Leibniz IPHT
LEIBNIZ INSTITUTE of PHOTONIC TECHNOLOGY

ABSTRACTS

DNA NANOTECHNOLOGY 2022

MAY 12 – MAY 14, 2022

Leibniz IPHT // Campus Beutenberg // Jena

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PROGRAM

THURSDAY, MAY 12, 2022

Leibniz IPHT, Beutenberg Campus
Public bus no. 10, 11, 12

12:00 **Workshop "DNA Mitteldeutschland" with short presentations //**
free to join, please send an e-mail to seier.florian@leibniz-ipht.de

12:00 SNACKS & GET-TOGETHER

12:30 **Presentations – ca. 16:45**

Phyletic Museum, Downtown

18:00 **Get-together**

18:30 **Guided Museum Tour**

19:30 DINNER

FRIDAY, MAY 13, 2022

Leibniz IPHT, Campus Beutenberg
Public bus no. 10, 11, 12

9:00 **Opening & Introduction / Session 1 //**
Wolfgang Fritzsche

Aptananozymes – A New Class of Aptamer-Modified Nanoparticles for Catalysis and Chemodynamic Medicine //
Itamar Willner (Jerusalem)

DNA Origami: From Devices to Materials //
Tim Liedl (Munich)

Metabolic DNA Systems Inspired from Life: Protocells and Systems with Lifecycles //
Andreas Walther (Mainz)

10:20 COFFEE BREAK

11:00 **Session 2 //** Itamar Willner

AFM Analysis of G-wire DNA Structure and Nanoparticle Decoration //
James Vesenka (Jena)

Molecular Electronics with DNA towards DNA Detection //
Danny Porath (Jerusalem)

Plasmonic Metastructures and Bio-Assemblies: Chirality, DNA-origami, and Hot Electrons //
Alexander Govorov (Athens, OH)

RNA-DNA Nanotechnology Identifies Native RNA with Nanopore Sensing //
Ulrich F. Keyser (Cambridge)

Poster Pitch Talks //
Ekaterina Podlesnaia

12:50 LUNCH & POSTER SESSION

14:00 **Session 3** // Tim Liedl

Surface Placement of DNA Origami for Self-Assembly of Inorganic Electronic Nanostructures //
Adam Woolley (Provo UT)

Metallic Nanowires Assembled by DNA Origami //
Artur Erbe (Dresden-Rossendorf)

Down-and-up: Combining DNA Self-assembly with Top-down Fabrication //
Jussi Toppari (Jyväskylä)

New Insights into the DNA Origami Silicification Reaction Mechanism by in Situ Small Angle X-ray Scattering //
Amelie Heuer-Jungemann (Munich)

15:40 **Poster Session**

17:00 ORCHID EXCURSION & BARBECUE DINNER

SATURDAY, MAY 14, 2022

Leibniz IPHT, Campus Beutenberg
Bus leaves 8:35 downtown

9:00 **Session 4** // Jussi Toppari

Hierarchical Self-assembly of DNA Origami Lattices at Solid-liquid Interfaces //
Adrian Keller (Paderborn)

Single-molecule SERS Measurements Enabled by DNA Origami Nanostructures //
Ilko Bald (Potsdam)

Orienting Single Molecules in DNA Origami Constructs //
Aleksandra Adamczyk (Fribourg)

Observing Single DNA Nanorobot Kinetics to Guide the Engineering of Molecular Torsion Springs //
Jonathan List (Munich)

10:40 COFFEE BREAK

11:00 **Session 5** // Adam Woolley

DNA Origami in Physiological Settings //
Veikko Linko (Helsinki)

Improving Single-molecule Detection: Polar Surface Array for Controlling the Adsorption of Plasmonic DNA Origami Nanostructures //
Zunhao Wang (Braunschweig)

Membrane Domain Sensing by Amphipathic Curved DNA Origami //
Henri Franquelim (Leipzig)

Target-induced Dual-signal Electrochemical Aptasensor for Sensitive and Accurate Detection of Aflatoxin B1 //
Long Wu (Jena)

12:40 END OF THE SESSION & LUNCH

LOCATION

LEIBNIZ INSTITUTE OF PHOTONIC TECHNOLOGY

Nanobiophotonics Department

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www.biophotonics4future.com/dna2022/

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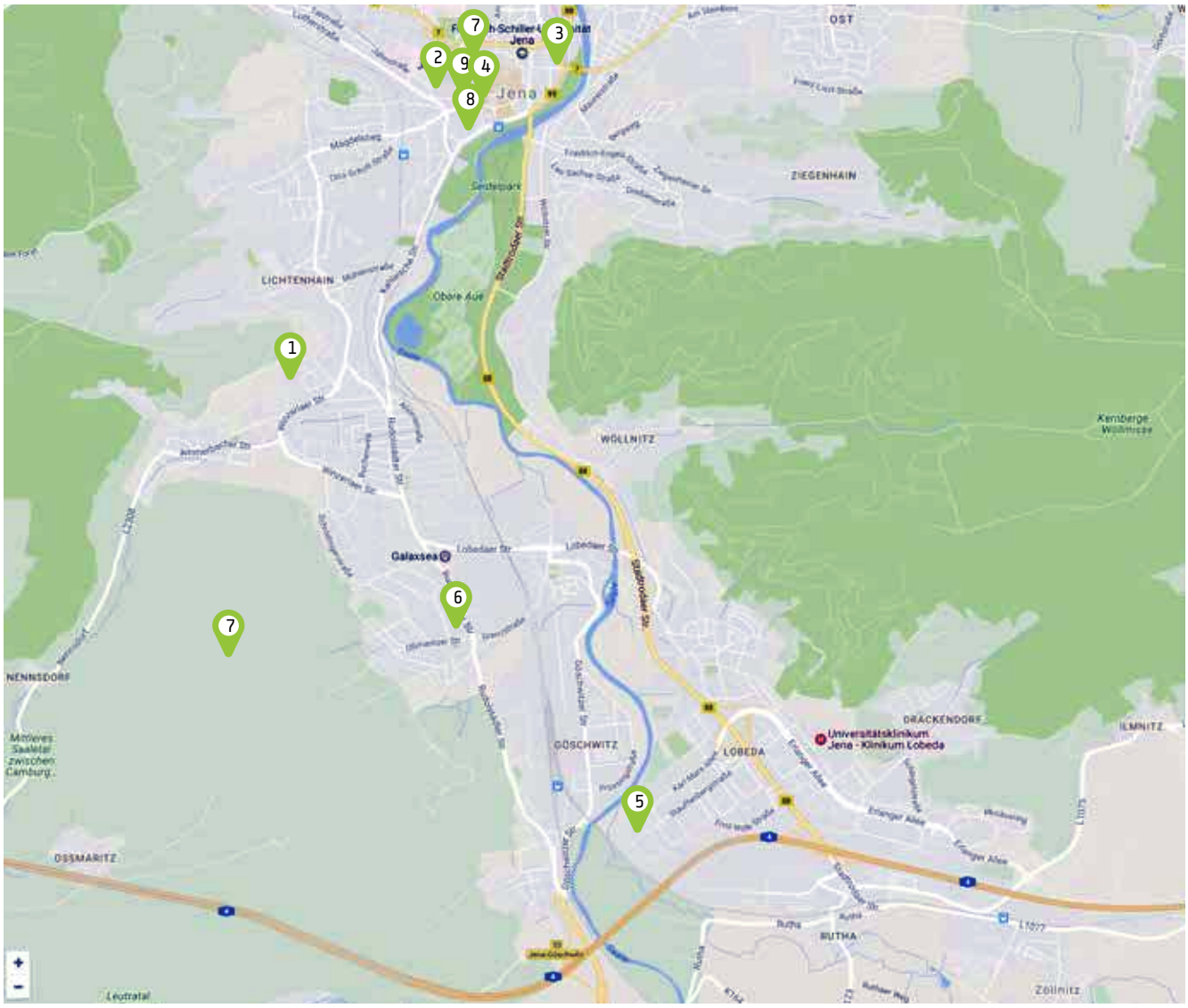
6 Hotel Best Western
7 Orchid Excursion
8 Phyletic Museum
9 Bus No. 10, 11, 12



TALKS

Aptananozymes – A New Class of Aptamer-Modified Nanoparticles for Catalysis and Chemodynamic Medicine

Itamar Willner, Yu Ouyang

The Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

The conjugation of DNAzymes or homogeneous catalysts to aptamer strands yields functional assemblies mimicking native enzymes by engineering active sites that concentrate the substrate in proximity of the catalytic site[1]-nucleoapzymes. Similarly, the conjugation of photocatalysts to an aptamer receptor that binds electron acceptor ligands yields organized assemblies emulating functions of the photosynthetic apparatus-photoaptazymes.[2]

The concept of catalyst-aptamer catalytic conjugates is now extended to include catalytic nanoparticles functionalized with aptamer receptor binding sites-aptananozymes.[3] These include Cu^{2+}-modified C-dots functionalized with the dopamine/tyrosinamide aptamer, polyadenine-stabilized Au NPs functionalized with the dopamine aptamer and Ce^{4+}-modified C-dots functionalized with the dopamine aptamer or the tyrosinamide aptamer. Key results that will be addressed include:

-Enhanced catalytic oxidation of the dopamine to aminochrome by H_2O_2 using a series of structurally modified aptananozymes. Structure-function relationships controlling the catalytic transformations will be presented.

-Chiroselective oxidation of L-/D-DOPA by the aptananozymes will be introduced.

-Catalyzed H_2O_2-driven oxygen insertion into tyrosinamide to yield the catechol product is followed by the formation of amidodopachrome by the Ce^{4+}-ion-functionalized C-dots aptananozymes in the presence of H_2O_2/ascorbate.

-Aerobic oxidation of glucose to gluconic acid and H_2O_2 by the polyA Au NPs and the Ce^{4+}-C-dots aptananozymes. The dual catalytic activities of the aptananozymes will be applied to engineer bioreactors for the cascaded aerobic oxidation of dopamine/tyrosinamide to aminochrome/amidodopachrome by glucose.

-Mechanistic aspects related to the generation of reactive oxygen species (•OH or $O_2^{\bullet-}$) by the different aptananozymes.

In addition, the synthesis of Ce^{4+}-ions-modified C-dots functionalized with the MUC-1 or the AS1411 aptamers will be described. The targeted and selective cytotoxic chemodynamic treatment of MDA-MB-231 breast cancer cells will be introduced.

References:

1. (a) *J. Am. Chem. Soc.* **2016**, *138*, 164-172. (b) *Adv. Funct. Mater.* **2019,** *29*, 1901484. (c) *ACS Catal.* **2018**, *8*, 1802-1809

2. Angew. Chem. Int. Ed. **2020,** *59*, 9163-9170.

DNA origami: from devices to materials

Tim Liedl, Gregor Posnjak, Irina Martynenko, Mihir Dass, Xin Yi

Center for NanoScience (CeNS), Ludwig-Maximilians-Universität, 80339 München, Germany

Inspired by the seminal DNA crystals design of Ned Seeman and Chengde Mao, which is based on a "tensegrity triangle" where three DNA duplexes are interconnected in a self-restricting over-under, over-under, over-under fashion, [1] our team set out to build 3D DNA origami crystals with lattice spacings approaching the wavelength of visible light. By adopting this design principle, we first presented a DNA origami tensegrity triangle that crystallizes into three dimensional, micrometer-scale assemblies. [2] With our goal to assemble functional DNA-based materials we have now expanded the range of lattice types to square and diamond lattices. Employing in-buffer silicification processes, we render our crystallites chemically and mechanically stable [3,4] and enable deposition of a variety of inorganic materials for potential applications in optics and catalysis.

1. J. Zheng *et al.* Nature 461, 74-77 (2009)
2. T. Zhang *et al.* Advanced Materials, doi: 10.1002/adma.201800273 (2018)
3. X. Liu *et al.* Nature 559, 593-598 (2018)
4. L. Nguyen *et al.* Angew. Chem. Int. Ed. 58, 912-916 (2019)

Metabolic DNA Systems Inspired from Life: Protocells and Systems with Lifecycles

Andreas Walther
A3BMS Lab, Active, Adaptive and Autonomous Bioinspired Materials, Department of Chemistry, University of Mainz, 55128 Mainz, Germany.
DFG Cluster of Excellence @ FIT "Living, Adaptive and Energy-Autonomous Materials Systems" (livMatS), Freiburg Center for Interactive Materials and Bioinspired Technologies, University of Freiburg, 79110 Freiburg, Germany.

Living self-organizing systems operate far-from-equilibrium and display energy-dependent adaptive functionalities that are orchestrated through feedback loops and metabolic reaction networks to allow tailored response in complex sensory landscapes. These principles serve as an inspiration to promote complexity and life-like functions in soft matter systems, which include for instance to pre-organize temporal behavior or install mechanisms for complex adaptative behavior. The pre-organization of the temporal fate of systems requires new types of internal control mechanisms, such as kinetic control over opposing reactions (built-up/destruction), the integration of feedback mechanisms, or the use of energy dissipation to sustain structures only as long as a chemical fuel is available. Even higher complexity and new functions are in reach by essentially embedding multi-sensory metabolic reaction networks into these systems.

In this talk, I will discuss two avenues towards autonomous and adaptive DNA active matter systems with simplistic metabolica reaction networks inside. On the one hand, I will discuss the formation of DNA-based protocell architectures with the ability to house abiotic catalysts driving downstream morphological adaptations. On the other hand, I will discuss the use of ATP as a chemical fuel to drive chemically fueled out-of-equilibrium systems using activation/deactivation networks. The latter allows to program self-assemblies and materials with lifetimes and programmable steady state dynamics.

Selected References:
Emerging area article: LH, AW "Approaches to Program the Time Domain of Self-Assemblies"10th year Soft Matter issue, 2015, 11, 7857. Review: AW "From Responsive to Adaptive and Interactive Materials and Materials Systems: A Roadmap" Adv. Mater. 1905111 (2020). Review: RM, AW "Materials learning from life: Concepts for active, adaptive and autonomous molecular systems". Chem. Soc. Rev. 2017, 46, 5588;

Selected References: Angew. Chem. Int. Ed. e202113477 (2022). Sci. Adv. 7, eabj5827 (2021). Nat. Commun. 14, 5132 (2021). Nat. Nanotechnol. 1856 (2020). Nat. Commun. 11, 3658 (2020). J. Am. Chem. Soc. 142, 685, (2020); J. Am. Chem. Soc. 142, 21102 (2020). Chem 6, 3329 (2020). Sci. Adv., eaaw0590, (2019). Angew. Chem. Int. Ed. 59, 12096 (2020); Nature Nanotech. 13, 730 (2018);

AFM analysis of G-wire DNA structure and nanoparticle decoration

James Vesenka, Marybeth Vesenka, Andrea Csáki, Wolfgang Fritzsche

University of New England, 11 Hills Beach Drive, Biddeford, Maine 04005, USA
Leibniz Institute of Photonic Technology, Albert-Einstein-Straße 9, 07745 Jena, Germany

Atomic force microscopy was used to characterize extended quadruplex DNA structures (G-wires) constructed from three different oligo building blocks adsorbed on two different substrate preparations. G-wires adsorbed onto freshly cleaved mica through magnesium cation bridge have a preferential orientation at 60° intervals after rinsing and drying, a characteristic of equilibrated adsorption [1]. These present a high degree of auto-orientation even after as little as 10 minutes of incubation, indicating rapid equilibration with the atomic structure of the mica surface. G-wires kinetically trapped onto amino acid treated-mica provide information regarding the flexibility of the G-wires in bulk solution [2]. Persistence length measurements indicate that G-wire flexibility about five time less than that of double stranded DNA. Progress in gold nanoparticle decorating of branched G-wires will be discussed.

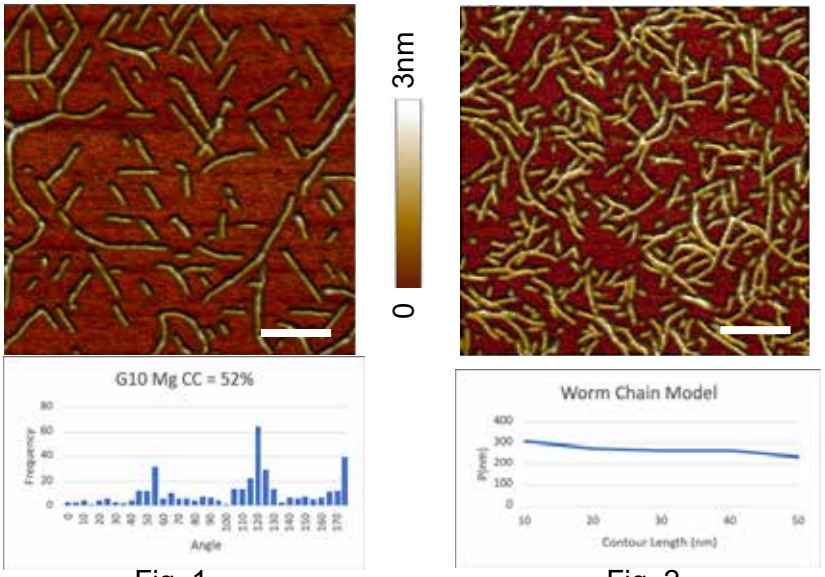


Fig. 1 Fig. 2

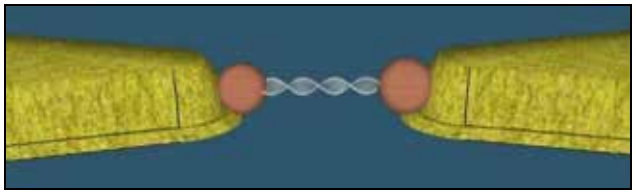
Fig. 1: G-wires equilibration adsorption on mica with autocorrelation function.
Fig. 2: G-wires kinetically adsorbed on amino acid treated mica with persistence length.
J. Vesenka, D. Bagg*, A. Wolff, A. Reichert, & W. Fritzsche, “Auto-Orientation of G-wire DNA on Mica”, Colloids and Surfaces B: Biointerfaces, 58, pp. 256-263 (2007).

[1] Vesenka et al. (2007). Colloids Surf. Sci. B: Biointerfaces, 58 256-263.
[2] Abels et al.(2006). Biophys. J, 88, 2737-2744.

“Molecular Electronics with DNA towards DNA Detection”

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. We recently reported a breakthrough in measuring charge transport in DNA (Nature Nanotechnology 2020) in a special configuration. This finding is of great importance by itself 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 and for a radically new method for DNA sequencing. Addressing these challenges is at the heart of the current pandemic as well as for early detection of cancer.

Selected relevant publications:

[1] *"Direct measurement of electrical transport through DNA molecules",* Danny Porath, Alexey Bezryadin, Simon de Vries and Cees Dekker*,* **Nature 403***,* 635 (2000).

[2] *"Charge Transport in DNA-based Devices"*, Danny Porath, Rosa Di Felice and Gianaurelio Cuniberti, **Topics in Current Chemistry** Vol. **237**, pp. 183-228 Ed. Gary Shuster. Springer Verlag, 2004.

[3] "*Long-range charge transport in single G4-DNA molecules",* Gideon I. Livshits et. al., **Nature Nanotechnology 9,** 1040 (2014).

[4] *“Advances in Synthesis and Measurement of Charge Transport in DNA-Based Derivatives”*. R. Zhuravel, A. Stern, N. Fardian-Melamed, G. Eidelshtein, L. Katrivas, D. Rotem, A. Kotlyar and D. Porath, **Advanced Materials 30**, 1706984 (2018).

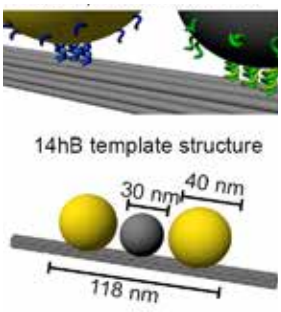
[5] *"Backbone charge transport in double-stranded DNA"*, R. Zhuravel et. al., **Nature Nanotechnology**, 15(10), 836 (2020).

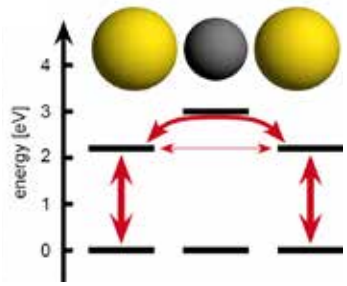
Plasmonic Metastructures and Bio-Assemblies:

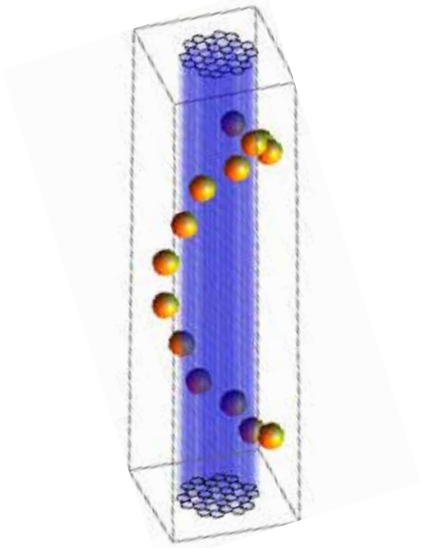
Chirality, DNA-origami, and hot electrons

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).

RNA-DNA nanotechnology identifies native RNA with nanopore sensing

Ulrich F. Keyser

Department of Physics, University of Cambridge, JJ Thomson Ave, Cambridge, UK

DNA nanotechnology is transformative for experiments that require molecular control over the shape of nanometer-sized objects. In combination with nanopores DNA self-assembly allows for novel experiments that reveal the physics of ions, and polymers on the single molecule level.

Nanopore sensing, best known for DNA sequencing, translates the three-dimensional structure of molecules into ionic current signals. Designed DNA molecules enable multiplexed protein sensing with an all-electrical approach [1]. Here, I will discuss our recent developments to detect and localise structures as accurately as possible along DNA molecules approaching super-resolution microscopy [2]. Based on our high-resolution measurements, I will show how to use the fundamental understanding for the identification of miRNA, RNA viruses and their variants [3], and RNA isoforms without reverse transcription or amplification [4]. In the future, our technology will enable to identify and quantify RNA structural elements and offer a strategy for the mapping of RNA binding proteins.

References

[1] N. A. W. Bell and U. F. Keyser. Digitally encoded DNA nanostructures for multiplexed, single-molecule protein sensing with nanopores. Nature Nanotechnology, 11:645-651, 2016. http://dx.doi.org/10.1038/nnano.2016.50

[2] K. Chen, I. Jou, N. Ermann, M. Muthukumar, U. F. Keyser, and N. A. W. Bell. Dynamics of driven polymer transport through a nanopore. Nature Physics, 17, 1043-1049, 2021. http://dx.doi.org/10.17863/CAM.69631

[3] F. Bošković, J. Zhu, R. Tivony, A. Ohmann, K. Chen, M. Alawami, M. Djordjevic, N. Ermann, J. Pereira Dias, M. Fairhead, M. Howarth, S. Baker, and U. F. Keyser. Simultaneous identification of viruses and SARS-CoV-2 variants with programmable DNA nanobait. medRxiv, 2021. https://medrxiv.org/cgi/content/short/2021.11.05.21265890v1

[4] F. Bošković and U. F. Keyser. Nanopore microscope identifies RNA isoforms with structural colors. bioRxiv, 2021. https://www.biorxiv.org/content/10.1101/2021.10.16.464631v1

Surface Placement of DNA Origami for Self-Assembly of Inorganic Electronic Nanostructures

Adam T. Woolley, Dulashani R, Ranasinghe, Chao Pang, Robert C. Davis, and John N. Harb

Brigham Young University, Provo, UT 84602, USA

DNA origami is a versatile platform for the self-assembly of nanomaterials for electronic applications [1]. We have focused on the controlled placement and characterization of inorganic nanowires on DNA origami, and have demonstrated the creation of metal-semiconductor junctions [2], as shown in Fig. 1.

To further improve on the creation of these nanostructures, we have been developing bottom-up formation of nanoscale metal islands to provide designed locations for positioning DNA origami on surfaces. We have used block copolymers and chemical etching to make nanoscale periodic gold dots on silicon surfaces, with their spacing and size controlled by the nature of the block copolymer. We are now using these arrays for site-selective placement of DNA origami, on which nanowires are self-assembled (Fig. 2).

In an effort to expand the range of semiconductor nanomaterials available for arrangement onto DNA origami, we are synthesizing CdS nanorods (Fig. 3). We are also using ligand exchange processes to coat the nanorod surfaces with DNA sequences for site-selective placement on DNA origami.

The combination of our surface localization approaches for DNA origami with these semiconductor nanomaterials opens up new possibilities for self-assembled inorganic nanostructures. These nanoscale architectures further offer excellent promise for well-controlled studies on nanoelectronic structures.

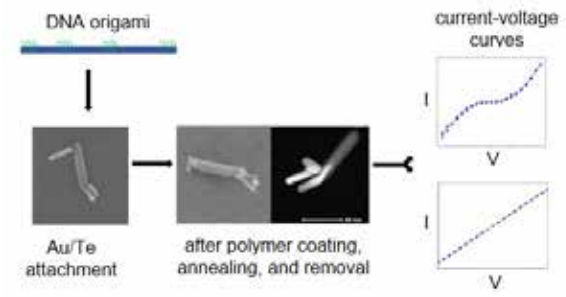


Fig. 1

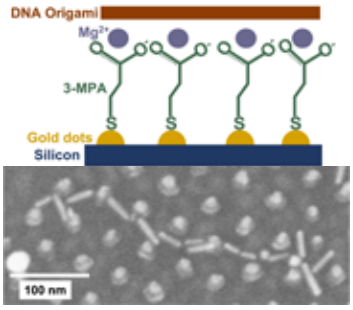


Fig. 2

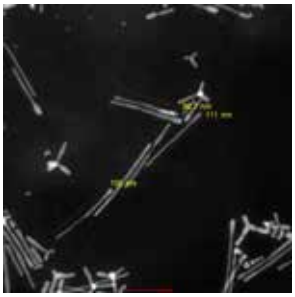


Fig. 3

Fig. 1: Polymer-constrained annealing to create Au-Te metal-semiconductor junctions
Fig. 2: DNA origami with attached nanorods assembled on gold nanodot arrays
Fig. 3: TEM image of synthesized cadmium sulfide nanorods

[1] C. Pang et al. (2021) Nanomaterials 11, 1655
[2] B.R. Aryal et al. (2021) ACS. Appl. Nano Mater. 4, 9094-9103.

Metallic nanowires assembled by DNA Origami

Artur Erbe[1], Archa Jain[1], Türkan Bayrak[1], Jingjing Ye[2], Ralf Seidel[2], Enrique Samano[3]
[1]Institute of Ion Beam Physics und Material Science, Helmholtz-Zentrum Dresden-Rossendorf, Germany
2Peter Debye Institute for Soft Matter Physics, Universität Leipzig, Germany
[3]Centro de Nanociencias y Nanotecnología-UNAM, Ensenada, B.C., México

The development of ever smaller electronic circuits may greatly benefit from the use of bottom-up strategies, which may lead to a more efficient and versatile construction of nanosized elements than by the traditional top-down approach. One of the most promising approaches is based on the self-recognition of DNA, which allows the construction of arbitrarily shaped nanoobjects with high reproducibility. Long DNA molecules, however, are poor conductors and thus not suitable for building electronics. Therefore, the nanostructures need to be functionalized with electrically conducting material, turning them into good wires or otherwise conducting circuit elements.

Here we demonstrate the formation of Au nanostructures based on DNA Origami structures, which are first formed by self-assembly and metallized in a subsequent step. Following one strategy, DNA nanomolds are employed, inside which gold deposition is employed by site-specific attached seeds. These structures can be subsequently continuously metallized by internal gold deposition. During this step the walls of the nanomolds serve as constraints. We were able to prove the metallic nature of these nanostructures by performing temperature-dependent charge transport measurements along the nanostructures [1]. These gold nanostructures can be used for contacting DNA assemblies, as well. Transport through these assemblies is strongly nonlinear and shows a decrease in conductance towards low temperatures [2].

Using a different approach, the shape of the nanowires can be controlled. We use DNA-origami templates which are functionalized on their surface in order to create desired shapes of the metallic nanostructures. Metallic nanoparticles are attached to the functional sites and enhanced through electroless deposition to form continuous conductive structures. Temperature dependent charge transport measurements reveal the dominating charge transport mechanisms along these wires [3].

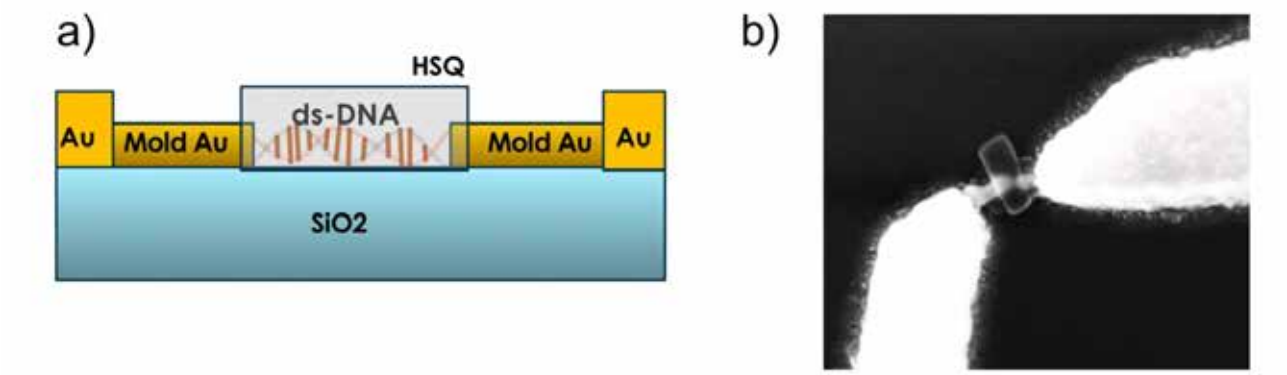


Figure 1 a) Sketch of Au-DNA-Au-nanojunction fabricated using DNA Origami molds. b) SEM image of DNA junction contacted by Au electrodes using electron beam lithography.

[1] T. Bayrak, *et al.* Nano Lett. **18**, 2116 (2018).
[2] J. Ye, *et al.* Advanced Materials **n/a**, 2100381 (2021).
[3] T. Bayrak, *et al.* Scientific Reports **11**, 1922 (2021).

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

Johannes Parikka, Kosti Tapio, Heini Järvinen, Nemanja Markesevic, 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 visualized to pose the next big leap in technology development. At a moment, DNA has proven to be a one of the most versatile and promising molecule for nanoscale fabrication [1], even for electrical and plasmonic purposes [2]. Since the self-assembly happens in a fully parallel manner, one can simultaneously fabricate huge amounts of devices. However, since the assembly happens in a solution, one needs a way to combine these bottom-up methods with some top-down method to - in the simplest case - position the assembled devices. Yet, combining the self-assembly directly with top-down methods during the fabrication, can yield even more versatile fabrication methods.

We have developed methods to trap and connect single molecular scale devices with other electrical circuitry [2, 3], and utilized this to study the conductance of several types of individual DNA nanostructures. For nanoscale optics, we have developed a novel method, 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 [4]. As a demonstration, we have fabricated optical bowtie antennas with a tunable plasmonic resonance in visible range. The method enables also fabrication of large optically chiral surfaces with high coverage.

To obtain the best possible optical response, we are developing a method to organize large lattices out of origami and fabricating metallic metamaterials out of that. So far, this kind of lattices have been fabricated mainly on mica [5], which is not compatible with almost any top-down methods. Thus, transporting the methods on silicon will open new avenues for DNA-based fabrication. In addition, we have found a way to fabricate rolled DNA-origami-lattices to form tubular geometries.

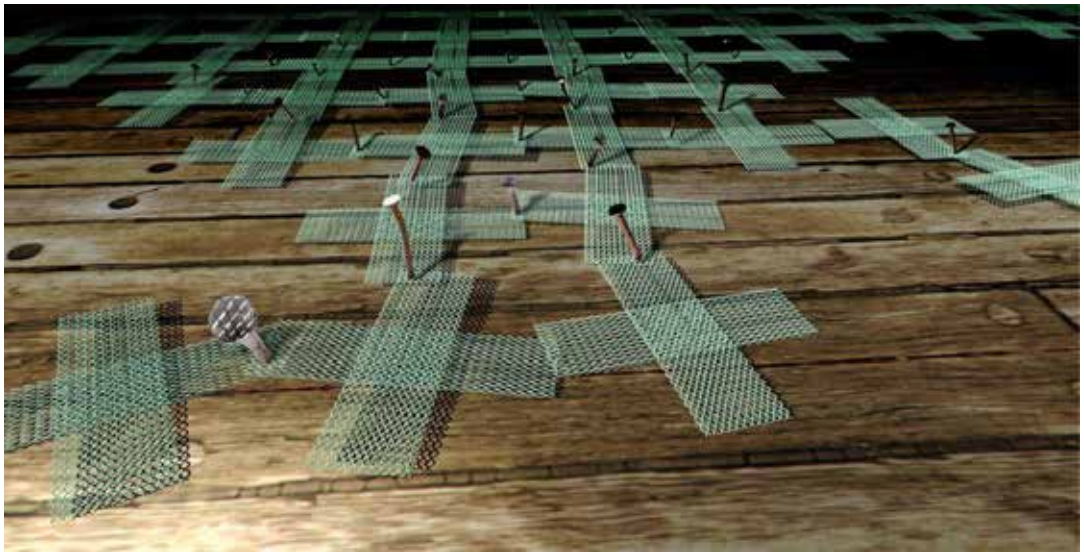


Fig. 1: Fabricating DNA-origami lattices.

[1] A.V. Pinheiro, et al. (2011). Nat. Nanotech. 6, 763; M. Madsen, K.V. Gothelf, (2019). Chem. Rev. 119, 6384.
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New insights into the DNA origami silicification reaction mechanism by *in situ* small angle X-ray scattering

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DNA origami allows for the formation of arbitrarily shaped nanostructures with nm precision control. However, many potential real-life applications have been hampered due to the instability of DNA origami in biological media, low salt buffers, in the presence of nucleases or at high temperatures. Silicification has been introduced as an excellent way of increasing the mechanical and thermal stability of DNA origami nanostructures as well as providing chemical protection [1-3]. However, so far, it remains unclear how silicification affects the internal structure of the DNA origami and whether the whole DNA framework is embedded or if silica just forms an outer shell. Especially for the formation of dielectrics or applications involving precise placement of guest molecules on/inside the DNA origami, this is essential knowledge. By using *in situ* small angle x-ray scattering (SAXS), we were able to show that silicification induces a substantial condensation of the DNA origami at early reaction times (Fig. 1) and that silica growth also occurs on the inner surface of the origami. The observed condensation is partly caused by depletion forces as water is slowly displaced by silica within the origami structure. Towards the end of the reaction, the overall size of the silicified origami increases again, which is in accordance with increased “shell thicknesses” observed by TEM and AFM in previous studies [1]. Remarkably, we found that thermal stabilization of the origami up to 60°C could already be observed for sub-nm silica deposition in the condensed state (Fig. 2). Furthermore, DNA origami objects with flat surfaces showed a greater tendency towards aggregation during silicification than origami with curved surfaces. Our studies provide novel insights into the silicification reaction and allow for the formulation of optimized reaction protocols based on DNA origami shape and design.

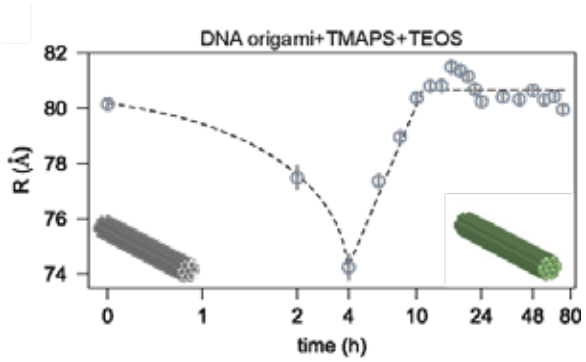

Fig. 1

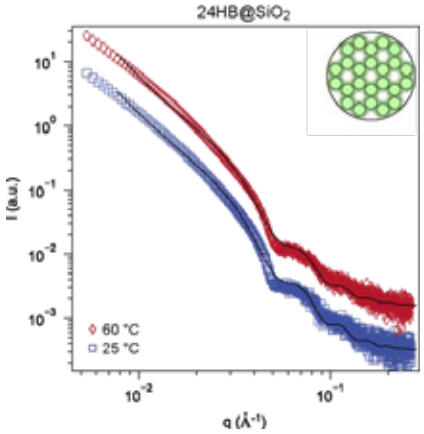

Fig. 2

Fig. 1: Overall cylinder radius (R) is reduced initially during silicification, then increases again.
Fig. 2: SAXS analysis shows that even at sub-nm silica coating, DNA origami remain stable when exposed to elevated temperatures for prolonged periods of time.

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Hierarchical self-assembly of DNA origami lattices at solid-liquid interfaces

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The hierarchical assembly of DNA origami nanostructures into ordered 2D lattices holds great promise for the fabrication of functional materials with applications in plasmonics, sensing, and biomedicine [1]. Among the different strategies that allow the fabrication of such lattices, surface-assisted hierarchical self-assembly at solid-liquid interfaces is a particularly promising approach, because it produces DNA lattices of astonishingly high order over macroscopic surface areas. This presentation will provide an overview of our recent activities that aimed at enhancing the size and order of such lattices. Using fast-scanning atomic force microscopy (AFM) and advanced image analysis techniques, we have monitored DNA origami lattice assembly at mica surfaces under different conditions and identified several parameters that influence lattice order [2-4]. By independently optimizing these parameters, we are able to obtain DNA origami lattices of unprecedented order and homogeneity (Fig. 1). Furthermore, we have also investigated the possibility of scaling up DNA origami lattice assembly and demonstrate the fabrication of a homogeneous, polycrystalline lattice over a total surface area of 18.75 cm² [5].

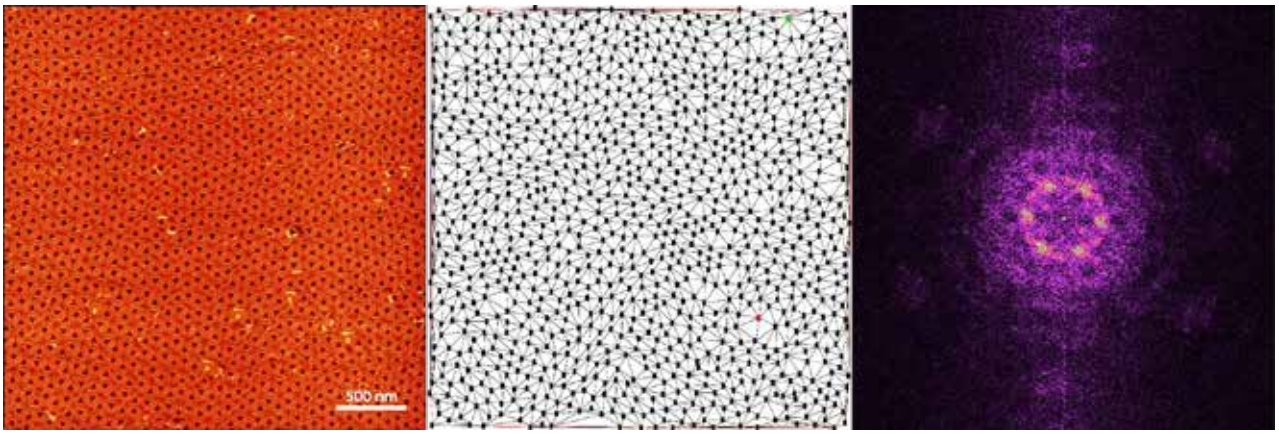

Fig. 1: Atomic force microscopy image (left), Delaunay triangulation (center), and Fast Fourier Transform (right) of a highly ordered DNA origami lattice on a mica surface.

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Single-molecule SERS measurements enabled by DNA origami nanostructures

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Surface-enhanced Raman scattering (SERS) is a powerful technique that allows for a sensitive detection of molecules and basically also for a monitoring of chemical reactions up to the single-molecule level. However, the signal enhancement is limited to nanoscale cavities in-between gold or silver nanoparticles, and it is inherently difficult to control the positioning of both, nanoparticles and analyte molecules. To tackle this challenge, we are using DNA origami nanostructures, which allow for the precise placement of functional entities such as metal nanoparticles,[1] and molecules such as proteins.[2] DNA origami nanostructures are formed from a long DNA scaffold strand and a suitable set of short staple strands, and through the specific choice of the set of staple strands, almost arbitrary 2D and 3D nanostructures can be formed.[3] Gold or silver nanoparticles can be attached to the DNA origami nanostructures to create dimeric or even more complex plasmonic nanostructures,[4] in which light is focused into nanoscale cavities creating intense hot spots for SERS. Using nanolenses made from three silver nanoparticles single streptavidin molecules could be detected by SERS.[2] We have recently designed a new and dedicated three-dimensional DNA origami nanostructure with the aim to create a versatile platform for reliable single-molecule SERS measurements.[5] Using this DNA origami nanostructure single molecule detection of three different dyes precisely placed into gold and silver nanoparticle dimers could be demonstrated. Furthermore, the label free single-molecule detection of two different proteins could be achieved by SERS.[5] In our most recent work, we could also extend this technique to monitor the molecular state of single hemin molecules, and follow chemical changes, i.e. a ligand-induced spin crossover on a single-molecule level by SERS.

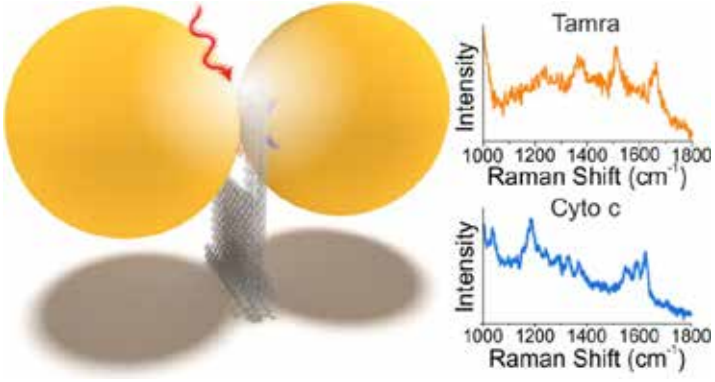

Fig. 1: Scheme of DNA origami nanoantenna (DONA) consisting of a Au nanoparticle dimer (left), which was used to collect single-molecule SERS spectra e.g. from the dye TAMRA and the protein cytochrome C (right).[5]

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Orienting single molecules in DNA origami constructs

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Over the last decade, DNA nanotechnology has been increasingly used to self-assemble functional nanostructures. One of the main advantages of this approach is that different species including colloidal nanoparticles and single photon emitters such as fluorophores can be positioned with nm precision and stoichiometric control [1]. This has been exploited for a growing number of nanophotonic applications [2]. While the relative distance between the hybrid species has been controlled up to the nanometer range, no control over the relative orientation has been exerted.

We present a method to both position and orient single photon emitters within DNA origami constructs. In particular, we exploit the ability of DNA origami to exert forces in order to “stretch” covalently incorporated dyes and deterministically align them with the orientation of double-stranded DNA helix they are located at (Fig 1.). We study the dye´s three dimensional orientation and wobbling using three independent techniques: polarization-resolved excitation measurement, point-spread function (PSF) analysis [3] and the four-polarization image splitting method [4] combined with a super-resolution (nanoscopy) measurement using the DNA-PAINT technique [5] to retrieve the orientation of the DNA origami “host” structure.

Our results show that by simply removing a number of nucleotides adjacent to both ends of the doubly-linked fluorophore, the dye transitions from a non-predictable orientation given by a combination of external factors to an orientation aligned with the predesign direction of the host ds-DNA helix. We believe this work shows a simple way to deterministically orient dyes which constitute the last degree of freedom required to manipulate the interaction of single photon emitters and fully control the coupling to other species.

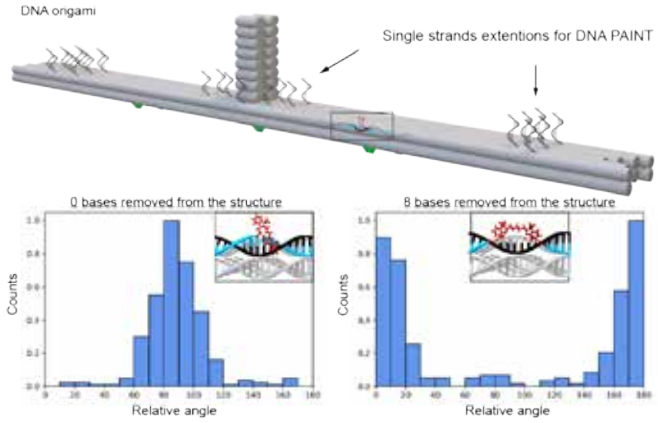

Fig. 1: Different orientation of a fluorescent dye in respect to DNA origami ‘host’ structure obtained for the structures with a different number of removed adjacent nucleotides.

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[3] Mortensen, K. et al., Nat. Methods(2010)
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Observing Single DNA Nanorobot Kinetics to Guide the Engineering of Molecular Torsion Springs

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A major challenge of bionanotechnology remains the development of nanoscale machines capable of manipulating and building biomolecules from ground up. Thereby the rapid development of manufacturing capabilities for self-assembled DNA nanoconstructs allows researchers to create increasingly powerful bionanomechanical devices which could lead the emerge of such molecular factories. Using DNA as a construction material, researchers already implemented various types of mechanisms known from mechanical engineering, including sliders[1], hinges[2] and rotary devices[3,4]. Such devices can be externally powered and controlled by employing stimuli like electric fields[5] with high speed and precision.

We perform motion analysis of individual nanomechanical robotic devices using single particle tracking microscopy in order to derive informed engineering guidelines for DNA based machine parts and optimize parameters for efficient electric actuation. One machine element developed with this strategy is a rotational joint including a torsion spring, which is implemented by two flexible DNA tethers wrapping around each other. Electric actuation thereby allows us to provide work against the torque of the torsion spring and study the machine behavior far from thermodynamic equilibrium to probe the mechanical properties of different torsion spring variants. The mechanical energy stored within the torsion spring can be released on demand, driving a directed motion of the nanorobotic arm towards its equilibrium state. To provide deeper insights in the molecular processes within the joint structure, we performed Brownian dynamics studies utilizing oxDNA with a simplified model of the nanorobotic arm.

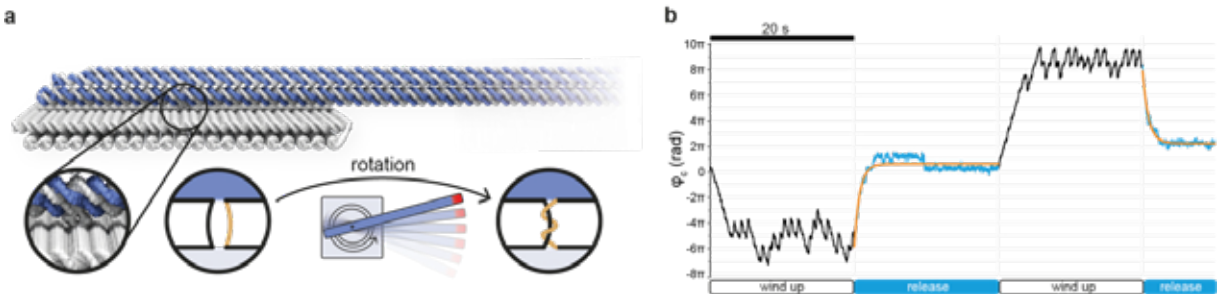

Fig. 1: a) Robotic DNA nanoarm fixed on a surface immobilized base plate. The connecting rotational joint is implemented in the form of two flexible DNA linkers which also act as a mechanical torsion spring. Upon rotation, the linkers wrap around each other and create a restoring torque b) Time trace of the angular position of an actuated nanoarm. The arm follows the rotating electric field until the restoring torque of the torsion spring cannot be overcome and the nanorobotic arm stalls, leading to a characteristic sawtooth pattern. Upon termination of the external field, the nanoarm rotates in a directed motion until the resting position is reached (blue). The same process is repeated in the opposite rotation direction.

*[1] Stömmer, P., et al. A synthetic tubular molecular transport system. Nat Commun **12,** 4393 (2021).*
*[2] Funke, J., et al. Placing molecules with Bohr radius resolution using DNA origami. Nature Nanotech **11,** 47–52 (2016).*
*[3] List, J., et al. Long-range movement of large mechanically interlocked DNA nanostructures. Nat Commun **7,** 12414 (2016).*
[4] Kosuri, P., et al. Rotation tracking of genome processing enzymes using DNA origami rotors. Nature 572, 136–140 (2019).
[5] Kopperger, E. et al. A self-assembled nanoscale robotic arm controlled by electric fields. Science 359, 296–301 (2018).

DNA origami in physiological settings

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LIBER Centre of Excellence, Aalto University, Finland

Nano-/subnanometer-precise DNA origami structures may serve as versatile high-resolution templates for engineering inorganic materials [1] and as components for bridging molecular and macroscopic scales (from nanometers to wafer scales) [2]. In biomedical settings, DNA origami could be integrated into sophisticated robotic devices [3], gene-editing tools [4], delivery vehicles [5] and diagnostic platforms [6]. However, under physiological conditions these structures may suffer from poor stability due to low-cation-induced denaturation and enzymatic degradation. Here, I present a few selected pieces of our very recent results dealing with these topics:

1) We have revealed some elemental structural features that govern the environment-dependent stability [7] and showed that DNA origami superstructure can be harnessed in rational design of drug release profiles [8] (Fig. 1).

2) We have developed static [9-11] and stimuli-responsive protective coating strategies [12] for DNA origami. With optically active protein coating we have demonstrated a controlled antigen targeting and binding (Fig. 2).

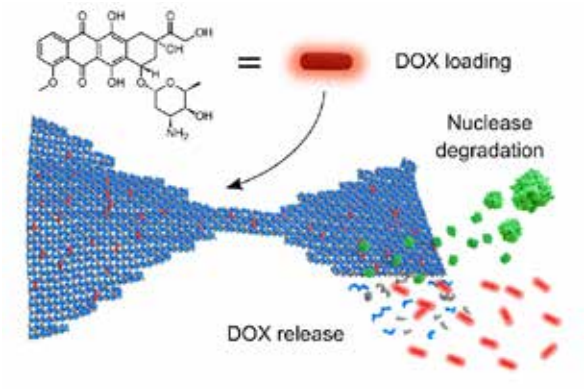

Fig 1.

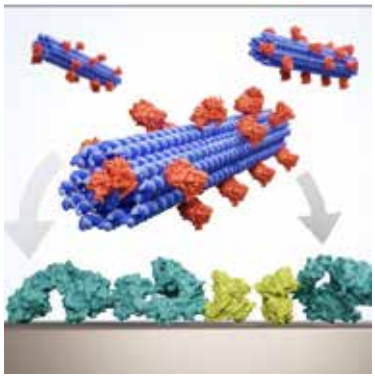

Fig 2.

Fig. 1: Doxorubicin is released from DNA origami upon nuclease degradation.
Fig. 2: Antibody-equipped DNA origami target antigens. Antibodies are displayed upon protective protein layer removal.

[1] A. Heuer-Jungemann, V. Linko (2021). ACS Cent Sci, 7 (12), 1969–1979
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[11] I. Seitz et al. (2022). In preparation
[12] I. Seitz et al. (2022). Submitted

Improving single-molecule detection: Polar surface array for controlling the adsorption of plasmonic DNA origami nanostructures

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The detection of specific single molecules in liquids, adsorbed on surfaces or in the gaseous state, is a long-desired goal in spectroscopy and requires spectroscopic techniques offering high spectral sensitivity. Single-molecule surface-enhanced Raman spectroscopy (SM-SERS) is one of these techniques that has been significantly developed in recent decades and now has the potential to detect the spectroscopic fingerprint of individual molecules using plasmonic-active materials. The gain of spectral sensitivity in SM-SERS can be achieved by utilizing DNA origami structures with plasmonic metal nanoparticles, forming complex nanostructures to capture single molecules for SM-SERS detection. However, such measurements are challenging since the nanostructures easily agglomerate when attached to surfaces.

Here, we introduce a promising approach to precisely control the adsorption position of DNA origami nanostructure on a flat substrate in the form of a polar surface array (PSA). The PSA surface is fabricated by physical vapor deposition (PVD) of specific organic molecules on a hydrophilic substrate, followed by electron-beam lithography (EBL) for surface patterning with nanometer precision. The polar surface pattern of the PSA substrate drastically reduces the agglomeration of DNA nanostructures, as shown by scanning electron microscopy (SEM) studies. Furthermore, we show the impact of incubation time and nanostructure density in the solution, as well as the impact of adsorption field size of PSA to improve the lateral isolation of a single DNA nanostructure for future SM-SERS measurements.

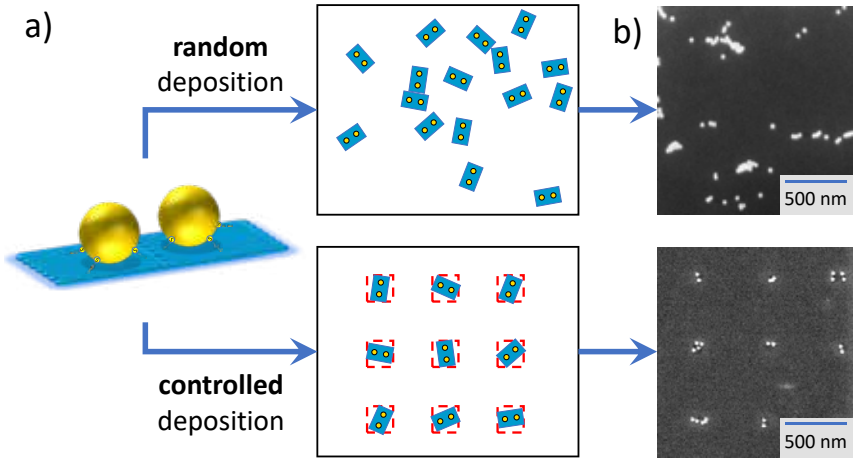

Fig. 1: a) Schematic representation of deposited DNA origami structure functionalized with two gold nanoparticles. b) SEM measurement of deposited nanostructures without (top) and with (bottom) polar surface array on the substrate.

[1] Kershner et al. (2009). Nature Nanotech. 4, 557–561 DOI: 10.1038/nnano.2009.220
[2] Tapio et al. (2021). ACS Nano 4 7065-7077 DOI: 10.1021/acsnano.1c00188
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Membrane domain sensing by amphipathic curved DNA origami

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In recent years, DNA origami has proven to be an exceptional engineering tool when combined with model membranes. The ability of DNA origami to provide custom nanoscale shapes, with defined curvature, oligomerization and precise placement of membrane-anchor moieties, enabled us to develop synthetic biomimetic structures [1, 2] that controllably bind, actuate and transform lipid membranes. One important feature of biological membranes is their compositional heterogeneity, which can give rise to so-called lipid rafts – ordered domains enriched in sphingolipids and cholesterol. By using membrane model systems, lateral lipid segregation can be mimicked and investigated using fluorescence microscopy.

In this work, we therefore set out to design a group of amphipathic DNA origami structures that interact with phase-separated lipid membranes, and controllably recognize fluid liquid-disordered (L_d) and/or raft-mimicking liquid-ordered (L_o) domains. By varying a) the intrinsic curvature of our DNA origami nanodevices (Fig. 1), b) accessibility of the hydrophobic cholesteryl anchors used for membrane binding, and c) $MgCl_2$ concentration in solution, a differential partitioning and tunable sensing towards the L_d vs. L_o membrane regions could be achieved.

Overall, our strategy opens new avenues for mapping membrane rigidity and lipid composition on biological membranes, demonstrating the unique advantages of DNA nanotechnology for targeting lipid membrane domains and studying their physicochemical properties.

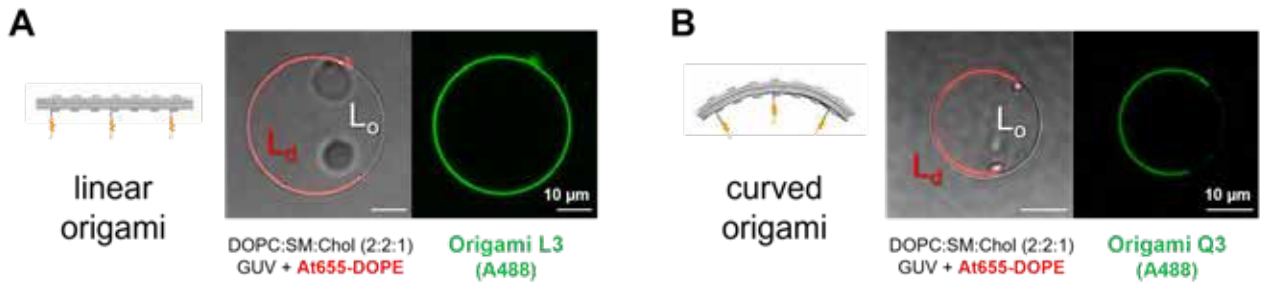

Fig. 1: Attachment of cholesterol-modified DNA origami nanostructures to phase-separated giant lipid vesicles as a function of origami curvature. A) Linear origami binds almost equally to L_d and L_o phases. B) Curved origami, on the other hand, binds selectively to the more fluid L_d phase.

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[2] Franquelim et al., 2021, Soft Matter, 17(2), 276-287

Target-induced dual-signal electrochemical aptasensor for sensitive and accurate detection of aflatoxin B1

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[3]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich Schiller University Jena, Helmholtzweg 4, Jena, Germany

Aflatoxin B1 (AFB1) is highly toxic and carcinogenic secondary metabolite produced by *Aspergillus flavus*. It remains a great challenge to develop an effective method for a rapid and sensitive detection of AFB1. It is reported that electrochemical aptasensors show the advantages of simple operation, high sensitivity, low cost as well as easy miniaturization. Moreover, increasing research have found that the use of signal amplification strategy can greatly improve the detection sensitivity and accuracy. Enzyme-free signal amplification has been widely used for constructing all kinds of biosensors via hybridization chain reaction (HCR).

Within this presentation, we present a dual-signal electrochemical aptasensor (DECA) for the detection of AFB1 based on HCR induced signal amplification. In the DECA system, ferrocene (Fc) and methylene blue (MB)-tagged DNA sequences acted as dual signals. Based on this, hairpin DNA (HS-H1) and MB-labelled auxiliary hairpin DNA (H1 and H2) were introduced (**Figure 1**). Specific interaction of AFB1 with Fc-labeled aptamer released MB-cDNA to trigger a hybridization chain reaction for MB signal amplification, resulting in the detection of an enhancement of the dual signal.

In the work, the signal amplification strategy and dual signals output mode can simultaneously improve sensitivity and accuracy of the electrochemical sensor. As a result, the sensor exhibited good sensitivity, specificity, and reproducibility, revealing a promising application in food safety monitoring.

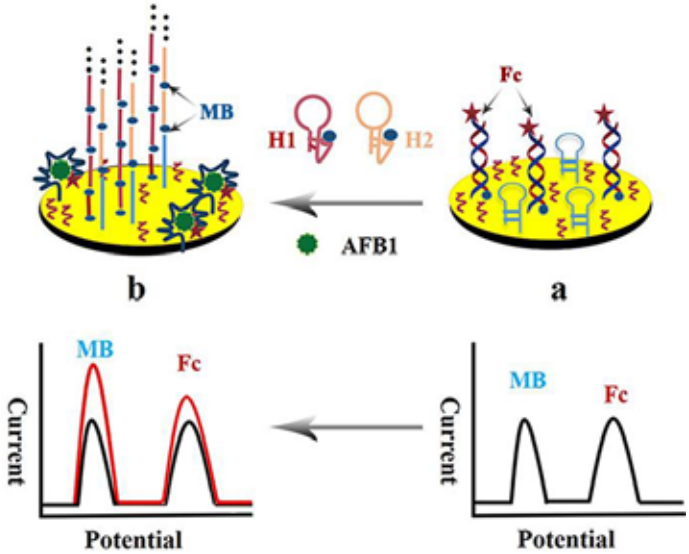

Fig. 1: Schematic presentation of electrochemical sensor based on hybridization chain reaction signal amplification for AFB1 determination.

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POSTER

Anion-Specific Structure and Stability of Guanidinium-Bound DNA Origami

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The "bottom-up fabrication" of DNA origami is an approach developed by Rothemund in 2006 for the fabrication of highly complex nanostructures by self-assembly, which allows the creation of 2D and 3D objects of arbitrary shape. DNA origami nanostructures are particularly well suited as substrates for the assembly of various functionalities such as proteins, nanoparticles, or specific DNA structures with unprecedented precision that can be assessed by numerous spectroscopic and microscopic techniques. In particular, the immobilization of individual proteins on DNA origamis appears attractive for the observation of conformational changes and folding by single-molecule techniques. Chemical denaturants such as urea and guanidinium chloride are commonly used to trigger such transitions in proteins. The effects of these chaotropic salts are well described for proteins, less so for DNA, and hardly at all for DNA origami nanostructures. Moreover, because of the unique properties of DNA nanostructures, their interaction with denaturants is of fundamental interest.

To reveal the interplay between DNA origami and chaotropic agents, atomic force microscopy (AFM) images of the denaturation process of DNA origami triangles with different guanidinium salts were compared with the corresponding circular dichroism spectra. The AFM data show an early break of the origami, while the hyperchromic shift indicates that the original melting process starts much later. Using principal component analysis, iterative target test factor analysis (ITTFA) and various methods of 2D correlation spectroscopy, the two processes can be correlated and explained with a thermodynamic model by the additional factor of a change in heat capacity. It is found that DNA-origami nanostructures undergo changes in secondary structure leading to breaks at the vertices. The reason for this could be due to the specific interaction of counter anions that shape the properties of the surrounding water structure and thus control the interaction between DNA and guadinium. These findings help to optimally tailor DNA-origami as a substrate for denaturant-induced folding dynamics of individual proteins while improving the fundamental understanding of the effects of water structure on DNA.

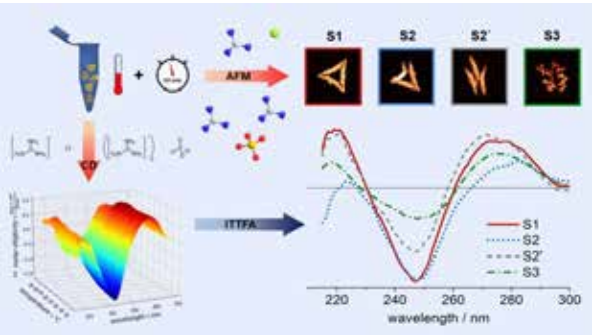

Figure 1. Experimental approach: The nanostructural integrity of DNA origami triangles after exposure to GdmCl and Gdm2SO4 for one hour is evaluated at selected temperatures by atomic force microscopy (AFM). Temperature-dependent DNA melting in the DNA origami triangles is assessed under equivalent conditions by circular dichroism (CD) spectroscopy. An iterative target test factor analysis (ITTFA) of the CD spectra primed with the fractions of intact and damaged DNA origami observed in AFM allows us to identify four different structural states (S1 – S4) occurring during DNA origami melting and their individual coun-teranion-independent component spectra.

Investigation of LEE-induced single-strand breaks using DNA origami nanostructures with radiosensitizers to support cancer radiotherapy

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Everyday radiation damages the DNA of living cells, which can lead to cancer or cell death (apoptosis). In cancer radiotherapy, the apoptosis effect is used to kill cancer tissue with the help of high-energy radiation. By ionising water molecules, highly reactive secondary species such as low-energy electrons (LEE), with an energy maximum at about 10 eV, can be generated.[1] Via dissociative electron attachment (DEA) involving negative ion resonances, LEEs can induce DNA strand breaks at certain energies. Using the DNA origami technique, we can determine absolute DNA strand break cross-sections at the molecular level on triangular DNA origami nanostructures (figure 1).[2,3] Using radiosensitizing therapeutics such as fluorinated nucleosides 2′-fluoro-2′-deoxycytidine (^{dF}C) the reactivity towards LEEs can be enhanced (figure 2). S. Vogel et al suspected that DNA strand breaks depend, among other things, on the irradiation energy.[4]

The present work shows results of quantitative investigations of LEE-induced single-strand breaks on well-defined DNA oligonucleotide sequences, each with fluorination on the sugar and the corresponding sequence without fluorination, which were irradiated at 7 eV, 15 eV and 20 eV.

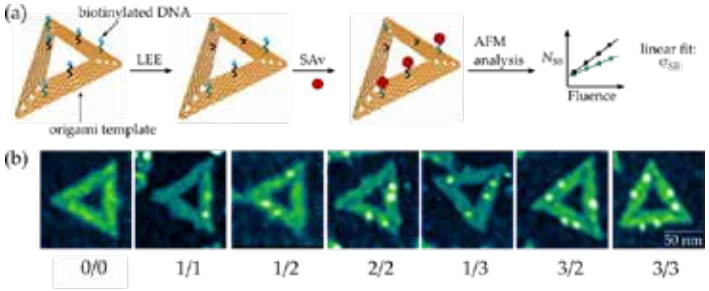

Fig. 1

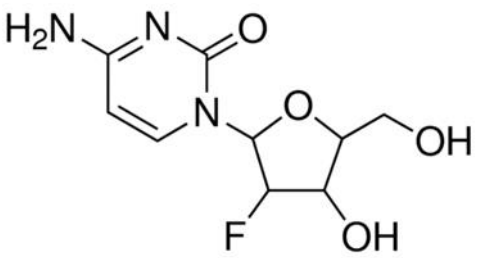

Fig. 2

Fig. 1: a) Scheme of the DNA origami structure and the experimental procedure to determine the absolute cross section of DNA strand breakage. Each DNA origami can carry two different (black and green) biotinylated target sequences. b) AFM images of DNA origami nanostructures
Fig. 2: Image of the radiosensitizer 2′-fluoro-2′-deoxycytidine (^{dF}C)

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[2] A. Keller et al (2012) ACS Nano 6 4392.
[3] J. Rackwitz et al (2016) Angew. Chem. Int. Ed. 55 10248-10252
[4] S. Vogel et al (2019) Phys. Chem. Chem. Phys. 21 1972-1979

DNA Origami Forks for Single Molecule SERS of Cytochrome C

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The field of DNA nanotechnology has had a remarkable impact on a number of areas such as biophysics, diagnostics, biomolecular structure determination and drug delivery. The core of this field is to take DNA out of its biological context and using its physical and chemical properties to create various nanostructures [1]. The specific base-pairing nature of DNA allows for rational design of self-assembled highly specific nanostructures [2].

In the past few years, DNA origami based structures have been successfully utilized in label-free detection using the principle of Surface Enhanced-Raman Spectroscopy (SERS). The field is still evolving, with a demand for more complex and highly specific structures. Our aim is to develop a DNA origami structure that could capture a single target molecule and then is coupled with nano-particles for SERS measurements. One could use such system for detection of dyes or biomolecules, an example would be heme containing proteins.
Hemeproteins or heme containing proteins are very important for proper function of various organisms. They are a large class of proteins that is highly abundant and have diverse biological functions [3]. One of these proteins is Cytochrome C which is found in plants, animals and some unicellular organisms, and it's highly conserved across the spectrum of these species. The heme in Cytochrome C is contained in a hemin moiety and it is essential for the electron transport chain in mitochondria [4].

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[2] N. C. Seeman, "Nanomaterials Based on DNA," Annu. Rev. Biochem., vol. 79, no. 1, pp. 65–87, Jun. 2010.
[3] C. J. Reedy, M. M. Elvekrog, and B. R. Gibney, "Development of a heme protein structure electrochemical function database," Nucleic Acids Res., vol. 36, no. Database, pp. D307–D313, Dec. 2007.
[4] "Cytochrome C." [Online]. Available: https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=54205.

Salting-out of DNA Origami Nanostructures by Ammonium Sulfate

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Over the last two decades, the DNA origami technique [1] gained increasing attention from a wide field of applications ranging from biophysics to biomedicine and molecular sensing [2]. Even though DNA origami nanostructures and genomic DNA both show high biocompatibility, biodegradability and are non-cytotoxic, their specific molecular interactions often differ significantly [3].
In this work [4], we investigated the salting-out effect of the prominent kosmotropic salt ammonium sulfate on different DNA origami shapes, i.e., quasi-1D six-helix bundles (6HBs), 2D triangles, and 3D 24-helix bundles (24HBs). The centrifugation of these different DNA origami shapes in the presence of 3 M ammonium sulfate lead to notable precipitation (see Fig. 1.), while double-stranded genomic DNA remained unaffected. However, we could find variations in the susceptibility of the employed shapes to salting-out. 6HBs were slightly less affected than the more compact triangles and 24HBs. Furthermore, all three shapes could be resuspended in ammonium sulfate-free buffer without showing any aggregation or loss in structural integrity. We finally utilized the selectivity of ammonium sulfate salting-out for DNA origami nanostructures to separate triangular shaped DNA origami from double-stranded genomic DNA in a complex solution. This novel method may offer a possibility for concentrating and purifying DNA origami nanostructures depending on their molecular weight and superstructure.

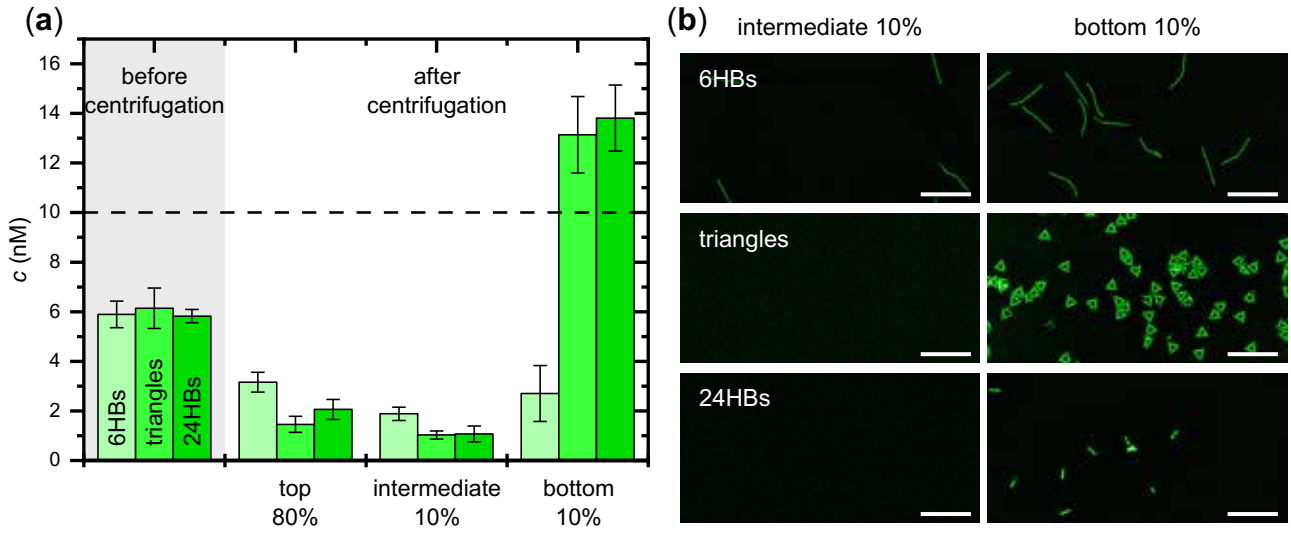

Fig. 1

Fig. 1: (a) Determined concentrations of DNA origami shapes before and after centrifugation in 3 M ammonium sulfate by UV absorption. (b) Representative AFM-images of DNA origami shapes in the intermediate 10 % and bottom 10 % fractions.

[1] P.W. Rothemund (2006). Nature, 440 (7082), 297–302.
[2] S. Dey et al. (2021). Nature Reviews Methods Primers. 1, 13.
[3] M. Hanke et al. (2020). Nanomaterials. 10 (11), 2200.
[4] M. Hanke et al. (2022). International Journal of Molecular Sciences. 23 (5), 2817.

LSPR-mediated detection of plasmid-based resistance genes in a single assay and a microarray format

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The development of rapid, simple, and accurate bioassays has received increasing demand in recent years. This includes the detection of nucleic acids, as well as of proteins for diagnostic purposes, biomonitoring, and research purposes [1, 2, 3, 4].
Here we used localized surface plasmon resonance (LSPR) spectroscopy as a suitable tool for the surveillance of antimicrobial resistance genes located on plasmids, which are extrachromosomal DNA elements that act as key drivers for the spread of antibiotic resistance in bacteria. As a *proof of principle*, we first focused on the detection of the bla_{SHV} gene, which confers resistance against a broad spectrum of beta-lactam antibiotics. By performing limit of detection experiments using a single assay format (ensLSPR), DNA target sequences down to 13 nM could be detected.
Furthermore, the setup also allowed studying the binding kinetics of target DNA molecules to the specific capture sequence in real-time [5]. Strikingly, performing LSPR measurements using a mutational hot spot of the bla_{SHV} gene revealed that sequences which only differ in a single nucleotide can be distinguished from the fully complementary sequence. The possibility to distinguish such sequences is of utmost importance in clinical environments, as it allows to identify mutations essential for enzyme function and thus, crucial for the correct treatment with antibiotics.
Ongoing work focuses on the transition to a microarray format, which will allow to detect and discriminate multiple DNA sequences in parallel. Adjusting various manufacturing conditions of the nanoparticle array sensors such as humidity and temperature, as well as nanoparticle concentration and volume resulted in a more homogeneous distribution of the nanoparticles overall, as well as the absence of a so-called 'coffee ring' effect [6]. Finally, a high signal-to-noise ratio during LSPR measurements was achieved.
Taken together, LSPR enables the rapid, cost-effective, and label-free detection of antimicrobial resistance genes. Combining this technique with a microarray approach will provide a robust, and cost-efficient analytical tool for the surveillance of antimicrobial resistance plasmids.

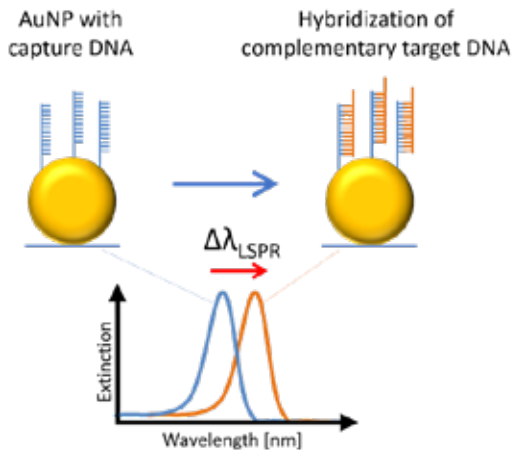

Figure 1: LSPR shift induced by the hybridization of targeted ssDNA to a complementary capture ssDNA, which is immobilized on the surface of gold nanoparticles.

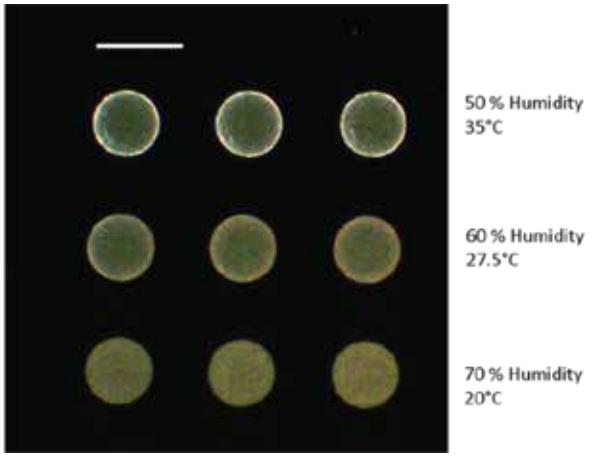

Figure 2: Dark-field microscope images of 160x concentrated AuNPs with 5 drops per spot. Different conditions of temperature and humidity were tested (20°C, 70% RH; 27,5°C, 60% RH, and 35°C, 50% RH). The white scale bar represents 200 µm.

[1] Kastner et al, Nature scientific reports 12, Article number: 836 (2022)
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[3] Zopf et al, ACS Sensors 4, 335–343 (2019)
[4] Thamm et al, DNA Nanotechnology: Methods and Protocols, 163-171 (2018)
[5] Barbir et al, ACS Langmuir 37, 1991-2000 (2021)
[6] Jatschka et al, Sensing and Bio-Sensing Research 7, 62-70 (2016)

AFM-assisted DNA origami patterning for lipid nanodisc studies

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DNA origami, as first presented by Rothemund [1], has become a versatile tool with great potential in many different research areas such as nanotechnology [2], biophysics and basic research at the single molecule level. By hybridizing a given single-stranded DNA scaffold with specially designed oligonucleotides one can achieve the self-assembly of almost arbitrary structures and offering the possibility of self-assemblies of regular patterns of chemical entities for various biomedical applications. The possibilities for novel approaches in membrane protein (MP) research is currently explored. Recent studies have immobilized MPs in lipid nanodiscs (NDs) on 2D streptavidin surfaces [3], without control over the lateral spatial arrangement of the NDs and the functionalization for supramolecular assembly into worm-like structures has been reported [4]. However, patterning of surfaces with NDs [5] by DNA origami may allow superior control of the final two or three-dimensional superstructures.

Here, we use DNA origami nanostructures as a substrate to create a defined arrangement of NDs in the origami lattice. We prepare highly hierarchical 2D lattices of triangular DNA origamis on solid support surfaces and analyze the filling of the cavities of the origami monolayers with NDs at different ND concentrations. Using Atomic Force Microscopy (AFM) over time, we find that the NDs fill and orient in the cavities without chemical or functional modifications. The AFM is not only used as an imaging method for the newly generated surface, it also serves at the same time as a tool for defined and controllable ND patterning. With increasing ND concentration, we find significant differences in the formation of the ordered surface structure of the newly generated ND origami monolayer system.

These proof-of-principle experiments demonstrate the applicability of the DNA origami structure as a highly hierarchically generated 2D lattice for the specific arrangement of large (several μm^2) ND-filled structured surfaces enabling quantitative AFM analysis of parallelized structural and functional studies of membrane proteins over long time scales.

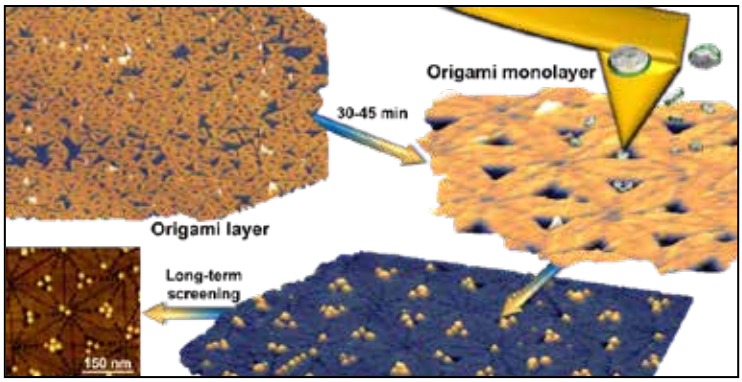

Fig. 1: Filling of NDs into cavities of a DNA origami monolayer forming large-scale 2D lattices.

[1] P. W. K. Rothemund, Nature 440 (2006) 297-302
[2] C. Kielar et al., ACS Appl. Mater. Interfaces 10 (2018) 44844-44853
[3] T. Haruyama et al., Structure 27 (2019) 152-160
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[5] T. H. Bayburt et al., Nano Letters 2 (2002) 853-856

Fabrication of large scale AuNP arrays as DNA LSPR sensors

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The label-free DNA sensing utilising localised surface plasmon resonance (LSPR) is a fast-growing application field that entirely relies upon the synthesis of plasmonic nanostructures. Here, gold nanoparticles (AuNPs) stand out given their size-/shape-tuneable plasmonic properties, biocompatibility, non-corrosivity, and amenability of the desired functionalisation. Moreover, well-ordered and close-packaged AuNPs can facilitate a plasmon coupling, critically increasing the hotspots' intensity and thus the signal enhancement. However, the fabrication of such sub-100 nm structures is not trivial nor affordable for many applications.

This work presents an affordable solution, illustrated in Figure 1, by synthesising AuNP arrays utilising porous anodic alumina (PAA) as a nanostructured template for solid-state dewetting of thin gold films. Transferring the AuNP array to a transparent substrate, better suited for sensing applications, is possible by sacrificing the aluminium template after a new substrate is cast over the AuNPs. Afterwards, the embedding substrate is etched away to reveal AuNPs' surface and enhance their sensitivity.

The periodic arrangement of the nanoparticles can be controlled over a large surface area (in the cm^2 range) in a homogenous way, with a precision approaching that of electron lithography, however, for only a fraction of the costs. The nanoparticles packing, namely the particle diameter / interparticle gap ratio, can be controlled, thereby the plasmon coupling between nanoparticles and effective plasmon field decay length of the array. This makes it a universal and powerful platform that can be optimised to detect variously long DNA probe/target segments.

Our previous work [1] demonstrated DNA detection (20 bases long) on such AuNPs-epoxy nanocomposites. Here, we present up-to-date technological improvements contributing to a dramatic increase in the stability, usability, and sensitivity of these sensors.

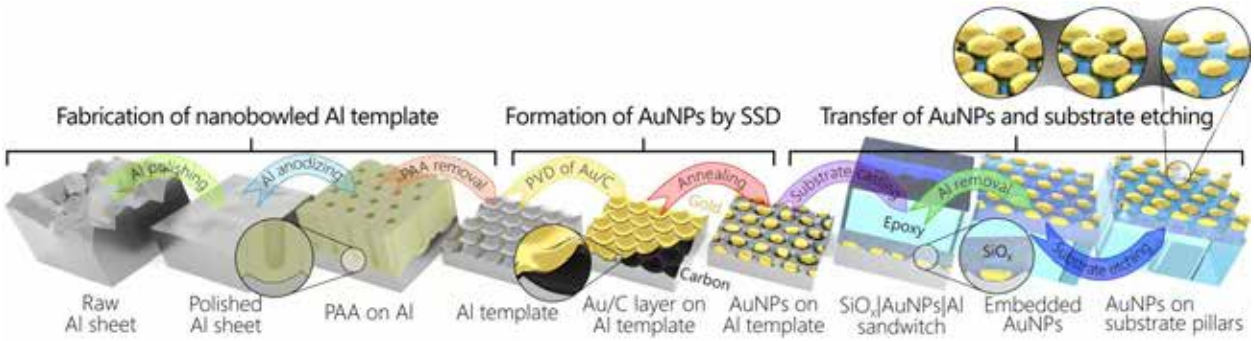

Fig. 1: The fabrication scheme utilising porous anodic alumina to synthesise well-ordered AuNP arrays and their transfer to a transparent substrate to serve as an LSPR sensor for DNA detection.

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Acknowledgement
CzechNanoLab project LM2018110 funded by MEYS CR is gratefully acknowledged for the financial support of the measurements/sample fabrication at CEITEC Nano Research Infrastructure.

Magnetic nanoparticle-assisted modification-free Surface Plasmon Resonance biosensor for early detection of Cytomegalovirus miRNA

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Surface Plasmon Resonance (SPR) is a well-known approach that is able to offer superb detection of biomolecules, such as proteins and nucleic acids [1]. However, nonspecific adsorption is a major concern in SPR biosensing. Herein, we developed a newly designed SPR biosensor for the detection of Cytomegalovirus (CMV)-specific microRNAs that does not involve extra care for receptor immobilization or treatment to prevent fouling on bare gold surfaces. The modification-free approach, which utilizes a poly-adenine [poly(A)]–Au interaction, exhibited a high affinity that was comparable to that of the gold–sulfur (Au–S) interaction. In addition, magnetic nanoparticles (MNPs) were used to separate the analyte from complex sample matrixes that significantly reduced nonspecific adsorption. Moreover, the MNPs also played an important role in SPR signal amplification due to the binding-induced change in the refractive index. Our platform successfully offered a sensitive detection for the CMV-associated miRNAs, UL22A-5p and UL112-3p, with a detection limit of 108 and 24 pM, respectively. In addition, our sensor is capable of discriminating between serum samples collected from healthy and CMV-infected newborns and the result were in agreement with those obtained from a commercial qRT-PCR kit. Taken together, we believe that our newly developed SPR biosensing platform is a promising alternative for the diagnosis of CMV-specific microRNA in clinical settings, and its application for the detection of other miRNAs may be extended further.

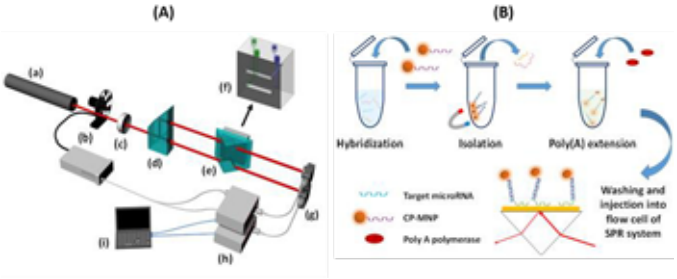

Fig. 1

Fig. 1: Schematic illustration of a modification-free, nucleic acid amplification-free SPR biosensor for the detection of CMV miRNA. (A) Configuration of the homemade SPR biosensing system. (B) Sensing platform

[1] Masson JF (2017) ACS Sens 2 (1):16-30.

SERS-based assessment of the pyrazinamide resistance of *Mycobacterium tuberculosis* in biological matrices

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Raman spectroscopy is a powerful tool for bioanalytical detection methods due to its molecular-specific fingerprint information. The limitation associated with the small Raman cross section can be overcome by the application of powerful plasmonic-active nanostructured sensing surfaces, i.e. by means of surface-enhanced Raman spectroscopy (SERS). Thus, analyte molecules in the µM range or lower can be detected with high specificity even in complex biological matrices. In this work, pyrazinoic acid (POA), the metabolite of the tuberculosis prodrug pyrazinamide (PZA), is detected in airway mucus culture supernatants by using specially designed gold nanoparticles coated with Prussian blue as a SERS sensor. As shown in Fig. 1, POA (1 mM) can be enriched on the Prussian blue-modified gold nanoparticles (AuPB), which results in a SERS spectrum with a good signal-to-noise ratio. In contrast, PZA (1 mM) shows no intense SERS spectrum by using the same sensing platform. Thus, the results illustrate the potential of the selective POA detection scheme for an assessment of the resistance of *M. tuberculosis* in cultures, as most resistant strains do not allow the metabolism from PZA to POA.

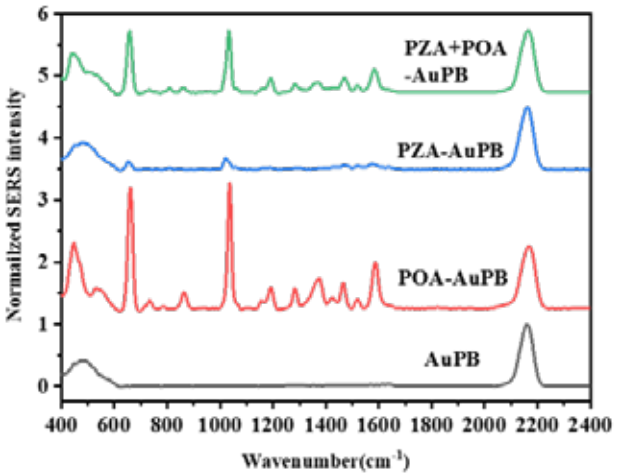

Fig. 1: SERS spectrum investigation of gold nanoparticles coated with Prussian blue (AuPB) as a SERS sensor in aqueous solution.

Acknowledgement: We thank the German Federal Ministry of Education and Research (BMBF) for supporting the InfectoGnostics project (13GW0096F).

[1] Hidi et al., Anal Bioanal Chem 408 (2016):8393-8401.
[2] Cialla et al., Anal Bioanal Chem 403 (2012):27-54.

Characterization of the sensing potential of plasmonic nanostructures

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Plasmonic metal nanostructures have garnered extensive research interest in the past decades for biological detection as they present unique optical properties due to their localised surface plasmon resonance (LSPR), which is responsible for the enhancement in the absorption and scattering of light. Hence nano-scale detections can be achieved by measuring the shift of the resonance wavelength of LSPR due to changes in the refractive index of the surrounding.

This work aims to design and fabricate hybrid plasmonic nanostructures as potential sensing components. As a starting point, a model system was used. A nanoarray of holes in PMMA was designed by Electron Beam Lithography (EBL), in which gold nanoparticles were immobilised. To obtain different hybrid nanostructures, the shape and dimension of the holes were varied. As a result, five different nanostructures of defined shapes could be fabricated, namely, single particles, dimers, prisms, particle ring hexamers and heptamers.

The single hybrid nanostructure spectra measured with a micro-spectroscopy setup corroborate with our simulations. We will further investigate the correlation between the colour information obtained in Dark field mode, the topography of the samples taken with the Atomic Force Microscope (AFM) as well as the single hybrid nanostructure spectra of the structures. In addition, we will determine which nanostructure is the most sensitive for bioanalytical applications.

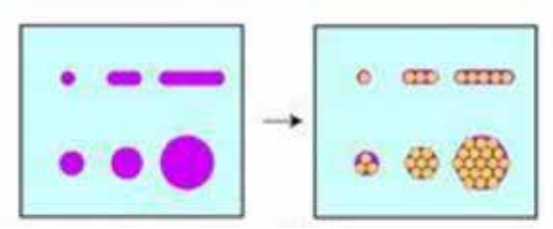

Fig. 1

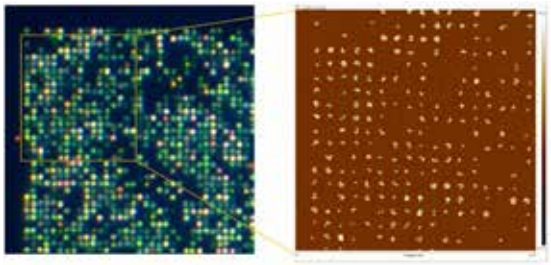

Fig. 2

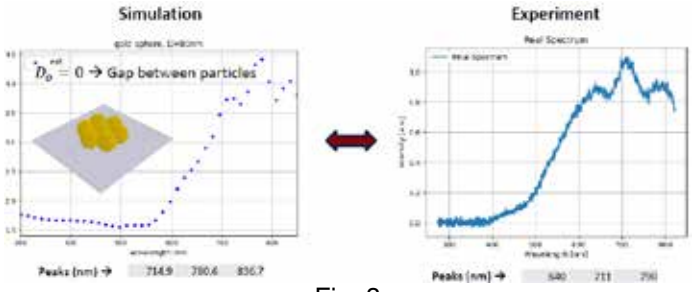

Fig. 3

Fig. 1: Immobilization of particles in linear and cylindrical slits
Fig. 2: Characterization of the hybrid nanostructures in Dark field mode and by AFM
Fig. 3: Spectra of a single heptamer

[1] A. Csáki, O. Stranik, and W. Fritzsche, 'Localized surface plasmon resonance based biosensing', *Expert Rev. Mol. Diagn.*, vol. 18, no. 3, pp. 279–296, Mar. 2018, doi: 10.1080/14737159.2018.1440208.

Tube-shaped assemblies of DNA-origami-lattices in solution

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In a past decade, DNA origami has become one of the most used building blocks in self-assembled materials in nanoscale [1]. Also, with different techniques large 2D lattices have been made using DNA-tiles [2]. In our recent work, we have shown that within certain ionic conditions the blunt-ended Seeman Tile (ST) [3] origami forms ordered 2D lattices, which then rolls into 3D tubular nanostructures as shown in figure 1. Via thorough studies, we have concluded that the process is heavily dependent on the concentration of both mono- and divalent cations, especially Ni^{2+}. Liquid-AMF *in-situ* imaging, i.e., imaging of the surface during the lattice formation, revealed that the DNA-tiles hardly diffuse on the surface, but already pre-formed lattice flakes are attaching and detaching the surface regularly. Our DNA origami deposition protocol employs high concentration of salts, which we hypothesized to result in a liquid-phase formation of the origami assemblies, despite the utilized weaker blunt-end stacking interactions.

We observed that in the liquid-phase assembly, it is possible to guide the lattice formation process into mono- and double layered sheets of the lattices. Since the origami structures collapse when attached on a surface, we needed to assess the possible tubular morphology of the observed ribbons (sheet vs tube) by cryo-electron microscopy (EM). The origami sample solution with correct ionic conditions was imaged using cryo-EM to determine the behavior of the system in liquid media and confirm the tube-like morphology, as shown in figure 1b. EM-imaging confirmed the formation of the structures already in solution. The imaged structures resembled tubular objects, but the exact morphology is hard to fully conclude, especially since the low height of the imaging volume at cryo-EM (< 200 nm) hindered the dimensions in our case. We noticed that width was quite uniform, but length was varying a lot. We are currently finalizing a joint publication about this finding of DNA origami tubes.

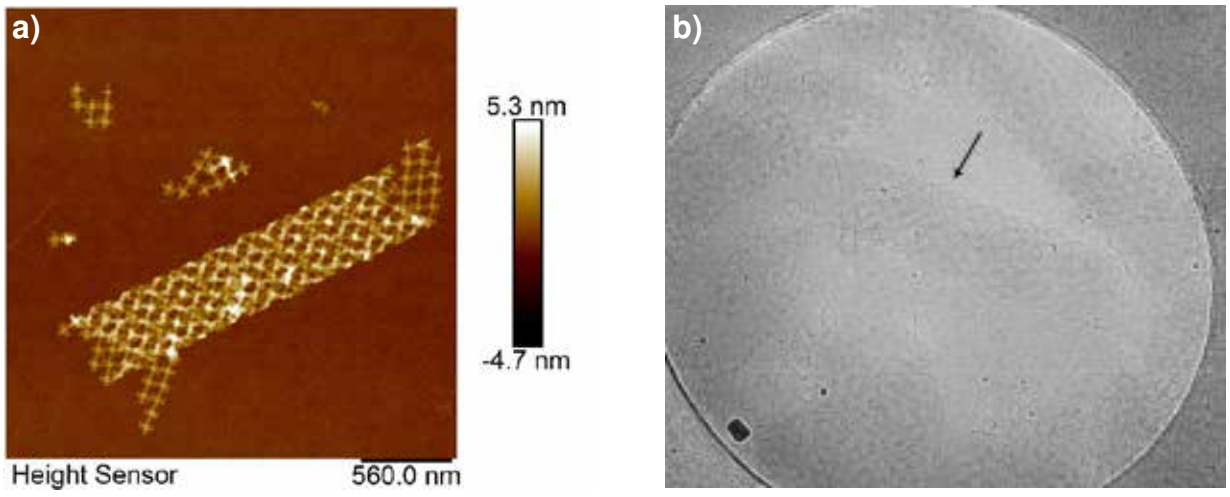

Fig. 1: ST-origami tube/ribbon imaged using a) AFM on dry Si-substrate and b) Cryo-EM in solution.

[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] A. Rafat, T. Pirzer, M. Scheible, et al., Angew. Chem. Int. Ed. 53, 7665 –7668 (2014).

DNA origami assisted gold dimers as SERS substrates on optical fiber tips for direct miRNA detection using hairpin probes

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MicroRNAs (miRNAs) serve an important regulatory role in various diseases and are emerging as novel class of biomarkers for early informative diagnostics of cancer, neurological and genetic disorders. However their intrinsic properties like low abundance and short length limits their use in clinical diagnostics. Hence it is crucial to develop new diagnostic strategies for miRNAs which overcome the limitations of conventional techniques. SERS has emerged as an exciting biodiagnostic tool over the past decade offering the detection limits up to single molecule regime. However the single molecule sensitivity is limited to the placement of target molecule in well-defined gaps between the plasmonic (gold, silver) nanotags referred as hotspots. A strong enhancement in Raman signal of a molecule is observed when it is precisely located in these hotspots. DNA origami offers a reliable strategy for precise self-assembly of nanostructures due to rich chemical functionalization possibilities. Our group have designed a versatile DNA origami termed as DNA origami nanofork antenna (DONA) with plasmonic dimers having optimized gap sizes for single molecule SERS detection for differently sized analytes like dye molecules and proteins [1]. A schematic view of DONA structure and AFM image of prepared nanoforks is shown in figure 1.

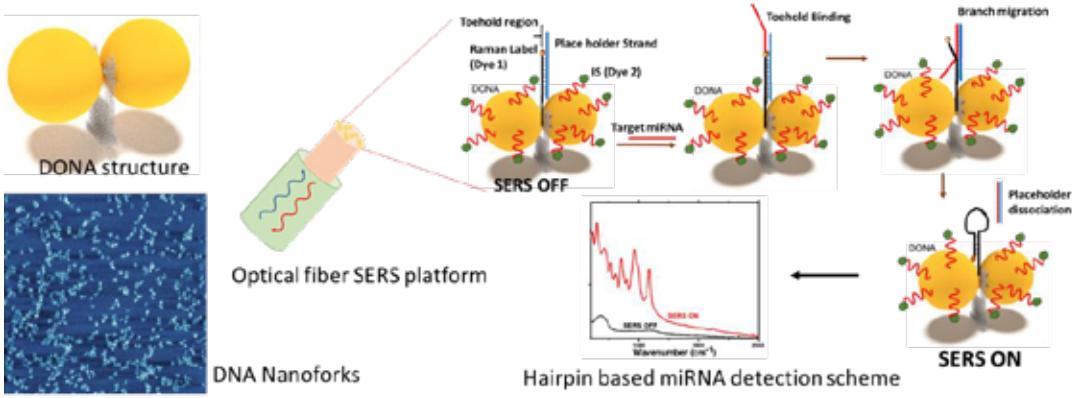

Fig. 1. Schematic of DNA origami assisted gold dimers (DONA) and miRNA detection on optical fiber SERS platform

For Based on this inventive DNA origami, we investigated a sensitive and specific ON-OFF SERS scheme for miRNA detection using this DONA structure integrated with hairpin DNA probes on optical fibers (figure 1). The biosensor is designed employing a stem-loop/hairpin DNA probe with Raman label at one end, attached at the DONA hotspot, as a target identification switch. The switch is initially kept OFF by a placeholder DNA strand which hybridize to the hairpin probe through a specific region. In the presence of target miRNA, the placeholder strand is displaced initiated by a toehold binding and branch migration mechanism [2]. This process finally releases the placeholder strand and the hairpin probe is closed bringing the Raman label in the SERS hotspot, enabling the switch ON. Thus, essentially the SERS signal of the Raman label turns OFF to ON in the presence of target miRNA enabling the direct detection of these biomarkers.

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Fabrication of optically active substrates using biotemplated lithography of inorganic nanostructures

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Self-assembling biotemplates such as the versatile DNA origami [1] provide an effective shortcut for fabricating nanoscale assemblies with intricate, sub-nanometer detail. Despite their convenience, then converting these biological constructs into equally accurate and small structures with more favorable physical properties is significantly more challenging. One recent approach to overcome this is DNA-assisted lithography (DALI) [2,3]. DALI integrates DNA origami as templates in negative-pattern lithographic masks and processes these with standard nanofabrication techniques to create metallic replicas of the template. Thus far, DALI has been used to pattern particles with sub-10 nm feature sizes from a limited selection of materials on silicon nitride and sapphire substrates [2].

Here, we present a remarkably more adaptable method that circumvents these material constraints with the incorporation of a sacrificial polymer layer into the process flow. The new technique, named the biotemplated lithography of inorganic nanostructures (BLIN) [4] (Fig. 1), enables the pattern transfer of engineered biotemplates, such as virus capsids and DNA origami, into inorganic structures from a multitude of materials and on less restricted substrates. Due to its versatile and parallel nature, BLIN provides an attractive alternative for patterning optically active devices and surfaces. To demonstrate this, we fabricate Ag bowtie nanoantennas on common glass substrates and characterize their optical response in the near-infrared regime. Lastly, we use the patterned substrates in surface-enhanced Raman spectroscopy (SERS) to augment the detection of Rhodamine 6G (R6G) (Fig. 2). [5]

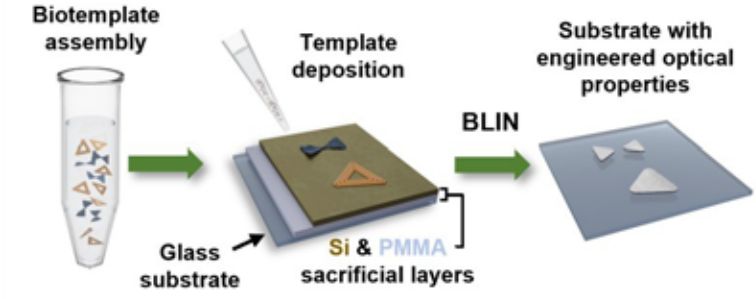

Fig. 1

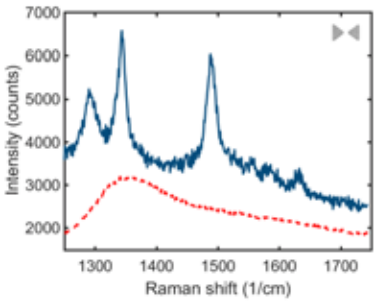

Fig. 2

Fig. 1: The basic principle of BLIN. The shape of a biotemplate is transferred into a SiO_x mask layer. The mask is then used in conjunction with conventional lithographic techniques to create nanostructures based on the templates. [4]
Fig. 2: The measured Raman intensity of R6G dye as a function of Raman shift: A blank substrate (red) *vs.* a substrate patterned with BLIN fabricated Ag bowties (blue). [5]

[1] S. Dey et al. (2021). Nat Rev Methods Primers, 1 (1), 13
[2] B. Shen, V. Linko et al. (2018). Sci Adv, 4 (2), eaap8978
[3] P. Piskunen et al. (2019). J Vis Exp, (151), e60313
[4] P. Piskunen, B. Shen et al. (2021). ACS Appl Nano Mater, 4 (1), 529–538
[5] K.M. Kabusure, P. Piskunen et al. (2022). Under review

Novel vancomycin-conjugated DNA origami-based nanoantibiotics

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Antimicrobial resistance has become a global burden because of the uncontrolled and increased use of antibiotics. Vancomycin is a cell wall-targeting glycopeptide antibiotic considered as a drug of last resort against various Gram-positive pathogens. However, since the 1980s, vancomycin resistance has spread around the globe and is now commonly observed in nosocomial infections. Previous studies have shown that vancomycin susceptibility can be restored in such pathogens by the multivalent presentation of vancomycin on nanoparticle surfaces.
In this study, we use DNA origami nanostructures to display defined nanoarrays of vancomycin molecules and investigate the effect of geometric vancomycin arrangement and vancomycin density on antibacterial activity. To this end, azide-modified vancomycin was coupled to selected amine-modified staple strands via copper-free click chemistry and incorporated into 2D DNA origami triangles.

Minimum inhibitory concentrations (MICs) against *Bacillus subtilis* were determined and show a clear effect of oligovalency, with larger numbers of vancomycin molecules per DNA origami resulting in lower MIC values compared to free vancomycin. DNA origami-based nanoantibiotics displaying optimized vancomycin nanoarrays thus have the potential to overcome vancomycin resistance in bacterial pathogens.

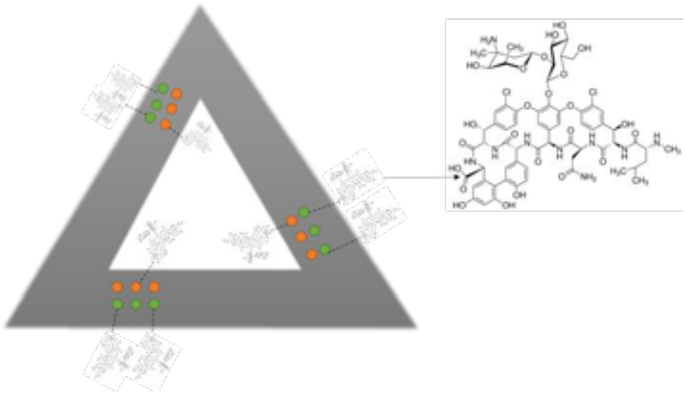

Fig. 1

Fig. 1: Schematic representation of the 2D DNA triangle origami presenting a vancomycin nanoarray.

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[2] Tanure, Luciana Coelho et al. (2020). In Open Forum Infectious Diseases, 7, S467-S467
[3] Gu, Hongwei et al. (2003). In Nano Letters, 3, 1261-1263

Crafting metamaterial surface using DNA origami

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Metasurfaces have garnered wide interest during past few decades. However, realization of such surfaces on wafer scale with feature size down to few tens of nanometer is challenging even for top-down lithography processes such as ultraviolet and electron beam lithographies. Meanwhile, many bottom-up techniques to assembly hierarchical nanostructures have surfaced past few decades, where one of the more promising candidates has been DNA due to its robust self-assembly and plethora of different functionalization options.

Fishnet-type, metallic metasurfaces (see Figure 1) feature unique optical properties like negative refractive index[1] which could pave way for applications such as superlenses[2]. Combination of DNA origami and DNA-assisted lithography (DALI)[3] offers cost effective and simple way to fabricate such surface on a wafer scale. One requirement for DALI is the formation of the DNA origami lattice on silicon surface. However, although there has been demonstration of DNA origami fishnet-lattices on mica using Seeman Tile (ST) origami[4], the realizations of such lattices on silicon surface remain still elusive. Here, we demonstrate that large scale lattice formation can be achieved also on silicon surface (see Figure 1). Additionally, the effects of the mono- and divalent cation concentration and temperature on the lattice formation are discussed.

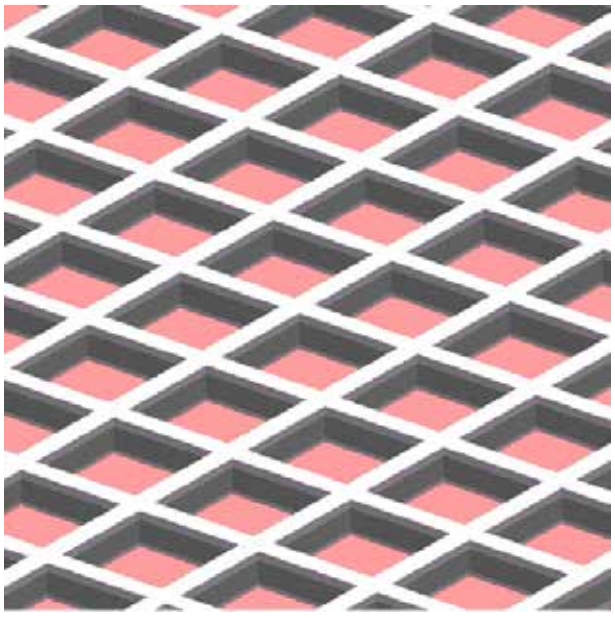

Fig. 1

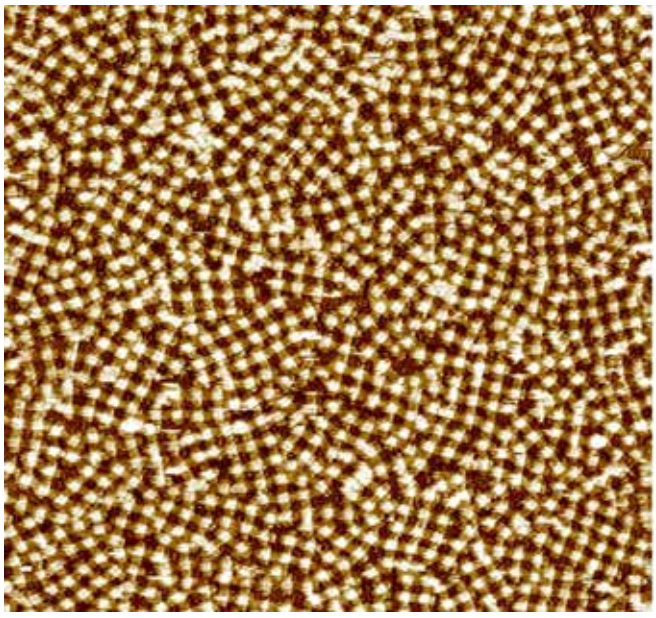

Fig. 2

Fig. 1: Fishnet-type metallic metasurface. The structure consists of three layers: bottom Al_2O_3 layer, middle Ag and top Al_2O_3.
Fig. 2: Seeman Tile origami lattice on silicon substrate.

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[2] X. Zhang and Z. Liu, Nat. Mater., 2008, 7, 435-441.
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[4] W. Liu et. al. , Angew. Chem. Int. Ed. , 2011, 50, 264-267.

A Highly Specific Isothermal Screening Platform for Pre-eclampsia-associated microRNA Based on Duplex Specific Nuclease and Rolling Circle Amplification

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Preeclampsia (PE), a common pregnancy complication characterized by high blood pressure, is one of the leading causes to higher risks of maternal and fetal morbidity and mortality. The clinical symptoms of preeclampsia often appear relatively late in pregnancy, while some abnormal interaction occurs much early during the first trimester.[1] Hence, there is a strong demand in early detection of preeclampsia. Herein, we chose miR-210 as the target biomarker to develop a new sensing platform that coupled an enzyme-linked Immunoassay (ELISA)-like detection method and a two-step isothermal signal amplification approach [duplex specific nuclease (DSN)-assisted amplification, followed by a rolling circle amplification (RCA)]. First, we modified the biotin-labelled DNA hairpin-probe on the wells of microplate pre-coated with streptavidin. The affinity between streptavidin and biotin allowed the hairpin probe to be immobilized on the microplate. An addition of miR-210-containing sample solution to the well allowed the hybridization of the hairpin probe with miR-210 to form a duplex that was further recognized by the duplex-specific nuclease (DSN). The DSN-cleaved probe remained attached to the well subsequently served as the primer for RCA reaction. G-quadruplexes were produced during RCA. In the presence of hemin, the hemin/G-quadraplex complex could function as a horseradish peroxidase (HRP)-mimicking DNAzyme to catalyze the oxidation of the substrate, 3.3',5,5'-Tetramethyl benzidine (TMB) with the presence of hydrogen peroxide, leading to the colorimetric detection of miR-210. This sensing platform has been proven its potential to achieve a sensitive detection of miR-210 in blood samples collected from patients diagnosed with preeclampsia.

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Tampering with microglia-mediated neuroinflammation by means of DNA nanostructures

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Neuroinflammation is one of the characteristic hallmarks of ALS and most other neurodegenerative diseases. In particular, microglial cells, the immune cells of the central nervous system, play a crucial role in this process and therefore in neurodegeneration. In fact, in early phases of chronic neurodegenerative diseases, microglia acquire an M2 phenotype, which is neuroprotective, while in advanced phases, microglia acquire the M1 phenotype, that turns out to be neurotoxic. The mechanisms that regulate the spreading of activation from activated microglial cells to non-activated ones is still unknown, nevertheless exosomes seem to be involved. We have previously demonstrated that activation of microglia stimulates the release of exosomes, also changing their content [1].
In Atomic Force Microscopy, the possibility to apply localized forces and to measure the resulting behavior of the probed system allows for the nanomechanical characterization of biological systems, including live cells. We have performed a nanomechanical analysis of live microglial cells both in resting an in activated cellular systems. We have shown that the inherent morphological changes also imply a measurable change in the mechanical properties of these cells, providing a measurement of activation alternative to end-point molecular characterizations.
As we have proved that microglia can spontaneously internalize tetrahedron-like DNA self-assembled nanostructures, we endowed such nanostructures with functional elements designed to interact with cellular RNAs that are involved in the microglial activation process. We recorded a reduction in the specific content of intracellular RNA, and we also verified a reduced expression of one of the protein biomarkers of neuroinflammation as a consequence of the uptake of the DNA nanostructures.
The effects of DNA nanostructures in modulating the nanomechanics of activated microglial cells can be assessed next.

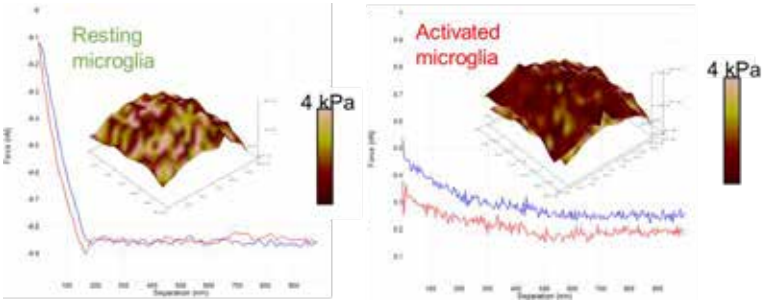

Fig. 1. Example AFM nanomechanical maps of resting and activated microglia cells

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Quantum dots from the kitchen table

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Quantum dots (QDs) are semiconductor particles of a few nanometers in size with special electronic and optical properties based on quantum effect and thus, different from larger particles. The so-called quantum size effect allows for the adjustment of the energy of the emitted light (and therefore, their wavelength) through the size during preparation. QDs can be utilized in transistors, solar cells, LEDs, lasers or quantum computers.
In the presented work, the preparation of carbon-based QDs is investigated. In contrast to conventional QDs, such carbon-based ones are non-toxic, are easier to prepare, and are less expensive. It was found that QDs solutions prepared by a mixture of citric acid and urea exhibited the best fluorescence properties under UV light excitation. The nitrogen doting by urea has not only a strong influence on the fluorescence intensity, but also on the emission wavelength (color). Already very small amounts of urea resulted in QDs with strong blue fluorescence. With increasing concentration of urea, a shift from blue to green emission was observed. In all other reaction mixtures with various carbon and nitrogen sources, only blue fluorescence was observed. Finally, the solutions of strong fluorescence were purified using chromatography.

Acknowledgements: This work was conducted in the framework of the German „Jugend forscht“ (Youth researches) – initiative, the biggest youth science and technology competition in Europe. The project was mentored by Kerstin Reichelt; and supported by Tom Fleischhauer, the Sierka family, Prof. Dr. Frank Müller, and Prof. Dr. Rainer Heintzmann.

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