistance for neuro surgery and rapid FD-FLIM measurements are shown to demonstrate the flexibility of the pco.flim camera system.

References: [1] Luminescence Lifetime Imaging of Chemical Sensors - A Comparison between Time-Domain and Frequency-Domain based Camera Systems, K. K. et al., Anal. *Chem.* **2019***, (acc. for publ.)*. [2] Widefield fluorescence lifetime imaging of protoporphyrin IX for fluorescence-guided neurosurgery: an ex vivo feasibility study, M. E., et al. *J. of Biophotonics* **2019**, *(acc. for. publ.)*. [3] Decay Widefield Frequency Domain Fluorescence Lifetime Imaging Microscopy (FD-FLIM) for Accurate Measurement of Oxygen Gradients within Microfluidic Devices, H.-M. W., et al. *Analyst* **2019**, *(acc. for. publ.)*

*Corresponding author: E-mail: gerhard.holst@pco.de

Biopolymer folding studies inspired by the works of Roger Tsien, Yitzhak Tor and Neal Devaraj

Nathan W. Luedtke*[a]

[a] *Department of Chemistry, McGill University, Montreal, Canada*

Research conducted at UCSD has had a profound influence on methods and applications of fluorescence in science and medicine. This talk will present three short stories about how the works of Roger Tsien, Yitzhak Tor, and Neal Devaraj have enabled the study of biopolymer synthesis and folding in living cells. For example, Roger Tsien and co-workers developed pro-fluorescent "biarsenical" dyes FlAsH and ReAsH that selectively label recombinant proteins in live cells containing the hexapetide CCPGCC through thiol-arsenic exchange reactions.[1-2] These reactions convert the non-fluorescent, 1,2-ethanedithiol (EDT) bound forms of FlAsH and ReAsH into highly fluorescent protein-bound complexes. These results inspired the idea that protein conformation, folding, and/or protein-protein binding could be monitored by replacing the Pro-Gly residues of the hexapeptide with a globular protein that, upon proper folding, would bring the two Cys-Cys pairs into close proximity to facilitate FlAsH or ReAsH binding.

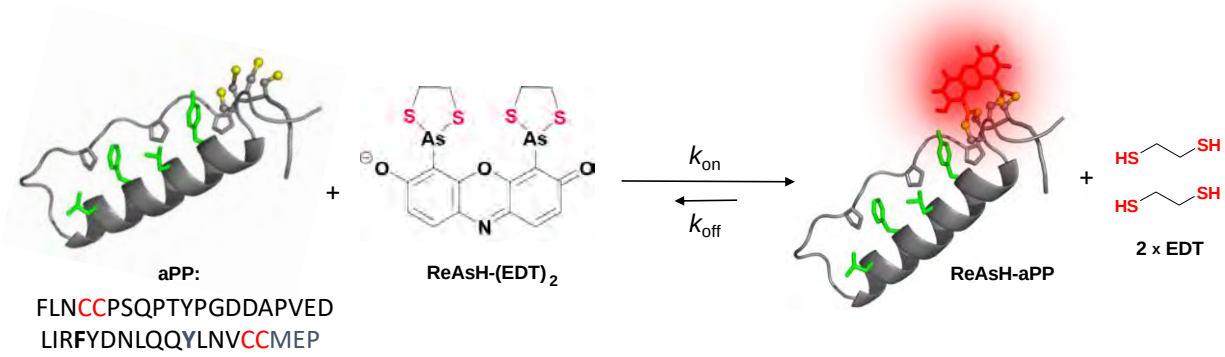


Figure. *Avian pancreatic polypeptide (aPP) containing a "split" tetracysteine motif binds to ReAsH to give a highly fluorescent complex that is detectable in cells.*[3]

Thiol-arsenic exchange reactions give fluorescent complexes with apparent binding affinities and quantum yields proportional to abilities of proteins to fold into their native structures. For example, a single point mutation in an aPP fusion protein was readily detected in living HeLa cells according to changes in ReAsH fluorescence intensity.[3] This strategy has subsequently been used to study amyloid-β aggregation,[4] epidermal growth factor receptor (EGFR) activation,[5] and α-synuclein fibril formation.[6]

Acknowledgments: This work was supported by the hard work of many talented students and postdocs who were also inspired by the research of many great scientists before them.

References: [1] Griffin, B. A., et al. *Science* **1998**, *28*, 269. [2] Martin, B. R., et al. *Nat. Biotechnol.* **2005**, *23*, 1308. [3] Luedtke, N. W., et al. *Nat. Chem. Biol.* **2007**, *3*, 779. [4] Lee, J., *Nat. Chem. Biol.* **2011**, *7*, 602. [5] Dhavale, D. D., et al. *J. Biol. Chem.* **2017**, *292*, 9034.

*Corresponding author: E-mail: nathan.luedtke@mcgill.ca

Do Perineuronal nets stabilize the engram of a synaptic circuit?

Varda Lev-Ram and Roger Y. Tsien

Department of Pharmacology, University of California San Diego, La Jolla, CA, USA

The PNN is a specialized form of extracellular matrix, initially deposited around selected neurons during critical periods of development in specific parts of the brain, interrupted by holes where synapses occur. We postulate that the PNN comprises a longer-lived structural template and that new memories are created by cutting new holes in the PNN or by expanding existing holes to enable formation of new synapses or to strengthen existing ones. A basic premise of this hypothesis is that the PNN, should undergo very low metabolic renewal from the first age at which memories are retained until senescence, whereas the active constituents of synapses turn over much more frequently and would therefore be poorer substrates for permanent information storage, unless they are equipped with incredibly accurate copying mechanisms (R.Y.Tsien PNAS 2013). Experimental tests of the hypothesis:

1. PNN longevity; using ^{15}N Spirulina diet for Stable Isotope Labeling in Mammals (SILAM) we compare the lifetimes of PNN proteins vs. synaptic components in Enriched Environment (EE) vs. Conventional Cages (CC), ending the pulse-chase by changing to ^{14}N diet at P45. Analysis by Multidimensional Protein Identification Technology (MudPIT) of four different brain areas indicate:
    a. Low turnover rate for PNN proteins while synaptic proteins were at the noise level of ^{15}N /^{14}N ratio.
    b. Higher turnover of PNN proteins in EE vs. CC cages
    c. Variability in the retention of ^{15}N in PNN proteins between brain areas.
2. Localization of the long-lasting proteins; Imaging of ^{15}N /^{14}N ratio using Nanoscale secondary ion mass spectrometry (nanoSIMS) localized and verified the MudPit finding that PNN turnover is very slow.
3. Spatial occupation of the PNN holes; 2 dimension electron microscopy (EM) and 3D volumes of Serial Block Face Scanning EM reveal that neurons engulfed in PNN have more than 95% of their plasma membrane surface occupied by PNN or synapses.
4. Inhibition of PNN holes modulation during strong memories acquisition; we examined the role and timing of matrix metalloproteinases (MMP) activity in memory consolidation using pharmacological inhibitors in a fear-conditioning paradigm. Our results demonstrate that MMP inhibition during fear induction:
    a. Does not affect acquisition
    b. Significantly impairs long-term memory (30 days)
    c. Is dose dependent
    d. That memory impairment increases with time.

So far the hypothesis is supported by the results of the above tests.

*Corresponding author: E-mail: vlevramellisman@ucsd.edu

Beating Nyquist limits for the measurement of fluorophore blinking rates using image correlation spectroscopy

Simon Sehayek,[a] Yasser Gidi,[b] Viktorija Glembockyte,[b] Gonzalo Cosa,[b] Paul W. Wiseman*[a,b]

[a] *Department of Physics, McGill University, 3600 University St., Montreal, QC H3A 2T8, Canada*
[b] *Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, QC H3A 0B8, Canada*

We present an image correlation spectroscopy ensemble technique for rapidly measuring on- and off-blinking rate constants of fluorescent probes from an image time series of the fluorophore emitters. Our combined imaging and fluctuation analysis method is significantly faster than single-molecule trajectory analysis of individual fluorophores. We demonstrate that the use of this technique allows for the extraction of characteristic blinking times which are faster than CCD camera detector exposure times, which cannot be accessed by threshold based single molecule approaches due to aliasing. We test our method on a wide set of computer simulations, as well as on TIRF image series of surface-immobilized labeled DNA. We analyzed DNA-Cy5 complex in the presence of varying concentrations of Ni(II) ions, which act as triplet-state quenchers. In general, we have found good agreement between our technique and a single-molecule based method, but with significantly reduced analysis times. Furthermore, we recover blinking times which are shorter than the detector exposure time for one of the datasets with more rapid blinking kinetics. Finally, we analyze STORM data, and show it is possible to recover the characteristic on-times, using this autocorrelation analysis.

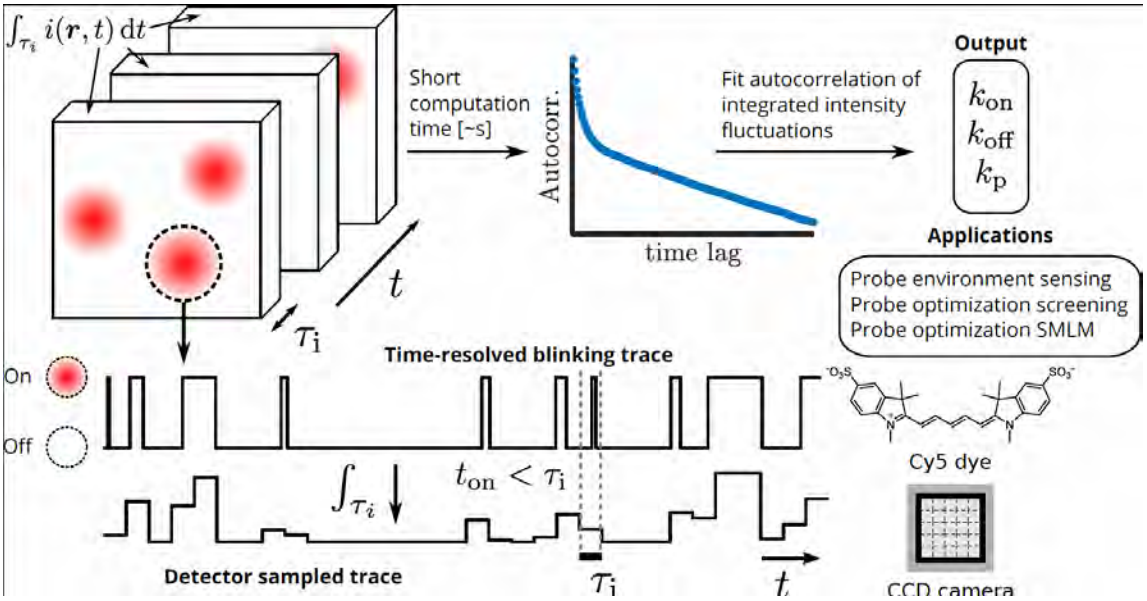


Figure. *Overview of image correlation based method for rapid measurement of fluorophore blinking rate constants*

Acknowledgments: This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada.

*Corresponding author: E-mail: paul.wiseman@mcgill.ca

Poster Presentation Abstracts

Poster Number	First Name	Surname	Abstract Title
P01	Aline	Acke	Expansion Microscopy (ExM) as a tool to unravel retroviral related questions at the nanoscale
P02	Sarita	Aryal	Investigating structural basis of Interaction between Cyclophilin A and Myb3 Transcription Factor in Trichomonas Vaginalis.
P03	Tom	Baladi	A Fluorescent Cytosine Analog in Gapmer Technology: Stealth Labeling vs. Conventional External Dyes
P04	Tom	Berkelman	Functionalized fluorescent polymer nanoparticles for flow cytometry and other biological applications
P05	Niels	Bisballe	Enabling water solubility of diazaoxatriangulenium through introduction of charged functional groups – a comparison of strategies
P06	Maidileyvis	Castro Cabello	Effect of water on the chemiexcitation step in peroxyoxalate chemiluminescence.
P07	Cecilia	Cerretani	A switchable dual emissive DNA-stabilized silver nanocluster
P08	Amitava	Chandra	Strategies and Development of Fluorescent Sensors for Signaling Phospholipids
P09	Sehoon	Chang	Probing Nanoparticles in Reservoir-on-a-Chip by Surface-Enhanced Fluorescence and Raman Scattering (SEF-SERS) Imaging
P10	Sune	Levin	A nanofluidic device for parallel single nanoparticle catalysis in solution
P11	Katia	Charov	One tool to probe them all: a solvatochromic method to monitor multiple protein-protein interactions of the bacterial acyl carrier protein
P12	Wei	Chen	Single-Molecule FRET Reveals Large-Scale Domain Motions in the Transcription Factor Protein NF-κB
P13	Ucheor	Choi	NSF-mediated disassembly of on- and off- pathway SNARE complexes and inhibition by complexin
P14	Li Hung Chloe	Chung	Time-resolved emission spectral studies of glycation effects on tyrosine fluorescence in protein under physiological conditions
P15	Nader	Danaf	Coordinative Binding of Polymers to Metal-Organic Framework Nanoparticles for Control of Interactions at the Biointerface
P16	Sune	Levin	High throughput size-determination and multiplexed fluorescence analysis of single biological vesicles in a nanofluidic device
P17	Laurens	D'Huys	Identifying microbial species through single-molecule, super-resolved DNA optical mapping
P18	Michelle	Digman	Correlating glioblastoma tumor subpopulation metabolism by Fluorescence-Lifetime Imaging Microscopy (FLIM) with growth rate and chromosome instability
P19	Chaoqing	Dong	Core-satellite Nanostructure Enhanced Fluorescence and its Applications in the Inhibition Assay on Receptor Dimerization
P20	Bilha	Fischer	Novel Styryl Quinolinium Fluorescent Probes for Imaging of Ribosomal RNA in Living Cells
P21	Dora-Luz	Flores	Identifying Phosphorescent Nanoparticles in Cancer Cells using Phasor Analysis
P22	Marcelo	Gehlen	Reactive phenanthrene derivatives as markers of amino groups in fluorescence microscopy
P23	Ulas	Coskun	ChronosDFD, a Fluorimeter for High Speed Time-Resolved Fluorescence Measurements
P24	Natalia	Grytsyk	Investigation of the interconversion kinetics of nucleic acid structures with time-resolved fluorescence in microfluidic droplets
P25	Daniel	Gudnason	Fluorescence Properties of Conjugated Polymers on DNA Origami
P26	George	Hamilton	Local and Global Dynamics in the Supertertiary Organization of Postsynaptic Density Protein 95
P27	Amani	Hariri	Independent Control of the Thermodynamic and Kinetic Properties of fluorescently labelled Aptamer Switches for biosensing applications.
P28	Xiangyi	Huang	Catalytic polystyrene-metalloporphyrin dot for highly effective chemiluminescence dynamic therapy
P29	Laura	Kacenauskaite	Probing solvation, ionpair formation and aggregation of fluorescent cationic dyes
P30	Sunil	Shah	VistaVision toolbox for quantitative multi-parameter analysis of single molecule dynamics
P31	Izabela	Kaminska	DNA origami-based nanopositioners for single-molecule studies on graphene
P32	Yusuke	Kawamoto	Synthesis of an Emissive Deoxyguanosine Analogue toward Studies of Non-canonical Nucleic Acids
P33	Narendar	Kolimi	A compact unbound state of p27 guides its specific binding with CyclinA/Cdk2

Poster Number	First Name	Surname	Abstract Title
P34	Venkata Rao	Krishnamurthi	Molecular Mechanism of Antimicrobial Activity of Low DC Voltage Against E. coli.
P35	Bo W.	Laursen	Bright long fluorescence lifetime dyad fluorophores for time-gated single molecule detection and imaging
P36	Xiaohua	Li	Syntheses and Applications of Fluorescent Probes for Redox-active Species in Living Cells
P37	Satoshi	Habuchi	Microfluidics-based single-molecule live-cell imaging reveals essential role of spatiotemporal dynamics of selectin ligands on blood stem-cell rolling
P38	Yulong	Li	Spy on neuromodulation with new genetically encoded fluorescent sensors
P39	Yao	Li	Monitoring the formation and degradation of cyclic dinucleotides using fluorescence
P40	Paul	Ludford	Fluorescing Isofunctional Ribonucleosides: Assessing Adenosine Deaminase Activity and Inhibition
P41	Patrick	Macdonald	Direct single-molecule imaging for diagnostic and blood screening assays
P42	Gerard	Masdeu	Shining light on enzyme immobilization
P43	Satoshi	Habuchi	Entangled polymer dynamics beyond reptation: a single molecule study
P44	Dannielle	Mccarthy	Stimulated Emission Depletion Microscopy of Programmed Death-1 in Melanoma Tissue
P45	Yves	Mely	Single particle imaging of upconversion nanoparticles in aqueous buffers
P46	Nicole	Mertes	A new class of indicators for fast and specific calcium imaging
P47	Krisztina Eszter	Treuerne Balazs	Rapid detection of pathogenic bacteria via fluorogenic enzyme substrate probes
P48	Krzysztof	Nawara	SAFE method for luminescence quantum yield determination
P49	Robert	Neely	Image search for the genome: the development and application of fluorescent single-molecule DNA barcodes
P50	Kevin	Nguyen	Fluorescent probes and analogues for staining bacterial cells
P51	Jesper	Nilsson	A Fluorescent Cytosine Analog in Gapmer Technology: Stealth Labeling of an Antisense Oligonucleotide
P52	Shuho	Nozue	Ultrasmall semicrystalline organic J-aggregate nanodots for single- and two- photon fluorescence microscopy
P53	Francesco	Palomba	p53 Oligomerization and Dynamics upon DNA Damage revealed by Fluorescence Fluctuations Imaging Techniques.
P54	Hubert	Piwonski	Millimeter-Deep Detection of Single SWIR Emitting Polymer Nanoparticles Through a Turbid Media
P55	Suman	Ranjit	Determination of the metabolic index using the fluorescence lifetime of free and bound NADH in the phasor approach
P56	Jicun	Ren	In situ studying protein kinase activity in single living cell by combining single molecule spectroscopy with activity-based protein profiling strategy
P57	George	Samaan	Structure and Properties of Fluorogenic Cytidine Analogues.
P58	Falk	Schneider	Deciphering free and hindered diffusion dynamics in the plasma membrane of living cells
P59	Lorenzo	Scipioni	AiryScan CCA provides structural and dynamic fingerprinting of subcellular compartments in living cells
P60	Maged	Serag	Probabilistic space filling: a new algorithm for single molecule tracking and analysis
P61	Caterina	Severi	New organic nanoantennas for FRET-based detection of nucleic acids
P62	Yi	Shen	Engineering Genetically Encodable Fluorescent Indicators to Probe Cellular Ion Dynamics
P63	Trevor	Smith	Time-resolved emission studies of photo-aggregated fluorophores
P64	Benedikt	Sohmen	Allosteric action in Hsp90: From nucleotide binding to domain rearrangement
P65	Hamid	Soleimaninejad	Fluorescence Anisotropy Imaging Microscopy (FAIM): A new approach with multiple applications from cell morphology to energy migration dynamics
P66	Di	Su	Determination of atrial fibrillation related exosomes by single molecule Fluctuation Correlation Spectroscopy on a microwell chip

Poster Number	First Name	Surname	Abstract Title
P67	Sarala	Tantirimudalige	Application of Fluorescence Cross Correlation Spectroscopy (FCCS) and Fluorescence Single Particle Tracking (SPT) to elucidate the role of GM1a as a possible receptor/attachment factor for Dengue Virus infection in live cells
P68	Azmain	Taz	Fluorescence Resonance Energy Transfer assay to study drug-induced conformation changes of NMDA receptor in live cells.
P69	Ewald	Terpetschnig	New NIR Emitting APC Tandem for Biomedical Applications
P70	Stuart	Thomson	Photophysical Characterization of Perovskite Quantum Dots
P71	Kateryna	Trofymchuk	Self-assembled plasmonic nanoantennas for sensitive diagnostics with a smartphone
P72	Marc	Turner	Spectroscopic Properties of Fluorescent 8-DEA-tC DNA-RNA Heteroduplexes
P73	Krystian	Ubych	Site-selective visualization of DNA methylation architecture in fixed cells
P74	Marisa	Vanheusden	Expanding the toolbox for the subcellular mapping of multi-omic biomarkers in tissues
P75	Tom	Vosch	Anti-Stokes Fluorescence Microscopy.
P76	Yong	Wang	Anomalous diffusive dynamics of individual H-NS proteins in live bacteria and their responses to silver ions
P77	Jerker	Widengren	Transient state (TRAST) imaging of local cellular redox conditions and intermittent protein-lipid interactions in cellular membranes
P78	Changge	Zheng	The environment-sensitive diketopyrrolopyrrole-based nucleoside fluorescent probe

Expansion Microscopy (ExM) as a tool to unravel retroviral related questions at the nanoscale

Aline Acke,[a] Flore De Wit,[b] Doortje Borrenberghs,[a] Susana Rocha,[a] Raffaele Vitale,[a] Volker Leen,[a] Kris P.F. Janssen,[a] Zeger Debyser,[b] Johan Hofkens*[a]

[a] *Molecular Imaging and Photonics, KULeuven, Celestijnenlaan 200F, 3001 Leuven, Belgium*
[b] *Molecular Virology and Gene Therapy, KULeuven, RK-Herestraat 49, 3000 Leuven, Belgium*

For some time, researchers have been interested in not only battling diseases induced by viral pathogens but also using these organisms to their advantage. Recently, use of viruses to treat genetic diseases is possible through gene therapy via viral vectors. A retrovirus called the Murine Leukemia Virus (MLV) is commonly used for this purpose. Its integration pattern is of great interest, since these vectors tend to integrate close to overly active regions or genes, which in turn can induce leukemia in treated patients. Therefore, studying integration patterns of safer mutants is highly beneficial[1]. As a starting point to visualize open and thus active chromatin sites, the epigenetic status of histone proteins is examined through fluorescent markers. Considering both histone-methylation and –acetylation correspond to open chromatin[2], fluorescent antibodies are used to highlight these markers and the interaction they display among different virus types through the use of fluorescence microscopy. However, since these processes tend to exceed the diffraction limit the sample is modified in order to obtain higher resolutions by physically expanding it, referred to as expansion microscopy (ExM). By infusing the biological sample with suitable monomers, a super-absorbent polymer can be formed throughout the sample, to which the biomolecules of interest are cross-linked such that their original geometry is preserved (Figure)[3]. For the first time, an accurate and high resolution study on the behavior of different MLV vectors by making use of conventional confocal microscopes seems within reach.

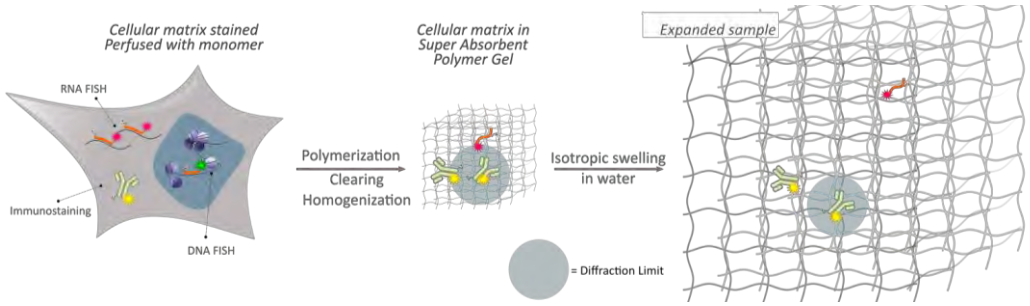


Figure. *Different biomolecules of interest (DNA, RNA, proteins) can be cross-linked into a super-absorbent polymer followed by isotropic expansion to improve resolution.*

Acknowledgments: This work was supported by grants from the Research Foundation - Flanders (FWO).

References: [1] El Ashkar, S., et al. *Mol. Ther. Nucleic Acids* **2017***, 7*, 231-245. [2] Tsompana, M., and Buck, M. J., *Epigenetics Chromatin.* **2014**, *7*, 33. [3] Chozinski, J. T., et al. *Nature Methods.* **2016**, *13*, 485-488.

*Corresponding author: E-mail: johan.hofkens@kuleuven.be

Investigating Structural Basis of Interaction Between Cyclophilin A and Myb3 Transcription Factor in *Trichomonas Vaginalis*.

Sarita Aryal[1,3], Yuan-Chao Lou,[1] Jung-Hsiang Tai,[1] Hsu Chuan Hua[2], Chinpan Chen[1]

[1] *Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan*
[2] *Department of Agricultural Chemistry, National Taiwan University, Taipei 106, Taiwan*
[3] *Department of Chemistry, National Tsinghua University, Hsinchu 300, Taiwan*

Trichomoniasis, caused by *Trichomonas vaginalis*, is the most common non-viral sexually transmitted disease in human and emergence of drug resistant strains poses a serious threat to public health in the context of elevated susceptibility to HIV and HPV transmission in diseased individuals. It has been reported that in *T. vaginalis, Tv* Cyclophilin 1 (*Tv*Cyp1) regulates the nuclear translocation of Myb proteins – Myb1 and Myb3 that control the expression of a 65-kDa malic enzyme which helps in cytoadherence to human vaginal epithelial cells. *T. vaginalis*, *Tv* Cyclophilin 2 (*Tv*CyP2) is similar with (*Tv*Cyp1) in addition to having extended N-terminus residues. We have previously reported X-ray structure of *Tv*CyP1 as a dimeric cyclophilin. In current study, we determined the unique structure of *Tv*CyP2 as a monomer where its N-terminus loop goes in to the catalytic pocket of its own monomer fitting well into the active site and auxiliary pocket, respectively. Interestingly, this N-terminus loop seems to mimic the nature of substrate as cyclosporine (CsA), where N-terminus residue valine -9 just fitted in to the hydrophobic core of its active site. Same interaction is also observed for CsA binding with cyclophilins. Moreover, analytical ultra-centrifugation (AUC) and gel filtration data showed that *Tv*Cyp2 is a monomer in solution. Also, we determined *Tv*CyP1 and *Tv*CyP2-binding sequence of Myb3 (Myb3 50–87) as the minimum binding motif in Myb3 using solution NMR, fluorescence polarization (FP) and isothermal titration calorimetry (ITC) experiments. Further NMR titration data revealed that the Myb3 fragment (Myb3 50–87) binds to the active site of *Tv*Cyp1 and *Tv*CyP2. To gain insights into interaction, we are currently carrying out the biophysical studies to determine the complex structures of *Tv*Cyp1 with Myb3 as well as *Tv*CyP2 with Myb3. Results from X-ray studies on these complexes will throw further light into the design of a specific inhibitor for *Tv*CyP1 and *Tv*CyP2. Since cytoadherence is one of the early steps in pathogenesis; we anticipate that *Tv*CyP1 and *Tv*Cyp2 might be a potent drug targets for designing a specific cyclosporine analogue inhibitors to control pathogenesis.

Acknowledgments: This work was supported by Academia Sinica and the Ministry of Science and Technology, Taiwan, ROC

*Corresponding author: E-mail: bmchinp@ibms.sinica.edu.tw

A Fluorescent Cytosine Analog in Gapmer Technology: Stealth Labeling *vs.* Conventional External Dyes

Tom Baladi,[a,b] Jesper R. Nilsson,[b] Emma Kay,[a] Kajsa Kanebratt,[a] Dzenita Bazdarevic,[c] Johan Meuller,[d] Malin Lemurell,[a] L. Marcus Wilhelmsson[b], Anders Dahlén[a]*

[a] *Medicinal Chemistry, Research and Early Development Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden.*
[b] *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Kemivägen 10, SE-41296 Gothenburg, Sweden.*
[c] *Bioscience, Research and Early Development, Cardiovascular, Renal and Metabolic, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden.*
[d] *Mechanistic Biology & Profiling, Discovery Sciences, R&D, AstraZeneca, Gothenburg, Sweden.*

Over the past 20 years the activity around oligonucleotides within the pharmaceutical industry has intensified significantly as new oligo-based drugs enter the market.[1] Many techniques to determine the properties of the oligos of interest have been developed, such as knockdown efficiency via qPCR, internalisation efficiency with commercially available kits and cell localisation with various dying protocols. It is also very common to make use of commercial fluorescent dyes such as AlexaFluor, Bodipy and cyanine-based dyes to visualise trafficking inside cells during imaging studies. However, these fluorescent dyes may change the overall properties of the oligo of interest since they are usually quite large and also contain multiple charges that may affect permeability, uptake, endosomal escape, trafficking inside the cell, accumulation in different cell compartments as well as the interaction with its target. Recently, our lab has made good use of fluorescent base analogs (FBAs) in several FRET studies[2, 3] of DNA and RNA and we recognized that these FBAs could also prove useful in imaging studies of oligos without affecting the knockdown or internalisation efficiency. To establish if results generated with commercial dyes are reliable, we decided to synthesize a number of naked and conjugated oligo gapmers containing either Cy3 or a fluorescent cytosine analog.

Acknowledgments: This work was conducted within the FoRmulaEx research consortium and supported by the Swedish Foundation for Strategic Research (SSF, grant No. IRC15-0065).

References: [1] Valeur, E. et al., *Angew. Chem. Int. Ed.* **2017**, *56*, 10294. [2] B. Dumat, M. Bood, M. S. Wranne, C. P. Lawson, A. F. Larsen, S. Preus, J. Streling, H. Gradén, E. Wellner, M. Grøtli, L. M. Wilhelmsson, *Chemistry – A European Journal* **2015**, *21*, 4039-4048. [3] M. Bood, A. F. Fuchtbauer, M. S. Wranne, J. J. Ro, S. Sarangamath, A. H. El-Sagheer, D. L. M. Rupert, R. S. Fisher, S. W. Magennis, A. C. Jones, F. Hook, T. Brown, B. H. Kim, A. Dahlen, L. M. Wilhelmsson, M. Grotli, *Chemical Science* **2018**, *9*, 3494-3502.

*Corresponding author: E-mail: anders.dahlen@astrazeneca.com.

Functionalized fluorescent polymer nanoparticles for flow cytometry and other biological applications

Tom Berkelman,*[a] Hong Chen[a], Matthew Mattson[a], Praveena Garimella[a], Christine Thornton[a], Dora Fonseca[a], Angie Green [a], Shane Petersen[a], Joseph Siino[a], Karen Zhu[a], Leila Rainis [a], Edwin Lozano[a], Chien-Hung Chiang[a], Yueping Xu[a], Lei Li[a], Jiango Yu[b], Xing-Hua Zhou[b], Yu Rong[b], Yi-Che Wu[b], Daniel T. Chiu[b]

[a]*Bio-Rad Laboratories, Life Science Group, 6000 James Watson Dr., Hercules, CA 94547*
[b]*Lamprogen, Inc., 4000 Mason Road, Suite 308D, Seattle, WA 98195*

Fluorescent polymer nanoparticles (Pdots) have several desirable qualities as fluorescent probes. They are exceptionally bright and photostable, and properties such as excitation wavelength, emission wavelength, size and biological compatibility can be precisely tailored for their intended application. Pdots are prepared from fluorescent semiconducting polymers by nanoprecipitation, and the composition of the polymer determines the optical properties and surface characteristics of the Pdot. The newest generation of Pdots prepared from single polymers comprised of monomers that serve as light absorbers, energy transfer units, terminal acceptors, as well as derivatized units conferring biocompatibility and facile bioconjugation[1,2]. We have developed a series of Pdots with UV, violet or blue excitation, with emission maxima covering the visible and NIR regions of the spectrum. The terminal acceptors for the longer-wavelength emitters are selected to give exceptionally narrow emission spectra. Detection reagents tagged with these Pdots are being commercialized under the StarBright brand.

These reagents have shown particular utility in flow cytometry applications, where materials with individually customized excitation and emission properties allow a high degree of multiplexing. They are being developed for other applications as well, such as multiplex Western blotting

References: [1] Rong, Y. et al., *ACS Nano* **2013***, 7,* 376. [2] Yu, J., et al. *Anal. Chem.* **2017**, *89*, 42.

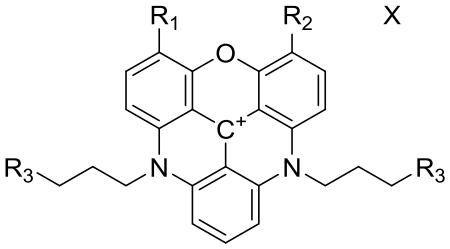
*Corresponding author: E-mail: Thomas_berkelman@bio-rad.com

Enabling water solubility of diazaoxatriangulenium through introduction of charged functional groups – a comparison of strategies

Niels Bisballe,[a] Bo W Laursen*[a]

[a] *Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 København Ø, Denmark*

While large aromatic organic molecules can be excellent fluorophores, their poor solubility and compromised emissive properties in water generally complicates their use in bioimaging. This is indeed also the case for diazaoxatriangulenium ($DAOTA^+$).[1,2] Water solubility could be achieved simply by pairing the cationic dye with a hydrophilic counterion, but further functionalization was needed to enhance solvation and prevent aggregation (Figure 1).



1: $R_1 = R_2 = R_3 = -H$, $X = Cl^-$
2: $R_1 = -SO_3^-$, $R_2 = R_3 = H$, X = n/a
3: $R_1 = R_2 = -SO_3^-$, $R_3 = H$, $X = K^+$
4: $R_1 = R_2 = H$, $R_3 = -N^+Me_3$, $X = 3Cl^-$
5: $R_1 = R_2 = H$, $R_3 = -N^+Me_2(CH_2)_3SO_3^-$, $X = Cl^-$
6: $R_1 = R_2 = -SO_3^-$, $R_3 = -N^+Me_2(CH_2)_3SO_3^-$, $X = Cl^-$

Figure 1. Structures of water soluble $DAOTA^+$ chromophores.

Functionalization directly on the aromatic system (**3**) lead to a reduced fluorescence lifetime (FLT = 12 ns) and quantum yield (QY = 0.28) in water. By restricting the functionalization to the side chains of $DAOTA^+$ (**4**, **5**), FLT (18 ns) and QY (0.56) in water were found to be comparable to those of the parent chromophore in acetonitrile.[3]

Acknowledgments: This work was supported by grants from the Independent Research Fund Denmark.

References: [1] Maliwal, B. P. et al., *Plos One* **2013**, *8*, e63043. [2] Bora, I. et al., *Org. Biomol. Chem.* **2016**, *14*, 1091-1101. [3] Bogh, S. A. et al., *ACS Omega* **2017**, *2*, 193-203.

*Corresponding author: E-mail: bwl@nano.ku.dk

Effect of water on the chemiexcitation step in peroxyoxalate chemiluminescence.

Maidileyvis Castro Cabello, Wilhelm Josef Baader*

Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes, 748, 05508-000, São Paulo, SP, Brazil

A wide variety of analytical and bioanalytical applications are based on the highly efficient peroxyoxalate chemiluminescence reaction. This system can be easily carried out by the reaction of an oxalic ester with hydrogen peroxide, catalyzed by a base and in the presence of an activator. Although, this transformation has been intensively studied in anhydrous medium, obtaining quantum yields of up to 50%,[1] there are only few mechanistic studies in aqueous conditions, where the efficiency of this reaction is much lower.[2,3] In this work we report our results of a study on the chemiexcitation step of the peroxyoxalate reaction using two oxalic esters with different reactivity in organic solvents like 1,2-dimethoxyethane, containing small amounts of water, in order to understand why the chemiexcitation quantum yields of this transformation are drastically lowered in aqueous media.

The quantum yields showed to increase in the presence of water up to a maximum value for a molar fraction of 0.06 of water, decreasing with still higher water content of the reaction medium. A possible explanation for these results can be given by assuming that small amounts of water stabilize the pair of radical ions formed within the solvent cage and the solvation initially contributes to keeping these species together, allowing the occurrence of the electron back-transfer step, responsible for the formation of states excited. From a certain limiting value of polarity the radical ions can be solvated individually by the water molecules avoiding the possibility of an electron back-transfer and leading to a decrease in the chemiexcitation quantum yields. The variation of the ACT concentration allows the study of the chemiexcitation step and the determination of the chemiluminescence parameters of this transformation, which showed to be critically influenced by the presence of small amounts of water. For three different water fractions the electron transfer coefficients α were obtained. The α value of 0.10 indicates an early transition state with respect to electron transfer and, presumably, also O-O bond cleavage, since these two steps should be simultaneous. In a preliminary conclusion it can be observed that the low chemiexcitation quantum yields obtained for the peroxyoxalate reaction in aqueous medium are not mainly due to concurrent oxalic ester hydrolysis but to the influence of water on the efficiency of the chemiexcitation step.

Acknowledgements: Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq, PhD scholarship for MCC), Coordenadoria de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), and Fundacão de Amparo à Pesquisa do Estado de São Paulo (FAPESP, WJB 2014/22136-4) for financial support.

References: 1.Ciscato, L. F. M. L. *et al.*,*Arkivoc* **2012,** 391–430 (2012). 2. Cabello, M. C., El Seoud, O. A. A. & Baader, W. J., *J. Photochem. Photobiol. A Chem.* **367,** (2018). 3. Souza, G. A., Peixoto, M. M. M., Santos, A. P. F. & Baader, W. J.,*ChemistrySelect* **1,** 2307–2315 (2016).

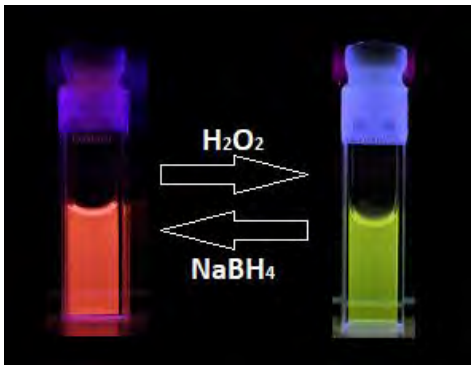
*Corresponding author: E-mail: wjbaader@iq.usp.br

A switchable dual emissive DNA-stabilized silver nanocluster

Cecilia Cerretani, Tom Vosch*

Nano-Science Center/Department of Chemistry, University of Copenhagen. Universitetsparken 5, 2100 Copenhagen, Denmark.

DNA-templated silver nanoclusters (DNA-AgNCs) are bright fluorophores formed by 2-30 silver atoms embedded in one or multiple single-stranded DNA oligomers.[1-3] According to models proposed by Schultz *et al.*[4] and Petty *et al.*,[5] DNA-AgNCs comprise a core of neutral Ag atoms surrounded by Ag^+ cations. The number of neutral atoms defines the emission wavelength of the clusters, while the Ag^+ cations *glue* the core and the DNA bases together.



Among the DNA sequences that stabilize emissive AgNCs, some can produce one specific emitter, while others host a range of different emitters. In this contribution, I will present results on a ss-DNA sequence (*5'-TTC CCA CCC ACC CCG GCC CGT T-3'*)[6] that can stabilize a red- and a green-emissive silver nanocluster. These two emitters can convert between each other in a reversible way. The change from red- to green-emitting DNA-AgNC can be triggered by addition of H_2O_2, while the opposite conversion can be achieved by addition of $NaBH_4$.[6]

Understanding the mechanism behind the remarkable conversion between the two emitters could lead to the development of a new range of DNA-AgNC-based ratiometric sensors.

Acknowledgments: We gratefully acknowledge financial support from the Danish Ministry of Science, Technology and Innovation (Grant 09-065274), the Villum Foundation (VKR023115) and the Carlsberg Foundation (CF14-0388, CF17-0470).

References: [1] Petty, J. T. et al., *J. Am. Chem. Soc* **2004**, *126*, 5207. [2] Schultz, D. et al., *ChemComm* **2012**, *48*, 5748. [3] Copp, S. M. et al., *J. Phys. Chem. Lett.* **2014**, *5*, 959. [4] Schultz, D. et al., *Adv. Mater.* **2013**, *25*, 2797. [5] Petty, J. T. et al., *J. Am. Chem. Soc.* **2016**, *138*, 3469. [6] Cerretani, C.; Vosch, T.; *ACS Omega*, **2019**, 4, 4, 7895.

*Corresponding author: E-mail: tom@chem.ku.dk

Strategies and Development of Fluorescent Sensors for Signaling Phospholipids

Amitava Chandra[1], Samsuzzoha Mondal[2], Ravindra Venkatramani[1], Ankona Datta[1]

1. Department of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India. 2. Department of Chemistry, University of Pennsylvania, Philadelphia, PA, United States.

Phospholipids are essential structural components of plasma membrane and also participate in a plethora of cell signaling events. Cytosolic proteins, driven by electrostatic and hydrophobic interactions, interact with the head-groups of these phospholipids mediating downstream signaling events with time-scales ranging from seconds to minutes. Dysregulation in the phospholipids signaling network results in fatal disorders like cancer, bipolar disorders, myopathy or even vicious invasion of deadly pathogens. (1) Hence there is a requirement for developing sensitive, selective and reversible sensors for these signal mediating phospholipids. These sensors can aid investigation of the molecular basis of signaling events. We have focused on the development of fluorescent probes for sensing two important classes of signaling phospholipids: phosphoinositides and phosphatidylserine. For tracking phosphoinositides we utilized a Forster Resonance Energy Transfer (FRET) based strategy. (1) We selected a phosphoinositide-(4,5)-bisphosphate (PI(4,5)P2) selective peptide Gel-20aa which undergoes coil to helix conformational change upon binding to PI(4,5)P2. We labeled the peptide with tryptophan and IAEDANS, a pair of donor-acceptor dyes, on suitable positions, based on molecular dynamics simulations performed on the coil and helix forms of the peptide. The resulting PI(4,5)P2 concentration dependent change in FRET was utilized to determine threshold concentration levels of PI(4,5)P2 required to induce conformational change in proteins which regulate cytoskeletal rearrangements in cells. We have also worked on the development of cell permeable, reversible and sensitive ratiometric sensors for another important class of signaling phospholipids, phosphatidylserine (PS). I will present the details of sensor development and photo-physical evaluation of the probes.

Acknowledgments: This work was supported by grants from the Department of Atomic energy (DAE), Govt. of India.

References: [1] Phosphoinositides in cell regulation and membrane dynamics, Paolo, G., D., Camili, P., D., Nature, **2006**, *443*, 651-657.

[2] Optically sensing phospholipid induced coil–helix transitions in the phosphoinositide binding motif of gelsolin, Mondal, S., Chandra, A., Venkatramani, R., Datta, A., *Faraday Discussions.* **2018***, 207*, 437-458.

Probing Nanoparticles in Reservoir-on-a-Chip by Surface-Enhanced Fluorescence and Raman Scattering (SEF-SERS) Imaging

Sehoon Chang, Wei Wang *

Aramco Services Company, Aramco Research Center-Boston, 400 Technology Square, Cambridge, MA 02139, USA

Nanofluid flooding is a new chemical enhanced oil recovery (EOR) technique whereby nanoparticles or nanocomposite fluids are injected into oil reservoirs to effect oil displacement or to improve injectivity. However, mechanism of the nanofluid flooding is still to be understood. In this research, dual-mode surface-enhanced fluorescence (SEF)-surface-enhanced Raman scattering (SERS) composite nanoparticles have been developed to study their transport phenomena and detectability in porous media, providing valuable information for understanding the role of nanoparticles in nanofluid EOR process. The composite nanoparticles are composed of Ag or Au metal cores, specific dye molecules, and a SiO_2 shell. To optimize geometry and morphology of the composite nanostructures for the maximum simultaneous signal enhancement of SEF and SERS, we studied the effect of distance between core metal nanoparticles and dye molecules on the signal enhancement by precise control of the distance through Langmuir-Blodgett technique. Based on the obtained information, we developed synthesis procedure to produce the composite nanoparticles, and demonstrated that the embedded dye molecules are detectable by both fluorescence and Raman spectroscopies generating dramatically enhanced detectability due to the strong SEF-SERS phenomena. The synthesized multifunctional composite nanoparticles have been applied to probe interfacial process of fluids at liquid-liquid interfaces and study the behavior of nanoparticles at liquid-solid interfaces by flooding in microfluidic chips using fluorescence and Raman microscopic imaging techniques.

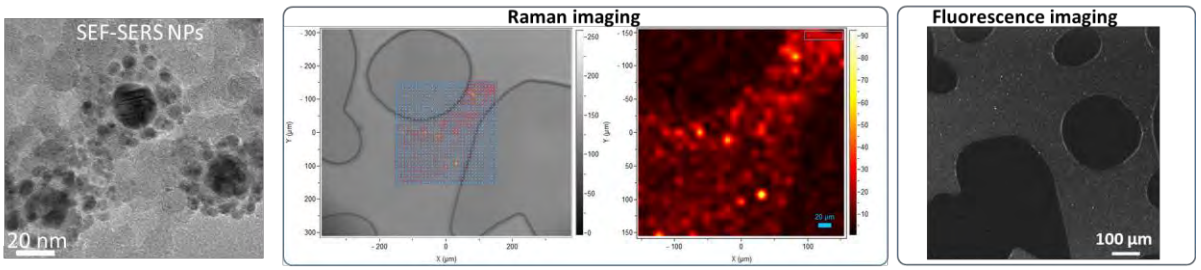


Figure. *Fluorescence and Raman imaging of dye embedded SEF-SERS nanoparticles in microfluidic reservoir-on-a-chip*

Acknowledgments: This work was supported by Aramco Services Company

References: Chang, S. et al., *J. Phys. Chem. C.* **2017**, *121*, 8070.

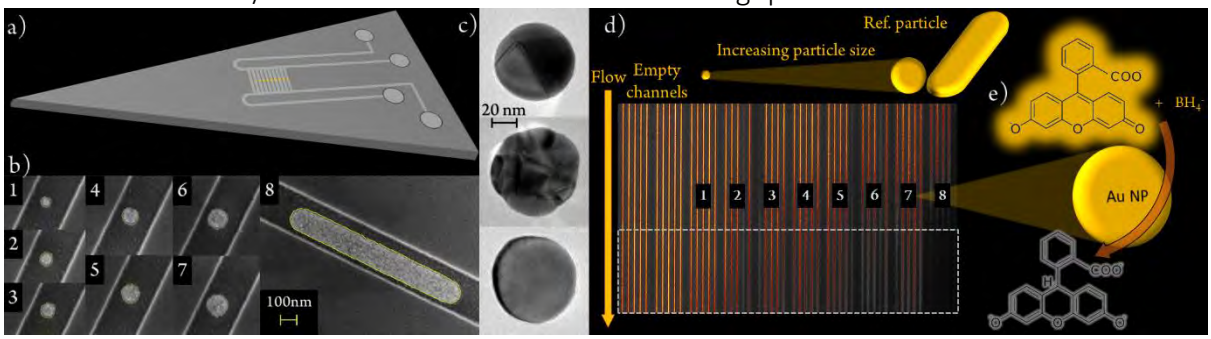
*Corresponding author: E-mail: wei.wang@aramcoservices.com

A nanofluidic device for parallel single nanoparticle catalysis in solution

Sune Levin,[a] Joachim fritzsche,[b] Sara Nilsson,[b] August Runemark,[c] Bhausaheb Dhokale,[c] Henrik Ström,[d] Henrik Sundén,[c] Christoph Langhammer,*[b] Fredrik Westerlund*[a]

[a] *Department of Biology and Biological Engineering, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden*
[b] *Department of Physics, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden*
[c] *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden*
[d] *Department of Mechanics and Maritime Sciences, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden*

In the field of nanoparticle catalysis, most measurements are performed on millions of particles and the results therefore always depict an average of the evaluated particles. Simultaneously, nanoparticles are known to be very heterogeneous resulting in large variations in reaction rate from particle to particle.[1] To answer this issue, several methods for studying single nanoparticle have emerged.[2] However, they all have limitations such as requiring low reactant concentrations and/or reaction rates or have a low throughput.[3]



We here present a new method utilizing nanofluidics and fluorescence to achieve measurements of highly parallelized single nanoparticle catalysis in solution. By employing reactions with a turn on or turn off in fluorescence while keeping the solution in focus, via the fluidic system, the conversion rate is easily monitored. In this work we show how the reduction rate of fluorescein by borohydride over an Au nanocatalyst varies for polycrystalline Au nanoparticles of similar size in the range of 64-129 nm in diameter. By scanning over µM fluorescein concentrations, we can observe the transition from the surface reaction limited regime to the fully mass transport limited regime. This concept provides a versatile platform for highly parallelized single particle catalysis in solution, at µM reactant concentrations and relevant turnover frequencies.

Acknowledgments: This work was mainly supported by grants from the Knut and Alice Wallenberg Foundation via project 2015.0055.

References: [1] Heiz, U., et al. *Nanocatalysis.* 2007. *[2]* Tachikawa, T., et al. *Langmuir.* 2012, *28,* 8933-8943. [3] De Cremer, G., et al. *Chem. Soc. Rev.* 2010, *39*, 4703.

*Corresponding authors: E-mail: fredrik.westerlund@chalmers.se
clanghammer@chalmers.se

One tool to probe them all: a solvatochromic method to monitor multiple protein-protein interactions of the bacterial acyl carrier protein

Katia Charov,[a] Michael D. Burkart*[a]

[a] *Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093*

Protein-protein interactions are ubiquitous to all domains of life and have gained recent interest as drug targets. However, many current methods to study protein-protein interactions can be costly and are low-throughput. Here, we demonstrate a solvatochromic tool based on the natural post-translational modification of the *Escherichia coli* acyl carrier protein (AcpP) used to visualize protein-protein interactions between AcpP and thirteen different partner enzymes from several biosynthetic pathways. We use this tool to confirm proposed interactions between AcpP and both catalytic and regulatory proteins. We also show the utility of this method towards detecting allosteric changes to partner enzyme structure and the validation of active site inhibitors. We anticipate the future adaptation of this assay into a high-throughput screen for antibiotic discovery.

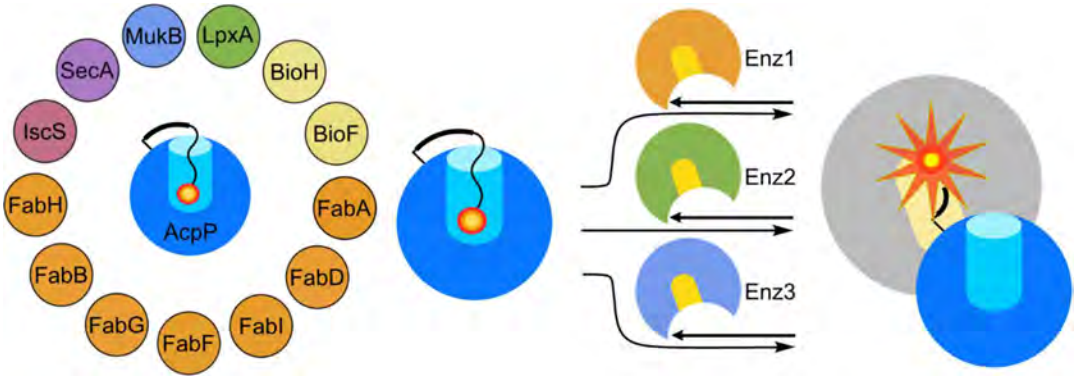


Figure. *A solvatochromic dye labels the acyl carrier protein and allows for the rapid analysis of multiple partner enzymes from various biosynthetic pathways.*

Acknowledgments: Research reported in this publication was supported by NIH grant RO1 GM095970. K.C. was supported by the National Science Foundation Graduate Assistance in Areas of National Need (NSF GAANN) Fellowship. We also thank E. Bouveret, PhD., V. Rybenkov, PhD., P. Zhou, PhD., and D. Oliver, PhD. for providing plasmids for the expression of IscS, MukB, LpxA and SecA. We thank J.T. Mindrebo for protein purification discussion, T.D. Davis, PhD. for synthetic chemistry advice, and M.H. Ostertag for assistance with data analysis.

*Corresponding author: E-mail: mburkart@ucsd.edu

Single-Molecule FRET Reveals Large-Scale Domain Motions in the Transcription Factor Protein NF-κB

Wei Chen[a] and Elizabeth A. Komives*[a]

[a] *Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California*

The NF-κB family of transcription factor is a central mediator of immune and imflammatory responses. NF-κB subunits form functional dimers and recognize DNA targets by the two N-terminal DNA-binding domains. It has been hypothesized that the two N-terminal DNA-binding domains in the NF-κB dimer can rotate and translate with respect to each other, but direct evidence of such motions is lacking. Previous studies by kinetic experiments, hydrogen-deuterium exchange mass spectrometry, and molecular dynamics simulations suggested that such motions are crucial in controlling DNA association and dissociation rates[1,2] and thus can keep gene expression under tight control. It was suggested that the various DNA-binding affinities of NF-κB dimers are a result of their different domain motions[1]. In addition, the inhibitor protein IκBα has been shown to accelerate NF-κB dissociating from DNA targets – a process termed molecular stripping.[2,3] Molecular dynamics simulations suggested that during molecular stripping, IκBα allosterically alters the relative orientation of NF-κB domains to lower the dissociation barrier for DNA[2]. All these studies strongly suggested that the N-terminal DNA-binding domains of NF-κB are highly dynamic. Here we show the first direct observation of NF-κB domain motions by single-molecule FRET. A donor-acceptor pair was introduced into the two domains using unnatural amino acids and copper-free click chemistry. The single-molecule time trajectories of immobilized NF-κB were obtained by TIRF microscopy. Large-scale domain motions of NF-κB were observed. The FRET efficiencies between the two N-terminal DNA-binding domains spanned from low to high, revealing a highly heterogeneous conformational ensemble. Transitions between different FRET states occurred on the time scale of few tens of seconds, indicating surprisingly slow motions. Our results provide direct visualization and quantitative characterization of large-scale domain motions of NF-κB and lay the foundation for understanding molecular mechanisms of NF-κB signaling.

Acknowledgments: This work was supported by National Institutes of Health (NIH) Grant P01GM071862 to E.A.K. W.C. acknowledges support from the Ministry of Education of Taiwan via a fellowship.

References: [1] Narang, D. et al., *J Mol Biol.* **2018,** *430,* 10. [2] Potoyan, D. A. et al., *Proc. Natl. Acad. Sci. U.S.A.* **2016,** *113,* 110. [3] Bergqvist, S. et al., *Proc. Natl. Acad. Sci. U.S.A.* **2009,** *106,* 19328.

*Corresponding author: E-mail: ekomives@ucsd.edu

NSF (N-ethylmaleimide sensitive factor), a member of AAA+ ATPase family, together with αSNAP (alpha soluble NSF attachment protein) disassemble SNARE (soluble NSF attachment protein receptor) complexes for recycling of the SNARE proteins for subsequent rounds of fusion. Neuronal SNAREs (synaptobrevin, syntaxin, SNAP-25) provide energy for membrane fusion, and they act together with the Ca^{2+}-sensor synaptotagmin, the regulator complexin, and the priming factors Munc13 and Munc18 for fast Ca^{2+}-triggered neurotransmitter release. The molecular mechanism of NSF regulated SNARE assembly and disassembly is largely unknown.

We developed a single molecule fluorescence approach of monitoring the spontaneous assembly and the NSF-mediated disassembly process of a single SNARE complex by NSF over multiple rounds. Similar to a previous chimeric SNARE design used for optical tweezer experiments, we covalently linked the components of the SNARE complex (syntaxin-1A, residues 181-262; full-length SNAP-25A, and synaptobrevin-2, residues 25-96) with spacer sequences, Sp1 and Sp2, and an Avi-tag sequence at the C-terminal end of synaptobrevin-2. We conducted single molecule FRET measurements with labeling sites attached to syntaxin-1A residue 249 and synaptobrevin-2 residue 82. This label pair is expected to produce high FRET efficiency when the SNARE complex is properly assembled.

We discovered that complexin reduces the efficiency of NSF-mediated disassembly of the SNARE complex in a concentration-dependent fashion. This observation suggests that complexin acts as a block to reduce undesirable disassembly of prefusion (i.e., *trans*-SNARE) complexes. After fusion, *cis*-SNARE complexes would be more readily disassembled by NSF since complexin may delocalize from the fusion site . Additionally we report a new structure of NSF/αSNAP/L-SNARE (L-20S) supercomplex using single-particle cryo-EM with the linked SNARE complex. Different from previous 20S structure with four αSNAP molecules wrapped around the truncated SNARE complex (Zhao et al., Nature 2015), only two αSNAP molecules were bound. The interfaces of the two αSNAP molecules overlap with the binding site of complexin on the SNARE complex, suggesting that it is competing with αSNAP and thereby reducing the disassembly efficiency. Furthermore, we show for the first time that NSF disassembles improperly assembled or misfolded SNARE complexes, thus acting as a protein quality control mechanism for efficient membrane fusion.

Time-resolved emission spectral studies of glycation effects on tyrosine fluorescence in protein under physiological conditions

Li Hung Chloe Chung,[a] Olaf J. Rolinski,[a] Vladislav Vyshemirsky,[b] Angelo Bella,[c] Maxim G. Ryadnov,[c] David J. S. Birch,*[a]

[a] *Department of Physics, SUPA, University of Strathclyde, 107 Rottenrow East, Glasgow, G4 0NG, United Kingdom*
[b] *School of Mathematics and Statistics, University of Glasgow, Glasgow, G12 8QQ, United Kingdom*
[c] *National Physical Laboratory, Hampton Road, Teddington, TW11 0LW, United Kingdom*

Protein misfolding alters the structure, stability and ultimately the function of native protein often leading to proteopathies. Insulin is an important protein that folds into a hexameric structure that is associated with glycaemia. Diabetic patients often have hyperglycemia, and the interaction between insulin and high glucose levels cause protein aggregation which leads to insulin resistance. Glycated protein is found in Lewy bodies[1] and β-amyloid[2] fibrillar deposits, which are related to Parkinson's and Alzheimer's diseases. Protein aggregation and amyloidogenesis can be monitored by fluorescence. However, amyloidogenic fibrils display a high degree of polymorphism which complicates the interpretation of fluorescence kinetics. With this in mind, we designed a synthetic protein that readily forms fibrous structures with homogenous growth kinetics. Both insulin and the synthetic protein have intrinsic tyrosine fluorophores and time resolved emission spectra (TRES) has been used to extract comparative information such as conformational changes and protein stability.[4,5] Understanding the kinetics of protein aggregation may provide an insight into the mechanism of insulin glycation and could enable better inhibitors of fibril formation.

Acknowledgments: This work was supported in part by the UK's Department for Business, Energy and Industrial Strategy and a NPL-Strathclyde Graduate Institute Studentship.

References: [1] Munch, G., et al. *J. Chem. Neuroanat.* **2000**, *20,* 253. [2] Sasaki, N., et al. *Am. J. Pathol.* **1998**, *153,* 1149. [3] Oliveria, L. M. A, et al. *BMC Biochemistry.* **2011**, *12*, 1. [4] Chung, L. H. C., et al. *Appl. Phys. Lett.* **2017**, *111*, 263701. [5] Chung, L. H. C., et al. *Methods Appl. Fluoresc.* **2019**, *(Submitted)*

*Corresponding author: E-mail: djs.birch@strath.ac.uk

Coordinative Binding of Polymers to Metal-Organic Framework Nanoparticles for Control of Interactions at the Biointerface

Andreas Zimpel,[a] Nader Al Danaf,[a] Benjamin Steinborn,[a] Jasmin Kuhn, [a] Miriam Höhn,[a] Tobias Bauer,[b] Patrick Hirschle,[a] Waldemar Schrimpf,[a] Hanna Engelke,[a] Ernst Wagner,[a] Matthias Barz,[b] Don C. Lamb,[a] Ulrich Lächelt,[a]* and Stefan Wuttke[a]*

[a] *Department of Chemistry and Pharmacy and Center for NanoScience (CeNS), LMU Munich, Butenandtstraße 11, 81377 Munich, Germany*
[b] *Institute of Organic Chemistry, Johannes Gutenberg-University Mainz, Duesbergweg 10-14, 55099 Mainz, Germany*

Metal-organic frameworks (MOFs) are a distinctive class of crystalline materials featuring an open framework established by coordination bonds between transition-metal cations and organic linkers. Due to their porous nature, MOFs have turned into an advanced research field that has proved to be useful in the implementation of various applications, such as storage, separation, catalysis, sensing, and energy technologies. In particularr, MOFs are of growing interest in the biomedical field and, due to their hybrid nature, they could display novel and enhanced properties compared to more established nanomaterials. The effective application of MOF nanoparticles (MOF NPs) depends strongly on their surface chemistry and understanding of their interactions at the biointerface. Using a self-assembly approach, we found that coating of Zr-*fumurate* MOF NPs with polymers is a convenient way for peripheral surface functionalization. Different polymers with biomedical relevance were assessed for the ability to self-assemble on the MOF surface. Carboxylic acid and amine containing polymers turned out to be potent surface coatings and a modulator replacement reaction was identified as the underlying mechanism. The strong binding of polycarboxylates was then used to shield the MOF surface with a polyglutamate-polysarcosine block co-polymer, which resulted in an exceptional high colloidal stability of the nanoparticles. The effect of polymer coating on interactions at the biointerface was tested with regard to cellular association and protein binding, which to the best of our knowledge, has never been discussed in literature for functionalized MOF NPs. We conclude that the used approach enables a high degree of chemical surface confinement, in which the physicochemical properties of MOF NPs in biological systems could be precisely controlled.

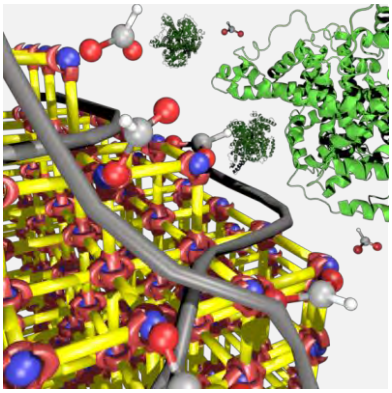


Figure1. *Self-assembly of different polymers on MOF nanoparticles for superior control of interactions at the biointerface*

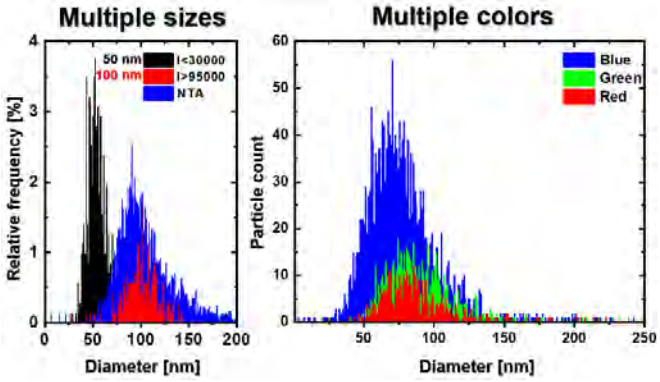
*Corresponding author: Email: stefan.wuttke@cup.lmu.de and ulaph@cup.uni-muenchen.de

High throughput size-determination and multiplexed fluorescence analysis of single biological vesicles in a nanofluidic device

Quentin Lubart,[a] Sune Levin,[a] Stephan Block,[b] Silver Jõemetsa,[c] Sriram Kesarimangalam,[a] Fredrik Höök,[c] Marta Bally,*[d] Elin K.Esbjörner,*[a] Fredrik Westerlund*[a]

[a] *Department of Biology and Biological Engineering, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden*
[b] *Department of Chemistry and Biochemistry, Freie Universität Berlin, DE-14195 Berlin, Germany*
[c] *Department of Physics, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden*
[d] *Department of Clinical Microbiology, Wallenberg Center for Molecular Medicine, Umeå University,* SE-*901 85 Umeå, Sweden*

Biological nanoparticles such as exosomes are potential targets for diagnostic and fundamental research, to fight against cancer and neurodegenerative diseases, or to help in tissue engineering and drug delivery.[1] Characterization of these particles is challenging due to their small size (30-100nm) and high heterogeneity[2] as well as samples often being relatively diluted and in small volumes. The main commercial techniques for characterizing biological nanoparticles are Electron microscopy, Nanoparticle tracking analysis and Flow cytometry. However, none of these techniques manages to achieve a combination of size determination, colocalization of particles and high throughput.



In this work we present a device for high throughput size-determination and multiplexed fluorescence analysis for single biological vesicles. This is achieved by flowing fluorescent vesicles through an array of 100 parallel nanochannels and determining their size by measuring each vesicle's size-specific one-dimensional diffusion constant.[3] Framerates of up to 50 fps are used as well as multiple fluorescence colour channels to attain detailed tracking of the particle movement and colocalize vesicles stained by multiple fluorophores. While the size determination accuracy is comparable with other commercial techniques, our method can distinguish complex size distributions and achieve size determination and colocalization of particles while maintaining a high throughput.

Acknowledgments: This work was mainly supported by grants from the Wenner-Gren Foundation.

References: [1] Sun. B., et al. *Rev. Neurosci.* 2018*, 29,* 531-546**.** *[*2] Rabanel. J., et al. *Nanoscale*. 2019, *11*, 383-406*.* [3] Block. S., et al. *Nature Com*. 2016, *7*, 12956.

*Corresponding authors: E-mail: fredrik.westerlund@chalmers.se
eline@chalmers.se, marta.bally@umu.se

Identifying microbial species through single-molecule, super-resolved DNA optical mapping

Laurens D'Huys[a], Arno Bouwens[a,b], Jochem Deen[b,c], Vince Goyvaerts[a], Adrien Descloux[b,c], Doortje Borrenberghs[a], Kristin Grussmayer[b,c], Tomas Lukes[c], Rafael Camacho[a], Jia Su[a], Cyril Ruckebusch[d], Theo Lasser[c], Dimitri Van De Ville[b,e,f], Aleksandra Radenovic[b,c], Kris Pieter Frans Janssen[a], Raffaele Vitale*[a,d], Johan Hofkens*[a]

[a] *Department of Chemistry, Katholieke Universiteit Leuven, 3000 Leuven, Belgium*
[b] *Institute of Bioengineering, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland*
[c] *School of Engineering, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland*
[d] *LASIR CNRS, Université de Lille, 59655 Villeneuve d'Ascq, France*
[e] *Center for Neuroprosthetics, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland*
[f] *Department of Radiology and Medical Informatics, Université de Genève, 1205 Geneva, Switzerland*

Microbiota have been part of a delicately balanced symbiotic system with animalia for about as long as they have co-existed. Assisting with the processing of e.g. host-indigestible carbohydrates or the production of vitamins, gut microbiota constitute a major factor in the etiology of complex disorders. They are implicated in obesity, cardiovascular disease, autoimmune diseases like multiple sclerosis, rheumatoid arthrosis and inflammation conditions such as inflammatory bowel disease (IBD) Crohn's (CD) or even neurological conditions such as Alzheimer's (AD).

Here, we present an approach for enterotype analysis via super-resolution optical genome mapping. Based on site-specific, enzyme-mediated introduction of fluorophores to the genome, so-called barcodes can be visualized through fluorescence microscopy (Fig. 1) and assigned to the origin species by applying resampling statistics. The long range genomic information unlocked by mapping will allow the envisioned platform to complement widely used NGS approaches by providing a more cost-effective alternative in diagnostic applications [1].

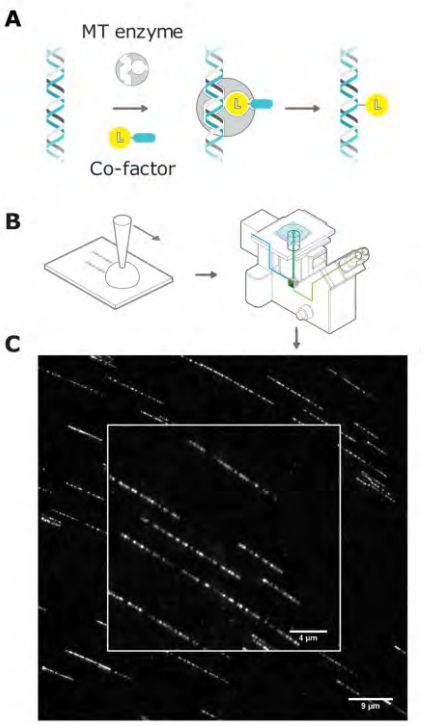


Fig. 1. DNA mapping flowchart.

Acknowledgments: This work was supported by Fonds voor Wetenschappelijk Onderzoek (FWO) Aspirant funding [No 11D3718N].

References: [1] Bouwens, Arno, et al. "Identifying microbial species by single-molecule DNA optical mapping and resampling statistics." *bioRxiv* (2019): 609412.

*Corresponding author: johan.hofkens@kuleuven.be, raffaele.vitale@kuleuven.be

Correlating glioblastoma tumor subpopulation metabolism by Fluorescence-Lifetime Imaging Microscopy (FLIM) with growth rate and chromosome instability

Andrew L. Trinh[1], Hongtao Chen[1], Yi-Hong Zhou [2], Michelle Digman[1]

[1]Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California Irvine, [2]UC Irvine Diabetes Center and Department of Medicine, UC Irvine Brain Tumor Laboratory and Department of Surgery, Irvine, CA, USA;

Glioblastoma multiforme (GBM) is a highly malignant type of primary brain tumor characterized by morphological heterogeneity in tumor cells. Inter-tumoral heterogeneity marked by different driver mutations in tumor suppressors and oncogene amplifications/over-expressions do not correlate with therapeutic responses. In addition, the number of Chr7 copies per cells is directly related to two key tumor cell subpopulations, stem-like tumor initiating cells (STIC) and tumor mass-forming cells (TMC). IN this study we used the phasor approach to fluorescence lifetime imaging microscopy (FLIM) to determine the fractional contribution of protein-bound and free NADH as a label-free indicator for inferring energy metabolism in living cells in these subpopulations. We observed high populations of bound NADH in one of the key functional subpopulations of GBM, namely stem-like tumor initiating cell (STIC), relative to other, namely tumor mass-forming cell (TMC). We compared changes in the bound and free NADH fractions, cellular respirations (OXPHOS and glycolysis), chromatin abnormality, and cell growth rate as the STIC population transforms to the TMC subpopulations. Our results indicate a distinct energy metabolism between the subpopulation of cells which correlate with enriched Chr7 copies and growth rate which is preceded by EGFR expression. This finding can potentially be used to non-invasively monitor chromosome instability-controlled tumor heterogeneity that may benefit overall tumor growth.

Core-satellite Nanostructure Enhanced Fluorescence and its Applications in the Inhibition Assay on Receptor Dimerization

Chaoqing Dong*, Xuejie Yang, Jicun Ren

School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

A stable and uniform fluorescence-enhanced media-Core-satellite (CS) gold Nanostructure were assembled using the click chemistry of 1,2,4,5-tetrazine (Tz) and trans-cyclooctene (TCO). The SPR peak of the nanostructure is tunable with the satellite number in the core to agree with the requirement of fluorescence enhancement for different dyes. And the nanostructure appears the fluorescence enhancement on the CY3 probe conjugated in the Her2 receptor in HeLa cells and it shows dependence on the number of satellite in Au Core. The fluorescence enhancement effect occurs in cell membrane was applied to the dimerization inhibition assay of HER2 receptor in living cell and an analytical method was established to assess dimerization inhibitor such as lapatinib.

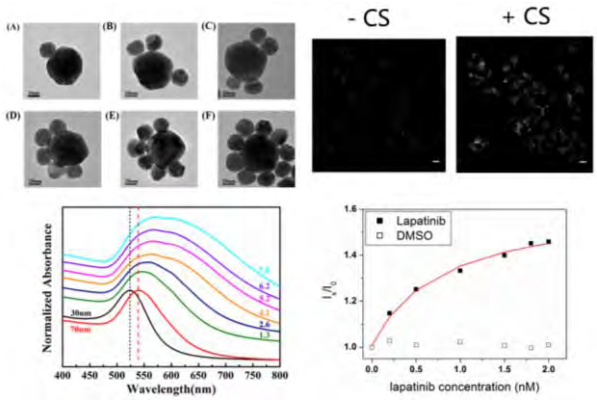


Figure. *TEM image of different CS nanostructure, absorption spectra, the enhanced fluorescence images of HeLa cell with CS, and the dependence of fluorescence intensity on dimerization inhibitor –lapatinib.*

Acknowledgments: This work was supported by grants from the National Natural Science Foundation of China.

References: [1] Cha H. et al., *ACS Nano* **2014**, *8*,8554. [2] Khatua, S., et al. *ACS Nano* **2014**, *8*,4440.

*Corresponding author: E-mail: cqdong@sjtu.edu.cn

Novel Styryl Quinolinium Fluorescent Probes for Imaging of Ribosomal RNA in Living Cells

Abed Saady[a], Eli Varon[b], Avi Jacob[b], Yaron Shav-Tal[b] and Bilha Fischer[a]*

[a] Department of Chemistry, Bar-Ilan University, Ramat-Gan 52900, Israel. [b] Faculty of Life Sciences & Institute of Nanotechnology, Bar-Ilan University, Ramat-Gan 52900, Israel.

The detection of subcellular domains in cells can be obtained by specific fluorescent markers. Here we report the use of novel styryl quinolinium dyes that selectively stain ribosomal RNA (rRNA) in nucleoli and in the cytoplasm. The dyes are cell permeable and can be applied to fixed and living cells and are non-toxic. With a view to develop new NIR emitting dyes for subcellular staining, we synthesized a group of 1-methyl-4-(substituted)styryl-quinolinium derivatives, (**12a–l**). For this purpose we developed a rapid and highly efficient microwave-assisted synthesis. This synthetic method requires only recrystallization to obtain the products in yields greater than 90%. These compounds in various solvents exhibited maximum absorbance at 500 - 660 nm, and a molar extinction coefficient of 25400–49000 $M^{-1}cm^{-1}$. Moreover, these compounds emitted at 630-715 nm. In glycerol quantum yields were 0.003-0.055. Furthermore, all the dyes were found to be highly photochemically stable. The cytotoxicity of the most promising dyes was evaluated on T cells, showing that certain dyes were not toxic. Particularly, compound **12e** exhibited unique photophysical properties in cells. The fluorescence of **12e** specifically stained nucleoli and the cytoplasm. Various tests showed the markedly higher affinity of the dye for RNA, specifically rRNA. We demonstrate that probe **12e** is an attractive staining reagent for visualizing and assessing rRNA in fixed and living cells.

*Corresponding author: E-mail: bilha.fischer@biu.ac.il

Identifying Phosphorescent Nanoparticles in Cancer Cells using Phasor Analysis

Dora-Luz Flores,[*a,b] Gustavo Hirata,[c] Prakhar Sengar,[c] Michelle Digman,[a]

[a] *Department of Biomedical Engineering, University of California Irvine, Irvine, USA*
[b] *Facultad de Ingeniería, Arquitectura y Diseño, Universidad Autónoma de Baja California, Ensenada Mexico*
[c] *Centro de Nanociencias y Nanotecnología, Universidad Nacional Autónoma de México, Ensenada, Mexico*

The nanoparticles have been used as nanocarriers for the administration of drugs must overcome numerous barriers including external, route and cellular aspects to finally reach their function that is the release of the drug in the indicated place. To trace the route of drug release with a nanocarrier, different biomedical imaging techniques[1] have been used, e.g. fluorescence-lifetime imaging microscopy (FLIM), from which a large amount of information is obtained. In this work, the phasor approach[2] is proposed to analyze data obtained using FLIM from phosphorescent nanoparticles in living cancer cells (Figure).

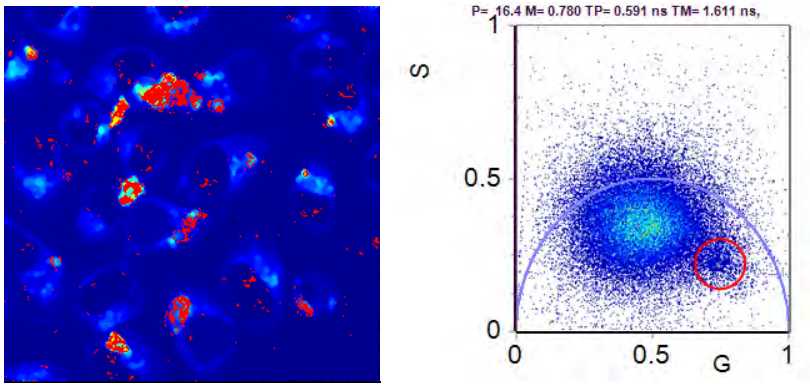


Figure. *Left: HeLa cells incubated with phosphorescent nanoparticles(NPs), red dots represent the NPs, right: phasor plot.*

Acknowledgments: This work was supported by grants from the COMEXUS-Fulbright García Robles and Consejo Nacional de Ciencia y Tecnología. The experiments reported in this publication were performed at the Laboratory for Fluorescence Dynamics (LFD) at the University of California, Irvine (UCI). The LFD is supported jointly by the National Institute of General Medical Sciences of the National Institutes of Health (2P41GM103540), and UCI. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References: [1] *Wolfbeis, O. S., Chem. Soc. Rev.* **2015**, *44,* 4743. [2] *Digman, M. A., et al Biophys J.* **2008**, *94,* L14.

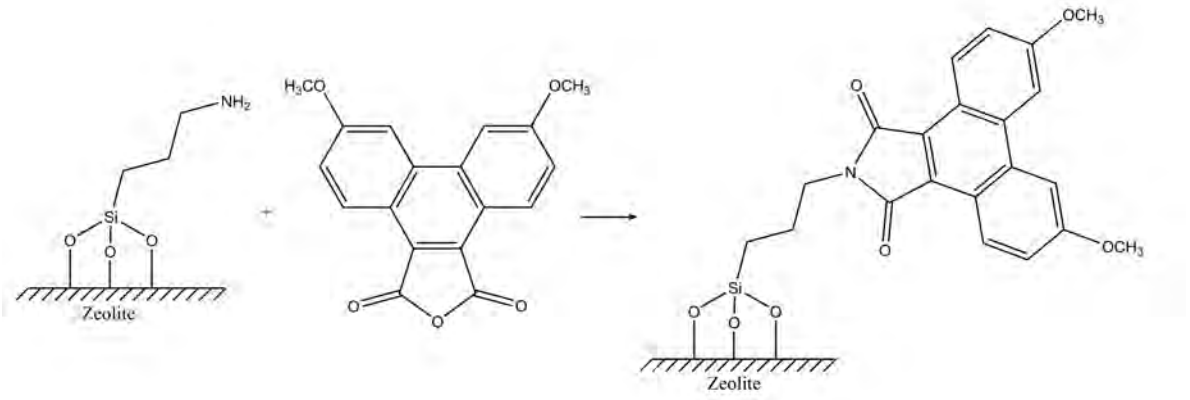
[*]Corresponding author: E-mail: dfloresg@uci.edu

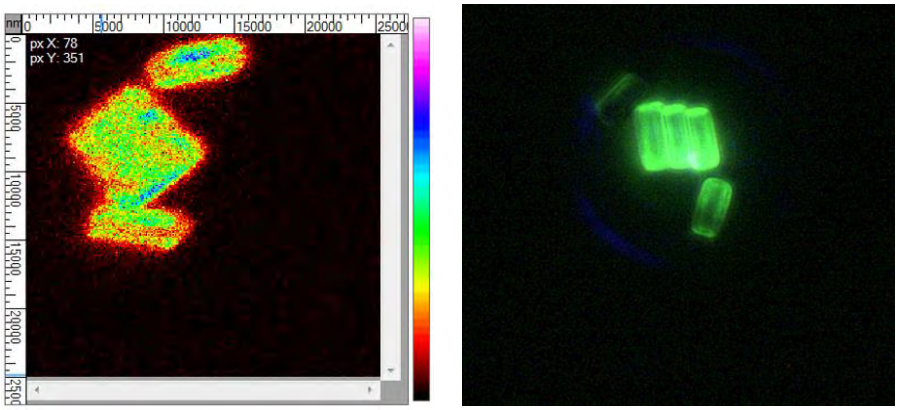
Reactive phenanthrene derivatives as markers of amino groups in fluorescence microscopy

Izadora F. Reis, Larissa S. Foltran, Marcelo H. Gehlen*

Institute of Chemistry of São Carlos, University of São Paulo, São Carlos – SP, Brazil

Two new reactive phenanthrene derivatives with high fluorescent quantum yields were prepared and used as fluorescent markers for free amino groups in fluorescence microscopy. In particular, modified μmZeolite-L containing free amino groups in the surface were labeled with the phenanthrene derivatives allowing good resolution imaging and spectroscopy in different conditions. The presence of a large Stokes shift of the probes due to the intramolecular charge-transfer character is an advantage of the compounds in confocal and wide-field laser fluorescence measurements. These results open up the possibility of using the method for visualization of Zeolite-based catalysts in chemical reactions.





Acknowledgments: This research was supported by projects CAPES 8887.334692/2019 (IFR), FAPESP 17660-7/2018 (LSF), FAPESP 22159-5/2018 and CNPq 303997/2017-6 (MHG).

*Corresponding author: E-mail: marcelog@iqsc.usp.br

ChronosDFD, a Fluorimeter for High Speed Time-Resolved Fluorescence Measurements

Yevgen Povrozin [1,2], Norin Redes [1], Mark Parsons [1] and Beniamino Barbieri [1]

[1] ISS, 1602 Newton drive, Champaign, IL 61822, USA
[2] Author to whom correspondence should be addressed: Yevgen Povrozin (Eugene.Povrozin@iss.com)
Address: 1602 Newton drive, Champaign, IL 61822, USA. Voice: 217-359-8681; Fax: 217-359-7879

ABSTRACT

Fluorescence techniques have moved in recent years from the research laboratory to the direct application in several fields of life sciences and material sciences; relatively inexpensive instrumentation has been developed for specific uses in medicine, diagnostics, pharmaceutical research, food quality control, agricultural process monitoring. The instrumentation is compact and the measurements relying on the acquisition of steady-state parameters (intensity, polarization, spectra) are fast. Yet, complex mixtures require sometimes the use of time-resolved measurements for the monitoring of a selected fluorophore; in this area the instrumentation is still comparatively expensive and the measurements acquisition is slow. We present the ChronosDFD; using the digital frequency domain (DFD) technique, this time-resolved fluorimeter allows for the acquisition of lifetime measurements on standard samples from 100ps to 100ms in less than one second. The fast data acquisition opens the development of monitoring instrumentation using lifetime measurements acquisition in high throughput screening, fast decay rates processes, monitoring of physiological metabolites and environmental markers.

Investigation of the interconversion kinetics of nucleic acid structures with time-resolved fluorescence in microfluidic droplets

Natalia Grytsyk,[a] Olivier Crégut,[a] Damien Cianfarani [a], Yves Mély,[b] Jérémie Léonard*[a]

[a] *Department of ultrafast optics and nanophotonics, Institut de Physique et Chimie des Matériaux de Strasbourg , 23 rue du Loess, 67200 Strasbourg, France*
[b] *Laboratory of Bioimaging and Pathologies, Faculty of Pharmacy, University of Strasbourg, 74 route du Rhin, 67401 Illkirch, France*

Förster resonance energy transfer (FRET) is a sensitive tool to monitor biomolecular interactions.[1] Nucleic acids undergo different structural interconversions that play an important role in transcriptional and translational regulation and also in repair and recombination.[2]
In this work we applied a new experimental approach that combines Time-Resolved Fluorescence (TRF) with Droplet Microfluidics (DMF)[3] to study the conversion kinetics of the FRET-pair labeled (-)DNA copy of the HIV-1 primer binding site (-)PBS stem-loop into extended (+)/(-)PBS duplex (Figure).

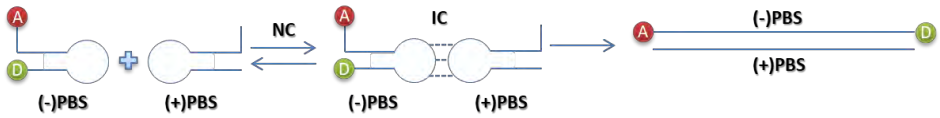


Figure. *Schematic representation of (+)/(-)PBS annealing reaction in the presence of Nucleocapsid protein p7 (NCp7).*

Droplet-based microfluidics is a promising tool that has been developed with success and applied to chemistry and biology.[4] Here, we implement it for the fast mixing (few ms) of (-)PBS with (+)PBS within the droplets in the presence of 3 equivalents of NCp7 protein. Preliminary results demonstrate the possibility to follow – by TRF detection - the kinetics of the hybridization reaction of the single-stranded (-)PBS into the extended duplex during the droplets propagation inside the microfluidics channel.

Acknowledgments: This work is supported by the ANR project PICO[2], (grant # ANR-15-CE11-0006-01).

References: (1) Liu, H.-W.; Cosa, G.; Landes, C. F.; Zeng, Y.; Kovaleski, B. J.; Mullen, D. G.; Barany, G.; Musier-Forsyth, K.; Barbara, P. F. *Biophysical journal* **2005**, *89*, 3470. (2) Kaushik, M.; Kaushik, S.; Roy, K.; Singh, A.; Mahendru, S.; Kumar, M.; Chaudhary, S.; Ahmed, S.; Kukreti, S. *Biochemistry and biophysics reports* **2016**, *5*, 388. (3) Maillot, S.; Carvalho, A.; Vola, J.-P.; Boudier, C.; Mély, Y.; Haacke, S.; Léonard, J. *Lab on a Chip* **2014**, *14*, 1767. (4) Casadevall i Solvas, X.; deMello, A. *Chemical Communications* **2011**, *47*, 1936.

*Corresponding author: E-mail: jeremie.leonard@ipcms.unistra.fr

Fluorescence properties of conjugated polymers on DNA origami

Daniel Aron Gudnason,[a,b] Mikael Madsen,[a,b] Kurt V. Gothelf, [a,b] Victoria Birkedal* [a,b]

[a] *Interdisciplinary Nanoscence Center, iNANO, Aarhus University, Gustav Wieds Vej 14, 8000, Aarhus C, Denmark*
[b] *Department of Chemistry, Aarhus University, Langelandsgade 140, 8000, Aarhus C, Denmark*

Conjugated polymers are used in OLEDS, solar cells and sensors. These polymers have interesting optical properties which are influenced by their morphology. Here, we used sequence specific DNA interactions to control polymer conformation and aggregation state. Fluorescence properties of DNA functionalized poly(Phenylene-Vinylene) polymers (poly(APPV-DNA))[1] were investigated using spectroscopy and single molecule microscopy.
We found, using AFM imaging, absorption and emission spectroscopy, that the aggregation state of poly(APPV-DNA) can be tuned in solution through divalent ion- and sequence specific DNA-interactions.[2] Using these interactions, we developed a DNA origami platform allowing to prepare different polymer conformations and aggregation states. These exhibit different photophysical properties, which were investigated using single molecule fluorescence microscopy. We found that these polymer DNA origami systems can be designed to show polymer intramolecular aggregation but also aggregation resistance.

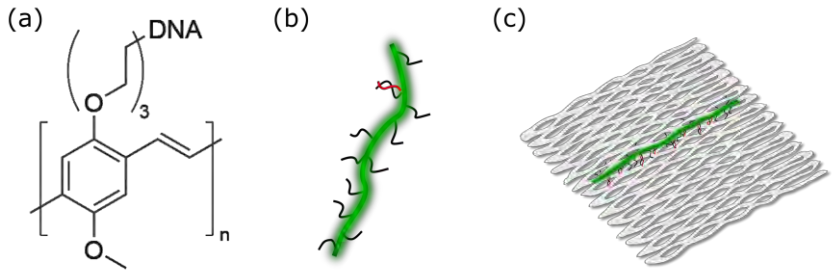


Figure. *A) Chemical structure of poly(APPV-DNA), B) cartoon of poly(APPV-DNA) including complementary DNA (cDNA) in red, C) poly(APPV-DNA) organized on a DNA origami platform.*

Acknowledgments: This work was supported by the Independent Research Fund Denmark and the Villum fonden.

References: [1] Knudsen, J. B. *et al.*, *Nat. Nanotechnol.*, **2015**, *10*, 892. [2] Gudnason, D. *et al.*, *Chem. Commun.*, **2018**, *54*, 5534.

*Corresponding author: E-mail: vicb@inano.au.dk

Local and Global Dynamics in the Supertertiary Organization of Postsynaptic Density Protein 95

George Hamilton[a], Nabanita Saikia[a], Justin Park[b], Jakub Kubiak[c], Claus AM Seidel[c], Mark Bowen[b], Feng Ding[a], Hugo Sanabria[a]

[a]Department of Physics and Astronomy, Clemson University, Clemson, SC, USA
[b]Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, NY, USA
[c]Department of Molecular Physical Chemistry, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

Postsynaptic density protein 95 (PSD 95) is a critical scaffolding protein in the excitatory postsynaptic density (PSD) with key functions in synaptic organization and regulation of synaptic strength. PSD-95 is a canonical multidomain scaffold protein comprised of three PDZ domains, an SH3 domain, and guanylate "psuedokinase" (GuK) domain connected in series by flexible linkers. The domains of PSD-95 show varying degrees of interaction and were suggested to partition into two independent supramodules[1]. It is known that PSD 95 exchanges between supertertiary configurations as part of its functional cycle[1]. Further, intermolecular interactions and posttranslational modifications can change the adopted conformation, directly affecting function. We recently characterized the first two PDZ domains, the PDZ tandem supramodule, as displaying weak interdomain interactions[2]. Additionally, others have shown that the third PDZ domain preferentially interacts with the SH3 and GuK domains in the PSG supramodule[1,3]. This suggests that internal dynamics may regulate PSD 95 function by changing the accessibility of binding sites for intermolecular interactions. In this work, we utilize a FRET network designed to probe pairwise combinations of the five PSD 95 domains. We used Multiparameter fluorescence detection[4] and filtered fluorescence correlation spectroscopy to identify interdomain interactions and resolve dynamics from picoseconds to milliseconds[5]. Global analysis of the entire FRET network allowed comparison of the timescales associated with interdomain interactions and local dynamics. Further, discrete molecular dynamics simulations[6] were performed to probe interdomain interactions in the PSG supramodule and screen experimental FRET distances against *in silico* conformations. The use of cross-linking allowed validation of identified interactions, describing how local and interdomain dynamics drive the supertertiary organization of PSD-95. Such descriptions provide a basis for understanding how conformational changes regulate the function of multidomain scaffold proteins.

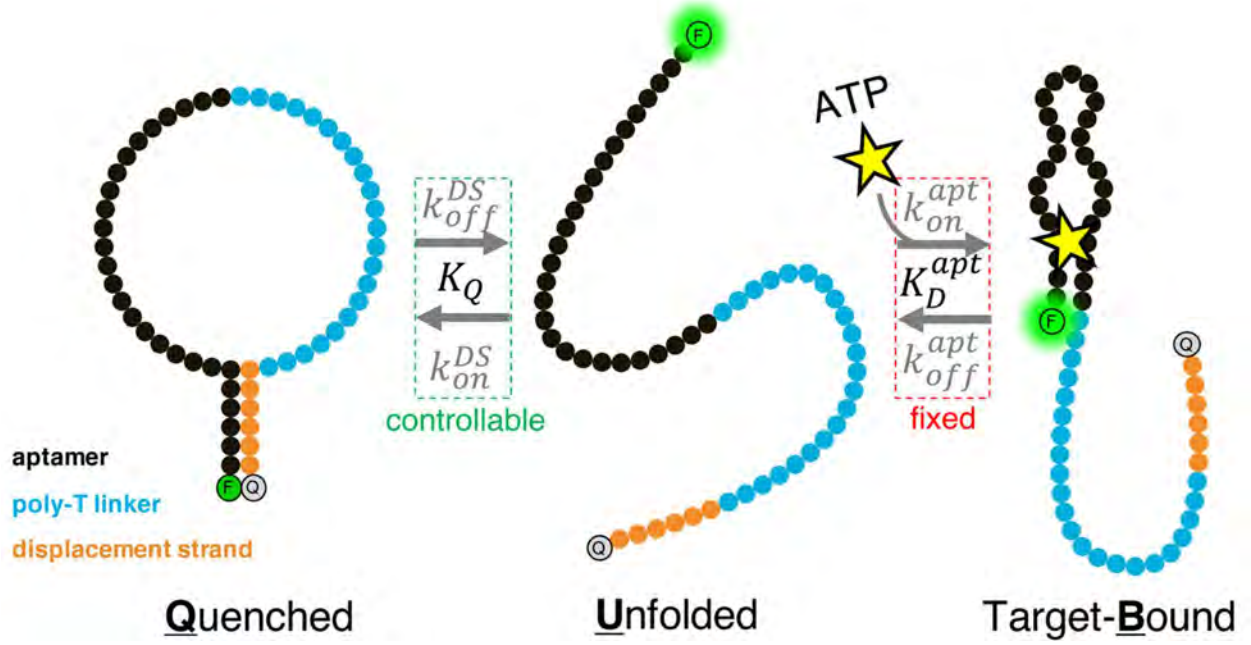
Acknowledgements: This work was supported by grants from NSF, NIH, and the Stay Connected@HHU program.

References: [1] McCann, J. et. al, PNAS 109 (39), 15775-15780 (2012). [2] Yanez-Orozco, I. et al., Nat. Comm. 9, 3724 (2018). [3] Pan, L. et. Al., J. Biol. Chem. 286 (46), 40068-40074 (2011). [4] Kühnemuth, R. et al, Single Mol. 2 (4), (2001). [5] Felekyan, S. et al., Chemphyschem 13(4), 1036-1053 (2012). [6] Shirvanyants, D. et. Al., J. Phys. Chem. B 116 (29), 8375-8382 (2012).

Independent Control of the Thermodynamic and Kinetic Properties of fluorescently labelled Aptamer Switches for biosensing applications.

Brandon D. Wilson, **Amani A. Hariri**, Ian A.P. Thompson, Michael Eisenstein, H. Tom Soh

Molecular switches that change their conformation upon target binding offer powerful capabilities for biotechnology and synthetic biology. In particular, aptamers have proven useful as molecular switches because they offer excellent binding properties, undergo reversible folding, and can be readily engineered into a wide range of nanostructures. Unfortunately, the thermodynamic and kinetic properties of the aptamer switches developed to date are intrinsically coupled, such that high temporal resolution (*i.e.*, switching time) can only be achieved at the cost of lower sensitivity or high background. Here, we describe a general design strategy that decouples the thermodynamic and kinetic behavior of fluorescently labelled aptamer switches to achieve independent control of sensitivity and temporal resolution. We used this strategy to generate an array of aptamer switches with effective dissociation constants (*KD*) ranging from 10 μM to 40 mM and binding kinetics ranging from 170 ms to 3 s—all generated from the same parent ATP aptamer. Our strategy is broadly applicable to other aptamers, enabling the efficient development of switches with characteristics suitable for diverse range of biotechnology applications.



Catalytic polystyrene-metalloporphyrin dot for highly effective chemiluminescence dynamic therapy

Xiangyi Huang

ABSTRACT

Placeholder

Probing solvation, ionpair formation and aggregation of fluorescent cationic dyes

Laura Kacenauskaite,[a] Junsheng Chen,[a] Amar H. Flood,[b] Bo W. Laursen*[a]

[a] *Nano-Science Center, Department of Chemistry, University of Copenhagen, Universitetsparken 5, Copenhagen, Denmark.*
[b] *Department of Chemistry, Indiana University, 800 East Kirkwood Avenue, Bloomington, Indiana 47405, USA.*

The fields of modern cell biology, biochemistry and even pharmaceuticals now heavily rely on quantitative, fluorescence-based measurements. Therefore, it is important to ensure that the same photophysical properties can be observed independently on the environment, especially when imaging highly complex mediums like cells or tissues.
All cationic dyes in nonpolar environments are expected to form tight ionpairs and/or aggregates, which often lead to significant quenching and spectral changes. To ensure physical separation of the fluorophores and their counteranions, we use pentagonal Cyanostar (CS) macrocycles (Figure) with specific shape and electron distribution that allow efficient trapping of anions like PF_6^- and BF_4^- in a sandwich-like structure [1]. Using ATOTA·PF_6 as a polarity and symmetry sensitive fluorophore [2], [3] we investigate ionpair formation in highly non-polar solvents.

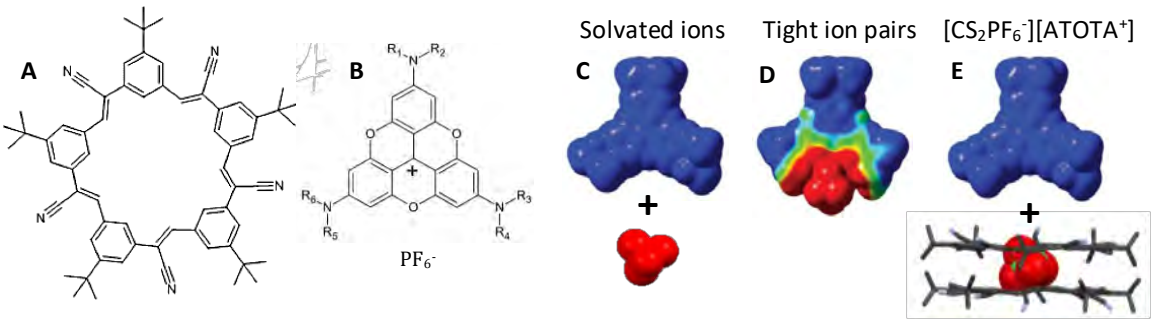


Figure. *Structure of Cyanostar (**A**) and ATOTA·PF_6 (**B**). Electrostatic potential surface plots for solvated ATOTA·PF_6 (**C**), its tight ionpairs (**D**) and [$CS_2PF_6^-$][$ATOTA^+$] complex (**E**).*

By applying both experimental spectroscopic methods and theoretical modeling we propose structure of fluorophore-CS_2 complex and show that fluorophore's photophysical properties relevant for most applications (like spectral shift, lifetime, quantum yield and absorption coefficient) can be revived even in the environments where an ionic dye would not be soluble otherwise.

References: [1] Lee, S. et al., *Nat. Chem.*, **2013**, 5, 704. [2] Laursen, B. W. et al., *Photochem. Photobiol. Sci.*, **2005**, *4*, 568. [3] Westerlund, F. et al., *Photochem. Photobiol. Sci.*, **2011**, 10, 1963.

*Corresponding author: E-mail: bwl@chem.ku.dk

VistaVision toolbox for quantitative multi-parameter analysis of single molecule dynamics

Yuansheng Sun [1,2], Sunil Shah [1], Shih-Chu (Jeff) Liao [1], Ulas C. Coskun [1] and Beniamino Barbieri [1]

[1] ISS, 1602 Newton drive, Champaign, IL 61822, USA
[2] Author to whom correspondence should be addressed: Yuansheng Sun (Yuansheng.Sun@iss.com).
Address: 1602 Newton drive, Champaign, IL 61822, USA. Voice: 217-359-8681; Fax: 217-359-7879

ABSTRACT

Single molecule imaging and particle tracking techniques open the observation of the dynamic behaviors of individual molecules, providing insight information of molecular processes that could be concealed by the ensemble average of the system. When combined with the time-resolved imaging technique, laser scanning confocal microscopy at the single molecule sensitivity allows quantitative multi-parameter analysis of single molecule dynamics. Here, we describe the single molecule imaging toolkit available in the ISS VistaVision software: FCS, FCCS, PIE-FCCS, FLCS, PCH, FLIM, steady-state and time-resolved FRET, steady-state and time-resolved anisotropy, burst analysis for single molecule FRET and stoichiometry, antibunching, etc. Measurement examples show how these techniques are used for studying photophysical properties and behaviors of single molecules, such as diffusion rate, molecule brightness, triplet time, rotational relaxation time, fluorescence lifetime and etc. By using donor-acceptor FRET pair-labeled proteins, we detect changes in protein conformation and dynamics by quantitatively measuring FRET efficiency, stoichiometry and lifetime. This quantitative multi-parameter analysis approach opens to researchers a wealth of opportunities for the study of single molecule dynamics.

DNA origami-based nanopositioners for single-molecule studies on graphene

Izabela Kaminska,*[a] Johann Bohlen,[b] Aleksandra Bednarz,[a] Sara Rocchetti,[b] Guillermo Acuna,[c] Philip Tinnefeld*[b]

[a]*Institute of Physical Chemistry of the Polish Academy of Sciences, 01-224 Warsaw, Poland*
[b]*Department of Chemistry and Center for NanoScience, Ludwig-Maximilians-Universität München, 80539 München, Germany*
[c]*Department of Physics, Université de Fribourg, Ch. du Musée 3, CH-1700 Fribourg, Switzerland*

Graphene has attracted a great attention since its discovery in 2004, due to its unique electronic, optical, thermal and mechanical properties.[1] One of the properties of graphene, which is exploited in the field of optical biosensors is fluorescence quenching due to the very efficient energy transfer, reaching nearly 100%. Graphene-based optical sensors have been applied to detect small biomolecules, DNA/RNA or proteins.[2,3] Notwithstanding, there is still a lot of room to explore the area of single-molecule biosensors based on graphene. The most common challenges and obstacles for their fabrication is a missing control of surface chemistry, sensor composition, and reproducibility.
We aim at expanding the field of application of hybrid graphene/optical nanoantennas-based biosensors, by addressing the aforementioned limitations through the use of DNA nanotechnology. The recently introduced DNA origami technique is used to fabricate hybrid constructs with nanometer precision and controlled stoichiometry.[4,5]
In order to connect the DNA origami constructs to the graphene layer, we use pyrene-modified DNA strands that are hybridized to the DNA origami on the one hand and interact with the graphene lattice via $\pi-\pi$ interactions on the other hand. This immobilization scheme provides stable DNA origami structures for different geometries and enable placing of single molecules and sensing moieties at defined distances to the graphene layer. We confirmed the d^{-4} dependence of energy transfer from the dye to graphene and determined the distance of 50% energy transfer: d_0 = 17.7 nm. The homogeneity of the population indicates that distances to graphene can be determined with high precision in a range of 5 – 30 nm. Presented strategy to immobilize DNA origami nanostructures on graphene opens new possibilities to develop graphene-based biophysics and biosensing.

Acknowledgments: IK is grateful for the support by the Foundation for Polish Science, within the program HOMING/2017-4/32 (POIR.04.04.00-00-42E8/17-00) and Polish National Science Center (MAESTRO, UMO-2016/22/A/ST4/00029).

References: [1] Ferrari, A. C. et al., *Chem. Rev.* **2014**, *7*, 4598. [2] Zhu, C., et al. *2D Mater.* **2015**, *2*, 32004. [3] Anichini, C., et al. *Chem. Soc. Rev.* **2018**, *47*, 4860. [4] Rothemund, P. W. K., et al. *Nature* **2006**, *440*, 297. [5] Douglas, S. M., et al. *Nature* **2009**, *459*, 414.

*Corresponding author: E-mail: ikaminska@ichf.edu.pl

Synthesis of an Emissive Deoxyguanosine Analogue toward Studies of Non-canonical Nucleic Acids

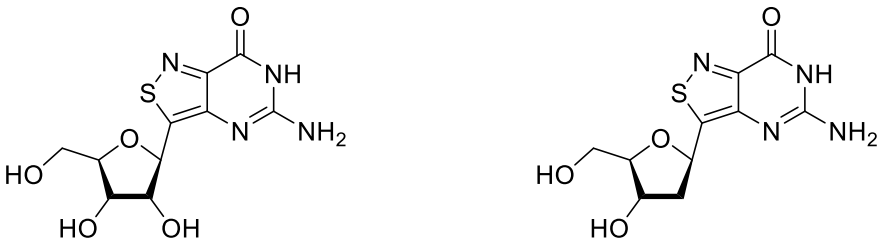
Yusuke Kawamoto, Paul T. Ludford III, Alexander R. Rovira, Yitzhak Tor*

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358, United States

G-quadruplex is one of the non-canonical nucleic acid structures formed by stacks of planer guanine tetrads. It is formed in guanine-rich DNA and RNA sequences, such as telomere repeats, and impacts gene expression, replication and genomic stability. Various fluorescent probes, such as nucleobases and G-quadruplex binding molecules, have been developed in order to investigate its function and structure.[1]

Our group has reported ribonucleoside alphabets consisting of emissive purines and pyrimidines.[2,3] In particular, **^{tz}G**, a nucleoside based on an isothiazolo[4,3-*d*]pyrimidine core, has the basic nitrogen corresponding to N7 in the purine nucleobases, which participates in forming G-quadruplexes.[3]

To employ this emissive guanine analogue for studying G-quadruplexes, we have prepared the corresponding emissive deoxyguanosine analogue **d^{tz}G**. Herein, we report its synthesis and photophysical properties. Further applications of the nucleobase analogue for oligodeoxynucleotides will be discussed.



^{tz}G **d^{tz}G**

Figure. *Emissive analogues of guanosine and deoxyguanosine*

Acknowledgments: This work was supported by the National Institutes of Health (grant no. GM 069773). YK is thankful for the Japan Society for the Promotion of Science Overseas Research Fellowships.

References: [1] Manna, S.; Srivatsan, S. G. *RSC Adv.* **2018***, 8*, 25673. [2] Shin, D.; Sinkeldam, R. W.; Tor, Y. *J. Am. Chem. Soc.* **2011**, *133*, 14912. [3] Rovira, A. R.; Fin A.; Tor, Y. *J. Am. Chem. Soc.* **2015**, *137*, 14602.

*Corresponding author: E-mail: ytor@ucsd.edu

A compact unbound state of p27 guides its specific binding with CyclinA/Cdk2

Maksym Tsytlonok[a], Katherina Hemmen[b,c], George Hamilton[d], Narendar Kolimi[d], Suren Felekyan[b], Claus A. M. Seidel[b], Peter Tompa[a], and Hugo Sanabria[b,d,*]

[a]*VIB Center for Structural Biology, Vrije Universiteit Brussel, Brussels, Belgium.*
[b]*Lehrstuhl für Molekulare Physikalische Chemie, Heinrich-Heine-Universität, Düsseldorf, 40225, Germany.*
[c]*Würzburg Universität, Würzburg, , Germany.*
[d]*Department of Physics and Astronomy, Clemson University, Clemson, SC, 29634, USA.*

p27, a prominent regulatory protein in eukaryotes and typical intrinsically disorder protein (IDP), regulates cell division by causing cell cycle arrest when bound in tertiary complex with cyclin dependent kinase (cdk) and cyclin (e.g. Cdk2/cyclinA). We present binding studies of p27 with cdk2/cyclinA complex by performing single molecule Fluorescence Spectroscopy and stopped-flow experiments. Our results suggest that the interaction of p27 with cdk2/cyclinA complex undergo conformational changes over a large dynamic range via highly specific and weak transient interactions that are facilitated by induced fit mechanism. Mutagenesis studies reveal that the domain 1 (D1) in p27 is the major driving force in the association kinetics and undergoes conformational rearrangement upon binding that causes compact to expansion. This indicates that p27 exhibits a limited binding surface in the apo form and prevents the multiple binding sites while interaction with cdk2/cyclinA complex. Furthermore, hydrophobic and charged amino acids in D1 of p27 play a major role in the interaction of cdk2/cyclinA complex. The outcome of this study would help to provide an additional information of IDP regions in the interaction pathways and regulatory mechanisms.

*Corresponding author: E-mail: hsanabr@clemson.edu

Molecular Mechanism of Antimicrobial Activity of Low DC Voltage Against E. coli.

Venkata Rao Krishnamurthi,[a] Ariel Rogers,[a] Janet Peifer,[a] Yong Wang*[abc]

[a] *Department of Physics,* [b] *Cell and Molecular Biology of Program,*
[c] *Microelectronics-Photonics Graduate Program, University of Arkansas, Fayetteville, AR, USA.*

Infections caused by drug-resistant bacteria have become a global threat,[1] which creates an urgent need for alternative strategies for combating pathogenic bacteria.[2] Physical agents such as low electric voltage/current have recently gained attention for antimicrobial treatment due to their bactericidal capability.[3,4] In this work, we applied super-resolution fluorescence microscopy to investigate the molecular mechanism of antimicrobial activity of low electric voltages/currents. We found that subjecting Escherichia coli (E. coli) bacteria to low DC voltage influences the distribution of histone-like structuring (H-NS) protein, a universal key protein for chromosome organization and genetic regulation. Clustering analysis based on Voronoi-tessellation[5] showed that the molecular density of H-NS proteins decreased, and bacteria formed fewer and smaller clusters of H-NS proteins after DC treatment. Furthermore, we found that the fraction of clustering proteins got lower when treating bacteria with low DC voltages. Our results suggest that nucleoid-associated proteins might play essential roles in response to exposure to low electric voltages/currents.

Acknowledgments: This work was supported by the University of Arkansas, the Arkansas Biosciences Institute (Grant No. ABI-0189, No. ABI-0226, No. ABI-0277), and the National Science Foundation (Grant No. 1826642).

References: [1] World Health Organization, World Health Statistics 2014. **2014**.
[2] Allen, H.K, et al. Ann. N. Y. Acad. Sci. **2014**, *1323*, 91. [3] Schmidt-Malan, S.M, et al. *Agents Chemother.* **2015**, *59*, 4610. [4] Brinkman, C.L, et al. *PLOS ONE.* **2016,** 11, e0168595.
[5] Levet, F, et al. *Nat. Methods.* **2015**, 10, 1038.

*Corresponding author: E-mail: yongwang@uark.edu

Bright long fluorescence lifetime dyad fluorophores for time-gated single molecule detection and imaging

Laura Kacenauskaite, Marco Santella, Rebecca Mucci, Tom Vosch and Bo W. Laursen*

Nano-Science Center & Department of Chemistry, University of Copenhagen, Denmark.

While long fluorescence lifetime fluorophores offer many advantages in time-resolved imaging, their inherently lower absorption cross-section has limited applications in single molecule imaging. Here we propose a generic approach to prepare bright, long fluorescence lifetime dyad fluorophores comprised of an absorbing antenna chromophore with high absorption cross-section linked to an acceptor emitter with a long fluorescence lifetime. We introduce a dyad consisting of a MPI perylene antenna and a triangulenium DAOTA emitter (Figure A) with close to 100% energy transfer from donor to acceptor. The dyad retained the long fluorescence lifetime (~17 ns) and high quantum yield (75%) of the triangulenium emitter but the perylene antenna increased the absorption coefficient 5 times. These triangulenium based dyads, can now be detected at the single molecule level and easily discriminated from 20-fold more intense auto-fluorescence by lifetime-based detection schemes (Figure B-D).

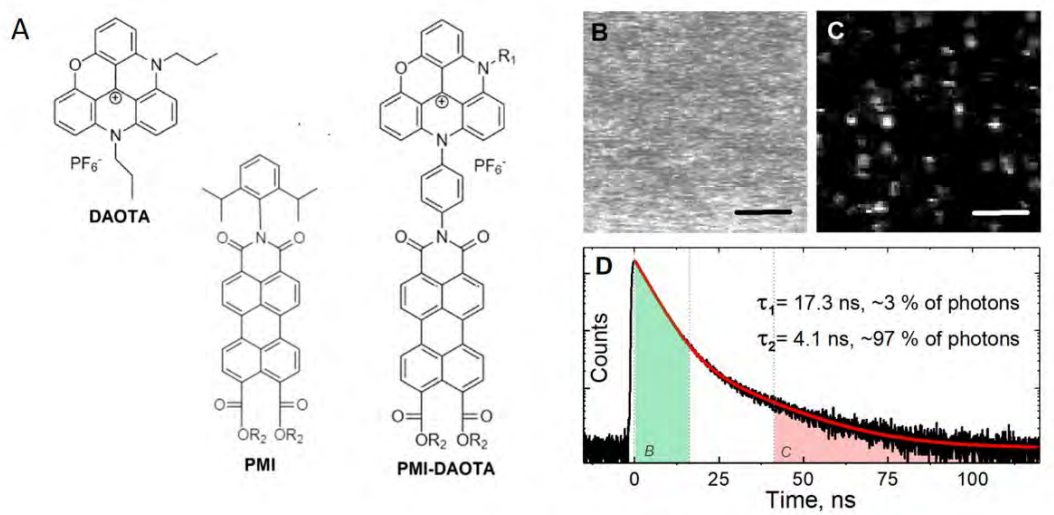


Figure. *(A) Structures of the reference chromophores and the dyad. (B-D) single dyad molecules blended with 20-fold excess of PMI: (B) Intensity image of from photons arriving during the first 17 ns after excitation (green time channel in graph D). (C) Time-gated image corresponding to the red time channel in graph D. (D) Fluorescence decay curve constructed from all the photon arrival times in the image.*

Acknowledgments: This work was supported by the Danish Council of Independent Research (DFF – 6111-00483).

*Corresponding author: E-mail: bwl@nano.ku.dk

Syntheses and Applications of Fluorescent Probes for Redox-active Species in Living Cells

Xiaohua Li,* and Huimin Ma*[a]

[a] *Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China.*

The intracellular dynamical redox balances are closely related with many physiological and pathological processes. Therefore, it is of great importance to establish efficient methods to monitor the in vivo redox states. However, challenges still exist in detecting redox-active species in living cells because of their native features, such as the short life-time of ROS, the ultralow level of redox-active emzymes and the similar reactivity of thiols. Herein, in the past several years, we have designed and synthesized a series of small molecular fluorescent probes for monitoring the redox-active species in living cells, which includes probes for •OH, thiols, monoamine oxidase and nitroreductase. By using these probes, we further explored the variation of their levels in different physio-/pathological conditions, and some new phenomena have been observed. These results are helpful to understand the biological functions of the above redox-active species and may be used in investigating the disease associated with redox imbances.

Acknowledgments: This work was supported by NSF of China, the Ministry of Science and technology of China and the Chinese Academy of Sciences.

References: [1] N. Soh, K. Makihara, E. Sakoda, T. Imato, *Chem. Commun*. **2004**, 496-497; [2] Y. Manevich, K. D. Held, J. E. Biaglow, *Radiat. Res.* **1997**, *148*, 580-591; [3] G. L. Newton, J. R. Milligan, *Radiat. Phys. Chem.* **2006**, *75*, 473-478;

*Corresponding author: E-mail: lixh@iccas.ac.cn; mahm@iccas.ac.cn

Microfluidics-based single-molecule live-cell imaging reveals essential role of spatiotemporal dynamics of selectin ligands on blood stem-cell rolling

Bader Al Alwan,[a] Karmen AbuZineh,[a] Shuho Nozue,[a] Aigerim Rakhmatulina,[a] Maged F. Serag,[a] Fajr Alesia,[a] Jasmeen S. Merzaban,[a] Satoshi Habuchi*[a]

[a] *Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), P.O. Box 4700 KAUST, Thuwal 23955-6900, Saudi Arabia*

Hematopoietic stem/progenitor cell (HSPC) homing occurs via cell adhesion mediated by spatiotemporally organised ligand-receptor interactions. Although molecules and biological processes involved in this multi-step cellular interaction with endothelium have been studied extensively, molecular mechanisms of this process, in particular the nanoscale spatiotemporal behaviour of ligand-receptor interactions and their role in the cellular interaction, remain elusive. Here, we introduce a microfluidics-based super-resolution (SR) and single-molecule fluorescence imaging platform[1] and apply the method to investigate the initial essential step in the homing, tethering and rolling of HSPCs in the presence of external shear stress that is mediated by selectins, expressed on endothelium, with selectin ligands (i.e. CD44 and PSGL-1) expressed on HSPCs. Our real-time imaging revealed the formation of membrane-tethers and slings (Figure 1), on which selectin ligands showed unique spatiotemporal dynamics that is distinct from those on the cell body. Our detailed analysis suggested that the spatial confinement of the selectin ligands together with the fast scanning of a large area by the selectin ligands increase the efficiency of selectin-ligands interaction during the rolling, resulting in slow and stable rolling of the cell on selectin.

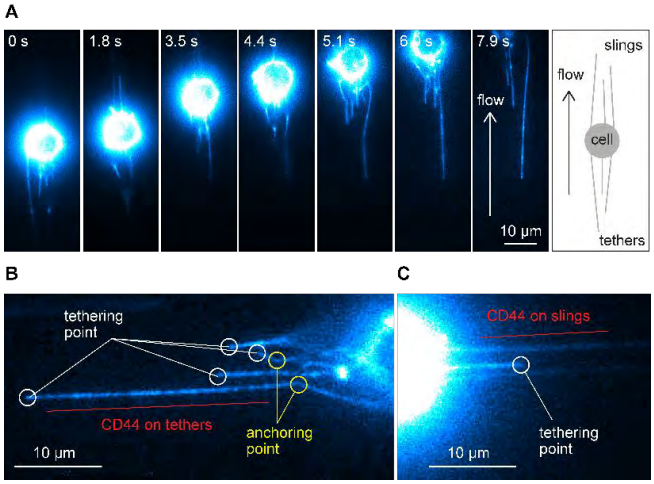


Figure 1. *(A) Time-lapse fluorescence images of a selectin ligand, CD44, on a KG1a cell (working model of HSPCs) captured during the cell rolling on the surface of microfluidic chamber whose surface was coated with E-selectin in the presence of external shear stress. Formation of multiple membrane tethers (B) and slings (C) is seen clearly in the enlarged views.*

Acknowledgments: This work was supported by King Abdullah University of Science and Technology (KAUST) and the KAUST Office of Sponsored Research (OSR) under Award No. CRG R2 13 MERZ KAUST 1.

References: [1] AbuZineh, K. et al., *Sci. Adv.* **2018**, *4*, eaat5304.

*Corresponding author: E-mail: satoshi.habuchi@kaust.edu.sa

Spy on monoaminergic neuromodulation with new genetically encoded fluorescent sensors

Yulong Li, [a,b]

[a] *Peking University School of Life Sciences,*
[b] *PKU-IDG/McGovern Institute for Brain Research, Beijing, P.R.China*

Dopamine (DA) is a central monoamine neurotransmitter involved in many physiological and pathological processes. A longstanding yet largely unmet goal is to measure DA changes reliably and specifically with high spatiotemporal precision, particularly in animals executing complex behaviors. Here, we report the development of genetically encoded GPCR-activation-based-DA ($GRAB_{DA}$) sensors that enable these measurements. $GRAB_{DA}$ sensors can resolve a single-electrical-stimulus-evoked DA release in mouse brain slices and detect endogenous DA release in living flies, fish, and mice. Similar strategies can be harnessed to develop a plethora of GRAB sensors for other important neurotransmitters/neuromodulators.

Acknowledgments: This work was supported by the National Basic Research Program of China (973 Program; grant 2015CB856402), the General Program of National Natural Science Foundation of China (project 31671118), the NIH BRAIN Initiative (NS103558), the Junior Thousand Talents Program of China, the grants from the Peking-Tsinghua Center for Life Sciences, and the State Key Laboratory of Membrane Biology at Peking University School of Life Sciences to Y. Li.

References:
[1] #Sun, F., #Zeng, J., #Jing, M., Zhou, J., Feng, J., Owen, S., ... & *Li, Y. (2018). A genetically-encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. Cell, 174(2), 481-496.
[2] *#Jing, M., #Zhang, P., Wang, G., Feng, J., Mesik, L., Zeng, J., ... *Zhu, JJ & *Li, Y. (2018). A genetically-encoded fluorescent acetylcholine indicator for in vitro and in vivo studies. Nature Biotechnology, 36(8), 726-737.*

*Corresponding author: E-mail: yulong@gmail.com

Monitoring the formation and degradation of cyclic dinucleotides using fluorescence

Yao Li,[a] Andrea Fin,[a] Paul T. Ludford III,[a] Alexander R. Rovira,[a] Yitzhak Tor*[a]

[a]*Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093, USA*

Cyclic dinucleotides (CDNs) are second messengers that have been found in all known bacteria. They play important roles in regulating central processes, including biofilm formation, motility and virulence. We report the enzymatic preparation, as well as biophysical applications of a novel group of CDN analogs. Two families of isomorphic emissive ribonucleotides, previously developed in our lab,[1,2] have been used to fluorescently monitor in real time the dinucleotide cyclase-mediated synthesis and phosphodiesterase (PDE)-mediated hydrolysis of CDNs (**Figure 1**). Our study demonstrates an effective method to probe the activities of two classes of bacterial enzymes that play critical roles in CDN signaling and metabolism.

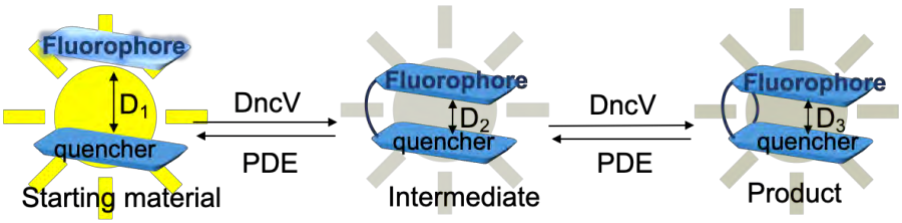


Figure 1*. Putative fluorescence changes during the biosynthesis and hydrolysis of emissive CDNs.*

Acknowledgments: This work was supported by the National Institutes of Health (grant number GM069773).

References: [1] Shin, D. et al., J. *Am. Chem. Soc.* **2011**, *133*, 14912–14915. [2] Rovira, A. R. et al., *J. Am. Chem. Soc.* **2015,** *137*, 14602–14605.

*Corresponding author: E-mail: ytor@ucsd.edu

Fluorescing Isofunctional Ribonucleosides: Assessing Adenosine Deaminase Activity and Inhibition

Paul Ludford, Alex R. Rovira, Andrea Fin, Yitzhak Tor*[a]

[a] *Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, 92093-0358, United States*

The conversion of isothiazolo[4,3-d]pyrimidine based adenosine (^{tz}A) and 2-aminoadenosine (^{tz}2-AA) analogues to the corresponding isothiazolo[4,3-d]pyrimidine based inosine (^{tz}I) and guanosine (^{tz}G) derivatives by Adenosine Deaminase (ADA) is evaluated and compared to the conversion of native adenosine to inosine (**Figure**).[1] A foundation for a high throughput screening assay is established and the efficacy of the assay showcased via fluorescence-based analysis of ^{tz}A conversion to ^{tz}I in the presence of known and newly synthesized inhibitors.

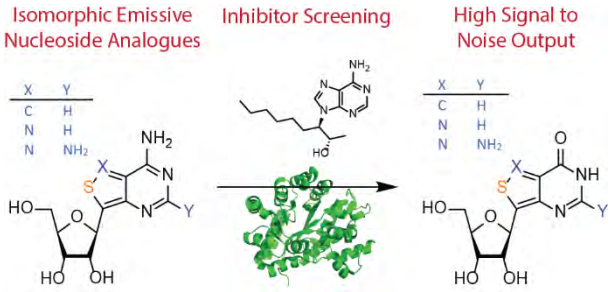


Figure. *Adenosine Conversion to Inosine Analogues in the Presence of Adenosine Deaminase.*

This work was supported by the National Institutes of Health (via grant number GM 069773).

References: [1] A. R. Rovira, A. Fin and Y. Tor, *J. Am. Chem. Soc.*, 2015, **137**, 14602–14605.
[2] P. T. Ludford, A. R. Rovira, A. Fin and Y. Tor, *ChemBioChem*, 2019, **20**, 718–726.

*Corresponding author: E-mail: ytor@ucsd.edu

Direct single-molecule imaging for diagnostic and blood screening assays

Qiaoqiao Ruan, Patrick J. Macdonald, Kerry M. Swift and Sergey Y. Tetin*

Applied Research and Technology, Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL 60064

Every year over a hundred million units of donated blood undergo mandatory screening for HIV, hepatitis B, hepatitis C and syphilis worldwide. Often, donated blood is also screened for other pathogens such as HTLV, Chagas, Dengue and Malaria. Several billion diagnostic tests are performed annually around the world to measure more than four hundred biomarkers for cardiac, cancer, infectious and other diseases. Considering such volumes, every improvement in assay performance and/or throughput has a major impact.

Here we show that medically-relevant assay sensitivities and specificities can be greatly improved by direct single-molecule imaging using regular epifluorescence microscopes. In current microparticle-based assays, an ensemble of bound signal-generating molecules is measured as a whole. Instead, we acquire intensity profiles to identify and then count individual fluorescent complexes bound to targets on antibody-coated microparticles. This increases the signal-to-noise ratio and provides better discrimination over non-specific effects. It brings the detection sensitivity down to the attomolar (10^{-18} M) for model assay systems, and to the low femtomolar (10^{-15} M) for measuring analyte in patient samples. Transitioning from counting single-molecule peaks to averaging pixel intensities at higher analyte concentrations enables a continuous linear response up to micromolar levels.

Emerging research for counting eluted labels in solution or on surfaces uses confocal or TIRF microscopy. Single-molecule ELISA also offers single-molecule sensitivity. However, these approaches require additional steps, microfluidic channels or nanowells. Direct imaging of single-molecules on microparticles is straightforward and thus more promising for clinical implementation. Moreover, single-molecule imaging-based detection simplifies the requirements for analyte capture and sandwich formation protocols. Highly sensitive assays can be performed faster with reduced or even with no washing steps because background and ambiguous peaks or clusters of pixels can be filtered out by image analysis algorithms. Additionally, our assays become insensitive to microparticle number and volume variations during the binding reaction, eliminating the main source of uncertainties in standard assays. Highly reproducible detection can be achieved by imaging fewer than two thousand microparticles. Altogether, these features allow for increased assay sensitivity, wide linear detection ranges, shorter incubation times, simpler assay protocols and minimal reagent consumption.

*Corresponding author: E-mail: sergey.tetin@abbott.com

Shining light on enzyme immobilization

Gerard Masdeu,*[a] Patrick Eiring,[b] Alexander Kuhlemann,[b] Gerti Beliu,[b] Milene Zezzi do Valle Gomes,[a] Anders Palmqvist,[a] Markus Sauer,[b] Marcus Wilhelmsson,[a] Björn Åkerman [a]

[a] *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, 41296 Göteborg, Sweden*
[b] *Department of Biotechnology and Biophysics, University of Würzburg, 97074 Würzburg, Germany*

The porous structure of mesoporous silica particles enables the co-localization of several enzymes from a cascade reaction in a very short distance.[1] Enhanced enzymatic activity on the CO_2 reduction was observed in our previous work, probably due to substrate channeling by direct substrate transporting among the enzymes.[2] Still, physical confinement of the enzyme may cause activity loss, undesired three-dimensional conformation, and mass transfer limitations. Understanding of the conjugated system becomes thus crucial to overcome these technical barriers for the industrial applicability of the catalyst.
Fluorescence spectroscopy is a powerful tool to gain insight into the support–enzyme interaction. In this work, its sensitivity to the local environment of the probe is being used to measure real-time kinetics on the immobilization of Cy3-labeled lipase into SBA-15 particles. The complexity of the modeling system lies on quantum yield variation when the probe is inside the pores, dye self-quenching, bleaching, and light scattering from the particles. Fluorescence-based confocal microscopy is currently ongoing to elucidate the immobilization mechanism, distinguishing the preferred binding sites for the enzyme, and the spatial distribution of the enzyme molecules inside the particle (Figure). The distribution will affect the transport behavior of substrate/product as well as the reaction kinetics.[3]

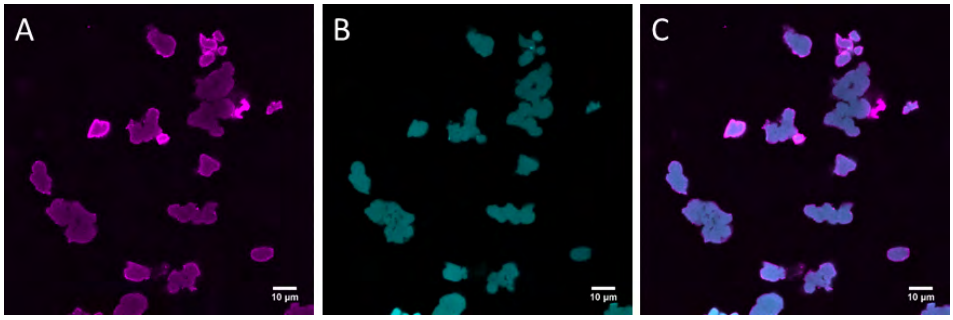


Figure. *Spatial distribution of cyanine-labeled lipase in Alexa532-labeled SBA-15 particles.*

Acknowledgments: Funding from the Swedish Research Council (Vetenskapsrådet; Project no. 21220128) is acknowledged.

References: [1] M. Hartmann, X. Kostrov, Chem. Soc. Rev. **2013**, 42, 6277–6289. [2] P. S. Nabavi Zadeh, M. Zezzi Do Valle Gomes, B. Åkerman, A. E. C. Palmqvist, ACS Catal. **2018**, 8, 7251–7260. [3] Y. Zhang, J. Ge, Z. Liu, ACS Catal. **2015**, 5, 4503–4513.

*Corresponding author: masdeu@chalmers.se

Entangled polymer dynamics beyond reptation: a single molecule study

Maram Abadi,[a] Maged F. Serag,[a] Satoshi Habuchi*[a]

[a] *Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), P.O. Box 4700 KAUST, Thuwal 23955-6900, Saudi Arabia*

Macroscopic properties of polymers arise from microscopic entanglement of polymer chains. Entangled polymer dynamics have been described theoretically by time- and space-averaged relaxation modes of single chains occurring at different time and length scales. Thus, scaling law analyses of experimentally obtained relaxation modes including those determined at the single chain level have provided useful tool to confirm the existing theory. However, theoretical and experimental studies along this framework provide oversimplified picture of spatiotemporally heterogeneous polymer dynamics. Characterization of entangled polymer dynamics beyond this paradigm requires a method that enables to capture motion and relaxation occurring in real space at different length and time scales. Here we develop new single-molecule characterization platform by combining super-resolution fluorescence imaging and recently developed single-molecule tracking method, cumulative-area tracking, which enables to quantify the chain motion in the length and time scale of nanometres to micrometres and milliseconds to minutes.[1] Using linear and cyclic dsDNA molecules as model systems, our new method reveals chain-position-dependent motion of the entangled linear chains, which is beyond the scope of current theoretical framework (Figure 1). We also demonstrate experimentally that the conformation and motion of cyclic chains under entangled conditions are governed mainly by topological constraint. Our results demonstrate that the new method developed in this study provides experimental platform to address key questions in the entangled topological polymer dynamics, which is relevant to a wide spectrum of researches in physics.

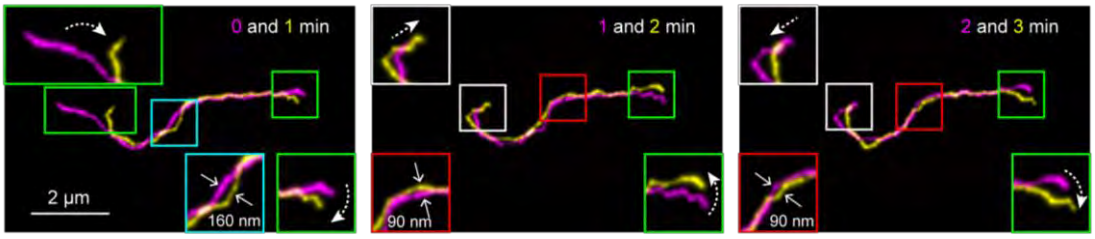


Figure 1. *Motion of a lambda DNA molecule under entangled conditions captured by 3D super-resolution fluorescence imaging technique.*

Acknowledgments: This work was supported by King Abdullah University of Science and Technology (KAUST) and the KAUST Office of Sponsored Research (OSR) under Award No. OSR-2015-2646-CRG4.

References: [1] Abadi, M. et al., *Nat. Commun.* **2018***, 9*, 5098.

*Corresponding author: E-mail: satoshi.habuchi@kaust.edu.sa

Stimulated Emission Depletion Microscopy of Programmed Death-1 in Melanoma Tissue

Dannielle G. McCarthy,[a] Colin J. Comerci,[b] Mehdi Nosrati,[c] Kevin B. Kim,[c] Mohammed Kashani-Sabet,[c] Stanley P. Leong,[c] W. E. Moerner*[a]

[a]Chemistry Department, Stanford University, Stanford, CA, USA
[b]Biophysics Program, Stanford University, Stanford, CA, USA
[c]Center for Melanoma Research and Treatment, California Pacific Medical Center Research Institute, San Francisco, CA, USA

T lymphocytes (T cells) are immune cells that are capable of completely eradicating cancer cells in an organism. Each T cell has its own T cell receptor (TCR) that recognizes specific antigens from certain cells within an organism. T cells raised against tumor specific antigens can identify the cancer cells within the tumor microenvironment through binding of the TCR with the recognized antigen bound to the major histocompatibility complex (MHC) on the cancer cells. In the absence of many deactivating signals, this binding interaction leads to killing of the cancer cell typically through the release of cytotoxic granules. However, some cancer cells can prevent this killing mechanism through binding of a class of molecules called immune checkpoint proteins with the appropriate receptor protein on the T cell. For example, many cancer cells express programmed cell death-1 ligands (PD-L1) that can bind to the programmed cell death-1 (PD-1) receptor proteins on T cells, which in turn can enable the cancer cell to evade the immune system. Immune checkpoint immunotherapy is a new class of cancer treatments that block these inhibitory binding interactions, like the PD-1/PD-L1 interaction, with antibodies, and thus help to enhance the immune system's natural ability to eradicate cancer cells. Although promising, this type of immunotherapy is currently only effective in about twenty percent of cancer patients. The variation in sub-cellular structure and density of PD-1 on T cells within the tumor microenvironment could perhaps correlate with response to immunotherapy treatment. We utilize two-color stimulated emission depletion (STED) microscopy to probe the nano-scale organization of PD-1 in metastatic melanoma tissue with ~60 nm resolution. Due to the large and heterogeneous nature of the tissue, we first map the distribution of PD-1+ cells in the tumor microenvironment using two-color confocal microscopy. We then choose promising regions of interest to image with STED at high resolution. We observe various degrees of nanoscale clustering of PD-1 on cells within the tumor microenvironment and describe additional findings.

Acknowledgments: This work was supported by NIH Grant R35-GM118067, Stanford Graduate Fellowship, NSF GRFP, Stanford ChEM-H Chemistry/Biology Interface Predoctoral Training Program.

*Corresponding author: E-mail: wmoerner@stanford.edu

Single particle imaging of upconversion nanoparticles in aqueous buffers

Oleksii Dukhno[1], Frédéric Przybilla[1], Verena Muhr[2], Markus Buchner[2], Thomas Hirsch[2], Yves Mély[1]

1. **Laboratory of Bioimaging and Pathologies (UMR 7021 CNRS), University of Strasbourg, 74 Route du Rhin, 67400 Illkirch, France**
2. **Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Universitaetsstrasse 31, 93040 Regensburg, Germany**

E-mail:yves.mely@unistra.fr

Keywords: upconversion nanoparticles, single particle microscopy, wide-field microscopy

Single-particle microscopy is a powerful method to extract information that is not accessible by ensemble methods. Upconversion nanoparticles (UCNPs) are attracting emissive labels for single-particle microscopy, because they offer very high signal to noise ratio, due their anti-Stokes emission. Moreover, they are extremely photostable and do not blink, giving the unique possibility to track events on time scales several orders of magnitude greater than conventional organic dyes [1]. However, ensemble measurements of diluted aqueous dispersions of UCNPs have recently shown luminescence instability over time due to particle dissolution-related effects [2, 3]. This can be especially detrimental for single-particle experiments, but this effect has never been estimated at the single particle level. To investigate this point, we used quantitative wide-field microscopy to monitor the luminescence response of individual UCNPs under aqueous conditions [4]. The particles were found to rapidly loose their luminescence and exhibit large changes in their spectral response, which led to considerable heterogeneity in their luminescence and band intensity ratio. These changes were clearly attributed to the dissolution of the particles, as a result of their low concentration in the buffer that forces the dissolution equilibrium towards total dissolution. The dissolution of the UCNPs and the subsequent development of their heterogeneity can be largely slowed down by adding millimolar concentrations of sodium fluoride in the buffer or addition of appropriate shells. Therefore, quantitative experiments based on intensity/spectral response from individual UCNPs in aqueous buffers need to be corrected for these effects or even better, be performed under conditions that carefully prevent these effects. Using optimized conditions, UCNPs were applied for tracking membrane receptors in live cells.

This work was supported by the ANR-11-LABX-0058-NIE within the Investissement d'Avenir program ANR-10-IDEX-0002-02, COST CM 1403 «The European upconversion network» and Institut Universitaire de France (IUF).

REFERENCES [1] Zhou, B.; Shi, B.; Jin, D.; Liu, X. Controlling Upconversion Nanocrystals for Emerging Applications. *Nat Nano* **2015**, 10, 924–936. [2] Lahtinen, S.; Lyytikäinen, A.; Päkkilä, H.; Hömppi, E.; Perälä, N.; Lastusaari, M.; Soukka, T. *J. Phys. Chem. C* **2017**, 121, 656–665. [3] Plohl, O.; Kraft, M.; Kovač, J.; Belec, B.; Ponikvar-Svet, M.; Würth, C.; Lisjak, D.; Resch-Genger, U. *Langmuir* **2017**, 33, 553–560. [4] Dukhno, O.; Przybilla, F.; Muhr, V.; Buchner, M.; Hirsch, T.; Mély, Y. Time-dependent luminescence loss for individual upconversion nanoparticles upon dilution in aqueous solution. *Nanoscale* **2018**, 10, 15904-15910.

A new class of indicators for fast and specific calcium imaging

Nicole Mertes,[a] Kai Johnsson*[a]

[a] *Department of Chemical Biology, Max Planck Institute for Medical Research, Jahnstr. 29, 69120 Heidelberg, Germany*

While cellular communication via large calcium waves is mostly understood, research on regionally limited calcium influxes (calcium microdomains) or even single calcium channel activity just began. Their detection is, due to the small size of these domains and/or their fast decay, challenging and appropriate tools are rare. For example, the widely used GCAMP6f (a genetically encoded calcium indicator) still lacks behind synthetic indicators in terms of speed, brightness and size; all critical criteria for fast fluctuations in calcium microdomains.
My work focuses on the development of a new class of calcium indicators (BOCA-tet) we specifically designed to facilitate the detection of these microdomains. The indicators are based on a BODIPY fluorophore combined with the well known calcium binding motifs BAPTA or APTRA. Unlike conventional synthetic calcium dyes, these indicators are localizable onto a reactive handle on a protein of interest via a biorthogonal click reaction. Like this, the advantages of synthetic dyes (speed, brightness) are combined with the localizability, hence improved resolution, of genetically encoded indicators. Even more these dyes receive a turn-on upon localization, making the non localized dye invisible and therefore reducing background fluorescence.
First *in vitro* and *in cellulo* experiments show the successful proof of principle, with a sufficient turn-on upon click reaction to realize no wash experiments. In the future, I plan to localize the indicator directly to the mouth of calcium channels. This will enable me to observe the behavior of single calcium channels and thus gives me an insight into the communication of subcellular compartments.

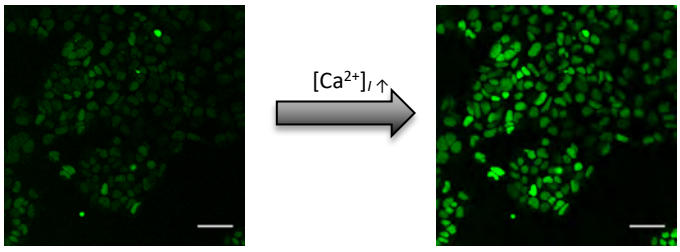


Figure. *Fluorescent images of BOCA-tet dyes localized to the nucleus (no wash). Upon calcium induction, fluorescence increases (right). Scale bar 50 µm.*

Acknowledgments: This work was supported by the Max Planck Society and a grant from Boehringer Ingelheim Fonds.

*Corresponding author: E-mail: johnsson@mr.mpg.de

Rapid detection of pathogenic bacteria *via* fluorogenic enzyme substrate probes

Presenters: Elias Y. Najib,[a] and Krisztina E. Treuerné Balázs[a]. Co-authors: David E. Hibbs,[a] John D. Perry,[b] Jonathan Iredell,[c] and Paul W. Groundwater[a]

[a] The University of Sydney School of Pharmacy, Camperdown Campus, Sydney, NSW 2006, Australia

[b]Microbiology Department, Freeman Hospital, Newcastle upon Tyne, UK

[c] Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research and Westmead Hospital, Westmead, Sydney, Australia

Among the challenges highlighted by the 2011 Annual Report of the UK Chief Medical Officer (CMO), Prof. Dame Sally Davies, was the need to improve diagnostic testing in order to ensure more tailored (or directed) therapeutic interventions.[1] This recommendation was further reinforced by the UK review on antimicrobial resistance (AMR),[2] which also recommended that, by 2020, it should be mandatory for antibiotic prescription to be informed by data and testing technology.

Of particular concern to the growing trend of AMR are the ESKAPE bacteria (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneuomniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and Enterobacter spp.). These multiple and pan drug resistant pathogens host a plethora of resistance mechanisms and contribute to the mortality of nosocomial infections.[3] We will describe here the design and synthesis of molecular probes incorporating amino acids and various fluorogens *via* self-immolative linkers, which target specific enzymatic activities of some of the ESKAPE pathogens. For example, using MacConkey media supplemented with one such probe (Figure) we have been able to differentiate *K. pneumoniae* from other coliform bacteria, with positive (PPV) and negative (NPV) predictive values of 92 and 95%, respectively.

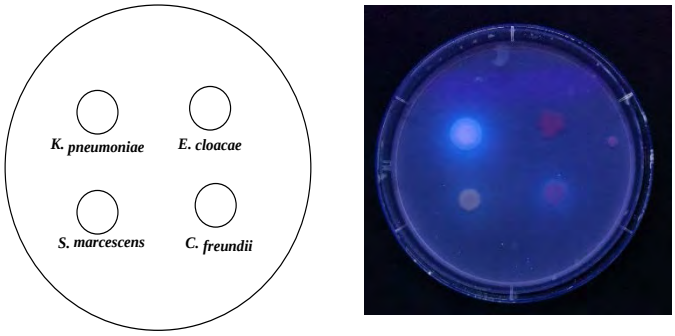


Figure. Differentiation of coliform bacteria using a fluorogenic enzymatic substrate.

References: [1] Davies, S. C., *et al*., *Lancet* **2013**, *381,* 1606. [2] O'Neill, J.. *Tackling drug-resistant infections globally: Final report and recommendations*, HM Government, Wellcome Trust, London, 2016 (https://amr-review.org/). [3] Santajit, S. and Indrawattana, N., *BioMed. Res. Int.* **2016**, 2475067.

SAFE method for luminescence quantum yield determination

Krzysztof Nawara,*[ab] Jacek Waluk [ab]

[a] Faculty of Mathematics and Science, Cardinal Stefan Wyszyński University, Dewajtis 5, Warsaw, Poland
[b] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, Warsaw, Poland

The determination of reliable luminescence quantum yield (QY) values of diluted dye solutions is still very challenging. The most common approach for QY determination is a relative method, which allows to establish QY of an unknown sample by comparison with a reference standard. The reference solution should be nonreactive, stable upon irradiation, and its emission should be temperature and excitation independent. In 2017 we have developed a SAFE (Simultaneous Absorption and Fluorescence Emission) method for relative determination of QY using single instrument: a spectrofluorometer equipped with transmittance detector[1]. This approach eliminates a number of errors as compared to the standard relative method. The SAFE approach allows also the determination of QY of poorly emitting compounds using well characterized high quantum yield standards, which is not possible while using the standard relative method[2]. Finally, we have adapted the SAFE method for the determination of QY values at the temperatures different than ambient[3]. This method has been used to study the temperature dependence of QY of the well-known standard solution: quinine sulfate. Its solution in 0.05M sulfuric acid was found to be temperature dependent near room temperature, whereas 0.1M perchloric acid solution shows no temperature dependence[3]. This suggests that quinine in sulfuric acid solution should no longer be used as the reference solution for QY determination.

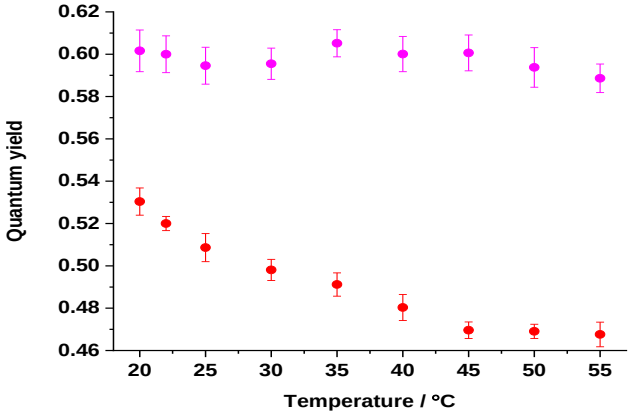


Figure. *The quantum yields of quinine in 0.05M sulfuric acid (red) and in 0.1M perchloric acid (magenta).*

Acknowledgments: This work was supported by grants from the Polish National Science Centre DEC-2017/01/X/ST4/00166 and DEC-2013/10/M/ST4/00069.
References: [1] Nawara, K., Waluk, J., *Anal. Chem.* **2017***, 89*, 8650. [2] Nawara, K. et al., *Anal. Chem.* **2018**, *90*, 10139. [3] Nawara, K., Waluk, J., *Anal. Chem.* **2019**, *91*, 5389.

*Corresponding author: E-mail: k.nawara@uksw.edu.pl

Image search for the genome: the development and application of fluorescent single-molecule DNA barcodes

Nathaniel O. Wand[a], Darren A. Smith[a], Andrew A. Wilkinson[a], Ashleigh E. Rushton[a], Stephen J. W. Busby[b], Iain B. Styles[c] and Robert K. Neely,*[a]

[a] *School of Chemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK*
[b] *School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK*
[c] *School of Computer Science, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK*

DNA sequence analysis is becoming increasingly affordable and accessible. Yet significant challenges remain in areas that sequencing can't readily address. For example, the analysis of sequence-specific binding events (by drugs, transcription factors, etc) is not possible using the current DNA sequencing technologies. Imaging experiments, by contrast, can be readily multiplexed.

We describe fluorescent DNA barcodes that allow us to identify specific DNA molecules within large, complex mixtures of genomes, using fluorescence microscopy. We build on previous work showing that DNA can be fluorescently labelled at specific sequence motifs (e.g. 5'-TCGA-3') using the DNA methyltransferase enzymes. This chemistry can be applied as a tool to produce molecular barcodes of DNA sequence which can be rapidly read, compared and addressed to search for specific regions of genome within a mixed sample.

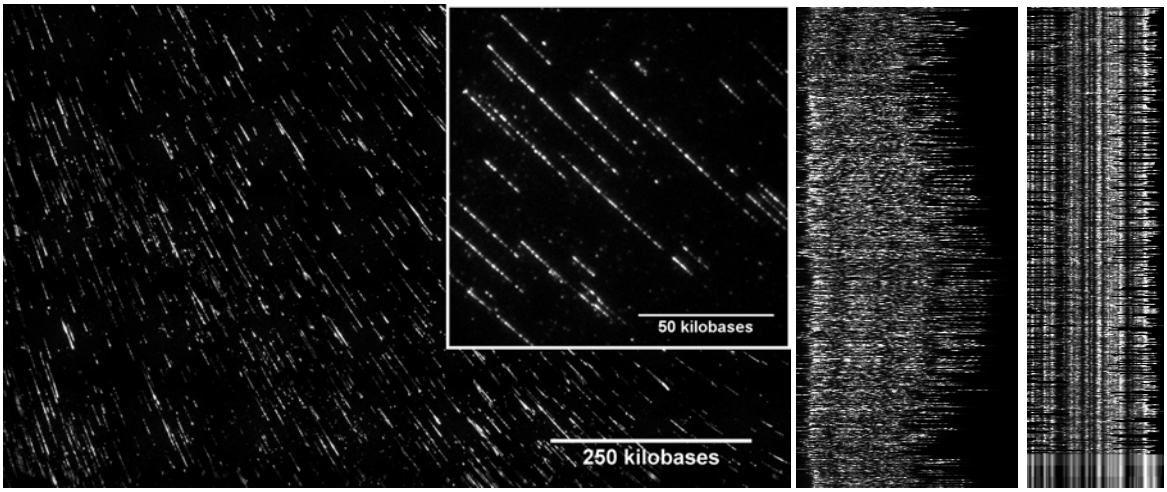


Figure. *Left to right: Microscope image of deposited DNA barcodes; extracted barcodes; filtered and aligned barcodes.*

Acknowledgments: This work was supported by grants from the EU H2020 (BeyondSeq: Grant 634890) and the Engineering and Physical Sciences Research Council, EP/N020901/1.

References: N. Wand et al, *bioRxiv*, 2018, 450809.

*Corresponding author: E-mail: r.k.neely@bham.ac.uk

Fluorescent probes and analogues for staining bacterial cells

Kevin Nguyen,[a] Sorachat Tharamak, [a] Jamie Lam,[a] Emmanuel Theodorakis*[a]

[a]*Department of Chemistry and Biochemistry, UC San Diego, 9500 Gilman Dr, La Jolla, CA 92093*

We report structure-property relationships for analogues of known amyloid-β staining agents (Figure 1) and their fluorescence application in bacterial cells. Using nitrogen substitution, the first set of analogues systematically varied the electronic structure of **ANCA-11** while preserving its overall size and shape. The second set modified both the steric and electronic structure of thioflavin T (**ThT**). We show that the fluorescence emission of **ANCA-11** was strongly blue- or red-shifted depending on the location of the nitrogen substitution in naphthalene. Similarly, (*aza*)-naphthalene analogues of **ThT** displayed analogous trends. Charged *N*-methylated **ThT** analogues effectively penetrate the cell membrane, while uncharged non-*N*-methylated analogues localize on the cell membrane surface. Overall, **ThT** and its analogues display significantly greater ability to stain bacterial cells, in contrast to **ANCA-11** and its analogues. These findings demonstrate the significance of certain structural and electronic features in standalone and cell-bound fluorescence properties.

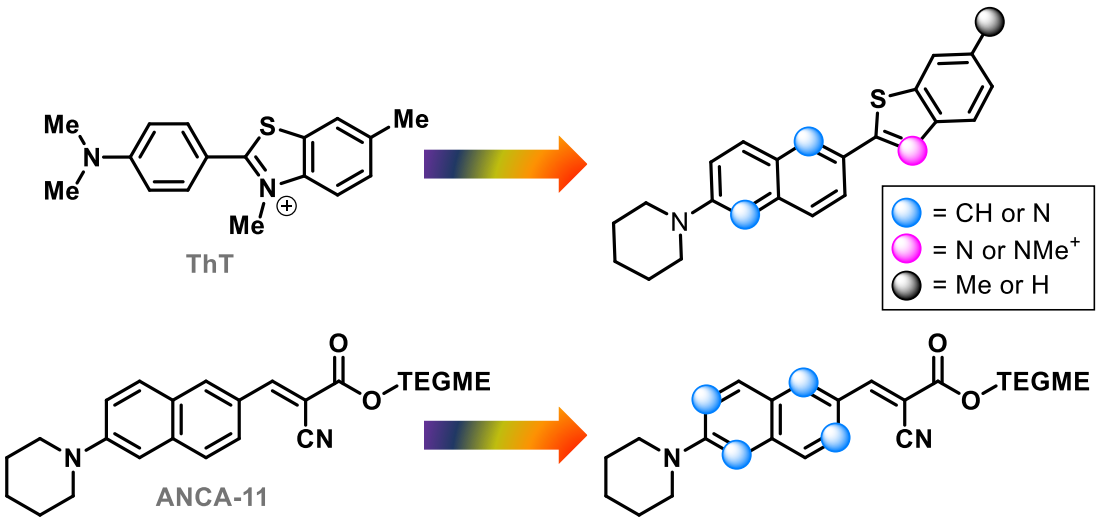


Figure 1. *Fluorescent amyloid-β staining agents and analogues*

Acknowledgments: This work was supported by a grant from the National Institute of Health.

*Corresponding author: E-mail: etheodorakis@ucsd.edu

A Fluorescent Cytosine Analog in Gapmer Technology: Stealth Labeling of an Antisense Oligonucleotide

Jesper R. Nilsson,[a] Tom Baladi,[a,b] Audrey Gallud,[c] Dzenita Bazdarevic,[d] Johan Meuller,[e] Malin Lemurell,[b] Elin K. Esbjörner,[c] L. Marcus Wilhelmsson,[a] Anders Dahlén *[b]

[a] *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Kemivägen 10, SE-41296 Gothenburg, Sweden.*
[b] *Medicinal Chemistry, Research and Early Development Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden.*
[c] *Department of Biology and Biological Engineering, Chalmers University of Technology, Kemivägen 10, SE-41296 Gothenburg, Sweden.*
[d] *Bioscience, Research and Early Development, Cardiovascular, Renal and Metabolic, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden.*
[e] *Mechanistic Biology & Profiling, Discovery Sciences, R&D, AstraZeneca, Gothenburg, Sweden.*

The emergence of RNA-based therapeutics in general, and antisense oligonucleotides (ASOs) in particular, is currently having a profound influence on the pharma industry.[1] A heavily researched ASO platform for selective gene knockdown is the "*gapmer*" – a short, chemically stabilized, nucleotide-based oligomer designed to catalytically induce degradation of its target RNA.[2] In this work, we show that robust fluorescence properties can conveniently be endowed to gapmers, gapmer-peptide conjugates, and gapmer-sugar conjugates, by exchanging cytosines in the gapmer sequence with a fluorescent tricyclic cytosine analog (**tC**, Figure). Critically, we herein establish that these "internal" modifications can be introduced without compromising the overall affinity towards the target RNA *in vitro* or the knockdown efficiency towards the target gene *in vivo*.

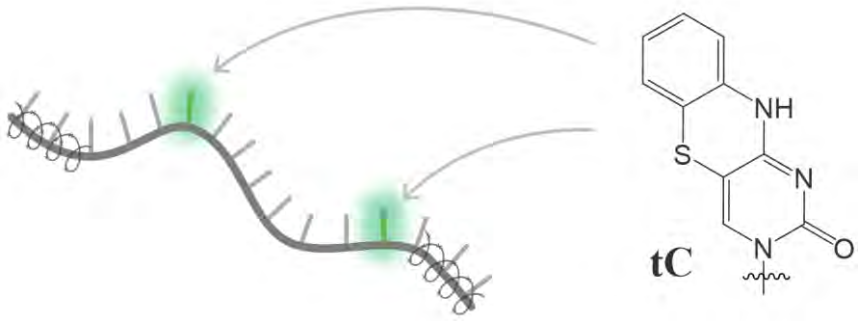


Figure. *A gapmer comprising the fluorescent cytosine analog* ***tC****.*

These findings constitute a significant addition to the toolkit for imaging- and fluorescence-based ASO research, effectively minimizing potential probe-induced pervasive effects.

Acknowledgments: This work was conducted within the FoRmulaEx research consortium and supported by the Swedish Foundation for Strategic Research (SSF, grant No. IRC15-0065).

References: [1] Valeur, E. et al., *Angew. Chem. Int. Ed.* **2017***, 56*, 10294. [2] Ämmälä, C. et al. *Sci. Adv.* **2018**, *4: eaat3386.*

*Corresponding author: Email: anders.dahlen@astrazeneca.com.

Ultrasmall semicrystalline organic J-aggregate nanodots for single- and two- photon fluorescence microscopy

Hubert M. Piwoński,[a] Shuho Nozue,[a] Tsuyoshi Michinobu,[b] Satoshi Habuchi*[a]

[a] *King Abdullah University of Science and Technology, Biological and Environmental Science and Engineering Division, P.O. Box 4700 KAUST, Thuwal 23955-6900, Kingdom of Saudi Arabia*
[b] *Tokyo Institute of Technology, Department of Material Science and Engineering, 2-12-1 Ookayama, Meguro-ku, Tokyo 152-8552, Japan*

Water miscible ultra-small luminescent nanostructures capable of efficient single and multi-photon excitation offer a broad range of applications in fluorescence microscopy, including bioimaging. To use fluorescent nanoparticles for imaging experiments in biological setting, several key characteristics are required: small size, high colloidal stability, large molar extinction coefficient and two-photon absorption cross-section, high fluorescence quantum yield, narrow spectral band width, and highly polarized fluorescence. Nanoparticles consisting of J-aggregates are a potential candidate for the application because of their very narrow spectral band width and spatially highly ordered alignment of the fluorophores inside the particles. However, J-aggregates nanoparticles that meet these requirements have not been developed to date.
Here we report quasicrystalline organic J-aggregate nanoparticles with a size range of 2-4 nm using 1.8–carbazole-benzothizole dimer ($(CBzBT)_2$), which exhibit a large molar extinction coefficient (8.1×10^6 $L\times mol^{-1}\times cm^{-1}$) and two-photon absorption cross-section (1.3×10^4 GM), narrow absorption (27nm) and fluorescence (57nm) spectral band width, bright, and highly polarized fluorescence (Figure). Our findings open an avenue for the development of new class of fluorescent nanoparticles based on organic J-aggregates.

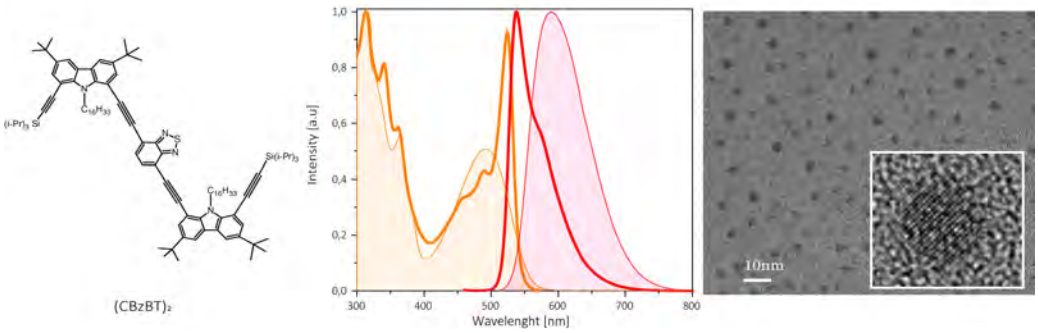


Figure. *(left) Chemical structure of $(CBzBT)_2$. (center) Absorption (orange) and fluorescence (red) spectra of $(CBzBT)_2$. The filled and solid lines are the spectra of $(CBzBT)_2$ in THF and the organic nanodots (ONDs) dispersed in water, respectively. (right) TEM micrograph showing spherical morphology of the ONDs. The inset show a HR-TEM of a single OND, which reveals a quasi-crystalline diffraction pattern with lattice fringes.*

*Corresponding author: E-mail: satoshi.habuchi@kaust.edu.sa

p53 Oligomerization and Dynamics upon DNA Damage revealed by pair-Correlation Function Imaging.

Francesco Palomba[a,b], Michelle A. Digman*[a,b]

[a] *Department of Biomedical Engineering, University of California Irvine,*
[b] *Laboratory for Fluorescence Dynamics, University of California Irvine,*

p53 is a tumor suppressor protein that regulates target genes involved in DNA damage migration and repair. If cells become stressed due to DNA damage, p53 will induce genes that trigger cell cycle arrest and/or apoptosis. Specific binding sites must be accessible to active either pathway. Thus, specific chromatin location as well as accessibility are required for stable tetramers of p53 to activate either response pathway. Evidence indicates the local chromatin structures can regulate the efficiency/pathway choice of DNA repair. Although evidence suggests spreading of repair and checks signals from the initial damage sites, experimental tools and analyses are currently limited to specifically interrogate their effects at damage sites and non-damage sites in the nucleus. Laser micro irradiation, which induces DNA damage in a confined region in the cell nucleus, allows clear distinction of molecular events that occur at damage sites and in the rest of the nucleus.

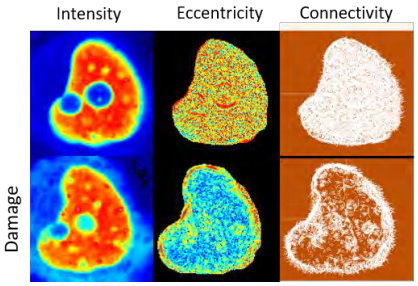


Figure. *Dynamics of p53-EGFP before and after DNA damage*

Previously we used the pair correlation function (pCF) analysis combined with laser micro irradiation to demonstrate the rapid changes of molecular flow of EGFP and the DNA end binding protein Ku fused to EGFP at damage sites and non-damage sites within the first few minutes of damage induction.[1] Here, we used the pCF and the Number/Brightness analysis (N&B)[2] to measure the changes in molecular connectivity and the oligomerization of EGFP-p53 to map the processes the of this tumor suppressor after DNA damage. Moreover, we compare the effects of a localized DNA damage respect to chemical damage on the p53-EGFP dynamics inside the cell.

Acknowledgments: NSF MCB-161570 and NIH grant P41 GM103540.

References: [1] Hinde E. et al., *Biophys J.*, **2014,** 107, 55. [2] Digman M. et. al, Biophys J., **2008**, 94, 2320

*Corresponding author: E-mail: mdigman@uci.edu

Millimeter-Deep Detection of Single SWIR Emitting Polymer Nanoparticles Through a Turbid Media

Hubert M. Piwoński, *[a] Tsuyoshi Michinobu,[b] Satoshi Habuchi[a]

[a] *King Abdullah University of Science and Technology, Biological and Environmental Sciences and Engineering Division, P.O. Box 4700 KAUST, Thuwal 23955-6900, Kingdom of Saudi Arabia*
[b] *Tokyo Institute of Technology, Department of Material Science and Engineering, 2-12-1 Ookayama, Meguro-ku, Tokyo 152-8552, Japan*

Achieving deep photon penetration while maintaining the high spatiotemporal resolution is one of the main objectives and challenges in luminescence-based optical imaging. This goal can be achieved by applying short-wavelength infra-red (SWIR, 1,000-1,700 nm) detection.[1] At this wavelength range, the low autofluorescence of biological tissues together with reduced attenuation from scattering and absorption by tissue components leads to an improvement in detection depth and imaging.[2] Recently, we demonstrated that donor-acceptor (DA) type of conjugated polymers are excellent materials for fabrication of bright and ultrasmall (< 5 nm) Pdots through proper control of reprecipitation processes.[3] Here we report single-particle fluorescence detection in scattering media with the unprecedented combination of over millimetre-deep penetration, high spatial resolution and fast acquisition afforded by nanoparticles made of DA type of SWIR emitting conjugated polymers (Figure). We demonstrate size independent emission properties of our polymer nanoparticles with fluorescence quantum yield reaching value of 0.31% and extreme brightness. Furthermore, we introduce great improvement in signal to background sensitivity of single-molecule imaging in turbid media by applying short-pulse laser excitation combined with time-gated fluorescence detection scheme.

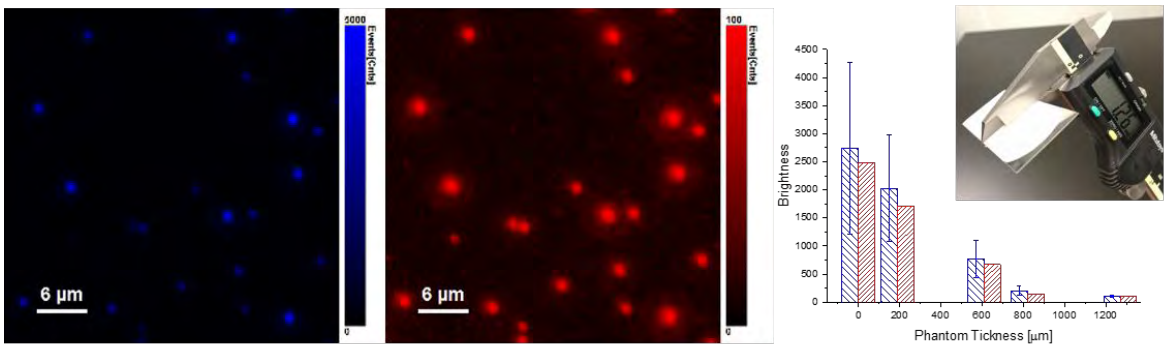


Figure. *Single particle-images of SWIR-emitting Pdots deposited on tissue-mimicking phantom recorded simultaneously by Si detector (blue) and InGaAs detector (Red) together with diagram of particle brightness for different phantom thickness.*

References: [1] Smith, A. M., et al., *Nat. Nanotechnol.* **2009**, 4, 710. [2] Cheng K, et al., *Nano Research* **2015**, 8, 3027. [3] Piwonski, H.M., et al., *Nat. Commun.* **2017**, 8, 15256.

*Corresponding author: E-mail: hubert.piwonski@kaust.edu.sa

Determination of the metabolic index using the fluorescence lifetime of free and bound NADH in the phasor approach

Suman Ranjit,[a,b] Leonel Malacrida,[a,c] Milka Stakic,[a] Enrico Gratton*[a]

[a] *Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California, Irvine, USA*
[b] *Department of Biochemistry and Molecular & Cell Biology, Georgetown University, Washington D.C. USA*
[c] *Departamento de Fisiopatología, Hospital de Clínicas, Universidad de la República, Montevideo, Uruguay*

Changes in metabolism in cells are often studied by the ratio of NAD+ to NADH. This ratio is correlated to free and enzyme bound NADH, and can be used as a of metabolic states in cells using fluorescence lifetime imaging (FLIM). Nicotinamide and adenine moieties of Nicotinamide adenine dinucleotide (NADH) separates from their collapsed structure in solution when they bind to enzymes and as a result, binding increase fluorescence quantum yield and lifetime. The extent of increase in fluorescence lifetime is dependent on the apoenzyme and presence of auxiliary ions. Reports from past studies show distinctive discrepancies in calculation of the bound NADH lifetime, often related to complications in sample preparation and limitations in data acquisition. In this work, we show that in presence of oxalic ion, proper preparation of lactate dehydrogenase (LDH) bound NADH has a lifetime of 3.4 ns and is positioned on the universal semi-circle of the phasor plot, representing mono-exponential lifetime. Improper preparation results in a mixture of species, with phasor positions inside the universal semi-circle. Measurement in cellular environment show similar trend and a linear trajectory between free NADH and cellular NADH components, which when extrapolated to the universal semi-circle shows a lifetime of 3.4 ns at the crossing point. These results suggest that 3.4 ns can be used as a bound NADH lifetime and phasor approach can correlate lifetime contributions to concentration fractions of free and bound species. The effects of different types of FLIM acquisitions are also discussed in context., CA, USA.

Acknowledgments: This work was supported by NIH grant P41-GM103540.

References: [1] Ranjit, S. et al., *Microsc. Res. Tech.* **2018***, 81*, 980.

*Corresponding author: E-mail: egratton22@gmail.com

In situ studying protein kinase activity in single living cell by combining single molecule spectroscopy with activity-based protein profiling strategy

Shengrong Yu, Zhixue Du, Chaoqing Dong, Jicun Ren*

School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

Protein phosphorylation is a very important regulatory mechanism in a majority of biological processes, and determination of protein kinase activity plays a key role in pathological study and drug development of kinase-related diseases. However, it is very challenging to in situ study endogenous protein kinase activity in single living cell due to shortage of in vivo efficient methods. In this work, we propose a new strategy for direct determination of protein kinase activity in single living cell by combining single molecule fluorescence correlation spectroscopy (FCS) with activity-based protein profiling (ABPP) strategy. Ribosomal S6 kinase-2 (RSK2) was used as a model, and the activity-based fluorescent probes (ABPs) were synthesized to specially label to active RSK2 in living cells. Conventional FCS and MEMFCS (maximum entropy method) single molecule techniques were used to in situ determine RSK2 activity in living cells based on the difference in molecular weight between free probes and probe-RSK2 complexes. Furthermore, wild-type and mutated RSK2 were fused with enhanced green fluorescent protein (EGFP) using lentivirus infection, and fluorescence cross-correlation spectroscopy (FCCS) was used to verify the selective binding of ABPs to RSK2-EGFP fusion protein in living cells. Finally, FCS with ABPP was applied for in situ monitoring the activation of endogenous RSK2 in the stimulation of short hairpin RNA, serum, epidermal growth factor, kinase inhibitor and ultraviolet irradiation, we observed that endogenous RSK2 showed different behaviors in the cytoplasm and nucleus in some stimulation. Our results document that FCS with ABPP is a very promising method for studying endogenous protein kinases in living cells.

Acknowledgments: This work was supported by grants from the National Natural Science Foundation of China.

References: [1] Liu, H, et al., *J. Am. Chem. Soc.,* **2014***, 136,* 2775. [2] Su,D, et al., *Anal. Chem.* **2017**, *89,* 9788. [3] Su,D, et al., *Anal. Chem.* **2018**, *90,* 1006. [4] Wang, J.J., et al., *Anal. Chem.* **2017**, *89,5230.* [5] Zhang, B.C. **2017**, *89,* 12609. [6] Du, Z.X., et al. *Anal. Chem.* **2018**, *90,*6144.
*Corresponding author: E-mail: jicunren@sjtu.edu.cn

Structure and Properties of Fluorogenic Cytidine Analogues.

George N. Samaan,[a] Byron W. Purse*[a]

[a] *Department of Chemistry and Biochemistry, San Diego State University, San Diego, California 92182, United states.*

Fluorescent nucleoside analogues are widely used in biophysical studies on the replication, maintenance, and expression of the genetic code, but it is not yet understood how and why they change their fluorescent properties in response to local environment, specifically base pairing and stacking. Since first made and studied by Matteucci and Wilhelmsson, tricyclic cytidine nucleoside analogues have been modified to create a family of compounds with a range of fluorescent properties and they have been used in nucleic acid studies including FRET applications, in DNA/RNA polymerase studies and in studying DNA/protein interactions.[1] One member of this family, 8-DEA-tC (Figure) is virtually nonfluorescent as a free nucleoside (Φ_{em} = 0.006), but the fluorescence increases up to Φ_{em} = 0.12 when the compound is base stacked and correctly base paired with adenosine.[2] Because the mechanism for this fluorescence increase is not fully understood, we are synthesizing a related compound, 8-DEA-tC^{O}, to compare its fluorescence with DEA-tC in response to duplex nucleic acid structure. The synthesis of 8-DEA-tC^{O} is completed in 7 steps in which the addition of the 4-diethylamino-2-aminophenol to protected 5-bromouridine, activated with $POCl_3$ and 1H-triazole, is the key challenging step. Understanding the structure-photophysical properties relationship will help in the design of very bright probes with predictable fluorescent responses to nucleic acid structure and dynamics.

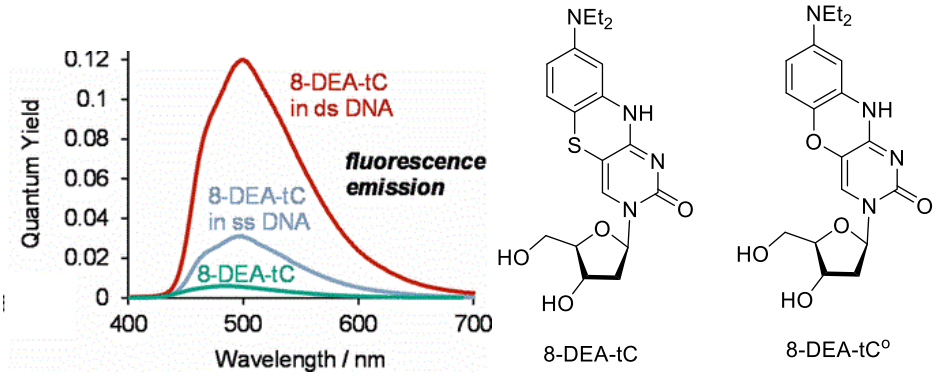


Figure.*8-DEA-tC and 8-DEA-tC°.*

Acknowledgments: This work was supported by grants from the National Science Foundation (CHE-1709796 and CHE-1800529 to BWP) and San Diego State University.

References: [1] Wilhelmsson, L, *Quart. Rev. Biophys.* **2010***, 43(2), 159*. [2] Purse, B. W., et al. *J. Am. Chem. Soc.* **2017**, 139, 1372.

*Byron Purse: bpurse@sdsu.edu

Deciphering free and hindered diffusion dynamics in the plasma membrane of living cells

Falk Schneider,[a] Erdinc Sezgin,[a] Dominic Waithe,[b,c] Marco Fritzsche,[d] Christian Eggeling*[a,b,e,f]

[a] MRC Human Immunology Unit, [b] Wolfson Imaging Centre Oxford and [c] MRC WIMM Centre for Computational Biology, Weatherall Institute of Molecular Medicine, University of Oxford, Headley Way Oxford, OX3 9DS, United Kingdom
[d] Kennedy Institute for Rheumatology, University of Oxford, Roosevelt Drive, Oxford OX3 7LF, United Kingdom
[e] Institute of Applied Optics Friedrich - Schiller - University Jena, Max-Wien Platz 4, 07743 Jena, Germany
[f] Leibniz Institute of Photonic Technology e.V., Albert-Einstein-Straße 9, 07745 Jena, Germany

The cellular plasma membrane is a highly heterogeneous structure organised on nano-scales, and it displays a crucial interaction platform for proteins, lipids and soluble ligands. Investigating the molecular membrane organisation by measuring diffusion dynamics offers a better understanding of its biological function[1].
Fluorescence correlation spectroscopy (FCS) is one of the prominent tools to elucidate these dynamics in living cells but can only report on the dynamics at one given spatial position at a time. Using scanning fluorescence correlation spectroscopy (sFCS), one can collect a multitude of FCS measurements at different spatial locations[2]. To obtain the modes of diffusion (e.g. free Brownian or hindered diffusion with nano-scale interactions) at these locations, we combined sFCS with super-resolution stimulated emission depletion (STED) microscopy introducing line interleaved scanning STED-FCS (LIESS-FCS)[3]. With LIESS-FCS we reveal homogeneously free diffusion for fluorescent phospholipids but strong local hindrance for sphingomyelin and GPI-anchored protein in the plasma membrane of living cells. However, it turns out that by using large data-sets and statistical analysis already the confocal (not super-resolved) sFCS data as obtained from a standard laser scanning microscope can be indicative for such diffusion modes[4].

Overall we are presenting a novel toolkit to investigate nano-scale molecular diffusion dynamics for shedding new light on membrane organisation and heterogeneity.

References:

1. E. Sezgin *et al.*, *Nat. Protoc.* **14**, 1054–1083 (2019).
2. Q. Ruan *et al.*, *Biophys. J.* **87**, 1260–1267 (2004).
3. F. Schneider *et al.*, *Nano Lett.* **18**, 4233–4240 (2018).
4. F. Schneider *et al.*, *ACS Nano*. **12**, 8540–8546 (2018).

*Corresponding author: E-mail: christian.eggeling@rdm.ox.ac.uk

AiryScan CCA provides structural and dynamic fingerprinting of subcellular compartments in living cells

Lorenzo Scipioni*, Enrico Gratton*

*Laboratory for Fluorescence Dynamics, University of California, Irvine (CA), USA

We will present our current advances in the development of the CCA (Comprehensive Correlation Analysis) technique using the Zeiss Airyscan detector, which consists of 32 GaAsP PMT arranged in a hexagonal pattern. Its fast temporal sampling (down to 1.28 µs per frame) and super-resolution capabilities (as per the ISM [1] principle) allows for the implementation of advanced state-of-the-art spatiotemporal correlation techniques.
The CCA technique features the parallel implementation of several correlation techniques in super-resolution such as spot-variation FCS [2], 2D-pCF [3] and iMSD [4] using this fast detector array. This simultaneous analysis of the same dataset provides a plethora of biophysical information regarding the fluorescent probe and the surrounding environment, such as diffusion coefficient, concentration, diffusion modality, environment organization, direction and anisotropy of molecular flows, diffusion connectivity and oligomerization state in a single analysis in a few seconds.
Using CCA we obtained a complete structural and dynamic characterization of several subcellular compartments of living NIH-3T3 cells. The parallel acquisition of numerous biophysical parameters opens the doors to new multidimensional characterization and classification methods that can be applied to virtually any fluorescent or fluorescently-labeled probe diffusing in complex environments.

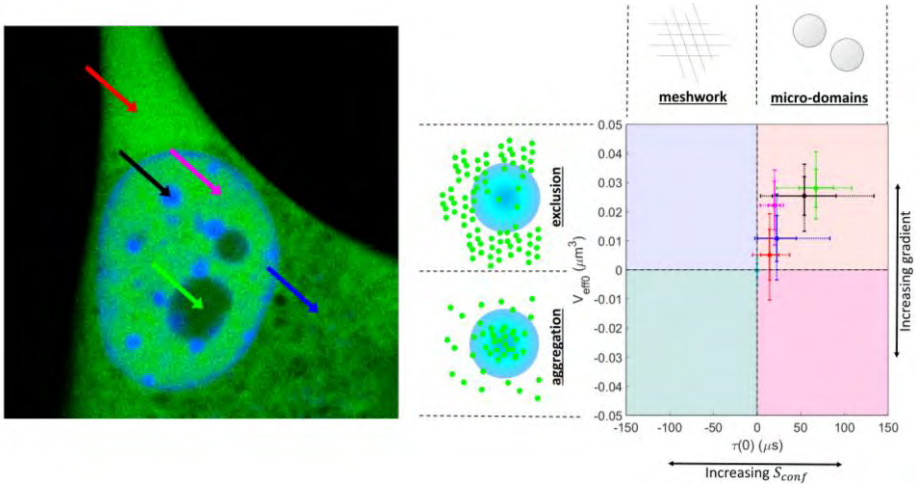


Figure. *Dynamic fingerprinting (right) of several subcellular compartments (left) after analysis with the CCA-Airyscan technique*

Acknowledgments: Work supported by NIH P41 GM103540 grant

References: [1] Sheppard et al *Opt. Lett.* (2013) [2] Wawrezinieck et al *Biophys. J.* (2005) [3] Hinde et al *PNAS.* (2010) [4] Di Rienzo et al *Nat. Comm.* (2014)

*Corresponding author: E-mail: lscipioni@uci.edu, scipioni.lorenzo@gmail.com

Probabilistic space filling: a new algorithm for single molecule tracking and analysis

Maged F. Serag,[a] Satoshi Habuchi*[a]

[a] *Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia*

We report the development of new theoretical framework and analytical tool that can capture modes of motion that could be averaged out during the mean squared displacement analysis of the diffusive motion of macromolecules. In this framework, we study the motion of single molecules with respect to a virtual latticed frame of reference with which we analyse how often the molecule steps into new lattice sites in a diffusion space during its motion (Fig. 1a). We used the probability distribution of finding a particle—moving in 1D space—(Fig. 1b) at different lattice site to derive the function that expresses the probability distribution in 2D space. We then used this function to fit the simulated and the experimental data to eventually obtain the probability of new visits at a specified time lag (Fig. 1c). We used the temporal profile of the new visits to reveal non-random sub-modes of motion of fluorescently-labeled DNA molecules that deviate from the expected random motion in the linear, diffusive regime. With our algorithm, we further show for the first time that the Brownian motion of DNA is governed by the interplay of these non-random dynamics. [1]

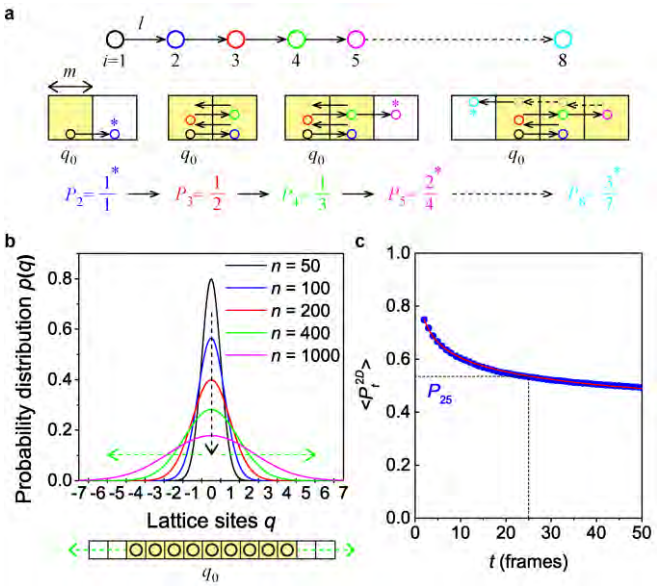


Figure1*. (a) Schematic diagram illustrating the motion of a single particle on 1D lattice frame. The probability of occurrence of visits to new lattice sites at time t (Pt) decreases as t increases. (b) Probability distribution (p) of finding a particle at different lattice sites after n. (c) Average probability of occurrence of visits to new lattice sites at time t.*

References: [1] Serag, M. F. et al., *Nature commun.* **2017**, *8*, 15675.

*Corresponding author: E-mail: Satoshi.Habuchi@kaust.edu.sa

New organic nanoantennas for FRET-based detection of nucleic acids

Caterina Severi,[a] Nina Melnychuk,[a] Andrey Klymchenko*[a]

[a] *Laboratory of Bioimaging and Pathologies, University of Strasbourg, Illkirch-Graffenstaden, France*

Dye-loaded polymeric nanoparticles are a powerful and interesting bioimaging tool because of their high brightness and capacity to bear multiple functional groups.[1] However when these nanoparticles are loaded with a dye at high concentrations, their brightness decreases because of Aggregation-Caused Quenching (ACQ) phenomenon. Our group addressed this issue by employing cationic dyes bearing hydrophobic groups paired with bulky hydrophobic counterions. Counterions are able to distance the fluorophores one from the other inside the nanoparticles and, moreover, they create a supramolecular organization in which the dyes behave cooperatively.[2] [3] This collective phenomenon was employed for creating a light-harvesting antenna nanoparticle system that feature Fluorescence Resonance Energy Transfer (FRET) from thousands of dyes (rhodamine B derivative) to few cyanine 5-based acceptors; obtaining a >1000-fold amplification of the acceptor emission.[4] The latter preparation of the nanoantenna based probe for amplified detection of nucleic acids.[5]

The aim of this work is to prepare bright antenna nanoparticles functioning in a spectral region which is compatible with an RGB camera. To this scope, DNA-decorated nanoparticles based on octadecyl rhodamine 6G ester (yellow emission) as a FRET donor and far red dye as acceptor were synthesized.
This system was tested in FRET assays for detection of nucleic acids both *via* spectroscopy and microscopy. It was found that the ratio between the light collected in the red and green channel of the RGB camera decreases significantly in response to the target DNA sequence. This enables biosensing using an RGB camera, similar to those used in conventional photography.

Acknowledgments: This work was supported by ERC consolidator grant BrightSens 648528.

References: [1] Reisch A. and Klymchenko, A. S. *Small* **2016***, 12*, 1968. [2] Reisch, A., et al. *Nat. Comm.* **2014**, *5*, 4089. [3] Reisch, A., et al. *ACS Nano.* **2015**, *9*, 5104. [4] Trofymchuk, K., et al. *Nat. Phot.* **2017**, *11*, 657. [5] Melnychuk, N. and Klymchenko, A.S. *J. Am. Chem. Soc.* **2018**, 140, 10856.

*Corresponding author: E-mail: andrey.klymchenko@unistra.fr

Engineering Genetically Encodable Fluorescent Indicators to Probe Cellular Ion Dynamics

Yi Shen,*[a] Sheng-Yi Wu,[a] Yurong Wen,[b] Rochelin Dalangin,[a] M. Joanne Lemieux,[b] Robert E. Campbell*[a,c]

[a] *Department of Chemistry, University of Alberta, Canada*
[b] *Department of Biochemistry, University of Alberta, Canada*
[c] *Department of Chemistry, School of Science, The University of Tokyo, Japan*

Genetically encodable fluorescent indicators for ions are indispensable tools for cell biology and neuroscience as they enable direct visualization of ion dynamics in live cells with spatiotemporal resolution. The present toolkit contains indicators for H^+, Ca^{2+}, Cl^-, Zn^{2+}, *etc*. Genetically encoded H^+ (pH) indicator superecliptic pHluorin (SEP) is an ideal probe for the cellular processes involving pH changes, including endocytosis and exocytosis.[1] When fused to synaptic vesicle luminal protein, SEP can be used to monitor vesicle fusion and neurotransmission. We present rationally improved SEP variants with better brightness, enhanced pH sensitivity, and better intracellular trafficking. In addition, we solved the structure of the new SEP variant which sheds light on the mechanism of its pH-sensitivity.

Among all the ion indicators, genetically encoded Ca^{2+} indicators (GECI) are the most widely used and arguably most important molecular tools. The development of GCaMP series represents the pinnacle of GECI engineering.[2] Besides GCaMP, the recent expansion of GECIs yielded many variants with spectral distinction, and altered affinities, which broadened the utilities of GECIs. It is widely recognized that the red and far-red GECIs will ultimately supplant GCaMPs due to the deeper tissue penetration associated with red light. Here we report the first GECI series with emission in the far-red region, FR-GECOs. We believe that the FR-GECO lineage will be embraced as the far-red GECI of choice for a broad cross-section of users in the neuroscience communities.

Despite their central role in essentially all aspects of cellular homeostasis of K^+, genetically encoded indicator for K^+ was unavailable until recently. We engineered the first single fluorescent protein-based K^+ indicator GINKO1, by insertion of a bacterial potassium binding protein Kbp into EGFP.[3] GINKO1, in combination with a red GECI, enables interrogation of K^+ and Ca^{2+} dynamics simultaneously in stimulated neuronal and glial cells. We now have further improved GINKO with better brightness and enhanced K^+ sensitivity using directed molecular evolution. We envision that these new K^+ indicators will usher a new era of imaging-based K^+ dynamics research.

Acknowledgments: This work was supported by grants from NIH, Brain Canada, NSERC, CIHR, & Alberta Innovates.

References: [1] Sankaranarayanan, S., et al. *Biophys. J.* **2000**, *79* (4), 2199–2208. [2] Chen, T.-W., et al. *Nature* **2013**, *499* (7458), 295–300. [3] Shen, Y., et al. *Commun Biol* **2019**, *2* (1), 18.

*Corresponding author: E-mail: yi.shen@ualberta.ca and robert.e.campbell@ualberta.ca

Time-resolved emission studies of photo-aggregated fluorophores

Trevor Smith,*[a] Jian Zhou,[b] Yang Xu[a]

[a]*ARC Centre of Excellence in Exciton Science and School of Chemistry, University of Melbourne, Parkville, 3010, Australiab*
[a]*College of Material, Chemistry and Chemical Engineering, Hangzhou Normal University, Hangzhou 310036, People's Republic of China*

The momentum of photons can be used to exert optical forces (pressure) on small particles. In addition to laser trapping ("tweezering") applications, photon pressure can be used to induce aggregation from solutions of polymers[1] and other materials. In this presentation, we will show how photon pressure has been used to trap particles and photo-aggregate from solution a series of photoluminescent materials, including conjugated polymers and polyelectrolytes containing tetraphenylethene (TPE) units.[2] These TPE-based polymers show emission properties that are dependent on factors that restrict their non-radiative deactivation pathways such as viscosity and aggregation. In particular their emission decay behaviour is sensitive to such changes, which enables the photon-induced aggregation of these fluorophores to be monitored by time-resolved fluorescence imaging techniques as the aggregates form. Structural differences in the polymer can lead to variations in the photo-induced aggregation.

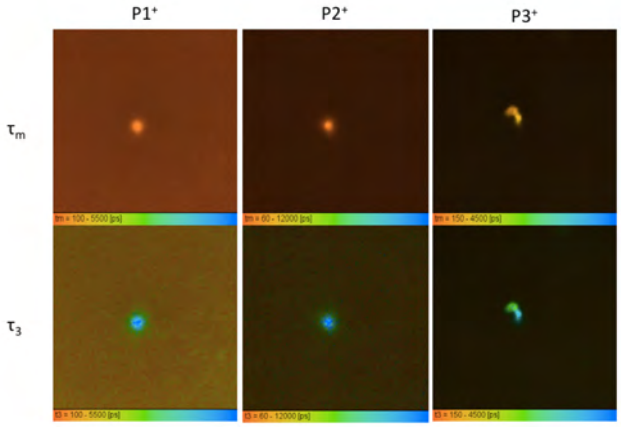


***Figure*.** Time-resolved fluorescence images of three conjugated polyelectrolyte samples represented as: (i) average fluorescence lifetime, τ_m, maps and (ii) the τ_3 component, after extended periods of focussed, IR laser irradiation.

Acknowledgement: Financial support from the Australian Research Council Centre of Excellence in Exciton Science is gratefully acknowledged.

References: [1] T. A. Smith, J.I.- Hotta, K. Sasaki, H. Masuhara, Y. Itoh, *J. Phys. Chem. B.* **1999,** *103,* 1660. [2] J. Zhou, Y. Wang, Y. Hong, B. Chen, M. Gao, B. Zhang, T.A. Smith, W.W.H. Wong, Z. Zhao. *J. Polymer Science, Part A: Polymer Chem.*, **2018,** *56*, 672

*Corresponding author: E-mail: trevoras@unimelb.edu.au

Allosteric action in Hsp90: From nucleotide binding to domain rearrangement

Benedikt Sohmen,[a] Steffen Wolf,[b] Johann Thurn,[a] Björn Hellenkamp[a,c], Gerhard Stock[b], Thorsten Hugel*[a,d]

[a] *Institute of Physical Chemistry, University of Freiburg, Germany*
[b] *Biomolecular Dynamics, Institute of Physics, University of Freiburg, Germany*
[c] *Engineering and Applied Sciences, Columbia University, New York, USA*
[d] *Signalling research centers BIOSS and CIBSS, University of Freiburg, Germany*

Small effectors like e.g. nucleotides can help highly dynamic proteins to cycle through their multitude of conformational states which in turn is the basis for molecular signaling. However, the underlying mechanism of information transfer from effector interaction to the signaling event is often unclear. Single-molecule experiments are ideal to dissect such allosteric actions on a wide range of timescales. [1,2]
Here, we present an integrative study combining single-molecule Förster resonance energy transfer (smFRET), fluorescence lifetime experiments and molecular dynamic (MD) simulations in atomic detail. This reveals nucleotide-triggered information transfer in the full 1300 amino acid Heat Shock Protein (Hsp90) dimer. As a chaperone Hsp90 is a key regulator in our cell interacting with several hundred substrate proteins and about 20 cochaperones.[3] We show that ATP stabilises an energetically disfavoured active folding state, while ATP hydrolysis initiates a sequence of consecutive structural changes which lead to the return of Hsp90 into a relaxed inactive state. Interestingly, the observed dynamics in the information transfer are of hierarchic nature. This might be a general molecular mechanism of intra-molecular information transfer. We anticipate that similar integrated approaches are promising to reveal the molecular details of allosteric action in other protein machineries.

Acknowledgments: This work was supported by the European Research Council through ERC grant agreement No. 681891 and by a grant of the German Research Foundation (No. INST 37/935-1 FUGG).

References: [1] Kalinin, S. et al., *J. Phys. Chem. B* **2010***, 114*, *7983-7995*. [2] Hellenkamp, B. et al., *Nat. Methods* **2017***, 14*, 174-180. [3] Schopf, F. H. et al., *Nat. Rev. Mol. Cell Bio.* **2017***, 18*, 345-360

*Corresponding author: E-mail: benedikt.sohmen@physchem.uni-freiburg.de

Fluorescence Anisotropy Imaging Microscopy (FAIM): A new approach with multiple applications from cell morphology[1] to energy migration dynamics[2]

Hamid Soleimaninejad

[1]*Biological Optical Microscopy Platform (BOMP), Bio21 Institute Node, The University of Melbourne, Victoria 3010 Australia. Email: hamid.s@unimelb.edu.au*

Fluorescence anisotropy measures the difference of fluorescence intensities emitted parallel and perpendicular to the polarisation of the excitation light, which reports on the rotational motion of the entire dye molecule within the excited-state lifetime. Fluorescence anisotropy provides information on the mobility and orientation of the molecule and has been used to investigate protein-protein interactions, ligand-receptor binding processes, membrane mobility, and protein fibril formation in cell dynamics studies.

We demonstrate the use of **FAIM** to map the fluorescence anisotropy in cells and monitor their change upon the increase of the macromolecular crowding effect. We developed a new strategy that allows the spatiotemporal visualization of the macromolecular crowding effect in cells. An amine-reactive aggregation-induced emission **(AIE)** fluorogen is used as molecular rotor to label proteins in the cytoplasm and the change in the protein mobility as well as local viscosity can be monitored by using fluorescence anisotropy imaging **(FAIM)** and fluorescence lifetime imaging **(FLIM)**, respectively[1].

Fluorescence anisotropy is an experimental observable for energy migration as well in disordered chromophores in condensed polymeric systems. In the absence of rotational diffusion of chromophores, fluorescence depolarization or loss of initial fluorescence anisotropy, results from the randomization of the initially photoselected emission transition dipole orientations through Förster-mediated energy migration.

We also demonstrate a systematic visualization of the unique photophysical and fluorescence anisotropic properties of polyfluorene coplanar conformation (β-conformation) using time-resolved scanning confocal fluorescence imaging (**FLIM**) and fluorescence anisotropy imaging microscopy (**FAIM**) measurements in PFO and PODPF polymers. We observe inhomogeneous morphologies and fluorescence decay profiles at various micrometer-sized regions within all types of polyfluorene β-conformational spin-coated films. β-conformational regions have larger fluorescence anisotropy for the low molecular rotational motion and high chain orientation, while the low anisotropy in glassy conformational regions shows more rotational freedom of the chain and efficient energy migration from amorphous regions to β-conformation as a whole[2].

1) Soleimaninejad, H., Chen, M. Z., Lou, X., Smith, T. A., & Hong, Y. (2017). Measuring macromolecular crowding in cells through fluorescence anisotropy imaging with an AIE fluorogen. *Chemical Communications*, *53*(19), 2874-2877.
2) Yu, M. N., Soleimaninejad, H., Lin, J. Y., Zuo, Z. Y., Liu, B., Bo, Y. F., ... & Wu, X. P. (2018). Photophysical and fluorescence anisotropic behavior of polyfluorene β-conformation films. *The journal of physical chemistry letters*, *9*(2), 364-372.

Determination of atrial fibrillation related exosomes by single molecule Fluctuation Correlation Spectroscopy on a microwell chip

Di Su,[a, b] Yuemei Hou, *[b] Xiangyi Huang,[a] Chaoqing Dong,[a] Jicun Ren*[a,c]

[a] *School of Chemistry & Chemical Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P. R. China.*
[b] *Shanghai University of Medicine & Health Sciences Affiliated Sixth People's Hospital South Campus, 6600 Nanfeng Highway, Shanghai, 201499, P. R. China.*
[c] *State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P. R. China.*

Cardiac cell specific exosomes play a critical role in the development of atrial fibrillation (AF) diseases. It is of importance to develop highly sensitive method for the analysis of AF related exosomes. When we combined fluorescence correlation spectroscopy (FCS) with microwell chip, the required sample volume can be reduced to 1 μL.[1,2] In this study, we demonstrated a new microwell chip - resonance light scattering correlation spectroscopy (MC-RLSCS) method for detecting the concentration and size of AF related exosomes without fluorescent label. Firstly, HL-1 cell was cultured and the AF research model was established by electrical stimulation. Microelectrode arrays (MEAs) system was used to monitor and stimulate HL-1 cell in real time. Then, AF related exosomes were extracted, and were characterized by transmission electron microscope (TEM), nanoparticle tracking analysis (NTA), and dynamic light scattering (DLS). Finally, AF related exosomes were detected by using MC-RLSCS and the characteristic diffusion time and concentration were obtained. Our proposed MC-RLSCS method has great potential in bioanalysis of diseases related exosomes.

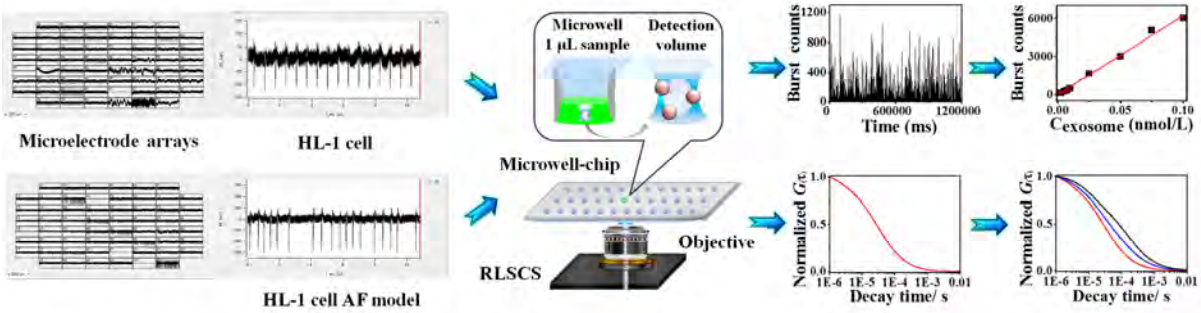


Figure. *The principle of determination of AF related exosomes by MC-RLSCS.*

Acknowledgments: This work funded by China Postdoctoral Science Foundation (Grant number 2018M640379) and the Youth Talent Sail Plan from Shanghai Committee of Science and Technology (Grant number 19YF1422000). This work was also financially supported by the NSFC (Grant Nos. 21327004, 21475087, 21675111 and 81670308).

References: [1] Su, D., et al. *Anal. Chem*. **2017**, 89, 9788-9796. [2] Ruan, L.G., et al. *Analyst* **2015**, 140, 1207-1214.

*Corresponding author: E-mail: jicunren@sjtu.edu.cn , houyuemei@sina.com

Application of Fluorescence Cross Correlation Spectroscopy (FCCS) and Fluorescence Single Particle Tracking (SPT) to elucidate the role of GM1a as a possible receptor/attachment factor for Dengue Virus infection in live cells

Sarala Tantirimudalige,[a] Thorsten Wohland,[*a, b]

[a] *Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117557*
[b] *Department of Chemistry, National University of Singapore, Block S8 Level 3, 3 Science Drive 3, Singapore 117543*

Dengue (DENV) is a positive sense RNA virus of the Flaviviridea family with four known serotypes (DENV1,2,3,4) which cause dengue fever. Viruses, which are obligate parasites, infiltrate and hijack cellular mechanisms in order to replicate their genome and are ultimately shed as infectious viral particles ready to infect another host. There are many reported entry pathways for DENV to enter cells, and exploring the receptors and attachment factors involved in infection can help understand the crucial step of virus entry into cells, so as to better find ways of reducing or halting virus invasion.

Here we investigate the possible involvement of sialic acid receptors on DENV early stage of infection. Sialic acid receptors are present in most mammalian cells, and have been previously reported as viral receptors (e.g. Influenza). We explore the function of GM1a as a possible virus receptor or attachment factor in DENV1 and DENV2 infection, by using Fluorescence microscopy, Fluorescence Cross Correlation Spectroscopy (FCCS) and Fluorescence Single Particle Tracking (SPT) as powerful single molecule techniques. We report that GM1a indeed colocalizes and interacts with DENV1 and DENV2 serotypes in live cell systems and we present this as a possible (previously unknown) receptor or attachment factor for DENV. We further explore the modes of motion of virus on the cell surface during the early stages of infection to decipher the relationship of the virus-receptor complex interactions. In the case of DENV2, once it attaches to the said receptor/attachment factor, it shows a slightly elevated diffusion coefficient as compared to virus on cell membranes lacking GM1a. In contrast, DENV1 lacks this trend and remains in an unchanged diffusion coefficient profile both when colocalized and non-colocalized with GM1a. We further expand our study to explore the relationship (if any) between GM1a and Heparan Sulfate Proteoglycans (HSPG) (a previously reported receptor/attachment factor) on live cells during infection. Our data indicates that GM1a could be an additional attachment factor or receptor for DENV1 and/or DENV2.

Acknowledgments: This work was supported by grants MOE2012-T3-1-008 and NRF-CRP19-2017-03, Singapore. We thank Lok Shee Mei and lab members (Duke-NUS Medical School, Singapore) for providing us with purified virus for this work.

[*]Corresponding author: E-mail: twohland@nus.edu.sg

Fluorescence Resonance Energy Transfer assay to study drug-induced conformation changes of NMDA receptor in live cells.

Azmain Taz,[a] Stephen F. Traynelis,[b] Dennis C. Liotta*[a]

[a] *Department of Chemistry, Emory University, Atlanta, GA, USA*
[b] *Department of Pharmacology and Chemical Biology, Emory University, Atlanta, GA, USA*

Dysregulation of ionotropic N-methyl-D-aspartate receptors (NMDAR) is implicated in numerous brain disorders driving the need for the development of subunit-specific modulators.[1] Electrophysiological recording in cells is a well-established functional assay for assessing drug activity but it provides limited information on the drug-induced conformation changes of NMDAR.[2] We aim to address this gap by developing a Fluorescence Resonance Energy Transfer (FRET) based assay to monitor conformation change of NMDAR in live cells. GluN1 and GluN2A constructs were mutated for site-specific fluorophore labeling of receptor via cysteine and co-transfected in HEK 293 cells.[3] The cells were labelled with maleimide forms of donor-acceptor dyes followed by fluorescence lifetime imaging. Donor fluorophore lifetime was measured in the presence (τ_{DA}) and absence (τ_D) of acceptor. The occurrence of FRET was indicated by $\tau_{DA}<\tau_D$. Three FRET pairs were assessed in this study, Cy3-Cy5, Alexa 555-Alexa 647 and Alexa 488-Alexa 555. Cy3-Cy5 caused cell toxicity, Alexa 555-Alexa 647 showed no significant difference in τ_{DA} and τ_D, and Alexa 488-Alexa 555 showed $\tau_{DA}<\tau_D$. Fluorescence lifetime of Alexa 555 being close to instrument response makes it unsuitable as donor fluorophore. Alexa 488 lifetime demonstrated the desired change in the presence on acceptor (τ_D ~3.2 ns, τ_{DA} ~1.1 ns). Thus, Alexa 488-Alexa 555 will be used in future experiments to study the conformational mechanism of action of NMDAR regulatory drugs.

References: [1] Traynelis, S. F., et al., *Pharmacol. Rev.* **2010,** *62*, 405.
[2] Lu, P. H., et al. *J. Am. Chem. Soc.* **2016**, *138*, 8789. [3] Jayaraman, V., et al. *J. Biol. Chem.* **2013**, *288*, 22555.

*Corresponding author: E-mail: dliottta@emory.edu

New NIR Emitting APC Tandem for Biomedical Applications

Dmitriy Kobzev,[a] Olga Semenova,[a] Anatoliy Tatarets[a] and Ewald A. Terpetschnig*[b]

[a] *Department of Luminescent Materials and Dyes, State Scientific Institution "Institute for Single Crystals" of the NAS of Ukraine, 60 Nauky Ave., Kharkiv, 61072, Ukraine*
[b] *SETA BioMedicals, LLC, 2014 Silver Ct East, Urbana, IL 61801, USA*

Fluorescent dyes having the same excitation and different emission wavelengths are important tools for use in simultaneous multiparametric analysis.

For multi-parameter analysis either a combination of dyes with different Stokes' shifts or FRET pairs of phycobiliproteins (phycoerythrin, R-PE, and allophycocyanin, APC) with dyes are utilized. Phycobiliproteins are routinely used at 488 nm (R-PE) and 633 nm (APC) excitation wavelengths in flow cytometers.

Previously we have produced several bright and photostable tandems of R-PE and APC (Seta-PE-670, Seta-PE-775, Seta-APC-705, Seta-APC-780) using our proprietary long-wavelength dyes having emission in the far red and NIR region. In order to further extend the range deeper into the NIR we now developed a new tandem, Seta-APC-820, and investigated its spectral properties and photostability.

The Seta-APC-820 tandem is highly fluorescent when excited at 633 nm having its maximum emission at 823 nm. The wavelength shift of 43 nm against Seta-APC-780, should allow its simultaneous use with other fluorescent reporters in flow cytometry and imaging applications.

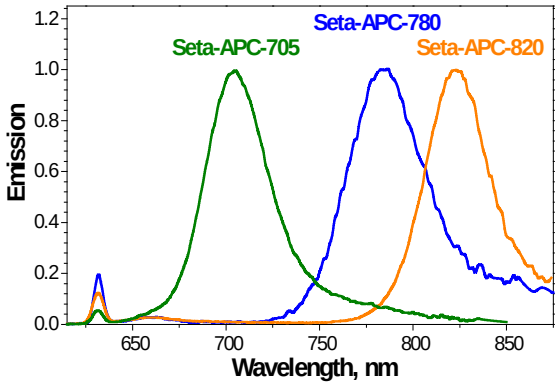


Figure. *Emission spectra of the APC tandems*

Acknowledgments: This work was supported by the Science and Technology Center Ukraine (STCU project P548a).

*Corresponding author: E-mail: ewaldte@setabiomedicals.com

Photophysical Characterization of Perovskite Quantum Dots

Stuart Thomson*

Edinburgh Instruments, Livingston, UK

Perovskite semiconductors have attracted immense attention due to their success as absorber layers in high-efficiency solar cells. The optoelectronic properties that make perovskites excel as absorber layers also make them excellent light emitters, and attention is now shifting towards their broader optoelectronic applications. Perovskite quantum dots, in particular, possess superb light-emitting properties; with highly tunable bandgaps, narrow emission profiles and high photoluminescence quantum yields and are therefore promising emitters for next-generation display technologies.

In order to move these materials towards technological applications, the fundamental material properties need to be better understood and quantum dot designs and synthesis routes optimized. Two of the most powerful techniques to characterize perovskite quantum dots and optimize their optoelectronic properties are photoluminescence and absorption spectroscopy. We demonstrate how a complete photophysical characterization; comprising of absorption spectra, photoluminescence spectra, photoluminescence lifetime, and quantum yield of perovskite quantum dots can be carried out using a compact spectrofluorometer.

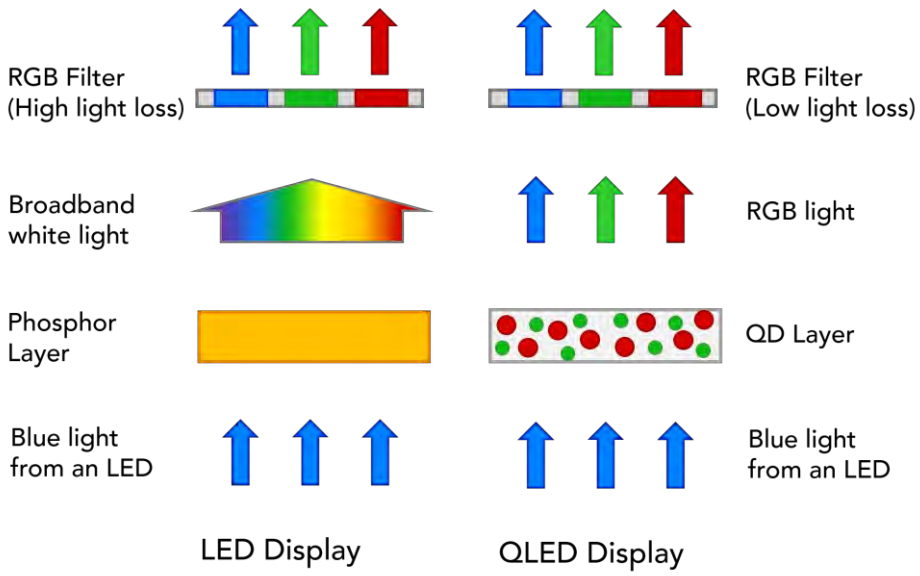


Figure. *Operating principle of LED & QLED displays.*

*Corresponding author: E-mail: stuart.thomson@edinst.com

Self-assembled plasmonic nanoantennas for sensitive diagnostics with a smartphone

Kateryna Trofymchuk, [a, b] Viktorija Glembockyte, [b] Florian Steiner, [b] Lennart Grabenhorst, [b] Birka Lalkens, [a] Aydogan Ozcan, [c] Philip Tinnefeld*[b]

[a] *Institute of Physical and Theoretical Chemistry, TU Braunschweig, BRICS - Braunschweig Institute for Systems Biology, Rebenring 56, 38106 Braunschweig Germany*
[b] *Department Chemie and Center for Nanosciences, Ludwig-Maximilians-Universität München, Butenandtstr. 5 – 13, 81377 München, Germany*
[c] *Electrical & Computer Engineering Department, Bioengineering Department, California NanoSystems Institute (CNSI), and Department of Surgery, University of California, Los Angeles, Los Angeles, California 90095, United States*

Today's health care system could be significantly improved with point-of-care diagnostics. In recent years, a lot of efforts has been made to integrate POC diagnostics on smartphones and to use their cameras as detectors.[1] Recently our group has demonstrated that monochrome smartphone cameras are capable to successfully detect emission of DNA-origami beads containing as few as 10 molecules of ATTO542 (Figure a).[2] In order to detect a single molecule, its emission should be enhanced, for example, by exploiting plasmonic nanostructures that act as optical nanoantennas. Using DNA origami as platform to precisely arrange a fluorescent dye in the plasmonic hotspot of metallic nanoparticles, we obtained enhancement of the fluorescent signal of ATTO647N reaching 500-fold (Figure b).[3] Recently we demonstrated successful incorporation of a molecular beacon in the hotspot of a silver nanoantenna for detection of Zika virus related nucleic acids.[4] We will discuss how these nanoantennas can be integrated into a smartphone-based microscope, paving the way for the development of inexpensive point-of-care diagnostic platforms.

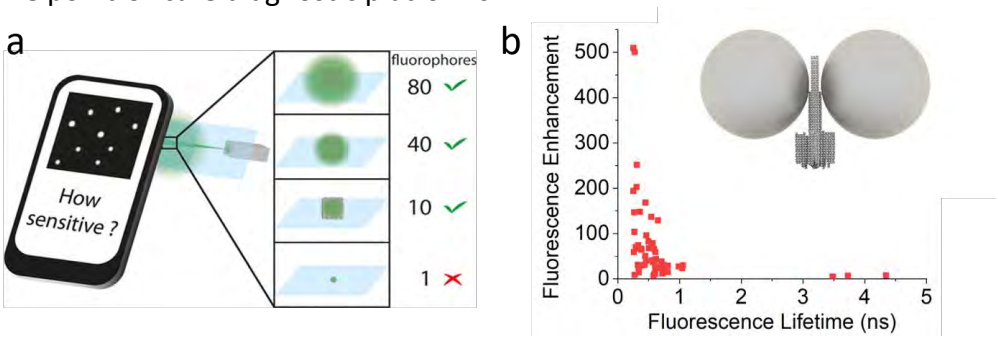


Figure: a) *Representation of the sensitivity of modern smartphone cameras tested with ATTO542 fluorescent dye DNA origami beads.* ***b)*** *Fluorescence enhancement of ATTO647N achieved in the hotspot of a DNA origami-based nanoantenna employing 80 nm silver nanoparticles.*

Acknowledgments: This work was supported by the BMBF (Grants POCEMON, 13N14336, and SIBOF, 03VP03891), and the European Union's Horizon 2020 research and innovation programme under grant agreement No. 737089 (Chipscope) and a Humboldt Research Fellowship.

References: [1] Roda, A. et al., *Trends Anal. Chem.* **2016**, *79*, 317. [2] C. Vietz et al., *ACS Omega* **2019**, 4, 1, 637. [3] Vietz et al., *ACS Nano* **2017**, 11, 4969. [4] Ochmann et al., *Analytical Chemistry* **2017**, 89, 13000.

*Corresponding author: E-mail: Philip.Tinnefeld@cup.uni-muenchen.de

Spectroscopic Properties of Fluorescent 8-DEA-tC DNA-RNA Heteroduplexes

Marc B Turner,[a] Jesus Ceja,[a] Byron W Purse*[a]

[a] *Department of Chemistry & Biochemistry, San Diego State University, 5500 Campanile Dr, USA*

Fluorescence in situ hybridization (FISH) has been an essential method for detecting specific sequences in biological samples, including specific mRNA, genomic DNA, or cloning vectors. This method can however yield false positives by hybridizing to similar sequences that may differ by 1-2 bases, yet exhibit similar thermostability as if perfectly complemented, highlighting the major limitation of FISH in that fluorescence is the direct result of preferential binding, not necessarily sequence recognition. Our lab's nucleoside analogue 8-diethylamino tricyclic cytidine (8-DEA-tC) can potentially discriminate sequences more accurately than current hybridization methods. 8-DEA-tC has low intrinsic fluorescence (Φ_{em}=0.010), however the emission can increase up to 0.12 when 8-DEA-tC pairs with guanosine in double-stranded DNA (dsDNA).[1] The fluorescence turn-on does not occur when 8-DEA-tC is mismatched with A, which may enable the use of 8-DEA-tC in higher fidelity probes.

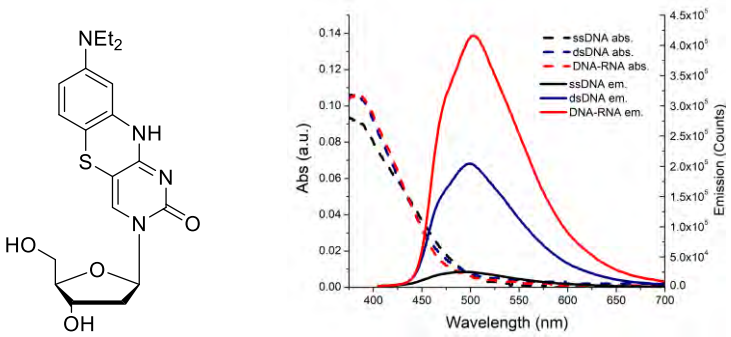


Figure 1. *Structure 8-DEA-tC nucleoside with emission spectra of 8-DEA-tC oligonucleotides.*

In this current work, we examine the fluorescent properties of 8-DEA-tC DNA-RNA heteroduplexes. The fluorescence turn-on is greater with RNA as compared with DNA (Fig 1) when using otherwise identical sequences. CD spectra indicate that the 8-DEA-tC DNA-RNA duplexes adopt the A-form conformation, which has a different geometry of stacking and changes the electronic interactions with neighboring bases. The major and minor grooves also differ geometrically, resulting in different solvation of the exposed edges of the bases. Here we present a detailed investigation of the enhanced fluorescence of 8-DEA-tC in these DNA-RNA heteroduplexes, including solvent isotope effects and time-resolved fluorescence studies.

Acknowledgments: This work was supported by the National Science Foundation (CHE-1709796 and CHE-1800529).

References: [1] Teppang, K. L.; et al. *Chem. - A Eur. J.* **2019**, *25*, 1249–1259.

*Corresponding author: E-mail: bpurse@sdsu.edu

Site-selective visualization of DNA methylation architecture in fixed cells

Krystian Ubych,[a] Paco Fernandez-Trillo,[a] Clare Davies,[b] Robert K. Neely*[a]

[a] *School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*
[b] *Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK*

DNA methylation plays a crucial role in epigenetic gene regulation and protein function modulation. Abnormal levels of CG methylation are found in cancer cells. For example, hypermethylation is found in regions of the genome rich in cytosine and guanine, so-called CpG islands; yet, across the entire genome hypomethylation is the dominant trait.[1] Even though the methylated cytosine residues can be detected with single nucleotide resolution using sequencing methods, little is known about the impact of DNA methylation in chromatin organization in nucleus.

Herein, we report a novel approach for the 3D visualization of DNA methylation architecture in mammalian cells. We employ methyltransferase-directed labelling of the epigenome using a synthetic cofactor analogue for these enzymes bearing a clickable moiety for fluorescent labelling of DNA (Fig. 1).[2] We combine this with expansion microscopy and inverted selective plane illumination microscopy (iSPIM) to reveal the organized structures of unmethylated regions of chromatin in healthy and cancerous cells.

The application of methyltransferase enzymes enables the site-selective labelling of the unmethylated DNA. Using the state-of-the-art microscopy techniques we were able to take images of its three dimensional architecture in cells.

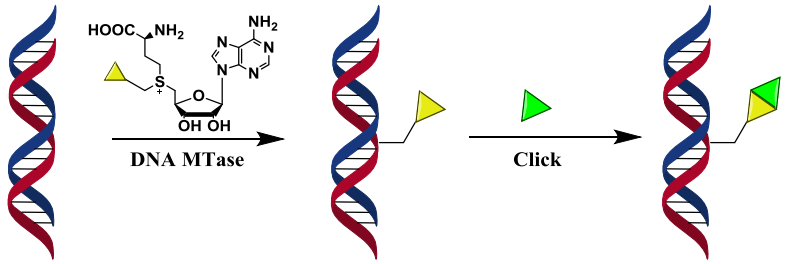


Figure 1. *Fluorescent labelling of DNA using methyltransferase enzymes and synthetic cofactors*

Acknowledgments: This work was supported by the EPSRC (EP/N020901/1).

References: [1] Ehrlich M., *Oncogene* **2002**, *21*, 5400-5413. [2] Deen J., et al. *Nucleic Acids Research*, **2018**, 46, e64

*Corresponding author: E-mail: R.K.Neely@bham.ac.uk

Expanding the toolbox for the subcellular mapping of multi-omic biomarkers in tissues

Marisa Vanheusden,[a] Aline Acke,[a] Raffaele Vitale,[a] Susana Rocha,[a] Kris Janssen,[a] Frederik De Smet,[b] Johan Hofkens*[a]

[a] *Department of Chemistry, KU Leuven, Celestijnenlaan 200F, Belgium*
[b] *Department of Imaging and Pathology, KU Leuven, O&N IV Herestraat 49, Belgium*

Cells are the fundamental functional units in all forms of life and subcellular processes are at the origin of most, if not all, disease processes. Proper cellular function requires the carefully coordinated action of numerous biomolecular actors, which are often much smaller than 200 nm. As a result, direct visualization to study these phenomena, e.g. through microscopy imaging is challenging. While conventional super resolution fluorescence microscopy approaches often uses isolated cells to map different biomolecules, expansion microscopy (ExM) addresses this issue in a different and elegant way (Figure 1).[1] By embedding cells or whole tissues in a suitable super-absorbent polymer, their size can be expanded, laying bare their subcellular structures. Due to the ability to image expanded samples on conventional, diffraction limited microscopes and the intrinsic optical clearing step, ExM is a very suitable approach to study cells in their original, multicellular environment.

Due to the versatility of ExM, the field is now rapidly evolving in a direction where it is combined with state-of-the-art methods for single cell genome, transcriptome and proteome analysis.[2,3] As cell function is determined by different DNA, RNA and protein markers, the next step will be to study a combination of the most important hallmarks in their spatial context via integrative techniques rather than full coverage techniques which measure only one type of molecule. In this effort, the Hofkens Lab is working towards an approach that allows for the simultaneous readout of different cellular biomarkers. We will expand the already existing toolbox with new, ExM-compatible labeling strategies to visualize the subcellular location of biomolecules, ultimately in the context of more complex tissues.

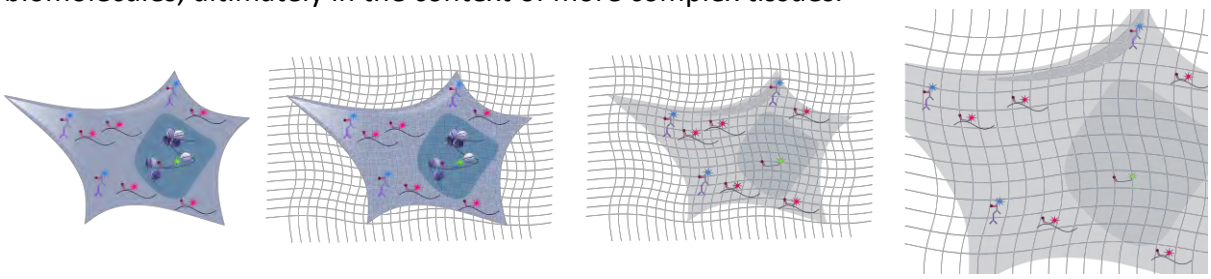


Figure 1: *Overview of the expansion process for different targeted biomolecules.*

Acknowledgments: This work was supported by a grant from the Fonds Wetenschappelijk Onderzoek (FWO).

References: [1] Chen, F., et al. *Science.* **2015***, 347*, 6221. [2] Chen, F., et al. *Nat. Methods* **2016**, *13*, 8. [3] Wang, D., et al. *Sci Rep.* **2018**, *8*, 4847.

*Corresponding author: E-mail: johan.hofkens@kuleuven.be

Anti-Stokes Fluorescence Microscopy.

Tom Vosch,*[a] Stefan Krause, [a] Cecilia Cerretani

[a] *Nanoscience Center & Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark.*

In this presentation I will discuss some newly developed fluorescence imaging techniques, based on the intriguing photophysical properties of DNA-stabilized silver nanoclusters. Using optically activated delayed fluorescence (OADF) and upconversion fluorescence (UCF), which both are on the high energy (Anti-Stokes) side of the read-out laser, one can remove unwanted autofluorescence. The presented methods could potentially also be applied on other emitters with similar photophysical properties, paving the way for background free fluorescence imaging.

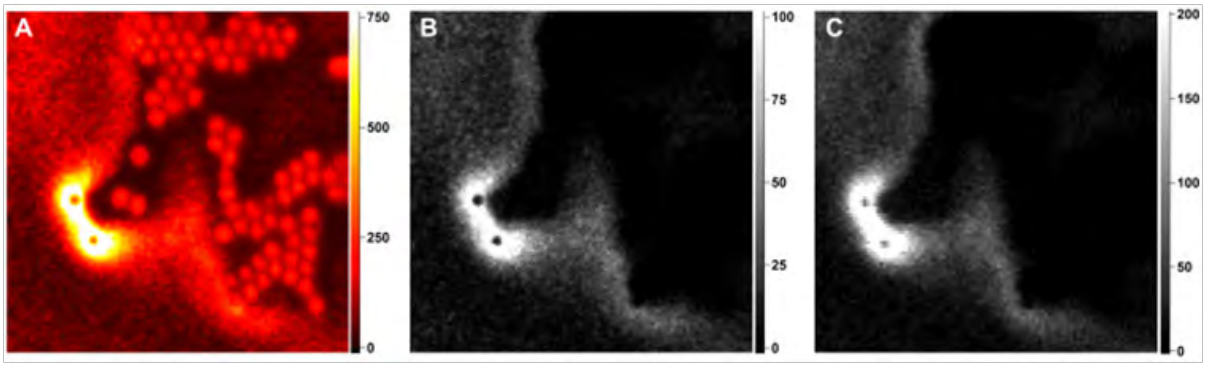


Figure 1. *Anti-Stokes fluorescence microscopy, using optically actived delayed fluorescence (OADF) and upconversion fluorescence (UCF). A) Normal fluorescence image, B) OADF image, C) UCF image. Image taken from Reference 1.*

Acknowledgments: This work was supported by grants from the Danish Ministry of Science, Technology and Innovation, the Villum Foundation and the Carlsberg Foundation.

References: [1] Krause, S.; Carro-Temboury, M. R.; Cerretani, C.; Vosch, T., Anti-Stokes fluorescence microscopy using direct and indirect dark state formation. *Chem. Commun.* **2018**, 54, 4569-4572. [2] Krause, S.; Carro-Temboury, M. R.; Cerretani, C.; Vosch, T., Probing heterogeneity of NIR induced secondary fluorescence from DNA-stabilized silver nanoclusters at the single molecule level. *Phys. Chem. Chem. Phys.* **2018**, 20 (24), 16316-16319. [3] Krause, S.; Cerretani, C.; Vosch, T., Disentangling optically activated delayed fluorescence and upconversion fluorescence in DNA stabilized silver nanoclusters. *Chemical Science* **2019**, 10 (20), 5326-5331.

*Corresponding author: E-mail: tom@chem.ku.dk

Anomalous diffusive dynamics of individual H-NS proteins in live bacteria and their responses to silver ions

Asmaa A. Sadoon,[a,b] Prabhat Khadka,[a] Ryan Manso,[c] Jack Freeland,[a] Jingyi Chen,[b,c] and Yong Wang[*,a,b,d]

[a] *Department of Physics, University of Arkansas, Fayetteville, AR, USA*
[b] *Microelectronics-Photonics Program, University of Arkansas, Fayetteville, AR, USA*
[c] *Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA*
[d] *Cell and Molecular Biology Program, University of Arkansas, Fayetteville, AR, USA*

We report our measurement on the diffusive dynamics of histone-like nucleoid structuring (H-NS) proteins in live *E. coli* bacteria using single-particle tracking photoactivated localization microscopy (sptPALM), as well as how the diffusive dynamics of H-NS proteins is affected when subjecting the bacteria to silver ions (Ag^+). We observed that the dynamics of H-NS proteins differed significantly from other molecules reported previously. A power-law distribution was observed for the diffusion coefficients of individual H-NS proteins. In addition, we observed a new distribution of displacements, which does not follow the Gaussian, Cauchy, or Laplace distributions, but the Pearson Type VII distribution. Furthermore, we experimentally measured the time/frequency dependence of the complex modulus of the bacterial cytoplasm, which deviates from the viscoelasticity of homogeneous protein solutions and shows a glass-liquid transition. We also observed that the dynamics of H-NS protein is cell-length/cell-age dependent. After treating the bacteria with Ag^+ ions, faster dynamics of H-NS proteins was observed. To understand the mechanism of the observed faster dynamics, we performed gel shift assay with purified H-NS proteins *in vitro* and found that Ag^+ ions weakened the binding between H-NS proteins. Furthermore, we examined the interaction between DNA and Ag^+ ions using our recently developed amplifiers based on bent DNA molecules and found that Ag^+ ions interfered with the hybridization of double-stranded DNA, providing a plausible mechanism for the faster dynamics of H-NS proteins in live bacteria when subjected to Ag^+ ions.

Acknowledgments: This work was supported by the University of Arkansas, the Arkansas Biosciences Institute (Grant No. ABI-0189, No. ABI-0226, No. ABI-0277), and the National Science Foundation (Grant No. 1826642).

[*]Corresponding author: Yong Wang; E-mail: yongwang@uark.edu

Transient state (TRAST) imaging of local cellular redox conditions and intermittent protein-lipid interactions in cellular membranes

Johan Tornmalm,[a] Joachim Piguet,[a] Elin Sandberg,[a] Jerker Widengren*[a]

[a]*Experimental Biomolecular Physics, Department of Applied Physics, Royal Institute of Technology (KTH), Albanova Univ. Center 106 91 Stockholm, Sweden*

In transient state imaging (TRAST), blinking kinetics of fluorescent molecules in a sample are determined from how the time-averaged fluorescence intensity varies upon differently modulated laser excitation [1,2]. TRAST combines sensitive detection of fluorescence from fluorophore markers, with monitoring of environment-sensitive dark state transitions within the same fluorophores, without the need of time-resolved or single-molecule detection conditions. TRAST imaging exploits the fact that transient dark states, such as triplet, photo-isomerized and photo-oxidized/reduced states, are long-lived (µs-ms), and therefore sensitive to the local environment. Via the kinetics of these states additional information can thus be provided, beyond that from regular fluorescence parameters. Here, we report on two categories of applications, where TRAST can offer such additional information.

First, TRAST was used to monitor transient, low-frequency protein-lipid interactions in live cell membranes [3]. Such interactions can modulate central cellular functions, are often transient in character, but typically occur at too low frequencies to be readily observable by fluorescence readouts. With TRAST imaging, monitoring the quenching of long-lived fluorophore triplet states by spin labels, low-frequency interactions in the membranes, too infrequent to occur within the fluorescence lifetime of fluorophores, can be readily followed. We applied this strategy to image the collisional triplet state quenching of NBD-lipid derivatives by spin-labelled G-Protein Coupled Receptors (GPCRs) in live cell plasma membranes. We could then resolve transient interactions between the GPCRs and different lipids, and how these interactions changed upon GPCR activation by a ligand agonist.

Second, we studied the autofluorescent coenzyme nicotinamide adenine dinucleotide (NADH) and its phosphorylated form (NADPH), major determinants of cellular redox balance [4]. While NAD(P)H fluorescence intensity and lifetime are extensively used as label-free readouts in cellular metabolic imaging studies, fluorescence blinking of NAD(P)H represents additional information, specifically sensitive to redox conditions and oxygenation, of particular relevance in cellular metabolic studies. We show that such dark state transitions in NAD(P)H can be quantified via TRAST, with a standard laser-scanning confocal microscope and two-photon excitation, in parallel with regular fluorescence lifetime imaging (FLIM) recording. Imaging of cells treated with a mitochondrial un-coupler or cyanide shows that the TRAST images can better distinguish the cells from each other and provides orthogonal information to FLIM.

Together, these studies suggest that imaging of highly environment-sensitive dark states can extend the information from traditional fluorescence readouts, and can be done in a widely applicable manner.

Acknowledgements: This work was supported by grants from the Swedish Research Council (VR-NT 2017-04057), and the Swedish Innovation Agency (Vinnova 2014-05038).

References: [1] Sandén T et al, *Anal. Chem.* 79(9), 3330-3341, 2007, [2] Rigler&Widengren *Eur. Biophys. J.* 47(4), 479-492, 2018, [3] Tornmalm J et al., *submitted*. [4] Tornmalm J et al, *under revision*

*Corresponding author: E-mail: jwideng@kth.se

The environment-sensitive diketopyrrolopyrrole-based nucleoside fluorescent probe

Jianquan Hong,[a] Yupeng Yang,[a] Yu Zhou,[a] Xiaoxiao Zhang,[a] Wei Zhang,[b] Shuai Huang,[a] Ge Fang,[b] Changge Zheng*[a]

[a] *Key Laboratory of Synthetic and Biological Colloids, Ministry of Education, School of Chemical and Material Engineering, Jiangnan University, Wuxi, Jiangsu 214122, P. R. China*
[b] *School of Chemical Engineering, Xinjiang Agricultural University, Urumqi 830052, Xinjiang Uygur Autonomous Region, P. R. China*

Fluorescence detection and bioimaging has still been a hot topic in recent decades, as a powerful method of disease diagnosing in medicine.[1,2] Fluorescent nucleoside analogues as probes have extensive application prospects with their good water solubility, biological compatibility and high sensitivity in different solution environments.[3]
Introducing DPP derivatives into cytidine by rigid and flexible triple bond, we design and synthesize two kinds of fluorescent nucleoside analogues. The photophysical properties of two modified fluorescent nucleoside analogues was improved obviously with a remarkable sensitivity to polarity and pH changes.[4]

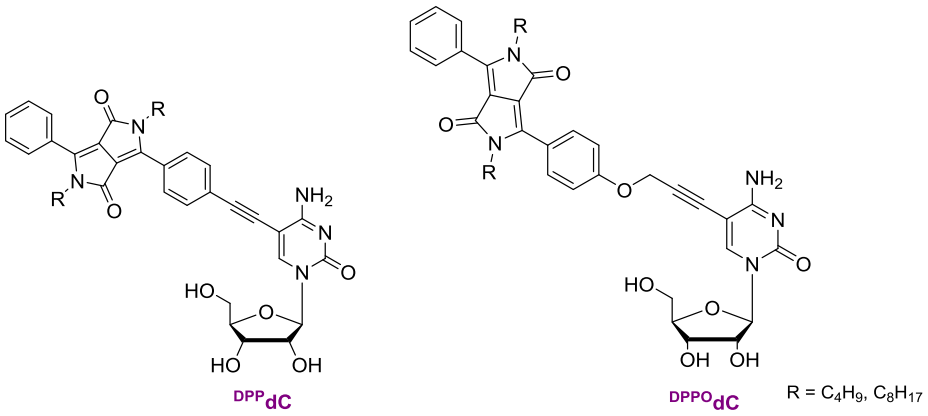


Figure. *Fluorescent DPP-based nucleoside analogs.*

A preliminary photophysical investigation has shown that two fluorescent nucleosides can also act as an environment-sensitive fluorescence-lifetime probe for DNA interactions and applications in live-cell microscopy with high fluorescence quantum yield, high brightness and long fluorescence lifetime.[5]

Acknowledgments: This work was supported by grants from the National Natural Science Foundation of China (Nos. 21562041)

References: [1] Bi, S., et al. *Chem. Soc. Rev,* **2017**, 46, 4281. [2] Zhang, K., et al. *Chem. Rev.* 2018, 118, 1770. [3] Sinkeldam, R. W., et al. *Chem. Rev.* **2010**, 110, 2579. [4] Kaur, M., et al. *Chem. Soc. Rev*, **2015**, 44, 58. [5] Dziuba, D., et al. *Angew. Chem. Int. Ed.* **2016**, 55, 174.

*Corresponding author: E-mail: cgzheng@jiangnan.edu.cn