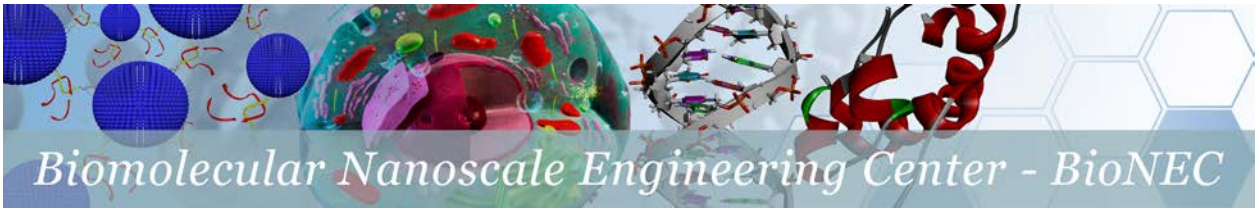
2018

4th BioNEC Mini-Symposium on Bio-molecular Synthesis and Nanotechnology

PROGRAM AND ABSTRACTS

Symposium Organizers: Knud J. Jensen and Jesper Wengel
BIOMOLECULAR NANOSCALE ENGINEERING CENTER,
UNIVERSITY OF COPENHAGEN AND UNIVERSITY OF SOUTHERN DENMARK
November 6, 2018



Welcome

It is a pleasure to welcome all participants of the Biomolecular Synthesis and Nanotechnology Mini-Symposium.

The synposium is supported by BioNEC (Biomolecular Nanoscale Engineering Center), a joint research center of excellence – funded by THE VILLUM FOUNDATION – involving the three Danish universities – University of Southern Denmark, University of Copenhagen and University of Aarhus.

We wish all participants a scientifically stimulating meeting.

The Organizing Committee

BIONEC

Biomolecular Nanoscale Engineering Center

Funded by THE VILLUM FOUNDATION

BioNEC Mini-Symposium on Biomolecular Synthesis and Nanotechnology

Tuesday november 6, 2018, DGI-byen, Tietgensgade 65, 1704 Copenhagen V

Program

12:45 – 13:00	Registration outside the meeting room
13:00 – 13:05	Welcome and Opening remarks by **Knud J. Jensen**
	Chairman: **Knud J. Jensen**
13:05 – 13:50	**Silvia Marchesan**, Department of Chemical & Pharmaceutical Sciences, University of Trieste, Italy: *Peptide nanostructure wonderland beyond the mirror*
13:50 – 14:35	**Poul M. Bendix**, Niels Bohr Institute, University of Copenhagen, Denmark: *Optical modulation of cell membranes for studying lateral organization and functional properties of membrane proteins*
14:35 – 15:00	- Coffee/tea break -
	Chairman: **Stefan Vogel**
15:00 – 15:45	**William M. Shih**, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, and the Department of Cancer Biology at the Dana-Farber Cancer Institute, Boston, USA: *DNA-origami barrels*
15:45 – 16:30	**Stefan Howorka**, Department of Chemistry, Institute of Structural Molecular Biology, University College London, UK: *Using DNA to cross membrane barriers*
16:30 – 16:40	Concluding remarks by **Jesper Wengel**

October 2018

Biographical Sketch: Professor Silvia Marchesan

Silvia Marchesan graduated in 2004 in chemistry at the University of Trieste, with a thesis in Prof. M. Prato's group on nanocarbon functionalization. She completed her PhD in 2008 at the University of Edinburgh (UK) on the synthesis of GDP-mannose derivatives for orthogonal modification of glycoproteins under the supervision of Dr. D. Macmillan. Her postdoc at the University of Helsinki (Finland) was on protein interactions mediating cell adhesion in the group of Prof. C. Gahmberg until 2010. She moved to Australia as a joint postdoc fellow between Monash University and CSIRO to work on biomaterials until late 2012. She returned to the University of Trieste in 2013, where she was appointed as assistant professor in 2015. She was awarded two national grants that allowed her to set-up her own lab to work on 1) peptide self-assembly for innovative therapeutic solutions and on 2) carbon nanostructure functionalisation towards composites for cancer diagnostics. In 2018 she received tenure as Associate Professor. She has published around 50 research papers, has an H-factor of 20 and has received 2 prizes. Her research explores design principles to assemble molecules and nanostructures into functional nanomaterials.

Peptide Nanostructure Wonderland beyond the Mirror

Silvia Marchesan*

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Nature assembles biomolecules into fascinating hierarchical architectures, ranging in size from the nano- to the macro-scale, and that can be recapitulated in the beauty of living systems. From a chemist's point of view, the molecular building blocks of life are mainly homochiral (*e.g.*, D-carbohydrates and L-proteins). Nature's well-known preference for homochirality has stimulated our research, as we challenge it with heterochiral systems.

Our scientific journey in this field starts from the design of simple tripeptides to define the rules of self-assembly for chemical systems of biological relevance. We introduce one or two D-amino acids in D,L-tripeptides, and analyse small libraries with different stereochemistry or sequence of amino acids.[1] We assess the role of chirality on self-assembly from single molecule, through nano-, micro-, and even to the macro-scale.

As Alice steps through the mirror and finds a wonderland of interesting characters,[2] we take inspiration from D-amino acids in D,L-peptides to identify a wonderland of nanostructures. This presentation illustrates our most recent findings on the conformation of molecules and their evolution *in continuo* from the nanoscale to hydrogels.[3] We have identified diverse morphological features, including 2 nm-wide water channels. We envisage applications spanning from biomaterials[4] to supramolecular catalysis,[5] ideally whereby functions can be switched on/off *on demand*.

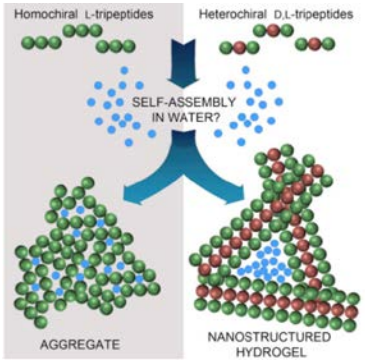

Figure: The pathway of homo- and hetero-chiral tripeptides diverges for assembly in water.[3]

1. S. Marchesan, *et al.*: *Chem. Commun.* 2012, *48*, 2195; *Nanoscale* 2012, *4*, 6752; *J. Mater. Chem. B* 2015, *3*, 8123.
2. L. Carroll. Alice's Adventures in Wonderland, New York, MacMillan (1865).
3. A. M. Garcia, *et al. Chem* 2018, *4*, 1862.
4. S. Marchesan, *et al. Biomaterials* 2013, *34*, 3678. M. Melchionna, *et al. Curr. Top. Med. Chem.* 2016, *16*, 2009. A. V. Vargiu, *et al. Chem. Commun.* 2016, *52*, 5912.
5. A. M. Garcia, *et al. Chem. Commun.* 2017, *53*, 8110.

October 2018

Biographical Sketch: Professor Poul M. Bendix

Poul M. Bendix graduated as a Ph.D. from the Niels Bohr Institute (NBI) in 2007 following a visit as a research scholar at Harvard University. During his postdoc period from 2007 to 2011 he worked at the Nanoscience Center at the University of Copenhagen and at Stanford University. In 2013 he became Associate Professor at the NBI where he received a permanent position in 2018. His main research includes biophysics and dynamics of cells surface structures and associated proteins in close collaboration with biologists and biochemists. Also, he has worked extensively with optical modulation of membranes and cells by combining optical trapping and thermoplasmonics. He received the Young Investigator Award in 2011 from the Villum Foundation and in 2015 he received the Sapere Aude research leader grant from the Danish Council for Independent Research.

Optical modulation of cell membranes for studying lateral organization and functional properties of membrane proteins

Poul M. Bendix

Niels Bohr Institute, University of Copenhagen

Investigations of cell surface dynamics is experimentally challenging due to the dynamic nature of nanoscopic membrane events**.** The cell surface environment exhibits constant shape changes driven by molecular complexes in the membrane and by supporting cytoskeletal structures. Molecular shapes and cooperativity are important functional properties of cells to support formation of essential nanostructures on the cell surface. Membrane proteins with bent shapes can sense curvature cues and undergo crystallization at high density which could play a regulatory role in a number of cell functions and cellular disorders. Here, I will present quantitative studies of protein-membrane interactions using optical shape modulation of the plasma membrane and also optically induced perturbation of the cell membrane integrity. We form high membrane curvatures in the membrane of living cells and cell-derived plasma membrane vesicles to test the affinity of both transmembrane and peripheral membrane proteins for high membrane curvatures. This allows us to test the preference of any cell expressed protein for negative and positive membrane curvature in the plasma membrane. Using thermoplasmonics, in parallel with imaging, we further investigate the cellular response to nanoscopic disruption of the membrane and test how curvature inducing proteins shape and repair the membrane of living cells. Additionally, plasmonic heating from optically trapped nanoheaters allows us fuse any membrane of choice which opens up an avenue of new possible studies of membrane-protein interactions and a few examples will be presented. These novel approaches are valuable for revealing mechanisms governing cell surface protein dynamics and mechanical properties of the surface of living cells.

October 2018

Biographical Sketch: Professor William M. Shih

William M. Shih is a Professor in the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School and the Department of Cancer Biology at the Dana-Farber Cancer Institute and a Core Faculty member at the Wyss Institute for Biologically Inspired Engineering at Harvard. William studied Biochemical Sciences at Harvard for his A.B. (1990–1994) and Biochemistry with Jim Spudich at Stanford for his Ph.D. (1994–2000) He did a postdoctoral fellowship with Jerry Joyce at The Scripps Research Institute (2001–2004) and has since been back at Harvard as a faculty member. William was a 2008 NIH Director's New Innovator Awardee, a 2013 Blavatnik National Award Finalist in the Physical Sciences, and the 2017 Foresight Prize Awardee in Experimental Nanotechnology. William is overseeing an effort to apply Synthetic Biology approaches to the development of self-assembling DNA nanostructures and devices for use in biomedical applications. In addition to carrying genetic information, DNA is increasingly being explored for its use as a building material. This new process is called DNA origami because a long strand of DNA can be programmed to fold in on itself to create specific shapes, much as a single sheet of paper is folded to create a variety of designs in the traditional Japanese art. Using long biologically produced DNA strands to construct particles with precisely specified shapes, William is able to approximate a level of complexity that rivals that of the molecular machinery found in cells. To achieve structures of even greater complexity, his laboratory is pioneering methods for hierarchical assembly of these particles into three-dimensional networks with site-specific control over chemical functionalization and mechanical actuation. This work could lead to breakthroughs in manufacturing and medicine. For example, these incredibly tiny forms could be used as cogs in a machine for molecular manufacturing, optical reporters for bioimaging, and carriers for delivery of cancer drugs deep inside the body.

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DNA-origami barrels

William M. Shih[1-3]

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[2]*Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA*
[3]*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, USA*

DNA origami, in which a long scaffold strand is assembled with a large number of short staple strands into parallel arrays of double helices, has proven a powerful method for custom nanofabrication. Although diverse shapes in 2D are possible, the single-layer rectangle has proven the most popular, as it features fast and robust folding and modular design of staple strands for simple abstraction to a regular pixel surface. Here we introduce a barrel architecture, built as stacked rings of double helices, that retains these appealing features, while extending construction into 3D. We demonstrate hierarchical assembly of a 100 megadalton barrel that is ~90 nm in diameter and ~270 nm in height, and that provides a rhombic-lattice canvas of a thousand pixels each, with a pitch of 9 nm, on its inner and outer surfaces. Complex patterns rendered on these surfaces were resolved using up to twelve rounds of exchange PAINT super-resolution fluorescence microscopy. We envision these structures as versatile nanoscale pegboards for applications requiring complex 3D arrangements of matter.

October 2018

Biographical Sketch: Professor Stefan Howorka

Stefan Howorka, born 1969 in Austria, studied biochemistry at the University of Vienna, Austria from 1988 to 1995. He obtained his PhD in 1999 on research about bacterial exoproteins conducted in the group of Prof Lubitz, Vienna and Prof Bayley, Texas A&M Univeristy. He received post doctoral training at Texas A&M Univeristy on protein engineering of membrane pores for biosensing. After returning to Austria in 2001 to work at a biotech start-up on ultransensitve biosensing and array technology, he re-joined academia in 2005 as assistant professor at University College London, Department of Chemistry. He studied bacterial exoprotein, protein pores, and biosesing for several years leading to the licensing of pore technology to Oxford Nanopore Technology for sequencing kits. He also initiated research in DNA nanotechnology to form synthetic membrane pores composed of DNA. In 2016 he was appointed full professor. He has published around 89 research papers, and has a H-factor of 39. The purpose of his research is to explore fundamental aspects of how DNA interacts with lipid bilayers and use the insight to create programmable and functional DNA nanoarchitectures for biosensing, cell research, and synthetic biology. He also has a research stake in developing nucleic acid probes and chromophores to enable new biological discoveries.

Using DNA to Cross Membrane Barriers

Stefan Howorka

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Semifluid membranes enclose biological cells and drug delivery vehicles. Crossing the barrier enables essential transport of molecular cargo. My talk presents synthetic transport channels made from DNA. Nucleic acids are easier to engineer than proteins of biological channels[(1)]. The artificial DNA channels are composed of interlinked duplexes. Attached lipid anchors hold the negatively charged structures in the membrane[(2,3,4)]. The DNA channels open and close in response to physical voltage stimuli, like natural templates[(3,4,5)]. One DNA version mimics ligand-gated channels[(3)] to help release drugs or build cell-like networks. The artificial pores can also be programmed into cytotoxic agents to kill cancer cells[(6)], or to create porous bionanoreactors[(7)]. Other rationally designed DNA nanostructures extend the functional range and can control, for example, bilayer shape[(8)]. The outlook concludes with an outlook of how DNA nanotechnology can help replicate biological functions to open up new applications in nanobiotechnology and synthetic biology.

References:

(1) *Nat. Nanotechnol. 2017* 12 619; *Nat. Rev. Chem. 2018* 2 113;
(2) *Nano Lett. 2013* 13 2351; *Angew. Chem. Int. Ed. 2013* 52 12069; *ACS Nano 2018* 12 3263;
(3) *Nat. Nanotechnol. 2016* 11 152;
(4) *ACS Nano 2015* 9 1117;
(5) *ACS Nano 2015* 9 11209; *Nat. Commun. 2017* 8 14784;
(6) *Angew. Chem. Int. Ed. 2014* 53 12466; *Nat. Chem. 2014* 7 17;
(7) *Angew. Chem. Int. Ed. 2016* 55 11106;
(8) *Science 2016* 352 890; *Nat. Chem. 2017* 9 611; *Nat. Commun. 2018* 9 1521;
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