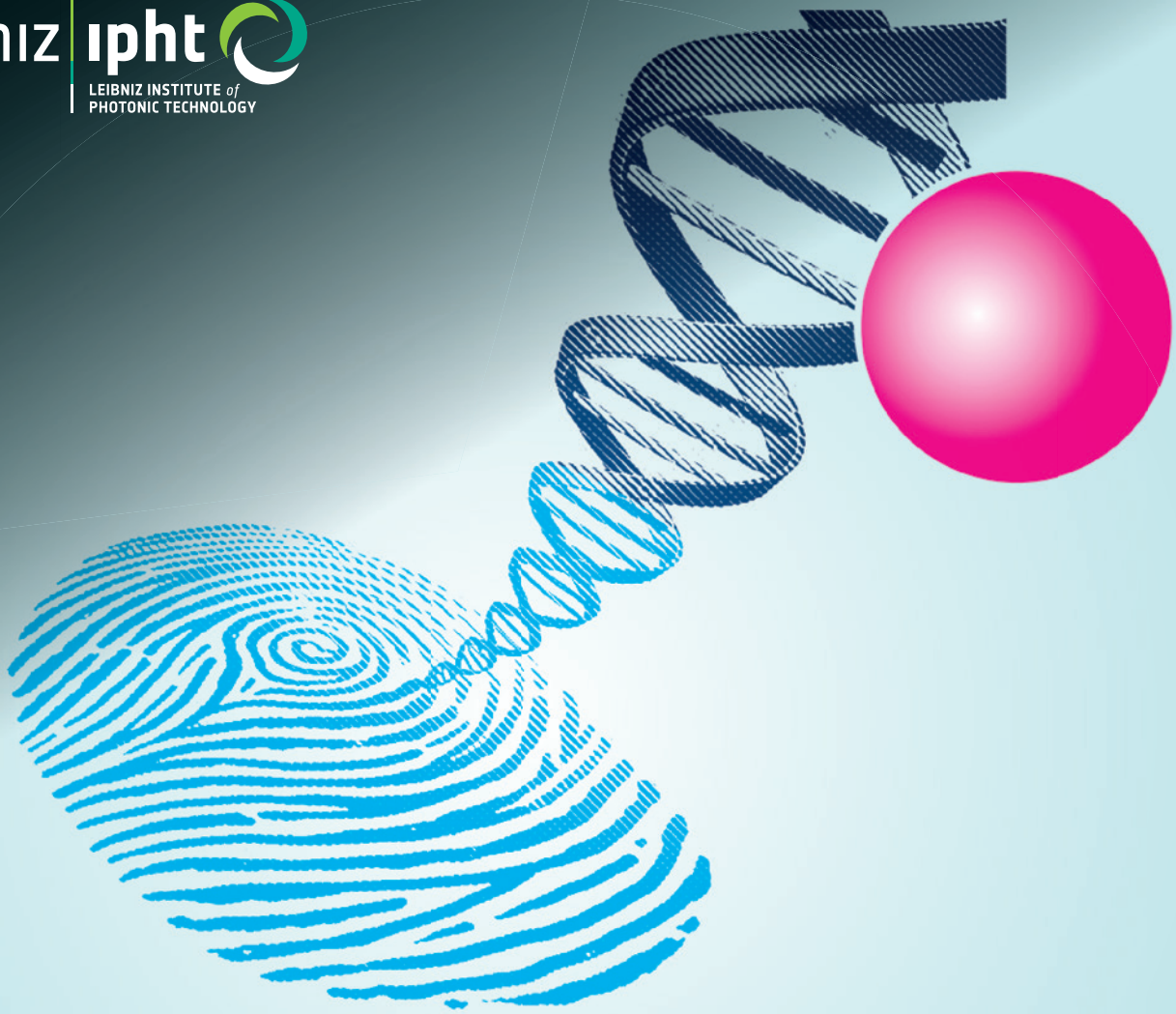
Leibniz IPHT
LEIBNIZ INSTITUTE of PHOTONIC TECHNOLOGY



DNA NANOTECHNOLOGY 2024

MAY 23 – MAY 25, 2024

Leibniz IPHT // Campus Beutenberg // Jena

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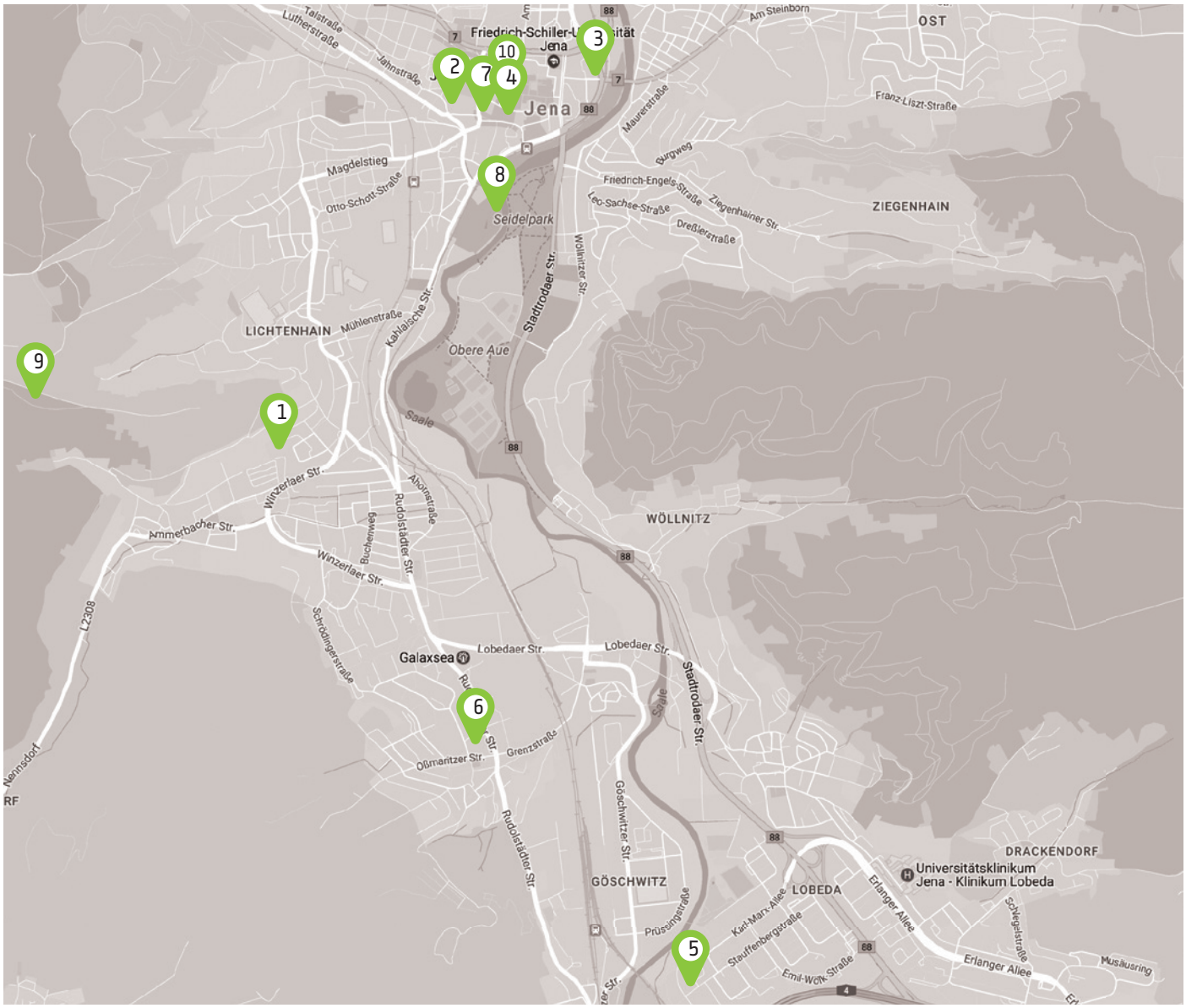
LEIBNIZ HEALTH TECHNOLOGIES

Leibniz Association

LOCATION

1 Leibniz IPHT, Campus Beutenberg
2 Dorint Hotel Esplanade Jena
3 Hotel Schwarzer Bär
4 Hotel Ibis
5 Hotel Maxx
6 Hotel Best Western
7 Bus No. 10, 11, 12
8 Paradies-Café
9 Ernst Haeckel Monument
10 Bus Stop "Nonnenplan"



LEIBNIZ INSTITUTE OF PHOTONIC TECHNOLOGY

Nanobiophotonics Department

Albert-Einstein-Str. 9 // 07745 Jena

Phone: 0049 3641 206-304 (Office), -371 (Conference Desk)

wolfgang.fritzsche@leibniz-ipht.de

https://www.biophotonics4future.com/dna2024/

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PROGRAM

THURSDAY, MAY 23, 2024

Leibniz IPHT, Campus Beutenberg – Bus no. 10, 11, 12

12:00 **Satellite-Workshop "DNA Mitteldeutschland" (free to join)** // Get-together

12:30 **Presentations**

17:00 END

Bus to downtown + 10 min walk, or appr. 40 min walk

Paradies-Cafe, Downtown – at Saale river, 6 min from IBIS Hotel

18:00 **Get-together**

19:00 **Welcome Lecture**

19:30 DINNER

FRIDAY, MAY 24, 2024

Leibniz IPHT, Campus Beutenberg – Bus no. 10, 11, 12

8:00 **Registration**

9:00 **Opening & Introduction** // Wolfgang Fritzsche

9:05 **Session 1**
Dynamic Dissipative, Transient DNA-Networks and Nanostructures: From Basic Concepts to Applications // Itamar Willner (Jerusalem)

DNA Nanomachines driven by RNA polymerization // Michael Famulok (Bonn)

DNA machines and super-assemblies // Friedrich Simmel (Munich)

10:20 COFFEE BREAK

11:00 **Session 2** // Itamar Willner
DNA origami stability and the role of superstructure // Adrian Keller (Paderborn)

The dependency of the single molecule SERS spectra on the molecular orientation // Sergio Kogikoski Jr. (Potsdam)

DNA Origami-Templated Silicon Nanoantennas: Distance Dependent Modulation of Emitter Decay Rate // Nicole Siegel (Fribourg)

Poster pitch talks // Ekaterina Podlesnaia

LUNCH & POSTER SESSION

14:00 **Session 3** // Michael Famulok

Development of aptasensors through gold nanoparticle-assisted SELEX // Young-Pil Kim (Seoul)

Designer DNA for sensing and nanomaterials // Andrew Pike (Newcastle)

DNA origami complexation with biomolecules // Veikko Linko (Tartu)

15:15 COFFEE BREAK

15:45 **Session 3a** // Andrew Pike

DNA-templated Nanofabrication of CdS-Au Heterojunctions with Electrical Characterization // Adam Woolley (Provo, UT)

DNA-based Assemblies of Plasmonic Nanocrystals, Chiral Photochemistry, The Origin of Chirality, And "What's so Hot about Electrons in Metal Nanoparticles?" // Alexander Govorov (Athens, OH)

Ultra Sensitive Detection of Nucleic Acids // Danny Porath (Jerusalem)

17:00 **Excursion** // Hike to Ernst Haeckel Monument

19:00 POSTER & BEER (& BARBECUE)

SATURDAY, MAY 25, 2024

Leibniz IPHT, Campus Beutenberg – Bus 10 leaves 8:35 a.m. downtown

9:00 **Session 4** // Adam Woolley

Stacking effects on mutation detection by T4 DNA ligation within dimeric DNA origami triangle barcodes for single-molecule nanopore analysis // Matteo Castronovo (Leeds)

Switchable DNA origami nanopores// Anna Baptist (Munich)

Nanotechnology in Diagnostics and Therapeutics // Annie Ho (Taipeh)

Down-and-up: Combining DNA self-assembly with top-down fabrication // Johannes Parikka (Jyväskylä)

10:40 COFFEE BREAK

11:00 **Session 5** // Jussi Toppari

Self-assembled DNA nanostructure applied on immunomodulation of microglia in neuroinflammation // Manon Libotte (Bologna)

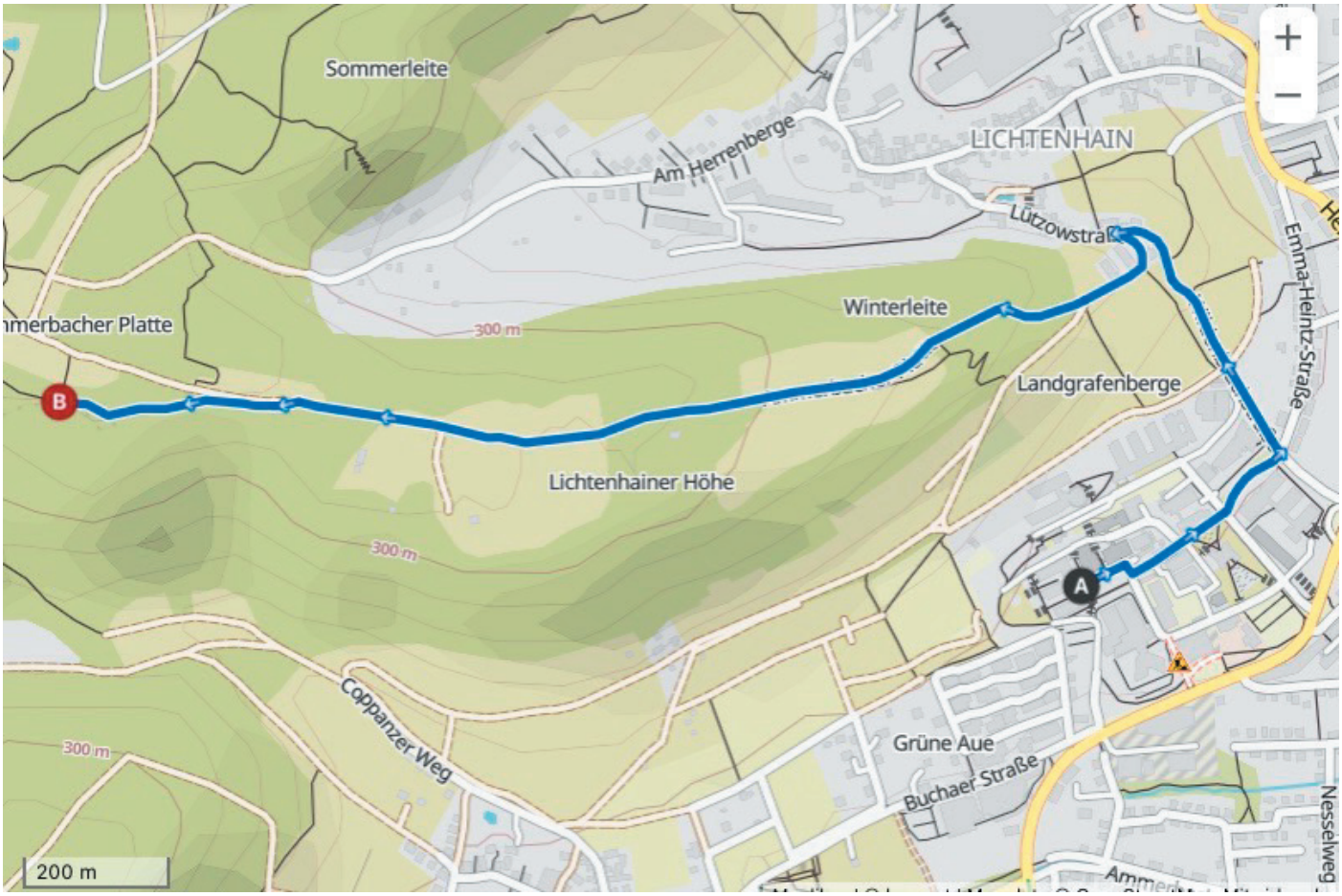
The Journey to a Controllable DNA Origami Drug Delivery System // Merle Scherf (Leipzig)

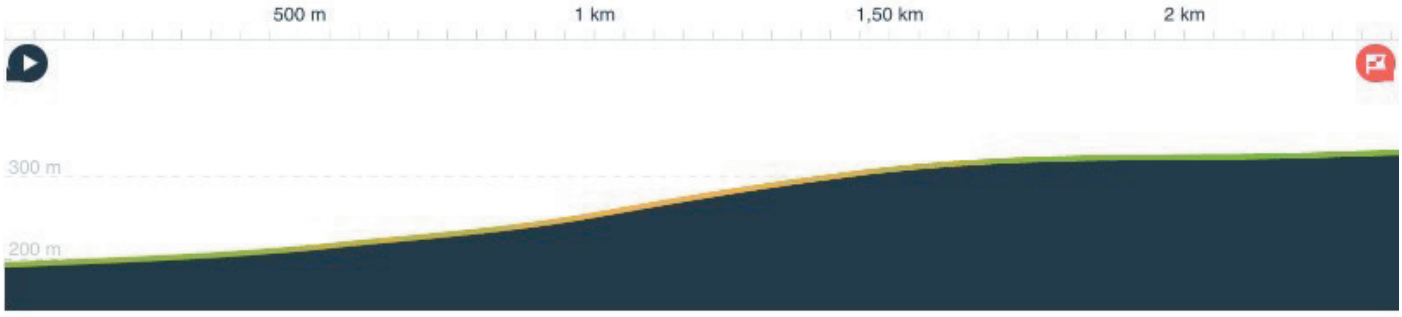
DNA origami nanostructures for antimicrobial photodynamic therapy // Jaime A. Garcia-Dorosa (Paderborn)

12:15 END OF THE SESSIONS & LUNCH

HIKE LEIBNIZ IPHT TO HAECKEL STONE

2.7 km roundtour, height differenc 140 m





△ **Höchster Punkt** 330 m

▽ **Niedrigster Punkt** 190 m

Ernst Haeckel (1834-1919) was a zoologist (and artist) active in Jena. He discovered and named thousands of new species, and coined many biological terms, such as ecology and phylogeny. He promoted and popularized Darwin's work in Germany. He suggested in 1866 that the cell nucleus was concerned with inheritance. His published artwork of (sea) animals (Art Forms of Nature) influenced the Art Nouveau artistic movement.

↔ 2,36 km ↗ 140 m

TALKS

Switchable DNA origami nanopores // Anna Baptist (Martinsried)

Stacking effects on mutation detection by T4 DNA ligation within dimeric DNA origami triangle barcodes for single-molecule nanopore analysis // Matteo Castronovo (Leeds)

DNA Nanomachines driven by RNA polymerization // Michael Famulok (Bonn)

DNA origami nanostructures for antimicrobial photodynamic therapy // Jaime A. Garcia-Diosa (Paderborn)

DNA-based Assemblies of Plasmonic Nanocrystals, Chiral Photochemistry, The Origin of Chirality, and „What's so Hot about Electrons in Metal Nanoparticles?" // Alexander O. Govorov (Athen, Ohio)

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DNA-templated Nanofabrication of CdS-Au Heterojunctions with Electrical Characterization // Adam T. Woolley (Provo)

Switchable DNA origami nanopores

Anna Baptist[1], Ze Yu[2], Susanne Reinhardt[1], Cees Dekker[3], Sabina Caneva[2], Amelie Heuer-Jungemann[1]

[1]Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, 81252 Martinsried, Germany
[2]Department of Precision and Microsystems Engineering, Delft University of Technology, Mekelweg 2, 2628 CD Delft, the Netherlands
[3]Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, 2629 HZ Delft, The Netherlands

Nanopores are nanoscale structures that form channels across membranes and enable the translocation of molecules. Inspired by naturally occurring pore-forming proteins, different types of artificial nanopores have been created. [1] The DNA origami technique allows for the fabrication of DNA nanostructures with precise control over shape and size that can be modified with a variety of functional molecules. Thus, DNA origami provides a platform for the customized design of nanoscale pores with different channel diameters that can be equipped with anchoring molecules for insertion into lipid membranes. [2] Such nanopores have potential applications in single-molecule sensing, sorting of molecules depending on their sizes or for the fabrication of artificial cells. However, most nanopores created so far are static with a fixed pore diameter.
Here, we present a large dynamic DNA origami nanopore that can be mechanically and reversibly switched between different conformations via strand displacement, offering three different pore sizes. After their successful insertion into the lipid bilayer, these nanopores form transmembrane channels with varying diameters depending on their conformation and can be used to control the transport of differently sized molecules across the lipid membrane. Such stimuli-responsive, actuatable nanopores are excellent mimics of complex natural occurring pores, while enabling a higher level of control and a more modular and easily adaptable design.

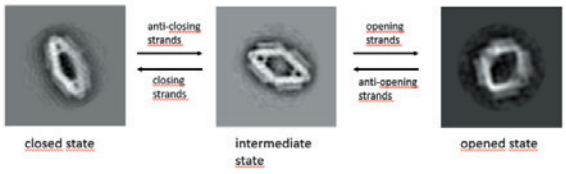


Fig. 1

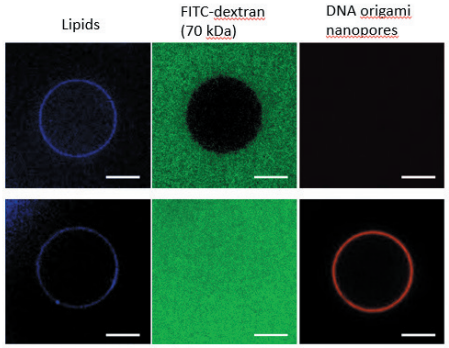


Fig. 2

Fig. 1: 2D class averages of TEM images for the DNA origami nanopore in its three different configurations
Fig. 2: Reconstitution of DNA origami nanopores in GUVs

[1] Langecker et al. (2012). Science, 338 (6109), 932-936
[2] Xing et al. (2022). Nature Nanotechnology, Vol 17, 708-713

Stacking effects on mutation detection by T4 DNA ligation within dimeric DNA origami triangle barcodes for single-molecule nanopore analysis

Dorothy Aboagye-Mensah[1,2,3], Samuel Confederat[4,5], Fahad Alammar[1], Lekshmi Kailas[5,6], Abimbola F. Adedeji-Olulana[1,3,7], Alex Stopar[3], Allen W. Nicholson[3], Neil H. Thomson[5,6,8], Paolo Actis[4,5] and Matteo Castronovo[1,5]

[1] School of Food Science and Nutrition, University of Leeds, LS2 9JT, Leeds, United Kingdom.
[2] College of Health and Science, University of Lincoln, LN6 7TS, Lincoln, United Kingdom.
[3] Department of Biology, Temple University, 1900 North 12th Street, Philadelphia, PA 19122, USA.
[4] School of Electronic and Electrical Engineering, Bragg Centre, University of Leeds, LS2 9JT, Leeds, United Kingdom.
[5] Bragg Centre for Materials Research, LS2 9JT, Leeds, United Kingdom.
[6] School of Physics and Astronomy, University of Leeds, LS2 9JT, Leeds, United Kingdom.
[7] Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Via della Ricerca Scientifica, Rome 000133, Italy.
[8] School of Dentistry, University of Leeds, LS2 9JT, Leeds, United Kingdom.

Solid-state nanopores represent an emerging technology for the highly sensitive detection of biomolecular markers, but nanopore detection of DNA point mutations is challenged by high noise levels. In contrast, barcoded DNA origami nanostructures can provide unique single-molecule nanopore fingerprints [1].

In this work we have integrated nanopore-barcoded DNA nanostructures with enzymatic DNA ligation, the latter of which is routinely applied in clinical protocols for DNA mutation detection [2]. We designed two triangular DNA origami variants containing three elongated staples that provide strand extensions on one side of the triangle, and that are complementary to a target sequence. Addition of the target sequence promotes formation of a DNA triangle dimer. T4 DNA ligase repairs a nick in a dsDNA segment only if there is Watson-Crick base-pairing at the nick. Thus, the two joined DNA triangles can be covalently linked only if the DNA sequence bridging the two triangles carries the mutation of interest at the ligation site.

We observe striking differences in detection of enzymatic ligation by gel electrophoresis, AFM, or quartz capillary nanopores. Stacking interactions between DNA triangles are enhanced by dimer formation, and results in formation of higher order nanostructures, which serve as gel electrophoretic readouts of enzymatic ligation provided by molecular weight amplification. Triangle-triangle stacking dynamics probably involves a clam-like folding mechanism, which is also detectable by quartz nanopore analysis, and with the stacking hindering enzymatic ligation.

These results provide the basis for development of rapid, highly sensitive, high-throughput approaches, using designed DNA nanostructures, for profiling genetic variation in point-of-care settings.

[1] Bell, N.A.W. et al. Digitally encoded DNA nanostructures for multiplexed, single-molecule protein sensing with nanopores. Nature Nanotechnology 11, 645-651 (2016)
[2] Aboagye-Mensah, D. et al., bioRxiv, https://doi.org/10.1101/2024.01.09.574918

DNA Nanomachines driven by RNA polymerization

Michael Famulok*, Mathias Centola, Nils G. Walter, Petr Šulc, Julián Valero, Yu Ze

*Chemical Biology and Medicinal Chemistry Unit, Life and Medical Sciences (LIMES) Institute, University of Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany

Molecular engineering seeks to create functional entities for modular use in the bottom-up design of nano-assemblies that can perform complex tasks. Such systems require fuel-consuming nanomotors that can actively drive downstream passive followers. Most artificial molecular motors are driven by Brownian motion, in which, with few exceptions, the generated forces are non-directed and insufficient for efficient transfer to passive second-level components. Consequently, the realization of efficient chemical-fuel-driven nanoscale machines capable of force transmission remains challenging.

This lecture describes the bottom-up design, generation, and characterization of various DNA-based nanomachines driven by RNA polymerization using the chemical energy of nucleoside triphosphates as fuel and their application for a variety of purposes, such as catalysis, directional walking, actuation, pulsation, or force transmission.

Since DNA-origami technology permits modular bottom-up construction of robust nanostructures with diverse properties that span from mechanically rigid to mechanically compliant structures, all allowing for combination into a single architecture, the versatility of mechanical power transmission by fuel-driven DNA nanomachines to other devices is high, and pilot-examples towards that end will be presented.

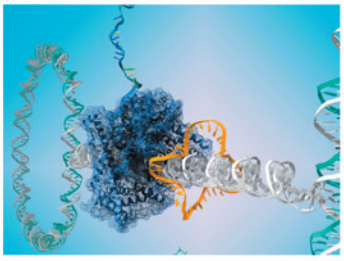


Fig. 1

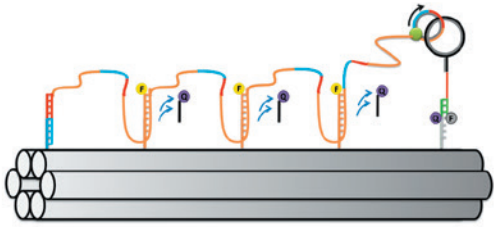


Fig. 2

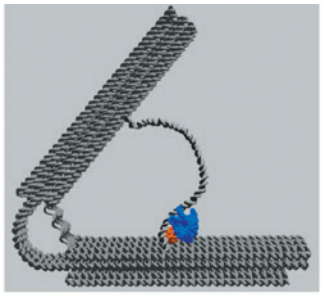


Fig. 3

Fig. 1: Self-Regulating DNA Rotaxane Linear Actuator
Fig. 2: DNA catenane walker
Fig. 3: Rhythmically pulsing leaf-spring DNA-origami nanoengine

DNA origami nanostructures for antimicrobial photodynamic therapy

Jaime A. Garcia-Diosa, Guido Grundmeier, Adrian Keller.

[1] Universität Paderborn, Technical and Macromolecular Chemistry, Paderborn, Germany

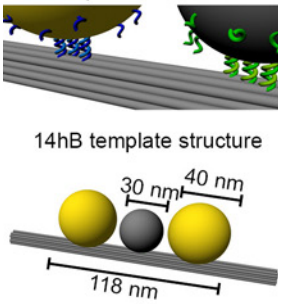
Photodynamic therapy (PDT) produces reactive oxygen species (ROS), including singlet oxygen, using a photosensitizer (PS) that is excited by absorption of light at determined wavelengths.[1] Among the various medical applications of PDT, the potential to treat antimicrobial resistant bacterial infections, without generating drug resistance, stands out.[1-3] However, for its clinical application, it is necessary to develop and implement methods to improve the target selectivity of the PS to avoid possible collateral damage to healthy cells.[4] In this aspect, DNA origami nanostructures (DONs) could provide a possible solution, since they have been highlighted as nano-vehicles for targeted drug delivery to due to their inherent properties such as excellent biocompatibility, high in-vivo stability, and low immunogenicity.[5-6] However, there are no reports of using DONs as vehicles for the targeted delivery of the PS in PDT. This presentation will evaluate the effect of different shapes of DONs on bacterial growth in dark conditions and during antimicrobial PDT, using methylene blue as a DNA-binding PS. Among the most outstanding results, two interesting phenomena were observed: (i) bacteria can use DONs as food source to promote bacterial growth, and (ii) DONs can scavenge the ROS generated during PDT. Both aspects need to be considered in possible applications of DONs in PDT.

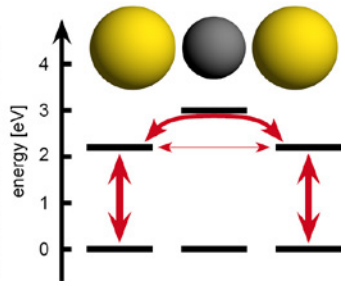
[1] M. R. Detty, S. L. Gibson, and S. J. Wagner. 2004. "Current Clinical and Preclinical Photosensitizers for Use in Photodynamic Therapy," J Med Chem, vol. 47, no. 16, pp. 3897–3915.
[2] L. S. Peloi, R. R. S. Soares, C. E. G. Biondo, V. R. Souza, N. Hioka, and E. Kimura. 2008. "Photodynamic effect of light-emitting diode light on cell growth inhibition induced by methylene blue," J Biosci, vol. 33, no. 2, pp. 231–237.
[3] L. L. Chen et al. 2018. "Laser-induced antibacterial activity of novel symmetric carbazole-based ethynylpyridine photosensitizers," ACS Omega, vol. 3, no. 4, pp. 3737–3743.
[4] S. Wang, R. Gao, F. Zhou, and M. Selke. 2004. "Nanomaterials and singlet oxygen photosensitizers: potential applications in photodynamic therapy," J Mater Chem, vol. 14, no. 4, p. 487.
[5] H. Bila, E. E. Kurisinkal, and M. M. C. Bastings. 2019. "Engineering a stable future for DNA-origami as a biomaterial," Biomater Sci, vol. 7, no. 2, pp. 532–541.
[6] K. E. Bujold, A. Lacroix, and H. F. Sleiman. 2018. "DNA Nanostructures at the Interface with Biology," Chem, vol. 4, no. 3, pp. 495–521.

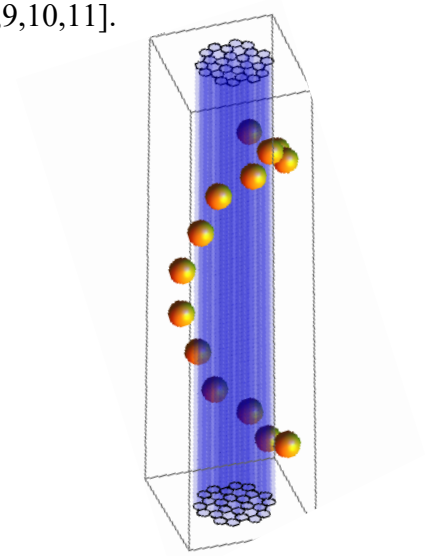
DNA-based Assemblies of Plasmonic Nanocrystals, Chiral Photochemistry, The Origin of Chirality, and "What's so Hot about Electrons in Metal Nanoparticles?"

Alexander O. Govorov
Department of Physics and Astronomy, Ohio University, Athens, USA; govorov@ohio.edu

Plasmonic nanostructures and metamaterials are very efficient at the absorption and scattering of light. The studies to be presented in this talk concern special designs of hybrid nanostructures with electromagnetic hot spots, where the electromagnetic field becomes strongly enhanced and spatially concentrated. Overall, plasmonic nanostructures with hot spots demonstrate strongly amplified optical and energy-related effects, and this talk will review some of such phenomena. (1) Using nanoparticle arrays made of different metals, one can transfer plasmonic signals coherently and with minimal losses [1]. (2) Plasmonic hot spots efficiently generate energetic electrons, which can be used for photochemistry and photodetection [2,3,4]. (3) Nanostructures with small interparticle gaps can strongly enhance heat's optical generation and confine high photo-temperatures in small volumes [5,6,7]. (4) Colloidal nanocrystal assemblies and metastructures with plasmon resonances allow us to strongly enhance the chiral optical responses (circular dichroism) of biomolecules and to induce chiral photo-chemical effects [8,9,10,11].







[1] E.-M. Roller et al., Nature Physics, 13, 761 (2017).
[2] A.O. Govorov, H. Zhang, H.V. Demir and Y. K. Gun'ko, Nano Today 9, 85 (2014).
[3] H. Harutyunyan et al., Nature Nanotech. 10, 770 (2015).
[4] L. V. Besteiro et al, Nano Today, 27, 120 (2019).
[5] A. O. Govorov and H. Richardson, Nano Today 2, 20 (2007).
[6] C. Jack et al., Nat. Commun. 7, 10946 (2016).
[7] X.-T. Kong et al., Nano Letters, 18, 2001 (2018).
*[8] A. O. Govorov et al., Nano Letters **10**, 1374–1382 (2010).*
[9] A. Kuzyk et al., Nature 483, 311 (2012).
[10] T. Liu et al., Nano Letters, 19, 1395–1407 (2019).
[11] K. Martens, et al., Nat. Commun., 12, 2025 (2021).

Nanotechnology in Diagnostics and Therapeutics

Annie Ho
Department of Biochemical Science and Technology/Department of Chemistry, National Taiwan University, Taipei 10617, Taiwan

Nanotechnology has emerged as a groundbreaking field with immense potential in diagnostics and therapeutics. The integration of nanotechnology into diagnostics and therapeutics has the great potential to revolutionize healthcare and significantly improve patient outcomes. Three strategies using nanotechnology in developing diagnostics and therapeutics were discussed herein. The first[1] entails a newly designed surface plasmon resonance (SPR) biosensor for CMV-specific microRNAs. This SPR biosensing platform was used successfully for the multi-detection of the microRNAs, UL22A-5p, and UL112-3p, which were associated with CMV, with the detection limits of 108 fM and 24 fM, respectively. In addition, this sensor is capable of discriminating between serum samples collected from healthy and CMV-infected newborns. The second[2] presents the externally thiolated hollow mesoporous silica nanoparticles (MSN-SH(E)) as a platform for improved bladder intravesical therapy. Enhanced mucoadhesive and permeation-enhancing effects of the thiolated nanovector are verified, and a fragmented distribution pattern of a tight junction protein, claudin-4, indicates the opening of tight junction. The antitumor activity of the mitomycin C (MMC)-loaded nanovector (MMC@MSN-SH(E)) is more effective than that of MMC alone in both in vitro and in vivo. The third[3] introduces autonomous surface-nucleolin-guided HCR that leads to the polymerization of G-quadruplex polymer chains, in which the ZnII-protoporphyrin IX is intercalated. We demonstrate that MDA-MB-231 (Triple Negative Breast Cancer cells, TNBC) with overexpressed surface nucleolin were able to induce HCR leading to the formation of the ZnII PPIX-loaded G-quadruplex polymer chains, while the M10 epithelial breast cells served as control. The ZnII PPIX-loaded nanowires allow the selective imaging of TNBC, and their permeation into the TNBC leads to selective cytotoxicity and guided photodynamic therapy toward the cancer cells due to structural perturbation of the membranes. The aptamer-guided HCR-generated G-quadruplex polymer chains may serve as a versatile tool to target TNBC featuring poor prognosis and high pathological risk of recurrence, thus offering a promising theranostic platform.

[1] Anal Chem. 2021 Jun 8;93(22):8002-8009.
[2] Adv Sci (Weinh). 2023 Mar;10(7):e2204643.
[3] Angew Chem Int Ed Engl. 2021 Sep 27;60(40):21673-21678.

DNA origami stability and the role of superstructure

Adrian Keller

Technical and Macromolecular Chemistry, Paderborn University, Paderborn, Germany

DNA origami technology has found numerous applications in various fields ranging from biomedicine to analytical chemistry to materials science. Many of these applications exploit the unprecedented addressability of DNA origami nanostructures and thus rely on their intact and well-defined 3D shapes [1]. However, such applications often expose the employed DNA origami nanostructures to denaturing or degrading environments with negative effects on their structural integrity. The relevant environments are highly diverse and may pose very different threats to DNA origami stability, for instance due to unfavorable ionic conditions, temperatures or pH values, the presence of DNA-digesting enzymes, or exposure to chemical denaturants. Understanding the underlying molecular mechanisms and how various extrinsic and intrinsic factors influence DNA origami stability under relevant conditions thus represents an important prerequisite for the rational engineering of DNA origami stability.

This presentation will summarize our activities aimed at elucidating the factors that govern DNA origami stability in various, application-specific environments. Over the last years, we specifically have assessed the effects of low-Mg^{2+} concentrations [2,3,4], active nucleases [4,5], and chaotropic agents [6] on the structural integrity of various 2D and 3D DNA origami nanostructures by atomic force microscopy and selected complementary analytical techniques. This allowed us to gain some fundamental insights on the specific role of superstructure in stabilizing DNA origami nanostructures under adverse conditions. Our work thus lays the ground for the rational optimization of DNA origami nanostructures on the design level to ensure their maximum stability in selected environments. Our results even hint at the possibly to achieve broad-spectrum stability under a variety of diverse conditions.

[1] Ramakrishnan et al. (2018). Comput. Struct. Biotechnol. J. 16, 342-349
[2] Kielar et al. (2018). Angew. Chem. Int. Ed. 57, 9470-9474
[3] Hanke et al. (2023). ChemBioChem 24, e202300338.
[4] Xin et al. (2022). Small 18, 2107393.
[5] Ramakrishnan et al. (2019). ChemBioChem 20, 2818-2823.
[6] Hanke et al. (2023). Nanoscale 15, 16590.

Development of aptasensors through gold nanoparticle-assisted SELEX

Jeong-Min Lee, Duc Loc Sai, Eun-Song Lee, Young-Pil Kim

Department of Life Science, Hanyang University, Seoul 04763, South Korea

To resolve time-consuming and imperceptible monitoring problems in the traditional systematic evolution of ligands by exponential enrichment (SELEX), we report gold nanoparticle-assisted SELEX (GNP-SELEX) as a visual, proofreading, and self-monitoring platform and its application to small molecule-binding single-stranded DNA (ssDNA) aptasensors. Through the colorimetric changes between rounds, GNP-SELEX enabled the rapid and proofreading determination of target-specific aptamer library enrichment with neither target modification nor extra monitoring process. We identified ssDNA aptamers with high selectivity and binding affinity by targeting two hormones (brassinolide; BL and bisphenol A; BPA) as a model. The rational design of selected aptamers by 3D molecular simulation increased their ability to detect BL or BPA in real samples as bioreceptors. These results suggest that GNP-SELEX is useful as a visual monitoring platform to discover ssDNA aptamers as well as to develop aptasensors for diverse targets in a rapid and simple way.

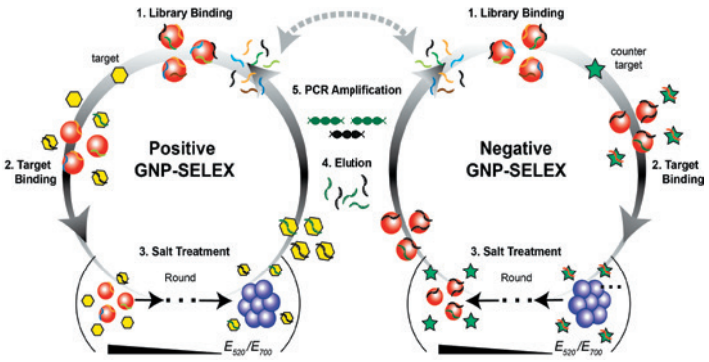


Fig. 1

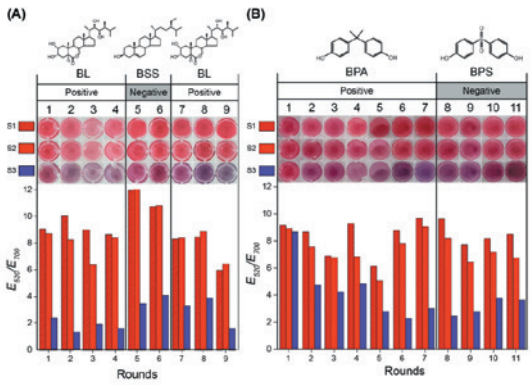


Fig. 2

Fig. 1: Schematic of the GNP-SELEX procedure
Fig. 2: GNP-SELEX for the discovery of ssDNA aptamers against BL (A) or BPA (B)

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The dependency of the single molecule SERS spectra on the molecular orientation

Yuya Kanehira[1], Aleksandra K. Adamczyk[2], Evgenii Titov[1], Peter Saalfrank[1], Guillermo P. Acuña[2], Sergio Kogikoski Jr[1], Ilko Bald [1]

[1] Institute of Chemistry, University of Potsdam, Potsdam, Germany.

[2] Department of Physics, University of Fribourg, Switzerland.

Modifying DNA sequences with diverse functional chemical groups is essential in using DNA origami nanostructures for few to single-molecule physical chemical studies. Herein, we used a DNA origami nanofork plasmonic nanoantenna [1] to study the effect of single molecule orientation on the surface-enhanced Raman spectra of such molecules. We used the strategy developed by the Acuña group [2] in removing or adding DNA bases to orient a single cyanine 5 (Cy5) dye in the plasmonic hot spot. In this way, we studied two different orientations at 0 or 90°, i.e., in relation to the DNA chain axis, the molecule is either parallel or perpendicular to it, Figure 1A. The obtained single-molecule SERS spectra show that the differences in the two orientations are relatively small. However, the distribution of Raman signals showed a dominance of vibrations related to the aromatic rings present at Cy5 when the molecule is oriented at 90°. We explain such behavior due to the possible closer proximity of this part of the molecule to the nanoparticle surface and the possible inhomogeneous plasmonic field of the hot spot. Using a different DNA origami plasmonic antenna with one oriented Cy5 dye in the hot spot, we could now observe the same difference in signal appearance. Still, we observed a clear peak shift for the molecule oriented at 90°. To explain such a difference, we simulated the Raman spectra of the Cy5 dye in the two orientations with an externally applied electric field with different strengths. The simulations confirmed that such peak shift is related to the different strengths of the electric field in the nanogap. This study shows for the first time the difference between two orientations of a molecule studied by SERS; such achievement was only possible due to the capabilities of DNA nanotechnology to assemble molecules in designed positions precisely. The SERS study showed significant differences in the spectra. With the help of spectra simulations, we could trace such differences to the electric field strength in the plasmonic hot spot.

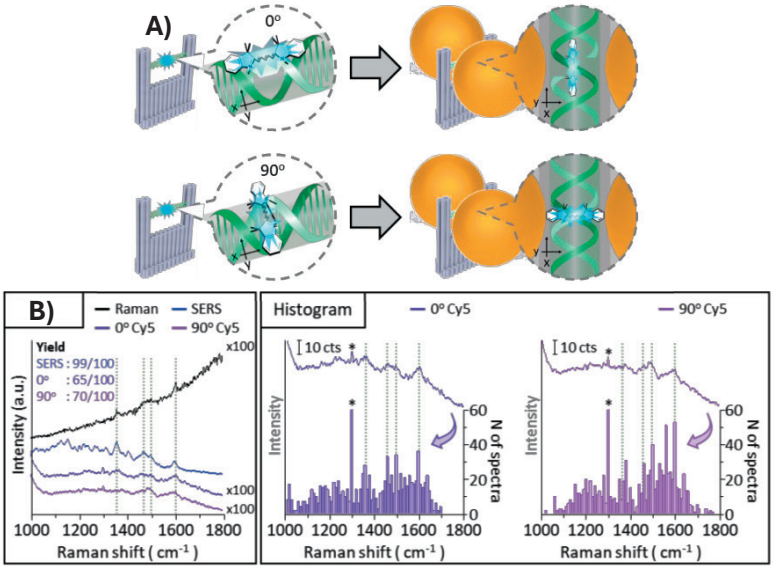

Fig. 1: A) Orientation of the Cy5 dye within the plasmonic hot spot using DNA origami nanoantennas. B) Comparison of the Raman, SERS, and oriented single molecule SERS spectra of Cy5; on the left side it is shown the histogram of band appearance distribution for the two orientations.

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Self-assembled DNA nanostructure applied on immunomodulation of microglia in neuroinflammation

Manon Libotte, Francesca Massenzio, Barbara Monti, Giampaolo Zuccheri

Department of Pharmacy and Biotechnology, Alma Mater Studiorum University Bologna, Via San Donato 19/2, 40127 Bologna, Italy

Alzheimer is one of the most common cognitive disorders affecting approximately 35 million people worldwide. Alzheimer results in part from the chronic neuroinflammation of the brain leading to the degradation of the neurons. This neuroinflammation is mainly due to accumulation of Aβ protein that induce the activation of the microglia, switching from a resting (M0) phenotype to a pro-inflammatory (M1) phenotype. It has also been shown that neuroinflammation promotes the accumulation of Aβ protein leading to a vicious cycle [1]. This chronic activation of microglia stimulates the release of cytokines, signal proteins, DNA and notably miRNAs.

DNazymes are catalytic DNA elements that can hydrolyze RNA. We can design such elements to specifically bind and cleave (albeit, with a moderate yield) a variety of RNA sequences.

The goal of our research is to produce DNA nanostructure (DNS) containing a DNAzyme targeting a miRNA responsible of the spreading of neuroinflammation. We engineered a tetrahedral DNS able of self-assembling, autonomous cell-uptake, biocompatible, non-toxic, and biodegradable [2]. These DNS offer many design options allowing to target different type of nucleic acid (such as long non-coding RNA) or, potentially, different types of cells (by the addition of a targeting system like aptamer). Based on previous studies [3] on mouse microglial cell model (N9), we chose to design a DNS capable to cleave mir34a, a miRNA reducing the level of TREM2, a protein involved in the clearance of Aβ accumulation [4].

We observed by fluorescence microscopy (Fig. 1) and polymerase chain reaction (PCR) (Fig. 2) an uptake of the NS into the cells. Moreover, the level of mir34a into the cells has been analyses by PCR (Fig. 3) and shows a decrease of mir34a level in activated microglia previously treated with the DNS. These promising results suggest that DNS could improve therapies against neurodegenerative disease.

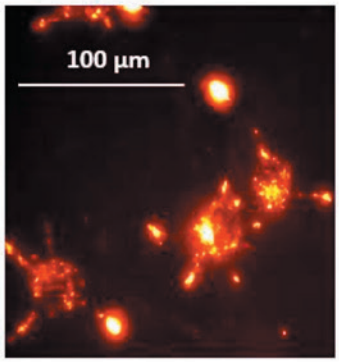

Fig. 1

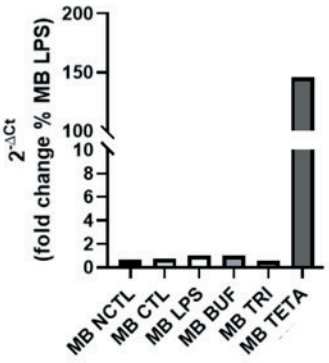

Fig. 2

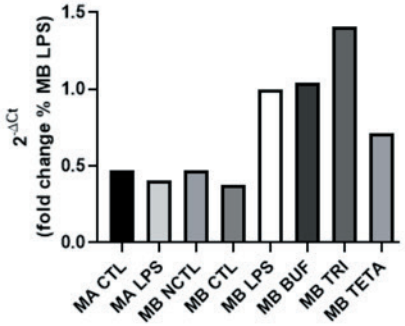

Fig. 3

Fig. 1: Fluorescence microscopy on the internalization of the Cy3-labbeled DNS in N9 cells.
Fig. 2: Analysis of NS abundance in N9 cells by qPCR (TETA=DNS).
Fig. 3: Analysis of miR-34a abundance in N9 cells by RT-qPCR (TETA=DNS).

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DNA origami complexation with biomolecules

Veikko Linko

University of Tartu, Institute of Technology, Nooruse 1, 50411 Tartu, Estonia
Aalto University School of Chemical Engineering, Kemistintie 1, 02150 Espoo, Finland

DNA origami is a powerful technique to rapidly fold [1] custom nanostructures that may further serve as templates for versatile biomolecular assembly. Here I present some of our recent results regarding the complexation of DNA origami with other biomolecules for gaining completely new functions and/or increasing the DNA nanostructure stability [2].

Intercalators for drug delivery: Previously, we have studied doxorubicin (DOX) loading into DNA origami and the subsequent release of DOX upon DNase I digestion [3]. We have now extended this work to a more detailed fluorescence anisotropy assessment of DOX-DNA origami interaction (Fig. 1, left) [4], and examined the feasibility (stability, uptake and toxicity) of such DOX-loaded DNA origami complexes for ocular drug delivery [5].

Protein coatings for stability and targeting & DNA origami-directed virus capsid polymorphism: We have extensively worked with electrostatic protein coatings for DNA origami protection and immunocompatibility [6]. We have also shown that the coatings can be composed of multiple proteins (serum albumins and antibodies) with stimuli-responsiveness and antigen targeting properties [7]. In another work, we have used DNA origami templates as "synthetic genome scaffolds" to direct virus capsid formation around them (Fig. 1, middle) [8]. The study shows successful formation of artificial virus capsids using multiple different DNA origami templates (varied geometry/topology) and capsid protein types.

Other complexation strategies: We have also recently showed how DNA origami can be complexed with phthalocyanines [9] and with lipids to form static [10] and stimuli-responsive assemblies (Fig. 1, right) [11].

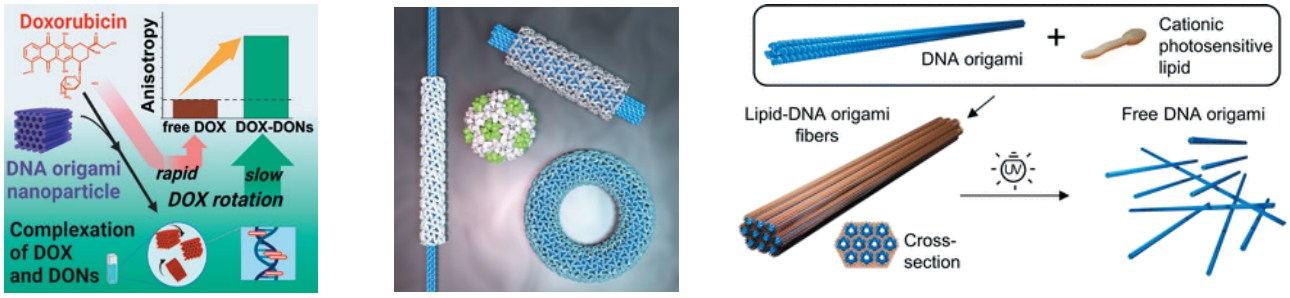

Fig. 1: Left: Fluorescence anisotropy analysis of the DOX-DNA origami interaction [4]. Middle: DNA origami-directed virus capsid polymorphism [8]. Right: Assembly and optically triggered disassembly of lipid–DNA origami fibers [11].

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Down-and-up: Combining DNA self-assembly with top-down fabrication

Johannes Parikka, Kosti Tapio, Heini Järvinen, Toni Rautio, Jussi Toppari

Nanoscience Center and Dept. of Physics, P.O. Box 35, 40014 University of Jyväskylä, Finland

The molecular electronics as well as molecular scale optics, i.e. plasmonics, have long been thought as the next big leap in technology development. However, the progress has been hindered by expensive and/or complicated fabrication. At a moment, DNA has proven to be a one of the most versatile molecules for nanoscale fabrication [1], even for electrical and plasmonic purposes [2]. Since the self-assembly happens in a parallel manner, one can simultaneously fabricate huge amounts of devices. However, since the assembly typically happens in a solution, one needs a way to combine these bottom-up methods with some top-down method – in the simplest case – just to position the assembled devices. Yet, combining the self-assembly directly with top-down methods during the fabrication, can yield even more versatile fabrication methods.

For nanoscale optics, we have developed a novel method, DNA assisted lithography (DALI), which takes advantage of the DNA origami constructions and together with conventional top-down nanofabrication processes enables fabrication of high-quality, sub-100 nanometer plasmonic nanostructures with desired shapes [3]. We can easily cover large areas, but the orientation of the structures has so far been mostly random. Though, to obtain the best possible optical response, we are extending DALI to organized large 2D-lattices out of origami [4,5]. With further processing these lattices can be turned even to a metamaterial. So far, this kind of DNA origami lattices have been mainly fabricated on mica [5,6], which is not compatible with almost any top-down methods. Due to this, we have transported the lattice formation method on silicon substrates [4], which will enable DALI as well as open new avenues for DNA-based fabrication. In addition, we have found a way to fabricate rolled DNA origami lattices to form tubular geometries [7]. Same origami with slight modifications can also be combined with nanoparticles to form controlled arrays of particles.

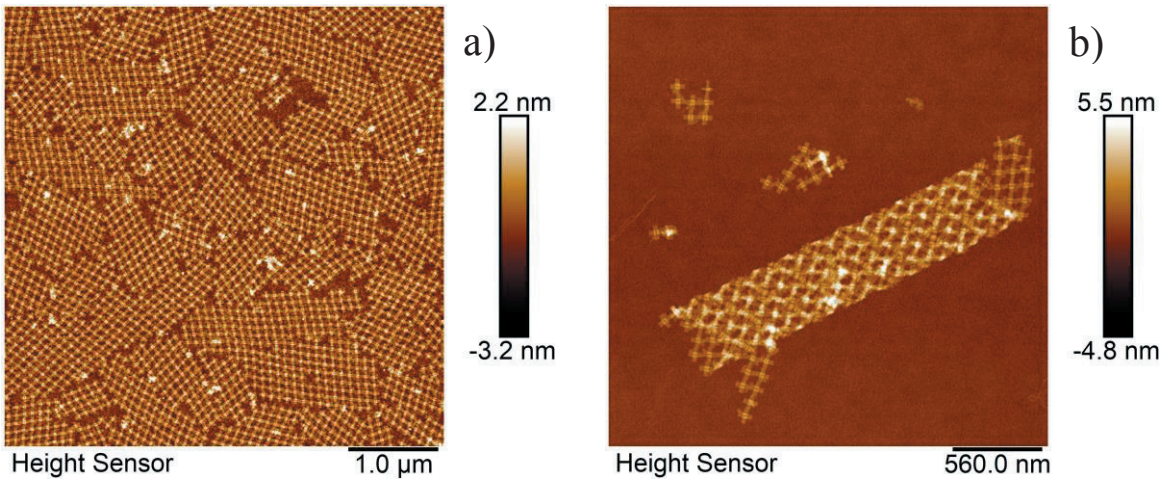

Figure: a) 2D-lattices formed by DNA origami structures and b) a tube formed by a rolled origami-lattice, both on a single crystal silicon substrate.

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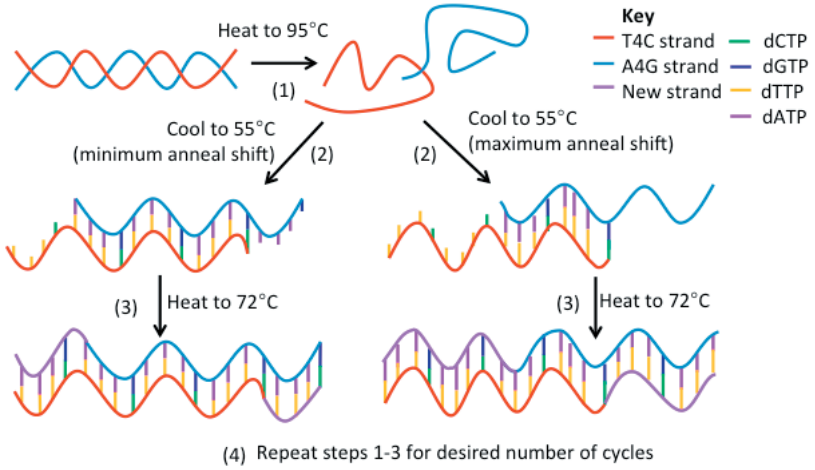
Designer DNA for sensing and nanomaterials

Lama Alharthi, Ellie Dimmick, Morag Grierson and Andrew R Pike
Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

DNA of user-defined sequences, and of lengths over 100 base pairs (bps) has not been easily accessible. Automated DNA synthesis affords great control over the DNA sequence, but longer strands (>100 bps) result in diminishing yields, and of only a single strand. Typically, polymerase-based routes for DNA synthesis are not limited to short DNA (<100 bps) but rely on primer-templates being available to give the sequence control. Additionally, non-standard modifications to DNA can lead to reduced yields and truncated sequences. This talk will present the recent developments from the Chemical Nanoscience Laboratories in Newcastle that address these shortcomings in an effort to produce off-the-shelf designer DNA.



We use a heat-cool cycle extension of oligo “seeds” to produce long DNA of controlled base pair composition, see figure above.[1] The oligoseeds are typically complementary repeat DNA sequences of the form $[W_aX_bY_cZ_d]_n$ where W, X, Y and Z represent any of the four standard nucleobases, and n(a+b+c+d) ≈ 20. Upon heating, the $[W_aX_bY_cZ_d]_n$ duplex denatures fully, which on cooling can re-assemble into displaced duplexes providing overhangs as templates for extension by polymerase enzymes. Multiple heat-cool cycles using a *Thermococcus gorgonarius* Family B polymerase exonuclease minus variant, Z3,[2] yields ever longer strands of the repeat sequence where n > 1,000 bases.

It is also possible to incorporate modified nucleotides into the extended DNA products, as well as load the DNA with fluorescent tags to produce a range of functional-DNA for use in nanomaterial and sensing platforms. This talk will cover several ways in which designer DNA can be synthetically modified and also used to specifically coordinate gold nanoparticles.[3]

Alkyne-modified DNA was produced to demonstrate facile modification via “click chemistry” with an azido ($N=N^+=N^-$)-fluorescein. The synthesis of thiolated-DNA allowed for metal with full characterization of the products by UV-Vis and IR spectroscopy, atomic force microscopy and fluorescence microscopy.

The synthesis of DNA with modifications situated at user-defined positions suggests that this method for the synthesis of long designer DNA may be a useful for the controlled fabrication of unique 1-D nanomaterials where composition control is required in extended sequences.

Funding from BBSRC, NunaBio Ltd., and Newcastle University is gratefully acknowledged.

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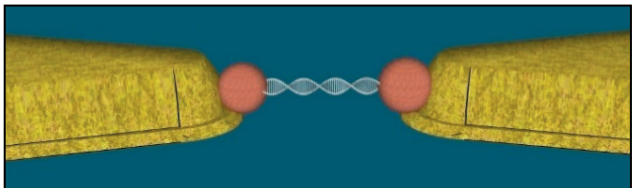
Ultra Sensitive Detection of Nucleic Acids

Danny Porath

Institute of Chemistry and Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, 91904 Israel



The DNA double-strand recognition, as well as the ability to manipulate its structure open a multitude of ways to make DNA useful for molecular electronics as well as for development of new single molecule sensors. Understanding charge transport in DNA molecules is a long-standing problem of fundamental importance across disciplines. We recently reported a breakthrough in measuring charge transport in DNA (Nature Nanotechnology 2020) in a special configuration. We demonstrated charge-transport measurements through single 30-nm-long double-stranded DNA (dsDNA) molecules with an experimental set-up that enabled us to address individual molecules repeatedly and to measure the current–voltage characteristics from 5 K up to room temperature. Strikingly, we observed very high currents of tens of nanoamperes, which flowed through both homogeneous and non-homogeneous base-pair sequences. Moreover, we showed that the presence of even a single discontinuity ('nick') in both strands that compose the dsDNA leads to complete suppression of the current, which suggests that the backbones mediate the long-distance conduction in dsDNA, contrary to the common wisdom in DNA electronics. These findings are of great importance for understanding electricity in DNA in particular, and for molecular electronics in general. However, it also paves the way for the design of new ultra-sensitive detectors for DNA and RNA. Addressing these challenges is at the heart of early detection of cancer, pathogens, emergency medicine as well as for pandemics like the COVID-19.

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The Journey to a Controllable DNA Origami Drug Delivery System – Improved Cargo Encapsulation and Stability

Merle Scherf [1,2], Florian Scheffler [1,2], Christopher Maffeo[3], Ulrich Kemper[2], Jingjing Ye[2,4], Aleksei Aksimentiev[3,5], Ralf Seidel[1,4], Uta Reibetanz[1]

[1]Institute for Medical Physics and Biophysics, Medical Faculty, University of Leipzig, Härtelstraße 16-18, 04107 Leipzig, Germany
[2] Peter Debye Institute for Soft Matter Physics, Faculty of Physics and Earth Science, University of Leipzig, Linneéstraße 5, 04103 Leipzig, Germany
[3] Department of Physics, University of Illinois at Urbana-Champaign, 1110 W Green St, Urbana IL 61801, USA
[4] Cluster of Advancing Electronics Devices Dresden, University of Dresden, Helmholtzstraße 18, 01069 Dresden, Germany
[5] Department of Physics and Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, 405 N Mathew Ave, Urbana, IL 61801, USA

A drug delivery system has to fulfill many criteria. Mainly, it has to avoid opsonization in the blood stream and has to be biocompatible as well as biodegradable in human body. While several different approaches have been investigated over time so far, DNA recently attracted attention, in particular folded as a DNA origami nanostructure. With this folding technique, it is possible to assemble a 3D-system with defined features in an easy way: Taking advantage of the capability of DNA being easily modified, forming strong hydrogen bonds and allowing electrostatic interaction of the negatively charged backbone, it is possible to adjust the system effectively to different transporting tasks. In our approach, we use a rectangular DNA tube with a cavity that can be filled with a defined number of cargo molecules by binding them to complementary ssDNA strands, which are attached in the inner walls of the tube. Two lids on each side of the tube specifically bind via 2nt overhangs to generate a closed cavity. Moreover, a detachment DNA strand was used to disconnect the cargo molecules, resulting in a freely movable cargo. The stability of the system was enhanced regarding extracellular and intracellular conditions by coating with positively charged polylysine-PEG blockpolymers resulting in electrostatic interactions with the negatively charged DNA. With that, a long time stable carrier system for a freely movable cargo was designed and tested against different media and DNAse1 resistance.

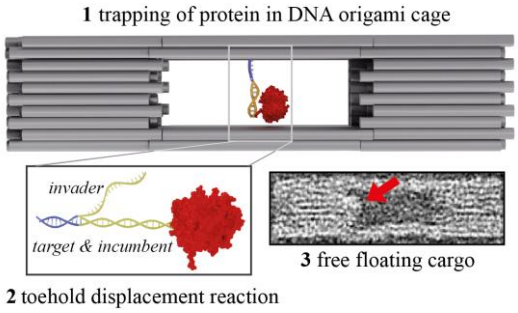

Fig. 1

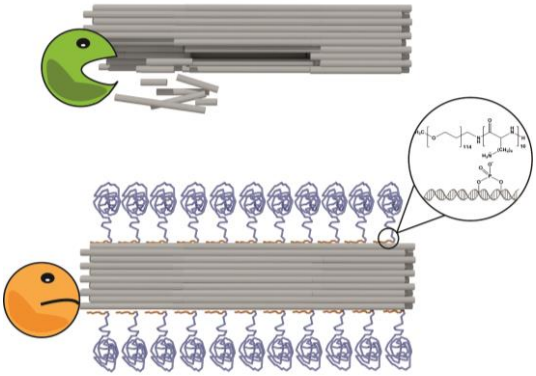

Fig. 2

Fig. 1: Encapsulation of a freely movable cargo inside DNA origami nanocages.
Fig. 2: Stability against DNAse1 of DNA Origami without coating and with polylysine-polyethylenglycol blockpolymer coating.

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DNA Origami-Templated Silicon Nanoantennas: Distance Dependent Modulation of Emitter Decay Rate

Nicole Siegel[1], Maria Sanz-Paz[1,2], Karol Kolataj[1], Hiroaki Hasabe[3], Javier Gonzales-Colsa[4], Hiroshi Sugimoto[3], Pablo Albella[4], Guillermo Acuna[1]

[1]Department of Physics, University of Fribourg, Fribourg CH 1700, Switzerland
[2] Sorbonne Université, Centre National de la Recherche Scientifique, Institut des NanoSciences de Paris, 75005 Paris, France
[3]Department of Electrical and Electronic Engineering, Graduate School of Engineering, Kobe University, Kobe 657-8501, Japan
[4]Department of Physics, University of Cantabria, Spain

Metallic nanoparticles (NPs) can enhance light-matter interactions in the visible range due to the strong electromagnetic near-fields they generate. However, such electric field enhancement is counteracted by the significant heat losses experienced in metallic NPs, which can actually hinder the radiation efficiency of nearby molecules. Conversely, high-index dielectric nanoparticles (HID-NPs), such as silicon NPs (SiNPs) are currently being researched as an interesting alternative due to their low heat losses and strong magnetic modes that can interact with the electric modes inside the material. For these reasons, dielectric NPs are great candidates to construct antennas with unique potential to enhance the decay properties of single emitters without decreasing their efficiency. Theoretical predictions show that, whereas dielectric and plasmonic antennas improve the radiative decay rate of dipolar emitters, silicon nanoantennas exhibit larger and more stable quantum efficiencies. This is because, unlike in plasmonics, the energy of the dipolar emitters in silicon nanoantennas is not dissipated by Ohmic losses.[1]

Here, we develop a technique to functionalized highly crystalline, monodisperse colloidal silicon nanospheres with single stranded DNA.[2] We exploit DNA origami to assemble Silicon optical nanoantennas (ONAs), with a nanometer precision unparalleled by top-down methods. This enables us to selectively couple a single fluorophore with a single silicon nanoparticle, allowing us to investigate the resulting modification of the emitter decay rate, known as the Purcell effect, as a function of the distance to the nanoparticle. Our results demonstrate that indeed changes in decay rates are mostly radiative, so that emission can be accelerated without significant changes in the efficiency. This will facilitate the use of HID-NPs for sensing applications, eliminating the need of spacing layers commonly emplyed in the case of metallic NPs.

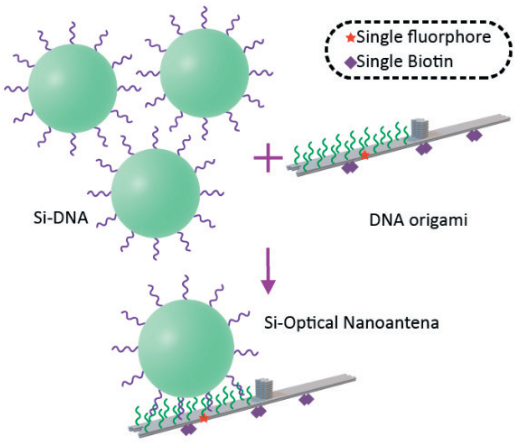

Fig. 1: Assembly of HID-ONAs with DNA origami

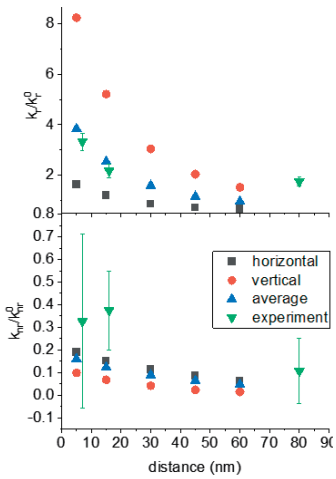

Fig. 2: Distance dependent variation of emitter decay rate

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DNA machines and super-assemblies

Friedrich C. Simmel

Technical University Munich, School of Natural Sciences, Am Coulombwall 4a, 85748 Garching, Germany

Over the past decades DNA nanotechnology has progressed significantly, today enabling the construction of almost arbitrarily shaped structures with nanometer precision. Among the forthcoming challenges are the creation of increasingly dynamic and machine-like assemblies, the efficient generation of complex super-assemblies from DNA-based building blocks, and the interfacing of nanostructures with biological systems. In the talk, we will discuss recent advances from our laboratory in these areas. Specifically, we will detail the development of electrically driven "nanorobotic" DNA devices and motors. Additionally, we will explore the realization of cell-scale compartment constructed from lipid-inspired DNA origami building blocks.

Dynamic Dissipative, Transient DNA-Networks and Nanostructures: From Basic Concepts to Applications

Itamar Willner

Institute of Chemistry and The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Many biological transformations, such as cell proliferation, motility and gene expression are driven by complex networks revealing adaptation, hierarchical adaptation, and characterized by complex reaction mechanisms, involving signal promoted switchable, oscillatory, bistable and branching process. Emulating these biological networks by artificial means would advance the areas of Systems Chemistry.

In the past years, we developed dynamic stimuli-responsive networks. While early studies emphasized the assembly of constitutional dynamic networks, CDNs, representing signal-triggered thermodynamically re-equilibrated networks, recent studies are directed to the development of transient, out-of-equilibrium, dissipative networks and circuitries. The talk will address the development of transient DNA frameworks and their applications:

1. A short survey of dynamically-reconfigured constitutional dynamic networks and their applications will be introduced.[1, 2]
2. The concept of transient, dissipative reaction circuits will be presented by introducing different triggers operating such dynamic frameworks (enzymes, DNAzymes, light).[3, 4] The application of such reaction modules for operating transient biocatalysis,[5] transient aggregation/deaggregation of nanoparticles.[6] In particular, coupling of transient rection modules to constitutional dynamic networks and the triggered operation of transient biocatalytic cascades will be presented. The concept will be applied for transient CND-guided orthogonally-modulated coagulation of fibrinogen to fibrin in the presence of thrombin.[7]
3. The engineering of transient transcription machineries will be introduced and the application of the transcription machinery as a functional framework for temporal modulation of thrombin-induced coagulation of fibrinogen to fibrin.[8]
4. The application of transient reaction modules for the assembly of DNA microdroplets and a “protocell” microdroplet framework will be introduced and the future directions of functional transient DNA materials will be addressed.

[1]. Wang, J.; Li, Z.; Willner, I., Dynamic Reconfigurable DNA Nanostructures, Networks and Materials. *Angew. Chem. Int. Ed.* 2023, **62,** e202215332.
[2]. Yue, L.; Wang, S.; Zhou, Z.; Willner, I., Nucleic Acid Based Constitutional Dynamic Networks: From Basic Principles to Applications. *J. Am. Chem. Soc.* 2020, **142,** 21577-21594.
[3]. Li, Z.; Wang, J.; Willner, I., Transient Out-of-Equilibrium Nucleic Acid-Based Dissipative Networks and Their Applications. *Adv. Funct. Mater.* 2022, **32,** 2200799.
[4]. Zhou, Z.; Ouyang, Y.; Wang, J.; Willner, I., Dissipative Gated and Cascaded DNA Networks. *J. Am. Chem. Soc.* 2021, **143,** 5071-5079.
[5]. Ouyang, Y.; Zhang, P.; Willner, I., Dissipative biocatalytic cascades and gated transient biocatalytic cascades driven by nucleic acid networks. *Sci. Adv.* 2022, **8,** eabn3534.
[6]. Ouyang, Y.; Zhang, P.; Manis-Levy, H.; Paltiel, Y.; Willner, I., Transient Dissipative Optical Properties of Aggregated Au Nanoparticles, CdSe/ZnS Quantum Dots, and Supramolecular Nucleic Acid-Stabilized Ag Nanoclusters. *J. Am. Chem. Soc.* 2021, **143,** 17622-17632.
[7]. Ouyang, Y.; Dong, J.; Willner, I., Dynamic DNA Networks-Guided Directional and Orthogonal Transient Biocatalytic Cascades. *J. Am. Chem. Soc.* 2023, **145,** 22135-22149.
[8]. Dong, J.; Willner, I., Transient Transcription Machineries Modulate Dynamic Functions of G-Quadruplexes: Temporal Regulation of Biocatalytic Circuits, Gene Replication and Transcription. *Angew. Chem. Int. Ed.* 2023, **62,** e202307898.

DNA-templated Nanofabrication of CdS-Au Heterojunctions with Electrical Characterization

Adam T. Woolley, Chao Pang, Benjamin T. Karlinsey, Megan Ward, Roger G. Harrison, and Robert C. Davis

Brigham Young University, Provo, UT 84602, USA

DNA-templated nanofabrication presents an innovative approach for self-assembly of metal-semiconductor interfaces that hold importance in advancing nanoelectronics. Herein, we report the successful contruction of n-type semiconductor–metal junctions using a DNA origami scaffold. The scaffold consists of a linear architecture, with a width of ~17 nm and length of ~410 nm; the DNA origami also contains protruding single-stranded DNA segments to allow localization of DNA-coated nanorods (NRs).

We used a competitive binding approach in arranging Au and CdS AuNRs onto these DNA origami on surfaces to form heterojunctions. We further carried out electroless Au plating on the assembled NRs to electrically connect the Au and CdS. We then utilized atomic force microscopy and scanning electron microscopy to characterize these nanostructures.

We performed electron beam induced tungsten deposition to create conductive connections between these DNA-templated nanostructures and micropatterned gold pads, to allow electrical testing. When we measured the current vs. voltage response of these Au-CdS nanostructures, we observed nonlinear, Schottky-like behavior. These nanostructures also sustained much higher electrical currents than the Au–Te junctions we created previously [1,2].

This research establishes the ability to assemble metal and n-type semiconductor NRs on DNA templates, with potential nanoelectronic functionality. This approach also offers considerable promise for future development to create DNA-based nanoscale circuits.

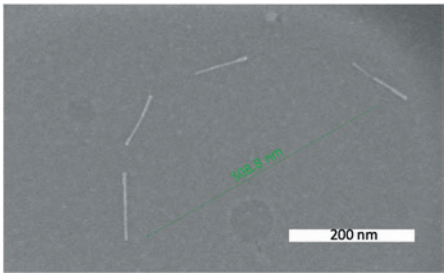


Fig. 1

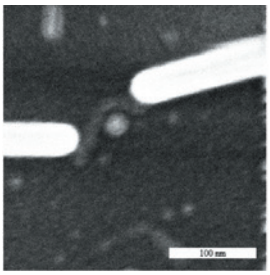


Fig. 2

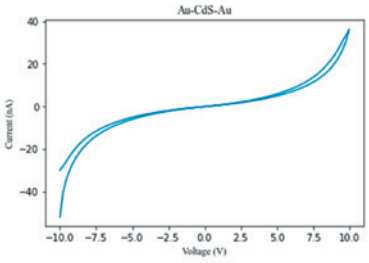


Fig. 3

Fig. 1: SEM of DNA-coated CdS NRs on DNA origami
Fig. 2: SEM of patterned traces to a Au-CdS-Au junction
Fig. 3: I-V curve for the Au-CdS-Au junction shown in Fig. 2

[1] B.R. Aryal et al. (2020) Nano Res. 13, 1419-1426.
[2] B.R. Aryal et al. (2021) ACS. Appl. Nano Mater. 4, 9094-9103.

POSTER PRESENTATIONS

01 **Probing few-molecule chemical reactions using DNA-origami-assembled nanoparticle dimers** // Christina Beresowski (Potsdam)

02 **Peroxidase mimicking DNazyme-based melatonin detection** // Burhan Bora (Izmir)

03 **The Design of Nucleic Acid-Amino Acid Hybrid Residues** // Alexander Corbett (Cambridge)

04 **Microfluidic PCR and network analysis reveals complex tick-borne pathogen interactions in the tropics** // Belkis Corona-Gonzalez (Havana)

05 **Investigation of the multivalency effect of vancomycin-conjugated nanoantibiotics on antibacterial activity** // Özge Coskuner (Paderborn)

06 **Highly Modified DNA That Can Be Read Accurately** // Ellie Dimmick (Newcastle)

07 **Development of aptamer-based multiplex assays for the on-site determination of micropollutants using plasmonic microarrays** // Lukas Dubbert (Jena)

08 **DNA Origami Enzymatic Nanofactories** // Manar Elnagar (Munich)

09 **Large-Scale Formation of DNA Origami Lattices on Silicon** // Heini Järvinen (Jyväskylä)

10 **Method for zinc finger protein-mediated of gold nanoparticle colorimetry and its application in target molecule detection** // Kyung-Min Kim (Seoul)

11 **Strand Displacement Reactions Beyond Room Temperature** // Naveen Kumar (Leipzig)

12 **Rapid discovery of hormone-specific ssDNA aptamers using gold nanoparticle-facilitated assembly of supernatant transfer** // Jeong Min Lee (Seoul)

13 **Long-Term Stable DNA Origami Nanostructures for Next Generation Molecular Data Storage (NEO)** // Xinyang Li (Paderborn)

14 **Surface Plasmon Resonance Biosensing of Biologically important nucleic acids using Exponational Amplification Reaction and pH-induced Triplex DNA Nanoswitches** // Pei-Ying Lin (Taipei)

15 **Membrane rigidity recognition by DNA origami nanostructures** // Rayehe Mamaghaniyeh (Leipzig)

16 **Thermally Switchable DNA Origami Nanopore – A Single Molecule Sensing Device** // Aleksei Overchenko (Leipzig)

17 **Ligand exchange on gold nanotriangle surface as an effective approach for the development of bioconjugation methods** // Olesia Petrova, Ekaterina Podlesnaia (Jena)

18 **Dynamics of infections in cattle and Rhipicephalus microplus: A preliminary study** // Elianne Piloto-Sardiñas (Havana)

19 Cation-Dependent Assembly of Hexagonal DNA Origami Lattices on SiO_2 Surfaces // Bhanu Kiran Pothineni (Paderborn)

20 Guided Evolution of DNA-Based Microdroplets Acting as Protocells // Yunlong Qin (Jerusalem)

21 Stability of DNA origami nanostructures in the presence of ROS // Lukas Rabbe (Paderborn)

22 Towards metasurfaces by DNA-assisted lithography // Toni Rautio (Jyväskylä)

23 High-intensity UV laser crosslinking for the study of DNA-protein interactions // Tino Schenk (Jena)

24 Characterization of DNA-Protein interactions utilizing localized surface plasmon resonance spectroscopy // Florian Seier (Jena)

25 Effect of hydrophobicity of lipid anchor functionalization on DNA origami binding to lipid membranes // Subhasini Singh (Leipzig)

26 Quantitative Analysis of Mg2+ Binding to DNA Origami Nanostructures // Emilia Tomm (Paderborn)

27 Tube-shaped assemblies made of DNA-origami-lattices // Jussi Toppari (Jyväskylä)

28 Evaluation of a duplex PCR assay for the detection of Salmonella spp. and Staphylococcus aureus in raw milk // Odalys Uffo-Reinosa (Havana)

29 Fully addressable, designer superstructures assembled from a single modular DNA origami // Johann Weck (Munich)

30 A DNA nanoreactor for the bioelectrocatalytic conversion of CO_2 into methanol // Xiaodan Xu (Paderborn)

Probing few-molecule chemical reactions using DNA-origami-assembled nanoparticle dimers

Christina Beresowski, Sergio Kogikoski, Ilko Bald
Institute of Chemistry, University of Potsdam, Karl-Liebknecht-Straße 24-25, 14476 Potsdam Germany

Due to its versatile characteristics, DNA origami structures demonstrate the capability to precisely position plasmonic metal nanoparticles in close proximity.These resultant dimeric configurations can subsequently serve as valuable tools for investigating plasmon-induced chemical reactions at the level of individual to few molecules. In this study, we employ a DNA origami nanofork as a plasmonic nanoantenna [1] to investigate the dimerization process of 4-nitrothiphenol. Notably, two distinct systems were examined, involving the construction of DNA origami nanoforks with 60 nm gold nanoparticles and 60 nm silver nanoparticles, respectively. The nanoparticles where modified with NTP and DNA in a ratio of 1:1.

The AuNP-DNA origami nanostructures (AuNP-DONAs), measured at a wavelength of 633 nm, revealed no notable spectral changes at 1.5 mW, implying an absence of NTP to DMAB dimerization.

In contrast, the Ag-DONAs, measured at 532 nm, exhibited spectral changes at a lower laser power of 850 µW, suggesting the potential occurrence of dimerization. Specific bands for DMAB where found at 1136 cm^{-1}, 1394 cm^{-1} and at 145 cm^{-1} [2]. Nonetheless, these alterations exhibited a transient nature, leading us to believe that molecular fluctuations occur on the surface and within the hotspot. This dynamic behavior is elucidated in the surface-enhanced Raman spectroscopy (SERS) spectrum, as depicted in Figure 2.

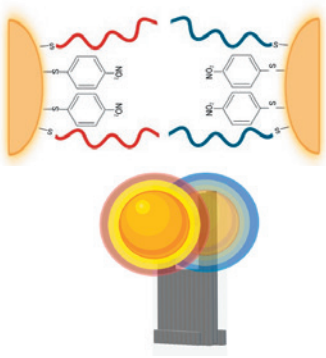


Fig. 1

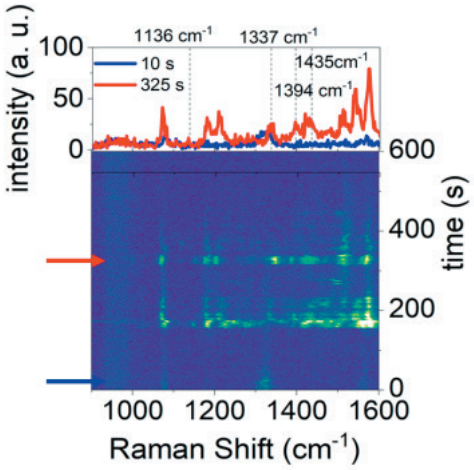


Fig. 2

Fig. 1: schematic figure of the DONA structure
Fig. 2: SERS spectra of a 60 nm Ag-DONA measured at 532 nm (0.85 mW)

[1] Tapio et al. ACS Nano 2021, 15, 7065−707.
[2] Choi et al.,J. Am. Chem. Soc. 2016, 138, 4673−4684.

Peroxidase mimicking DNazyme-based melatonin detection

Burhan Bora, Elvin Turgut

Ege University Faculty of Science Department of Biochemistry, İzmir, Türkiye

Melatonin is an immun-modulator, anti-oxidant and anti inflammatory indol hormone. Regulation of circadian rhythm, tumour suppression, regulation of blood pressure are some of the biological activities of melatonin hormone. It was also evidenced that melatonin has a broad range of radical scavenging activity. Melatonin is the most reliable biomarker for circadian rhythm sleep disorder. Some animal and vegetable foods are rich in melatonin content. Regarding the biological activities mentioned above, reliable detection of melatonin in biological samples is of great importance. Currently available detection methods are restricted to LC-MS, HPLC and ELISA methods. Aptamers are single-stranded DNA or RNA molecules that are able to recognize their target with good affinity and selectivity. Metal ions, small organic molecules, proteins and cells are some of the targets that aptamers have been developed for. Recently, aptamer gold nanoparticle based assay for melatonin detection in saliva samples was developed. Peroxidase mimicking DNazyme activity is also an attracting assay for development of DNA-based colorimetric, fluorometric or electrochemical biosensors for detection of analytes in different samples.

In this study, we have tested intrinsic peroxidase-mimicking DNazyme activity of melatonin aptamer. Surprisingly, melatonin aptamer showed a light response to melatonin levels in buffer solution. In order to improve the response of peroxidase-mimicking activity of melatonin aptamer, we have designed novel melatonin aptamer-G Quadruplex structures. Among the designed structures, we were able to develop a colorimetric method to sense melatonin down to sub-micromolar range. DNazyme-based colorimetric assay was also available to determine melatonin levels in food samples. Even though currently developed method is still not sensitive enough to detect biologically available levels of melatonin, this is the first example of modifying intrinsic peroxidase-mimicking DNazyme activity to detect the target analyte. This method can be improved to modify the intrinsic DNazyme activity of aptamers and can also be manipulated for the selection of intrinsic DNazyme activity aptamers.

The Design of Nucleic Acid-Amino Acid Hybrid Residues

Alexander L. Corbett, Ljiljana Fruk
Department of Chemical Engineering and Biotechnology, Cambridge, UK

Nucleic acids have played an important role in the development of useful bionano tools due to their ability to regularly self-assemble into higher order structures thanks to their complimentary bases. Although useful as structural elements, these bases provide very little diversity in terms of their chemical functionality thus limiting the applications of artificial nucleic acid nanostructures. One way to engineer novel functional DNA nanostructures is to expand the functionalities of the main building blocks.

In an attempt to introduce new functional groups to core nucleic acids, we employed a Corey-House inspired non-catalytic organocuprate reaction to attach aliphatic side chains to the 1' position of deoxyribose. Contrary to previously employed strategies, this reaction is straightforward, including fewer steps [1] or using materials that are significantly less hazardous [2]. This greener procedure has allowed us to create a small library of nucleoside analogue structures with potential to further expand the toolbox.

These new residues, which can be incorporated within synthetic DNA strands, will allow us to design and study new DNA nanostructures. For example, the introduction of natural and artificial amino acids side chains might results in three-dimensional mimics of enzyme active sites further expanding the DNAzymes space.

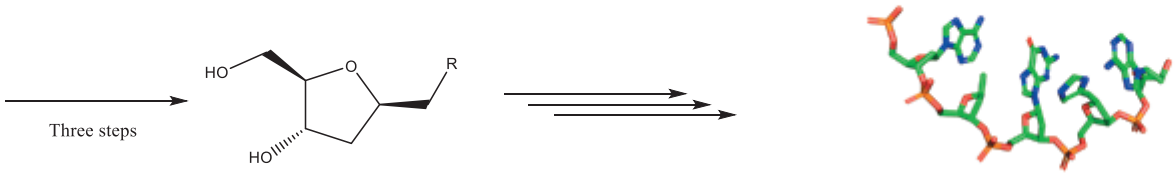


Fig1. Structure of aliphatic nucleic acid residue analogues individually and conceptually in a DNA strand.

[1] Weizman & Tor (2001) J Am Chem Soc, 123, 3375-3376.
[2] Chaudhuri & Kool (1995). Tetrahedron Lett, 37, 1795-1798

Microfluidic PCR and network analysis reveals complex tick-borne pathogen interactions in the tropics

Cristian Diaz-Corona[1,2,] Lisset Roblejo-Arias[1], Elianne Piloto-Sardiñas[1,2], Adrian A. Díaz-Sanchez[3], Angelique Foucault-Simonin[2], Clemence Galon[2], Alejandra Wu-Chuang[2], Lourdes Mateos-Hernandez[2], Zbigniew Zając[4], Joanna Kulisz[4], Aneta Wozniak[4], Maria Karla Castro-Montes de Oca[1], Evelyn Lobo-Rivero[1], Dasiel Obregon[5], Sara Moutailler[2], Belkis Corona-Gonzalez[1], Alejandro Cabezas-Cruz[2]

[1] Direction of Animal Health, National Center for Animal and Plant Health, Carretera de Tapaste y Autopista Nacional, Apartado Postal 10, 32700 San José de Las Lajas, Mayabeque, Cuba.
[2] UMR BIPAR, Laboratoire de Sante Animale, ANSES, INRAE, Ecole Nationale Veterinaire d'Alfort, 94700 Maisons-Alfort, France.
[3] Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK S7N 5E2, Canada.
[4] Department of Biology and Parasitology, Medical University of Lublin, Radziwiłłowska 11 St, 20-080 Lublin, Poland.
[5] School of Environmental Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada.

Ixodid ticks, particularly *Rhipicephalus sanguineus* s.l., are important vectors of various disease causing agents in dogs and humans in Cuba. However, our understading of interactions among tick-borne pathogens (TBPs) in infected dogs or the vector *R. sanguineus* s.l. remains limited. This study integrates microfluidic-based high-throughput real-time PCR data, Yule's Q statistic, and network analysis to elucidate pathogen-pathogen interactions in dogs and ticks in tropical western Cuba. A cross-sectional study involving 46 client-owned dogs was conducted. Blood samples were collected from these dogs, and ticks infesting the same dogs were morphologically and molecularly identified. Nucleic acids were extracted from both canine blood and tick samples. Microfluidic-based high-throughput real-time PCR was employed to detect 25 bacterial species, 10 parasite species, 6 bacterial genera, and 4 parasite taxa, as well as to confirm the identity of the collected ticks. Validation was performed through end-point PCR assays and DNA sequencing analysis. Yule's Q statistic and network analysis were used to analyse the associations between different TBP species based on binary presence-absence data. The study revealed a high prevalence of TBPs in both dogs and *R. sanguineus* s.l., the only tick species found on the dogs. *Hepatozoon canis* and *Ehrlichia canis* were among the most common pathogens detected. Co-infections were observed, notably between *E. canis* and *H. canis*. Significant correlations were found between the presence of *Anaplasma platys* and *H. canis* in both dogs and ticks. A complex co-occurrence network among haemoparasite species was identified, highlighting potential facilitative and inhibitory roles. Notably, *H. canis* was found as a highly interconnected node, exhibiting significant positive associations with various taxa, including *A. platys*, and *E. canis*, suggesting facilitative interactions among these pathogens. Phylogenetic analysis showed genetic diversity in the detected TBPs. Overall, this research enhances our understanding of TBPs in Cuba, providing insights into their prevalence, associations, and genetic diversity, with implications for disease surveillance and management.

Investigation of the multivalency effect of vancomycin-conjugated nanoantibiotics on antibacterial activity

Özge Coskuner,[1] Ulrike Hofmann,[2] Bhanu Kiran Pothineni,[1] Christin Möser,[3] Guido Grundmeier,[1] David M. Smith,[3] Yixin Zhang,[2] Adrian Keller[1]

[1]Technical and Macromolecular Chemistry, Paderborn University, Warburger-Straße 100, 33098 Paderborn, Germany

[2]B CUBE – Center for Molecular Bioengineering, Technische Universität Dresden, Arnoldstr. 18, 01307 Dresden, Germany

[3]DNA Nanodevices Unit, Department DiagnosticsFraunhofer Institute for Cell Therapy and Immunology IZI, 04103 Leipzig, Germany

Vancomycin is a widely used glycopeptide antibiotic against multidrug-resistant Gram-positive bacteria [1]. Its effectiveness against Gram-positive bacteria stems from preventing the cross-linking of the precursor peptidoglycans by the transpeptidase enzyme via binding with five hydrogen bonds to the C-terminal D-Ala-D-Ala precursor of peptidoglycan [2]. Due to the emergence of vancomycin-resistant strains in the 1980s, many studies focused on nanoparticle formulations of vancomycin [1].

The multivalency phenomenon plays a crucial role in improving new formulations to create molecules to have multiple binding sites to interact with targets [3] In a previous study vancomycin-conjugated magnetic nanoparticles (NPs) with high local vancomycin density showed an increase in affinity to the bacterial binding targets compared to free-vancomycin [4].

In this study, we present the vancomycin decoration of the Rothemund triangle DNA origami (RTO). DNA origami is an ideal engineering tool to study multivalent interactions and super-selective binding in low-valency systems with several advantages such as precise and multivalent spatial control, customizability, high resolution, stability, rigidity, and biocompatibility [3]. Conjugation was achieved by coupling azide-vancomycin to selected amine-modified staple strands using SPAAC and integrating them into RTO. Conjugation was confirmed with Native PAGE, UV-Vis analysis, and HPLC. Designs ranged from octodocavalent to hexatriacontavalent (Figure 1). Minimum inhibitory concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) against *Bacillus subtilis* of 0.6 µg/mL were determined for each design, representing half of the MIC and a quarter of the MBC of free vancomycin. This shows that the effect of multivalency can be used to increase the potency of established antibiotics.

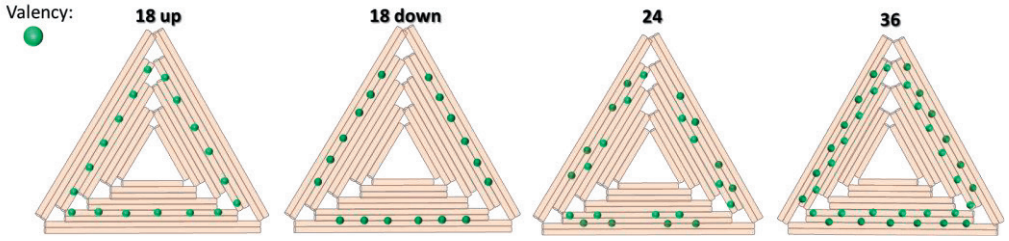


Fig. 1

Fig. 1: Schematic from octodo- to hexatriacontavalent designs.

[1] Pothineni et al. (2023). Adv. NanoBiomed Res, 3, 2200134
[2] Ashford et al. (2012). Chem Soc Rev, 41, 957-978
[3] Bila et el. (2022). J. Am. Chem. Soc, 144, 21576-21586
[4] Hassan et al. (2017). Bioconjugate Chem, 28, 353-361

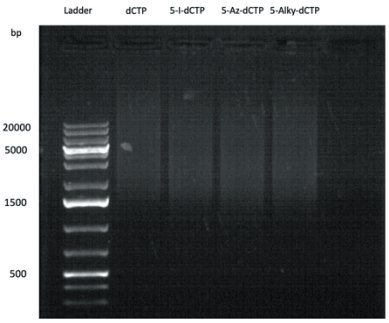
Highly Modified DNA That Can Be Read Accurately

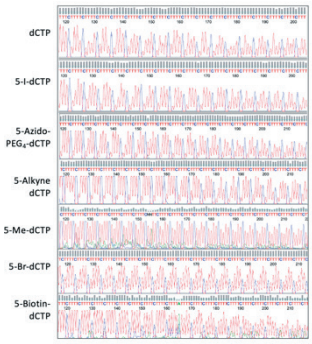
Ellie Dimmick, Tom McAllister and Andrew Pike

Newcastle University Department of Chemistry, United Kingdom

Most sequencing techniques are not capable of capturing highly modified DNA strands, but single-molecule techniques such as nanopore sequencing, which measure a change in current across a pore when DNA is translocated through, have the potential to retrieve modified base information (Burdick et al., 2023). Nanopore sequencing records a signal for four bases simultaneously but using defined 'training' sequences these can be interpreted at single-base resolution. The location of endogenous base modifications such as cytosine methylation can be detected already (Simpson et al., 2017), but this does not extend to non-canonical bases such as 5-iodo-dCTP or 5-azido-PEG_4-dCTP.

The extension method, outlined by Whitfield et al., uses an oligo-seed approach to produce large, highly repetitive, defined DNA sequences. These contain regularly spaced modified cytosine bases which can act as training sets to develop an algorithm for modified base decoding of nanopore datasets. $[A_4G]/[T_4C]_n$ is of primary interest currently, but we hope to expand to more complicated sequences in the near future.





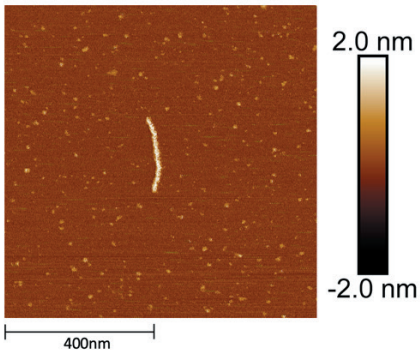


Fig. 1 Fig. 2 Fig. 3

Fig. 1: 1% agarose gel showing successful extension of $[A_4G]/[T_4C]_n$ with modified bases. Lengths ranging from 700bp-14kb after 10 heat-cool cycles.
Fig. 2: Sanger sequencing data from Source Bioscience showing modified bases are base called as regular cytosines.
Fig. 3: AFM Image of an extended DNA molecule, of sequence $[A_4G]/[T_4C]_n$ with regular cytosine bases.

[1] Burdick, J.T., Comai, A., Bruzel, A., Sun, G., Dedon, P.C., Cheung, V.G., 2023. Nanopore-based direct sequencing of RNA transcripts with 10 different modified nucleotides reveals gaps in existing technology. G3: Genes, Genomes, Genetics.

[2] Simpson, J.T., Workman, R.E., Zuzarte, P.C., David, M., Dursi, L.J., Timp, W., 2017. Detecting DNA cytosine methylation using nanopore sequencing. Nat Methods 14, 407–410.

[3] Whitfield, C.J., Turley, A.T., Tuite, E.M., Connolly, B.A., Pike, A.R., 2015. Enzymatic Method for the Synthesis of Long DNA Sequences with Multiple Repeat Units. Angewandte Chemie 127, 9099–9102.

Development of aptamer-based multiplex assays for the on-site determination of micropollutants using plasmonic microarrays

Lukas Dubbert, Andrea Csáki, Wolfgang Fritzsche,
Leibniz Institute of Photonic Technology, Albert-Einstein-Straße 9, 07745 Jena, Germany

We describe the development of an innovative detection method for micropollutants in water based on plasmonic microarrays using aptamers.
Micropollutants are persistent contaminants in a very low concentration found in water bodies, posing risks to both human health and the environment. These pollutants can originate from various sources including industrial processes, agricultural wastewater, pharmaceuticals, and personal care products. Due to their size and chemical composition, micropollutants can be difficult to remove through conventional water treatment methods [1], necessitating advanced technologies and rigorous monitoring to mitigate their impact.
Aptamers are short, single-stranded DNA or RNA molecules that can bind to specific target molecules with high affinity and specificity, similar to antibodies. Aptamers are produced *in vitro* in a simple and inexpensive process called SELEX. This eliminates the need for animal testing, which means that toxic or less immunogenic target structures and non-physiological conditions can also be used. [2]

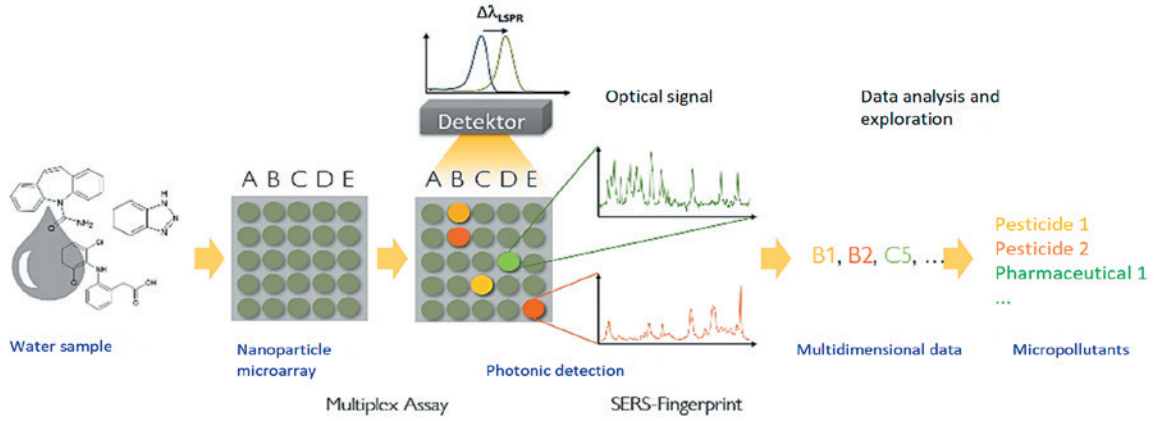


Figure 1: Concept of the Thesis

In LSPR-based biosensors, the transducer typically consists of metallic nanoparticles, such as gold nanoparticles, that exhibit localized surface plasmon resonance. When the recognition element (or receptor, immobilized on the nanoparticles) binds the target analyte present in solution, it induces a change in the refractive index near the metallic nanoparticles, leading to a shift in the LSPR wavelength. This shift is measured as sensor signal [3]
Together with partners at Comenius University Bratislava, we have combined LSPR with QCM [4]. QCM (Quartz Crystal Microbalance) and LSPR (Localized Surface Plasmon Resonance) are both sensing techniques used in biosensing applications. While QCM measures changes in mass by detecting frequency shifts in a quartz crystal, LSPR relies on localized electromagnetic field changes near metallic nanoparticles to detect biomolecular interactions. The combination allows for the simultaneous measurement of this complementary analytical information provided by both methods.

[1] Schmoldt, A.; Benthe, H. F.; Haberland, G. (1975): Digitoxin metabolism by rat liver microsomes. In: Biochemical pharmacology 24
[2] Zhou, Jiehua; Rossi, John (2017): Aptamers as targeted therapeutics: current potential and challenges. In: Nature reviews. Drug discovery 16 (3), S. 181–202. DOI: 10.1038/nrd.2016.199.
[3] Kastner, Stephan; Dietel, Anne-Kathrin; Seier, Florian; Ghosh, Shaunak; Weiß, Daniel; Makarewicz, Oliwia et al. (2023): LSPR-Based Biosensing Enables the Detection of Antimicrobial Resistance Genes. e2207953. DOI: 10.1002/smll.202207953.
[4] Spagnolo S, Muckley ES, Ivanov IN, Hianik T. Application of Multiharmonic QCM-D for Detection of Plasmin at Hydrophobic Surfaces Modified by β-Casein. Chemosensors. 2022; 10(4):143. https://doi.org/10.3390/chemosensors10040143

DNA Origami Enzymatic Nanofactories

Manar Elnagar, Amelie Heuer-Jungemann
Max Planck Institute for Biological Intelligence, Am Klopferspitz 18, 82152 Planegg, Germany

Precise addressability of DNA nanostructures enables self-assembly of functional complex systems. Particularly, using the DNA origami fabrication method has gained special interest as it allows for the production of complex structures with different sizes, shapes, and features. As such, DNA origami is used as host for enzyme molecules in order to modulate their activity [1]. This offers a platform to explore biological mechanisms and utilize their potential for various applications such as catalysis. Here we show the effect of immobilization and encapsulation on the activity of α.chymotrypsin (α.chy) in silicified DNA origami barrels, and discuss how biological activity could be enhanced via microenvironment engineering.

To test α.chy activity change in its free state vs within DNA barrels, we used a strand displacement reaction, releasing the enzyme from the DNA. Activity assessment based on the proteolytic cleavage of a short peptide substrate [2] showed an impressive activity difference of 30.08 % ± 7.9. Encapsulation in silicified barrels, interestingly showed an even higher increase in activity.

It has been proposed in literature that enhanced activity of immobilized enzymes in DNA origami could be attributed to the acidic microenvironment contributed by the negative charge of DNA surface [3]. However, contrasting to this, we found that α.chy has a preferential basic pH of 9, strongly suggesting that the effect of pH or surface charge inferred by the silicified DNA origami is not the main factor resulting in the enhanced enzymatic activity.

Instead, the overall confinement effect appears to be a major factor, as can be seen when comparing enzymes immobilized on a one-layer sheet origami vs. encapsulation in barrel-shaped structures.

All in all, our studies aid to shed light on the effect of encapsulation in silicified DNA origami on the enhancement of enzymatic activity.

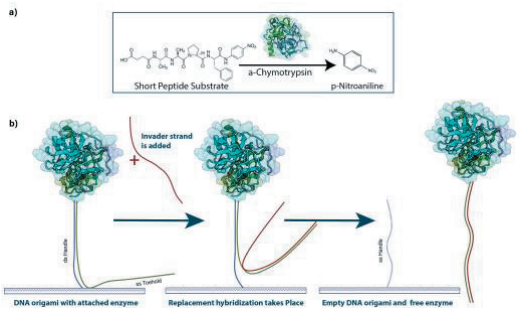


Fig. 1

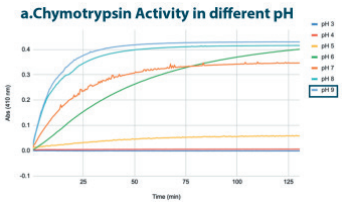


Fig. 2

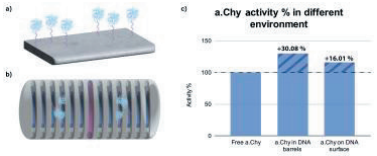


Fig. 3

Fig. 1: a) a.chy proteolytic cleavage of short peptide substrate, releasing p-nitroaniline that is calorimetrically measured via microplate reader, b) releasing immobilized a.chy from DNA origami via strand displacement reaction.
Fig. 2: pH dependence of α.chy activity shows highest activity at pH 9.
Fig. 3: a) a.chy assembly on DNA origami one layer sheet, b) a.chy immobilization in DNA origami barrels, c) activity difference of a.chy measured kinetically showing double activity difference for a.chy when immobilized in DNA barrels vs on 1LS.

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Large-Scale Formation of DNA Origami Lattices on Silicon

Kosti Tapio[1], Charlotte Kielar[2], Johannes M. Parikka[1], Adrian Keller[3], Heini Järvinen[1], Karim Fahmy[2], Jussi J. Toppari[1]

[1]Department of Physics and Nanoscience Center, 40014 University of Jyväskylä, Finland
[2]Institute of Resource Ecology Helmholtz-Zentrum Dresden-Rossendorf (HZDR), 01328 Dresden, Germany
[3]Technical and Macromolecular Chemistry, Paderborn University, 33098 Paderborn, Germany

In recent years, hierarchical nanostructures have found applications in fields like diagnostics, medicine, nano-optics, and nanoelectronics, especially in challenging applications like the creation of metasurfaces with unique optical properties [1]. One of the promising materials to fabricate such nanostructures has been DNA due to its robust self-assembly properties and plethora of different functionalization schemes [2].

Here, we demonstrate the assembly of a two-dimensional fishnet-type lattice on a silicon substrate using cross-shaped DNA origami as the building block, *i.e.*, tile [3]. The effects of different environmental and structural factors are investigated under liquid atomic force microscopy (AFM) to optimize the lattice assembly. Effects of incubation time and sodium concentration at 35 °C temperature is shown in figure 1. Furthermore, the arm-to-arm binding affinity of the tiles is analyzed, revealing preferential orientations. From the liquid AFM results, we developed a methodology to produce closely spaced DNA origami lattices on silicon substrate. Similar lattices have been formed on the common mica substrate [4], but the special importance of silicon is that it allows further nanofabrication process steps, such as metallization. This formed polycrystalline lattice has high surface coverage and is extendable to the wafer scale with an average domain size of about a micrometer. Further studies are going on to increase the domain size toward a single-crystalline large-scale lattices. Furthermore, with slight modifications, we have used this approach to make the lattice act as a spacer for creating an ordered array of metallic nanoparticles to be used in LSPR measurements and sensor applications [5].

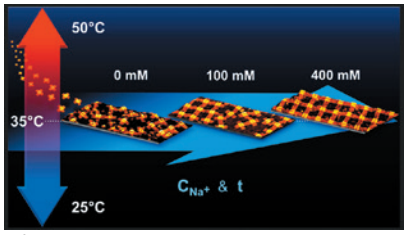

Fig. 1

Fig. 1: Folding ST lattice formation with increasing time and sodium concentration.

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Method for zinc finger protein-mediated of gold nanoparticle colorimetry and its application in target molecule detection

Kyung-Min Kim, Chae Yeon Kim, Yeonju Lee, Jeong Min Lee, Sangwoo Park, and Young-Pil Kim

Department of Life Science, Hanyang University, Seoul, Korea

Gold nanoparticle scatters visible light at a specific range of wavelength due to the inherent plasmonic effect. And the localized surface plasma resonance (LSPR) is used to evaluate the stability and the change of surface properties. Under the changes, it scatters light of longer wavelengths. For example, the increase of salt concentration reduces the surface charge, decreasing stability of the gold nanoparticle and causes colloid aggregation. The stability of the gold nanoparticles may be evaluated through a color change caused by an increase of LSPR wavelength due to aggregation. The stability against salt is changed by the affinities of surfactants constituting the surface of gold nanoparticle or by some biomolecules capping gold nanoparticle. And based on this property, colorimetric bioassays were developed for detection of specific molecules.

It is known that a sensitivity of colorimetric DNA assay is increased under specific salt concentration conditions as the affinity between the surfactant and the gold nanoparticles weakens. However, weak affinity of surfactants increases the possibility of interaction between target molecule and gold nanoparticles, changing the range of salt conditions in which the gold nanoparticles are stabilized. Also, not only target, but also blocking agents reducing non-specific interaction can interfere to the system, making complicated assay environment. And surfactants with low stability suffer from aging during the storage process.

Here, we analyzed the interaction between proteins and gold nanoparticles and tried to apply the zinc finger protein, which effectively aggregates gold nanoparticles strongly stabilized by surfactants, to the colorimetric assay. In addition, through analysis of factors affecting aggregation and molecular dynamic simulation, the interaction mechanism was understood and a method of controlling it was studied.

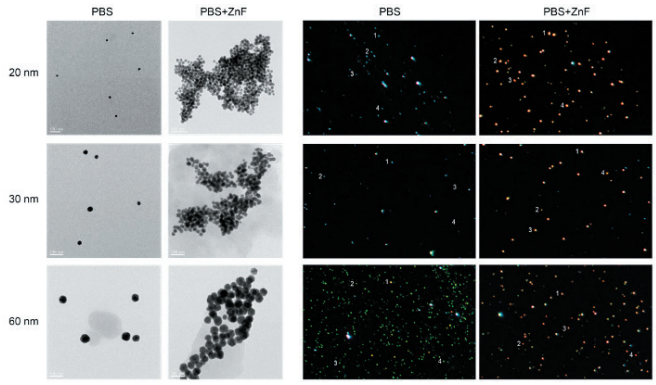

Fig. 1

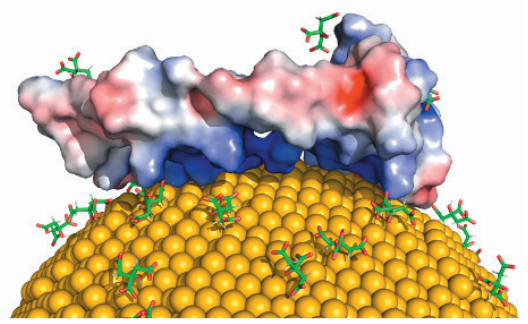

Fig. 2

Fig. 1: TEM and dark-field imaging of aggregated AuNP with zinc finger protein
Fig. 2: Molecular dynamic simulation of interaction between zinc finger and citrate-capped AuNP.

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Strand Displacement Reactions Beyond Room Temperature

*Naveen Kumar[1], Patrick Irmisch[1], Henri G Franquelim[*1], and Ralf Seidel[*1]*

[1]Peter Debye Institute for Soft Matter Physics, University of Leipzig, Leipzig, Germany
Emails: naveen.kumar@uni-leipzig.de, patrick.irmisch@uni-leipzig.de
ralf.seidel@physik.uni-leipzig.de, henri.franquelim@uni-leipzig.de

Toehold-mediated DNA strand displacement reactions have been widely applied to realize dynamic nano-devices and chemical reaction networks [1]. In these reactions, an invader strand binds to the toehold overhang of a target strand, replacing a target-bound incumbent strand (Figure 1A). Typically, a strand displacement reaction system transits into a thermodynamic final state due to the irreversible replacement of the incumbent strand by the invader strand.

Recently, there has been a growing interest in driving these reactions out-of-equilibrium to mimic reaction networks of living systems [2]. This involves incorporating enzymatic processes, such as the enzymatic degradation of nucleic acids strands [3],which often function optimally at physiological temperatures. Notably, most currently established principal reactions and complex reactions networks operate at room temperature. To design and realize complex reaction networks that include dissipative as well as temperature-responsive elements, an extensive understanding of the temperature-dependence of strand displacement reactions is essential, which at present is neither systematically characterized nor completely comprehended.

Here, we systematically investigated the temperature dependence of the strand displacement process by using bulk fluorescence experiments. Interestingly, the temperature dependence of the strand displacement rate constant initially increases but subsequently decreases with rising temperature (see Figure 1B). By employing simple thermodynamic modeling based on a previously established Markov chain model [4], we demonstrate that the temperature dependence is dictated by a dynamic interplay between reversible toehold hybridisation and irreversible strand replacement. We anticipate that our results will be particularly valuable when designing DNA-based chemical reaction networks at elevated and/or changing temperatures.

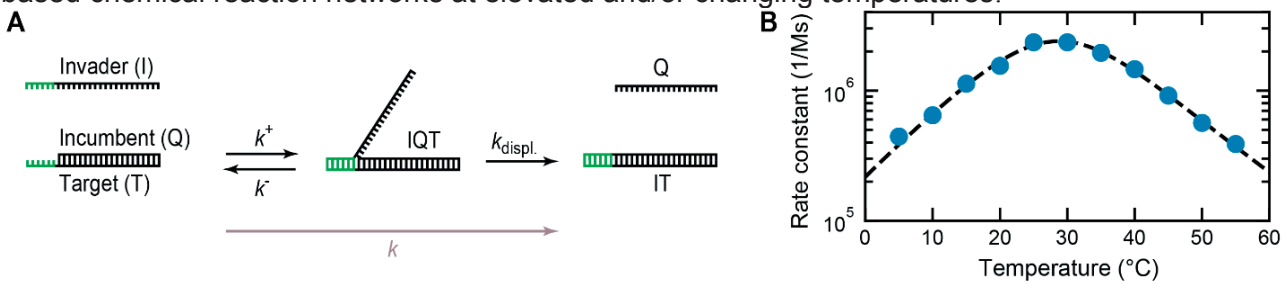

Figure 1: Temperature dependence of the toehold-mediated DNA strand displacement reaction. (**A**) Schematic representation of the strand displacement process. The invader strand reversibly binds to the toehold overhang of the preformed incumbent-target duplex. Displacement of the incumbent leads to the irreversible formation of the invader-target duplex. The displacement reaction can be described by a single second-order rate constant, or by a three state system using three rate constants. (**B**) Second-order rate constant of the strand displacement reaction involving a 6-nucleotide long toehold measured at different temperatures (solid symbols). The dashed line represents a fit of a simple thermodynamic model to the experimental data.

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Rapid discovery of hormone-specific ssDNA aptamers using gold nanoparticle-facilitated assembly of supernatant transfer

Jeong Min Lee, Sai Duc Loc, Eun-Song Lee, Young-Pil Kim

Department of Life Science, Hanyang University, Seoul 04763, Republic of Korea

Single-stranded DNA (ssDNA) aptamers serve as the bioreceptors capable of binding to target molecules via systematic evolution of ligands by exponential enrichment (SELEX) process. We previously reported gold nanoparticle-assisted SELEX (GNP-SELEX) as a visual monitoring platform in/between SELEX rounds, enabling rapid determination of target-specific ssDNA aptamer enrichment without the need for target modification. Despite the advantages of GNP-SELEX, GNP-absorbing ssDNA libraries can often impede the optimal selection of target-specific aptamers. To address this challenge, we introduce a novel platform called GNP-facilitated assembly of supernatant transfer (GNP-FAST). This approach involves pre-screening and pre-selection process preceding the GNP-SELEX to enhance the identification of positive ssDNA aptamer library pools. GNP-FAST entails serial dilution of ssDNA libraries with copious target molecules, employing centrifugation alongside the continuous addition of GNP. Subsequently, target-bound ssDNA libraries remain in the supernatant while unbound ssDNA libraries pellet with the GNP. This process is easily monitored by observing color changes in the pelleted GNP induced by the salt. To demonstrate the usefulness of this method, we employed cortisol, a stress hormone, as a target molecule throughout the GNP-FAST and GNP-SELEX processes. This approach successfully yielded a high-affinity anti-cortisol aptamer, which we then utilized to develop a biosensor. We suggest that this platform offers a practical and efficient strategy for discovering ssDNA aptamers against a wide range of small molecules.

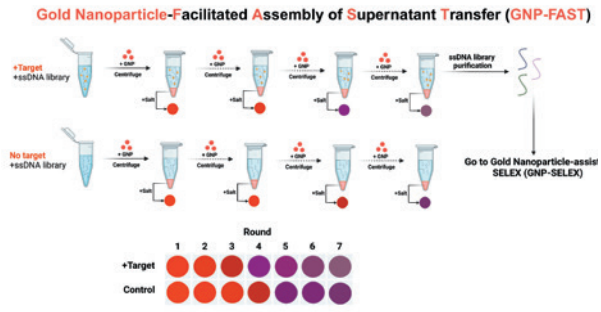

Fig. 1

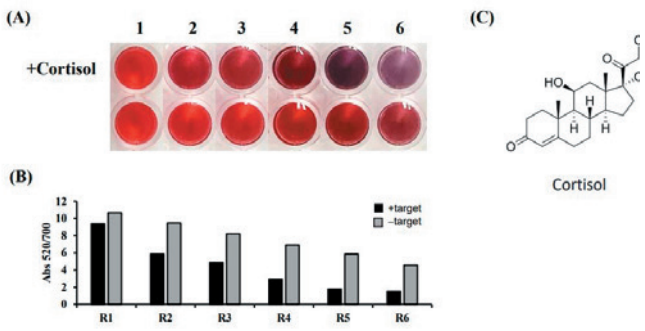

Fig. 2

Fig. 1: Schematic illustration of GNP-FAST.
Fig. 2: Enrichment of the cortisol-bound ssDNA library via the GNP-FAST process. (A) Photograph illustrating the rounds in GNP-FAST in the presence and absence of cortisol. (B) Changes in extinction ratio of GNPs across rounds. (C) Chemical structure of cortisol.

Long-Term Stable DNA Origami Nanostructures for Next Generation Molecular Data Storage (NEO)

Xinyang Li,[1] Lukas Benedict Rabbe,[1] Emilia Tomm,[1] Prof. Guido Grundmeier,[1] Dr. Adrian Keller [1]

[1] Paderborn University, Technical and Macromolecular Chemistry, Warburger Str. 100, 33098 Paderborn, Germany

As the digital era progresses, the exponential growth of data generation presents a significant challenge for traditional storage media, necessitating innovative solutions. The NEO project, a collaborative initiative among Paderborn University, Imperial College London, University of Surrey, KU Leuven, and TU Graz, addresses this challenge by aiming to revolutionize data storage through DNA nanotechnology. At Paderborn University, we explore novel approaches to enhance storage density, efficiency, and longevity. Our tasks focus on optimizing DNA origami [1] designs for higher bit density, refining folding protocols to reduce costs, employing barcoded and arrayed nanostructures for scalable storage solutions, and pioneering methods for long-term dry and cryostorage.

We have designed a rectangular DNA origami nanostructure specifically designed for data storage, that can carry 200 individual bits in the form of bound streptavidin proteins (see Figure 1). However, we encountered challenges in achieving full occupancy of these origami structures with bound proteins. This has underscored the importance of robust statistical analysis and error correction algorithms to ensure data storage reliability and retrieval accuracy. Currently, this design is undergoing initial stability testing, a crucial step to assess its viability for real-world data storage applications. These activities will focus in particular on the stability of the bound proteins during storage, as dissociation or denaturation of the proteins may introduce errors in the read-out of the stored data.

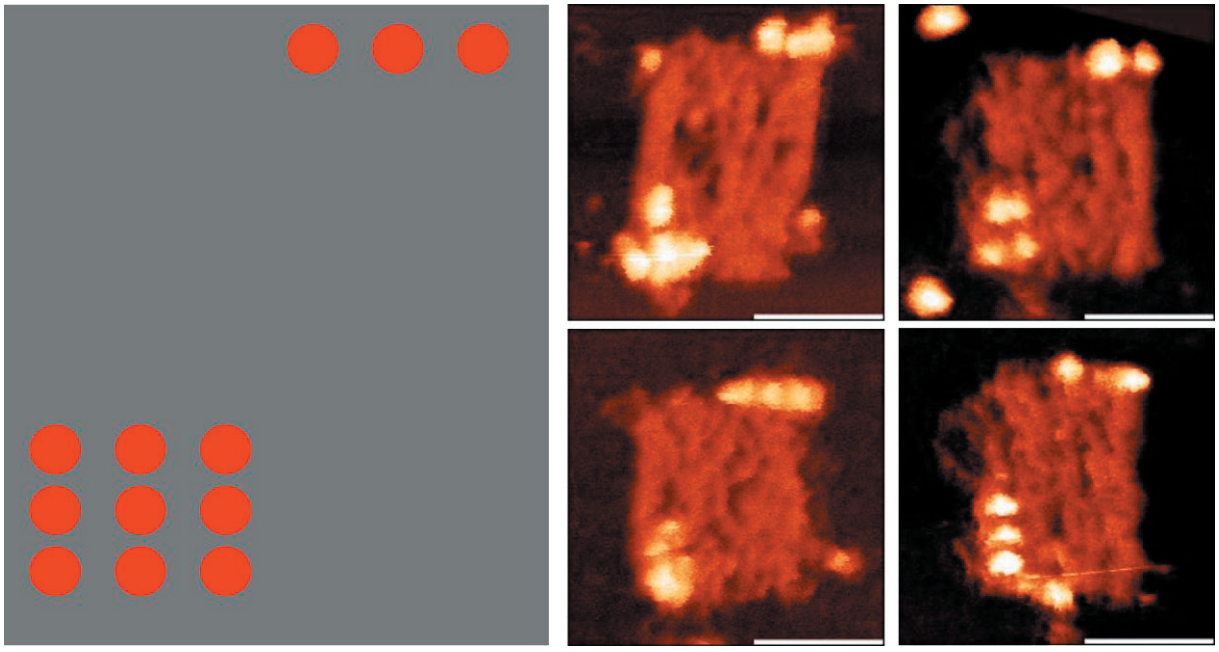

Figure 1: Preliminary Design of Rectangular DNA Origami for Enhanced Data Storage, 50 nm scale bar.

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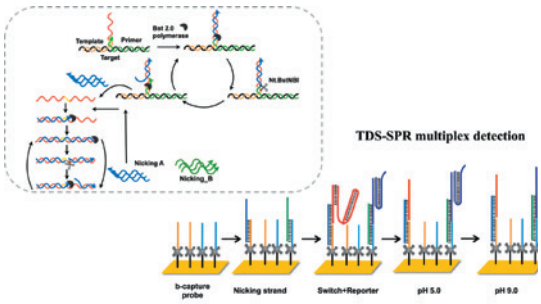
Surface Plasmon Resonance Biosensing of Biologically important nucleic acids using Exponational Amplification Reaction and pH-induced Triplex DNA Nanoswitches

Pei-Ying Lin,[1] Ying-Feng Chang,[2] Ja-an Annie Ho[1,3,4 *]

[1]BioAnalytical Chemistry and Nanobiomedicine Laboratory, Department of Biochemical Science and Technology, National Taiwan University, Taipei, Taiwan
[2] Artificial Intelligence Research Center, Chang Gung University, Taoyuan, Taiwan
[3]Department of Chemistry, National Taiwan University, Taipei, Taiwan
[4]Center for Emerging Materials and Advanced Devices, National Taiwan University, Taipei, Taiwan
*Correspondence

In this study, we introduced triplex DNA nanoswitches (TDS) with distinct pH responsiveness to establish a multiplexed surface plasmon resonance (SPR) biosensing platform (TDS-SPR assay) that could detect two biologically important pieces of nucleic acids. First, a three-way junction (3WJ) probe was employed for target nucleic acid capture, coupled with an Exponential Amplification Reaction (EXPAR) for rapid nucleic acid amplification at a constant temperature. We optimized the primer sequence, enzyme concentration, reaction temperature, and reaction time to achieve the one-pot amplification reaction. Subsequently, pH-responsive TDS were incorporated, enabling the detection of two pieces of nucleic acids based on SPR signal changes at corresponding pH values. This one-pot reaction streamlined experimental steps and time, demonstrating the potential for efficient and dual-target nucleic acid detection. This platform technology, using TDS-assisted SPR systems, presents a user-friendly and promising approach for diverse biomedical applications.



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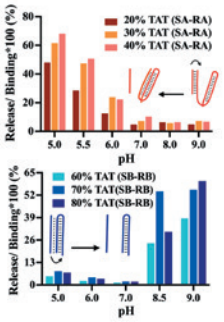

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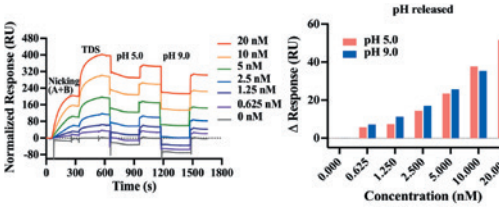

Fig. 3

Fig. 1: Schematic of the TDS-SPR biosensing platform combined with EXPAR for nucleic acids detection
Fig. 2: Optimization of pH-responsive Triplex DNA nanoswitches
Fig. 3: The dose-response of TDS-SPR multiplex detection assay (without EXPAR)

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Membrane rigidity recognition by DNA origami nanostructures

Rayehe Mamaghaniyeh and Henri. G. Franquelim
Leipzig University, Interfaculty Center for Bioactive Matter (b-ACTmatter), Deutscher Platz 5, 04103, Leipzig, Germany

Biological lipid membranes are heterogeneous in composition and are often modeled as combination of liquid-ordered and liquid-disordered lipid phases which differ in rigidity. A variety of cell functions, such as signaling, membrane trafficking, and viral infection, rely under physiological conditions on the existence of lipid domains with specific phase behavior. Additionally, many proteins function at lipid interfaces and their geometrical features are related to their appropriate localization. It is therefore advantageous to controllably target such lipid domains in model and biological membranes for better mimicking and studying cell functions. In this work we propose to develop fluorescently-DNA origami nanostructures modified displaying different types and numbers of lipophilic moieties with selective affinity for specific lipid phases and hence, different lipid environment rigidities. Using confocal laser scanning microscopy, membrane binding of the various modified DNA origami nanostructures was detected on giant unilamellar vesicles (GUVs), which are excellent models for biological membranes with four levels of rigidity. Effective factors such as number of moieties, stiffness of lipid compositions, lipid chain lengths, and DNA origami concentration were studied and efficient nanostructures with domain selection capability were developed. To conclude, these results, will help us to understand the physicochemical rules that govern lipid phase selectivity, and to design useful toolkits for detecting membrane properties for a variety of applications within cell membrane studies.

Thermally Switchable DNA Origami Nanopore – A Single Molecule Sensing Device

Naveen Kumar[1], Aleksei Overchenko[2], Henri G Franquelim[*1], Frank Cichos[*2] and Ralf Seidel[*1]

[1]Peter Debye Institute for Soft Matter Physics, University of Leipzig, Leipzig, Germany
[2]Peter-Debye-Institut für Physik der weichen Materie, University of Leipzig, Leipzig, Germany
Emails: naveen.kumar@uni-leipzig.de, aleksei.overchenko@uni-leipzig.de, henri.franquelim@uni-leipzig.de, cichos@physik.uni-leipzig.de ralf.seidel@physik.uni-leipzig.de,

Nanopore sensing and sequencing hold promise for high-resolution detection and analysis at the single-molecule level, emerging as a competitive and portable technology [1]. Nanopore sensing entails passing molecules through a nanoscale pore while measuring changes in electrical current or optical readouts [2]. As molecules traverse the pore, their unique signatures in current or optical profiles enable applications such as single-molecule detection and DNA sequencing. However, a key problem with DNA sequencing technology using nanopores is that molecules pass through the pore too quickly [3]. Here, DNA Origami and its versatility of nanostructure formation [4,5] offers novel avenues for solving the challenges of nanopore technology.

The DNA Origami structures have relatively rigid characteristics. Control of local temperature in the Origami may allow to modulate the conformation of parts of the Origami and thus the mobility of molecules through pores [6]. To design and realize a hybrid DNA Origami nanopore sensor that includes temperature sensitive elements, an extensive understanding of the effects of the temperature on such systems is essential, which at present is neither systematically characterized nor completely comprehended.

Our study introduces a novel approach to enhance the functionality of DNA Origami nanopores through a thermally switchable mechanism. The proposed concept capitalizes on the programmable nature of DNA Origami to create devices with tunable thermal responsiveness, allowing a precise and triggerable control over the translocation of molecules through the nanopore. As an initial step, we investigated the denaturation of various fluorescently labeled DNA duplexes, using bulk fluorescence experiments, to select best sequences with desired melting temperature ranges. Subsequently, we applied this knowledge to our DNA Origami nanopores to study the release of cargo molecules at the single-molecule level. Overall, we anticipate that our results will be particularly valuable for the design of thermally switchable hybrid plasmonic DNA Origami nanopores devoted to single molecule sequencing technology.

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Ligand exchange on gold nanotriangle surface as an effective approach for the development of bioconjugation methods

Podlesnaia Ekaterina, Petrova Olesia, Csáki Andrea, Fritzsche Wolfgang

Leibniz Institute of Photonic Technology, Albert-Einstein-Straße 9, 07745 Jena, Germany

In recent decades, plasmonic nanoparticles have garnered substantial interest among researchers owing to their unique electronic and optical properties [1]. Thanks to the phenomenon of localized surface plasmon resonance (LSPR), nanoparticles of noble metals are used as optical transducers in sensorics [2-5]. Of particular interest in this field are nanoparticles of anisotropic shapes, such as gold nanotriangles (AuNTs) [6]. Nanoparticles of this shape are promising in increasing the sensitivity due to the generated hotspots of electron density on their sharp vertices. Recently, we reported a time-optimized protocol [7], and developed a procedure utilizing microfluidic platform for the colloidal synthesis of AuNTs [8]. For such syntheses, surfactants are necessarily used to direct the shape formation and stabilize colloids against aggregation. On another hand, the presence of densely packed surfactant bilayer hinders the interaction of AuNTs with biomolecules (e.g., DNA). This motivated us to focus on a shift towards more suitable and biocompatible ligands. Hence, we developed two methods for replacing cetyltrimethylammonium chloride (CTAC) surfactant on the surface of AuNTs with polystyrene sulfonate (PSS) and citrate (Cit) [9]. The first approach involves a one-step procedure for replacing CTAC with PSS polymer, while the second method entails a two-step exchange. The latter employs an ultrathin silver layer capped by a mix of polyvinyl pyrrolidone and polyvinyl alcohol (PVP / PVA) polymeric molecules, followed by a subsequent exchange to biocompatible citrate anions. The results of ligand exchange were examined with ultraviolet–visible spectroscopy (UV-Vis), Fourier-transform infrared (FTIR) spectroscopy, scanning transmission electron microscopy (STEM), dynamic light scattering (DLS), and zeta potential measurements. This functionalization allowed assessing bulk refractive index sensitivity of AuNTs, which highlighted their potential in LSPR-based sensing when compared to previously studied materials. The gained experimental results will serve as a great tool for bioconjugation of AuNTs with single-strand DNA macromolecules or their immobilization on glass-modified substrates for the use in LSPR-based sensing.

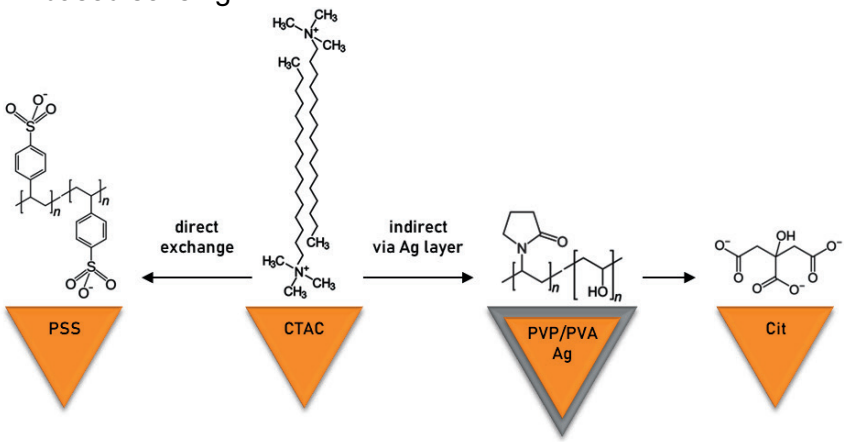

Fig. 1: The pathways of ligand exchange on gold nanotriangle surface

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Dynamics of infections in cattle and *Rhipicephalus microplus*: A preliminary study

Elianne Piloto-Sardiñas[1,2]; Angélique Foucault-Simonin[2]; Alejandra Wu-Chuang[2]; Lourdes Mateos-Hernández[2]; Roxana Marrero-Perera[1]; Lianet Abuin-Denis[2]; Lisset Roblejo-Arias[1]; Cristian Díaz-Corona[1]; Zbigniew Zając[3]; Joanna Kulisz[3]; Aneta Wozniak[3]; Sara Moutailler[2]; Belkis Corona-González[1]; Alejandro Cabezas-Cruz[2].

[1] Direction of Animal Health, National Center for Animal and Plant Health, Carretera de Tapaste y Autopista Nacional, Apartado Postal 10, 32700 San José de las Lajas, Mayabeque, Cuba.
[2] ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, France.
[3] Department of Biology and Parasitology, Medical University of Lublin, Radziwiłłowska 11 st, 20-080, Lublin, Poland.

Tick-borne pathogens (TBPs) pose a significant threat to livestock, including bovine species. This study aimed to investigate TBPs in cattle and ticks across four sampling points, utilizing real-time microfluidic PCR. The results revealed that *Rhipicephalus microplus* ticks were found infesting all animals. Among the detected TBPs in cattle, *Anaplasma marginale* was the most frequently identified, often as a single infection, although mixed infections involving *Rickettsia felis*, uncharacterized *Rickettsia* sp., and *Anaplasma* sp. were also observed. In ticks, A. marginale was predominant, along with *R. felis*, *Rickettsia* sp., and *Ehrlichia* sp. It is noteworthy that although *A. marginale* consistently infected all cattle during various sampling times, this pathogen was not detected in all ticks. This suggests a complex dynamic of pathogen acquisition by ticks. A phylogenetic analysis focused on the identification of *Anaplasma* species using amplified 16S rDNA gene fragments revealed the presence of *A. marginale*, and *Anaplasma platys* strains in bovines. These findings underscore the presence of multiple TBPs in both cattle and ticks, with *A. marginale* being the most prevalent. Understanding the dynamics and phylogenetics of TBPs is crucial for developing effective control strategies to mitigate tick-borne diseases in livestock.

Cation-Dependent Assembly of Hexagonal DNA Origami Lattices on SiO_2 Surfaces

Bhanu Kiran Pothineni,[1] Guido Grundmeier,[1] Adrian Keller

[1]Technical and Macromolecular chemistry, Paderborn University, Warburger Str. 100, 33098 Paderborn, Germany

DNA origami nanostructures [1] have emerged as functional materials for applications in various areas of science. In particular, they are promising templates for the synthesis of functional materials [2]. Here, we investigate the assembly of hexagonal DNA lattices from DNA origami triangles on silicon wafers with hydroxylated surface oxide by time-lapse atomic force microscopy (AFM) [3]. Lattice assembly on the SiO_2 surface is achieved by a competition of monovalent and divalent cations at elevated temperatures. Ca^{2+} is found to be superior to Mg^{2+} in promoting the assembly of ordered lattices, while the presence of Mg^{2+} rather results in DNA origami multilayer formation at the comparably high Na^+ concentrations of 200 to 600 mM. However, even under optimized conditions, the DNA origami lattices assembled on the SiO_2 surface exhibit a lower degree of order than comparable lattices on mica surfaces.

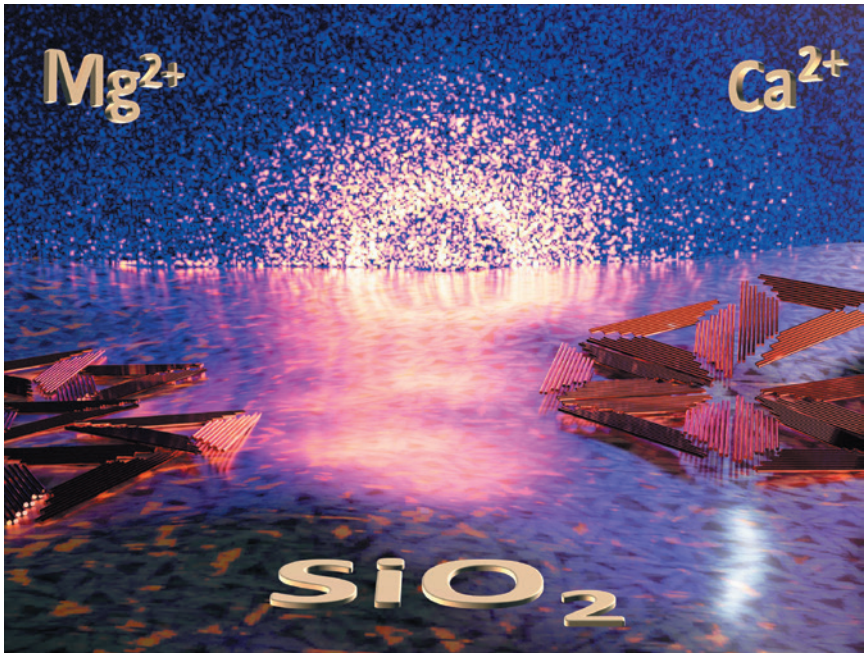

Fig. 1: Ca^{2+} but not Mg^{2+} promote the arrangement of DNA origami triangles into a hexagonal lattice on SiO_2 surfaces.

[1] P.W. Rothemund (2006). Nature, 440 (7082), 297–302.
[2] W. Jiang et al. (2023). Small Struct, 2200376.
[3] B.K. Pothineni et al. (2023). Nanoscale, 15, 12894-12906.

Guided Evolution of DNA-Based Microdroplets Acting as Protocells

Yunlong Qin,[1] Yang Sung Sohn,[2] Shijun Xu,[3] Fujian Huang,[3*] Fan Xia,[3*] Itamar Willner[1*]

[1]Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
[2]Institute of Life Science, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
[3]Falulty of Materials Science and Chemistry, China University of Geosciences, Wuhan 430074, China

Recent research efforts are directed towards the development of phase-separated microdroplets as synthetic models of native cells-Protocells. The information encoded in the base sequences of oligonucleotides and to base-dictated base pairing and strand displacement principles, their base-controlled switchable properties, and the sequence-controlled reactivity functions provide versatile tools to apply oligonucleotides as functional components to assemble functional phase-separated DNA-based microdroplets as protocell nanostructures.

1. We describe the novel method to assemble barcode-functionalized cyanuric acid/polyadenine triplex stabilized phase-separated microdroplets (MDs). The spatiotemporal kinetically-controlled compartmentalization by fluorescent “invading” nucleic acids of different lengths is demonstrated, Figure 1. By applying o-nitrobenzyl phosphate ester “caged” invading strands, light-triggered reorganization of the MDs is introduced and spatiotemporal catalytic DNAzymes or transcription machinery-guided reconfiguration of MDs compartments are highlighted, Figure 2(A) and Figure 2(B). Moreover, by mixing two kinds of functionalized phase-separated MDs, aggregated MDs revealing triggered, transcription machinery guided, intercommunication between MDs compartments is demonstrated. This latter system emulates intracellular signaling pathways across submembrane organelles, such as mitochondria or lysosomes.

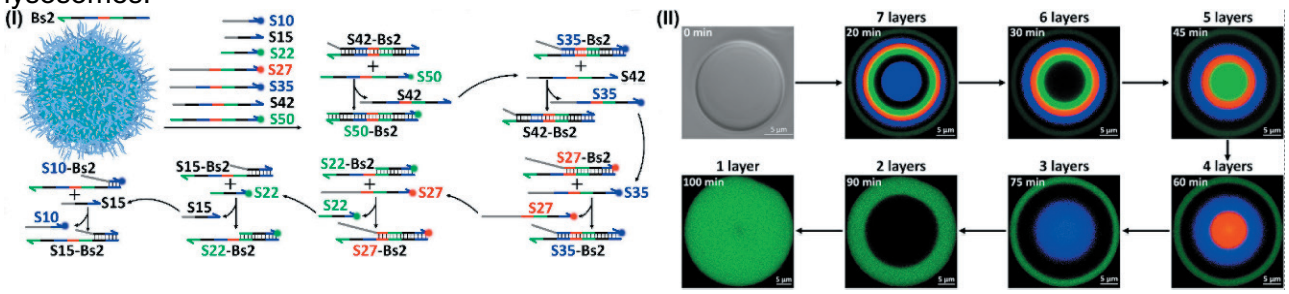

Fig. 1: Competitive and temporal assembly of compartmentalized MDs using seven invading strands.

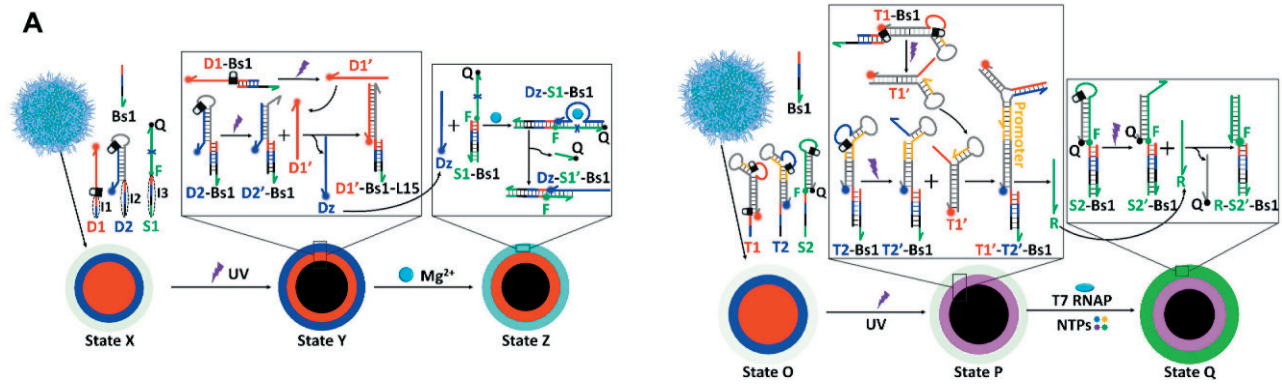

Fig. 2: Coupled light/DNAzyme-stimulated (A) or light/transcription machinery-guided dynamic compartmentalization and reconfiguration of MDs

Stability of DNA origami nanostructures in the presence of ROS

Lukas Rabbe,[1] Jaime Garcia-Diosa,[1] Guido Grundmeier,[1] Adrian Keller[1]

1 Technical and Macromolecular Chemistry, Paderborn University, Warburger Str. 100, 33098 Paderborn, Germany

The development of drug resistance in medical treatments has increased the interest in innovative therapeutic approaches. In this context, the combination of photodynamic therapy (PDT) with the DNA origami technique has gained in importance and has become the focus of various studies for example for cancer therapy [1, 2]. However, little is known about the stability of the DNA origami nanostructures during treatment, which is characterized by the continuous production of reactive oxygen species (ROS). In this work, DNA origami triangles and six-helix bundles (6HBs) are loaded with the clinically employed photosensitizer methylene blue and irradiated with light of the wavelength of 660 nm. The ROS-induced damage to the DNA origami structures is investigated using three environments with different ionic compositions. Tris-HCl buffer supplemented with Mg^{2+}, supplemented with K^+ and Na^+, and without supplemented ions was used for this purpose. Firstly, the influence of the photosensitizer and the influence of irradiation on the DNA origami nanostructures was evaluated. Subsequent investigations in which the loaded nanostructures are irradiated for ROS production then provide information on the stability behaviour. It shows that Mg^{2+} has a strong protective effect against ROS-induced damage. Further investigations revealed that post-irradiation addition of Mg^{2+} is able to heal some of the damage obtained in the absence of Mg. In addition, the 6HBs were found to be more resistant toward ROS-induced damage, compared to the triangles (see Fig. 1). The results of this research will help to gain a more detailed insight into the treatment combining PDT and DNA origami and thus provide guidance for further research regarding the selection of a design and its environmental conditions.

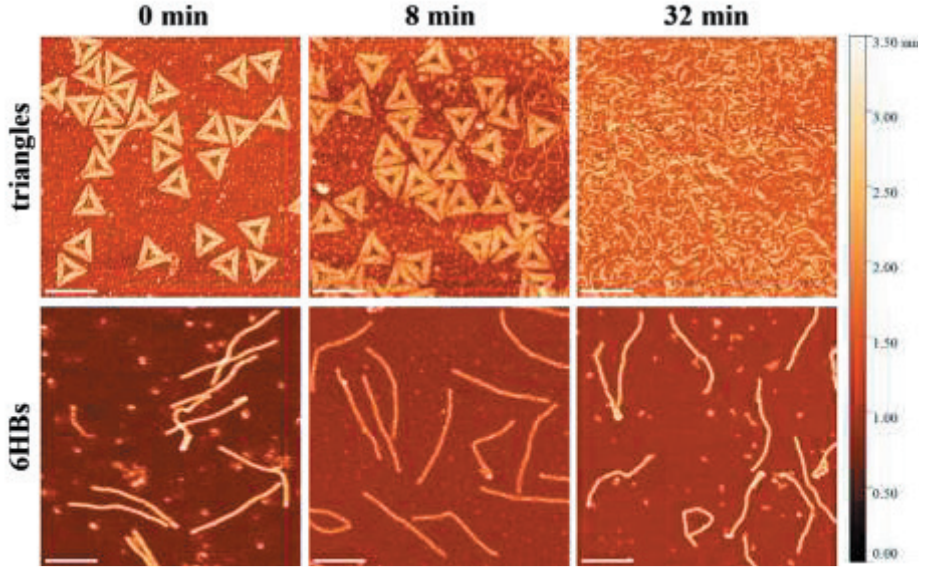

Fig. 1: AFM images of MB-loaded DNA origami triangles and 6HBs in non-supplemented Tris-HCl before and after irradiation for 8 min and 32 min. After irradiation, the DNA origami nanostructures were incubated for another 5 min in the presence of 10 mM Mg^{2+}. Scale bars are 200 nm.

[1] Hanke, M., Grundmeier, G., and Keller, A. 2022. Direct visualization of the drug loading of single DNA origami nanostructures by AFM-IR nanospectroscopy. *Nanoscale* 14, 32, 11552–11560
[2] Kollmann, F., Ramakrishnan, S., Shen, B., Grundmeier, G., Kostiainen, M. A., Linko, V., and Keller, A. 2018. Superstructure-Dependent Loading of DNA Origami Nanostructures with a Groove-Binding Drug. *ACS Omega* 3, 8, 9441–9448.

Towards metasurfaces by DNA-assisted lithography

Toni Rautio, Johannes M. Parikka, Heini Järvinen, J.Jussi Toppari

Nanoscience Center and Department of Physics, 40014 University of Jyväskylä, Finland

In a past decade, DNA origami has become one of the most used building blocks in self-assembled materials in nanoscale [1]. Using DNA origami as tiles large 2D lattices have been assembled with different techniques [2]. In our recent work, we have demonstrated that within certain ionic conditions the blunt-ended Seeman Tile [3] origami forms ordered 2D lattices [4] on top of silicon, as shown in figure 2b. These can be further utilized in lithography processes, like in DNA assisted lithography (DALI) [5] shown in figure 1. Our final goal is to fabricate a layered metamaterial surface like the one in reference [6].

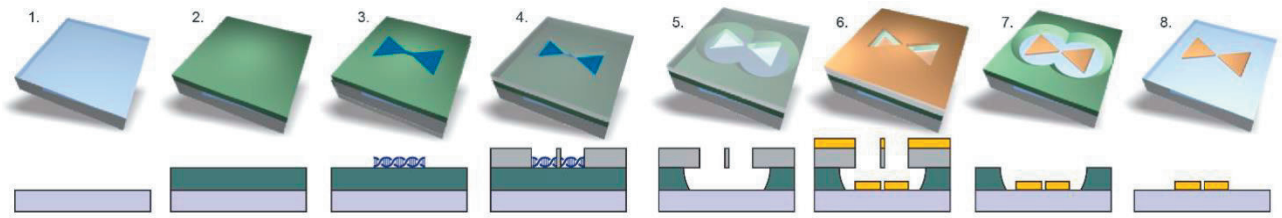

Fig. 1. DALI fabrication steps [5]: 1. Transparent substrate, 2. Silicon growth by Chemical Vapor Deposition, 3. Origami deposition, 4. SiO2 growth by the special CVD, 5. Isotropic Si etching by Reactive Ion Etching (RIE), 6. Au depositions by evaporation, 7. Lift-off by HF:HCl wet etching, 8. Removing of Silicon by RIE.

We have already successfully demonstrated the DALI up to step 5, *i.e.*, we have been able to grow silicon dioxide using the DNA lattice as a mask [5] shown in figure 2c. We have tried etching the silicon layer using the SiO_2 layer as a mask and imaged the results (figure 2d). We require a high precision etch process with large Si/SiO_2 selectivity and anisotropic etch profile (no undercut) to avoid the collapse of the silicon dioxide mask. We still need to optimize the etching process to get higher etching rate while keeping the profile (figure 2a). Therefore, the etch is done in cryogenic temperatures using SF_6 and O_2 as precursors. Using HBr instead of SF_6 is also a possibility and needs more research with help of larger e-beam lithography structures and DNA lattice samples. HBr etching is done in higher temperatures. After etching is optimized, the next step is to use etched pattern as an evaporation mask to make the metallic nanostructures (step 6).

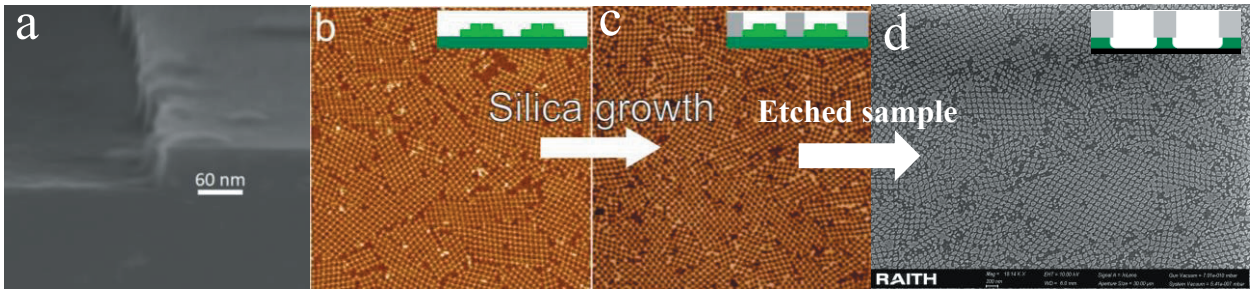

Fig. 2: a) SEM image for one e-beam lithography sample. b) AFM image of Seeman Tile fishnet-type lattice deposited on a silicon substrate. c) a Similar DNA lattice after silica growth, where origami shaped holes can be observed. d) SEM image of etched DNA lattice.

[1] F. Hong, F. Zhang, Y. Liu, et al., Chemical reviews 117, 12584-12640 (2017).
[2] J.M. Parikka, K. Sokołowska, N. Markešević, and J.J. Toppari, Molecules 26, 1502 (2021).
[3] W. Liu, et al., Angew. Chem. Int. Ed. 50, pp.264-267 (2011); A. Rafat, et al., Angew. Chem. Int. Ed. 53, 7665 –7668 (2014).
[4] K. Tapio, C. Kielar, J.M. Parikka, A. Keller, H. Järvinen, K. Fahmy, and J.J. Toppari, Chem. Mat. 35, 1961–1971 (2023).
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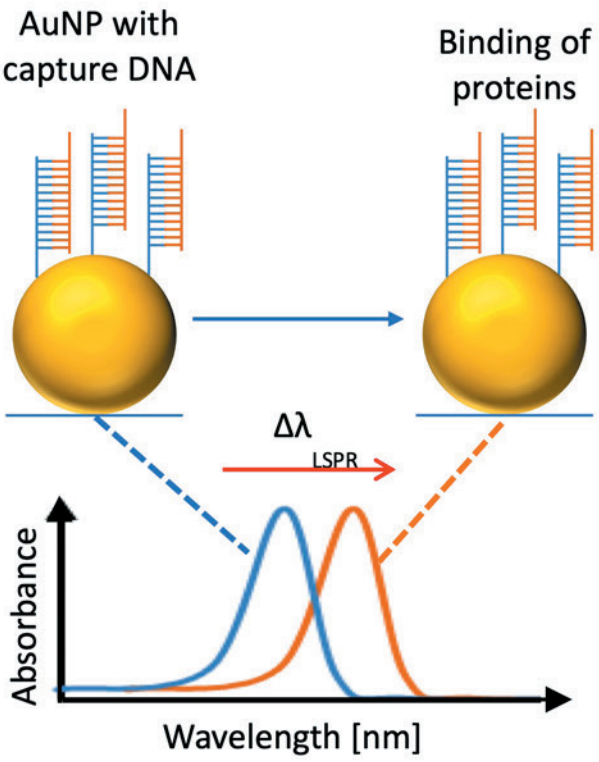
Characterization of DNA-Protein interactions utilizing localized surface plasmon resonance spectroscopy

Florian Seier[1], Dhaarna Gobin[1], Tino Schenk[2], Walter Koopman[3], Andrea Csáki[1], Wolfgang Fritzsche[1]

[1]Leibniz Institute of Photonic Technology, Albert-Einstein-Straße 9, 07745 Jena, Germany
[2]Universitätsklinikum Jena, Hans-Knöll-Strasse 2, 07745 Jena, Germany
[3]Institut für Physik und Astronomie, Universität Potsdam, Karl-Liebknecht-Str. 24/25, 14476 Potsdam, Germany

Nucleic acids and proteins are biopolymers commonly found in nearly every cell. Both polymers have the capability of interacting specifically with other molecules. In biology there are many reported interactions between nucleic acids, such as DNA, and proteins. One of the most important biological process is the transcription of DNA. Two examples of these interactions are the interaction of single-stranded DNA-binding (SSB) proteins with ssDNA and the binding of BCL6 as a transcription factor needed for the attachment of the DNA polymerase. SSB proteins are responsible for keeping the freshly separated sense and anti-sense strand single-stranded, avoiding their rehybridization. BCL6 is a protein mediating the binding of zinc-finger transcription factors to DNA. By studying the interaction mechanisms, new opportunities for treating cancer or antimicrobial resistant microbes by interfering with these essential processes can be developed. The utilized method, localized surface plasmon resonance spectroscopy, utilizes the collective oscillation of the free electron cloud confined in a noble metal nanoparticle caused by incident light with a wavelength larger than the particles diameter. As a result of this oscillation, an electric dipole moment at the particles surface is created, alternating at a certain frequency by the back-and-forth movement of the free electron cloud. Light is scattered and maximally absorbed at the peak plasmon resonance frequency. This effect depends on the refractive index at the surface of the particles, which is changed upon molecular binding or dissociation, and therefore allows to study these processes by a time resolved spectrometer.
The sensing mechanism relies on the interaction of the nanoparticles with light. To study the mechanism of this interaction, experiments are conducted to investigate the behavior of fluorescent dyes in the vicinity of in resonance pulsed laser irradiated particles.



Effect of hydrophobicity of lipid anchor functionalization on DNA origami binding to lipid membranes

Subhasini Singh and Henri. G. Franquelim
Interfaculty Centre for Bioactive Matter (b-ACTmatter), Leipzig University, Deutscher Platz 5 (BBZ), 04103 Leipzig, Germany

Recently, DNA origami has been demonstrated to be an exceptional engineering tool when combined with lipid membranes by exploiting their self-assembly nature for biomimicking the membrane-associated biological processes. Most reported membrane-active DNA nanostructures, however, rely on the use of commercially available cholesterol modifications to achieve efficient binding to cell and model lipid membranes. In this work, we aim to investigate new alternative strategies to bind DNA origami to lipid membranes, especially via weakly lipophilic anchors comprising of aromatic and short aliphatic moieties of different hydrophobicity (cLogP) values.
As model DNA origami, we used a linear Atto488-labeled 20-helix bundle functionalized with lipophilic moieties at the bottom facet. The binding affinity of DNA origami displaying different numbers and types of strongly to weakly lipophilic anchors were quantitatively studied on various lipid model systems (GUVs, LUVs, …) using primarily confocal laser scanning and other biophysical detection tools. Furthermore, the interference of weak anchors on the membrane phase localization property of origami were analyzed by determining the cooperativity effect in conjunction with cholesterol as a strong anchor. Simultaneously, we were able to distinguish the minimal hydrophobicity required for binding DNA nanostructures to homogeneous membranes, depending on the class of lipophilic anchor, along with the influence of combined anchors on the membrane phase partitioning properties.
In the end, the effect of charge density of DNA origami due to the presence of highly negative charge on phosphate backbone was determined by their surface passivation with positively charged poly-lysine molecules in order to improve membrane binding efficiency. Overall, our strategy opens new avenues for mapping membrane vesicle properties, demonstrating the unique advantages of DNA nanotechnology for future membrane trafficking and biosensing applications.

Quantitative Analysis of Mg^{2+} Binding to DNA Origami Nanostructures

Emilia Tomm, Marcel Hanke, Guido Grundmeier, Adrian Keller

Technical and Macromolecular Chemistry, Paderborn University, Warburger Straße 100, 33098 Paderborn, Germany

The structural stability and integrity of DNA origami nanostructures are fundamentally reliant on interactions with divalent cations, notably Mg^{2+}. These ions are crucial in facilitating the origami folding process and preserving structural integrity through the mitigation of electrostatic repulsion.[1] However, the precise quantification of Mg^{2+} ions associated with DNA origami remains elusive, underscoring a significant gap in current understanding. Despite the acknowledged importance of these interactions, the exact amount of Mg^{2+} that is bound to DNA origami structures has not been adequately quantified. This investigation seeks to determine the actual concentration of Mg^{2+} associated with DNA origami, distinguishing between ions bound directly to the DNA and those present in the bulk solution under conditions of micromolar-range Mg^{2+} concentrations. Through the application of fluorescence measurements utilizing Magnesium Green as a Mg^{2+} sensitive probe, this study quantitatively evaluates the binding of Mg^{2+} to different 2D and 3D DNA origami structures, exploring the hypothesis that DNA-Mg^{2+} interactions may be dependent upon the structural design.[2] In parallel, Atomic Force Microscopy is employed to assess the structural integrity of the nanostructures at various Mg^{2+} to phosphate ratios.

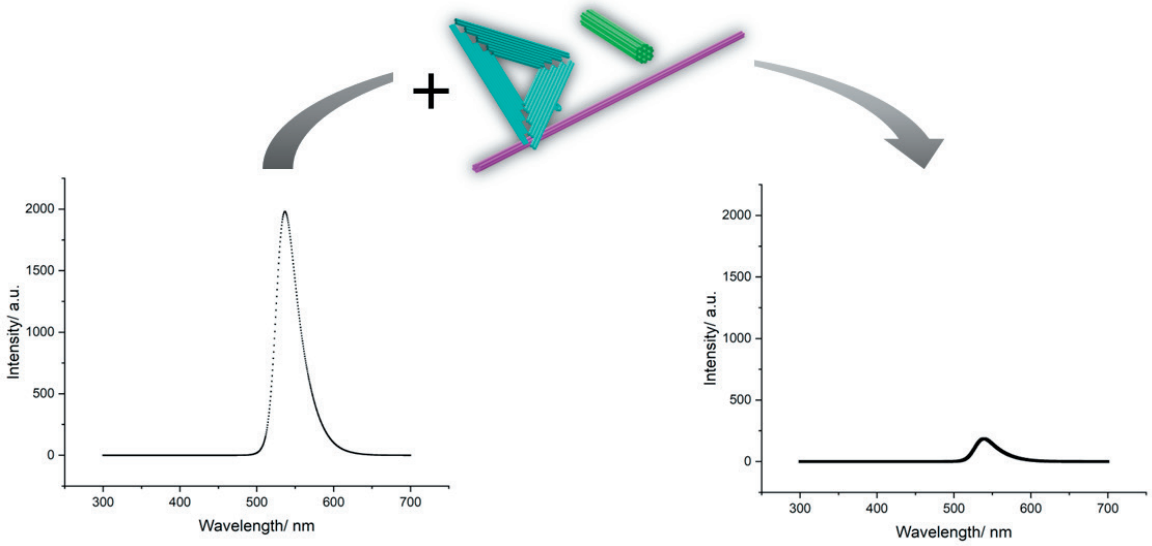


Fig. 1: Reduction of Mg^{2+}-Magnesium Green Fluorescence Intensity upon Introduction of DNA Origami Nanostructures

*[1] C. Kielar et al. (2018). Angew. Chem., **130 (30)**, 9614–9618.*
*[2] M. Hanke et al. (2023). ChemBioChem., **24 (12).***

Tube-shaped assemblies made of DNA-origami-lattices

Johannes Parikka[1], Heini Järvinen[1], Karolina Sokołowska[1], Visa Ruokolainen[2], Nemanja Markešević[1], Ashwin Natarajan[3], Maija Vihinen-Ranta[2], Anton Kuzyk[3], K. Tapio[1,3], Jussi Toppari[1]

Nanoscience Center & Department of Physics[1] or Department of Biological and Environmental Science[2], 40014 University of Jyväskylä, Finland
[3]Department of Neuroscience and Biomedical Engineering, Aalto University, 00076 Aalto, Finland

Hierarchical self-assembly of nanostructures with addressable complexity has been a promising route for realizing novel functional materials. In particular, bottom-up methods using molecules like DNA have gained attention [1] due to the advantages of low fabrication costs, high resolution, and simplicity in an extension of the methods to the third dimension [2]. One of the more promising bottom-up techniques is DNA origami due to the robust self-assembly of arbitrarily shaped nanostructures with feature sizes down to a few nanometers [3].

Here, we show that under specific ionic conditions of the buffer, the employed plus-shaped, blunt-ended Seeman tile (ST) [4] DNA origami structures forms elongated, ordered 2D lattices, which are further rolled into 3D tubes in solution [5]. Imaging structures on a surface by atomic force microscopy reveals ribbon-like structures, with single or double layers of the origami lattice. Further studies of the double-layered structures in a liquid state by confocal microscopy and cryo-TEM revealed elongated tube structures with a relatively uniform width but with a varying length. Through meticulous study, we concluded that the assembly process of these 3D DNA origami tubes is heavily dependent on the concentration of both mono- and divalent cations. In particular, nickel seems to act as a trigger for the formation of the tubular assemblies in liquid, as depicted in figure below. By DLS, we can also follow formation of the assemblies as well as the disassembly of them after removing Ni^{2+} ions by addition of ethylenediaminetetraacetic acid (EDTA).

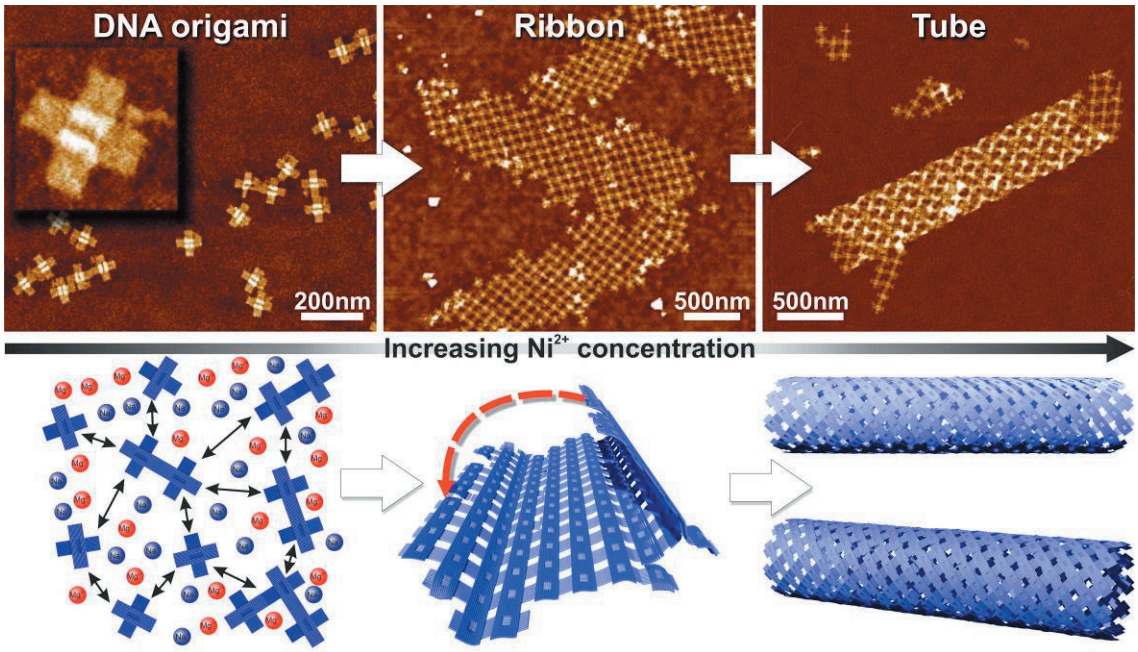


Figure 1: ST assembly formation with increasing nickel concentration

[1] F. Hong, F. Zhang, Y. Liu, et al., (2017), Chemical reviews 117, 12584–12640.
[2] T. Zhang, et al., (2018) Adv. Mat. 30, 1800273; S.H. Park, et al., (2023) Nanophotonics 12, 2611–2621.
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[4] W. Liu, et al., Angew. Chem. (2011), 50, 264–267; A. Rafat, et al., (2014), Angew. Chem. 53, 7665–7668.
[5] J.M. Parikka, H. Järvinen, K. Sokołowska, et al., (2023), Nanoscale 15, 7772–7780.

Evaluation of a duplex PCR assay for the detection of *Salmonella* spp. and *Staphylococcus aureus* in raw milk

Yuneilys Aleli Martínez-García[1], Yamilka Riverón-Alemán[1], José Antonio Agüero-Fernández[1], Ailin Martínez-Vasallo[1], Odalys Uffo-Reinosa[1]

[1] Direction of Animal Health, National Center for Animal and Plant Health, Carretera de Tapaste y Autopista Nacional, Apartado Postal 10, 32700 San José de las Lajas, Mayabeque, Cuba.

Infectious and toxigenic pathogens transmitted through food have been recognized as the cause of Foodborne Diseases (FBD). The main pathogens of concern are *Salmonella* spp. and *Staphylococcus aureus*. These bacteria are associated with acute gastrointestinal outbreaks caused by contaminated food. One of the causes of this contamination is the poor hygiene during the processing and handling of food. Foodborne outbreaks caused by milk and dairy products contaminated reach up to 6 %. The objective of the present work was to develop and standardize a duplex PCR assay for the detection of *Salmonella* spp. and *Staphylococcus aureus* in raw milk. The principle of the method is the simultaneous amplification of the genes *yfiR* for *Salmonella* spp. and ARNr 23S for *Staphylococcus aureus*. The thermodynamic properties of each primer were determined and the main critical parameters of the dPCR assay were optimized. The duplex PCR demonstrated its specificity for the target microorganisms and the detection limit of the assay was up to 1 pg/µL of DNA and 10_2 cfu/mL in unpasteurized milk. In the milk samples analyzed, 46.7 % were contaminated with *S. aureus* and none of the samples were positive to *Salmonella* spp. The results suggest that the developed duplex PCR is sensible and specific for the simultaneous detection of both pathogens from raw milk.

Fully addressable, designer superstructures assembled from a single modular DNA origami

Johann M. Weck, Amelie Heuer-Jungemann

Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany and Center for Nanoscience, Ludwig-Maximilians University, Munich, Germany

Intricate self-organization is essential in many biological processes, underpinning vital functions and interactions. In an effort to mimic such processes, synthetic biology aims to engineer dynamic structures with controllable functions using nanotechnological tools.

A key requirement of engineered building blocks is the ability to assemble and disassemble superstructures with precision. Making use of the highly controllable nature of DNA self-assembly, we here present the moDON[1], a modular DNA origami nanostructure, which is capable of assembling into 18,225 distinct monomers, forming complex and controlled superstructures with connections in three dimensions. While shape and addressability of DNA origami are nearly arbitrary, its overall size is limited by the length of the scaffold. Previous methods of extending the size of DNA origami (e.g. hierarchical assembly[2], modified scaffolds[3], etc.), either led to loss over control of shape and addressability beyond a single monomer or led to proportionally increased cost and design effort if a plethora of distinct monomers had to be formed.

With the moDON we are able to overcome both issues. The modular design combines xy- and z-plane assembly methods, enabling the one-step assembly of finite, fully addressable structures of > 40 MDa and periodic structures reaching several µm in size and > 1 GDa mol. Weight (see Fig 1). We demonstrate complete xy-z orthogonality, by enabling controlled selective or parallel assembly and disassembly using distinct orthogonal triggers. While xy assembly and disassembly are triggered by an increase or decrease in MgCl2 concentration, assembly and disassembly in the z direction are triggered by the presence of linker or invader strands. Interestingly, the kinetic profile of assembly and disassembly aligns with biological time scales, paving the way for applications in dynamic nanomachinery and advanced biomaterials. Finally, we showcase the conjugation of gold nanoparticles to specific positions within superstructures, underscoring the efficacy of this approach for creating intricate and orthogonal nanoscale architectures with preserved site-specific addressability. The moDON thus offers an efficient, cost-effective solution for constructing large, precisely organized, and fully addressable structures with vast potential in synthetic cellular systems design and beyond.

A DNA nanoreactor for the bioelectrocatalytic conversion of CO_2 into methanol

Xiaodan Xu, [1] Marcel Hanke, [1] Xinyang Li, [1] Guido Grundmeier, [1] Adrian Keller [1]

[1] Paderborn University, Technical and Macromolecular Chemistry, Warburger Str. 100, 33098 Paderborn, Germany

As biocatalysts, enzymes exhibit remarkable selectivity and activity under mild conditions, such as ambient pressure and room temperature, while being readily available in abundance from natural sources [1]. Dehydrogenases, for instance, can catalyze the reduction of fuel primary product CO_2 when electrons and protons are supplied by sacrificial cofactors or coenzymes [2]. However, the reliance on such components adds to the overall cost and introduces challenges related to instability and separation processes. DNA nanotechnology has emerged as a promising avenue due to the programmability of DNA hybridization and the versatility of DNA-biomolecule conjugation strategies [3, 4]. Here, we present the assembly of a DNA origami-templated enzymatic cascade involving, formate dehydrogenase (FateDH), formaldehyde dehydrogenase (FaldDH) and alcohol dehydrogenase (ADH) on a gold electrode to facilitate the reduction of CO_2 to methanol (see Fig. 1). Herein, a DNA origami is immobilized onto the gold electrodes via Au-S chemistry. The assembly of the enzyme cascade on the DNA origami and the subsequent immobilization of the DNA origami onto the gold substrate are characterized using atomic force microscopy (AFM). The detection of the reduction product will be performed using mass spectrometry (MS).

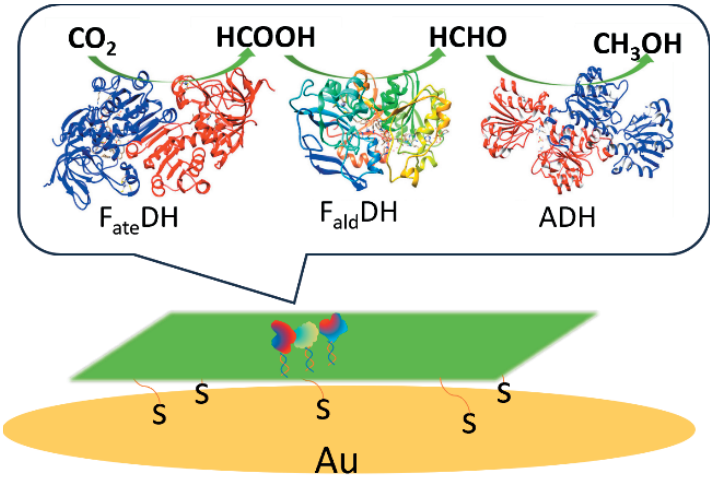

Fig. 1: Schematic conversion of CO_2 by an enzyme cascade assembled on the DNA origami immobilized onto the gold electrode.

[1] S. Schlager et al. 2016. ChemSusChem. 9: 631-635.
[2] D. Mandler and I. Willner. 1988. Journal of the Chemical Society. Perkin Transactions 2: 997-1003.
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