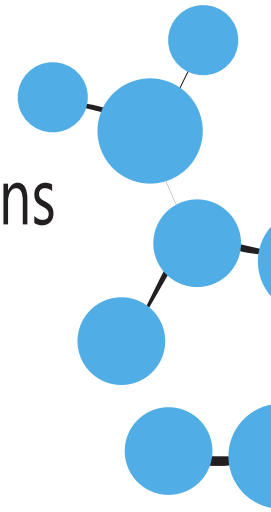
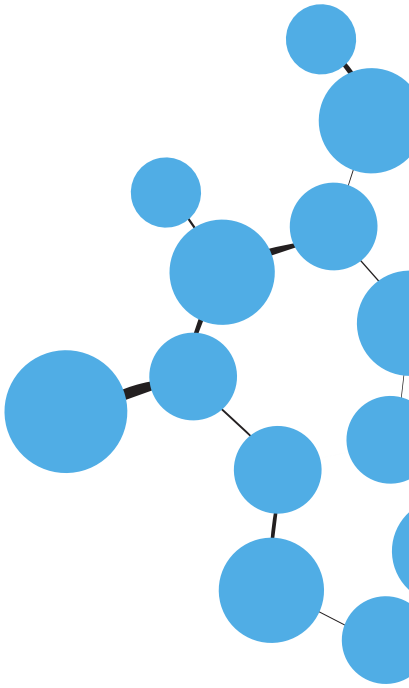
NANTECH 2019

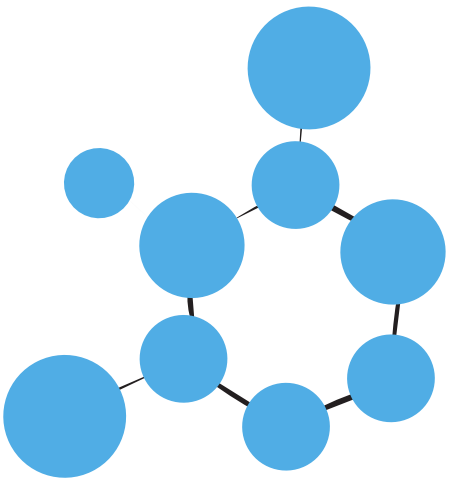
Nucleic Acid Nanotechnology:
from algorithmic design to biochemical applications



Programme

Aalto University
27-29 May 2019





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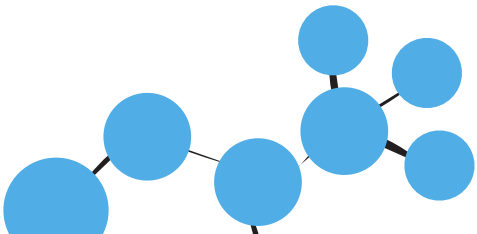

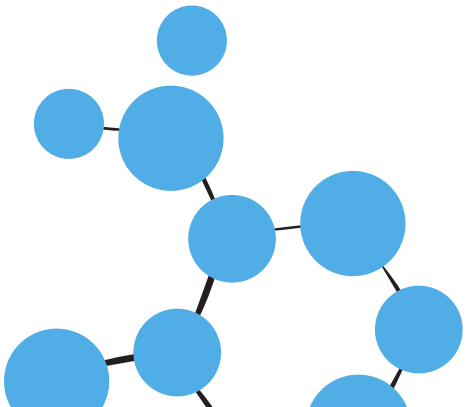

Programme – Nucleic Acid Nanotechnology: from algorithmic design to biochemical applications

Invited talk: **25 min** + 5 min questions/discussion
Contributed talk: **15 min** + 5 min questions/discussion

Location: Lecture hall *"Jeti"* in the "*A Grid"* hub, Aalto University, Otakaari 5, Espoo

Monday 27.05.

08:15 *Registration opens*
09:15-09:30 **Organisation and programme committee** — *Opening of NANTECH 2019*

-- **Session 1. Chair: Philip Tinnefeld** --

09:30-10:00 **Tim Liedl** — Ludwig Maximilian University of Munich, Germany
Tools and materials assembled from DNA

10:00-10:20 **Thorsten Schmidt** — Kent State University, USA
DNA-assembled plasmonic waveguides for nanoscale light propagation to a fluorescent nanodiamond

10:20-10:40 **Jussi Toppari** — University of Jyväskylä, Finland
DALI: DNA-assisted lithography for plasmonic nanostructures

10:40-11:30 *Coffee break*

-- **Session 2. Chair: Tim Liedl** --

11:30-12:00 **Ralf Jungmann** — Max Planck Institute of Biochemistry and LMU Munich, Germany
Super-resolution microscopy with DNA molecules: Towards localizomics

12:00-12:30 **Philip Tinnefeld** — Ludwig Maximilian University of Munich, Germany
Sensing enhanced by DNA nanotech

12:30-12:50 **Ilko Bald** — University of Potsdam, Germany
FRET nanoarrays in DNA origami platforms and their application as ratiometric sensor

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12:50-14:30 *Lunch (at your own cost)*

-- **Session 3. Chair: Jonathan Doye** --

14:30-15:00 **Damien Woods** — Maynooth University, Ireland
Diverse and robust molecular algorithms using reprogrammable DNA self-assembly

15:00-15:30 **Luca Cardelli** — University of Oxford, UK
Sequenceable DNA algorithms

15:30-15:50 **Carlo Spaccasassi —** Microsoft, UK
A logic programming language for computational nucleic acid devices

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15:50-16:30 *Coffee break*

-- **Session 4. Chair: Damien Woods** --

16:30-17:00 **Roman Jerala** — National Institute of Chemistry, Slovenia
Designed coiled-coil protein origami nanostructures

17:00-17:30 **Jonathan Doye** — University of Oxford, UK
Coarse-grained modelling for DNA nanotechnology with oxDNA

17:30-17:50 **Eugen Czeizler** — Åbo Akademi University, Finland
Rule-based modeling of DNA multi-strand dynamics

17:50-19:30 **Poster session 1**, discussions, *refreshments*

Tuesday 28.05.

-- **Session 5. Chair: Björn Högberg** --

09:00-09:30 **Kurt Gothelf** — Aarhus University, Denmark
Self-assembly and optical properties of single molecule polymers on DNA origami

09:30-10:00 **Hendrik Dietz** — Technical University of Munich, Germany
Designing biomolecular devices and machines

10:00-10:20 **Masayuki Endo** — Kyoto University, Japan
Characterization of DNA origami nanospace using G-quadruplex and i-motif structure as a molecular probe

10:20-10:40 **Adrian Keller** — University of Paderborn, Germany
Real-time observation of superstructure-dependent DNA origami digestion by DNase I using high-speed AFM

10:40-11:30 *Coffee break*

-- **Session 6. Chair: Kurt Gothelf** --

11:30-12:00 **Björn Högberg** — Karolinska Institutet, Sweden
DNA origami reveals the spatial tolerance of antibodies

12:00-12:20 **Jørgen Kjems** — Aarhus University, Denmark
APTA-SHAPE technology – an unbiased identification of disease biomarkers in biofluids

12:20-12:40 **Michael Mertig** — Technical University of Dresden, Germany
DNA origami-based nanostructures in stable motion

12:40-14:30 *Lunch (at your own cost)*

-- **Session 7. Chair: Hendrik Dietz** --

14:30-15:00 **Francesco Ricci** — University of Rome Tor Vergata, Italy
DNA-based nanodevices controlled by purely entropic domains

15:00-15:30 **Stefan Howorka** — University College London, UK
Crossing boundaries with DNA: Sequencing and biosensing with nanopores

15:30-15:50 **Caroline Rossi-Gendron** — Ecole Normale Supérieure, France
Isothermal formation of DNA origamis at room temperature in a saline buffer going through multiple folding pathway

15:50-16:10 **Ralf Strasser** — Dynamic Biosensors GmbH, Germany
Preparation of well-defined protein-DNA conjugates

16:10-16:20 ***Group photo***

16:20-18:00 **Poster session 2**, discussions, *refreshments*

19:00- ***Conference dinner***, *poster prizes*
"Restaurant Kaarre", Kaivokatu 3, Helsinki

Wednesday 29.05.

-- **Session 8. Chair: Friedrich Simmel** --

09:00-09:30 **Barbara Saccà** — University Duisburg-Essen, Germany
DNA origami tools to explore biological processes

09:30-10:00 **Ebbe Andersen —** Aarhus University, Denmark
Towards RNA origami devices in cells

10:00-10:20 **Matteo Castronovo —** University of Leeds, UK
DNA origami-protein interactions and steric hindrance control

10:20-10:40 **Peter Unrau —** Simon Fraser University, Canada
Accurate reporting of relative orientations using FRET and rigid fluorogenic RNA aptamers

10:40-11:00 **Ivan Barisic —** AIT Austrian Institute of Technology, Austria
An approach towards catalytic DNA nanostructures

11:00-11:30 *Coffee break*

-- **Session 9. Chair: Barbara Saccà** --

11:30-12:00 **Friedrich Simmel** — Technical University of Munich, Germany
Using strand displacement in RNA-based gene circuits

12:00-12:20 **Si-Ping Han —** City of Hope and California Institute of Technology, USA
Development and optimization of strand displacement based conditional small interfering RNAs for operation inside mammalian cells

12:20-12:40 **Leo Chou —** University of Toronto, Canada
A composable cell-free gene regulatory architecture using nucleic acid transcription factors and semi-synthetic RNA polymerase

12:40-13:00 **Organisation and programme committee** — *Closing remarks*

13:00 *Lunch (at your own cost)*

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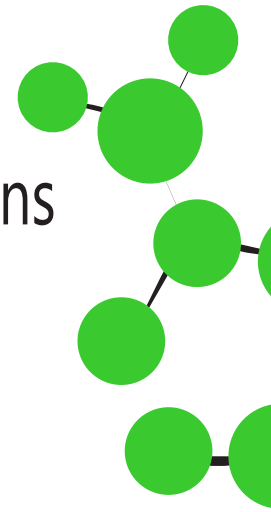
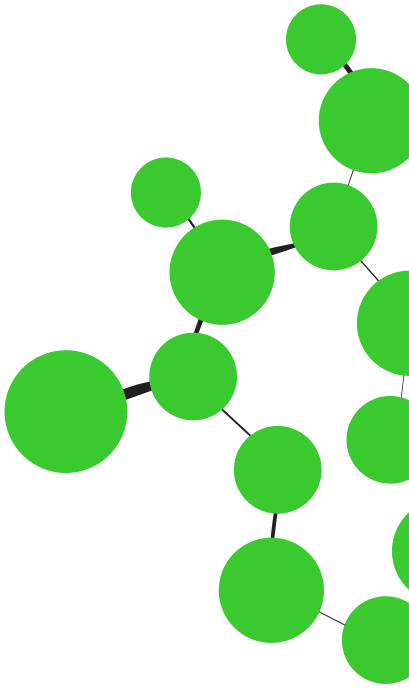
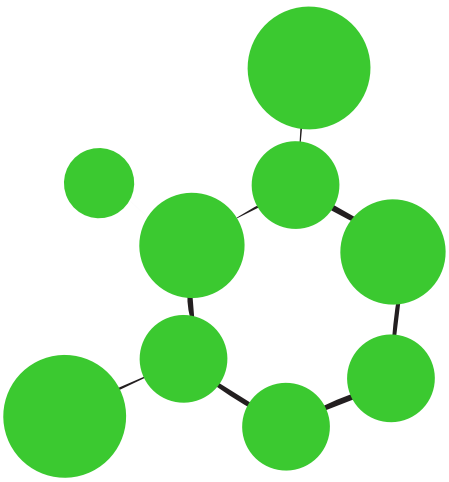


Talk Abstracts





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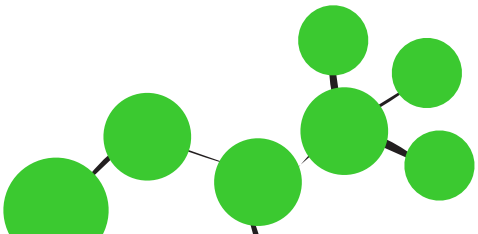

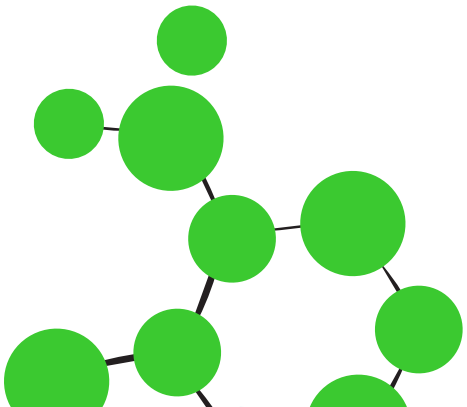

Tools and materials assembled from DNA

Tim Liedl[1]

[1] *Fakultät für Physik and Center for NanoScience, Ludwig-Maximilians-Universität München, Germany*
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DNA has proven to be an excellent choice of molecule for programmable self-assembly. In recent years, DNA self-assembly has surpassed its early stages and today is routinely used for constructing functional two- and three-dimensional nanomachines and materials [1,2].
By defining attachment sites for active components on DNA structures, our group has realised complex and nanometer-precise assemblies of biomolecules, organic fluorophores and inorganic nanoparticles [3]. We employed these devices as autonomous force spectrometers [4] and to create new plasmonic effects. These effects, in turn, enable the selective and sensitive detection of proteins and virus-derived RNA molecules [5].
The initial thrust catalyzing the rapid development of DNA nanotechnology has been to arrange periodic DNA frameworks to host guest molecules for crystal structure analysis. Despite enormous efforts and fundamental progress, placing guest molecules in designed DNA crystals remains a challenging goal. By adopting design principles of Ned Seeman and Chengde Mao [6], we are now able to crystallise DNA origami structures that grow into three dimensional, micrometer-scale assemblies [7]. Silicification of these crystals leads to designer nanomaterials that withstand drying without structural deformation [8].
Our results demonstrate the assembly power of DNA and our ability to fabricate functional devices and 3D materials that are designed on the molecular level while reaching macroscopic dimensions.

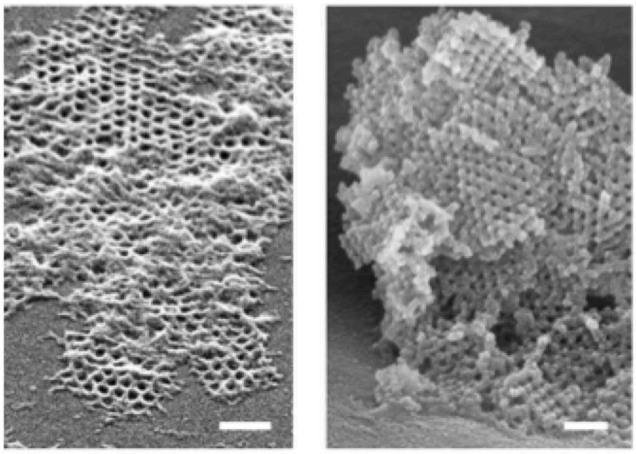

Figure 1: Silica growth on DNA origami crystals. Left: 60° tilted view of bare DNA origami crystals. The crystals flatten upon adsorption to the grids. Right: 60° tilted view of silica coated DNA origami crystals. The 3D structure is preserved. Scale bars are 200 nm..

References

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[3] R. Schreiber et al. Nature Nanotechnology 9, 74-78 (2014)
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[6] J. Zheng et al. Nature 461, 74-77 (2009)
[7] T. Zhang et al., Adv. Mat. 30, 1800273 (2018)
[8] L. Nguyen et al. Angew. Chem. Int. Ed., 58, 912-916 (2019)

DNA-assembled Plasmonic Waveguides for Nanoscale Light Propagation to a Fluorescent Nanodiamond

F. N. Gür, C. P. T. McPolin, F. W. Schwarz, J. Ye, S. Diez, S. Raza, M. Mayer, D. J. Roth, A. M. Steiner, M. Löffler, A. Fery, M. L. Brongersma, A. V. Zayats, T. A. F. König & T. L. Schmidt*

*Department of Physics, Kent State University, Kent, OH 44242, USA

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Plasmonic waveguides consisting of metal nanoparticle chains can localize and guide light well below the diffraction limit, but high propagation losses due to lithography-limited large interparticle spacing have impeded practical applications. We previously demonstrated a robust DNA-origami-based self-assembly pipeline of monocrystalline gold nanoparticles.[1] More recently,[2] we demonstrate that this method allows the interparticle spacing to be decreased below 2 nm, thus reducing propagation losses to 0.8 dB per 50 nm at a deep subwavelength confinement of 62 nm (~λ/10). We characterize the individual waveguides with nanometer-scale resolution by electron energy-loss spectroscopy. Light propagation towards a fluorescent nanodiamond is directly visualized by cathodoluminescence imaging spectroscopy on a single-device level, therefore realizing nanoscale light manipulation and energy conversion. Simulations suggest that longitudinal plasmon modes arising from the narrow gaps are responsible for the efficient waveguiding. With this scalable DNA origami approach, micrometer-long propagation lengths could be achieved, enabling applications in information technology, sensing and quantum optics.[2]

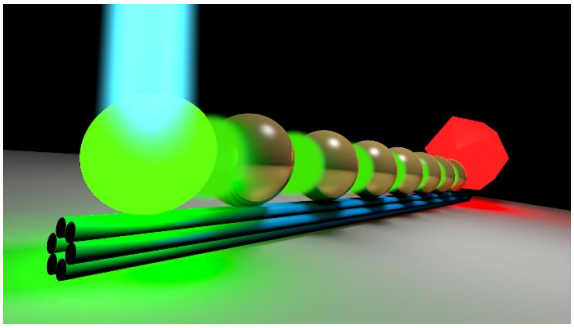

Figure 1: Artistic representation of energy transfer to a fluorescent nanodiamond.

Reference

[1] Gür, F. N.; Schwarz, F. W.; Ye, J.; Diez, S.; Schmidt, T. L. Toward Self-Assembled Plasmonic Devices: High-Yield Arrangement of Gold Nanoparticles on DNA Origami Templates. *ACS Nano* **2016**, *10* (5), 5374–5382.

[2] Gür, F. N.; McPolin, C. P. T.; Raza, S.; Mayer, M.; Roth, D. J.; Steiner, A. M.; Löffler, M.; Fery, A.; Brongersma, M. L.; Zayats, A. V.; et al. DNA-Assembled Plasmonic Waveguides for Nanoscale Light Propagation to a Fluorescent Nanodiamond. *Nano Lett.* **2018** *18* (11), 7323-7329.

DALI: DNA-assisted Lithography for Plasmonic Nanostructures

Boxuan Shen[1,2], Veikko Linko[2], Kosti Tapio[1], Siim Pikker[1], Tibebe Lemma[1], Ashwin Gopinath[3], Kurt Gothelf[4], Mauri Kostiainen[2] and Jussi Toppari[1]

[1] *Nanoscience Center and Department of Physics, University of Jyväskylä, Finland*
[2] *Biohybrid Materials, Department of Bioproducts and Biosystems, Aalto University, Finland*
[3] *Department of Bioengineering, California Institute of Technology, Pasadena, California, USA*
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Programmable self-assembly of nucleic acids enables the fabrication of custom, precise objects with nanoscale dimensions, which can be further harnessed as templates to build novel materials and nanodevices with diverse functionalities [1]. Metallic nanostructures are widely used and explored because of their unique optical properties, such as selective field enhancement via plasmonic resonances, and their potency to serve as components of novel metamaterials [2]. However, the currently available fabrication techniques are not feasible for creating complex and sufficiently small metallic shapes for metamaterials functioning at the visible wavelength range. The common wet chemical methods merely yield geometrically limited structures, whereas the standard lithography that allows arbitrary shapes does not provide the required spatial accuracy. Here the DNA nanotechnology would enable many new possibilities. However, approaches to transfer the spatial information of DNA constructions to metal nanostructures remain a challenge.

We have developed a DNA-assisted lithography (DALI) method that combines the structural versatility of DNA origami with conventional lithography techniques to create discrete, well-defined, and entirely metallic nanostructures with designed plasmonic properties [3]. DALI is a parallel, high-throughput fabrication method compatible with transparent substrates, thus providing an additional advantage for optical measurements, and yields structures with a feature size of ~10 nm. We demonstrate its feasibility by producing metal nanostructures with a chiral plasmonic response and bowtie-shaped nanoantennas for surface-enhanced Raman spectroscopy. We envisage that DALI can be generalized to large substrates, which would subsequently enable scale-up production of diverse metallic nanostructures with tailored plasmonic features.

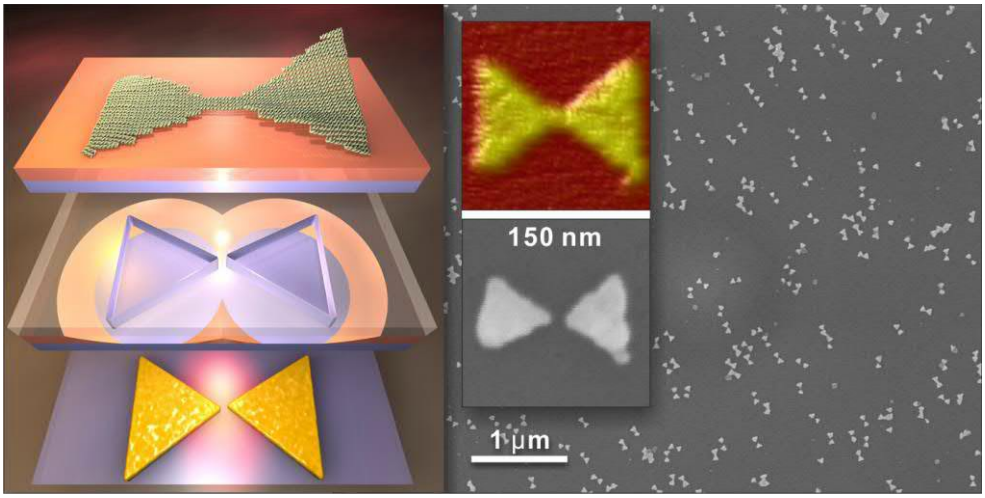

Figure 1: Schematic presentation of the DALI process. AFM image of a bowtie origami on a silicon surface and SEM image of a ready plasmonic bowtie antenna. Large surface covered with metallic bowties from a single run.

Funding by the Academy of Finland, Jane and Aatos Erkko Foundation, the Finnish Cultural Foundation, the Finnish Academy of Science and Letters, the Emil Aaltonen Foundation, and the U.S. Office of Naval Research is greatly acknowledged. The work was carried out under the Academy of Finland Centres of Excellence Programme (2014–2019).

References

[1] M.R. Jones, N.C. Seeman, C.A. Mirkin, Science 2015, **347**, 1260901; N.C. Seeman, H.F. Sleiman, Nature Rev. Mat. 2017, **3**, 17068; N. Liu, T. Liedl, Chem. Rev. 2018, **118**, 3032-3053.
[2] V. M. Shalaev, Nat. Photonics 2007, **1**, 41–48; Z. Wang, et al., Nanotechnology 2016, **27**, 412001.
[3] B. Shen, V. Linko, K. Tapio, S. Pikker, T. Lemma, A. Gopinath, K. V. Gothelf, M. A. Kostiainen, J. J. Toppari, Science Adv. 2018, **4**, eaap8978.

Super-resolution Microscopy with DNA Molecules: Towards Localizomics

Ralf Jungmann[1,2]

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[2] *Max Planck Institute of Biochemistry, Martinsried, Germany*
Email: jungmann@biochem.mpg.de

Super-resolution fluorescence microscopy is a powerful tool for biological research. We use the transient binding of short fluorescently labeled oligonucleotides (DNA-PAINT) for easy-to-implement multiplexed super-resolution imaging that technically achieves sub-5-nm spatial resolution[1].

To translate this resolution to cellular imaging, we introduce Slow Off-rate Modified Aptamers (SOMAmers) as efficient and quantitative labeling reagents. We demonstrate the achievable image resolution and specificity by labeling and imaging of transmembrane as well as intracellular targets (**Figure 1**) in fixed and live cell-specimen[2].

Apart from ever increasing spatial resolution, efficient multiplexing strategies for the simultaneous detection of hundreds of molecular species are still elusive. We introduce a new approach to multiplexed super-resolution microscopy by designing the blinking behavior of targets with engineered binding frequency and duration. We assay this kinetic barcoding approach in silico and in vitro using DNA origami structures, show the applicability for multiplexed RNA and protein detection in cells and finally experimentally demonstrate 124-plex super-resolution imaging within minutes[3].

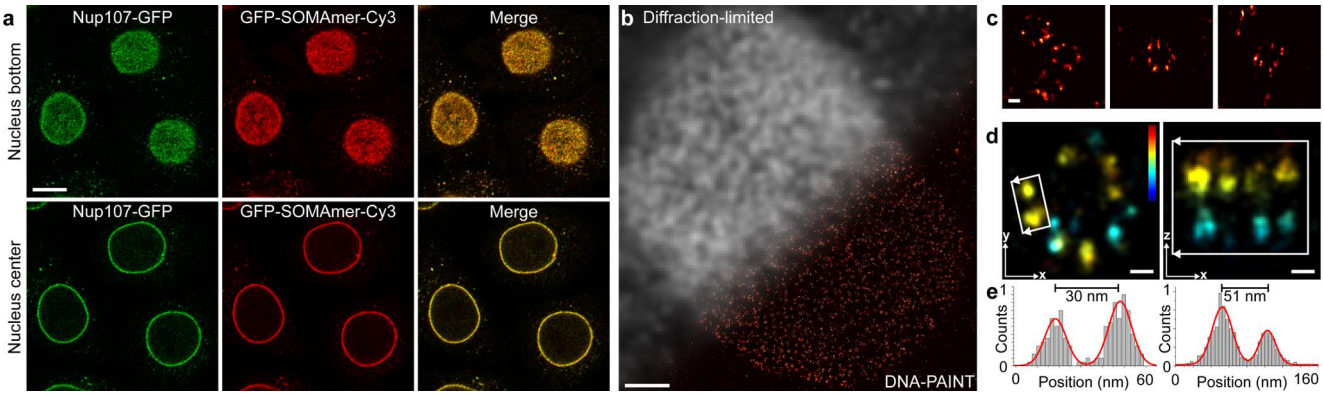

Figure 1: Intracellular labeling of GFP-tagged Nup107 for DNA-PAINT imaging using GFP–SOMAmers.

References

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Sensing enhanced by DNA nanotech

Philip Tinnefeld[1], Carolin Vietz[2], Birka Lalkens[2], Kateryna Trofymchuk[1,2], Hakan Inan[1], Ece Büber[1], Sarah Ochmann[1], Lennart Grabenhorst[1], J. Michael Scheckenbach[1], Guillermo P. Acuna[3], Florian Selbach[1], Ija Jusuk[1], Mario Raab[1], Viktorija Glembockyte[1]

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[2]*Institute for Physical & Theoretical Chemistry, and Braunschweig Integrated Centre of Systems Biology (BRICS), and Laboratory for Emerging Nanometrology (LENA), Braunschweig University of Technology, Rebenring 56, 38106 Braunschweig, Germany.*
[3]*Department of Physics, University of Fribourg, Chemin du Muse´e 3, Fribourg CH-1700, Switzerland.*
Email: Philip.tinnefeld@cup.uni-muenchen.de

In recent years, DNA nanotechnology has matured to enable robust production of complex nanostructures and hybrid materials. We have combined DNA nanotechnology with sensitive optical detection to create functional single-molecule devices that enable new applications in single-molecule biosensing and superresolution microscopy. Starting with superresolution nanorulers and brightness reference samples we determined the resolving power of superresolution microscopes and evaluated the sensitivity of smartphone cameras[1-3]. To improve the sensitivity, we created DNA origami optical antennas for metal enhanced fluorescence[4]. The unique ability of our DNA origami nanoantennas to place molecular assays specifically in the plasmonic hotspot is used for detecting Zika-virus and antibiotic resistance related nucleic acids[5, 6]. Furthermore, DNA origamis are used in biophysical assays to work at locally increased concentrations[7], to apply forces as well as to sense further physical parameters[8].

References

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[7] D. Wang, C. Vietz, T. Schroder, G. Acuna, B. Lalkens, and P. Tinnefeld, "A DNA Walker as a Fluorescence Signal Amplifier," Nano Lett 17, 5368-5374 (2017).
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FRET Nanoarrays in DNA Origami Platforms and their Application as Ratiometric Sensor

Ilko Bald[1,2], Youngeun Choi[1,2], Lisa Kotthoff[2], Lydia Olejko[1], Ute Resch-Genger[2]

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[2] *Federal Institute for Materials Research and Testing (BAM), Berlin, Germany*
Email: bald@uni-potsdam.de

DNA origami nanostructures [1] are excellent platforms to arrange dye molecules with nanoscale accuracy. This allows for assembly of multiple fluorophores whilst avoiding dye-dye aggregation. With the aim of developing a bright and sensitive ratiometric sensor, the optical properties of nanoarrays built on DNA origami nanostructures were systematically studied. Here, dye molecules were arranged at distances where they can interact efficiently *via* Förster resonance energy transfer (FRET). First, the brightness as well as FRET efficiencies of nanoarrays of different sizes and patterns were studied using fluorescein (FAM) and cyanine 3 (Cy3) as the FRET donor and acceptor, respectively. Utilizing the nanoarray giving the optimum FRET efficiency and brightness, a ratiometric pH nanosensor was subsequently designed where coumarin 343 was used as the analyte unresponsive FRET donor and FAM as the pH responsive acceptor. Our results showed that the sensitivity of a ratiometric sensor could be improved by simply arranging the dyes into a well-defined array [2]. The fabrication process used here allows for easy replacement of the dyes with other analyte-responsive dyes, demonstrating the huge potential of our design for further sensing applications.

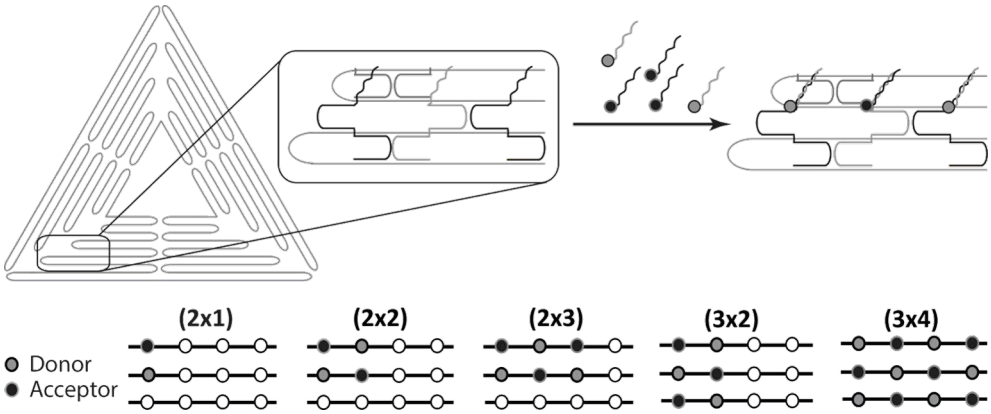

Figure 1: Illustration of the DNA origami nanostructure based Förster resonance energy-transfer (FRET) nanoarrays. Upper panel shows the fabrication process allowing for the arrangement of the dyes in a well-defined array, as well as providing high versatility. Bottom panel illustrates the different nanoarrays built for optimization of FRET efficiency as well as brightness.

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Diverse and robust molecular algorithms using reprogrammable DNA self-assembly

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Algorithmic self-assembly is a form of molecular computation where molecules attach to a growing nanostructure and where each attachment event executes a logical instruction step of the computation. In recent work [1] we have designed a reprogrammable set of 355 DNA strands, or tiles, capable of implementing a wide variety of self-assembly algorithms. Each algorithm is programmed by choosing a subset of the DNA strands. In this way, we implemented a total of 21 6-bit algorithms, including bit-copying, sorting, recognizing palindromes and multiples of 3, random walking, obtaining an unbiased choice from a biased random source, electing a leader, simulating cellular automata, generating deterministic and randomised patterns, and serving as a period 63 counter. The average per-tile error rate over the 21 different algorithms was less than 1 in 3000.

Having a reprogrammable architecture enabled programming while at the bench: we could come up with a new algorithm and implement it on the same day. Just like laptops can be reprogrammed using high-level programming languages, and without knowledge of the underlying device physics or processor instruction sets, users of our self-assembly system can design and run molecular algorithms without needing to know the intricacies of our design pipeline. Development of multipurpose molecular machines, reprogrammable without knowledge of the machine's physics, could establish a creative space where high-level molecular programmers can flourish.

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Sequenceable DNA Algorithms

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We give an introduction to DNA Strand Displacement: a technique that is used to program interactions between DNA strands in such a way, e.g., as to emulate the kinetics of an arbitrary finite network of chemical reactions. We discuss current capabilities and trends in DNA nanotechnology, including "high throughput" equipment that can read and write DNA massively in parallel.
High throughput DNA synthesis and sequencing render easily feasible a new class of algorithms that use O(n^2) structures in input and output. We give two examples of such algorithms, for detecting the coincidence of events, and for detecting the preorder of evens, over the course of an experiment in a biochemical soup.

A Logic Programming Language for Computational Nucleic Acid Devices

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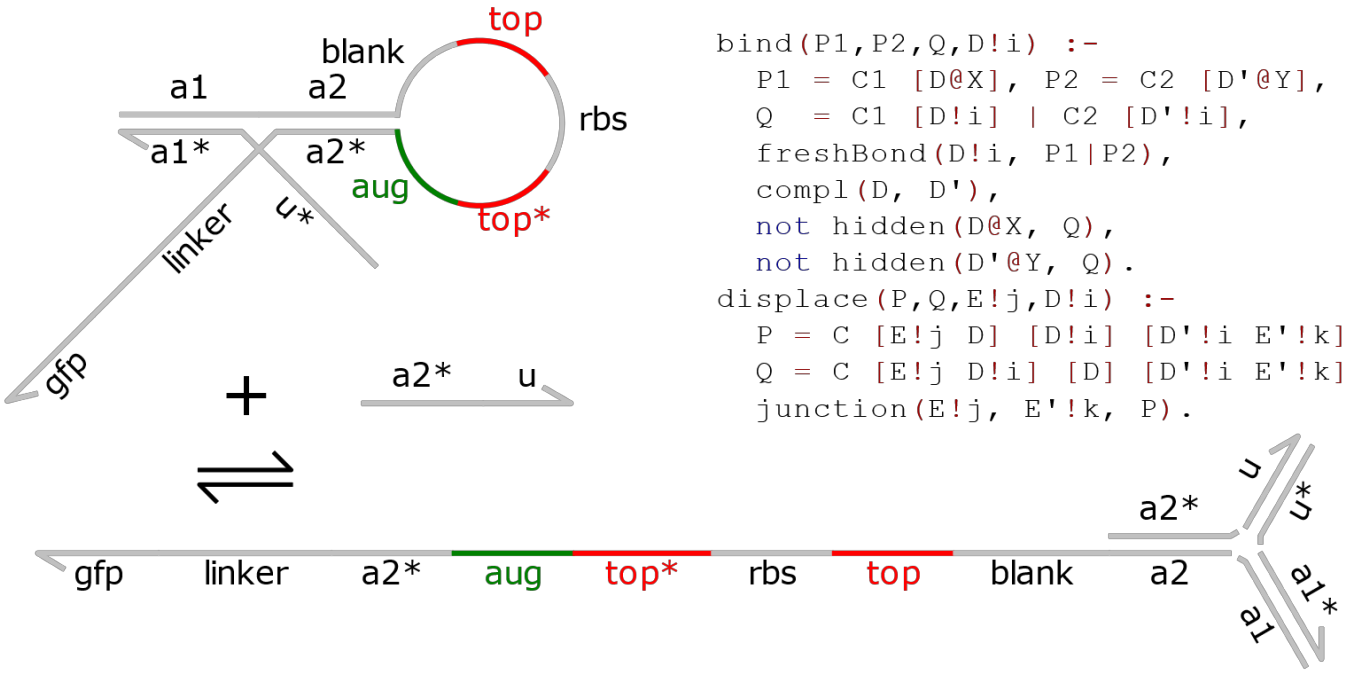
Computational nucleic acid devices show great potential for enabling a broad range of biotechnology applications, including smart probes for molecular biology research, in vitro assembly of complex compounds, high-precision in vitro disease diagnosis and, ultimately, computational theranostics inside living cells. This diversity of applications is supported by a range of implementation strategies, including nucleic acid strand displacement, localization to substrates, and the use of enzymes with polymerase, nickase, and exonuclease functionality. However, existing computational design tools are unable to account for these strategies in a unified manner.

We present a logic programming language[3] that allows a broad range of computational nucleic acid systems to be designed and analysed. The language extends standard logic programming with a novel equational theory to express nucleic acid molecular motifs. It automatically identifies matching motifs present in the full system, in order to apply a specified transformation expressed as a logical rule. The language supports the definition of logic predicates, which provide constraints that need to be satisfied in order for a given rule to be applied.

Our language can encode the semantics of nucleic strand displacement systems with complex topologies, previous extensions to the Visual DSD language[2], as well as new extensions including the encoding of kinetic rate hypotheses, together with computation performed by a broad range of enzymes. More importantly, our approach is extensible in that new nucleic acid implementation strategies can be encoded simply by defining new logic predicates. Thus, our approach lays the foundation for a unifying framework for the design of computational nucleic acid devices.




```
bind(P1,P2,Q,D!i) :-
  P1 = C1 [D@X], P2 = C2 [D'@Y],
  Q  = C1 [D!i] | C2 [D'!i],
  freshBond(D!i, P1|P2),
  compl(D, D'),
  not hidden(D@X, Q),
  not hidden(D'@Y, Q).
displace(P,Q,E!j,D!i) :-
  P = C [E!j D] [D!i] [D'!i E'!k]
  Q = C [E!j D!i] [D] [D'!i E'!k]
  junction(E!j, E'!k, P).
```

Figure 1: Part of a chemical reaction network for a ribocomputing AND gate[1], and corresponding logic program code to express domain binding and strand displacement.

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Designed Coiled-coil Protein Origami Nanostructures

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Proteins are the most complex functional nanostructures, defined by the linear sequence of amino acids that can self-assemble into complex folds. Natural proteins are defined by a large number of weak cooperative long range interactions. In analogy to DNA nanostructures, based on strand complementarity, we devised coiled-coil protein origami (CCPO) which is based on the well-understood specificity of coiled-coil dimers (CC). CC dimer forming modules were concatenated into a single long polypeptide chain, where the CC modules form the edges of the self-assembling polyhedral scaffold. The principle was first demonstrated by the construction of a nanoscale tetrahedral cage from a single polypeptide chain composed of 12 coiled-coil forming segments1. In this case 6 edges of the polyhedron were defined by orthogonal CC dimers, measuring 5 nm. Design of the new CC building modules2 and computational design platform CoCoPOD enabled the design of protein origami cages that are able to self-assemble in vivo. The CCPO cages were extended to more than 700 residue proteins with tetrahedral, four-sided pyramid, triangular prism and trigonal bipyramid that fold efficiently, with kinetics and stability comparable to globular proteins3. Diverse chemical properties of amino acids enable introduction of functional sites and regulation of CC assembly. CC modules have been used to design artificial signalling pathways and fast information processing circuits in mammalian cells4. Additionally, in vivo folding of protein origami opens the prospects for new applications of this new type of designed nanostructures.

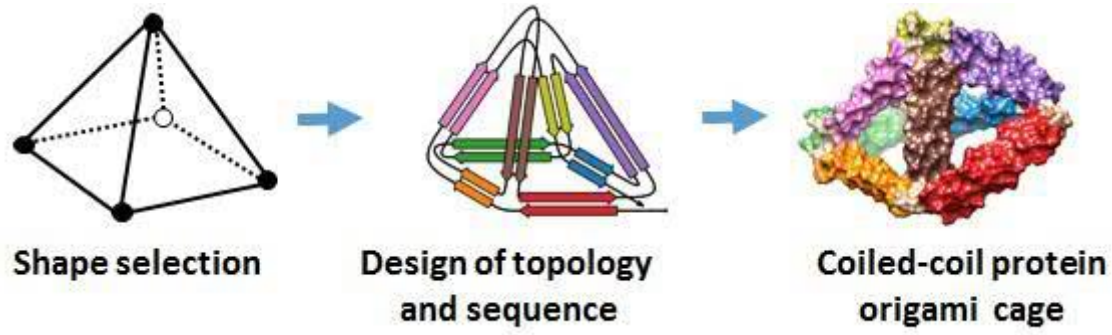

Figure 1: Principle of the design of coiled-coil protein origami nanoscale polyehedra composed of concatenated polypeptide modules.

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Coarse-grained modelling for DNA nanotechnology with oxDNA

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OxDNA is a coarse-grained model of DNA at the nucleotide level that has been parameterized to reproduce the structural, mechanical and thermodynamic properties of DNA [1]. The code that we use to simulate the model (including both molecular dynamics and Monte Carlo) is publicly available [2] and the model has also been incorporated into LAMMPS [3]. The model has been used to explore a wide range of biophysical properties of DNA and many DNA nanotechnology systems. In this talk I will review some of the recent applications of oxDNA to DNA nanotechnology. This will include characterizing the basic structural properties of DNA origami [4], and example applications to more complex origami, including those with flexible components [5] and internal stresses. The model can also be used to characterize the mechanical properties of DNA nanostructures, both in the elastic regime and their modes of failure under tension [6]. The model is also able to provide insights into the self-assembly dynamics of DNA nanostructures from the complete assembly of small DNA tetrahedra to the details of staple binding in origami [7] and even the oligomerization of DNA origami (see Figure 1). Combined with classical density functional theory, oxDNA has also been used to predict the cholesteric liquid-crystalline properties of chiral elongated DNA origami [8].

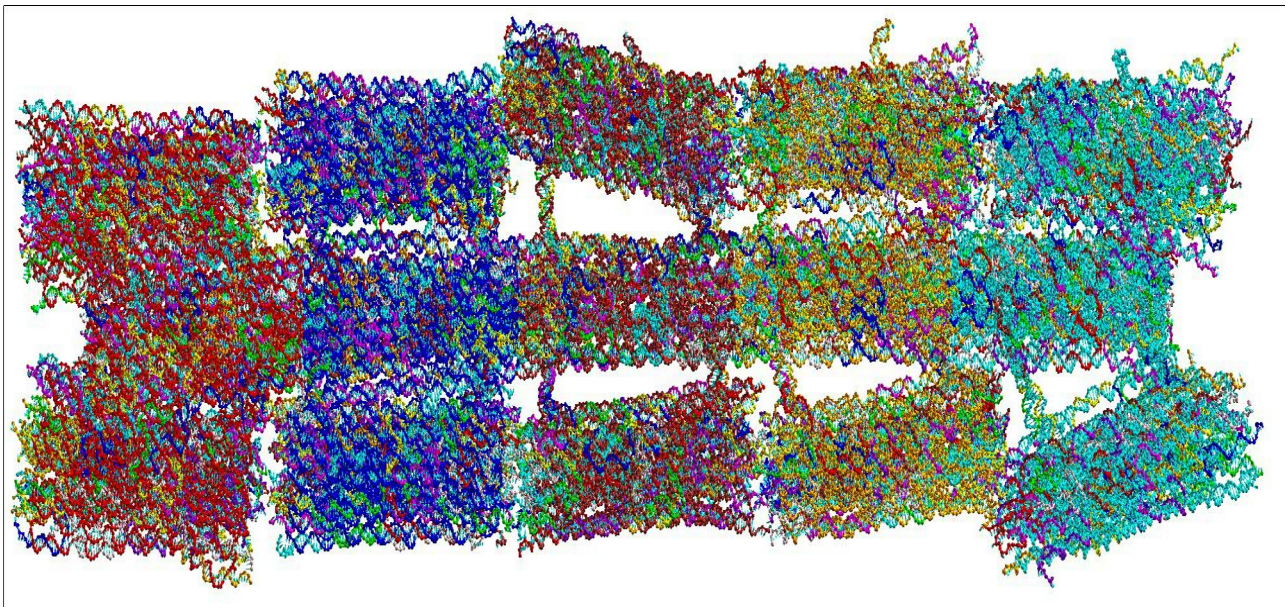

Figure 1: An oxDNA representation of a 5-mer of a DNA origami designed by the group of Lawrence Lee to undergo self-limited oligomerization due to the build-up of stress.

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Rule-based modeling of DNA multi-strand dynamics

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The dynamics of nucleic-acids dynamical systems is intrinsically based on local interaction. The major acting mechanisms are that of Watson-Crick complementarity on one-hand, generating binding events, and thermal energy on the other, generating random motion and un-binding. It is thus predictable that such systems could be captured successfully by computational modeling paradigms based on local interactions, such as the rule-based modeling methodology [1, 2].

Using the BioNetGen Language (BNGL) formalism [3] and the NFsim computational platform [4], we created a computational modeling framework for DNA multi-strand dynamics. The system takes as input populations of isolated (or partially bounded) oriented single-stranded DNA molecules (ssDNAs), and simulates their binding and dissociation reactions. At the core of this model implementation lie 9 binding and un-binding local interaction rules, each with its own kinetic rate constant, and each implemented through one or several rule-based reactions. Using these reactions/rules, we capture the dynamics of the DNA-dynamical system, by modeling: - the initial binding of short toeholds, here implemented as length-3 short complementary and opposite-oriented sub-sequences, - the "breathing" dynamics in-between bounded ssDNAs, - random un-binding events (between one pair of nucleotides), - as well as the un-binding of loosely connected ssDNAs.

Since the BNGL formalism is not suited for tracking and reporting the global mapping of the components within a heterogeneous complex, as it focuses on the count of local patterns, we have developed distinct subroutines for this purpose. Thus, the content of the dynamical system is unloaded at various time-points within the simulation, and it is re-assembled for visualization and further numerical analysis. A simple 2D graphical representation of the assembled complexes is also generated, where one can track the ssDNAs within the complex as well as all the binding interactions. Note, the strands are not displayed as per their relative positions, but rather listed one under another. Nevertheless, the visualization is highly useful for assessing the overall assembly status and the stability of one complex.

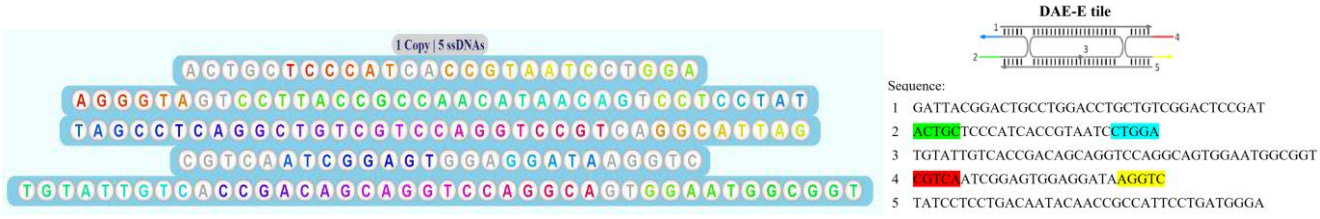

Figure 1. Left: fraction of the generated output of a DNA dynamical system simulation consisting of 5 ssDNAs which assemble into a DAE-E tile; nucleotides that are bound by hydrogen bonds are colored similarly. Right: the design scheme and nucleotide sequences of the 5 strands assembling the DNA tile; taken from [5].

Different from other computational modelling frameworks for DNA strand assembly, we can modify some of the systems parameters, such as the temperature of the system, during the system simulation. Thus, we can model also an entire annealing process for the formation of a DNA structure. Many other parameters can also be specified (or adjusted mid-simulation); e.g., we can define specific binding/un-binding kinetic rates for each different length-3 subsequence.

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Self-assembly and optical properties of single molecule polymers on DNA origami

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We are using DNA as a programmable tool for directing the self-assembly of molecules and materials. The unique specificity of DNA interactions and our ability to synthesize artificial functionalized DNA sequences makes it the ideal material for controlling self-assembly and chemical reactions of components attached to DNA sequences [1]. In particular we are using DNA origami, large self-assembled DNA structures as a template for positioning of materials such as organic molecules, polymers and biomolecules.
In recent years we have developed methods for functionalizing conjugated polymers with multiple DNA strands in a graft type fashion [2-4]. We have prepared long phenylene-vinylene and fluorene polymers and synthesized DNA strands extending from most of the repeat units of the polymers. The polymers self-assemble on tracks of complementary DNA strands extending from DNA origami structures and in this way the routing of the individual polymers can be controlled. By immobilizing fluorescent dyes along the polymer we have investigated the properties of the polymers as single molecule optical wires (Figure 1).

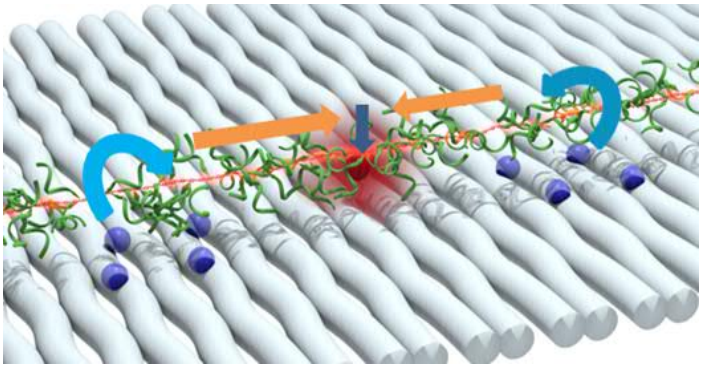

Figure 1: Transfer of excitation energy from a donor dye to an acceptor dye via a conjugated polymer immobilized on DNA origami.

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Designing biomolecular devices and machines

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Inspired by the rich functionalities of natural macromolecular assemblies such as enzymes, molecular motors, and viruses, we investigate how to build increasingly complex molecular structures. Our goal is to build molecular devices and machines that can execute a variety of user defined tasks, ranging from the positioning and processing of other molecules to drug delivery. A central obstacle in our work is the difficulty of constructing complex and accurate molecular structures. Another problem relates to an insufficient understanding of the mechanisms necessary to achieve a desired function. Currently we mainly focus on molecular self-assembly with DNA to build de novo structures. DNA origami in particular enables building nanodevices that can already be employed for making new discoveries in biomolecular physics and protein science. In the longer term we hope to contribute to the creation of molecular machines and systems that have practical benefits for everyday life through uses in medicine – for diagnosis and therapy – and synthetic enzymes for biologically inspired chemistry.

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Characterization of DNA origami nanospace using G-quadruplex and i-motif structure as a molecular probe

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Physical properties of biomolecules such as thermal stability and folding kinetics change depending on the environment. For investigating the properties of biomolecules in a confined space, precise design of a nanoscale space to place the molecules is critical. We created a nano-sized space using DNA origami, and found that G-quadruplex placed in the nanospace was thermodynamically stabilized and folding occurred rapidly [1]. We investigated the influence of the nano-sized space on the physical properties of the i-motif (iM) and G-quadruplex (GQ) structure. Mechanical unfolding of the iM and GQ in the nanocages was performed using optical tweezers. The nanocages including iM and GQ were prepared by incorporation of iM- and GQ-containing strand into a half-opened nanocage and subsequent closing. In the nanocage, iM structure was formed stably even at a pH close to neutral [2]. By using four different sizes of nanocages, we found that the mechanical and thermodynamic stability of iM and GQ increased with decreasing size of nanocages. It was also found that the water activity reduced by decreasing the size of nanocages. These results revealed that the stability of iM and GQ in nanocages is correlated with the decrease in water activity.

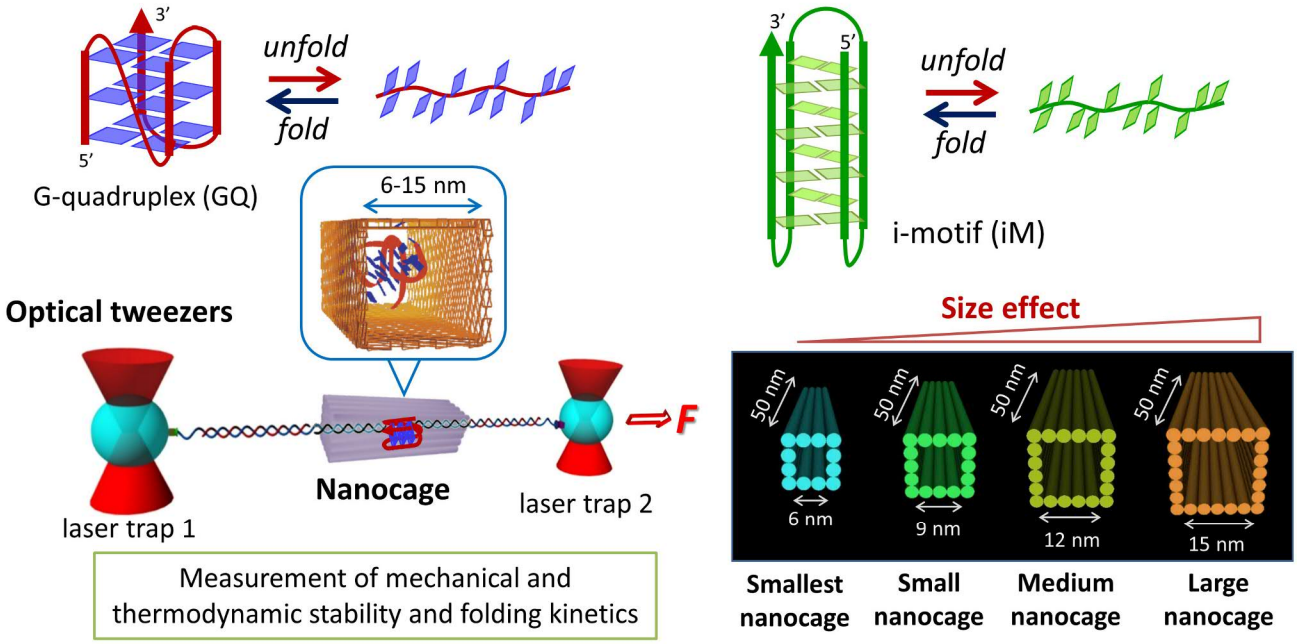

Figure 1: Schematic illustration of G-quadruplex (GQ) and i-motif (iM) in the DNA nanocage and the method to unfold and fold GQ and iM structure inside the four different sizes of nanocages using optical tweezers.

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Real-Time Observation of Superstructure-Dependent DNA Origami Digestion by DNase I using High-Speed AFM

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DNA origami nanostructures are promising tools for numerous biomedical applications, ranging from diagnostics to drug delivery and targeted therapy [1]. They are not only intrinsically biocompatible, biodegradable, and noncytotoxic, but can be synthesized in a wide variety of different shapes and sizes, and further functionalized in a precisely controlled manner with various organic and inorganic species. This enables their defined loading with therapeutic cargos and may be further exploited to facilitate cell targeting, cellular uptake, and cargo release. The performance of such DNA origami vehicles strongly depends on their structural and shape integrity. Unfortunately, previous studies have observed that DNA origami nanostructures are rapidly degraded in biological media, which poses serious limitations for their application in such environments [2]. Two major factors have been identified to contribute to the limited stability of DNA origami nanostructures in biological media: low Mg^{2+} concentrations and the presence of nucleases. While the former issue may be at least partially circumvented by rational design choices that ensure sufficient stability in selected media [3], DNase attack so far appears to be more difficult to control.

In this work [4], we study the degradation of four well established and structurally distinct 2D DNA origami designs (different lattice types, different edge types, and different flexibility) under the attack of DNase I using high-speed atomic force microscopy (HS-AFM). The temporal resolution in our experiments ranged from 5 to 10 seconds per frame, thus allowing a real-time observation of the digestion process. Our results reveal that digestion of the different DNA origami exhibits a superstructure dependence (see Fig. 1). Furthermore, we could identify structural features of each DNA origami design that are most susceptible and most resistant to DNase I digestion, respectively. The results acquired for DNA origami nanostructures immobilized at a solid surface are compared to digestion profiles obtained under identical conditions in bulk solution. It is found that DNA origami designed on the square lattice without twist-correction show remarkably different digestion profiles in bulk solution and at the solid-liquid interface, which is attributed to adsorption-induced shape distortions and strain build-up. Our findings may thus not only help in creating more resilient DNA origami nanostructures, but could also be applied in designing structures with building blocks possessing distinct susceptibilities to nucleases.

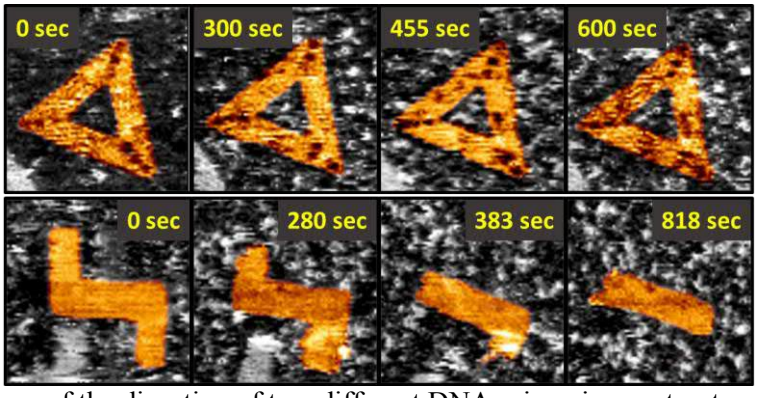

Figure 1: HS-AFM images of the digestion of two different DNA origami nanostructures by DNase I.

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DNA origami reveals the spatial tolerance of antibodies.

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Rigidly organized protein patterns are typically either foreign or intracellular in most mammals and the immune system has thus evolved an efficient response to such structures. Notably, both the HPV and the HBV vaccines employ a particle display of dense arrangements of proteins to elicit a strong immune response. However, the molecular understanding for why antigen positioning appears important in early immune response remains largely unanswered. By precisely controlling the spatial display of antigens, coupled to a dynamic model that is able to recreate the data, we are now able to rigorously dissect what is bivalent binding, what is monovalent binding, what are the rates for mono- to bivalent, and in particular; how does the distance between antigens affect the affinity of different isotypes and classes of Abs.
We introduce a method where molecularly precise nanoscale patterns of antigens are displayed using DNA origami and immobilized in a surface plasmon resonance (SPR) setup. Using human antibodies where all carry identical binding domains, we find that all subclasses and isotypes studied here, bind bivalently according to a unique separation distance dependent curve spanning 3-17 nm with a distinct preference for antigens separated by approximately 16 nm, and that considerable differences in this *spatial tolerance* exist between IgM and IgG and between low and high affinity antibodies.

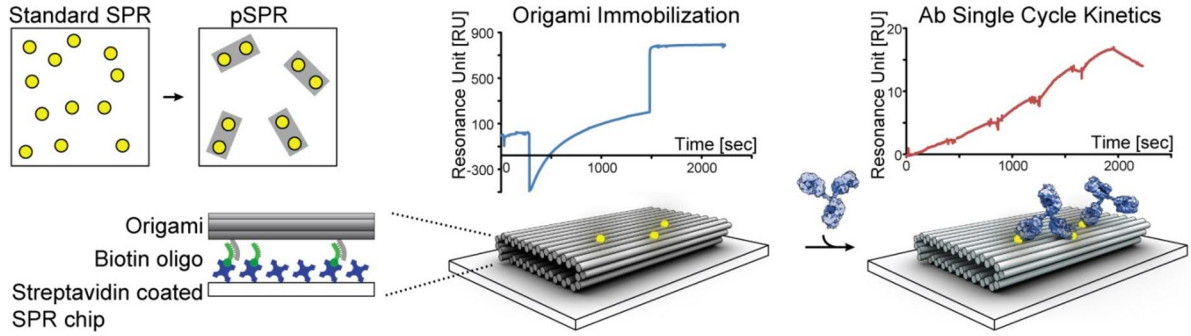

Figure 1: **The Patterned Surface Plasmon Resonance method.** In contrast to conventional SPR, which randomly arranges its ligands on the surface, the PSPR method utilizes DNA origami to pre-pattern the molecule of interest prior to immobilization. The antigen nanopatterns were fabricated using different combinations of antigen-decorated staple oligonucleotides. The antigen nanopatterns were immobilized onto a streptavidin-biotinlylated oligonucleotide surface via oligo hybridization to sequences protruding from the bottom of the origami, followed by an injection of increasing antibody concentrations, and finally a dissociation phase and the ensemble kinetic data for each pattern or distance can be obtained by fitting the binding curves.

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APTA-SHAPE technology – an unbiased identification of disease biomarkers in biofluids

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The molecular signature of blood mirrors the physiology and health state of an individual and carries information about our age, life-style, diseases and more. I will present a new method termed, "APTA-SHAPE", which has been developed to translate the molecular signature of a complex mixture of targets, such as a blood sample, into a digital fingerprint using large aptamers libraries and machine-learning algorithms. The method can, in principle, be used to "fingerprint" the constituents of any aqueous solution including body fluids, beverages and waste water.
In proof-of-concept experiments, we addressed the utility of APTA-SHAPE for analyses of plasma and urine samples and found a robust potential for distinguishing healthy individuals from diseased patients. We further demonstrated the applicability of the method to categorize beverages and contaminations in food products.

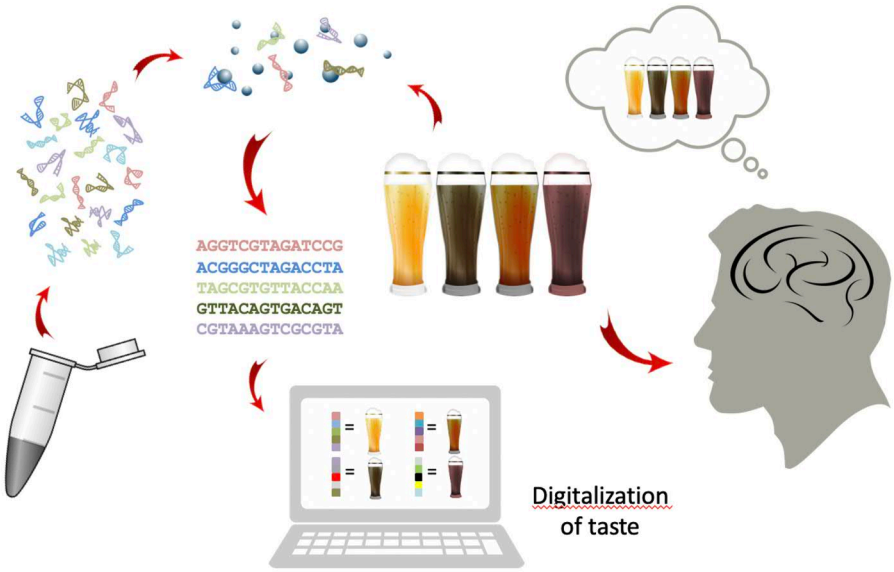

Figure 1: The sensing principle in the DNA-SHAPES method is here exemplified with beer. A large number of nano-meter sized biosensors are synthesised in a test tube and added to various types of beer. Biosensors that are triggered by beer ingredients are separated from non-responsive variants and characterised in the form of a large set of DNA sequences. The aim of the project is to align the data with human taste perceptions we can "teach" the computer to recognise specific ingredients and taste.

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DNA origami-based nanostructures in stable motion

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We have studied the electrically induced switching behavior of different rod-like DNA origami nanolevers (Figure 1) [1-3] and compare that to the actuation of simply double-stranded DNA nanolevers. The measurements reveal a significantly stronger response of the DNA origami to switching of electrode potential, leading to a smaller potential change necessary to actuate the origami, and subsequently, to a long-term stable movement. The mechanical response time of a 100 nm long origami lever to an applied voltage step is less than 100 µs, allowing for a highly dynamic control of the induced motion. Moreover, through voltage-assisted capture, the origamis can be immobilized directly from folding solution without purification, even in the presence of a large excess of staple strands. Dynamic measurements in buffer solutions with different Mg^{2+} content show that the levers do not disintegrate even at very low ion concentration and constant switching stress, and thus, provide stable actuation performance. The latter will pave the way for many new applications without largely restricting application-specific environments. In particular, using DNA origami with low concentrations of Mg^{2+} ions in solution qualifies them for a broad range of biomedical and biophysical applications.

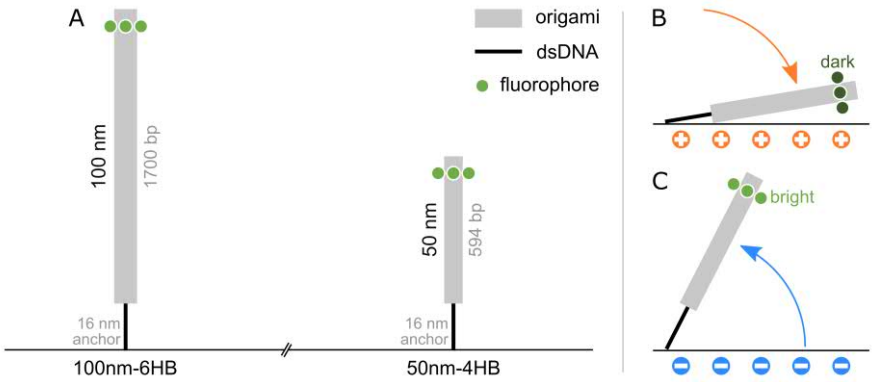

Figure 1: (A) Schematic of the investigated origami attached to a gold electrode: 100 nm long six-helix bundle and 50 nm long four-helix bundle. (B, C) Electrically induced orientation switching of origami rods: When positive potentials are applied to the electrode, the rods are attracted to the electrode and the fluorescence emission from dyes attached to their top ends is quenched by the metal surface. When negative potentials are applied, the rods are repelled from the surface and the fluorescence emission is high.

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DNA-based nanodevices controlled by purely entropic domains

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Many proteins employ conformational entropic contribution of domains that are not directly involved in the recognition event to better control their activity. Such property allows a fine regulation of proteins response and activity in a very versatile and precise way.
Inspired by this mechanism, we report a convenient and versatile approach to control the activity and response behavior of synthetic molecular recognition systems by rationally designing intrinsically disordered domains. To do so and to highlight the versatility and generality of this approach, we have rationally re-engineered three DNA-based receptors: a clamp-like DNA-based switch that recognizes a specific DNA sequence, an ATP-binding aptamer and a pH-sensitive switch.

We demonstrate that, similarly to intrinsically disordered proteins, it is possible to finely modulate the activity of such molecular nanodevices through a purely entropic contribution.
This approach appears as a versatile and general approach to finely control the activity of synthetic receptors in a highly predictable and controllable fashion.

Using DNA to Cross Membrane Barriers

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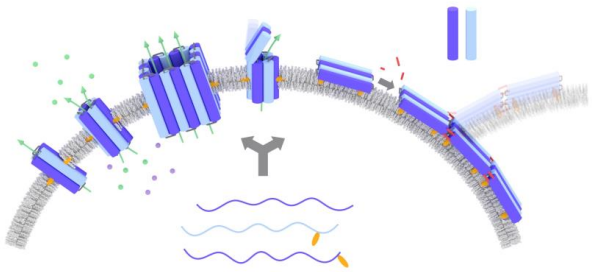
Semifluid membranes enclose biological cells and drug delivery vehicles. Crossing the membrane barrier enables essential transport of molecular cargo. My talk presents routes to cross the barrier with synthetic transport channels made from DNA. Nucleic acids are easier to engineer than proteins of biological channels[(1)]. The artificial DNA channels are composed of interlinked duplexes. Attached lipid anchors hold the negatively charged structures in the membrane[(2,3,4)] based on rational design rules[(5)]. The DNA channels open and close in response to physical voltage stimuli, like natural templates[(3,4,6)]. The DNA versions can also mimic ligand-gated[(3)] and temperature-gated channels[(7)] to help release drugs or build cell-like networks. The artificial pores can be programmed into cytotoxic agents to kill cancer cells[(8)], or to create porous bionanoreactors[(9)]. Other rationally designed DNA nanostructures extend the functional range and can control, for example, bilayer shape[(10)]. The presentation concludes with an outlook of how DNA nanotechnology at membrane interfaces can help replicate biological functions to open up new applications in nanobiotechnology and synthetic biology.



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Isothermal self-assembly of DNA origamis at room temperature in an unchanging buffer going through multiple folding pathways

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When it comes to DNA origami formation, the large majority of research papers use the same method: the M13 template and the staples of a given shape are mixed together in a magnesium-containing buffer (usually TAE 1x + ~10 mM MgCl2) and submitted to a temperature ramp starting with a denaturing step at high temperature (typically 90 °C) and going down to 20 °C. This temperature ramp allows the system to go through several distinct equilibrium states, each of which stable at a given temperature. But what happens when the primitive system (meaning the M13 template and the staples in the buffer, before annealing) is incubated at a constant temperature? Can the system self-organize to create a flawless origami structure after a certain incubation time? In other words, is it possible to form DNA nanostructures by replacing the thermal annealing with an isothermal annealing? A few isothermal protocols have been developed, but they are based either on the addition of organic denaturing agents [1–4] or on the use conventional saline buffers but at a high temperature [5,6].

In this presentation, I will describe experimental results on a new original method for the isothermal formation of DNA nanostructures at room temperature, that doesn't involve any structural modification of the DNA, nor any environmental modification before, during or after the isothermal folding process. I will demonstrate the versatility of this method by describing the formation of different origami shapes at room temperature (25 °C) and at 30 °C in an unchanging buffer, I will describe the kinetics of the folding process and, as the most significant result, I will show the first in situ observation by AFM of the isothermal folding process at room temperature. This constitutes the first direct characterization and visualization of the folding mechanism(s) [7] of DNA origamis, thus bringing new insights on the matter.

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Preparation of well-defined protein-DNA conjugates

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Conjugates of proteins and DNA are becoming increasingly important for their use in DNA nanotechnology. We have developed a generic solution for the preparation, purification, and analysis of well-defined conjugates of proteins and oligodeoxynucleotides in a simple two-step workflow.
First, oligodeoxynucleotides are linked to proteins via amine-reactive or thiol-reactive chemistries, provided as ready-to-use coupling kits. In a second step, the reaction products are analysed, purified, and automatically collected by the proFIRE ion-exchange chromatography system. The reaction conditions are optimized for high yield while maintaining a 1:1 protein:DNA stoichiometry. Further, the purification step ensures that only mono-conjugates are being selected for subsequent experiments.
Key quality parameters such as purity (%), amount (pmol), and concentration (nM) of the selected fractions are quantified automatically by the instrument, which renders the utilization of the protein-DNA conjugates in following experiments straightforward.

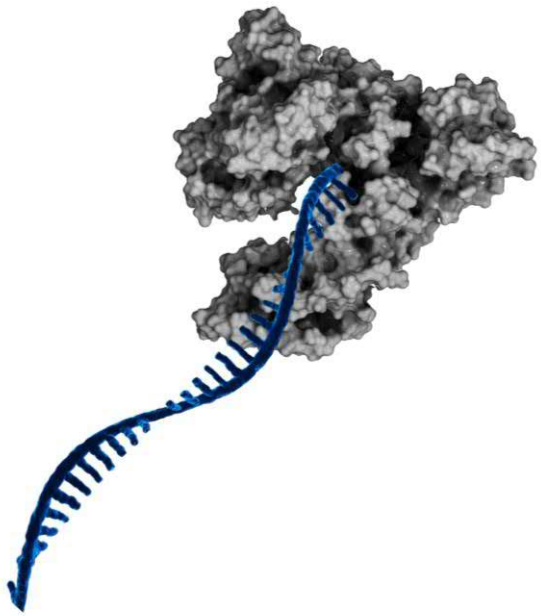

Figure 1: A 1:1 protein-DNA conjugate.

DNA origami tools to explore biological processes

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Structural DNA nanotechnology is probably one of the most successful chemical methods of the past 40 years to achieve control of matter distribution at the nanometer scale [1]. In particular, the DNA origami approach demonstrated to be a robust and versatile method for the construction of DNA objects of almost any desired shape and size, thus offering numerous opportunities in diverse scientific disciplines [2]. We employ DNA origami tools to construct simplified models of complex biological systems, where single structural and functional parameters can be manipulated in a completely predictable fashion. Our scientific ambition is to gain a better understanding of fundamental aspects of biological self-assembly and to use this knowledge for the generation of biomimetic materials with customized properties. We are currently focusing on three aspects of natural self-assembling systems (Figure 1): (i) their capacity to self-assemble into hierarchical high-ordered structures [3]; (ii) their capability to respond to the external environment by changing their shape [4]; and finally (iii) their role as encaging systems, to control the spatio-temporal location and possibly the energetics of reactions [5]. Here, I will present our recent achievements in each of these areas.

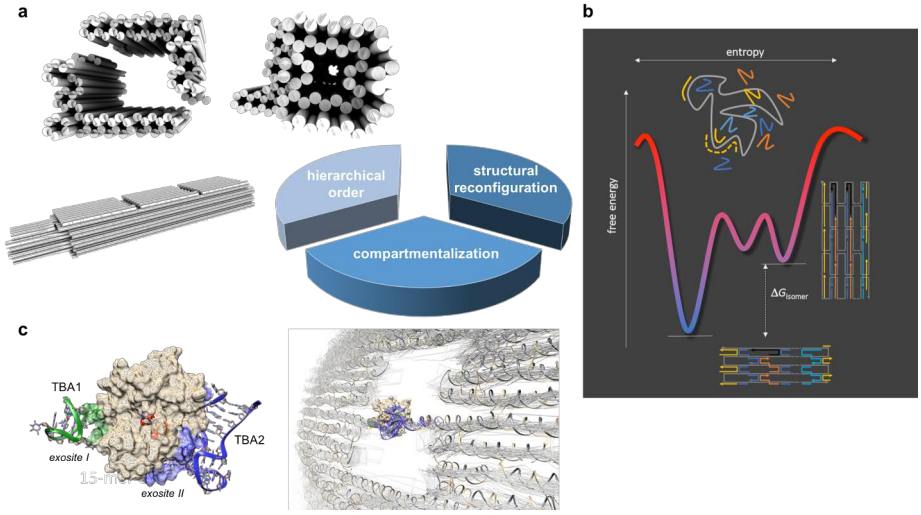

Figure 1. DNA origami tools to explore biological mechanisms. DNA origami tools can be employed to mimic and better understand fundamental properties of biological systems, such as (**a**) hierarchical order, (**b**) structural adaptation to external changes and (**c**) spatial confinement.

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Modular design of RNA origami scaffolds and devices

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Single-stranded RNA origami is an architecture enabling the design of genetically expressible RNA scaffolds that assemble cotranscriptionally. Through the addition of RNA aptamer modules, such scaffolds can control the relative position and orientations of small molecules and proteins in three dimensions (3D). The design of large and complex RNA origami has been limited by a lack of modeling and design tools. Here we expand the complexity of RNA origami architecture, and provide software to automate the 3D modeling of structures and thermodynamic design of sequences. This enables the scale and diversity of RNA origami scaffolds to be greatly extended, as exemplified by a series of 14 different shapes of increasing size and complexity. Nanometer-scale positioning of small-molecule and protein binding modules is demonstrated using fluorescence resonance energy transfer (FRET) for both light-up aptamers and fluorescent proteins. The RNA origami FRET systems are further used to design and characterize a set of 6 dynamic devices that switch conformation in response to small molecule, RNA and protein inputs. Finally, FRET output is used to demonstrate scaffolding of fluorescent proteins on RNA origami scaffolds when expressed in *E. coli* cells. This study opens the door for the modular design of functional and switchable RNA scaffolds with potential to control molecular systems in the cell ranging from signaling pathways to enzyme cascades.

DNA origami-protein interactions and steric hindrance control

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Here, we characterize the structure and dynamics of a triangular-shape DNA origami with the use of Langevin-type molecular dynamics simulations, using the coarse-grained OxDNA model. In particular, we focus on two different network geometries and analyse the different fluctuations properties to characterize the digital behaviour of restriction enzymes over sites contained in the folded M13 sequence, which we recently reported [1]. We link the local and global conformational changes, and the local DNA network properties, to the binding propensity of restriction enzyme HinP1I. Finally, we will attempt to use the Michaelis-Menten model to connect the prediction of molecular dynamics simulations with gel electrophoresis experiments.

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Orientation and Distance Measurements using FRET and rigid fluorogenic RNA aptamers

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The angle dependence of fluorescence resonance energy transfer (FRET) has many useful applications for the study of conformationally active RNA structures. Angle dependent FRET has been difficult to study as it is hard to ensure that the donor and acceptor dipoles of the fluorescent system precisely track the orientation of the structure under study. Chemically coupling fluorophore reporters directly to nucleic acid partially solves this problem, but the fluorophores are not fully oriented [1]. Guided by crystal structures of RNA Spinach [2, 3] and Mango I [4] and the orthogonal fluorophore binding potential of these two aptamers [5], it was recently demonstrated that a fluorogenic aptamer-based FRET signal can be modulated by a small molecule, or invader nucleic acid strands [6]. Structural data, however, suggest the fluorophore binding core of Mango I is flexibly connected to external sequence4 presumably precluding precise angular measurements.

Recently we developed three new RNA Mango aptamers [7]. Like Mango I, all of these aptamers connect to external RNA helices. Using Broccoli/DFHBI-1T as a donor and the far red-shifted Mango III/YO3-Biotin complex as an acceptor, we measured FRET efficiency using an RNA duplex of variable length between the two aptamers. FRET was dependent on the length of the joining RNA duplex, and oscillated in intensity precisely with the predicted twist of the duplex. In contrast, replacing Mango III with Mango I resulted in a FRET signal that was substantially rotationally averaged. This finding is consistent with a recent crystal structure of Mango III (Trachman et al., in press) that indicates that the fluorophore binding domain of Mango III is rigidly connected to its closing helix. As aptamer-tagged RNA constructs can be transcribed in living cells, and since the fluorogenic dyes used are cell permeable and non-toxic, we believe that rigid fluorogenic aptamers offer the prospect of building reliable biological FRET reporter systems.

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An approach towards catalytic DNA nanostructures

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In the last years, DNA nanotechnology has inspired researchers from a wide range of disciplines to develop novel technologies. The possibility to design objects in the nanoscale facilitated very promising applications as e.g. therapeutics and catalytic compartments [1,2]. In the highly ambitious H2020 FETopen project MARA, we are refining such applications and are developing DNA nanostructures that comprise catalytic sites of proteins [3]. For the design of such structures, the new software tool ADENTIA was developed. It is implemented in the SAMSON Connect framework that enables the atomic-based modelling and simulation of nanosystems using a GUI and personal computer. An outstanding feature is that PDB-files from e.g. proteins can be imported and modified in our DNA nanostructure editor (Fig, 1). The tool can load Cadnano designs and has implemented the Daedalus algorithm for the design of wireframe structures. In addition to a new multiscale visualisation concept (incl. 1D, 2D, 3D) that facilitates the inspection and modification of DNA nanostructures [4], a data model was developed that can be exported for oxDNA simulations. Thus, we are convinced that our tool should be useful for many researchers working with DNA and DNA nanostructures (Fig 1).

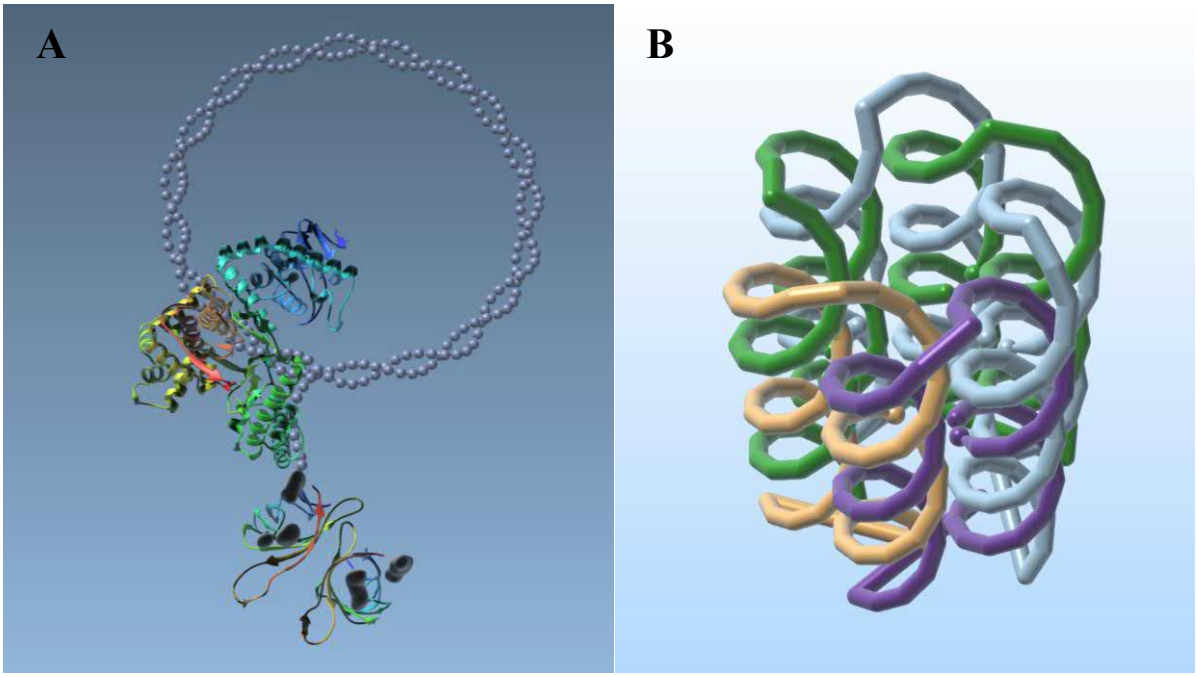

Figure 1: Adenita screenshots illustrating a (A) biosensor surface with neutravidin, an oligonucleotide, DNA polymerase and circularised padlock probe and (B) a DNA pore.

In addition to Adenita, we will present in our talk the first designs of DNA nanostructures comprising the catalytic site of the FlaI motor protein of the archaeallum and preliminary results concerning enzymatic activity of these structures.

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Using strand displacement in RNA-based gene circuits

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Nucleic acid strand displacement reactions are key processes of dynamic DNA nanotechnology. They enable the removal of nucleic acid strands from duplexes or the invasion of secondary structures formed by self-complementary nucleic acid sequences. This in turn allows the realization of nucleic acid based molecular devices that are driven by hybridization/strand displacement cycles. A promising area of application for strand displacement processes is found in the context of RNA-based gene regulation processes such as transcriptional and translational riboregulators, or CRISPR/Cas mechanisms [1].
In this talk we will discuss several of such applications: We will show how RNA-based strand displacement can be used to switch the conformation of guide RNAs for the CRIPSR associated nuclease Cas12a (formerly known as Cpf1). Such strand displacement gRNAs (SD gRNAs) can be used to activate the action of Cas12a via appropriate trigger RNAs. It is also possible to create multi-input AND gates for such SD gRNAs, and also to operate them in the context of bacterial gene expression (using the catalytically inactive dCas12a) [2].
Another class of RNA regulators are toehold switch riboregulators, previously developed by Green et al. [3], which are based on the sequestration of the ribosome binding site of an mRNA inside of a hairpin structure, which can be broken via toehold-mediated strand invasion by trigger RNA molecules. We demonstrate how the action of toehold switches can be further modulated via strand displacement processes using antisense trigger RNAs, which can be used, e.g., to implement a genetic XOR gate in bacteria.

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Development and optimization of strand displacement based conditional small interfering RNAs for operation inside mammalian cells

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A longstanding goal for nucleic acid nanotechnology[1, 2] and biomolecular computing[3] is the development of conditionally activated oligonucleotide therapeutics that can detect and respond to cellular expression of specific genes[2, 3].

Nucleic acid switches based on toehold mediated strand displacement[4] have executed logic operations and detected RNA transcripts in both bacteria[5] and mammalian cells[2, 6], but the conditional activation of oligonucleotide drugs by RNA transcripts in mammalian cells has not been convincingly demonstrated. Significant challenges include poorly suppressed background drug activity, weak ON state drug potency, input and output sequence overlap, high design complexity, short device lifetimes (< 24 hours) and high required device concentrations (> 10 nM).

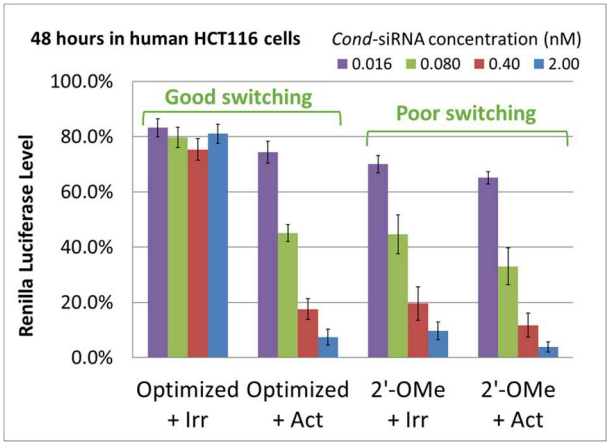

Figure 1: RNAi activity of *Cond*-siRNAs with fully optimized sensors or 2'-OMe only sensors in human cells expressing irrelevant (Irr) or correct (Act) RNA inputs. Data is from three biological replicates. Expression levels of the target (Renilla Luciferase) are normalized to cells with no transfected siRNA.

We have now overcome all of these problems to develop a programmable, *conditionally activated* small interfering RNA (*Cond*-siRNA). These simple riboswitches can maintain their integrity over days in the mammalian cytosol, and detect RNA transcripts from specific **input genes** via toehold mediated strand displacement. Upon input detection, they can release potent RNAi triggers[7] silencing specified **target genes** with *completely independent sequences* from the input. We tested the switching activity of dozens of *Cond*-siRNA variants in human adherent cells to identify necessary and sufficient chemical modification motifs that allow good device performance over diverse input: output combinations. Some optimized *Cond*-siRNAs achieved more than 90% silencing of target genes (protein expression versus baseline) in cells expressing sequence-matched RNA transcripts, and strongly suppressed background RNAi activity (< 25% knockdown) in cells expressing mismatched inputs (e.g. Fig. 1).

Our findings provide a set of clear and simple guidelines for substantively improving the performance of strand displacement switches in live mammalian cells. The *Cond*-siRNA technology provides a plausible platform for gene expression activated RNAi smart drugs.

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A composable cell-free gene regulatory architecture using nucleic acid transcription factors and semi-synthetic RNA polymerase

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Living cells use information encoded in biochemical circuits to make complex decisions and perform sophisticated tasks. Inspired by their rich functionality, synthetic gene circuits are currently being developed to model biology and engineer organisms for various applications. Recently, there have also been increasing interests in developing in vitro gene circuits that operate using reconstituted molecular components.[1] Compared to cellular devices, these cell-free devices have the advantages of being more portable and accessible. These features are being explored for a number of applications ranging from point-of-care diagnostics, reconfigurable materials, artificial cells, to education. However, as with cellular devices, a principle challenge in scaling up the complexity of gene circuits is the lack of a sufficiently large gene regulatory toolset for "wiring up" genetic elements without introducing cross-talk. In living cells, the specificity of circuit wiring is achieved via the interactions between distinct protein regulatory assemblies called transcription factors (TFs) with *cis*-regulatory elements distributed throughout the genome. The molecular properties of TFs enable sophisticated self-assembly-mediated regulatory behaviors, including specific promoter recognition, combinatorial binding, and signal integration via multicomponent assembly. Engineering these properties has been a rate-limiting step in gene circuit fabrication.[2]

Here we use concepts from DNA nanotechnology to synthetically recapitulate features of TF-mediated gene regulation in cell-free gene networks actuated by the relatively primitive T7 phage RNA polymerase (RNAP). Our architecture controls gene expression via programmable nucleic acid hybridization interactions between an oligonucleotide-tethered T7 RNAP with genetic templates displaying single-stranded DNA (ssDNA) regulatory domains, and auxiliary nucleic acid assemblies serving as "artificial TFs" (Fig. 1A). By relying on nucleic acid hybridization, we demonstrate the ability to computationally design de novo regulatory assemblies that emulate features of protein-based TFs (Fig. 1B) while offering unique advantages such as programmability, chemical stability, and scalability. We show how synthetic, nucleic-acid based TFs can be used to implement transcriptional logic, cascade, feedback, and multiplexing (Fig. 1C). Finally, we also demonstrate the integration of this regulatory mechanism with engineered DNA nanostructures to spatially localize gene expression. The composability of this gene regulatory architecture lends itself to design abstraction, standardization, and scaling. We therefore imagine this new regulatory architecture to enable rapid prototyping of increasingly complex in vitro genetic devices for various applications.

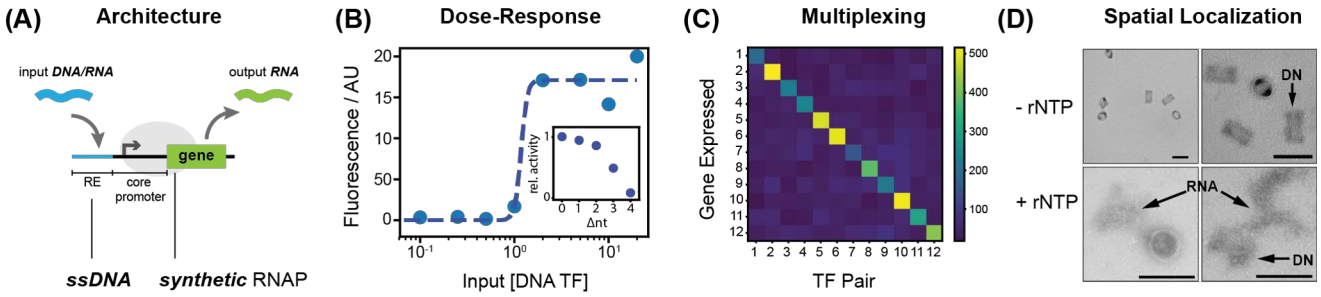

Figure 1: (A) Schematic of gene regulatory architecture. RE = regulatory element (B) Dose-response of an inducible gene system as a function of nucleic acid TF input. Inset shows how the response changes with deletion mutations on the nucleic acid TF. (C) Example of transcriptional multiplexing, showing independent control over the expression of twelve genes in a pooled format. (D) Transmission electron micrographs showing rolling-circle transcription from within the lumen of a 3D DNA cylinder. DN = DNA nanostructure.

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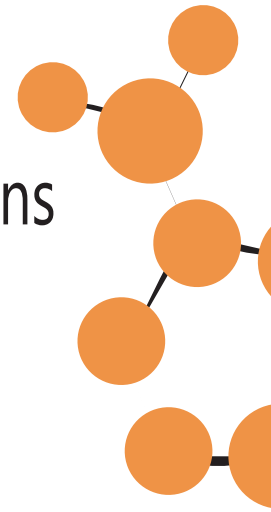
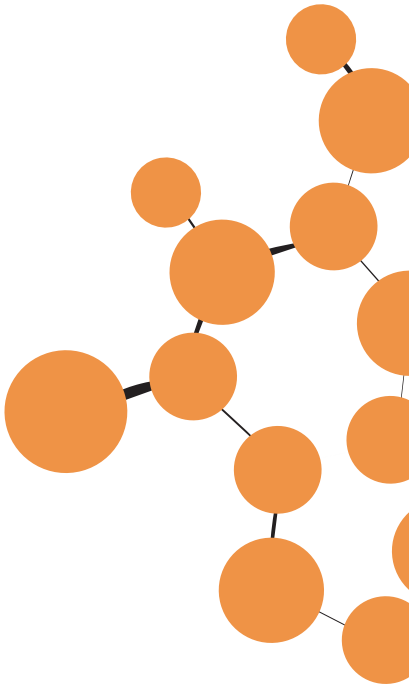
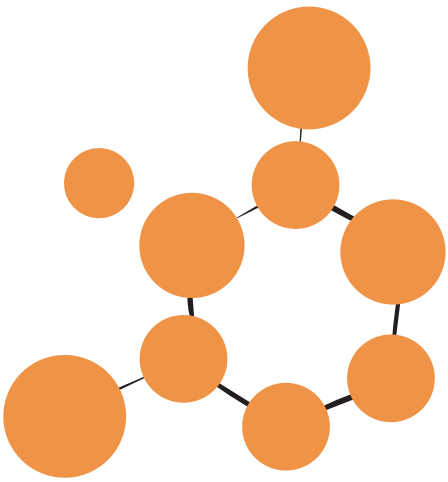
NANTECH 2019

Nucleic Acid Nanotechnology:
from algorithmic design to biochemical applications



Poster Abstracts





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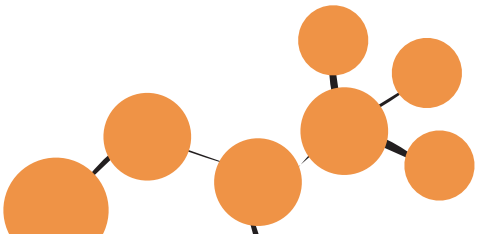

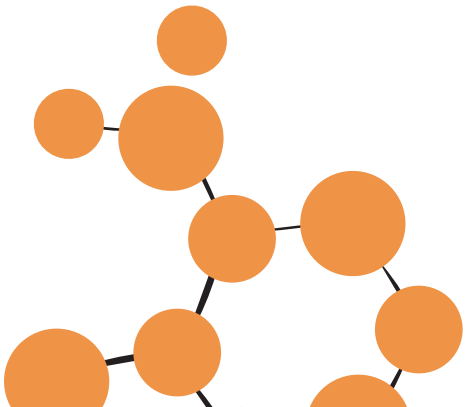

Poster No. 1

Solubilizing DNA origami in organic solvents

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DNA nanotechnology enables the self-assembly of DNA into shapes of variable aspect ratios. These nanometer-sized objects can be site-specifically functionalized with a large variety of materials.[1] However, due to the high-polar nature of the negatively charged phosphate backbone of DNA, all the benefits are only available in high-dielectric environments like water. Herein, we present a robust, modular and reversible method to overcome this restriction, by making DNA nanostructures soluble in organic solvents with the help of cationic poly(ethylene glycol)-polylysine block copolymer.[2] Towards that, we have optimized this method across several organic solvents that are compatible with the poly(ethylene glycol) (PEG) shell, and we also tested the stability and activity of bulky functionalities such as inorganic nanoparticles (gold), biomolecular functionalities (streptavidin, HaloTag, and antibodies) and fluorescent molecules (Cy3 and Cy5). Furthermore, we also carried out a conjugation reaction in the organic solution on the DNA origami. Since the solubility of the complex is a function of the outermost shell that is exposed to the solvent, this method is, therefore, compatible with any solvent. With this, we expand the property and versatility of DNA with possible applications in therapeutics and diagnostics such as gene-therapy. Other possible applications also included DNA-templated synthesis which could potentially allow synthesis of small molecules and polymers.

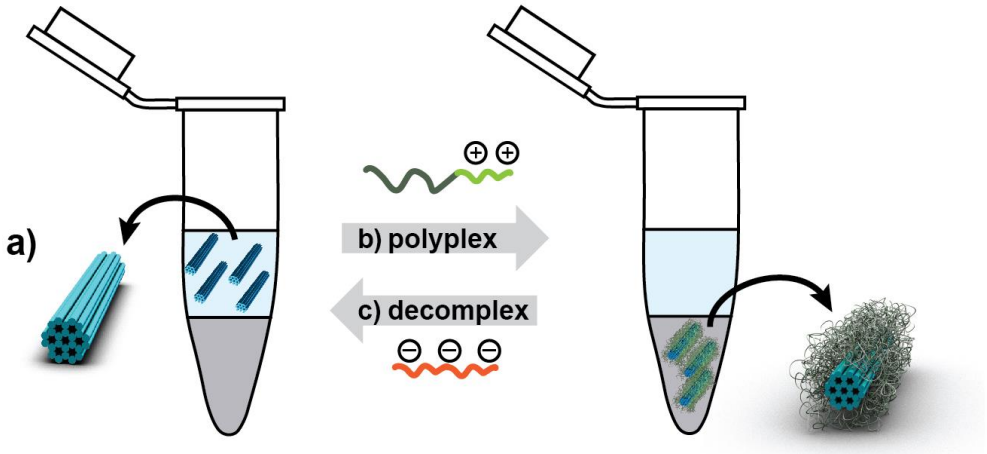

Figure 1: Overview of the method to make DNA origami soluble in organic solvents. a) In native conditions, the DNA origami resides only in the aqueous phase. b) On the addition of the block copolymer, the DNA origami forms polyplex micelles with PEG shell exposed to the environment which has a higher solubility in the organic solvent (eg: Chloroform). c) This process is reversible as the electrostatically bound block copolymers can be displaced from the DNA origami with the help of a highly negatively charged dextran sulfate.

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Poster No. 2

A self-assembled, modular nucleic acid-based nanoscaffold for multivalent theranostic medicine

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The field of personalized medicine aims to tailor treatment precisely to individual patients by targeting the specific molecular characteristics of the diseased cells [1]. For these applications, a drug delivery scaffold must be able to combine specific ligands for different disease markers with potent drugs and imaging agents, while also ensuring a sufficiently long half-life and stability in the body. In this respect, modular systems are advantageous, as a smaller number of functional modules can be synthesized and subsequently combined to produce different functional structures that fit specific disease profiles in individual patients.

We have developed a modular, nanoscale (4-5 nm) nucleic acid platform for bioimaging, targeted drug delivery and combinatorial screening. The four oligonucleotide modules of the device consist entirely of chemically modified nucleotides, including locked nucleic acids (LNA) [2], rendering it low-immunogenic and highly stable in biological fluids. A chemical handle on each module is used for facile and efficient conjugation to different functional molecules, including targeting ligands, imaging agents, modulators of pharmacokinetics, and therapeutic payloads. Stoichiometric amounts of the oligos rapidly self-assemble into a four-armed Holliday Junctions (HJs) with high purity. We have shown that *in vivo* circulation times and biodistribution can be efficiently controlled by functionalization with polyethylene glycol and albumin-binding fatty acids, and we have observed a high extent of uptake in liver cells in *vitro* and *in vivo* with multivalent displays of tri-antennary galactosamine (triGalNAc) moeities.

We are currently exploring the use of the scaffold to assemble different classes of targeting molecules, such as aptamers, to create specific HJs for PET-imaging and radiotherapy to further demonstrate the potential of the HJ to function as both a multifunctional bioimaging- and drug delivery platform.

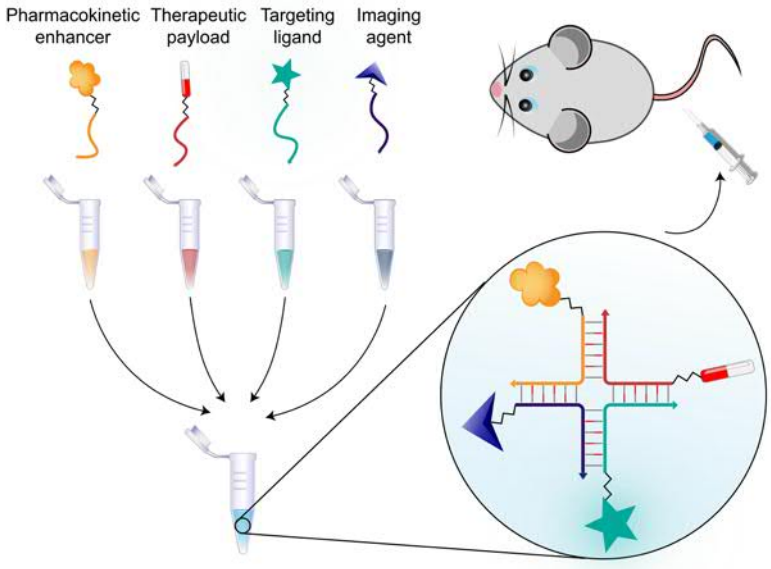

Figure 1: The four oligonucleotide modules are conjugated individually and assembled in different configurations to form multifunctional HJs carrying different combinations of therapeutically relevant functionalities.

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Poster No. 3

Responsive coating and stabilization of liposomes by clathrin-inspired DNA self-assembly

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The self-assembly of the protein clathrin supports multiple processes of endocytosis in biological systems and fascinates by the high structural order. By coating liposomes with biomaterials, architecturally mimicking clathrin self-assemblies, new classes of composite carriers can be derived. DNA represents a versatile material offering high controllability of nanoscale structure assembly, excellent biocompatibility, and multiple ways of functionalization to allow for interaction with lipid bilayers [1]. While many such DNA-superstructures are yet either limited to 2D supported lipid bilayers or deform and even destroy liposomes when assembled on such curved surfaces [2], this work presents a fabrication method achieving protective DNA coating. Utilizing a two-step assembly process, aided by hydrophobic anchoring, a DNA network was grown on the liposome surface mimicking the structure of clathrin self-assemblies. Dynamic light scattering (DLS), ζ-potential and cryo-electron microscopy demonstrate successful DNA coating. Moreover, the DNA coating enhances the mechanical stability, as revealed by atomic force microscopy, and hinders liposome rupture upon detergent treatment, as shown by DLS. Triggered by a toehold-mediated displacement reaction, we also enable the reversal of the coating process by disassembling the DNA coating. With this study, we describe a straightforward, versatile, and reversible way of coating lipid vesicles by an interlaced DNA network, offering potential for applications such as drug delivery or arrangement of further functionalities on the liposome surface.

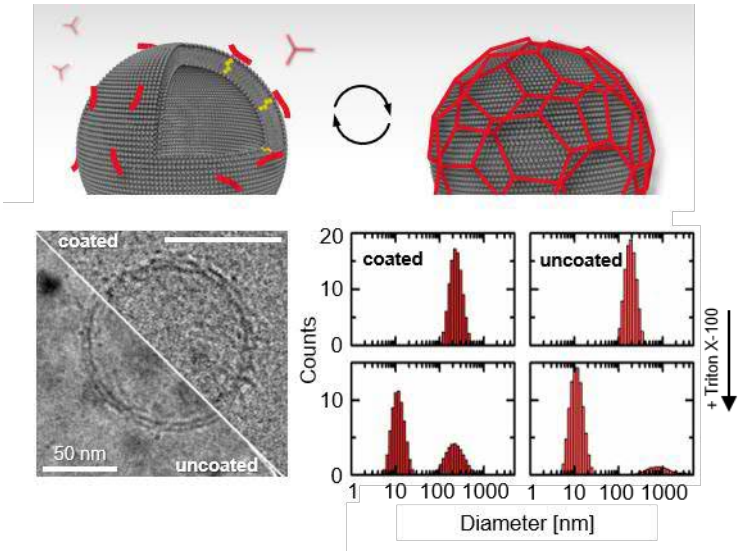

Figure 1: Reversible clathrin-inspired DNA coating of large unilamellar vesicles. Cryo-electron microscopy and the addition of triton to the uncoated and coated stage respectively (DLS) evidence the coating and uncoating success.

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Poster No. 4

Functionalized DNA Origami Nanostructures for Molecular Electronics

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The DNA origami method provides a programmable bottom-up approach for creating nanostructures of any desired shape, which can be used as scaffolds for nanoelectronics and nanophotonics device fabrications. Based on this technique, the precise positioning of metallic and semiconducting nanoparticles along DNA nanostructures can be achieved. In this study, various DNA origami nanostructures (nanomolds[1] and nanosheets[2]) are used for the fabrication of nanoelectronic devices. To this end, gold nanoparticles, semiconductor quantum dots/rods are used in/on the DNA origami structures to create nanowires and transistor-like devices. The DNA origami nanowires and transistors were electrically characterized from room temperature (RT) down to 4.2K. Temperature-dependent characterizations of wires were performed in order to understand the dominant conduction mechanisms. Some nanowires showed pure metallic behavior. Transistor like devices showed Coulomb blockade behavior at RT. The study shows that self-assembled DNA structures can be used for nanoelectronic patterning and single electron devices.

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Poster No. 5

Evolved ecosystems - A simulation of an emerging complex adaptive system

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This abstract introduces *Grafiliv*, a model of *artificial life*[1] based on a particle simulation run on a general-purpose graphics card. The model was created with the purpose of studying self-assembly and the construction of open-ended evolution, its principles and possibilities, in complex adaptive systems. The organisms in the model consist of cell particles determined through pattern-producing networks that evolve over time, in a manner inspired by the CPPN-NEAT method proposed by Stanley[2]. Starting with a simple initial organism designed to be able to survive and reproduce, the simulation was left running for 14-days. After 1,160,000 timesteps and over 250 generations, the result includes several distinctly adapted lineages originating from a single initial organism. Categorised trophic levels include plants, decomposers and predators, with examples from each level described and compared. While issues remain in determining an appropriate modelling level and environment, this work could still be considered a step on the way towards an understanding of the nature of living systems, their characteristics and their limitations. It may also provide inspiration for the physical realisation of evolvable synthetic systems based on nanoscale and microscale construction techniques such as DNA nanofabrication.

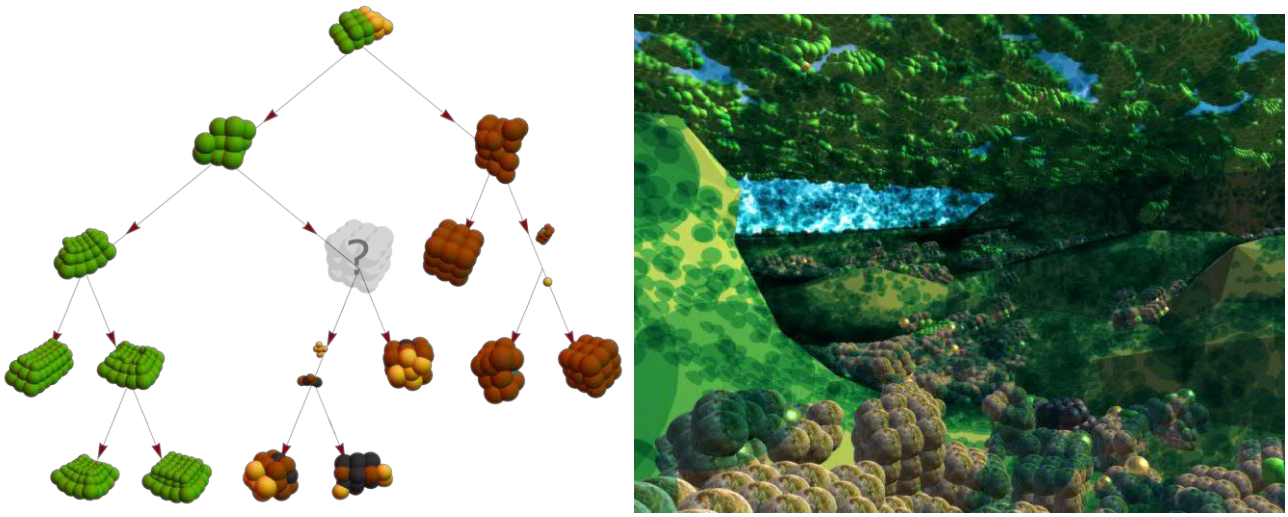

Figure 1: **(left)** Family tree of evolved organisms. Three organisms from each identified trophic level are represented as leaf nodes, with their most recent common ancestors represented as parent nodes. The root node is the initial organism. Note that the organism visualisations are taken from saved timesteps and that the organisms are at different stages of decay. One ancestor was not found on any saved timestep on record. **(right)** Rendered view of *decomposers* living off eating the dead cells falling from the surface-level *plants*.

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Amphiphilic DNA crystals with embedded functionality

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Periodically structured nanoscale frameworks are crucial for many emerging technologies including plasmonics, molecular filtration, and sensing. We recently reported on a new method for the production of such crystalline materials using amphiphilic cholesterol-functionalised branched DNA junctions, C-stars, as building blocks which combines the design freedom and nanoscale structural control offered by DNA nanotechnology with the robustness of hydrophobic interactions (**Fig 1a**).[1] In contrast to approaches reliant on Watson-Crick interactions alone,[2,3] here long-range order emerges from the frustrated phase separation between the hydrophobic cholesterol and hydrophilic DNA. This mechanism is controlled uniquely by the topology and symmetry of the flexible branched DNA motif, which makes C-star self-assembly robust against substantial structural variations which preserve these features. For example, the lattice parameter of the self-assembled crystals can be precisely and continuously defined over a range of more than 15 nm simply by changing the arm length of the DNA moieties.[4] By exploiting the inherent amphiphilic properties of these materials along with the controllable mesh size, we have additionally demonstrated the ability to fine-tune the partitioning of macromolecules within the crystals based on size and hydrophobicity.

Here, we show how the resilience of this approach to design changes in combination with the high porosity of these materials can be utilised to prepare crystalline frameworks with embedded functionalities. By incorporating a responsive DNA motif, we have been able to demonstrate isothermal dissolution of the frameworks induced solely through the addition of a specific DNA strand (**Fig 1b**). To further demonstrate the versatility of this approach, we produced C-star frameworks modified with a protein binding ligand, NTA, which enables the controllable and reversible binding of a recombinant protein carrying a specific amino acid residue (**Fig 1c**).

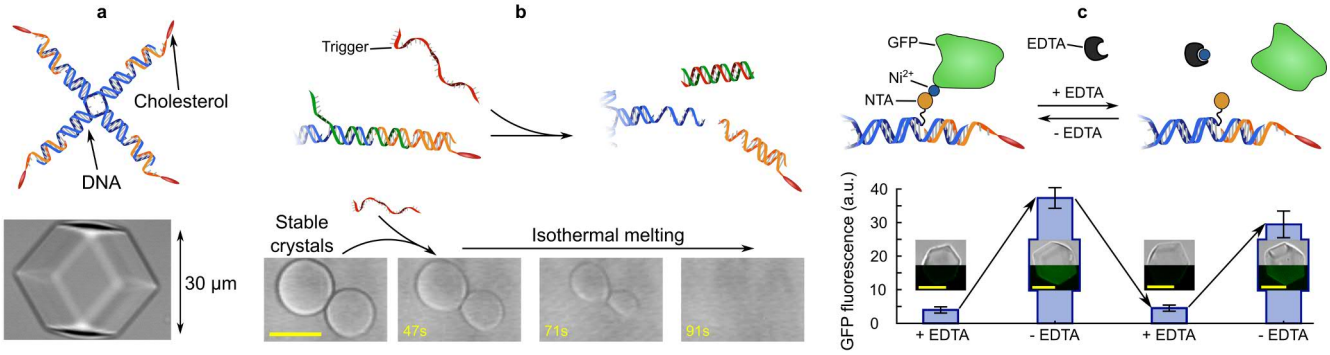

Figure 1: a, Cholesterol modified DNA nanostars, *C-stars*, self-assemble from 4 cholesterol-functionalised strands (orange) and 4 non-functionalised strands (blue). Under suitable conditions, C-stars form micron scale single crystals. **b,** C-star frameworks can be designed to undergo isothermal dissolution in the presence of a target oligonucleotide through the incorporation of a responsive DNA motif within the C-star arms. Material dissolution is followed with bright field microscopy. Scale bar 10 µm. **c,** Protein binding functionality can be introduced within C-star networks by modification of nanostar arms with the binding ligand NTA. In the presence of Ni^{2+} ions, recombinant Green Fluorescence Protein (GFP) binds to the NTA molecule. Removal of Ni^{2+} ions through addition of the chelating agent NTA causes GFP release. GFP binding was quantified using confocal fluorescence microscopy. NTA modified crystals are shown in bright field (insets: top half) and confocal fluorescence microscopy (insets: bottom half). Scale bars 10 µm.

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Poster No. 7

Single-molecule DNA Origami arrays

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Placement of single molecules in spatially defined arrays is a fundamental issue for studying biomolecular behavior. DNA nanotechnology has enabled the construction of DNA nanostructures scaffolds that allow placement of functional moieties with nanometric precision. Based on the combination of both bottom-up and top-down strategies, our group recently demonstrated the fabrication, via a 1-step lithographic process, of DNA origami nanoarrays to position multiple nano-objects [1], as well as, multivalent ligands to investigate cancer cell spreading [2]. In this poster, we will discuss the fabrication of high-yield nanoarrays of DNA origami for the assembly of single-molecule fluorophores. Nanoapertures in metal-coated glass were patterned with focused-ion beam lithography, and functionalized DNA Origami were organized in nanoarrays. Photobleaching experiments indicated that 97% of the wells contain a single Origami, compared to 3% exhibiting double occupancy. This single-molecule platform we fabricated will enable us to study biomolecular events related to biosensing, with single-molecule resolution and multivalent control.

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Poster No. 8

DNA origami nanoparticles to study Dendritic Cells activation

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Vaccination is one of the greatest achievements in the history of medical treatment and the quest to manipulate the immune system to generate optimally effective immunity against different pathogens can be considered the "grand challenge" of modern immunology. Although adjuvants have been used in vaccines to enhance immune response to antigens, there is still limited knowledge on the detailed mechanism of actions of most of the adjuvants. [1]

The activation of dendritic cells (DCs) as consequence of their ability to detect "danger signals" is the first step to trigger T cell response in vaccination. Current immuno-therapeutic approaches suffer from a lack of detailed understanding in the complex interplay of immune ligand-receptors interactions. Understanding what combination and arrangement of signals are required for activating DC-T cell interactions is important to develop potent immune therapies that are highly specific and limit toxic or even lethal off-target effects. To fully understand cellular processes, we need novel synthetic particles with a level of control in size, shape and function that is currently not attainable in any nanoparticle delivery systems for antigens and danger signals. [2]
DNA has emerged as a key player in material science and could lead to breakthrough advances in immune-engineering: DNA origami is an ideal platform to display with unprecedented level of uniformity the presentation of ligands for cellular activation and allow the detailed study of ligand receptor interaction in the immune response. DNA offers unprecedented control over the precise positions of ligands in a nanostructure since each staple has a unique sequence, therefore ligands can be positioned as pixels on the structure. [3]

In this work, we present a highly controlled library of danger-signal presenting DNA-origami based nanoparticles to study dendritic cell activation. By analysing ligand-receptor interactions, we hope to understand the best spacing and features of ligand presentation of adjuvants for the development of improved vaccines. State-of-the-art nanoparticle research has started to target these pathways, however, the focus remains limited to general ligand function instead of utilizing the unique spatial control given by the DNA platform to truly understand the relation between spatially defined ligand presentation and cellular activation intensity. Previous studies performed with DNA origami have used DNA nanoparticles that lack cellular stability. For the application of this promising technique to biological studies, stability of the DNA structures needs to be safeguarded. In the previous years, methods to solve the weak stability of DNA origami in cell media and *in vivo* have been developed. [4]

Here, we address the stability issues by applying two new strategies to increase the *in vivo* stability of DNA origami: (1) UV-induced covalent crosslink reaction between neighbouring T developed by Dietz and coworkers [5]; (2) Oligolysine-PEG based coating developed by Shih lab, in which the positive changes of the lysines interact with DNA phosphate backbone to protect DNA origami in low salts environments and the PEG shield from the attack of nucleases [6]. Our stable precision materials allow us for the first time to quantitatively study how the amount and spacing of ligands affect the stimulation of DCs. The possibility to control these features with nanoscale precision is a great advantage compared to common polymeric nanoparticles, which not only largely vary in size (high polydispersity) but display ligands in a random disposition on their surface. These tools allow to unravel the complexity of our immune system and to set the next steps for DNA-origami to become functional structures in medicine.

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Poster No. 9

Electron-induced DNA strand breaks probed by DNA origami platforms

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For cancer treatment high-energy radiation is used in combination with radiosensitizing therapeutics. Therefore halogenated nucleosides are used which are able to enhance the reactivity towards secondary low-energy electrons (LEEs) generated along the radiation track of high-energy primary radiation [1]. The high reactivity results in an efficient fragmentation by dissociative electron attachment (DEA). Recently, we have studied low-energy electron induced single strand breaks in DNA modified with 2'-Fluoroadenine [2] and 8-Bromoadenine [3] using a DNA origami based technique (Figure 1). This method provides access to efficient and systematic determination of electron induced DNA strand break cross sections [4].
For non-modified oligonucleotides recent experiments suggest that electron induced strand breaks depend on the nucleotide sequence and its length [5]. Our approach using DNA origami templates carrying different oligonucleotide target sequences provides access to efficient and systematic determination of electron induced DNA strand break cross sections. Here we present the strand break cross sections for polyA_n (n = 4, 8, 12, 16, 20) DNA-strands at different electron energies (5 - 10 eV).
Nevertheless, the investigation of double strand breaks is more relevant than single strand breaks. Therefore, we present first results on the modification of DNA origami templates with double stranded target sequences and their irradiation with electron energies in the range of 5 – 10 eV.

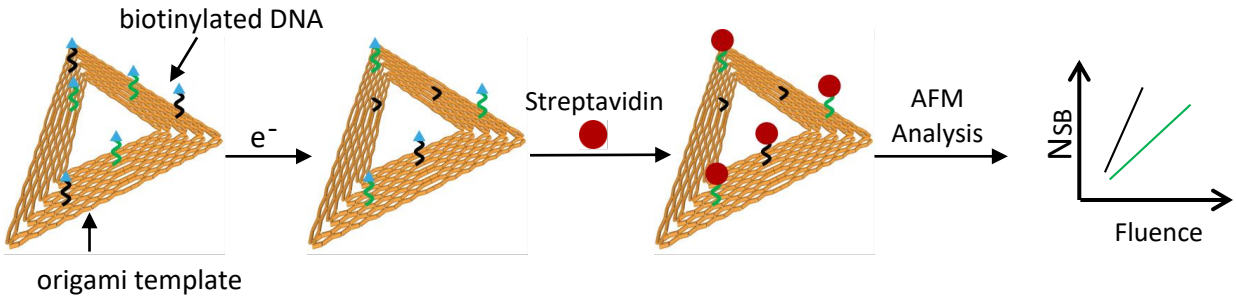

Figure 1: Scheme of the DNA origami technique to determine absolute cross-sections for irradiation-induced DNA strand breaks. DNA target strands are placed onto DNA origami platforms. After irradiation and rinsing the intact target strands are labelled with streptavidin to visualize them in AFM images. Analysis of AFM images obtained at various irradiation conditions yields absolute cross-sections for strand breaks.

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Introducing handhold-mediated strand displacement: A new template-catalysed reaction for DNA nanotechnology

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Introduction

DNA nanotechnology allows us to encode a plethora of dynamic systems by harnessing two fundamental DNA reactions: hybridisation and strand displacement. The latter consists in the spontaneous replacement of one DNA or RNA strand in a duplex by an "input" strand. This input strand hybridises with a "target" stand in the duplex, while liberating an "output" strand. This strand exchange is extremely slow for blunt-ended duplexes, unless the reaction rate is boosted by adding to the target a small single-stranded overhang known as "toehold" [1]. Even though the use of toehold-mediated strand displacement has been extremely successful, it only relies on the key step of input-toehold binding to occur, obviating any input-output complementarity. The present work introduces an orthogonal fundamental reaction for DNA nanotechnology, "handhold-mediated strand displacement". "Handholds" are analogues of toeholds located in the output strand, effectively allowing the output strand to template the input-target association. This study introduces a systematic characterisation of handhold contribution in strand displacement reactions that will allow rational engineering of handhold-mediated systems.

Methods

Strand sequences were rationally generated with the software NUPACK [2]. A reporter DNA duplex labelled with a fluorophore/quencher pair monitored the progress of the reaction. The fluorescent data was fitted to an ODE-based model of the reaction sub-steps to extract the dependence of reaction rate constants on system parameters. PAGE and an additional fluorescent reporter quantified output strand detachment.

Results

Data evidence a clear correlation between handhold length and the rate of handhold-mediated strand displacement reactions. Shorter handhold lengths increase the reaction rate by several orders of magnitude, while still allowing the eventual detachment of the output strand.

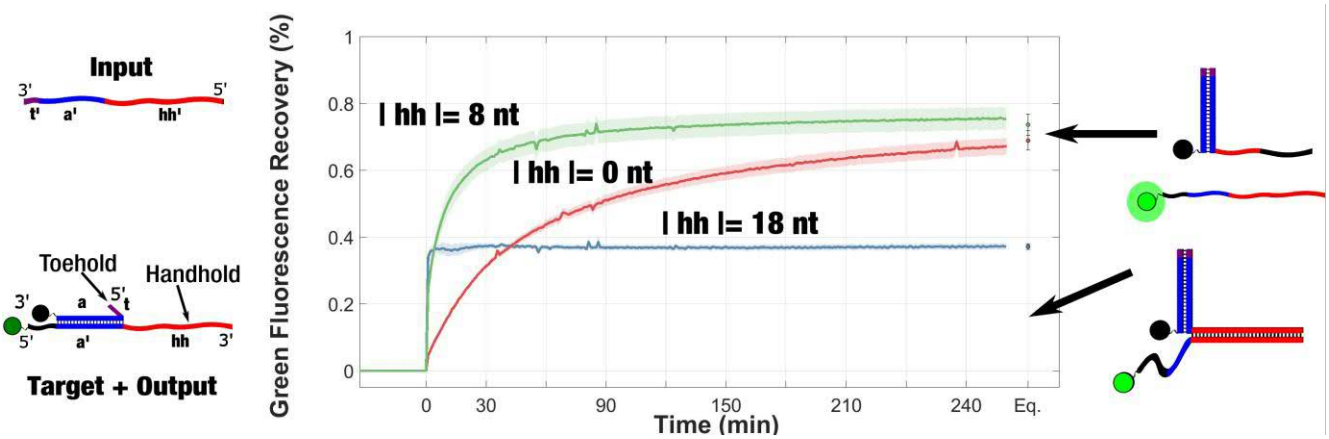

Figure 1: **Handhold-mediated strand displacement proof of concept.** *Conditions: 37 °C, Target + Output duplex (200 nM). Time 0 marks the addition of Input strand (150 nM).* When compared with conventional strand displacement (|hh|=0 nt), complementarity between input strand and output's handhold increases significantly the speed of the reaction (|hh|=8 nt). However, a strong complementarity between input strand and handhold (|hh|=18 nt) prevents the dissociation of the output strand.

Conclusion

Handholds can modify strand displacement reaction rate constants by orders of magnitude. The reaction entails transitory sequence complementarity between input strands and handhold. The handhold can therefore be recovered, acting as a catalyst, a functionality not present in toehold-mediated reactions. Future work will aim to rationally exploit handhold-mediated strand displacement motifs to demonstrate complex catalytic behaviour.

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Poster No. 11

Design of DNA strand displacement systems resistant to spuriously triggered leakages

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One of the goals of DNA nanotechnology is to design enzyme-free DNA-based molecular systems with programmable dynamic behaviors. The invention of Toehold-mediated Strand Displacement (TSD) [1] provides a basic building block for designing DNA Strand Displacement (DSD) systems. In general, an arbitrary Chemical Reaction Network (CRN) can be converted into a DSD system of approximately equivalent behavior.

A typical DSD system comprises of several DNA complexes having their one end as a designated toehold binding site and the other blunt end is ideally non-reactive. In practice, however, an eventual fraying in the blunt ends of two DNA complexes can trigger a TSD, which ultimately ends up releasing output in the absence of input, and thus causing leakages [2]. A variety of DSD systems have been demonstrated both theoretically and experimentally, but their scalability and predictability are limited by these leakages [3].

We introduce a leakless design for DSD systems based on especially designed DNA complexes with two toeholds. The TSD in a DNA complex is triggered by a cooperative binding of an invading DNA strand at both the toeholds. We demonstrate that the leakless design approach is generalized, and it strongly suppresses spuriously triggered leakages.

Figure 1 illustrates a domain level conceptual design of a leakless translator (Y := X). Complementary domains are marked by asterisks, and toehold domains are shown by an additional alphabet ´T´ in their subscripts. In a DNA strand, 3' and 5' ends are represented by arrowhead and non-arrow, respectively. The translator system consists of input X, output Y and two specially designed fuel DNA complexes, F1 and F2, each having two toehold binding sites. The fuel DNA complexes are designed to ensure a co-localized toeholds binding triggered displacement of bulge forming DNA strands.

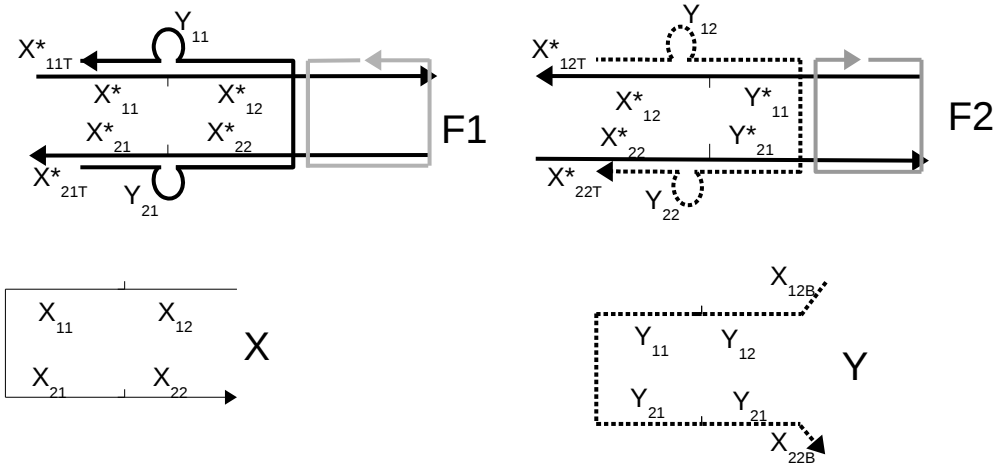

Figure 1: The design of leakless translator system. Domain level representation of DNA fuel complexes (F1 and F2), input (X) and output (Y).

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Poster No. 12

UV-Point Welding of DNA Nanostructures and Reversible Covalent Stabilization of Stacking Contacts in Higher-Order DNA Assemblies

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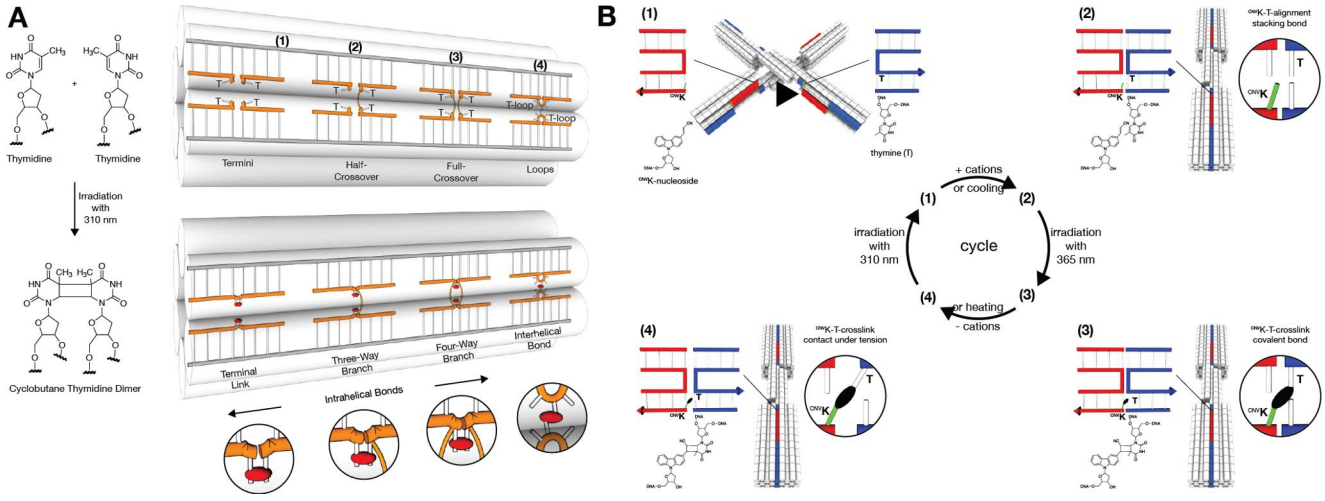
I will present two methods that can be used to either increase the structural stability of multilayer DNA objects or the stability of stacking contacts within higher-order DNA assemblies. The first method relies on introducing additional, un-paired thymidines in close proximity within DNA nanostructures at user-defined sites (see figure A) [1]. Upon ultraviolet irradiation, these nearby thymidines can be covalently linked to one another by forming predominantly cyclobutene pyrimidine dimers. The thus created covalent bonds may be used to link free strand termini, to bridge strand breaks at crossover sites, and to form additional interhelical connections. Multilayer DNA origami objects designed with un-paired thymidines and irradiated with UV light to induce covalent bonding preserve their global shape up to temperatures of 90°C and in pure double-distilled water with no additional cations present. This is a general, site-selective method that can be scaled in a biotechnological fashion.
The second method relies on modifying the terminal base of one blunt-ended helix of the stacking contact with the 3-cyanovinylcarbazole (^{cnv}K) moiety (see figure B) [2]. In addition, a thymidine residue is positioned at the other corresponding blunt-end of the stacking contact. In the bound state, the two blunt-ended helices are stacked to one another, resulting in a co-localization of ^{cnv}K and the thymidine. Upon ultraviolet irradiation, a covalent bond can be created across the stacking contact linking the ^{cnv}K moiety to the thymidine. This bond can also be cleaved again upon irradiation with UV light of shorter wavelength, allowing repeated formation and cleavage of the same covalent bond on the timescale of seconds. Using this method, stacking-bond stabilized higher-order DNA assemblies can be covalently linked in their fully assembled state. Once covalently stabilized, they can be subjected to purification and enrichment procedures to produce pure and dense solutions. These procedures typically require low ionic strength to obtain satisfying recovery yields. At these conditions, higher-order assemblies would fall apart into their individual building blocks unless further stabilized.
Taken together, both methods open up new avenues for applications of DNA nanostructures in a wider range of conditions, which may result in a broader applicability of DNA-based nanotechnology in general.



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Poster No. 13

Molecular program for the isothermal digital detection of microRNAs

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Molecular programming is an emerging discipline that involves the design of artificial biomolecular circuits. These architectures, capable of information-processing tasks, have been implemented in Boolean computation, reaction-diffusion systems or matter shaping and are now expected in biosensing applications. Here, we report a molecular program performing isothermal target-triggered signal amplification. As a proof of concept we demonstrate the ultrasensitive detection of microRNAs, short RNA strands involved in the regulation of gene expression and emerging as promising disease-specific biomarkers. We used a versatile molecular programming language named PEN-DNA toolbox (Polymerase Exonuclease Nickase Dynamic Network Assembly) [1]. It employs a set of short oligonucleotides (template) encoding the topology of a network of reactions, which are catalyzed by a mixture of enzymes. Each template thus senses the presence of input strands an in turn produces output strands that act on other nodes of the system. The connectivity of the microRNA detection circuit is presented in Figure 1a: the universal signal amplification occurs in the form of a bistable node composed of two templates: an autocatalytic template (aT), made of a dual-repeat sequence catalyzing the exponential replication of a 12-mer oligonucleotide (trigger) via a polymerization/nicking process; and a pseudotemplate (pT) that absorbs the trigger produced by spurious reactions (leak) on the aT, essential to avoid unwanted amplification [2]. A converter template (cT) is connected upstream to the aT: upon binding of the target to the input part of the cT, the latter linearly produces trigger strands, which in turn activate the autocatalytic reaction on the aT. Downstream to the aT, a reporting template (rT) captures the amplified signal strands to produce a fluorescence signal (Figure 1b). The leakless amplification switch makes possible the detection of single molecules compartmentalized in water-in-oil droplets and thus enables digital quantification (Figure 1c) [3]. In perspective, we present a multiplex and digital detection format relying on the compartmentalization of DNA-grafted particles specific for each target.

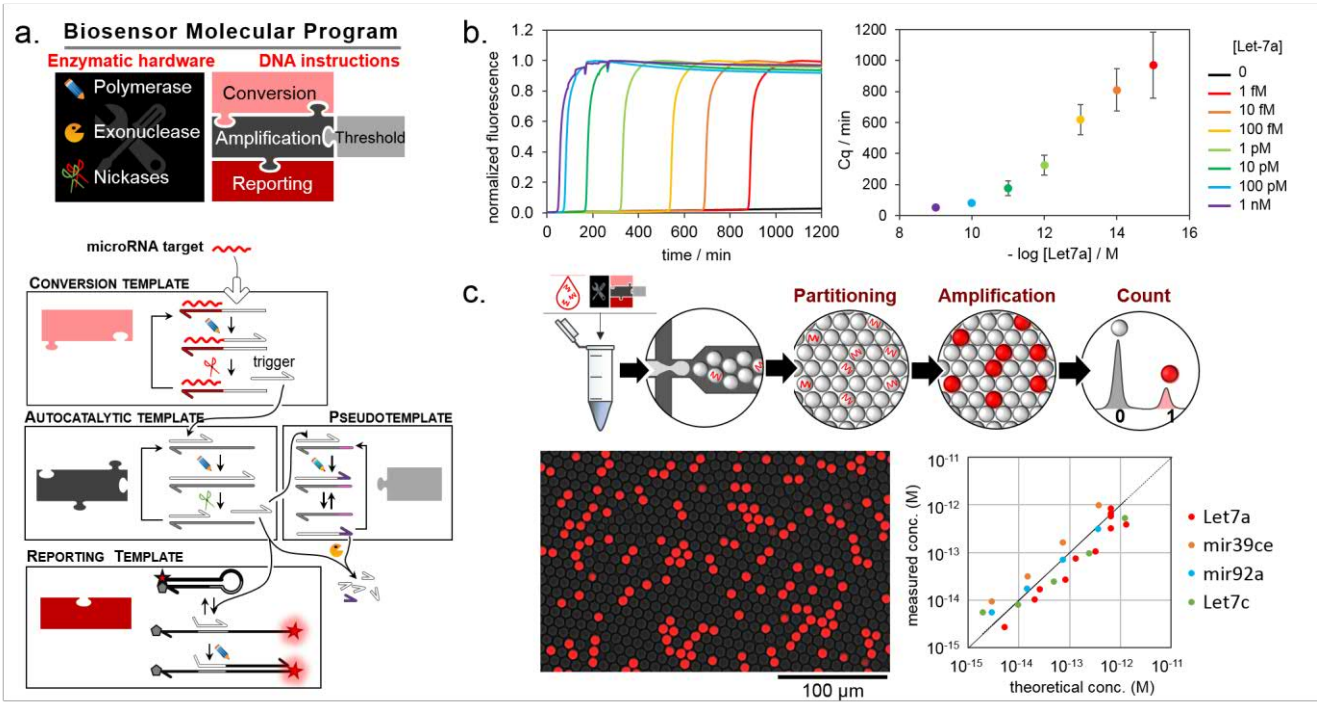

Figure 1: Molecular programming for the digital detection of microRNA biomarkers. **a.** Molecular program design. **b.** Bakground-free detection of Let7a target. **c.** Droplet digital format enables absolute target quantification.

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Poster No. 14

Self-Assembled Plasmonic DNA Origami Nanoantennas for Diagnostic Applications

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Signal amplification strategies are essential for improving sensitivity, speed, and robustness of sensing assays for point-of-care diagnostic applications. One strategy to achieve this relies on physical fluorescence signal amplification by plasmonic nanostructures that act as optical nanoanntenas concentrating the incident excitation light into zeptoliter volumes and enhancing the radiative decay rate of fluorescent molecules. Using a DNA origami platform to precisely arrange fluorescent dyes in the plasmonic hotspots of metallic nanoparticles, optical DNA origami nanoanntenas reaching up to 5000-fold fluorescence enhancement have been previously achieved in our group.[1-3] This contribution will describe the ongoing research efforts to utilize these plasmonic nanoanntenas for fluorescence-based sensing applications.[4-5] With the help of unique positioning precision and addressability of DNA origami we designed plasmonic nanoantennas with diagnostic assays incorporated directly in the plasmonic hotspots of gold and silver nanoantennas (Figure 1). Using fluorescence-quenched hairpin and sandwich binding assays we were able to detect DNA and RNA sequences specific to the Zika virus as well as antibiotic resistant bacteria. Utilizing the plasmonic nanoantennas we show that fluorescence enhancements reaching several hundred fold could be achieved in these fluorescence sensing assays. Finally, we will also discuss the ongoing work towards utilizing these DNA origami antennas to enable single-molecule detection and sensing *via* smartphone-based devices which could lead to the development of inexpensive point-of-care diagnostic platforms.[6]

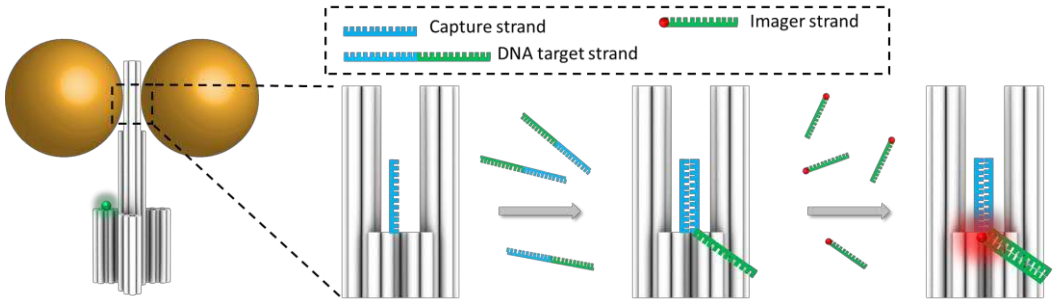

Figure 1: Sketch illustrating the sandwich binding assay incorporated in the hotspot of plasmonic gold nanoantenna. DNA origami platform is utilized to position two gold nanoparticles in close proximity creating a plasmonic hotspot for fluorescence enhancement. The capture strand is placed directly in the hotspot which allows to capture a specific target DNA and its further imaging with dye labelled imager strand. The fluorescence of the imager dye is enhanced up to several hundred fold allowing for direct signal amplification.

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3D DNA Hollow Nanocylinders For Reversible Step-Growth Polymerization into 1D Fibrils

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The design of hierarchical structures with pre-defined functionalities is of high interest to successfully mimic complex processes and systems present in nature. Self-assembly over a wide range of length scales and hierarchical levels calls for monodisperse building blocks with precisely defined anisotropic interaction patterns ('patches'). Polymer-based nanoparticles have been shown to co-assemble into intricate patterns by precise positioning of attractive and repulsive patches.[1] The easy programming and handling of 3D DNA origami recommends them as precision patchy particles for creating continuous self-assembled systems such as fibrils or networks.[2,3]

Previously, our group reported 3D DNA origami cuboids which self-assemble by base-pairing of complimentary ssDNA overhangs creating defined attractive patches. The stability of the system is shown to be due to multivalency during self-assembly of 3D DNA origami cuboids into 1D fibrils. Even in presence of excess connectors, fibrils form as the assembly is guided through cooperative and entropic effects. The amount of connector strands governs the extent of this step-growth polymerization. Furthermore, temperature is applied as external switch to reversibly disassemble the fibrils into single particles.[3]

In a new approach, we now changed the interaction patterns by using ssDNA overhangs on T-passivated 3D DNA hollow nanocylinders. Fibrillation is achieved by addition of a fuel strand (see figure 1A). This design allows for highly flexible reprogramming of self-assembly by changing the number of overhangs and the length of the fuel strand. The melting temperature of the fuel strand hybridization has a high impact on fibrillation and can readily be adjusted by sequence and sequence length. Moreover, the system can be switched reversibly from single particles to elongated 1D fibrils by using a toehold-mediated re-hybridization of the fuel-strand upon addition of antifuel (see figure 1B). This switching from monodisperse single building blocks to 1D fibrils can be repeated multiple times. In this way, use of DNA origami is extended from the area of DNA nanotechnology to soft matter and colloidal science, thus showing how DNA origami as soft colloidal building blocks allow for self-assemblies with controllable and tunable periodicities.

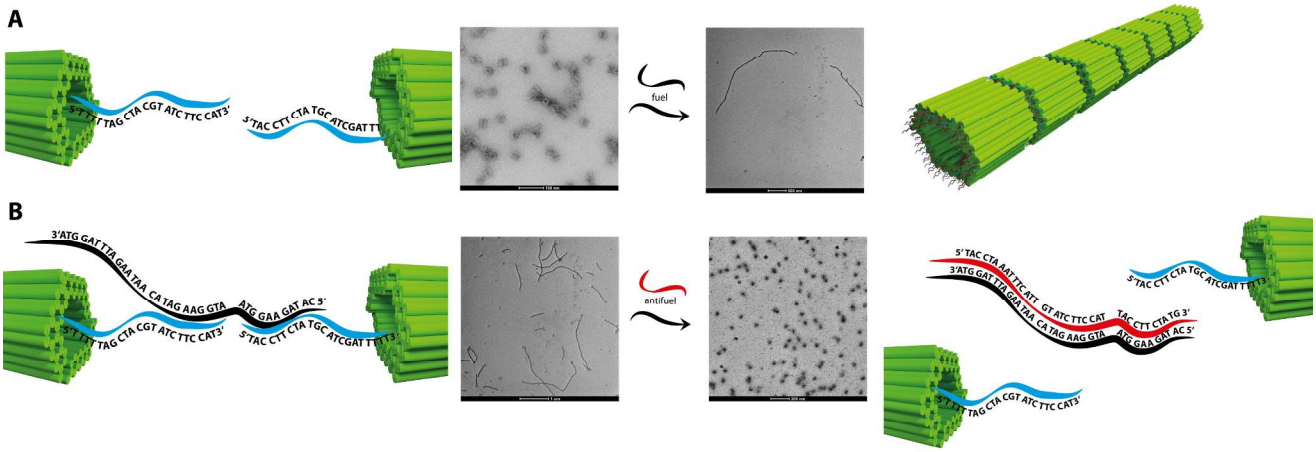

Figure 1: Tunable and switchable self-assembly of 3D DNA hollow nanocylinders as patchy particles. A: Up to 24 connector overhangs (blue) create precise patches for controllable self-assembly. Addition of the fuel strand (black) leads to formation of fibrils. Scheme and negative stain TEM images. B: By programming a fuel strand with a toehold, antifuel strands (red) can break down fibrils to regain the single DNA origami building blocks. Scheme and negative stain TEM images.

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Poster No. 16

Enzymatic Modification of DNA Nanostructures

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Self-assembly of DNA into complex shapes has enabled the construction of complex, discrete nanostructures with defined geometries. In many applications, the assembled DNA nanostructure does not fulfil a function by itself, but serves as a structural scaffold, onto which functional moieties are placed at defined relative positions, much as on a molecular breadboard. Since synthetic oligonucleotides with a broad range of chemical modifications are commercially available, the incorporation of chemical moieties usually is straight forward. If many chemically modified strands are to be incorporated, for example, as in a molecular beacon with many fluorescent labels, this procedure results in high manufacturing costs. Enzymatic modification of DNA nanostructures could lead a way around this issue. We will present our results evaluating the potential for an enzymatic post-assembly modification of DNA origami nanostructures.

DNA origami-templated silica growth by sol-gel chemistry

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DNA nanotechnology allows for the bottom-up synthesis of nanometer-sized objects with high precision and selective addressability due to the programmable hybridization of complementary DNA strands. The introduction of DNA origami[1] has resulted in a plethora of objects of different shapes and sizes, many of which have been site-specifically modified with a variety of functional moieties such as proteins or nanoparticles.[2, 3] Potential fields of applications of these DNA nano-objects range from plasmonic metamaterials to nanomedicine. However, improving the stability of DNA origami structures with respect to thermal, chemical, and mechanical demands will be essential to fully explore the real-life applicability of DNA nanotechnology. By encapsulating DNA origami in a protective silica shell using sol–gel chemistry, we developed a strategy to increase the mechanical resilience of individual DNA origami objects and 3D DNA origami crystals[4] in solution as well as in the dry state.[5] This allowed for a detailed structural analysis of the crystals in a dry state, thereby revealing their true 3D shape without lattice deformation and drying-induced collapse. Our synthetic approach (Figure 1a) is based on the well-known Stöber method.[6] However, the Silica species involved in this reaction are negatively charged, which poses a problem when considering reactions with anionic DNA. Therefore we employed a positively charged co-structure directing agent TMAPS (*N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride), which is capable of electrostatic attraction to the polyanionic DNA backbone. Co-condensation with the silica precursor tetraethoxy orthosilicate (TEOS), then enabled uniform silica growth directly on the DNA structure (Figure 1b and c). The shell thickness could be tightly controlled by means of reaction time as well as reactant concentrations. Resulting silicified DNA origami structures displayed not only excellent mechanical and thermal stability, but were also protected from degradation by nucleases. All in all our encapsulation strategy facilitates shape-controlled bottom-up synthesis of designable biomimetic silica structures through transcription from DNA origami.

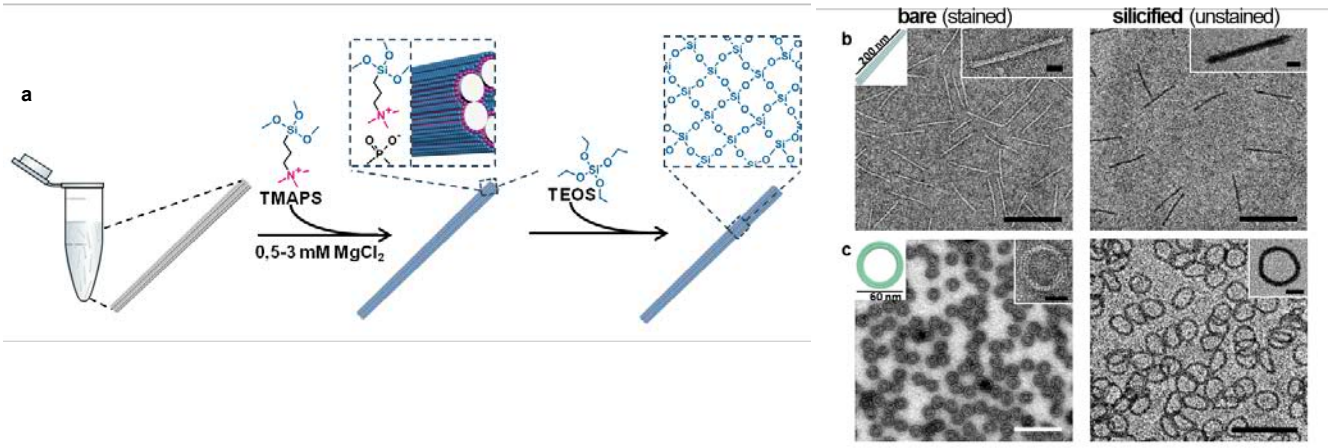

Figure 1: a Schematic of DNA Origami-templated Silica structures. **b** shows a TEM image of 14 helix-bundle DNA origami rods stained with Uranyl Formate (left) and the same rods after silica coating (right). Due to the higher electron density of silica, no staining was required for visualization. **c** depicts a TEM image of 14 helix-bundle DNA origami rings stained with Uranyl Formate (left) and the same rings after silica coating (right). Scale bars are 300 nm for a and b and 30 nm for insets.

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Poster No. 18

A computational framework for DNA sequencing-based microscopy

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We present a theoretical framework and proof of principle for a method of 2D image construction from DNA sequencing data. DNA polony amplification techniques have enabled the generation of uniquely barcoded patches of DNA to be grown in spatialyl restricted patches on surfaces [1]. By growing these patches to the point of saturation such that adjacent polonies are in contact with one another, and by subsequently crosslinking polonies that are in contact with one another, it is possible to generate edge data or the association of two unique barcodes. Edge data can be used to reconstruct a fully interconnected network. We show that by exploiting the random distribution of polonies on a surface, a symmetric circular boundary condition of the region of interest, and the inherent planarity of the surface, it is possible to reproduce with high fidelity the original relative arrangement of polonies using the Tutte embedding algorithm. We also show that a non-deterministic solution with similarly high fidelity can be achieved using a spring relaxation technique. By using polonies as a kind of pixel, able to capture molecules of interest and transmit those associations to the mesh reconstruction, this principle can be used to generate whole images from sequencing data alone. We characterize the distortions in reconstructed images and show that they are local and most prevalent near boundaries but do not extend over the length scale of the reconstructed image.

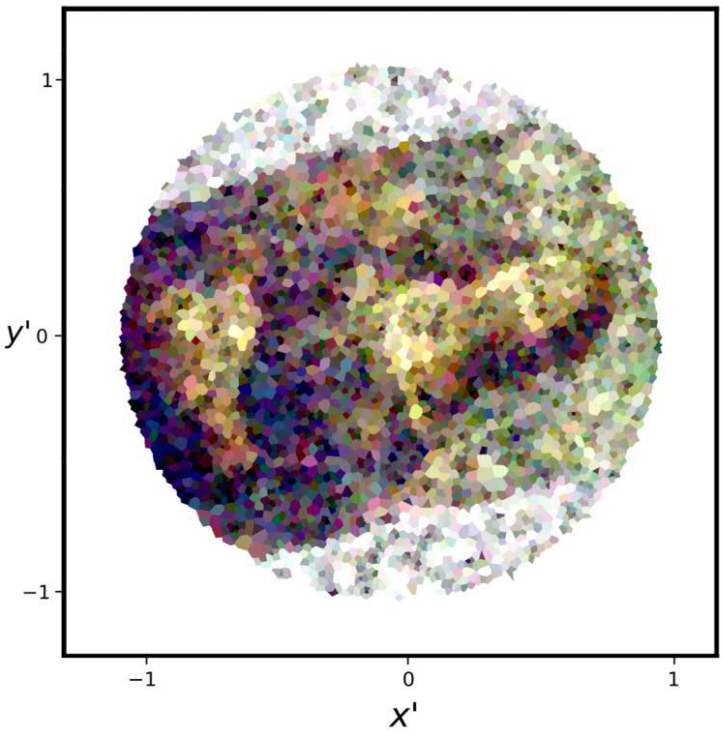

Figure 1: Image reconstructed using Tutte embedding to arrange purely topological, pairwise association data alone – data which could be transmitted through the pairing of adjacent surface-grown barcoded polonies and subsequent next gen sequencing.

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Poster No. 19

A DNA Origami-based Chiral Plasmonic Sensing Device

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Accurate and reliable biosensing is crucial for environmental monitoring, food safety, and diagnostics. Various materials and biochemical entities hold potentials for biorecognition and/or transduction in sensors. The difficulty often lies in the integration of them for realizing efficient and reliable biosensing, which is especially challenging at nanoscale. We engineered a nano-sensing device that combines the beneficial properties of DNA origami technique, chiral plasmonics, and aptamers. This combination enables selective and sensitive detection of targets.

The nanosensor was composed of aptamer-based locks for biorecognition, a reconfigurable DNA origami for transduction, and gold nanorods (AuNRs) for optical detection. The state of the aptamer-based lock changes upon target binding. The DNA origami structure transduces the state of the lock (closed/open) into two spatial configurations of the AuNRs dimer (chiral/relaxed), which exhibit distinct circular dichroism (CD) responses. In this work [1], the adenosine molecule was used as a model analyte, and corresponding aptamer sequence was inserted into the DNA origami through Watson-Crick base pair. The AuNRs-origami construct was assembled through thiol-functionalized oligonucleotides. Transmission electron microscopy (TEM) and CD spectroscopy were used to characterize the nanosensor [2].

Two types of locks were designed for optimizing the system. The double stranded (ds)-lock consisted of an aptamer and a complementary strand and undergoes closed to open transition upon analyte binding. The split aptamer (sp)-lock consisted of partial aptamer strands and closes upon target binding. The AuNRs were properly assembled onto the DNA origami and the construct with ds-lock or sp-lock generated CD signal decrease or increase, respectively, in addition to adenosine while remained the same in the presence of control molecules. After combing the two locks, the nanosensor demonstrated significant CD responses corresponding to the concentration of adenosine in the range from 30 μM to 10 mM (Figure 1). In addition, the nanosensor enables optical detection in environments with strong optical extinction, which would simplify preparation procedures of biologically relevant samples.

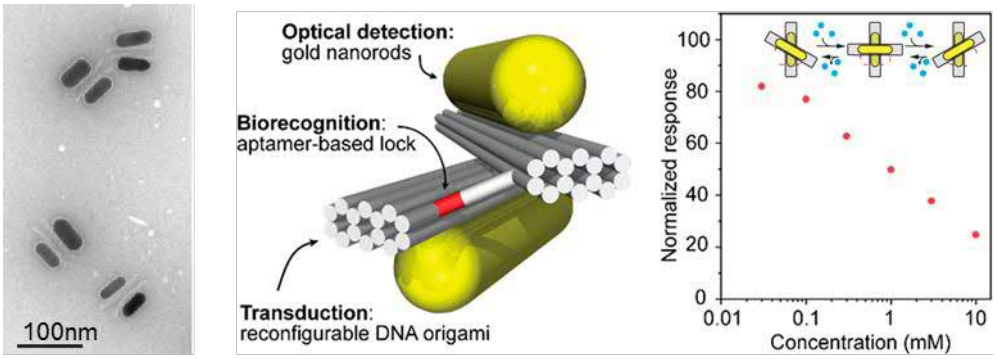

Figure 1: The schematics and characterizations of the nanosensor.

We developed a highly programmable plasmonic nano-sensing device, which exhibited selectivity and sensitivity in a wide detection range. By manipulating the properties of the lock and the characteristics of the AuNRs, the nanosensor can be developed for various applications. We also expect that the presented sensing scheme can be adapted to a wide range of analytes and tailored to specific needs.

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Reconfigurable and Programmable pH-Responsive DNA Origami Nanocapsule for Loading, Encapsulation and Displaying of Cargo

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DNA nanotechnology provides a toolbox for creating custom and precise nanostructures with nanometer-level accuracy. In the recent years, a growing interest to add dynamic properties to these otherwise static nano-objects has led to the introduction of various examples of dynamic nanoscale devices based on the DNA origami technique [1]. Devices capable of shielding and transporting cargo until receiving a specified external trigger for cargo display or release can have intriguing prospects in drug delivery. One such example of a biologically relevant trigger is a pH change in the environment – such as an elevated cytoplasmic pH, a distinct characteristic of cancer cells [2]. Here we present a dynamic, pH-responsive DNA origami nanocapsule for the encapsulation and display of various types of molecular cargo [3] (see Figure 1). The design has been functionalized with multiple "pH latches" – triplex-forming pairs of a DNA double helix and a single-stranded DNA that lock the capsule in a closed state when pH is below the pK_a of the system (adjustable by the T-A·T base triad content of the latch strands [4,5]). By using Förster resonance energy transfer (FRET) based measurements, we have shown that opening and closing can be triggered repeatedly in physiologically relevant conditions [6]. By applying both gold nanoparticles and enzymes as cargo mimics, we have demonstrated that this can be appended into a full cycle of cargo loading, encapsulation, and display, while preserving the functionality of the enclosed molecules.

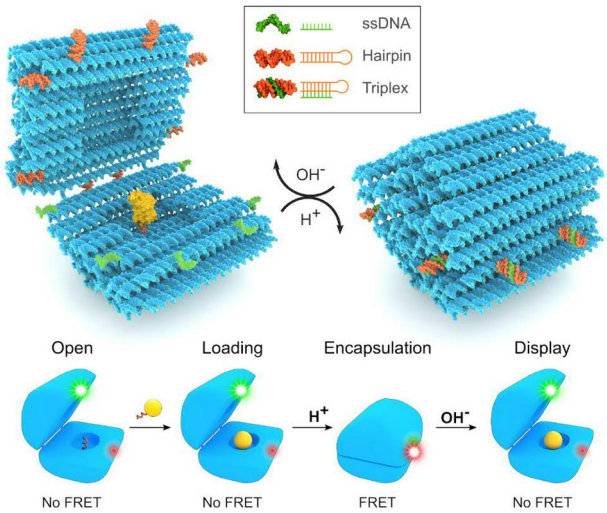

Figure 1: DNA origami nanocapsule design and function. Top panel: The hinged nanocapsule with a horseradish peroxidase (HRP) payload is equipped with eight programmable pH-responsive DNA triplexes, which hold the nanocapsule closed when pH < pKa. Bottom panel: Cargo loading cycle of the nanocapsule, Open/closed state of the structures during each step of the cycle is determined by FRET analysis of fluorescent dyes (green: energy donor, red: energy acceptor) positioned into the opposing halves.

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DNA-Encircled Lipid Bilayers

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Lipid bilayers and lipid-associated proteins play crucial roles in biology. As in vivo studies and manipulation are inherently difficult, membrane-mimetic systems are useful for the investigation of lipidic phases, lipid-protein interactions, membrane protein function and membrane structure in vitro [1]. Controlling the size and shape or introducing functional elements in a programmable way is, however, difficult to achieve with common systems based on polymers, peptides or membrane scaffolding proteins. In this work [2], we describe a route to leverage the programmability of DNA nanotechnology and create DNA-encircled bilayers (DEBs). DEBs are made of multiple copies of an alkylated oligonucleotide hybridized to a single-stranded minicircle, in which up to two alkyl chains per helical turn point to the inside of the toroidal DNA ring. When phospholipids are added, a bilayer is observed to self-assemble within the ring such that the alkyl chains of the oligonucleotides stabilize the hydrophobic rim of the bilayer to prevent formation of vesicles and support thermotropic lipid phase transitions. The DEBs are completely free of protein and can be synthesized from commercially available components using routine equipment. The diameter of DEBs can be varied in a predictable manner. The well-established toolbox from structural DNA nanotechnology, will ultimately enable the rational design of DEBs so that their size, shape or functionalization can be adapted to the specific needs of biophysical investigations of lipidic phases and the properties of membrane proteins embedded into DEB nanoparticle bilayers.

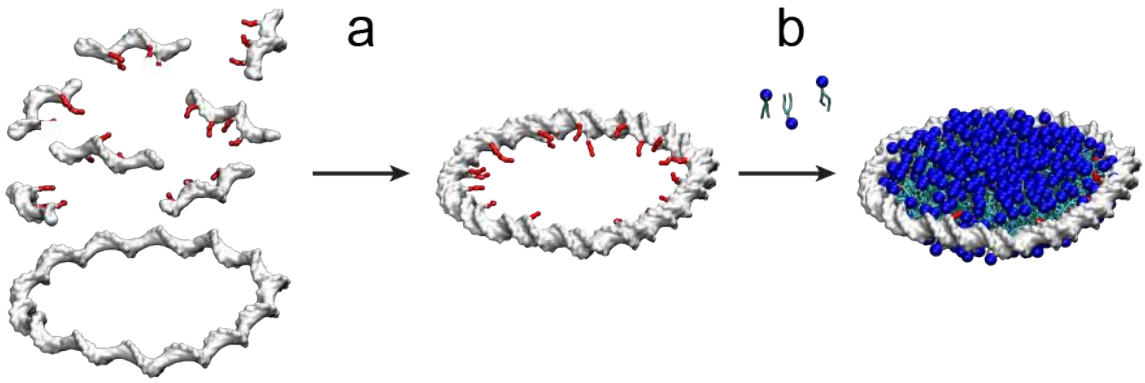

Figure 1: Assembly of the DEBs: a. ssDNA MCs (bottom) react with 7 complementary strands, each carrying 4 alkyl groups in red (top) forming a double-stranded DNA MC b. in the presence of lipids, bilayer is formed inside the ds DNA MC, resulting in DEB formation

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Rationally designed DNA-origami hosts for protein guests

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Host-guest complexes rely on non-covalent interactions between a concave host surface, displaying convergent ligands, and a convex guest surface, exposing divergent binding sites. We recently applied this principle to hold large DegP oligomers within the cavity of a DNA origami structure [1] (Figure 1a). Our approach is based on the decoration of the inner surface of the DNA host with multiple identical ligands converging to the corresponding binding sites exposed over the surface of the symmetric protein. The enhanced binding avidity of this ligands' configuration has been demonstrated for specific protein recognition motifs and amino-acid selective tweezers, leading to formation of 1:1 DNA:protein complexes even for relatively weak affinity constants. However, the apparent entropic advantage of symmetric encapsulation systems [2] is counterbalanced by the lack of control over the relative orientation of the protein cargo: a property that could be instead useful for single-molecule studies. With the aim to overcome this drawback and better understand the role of compartmentalization on the binding events, we are currently investigating the asymmetric functionalization of a modular DNA host for the directionally oriented encapsulation of a symmetric protein, the VCP/p97 segregase [3] (Figure 1b-d). We envisage that single-molecule imaging of DNA-p97 coaxial complexes will help to unravel the working principle of this protein, involved in several ubiquitin-related physiological processes.

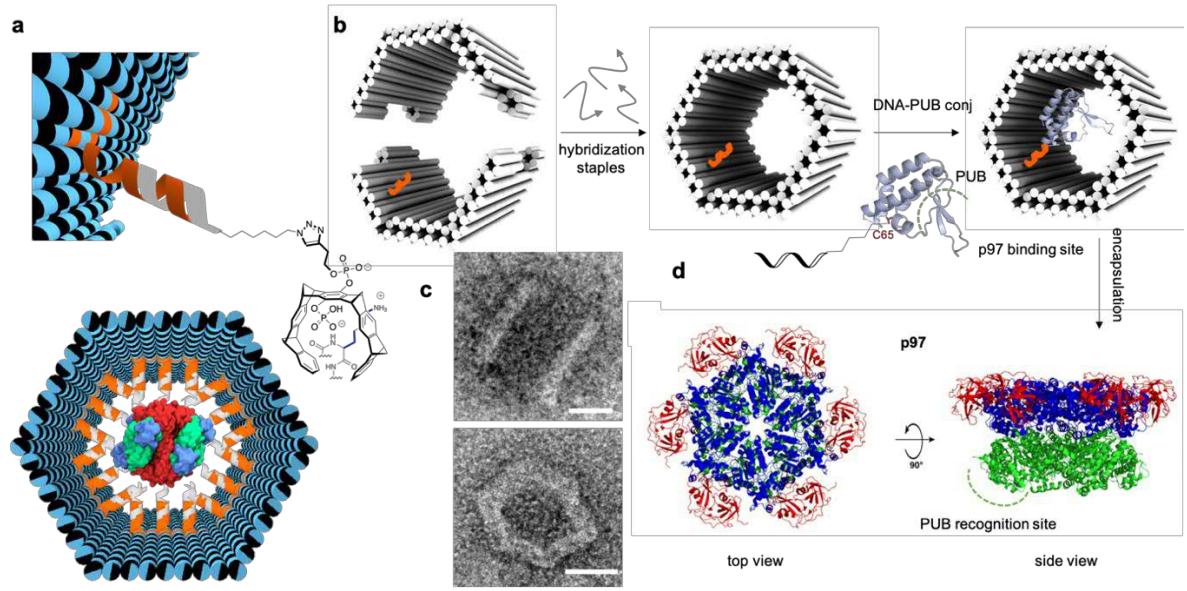

Figure 1. Symmetric and asymmetric strategies for binding proteins guests into DNA-origami hollow hosts. a) Previous studies performed on the DegP protein, using peptide ligands and Lys-selective molecular tweezers. **b**) Applying a modular design principle, hindered molecular diffusion through the host cavity can be circumvented. **c**) TEM images of fully assembled cages. Scale bars are 20 nm. **d**) Encapsulation of a PUB motif within the DNA cavity will enable recognition of p97 at its C-terminus, thus favoring the coaxial orientation of the protein along the central axis of the cage.

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Poster No. 23

Programmable Functionalization of Surfactant-Stabilized Microfluidic Droplets via DNA-Tags

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Droplet-based microfluidics has emerged as a powerful tool in synthetic biology[1,2]. For many applications, chemical functionalization of the droplets is a key process. Therefore, we developed a straight-forward and broadly applicable approach to functionalize the inner periphery of microfluidic droplets with diverse reactive groups and components. Instead of covalent modification of the droplet-stabilizing surfactants, our method relies on cholesterol-tagged DNA that self-assembles at the droplet periphery. The cholesterol-tagged DNA serves as an attachment handle for the recruitment of complementary DNA. The complementary DNA can carry diverse functional groups. We exemplify our method by demonstrating the attachment of amine groups, DNA nanostructures, microspheres, a minimal actin cortex and leukemia cells to the droplet periphery. We further show that the DNA-mediated attachment to the droplet periphery is temperature-responsive and reversible. We envision that droplet functionalization via DNA handles will help to tailor droplet interfaces for diverse applications – featuring programmable assembly, unique addressability and stimuli-responsiveness[3].

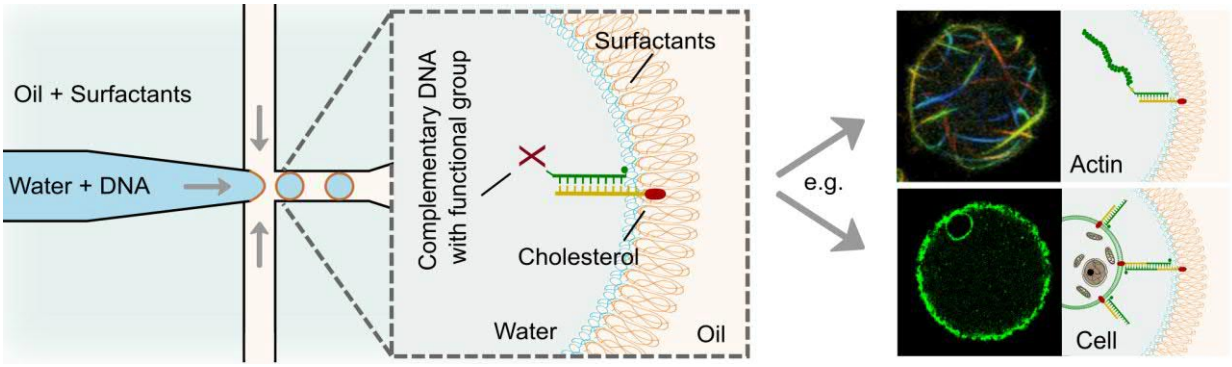

Figure 1: *Left:* Illustration of the concept for the chemical functionalization of surfactant-stabilized microfluidic droplets via DNA handles. *Right:* Confocal fluorescence images and illustrations demonstrating the attachment of an actin cortex and a living cell to the periphery of microfluidic droplets.

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Poster No. 24

Spatially Controlled DNA-Based Communication in Populations of Synthetic Protocells

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The molecules of a cell, and at a higher length scale, cells in an organism are nodes in an interwoven communication network, collectively transmitting and receiving, coding and decoding information. Using a constructive approach, synthetic biologists have started to unravel the design principles behind intercellular communication using genetically modified cells [1]. However current experimental tools are insufficient to understand the behavior comprehensively and quantitatively. Therefore, the design and construction of simple model systems featuring compartmentalized signaling is highly desirable. Here, we present a general and scalable platform to engineer distributed multichannel molecular communication between populations of non-lipid semipermeable microcapsules [2,3]. Our method uses proteinosomes [4] as synthetic protocells, short DNA oligomers as signaling molecules and the intra-protocellular signal processing logic is implemented using enzyme-free DNA strand-displacement circuitry (Figure 1). We engineer a variety of biochemical communication networks capable of cascaded amplification, bidirectional communication and distributed computational operations. The systems are characterized in microfluidic trap arrays where the positioning and density of protocells can be precisely controlled and the DNA strand-displacement reactions are monitored using confocal microscopy. We have further extended the system by incorporating light-activated DNA gates, allowing for specific triggering of individual protocells and spatially controlled reaction networks. While protocell-scale systems do not display the same levels of information-processing properties as living cells, their minimal and streamlined design could yield simple multicellular model systems with a high degree of control and the potential to uncover generalizable concepts.

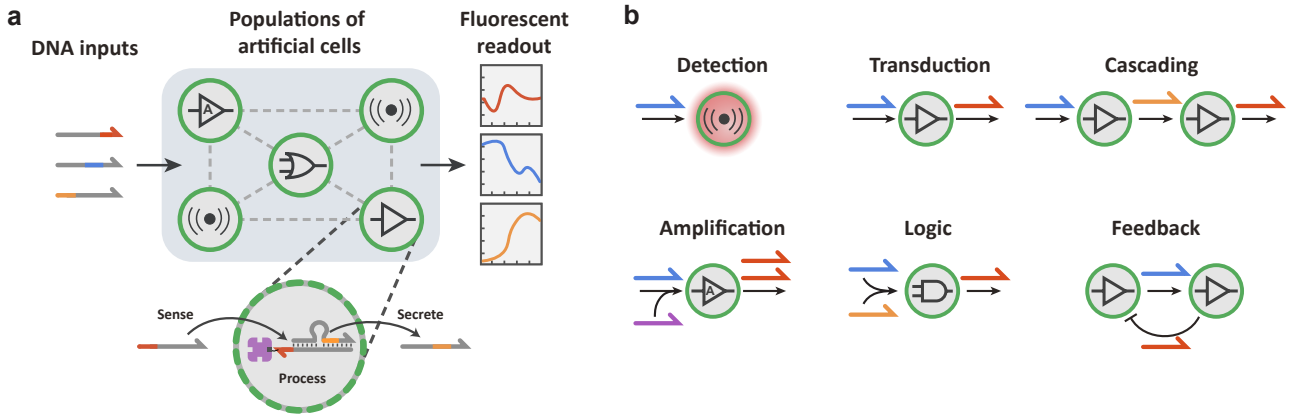

Figure 1 | Design elements for biomolecular implementation of protocellular communication. a, General strategy of the platform. Protocells with encapsulated DNA gate complexes are localized on a 2D spatial grid and can sense, process and secrete short ssDNA-based signals. The system is initiated by adding of ssDNA inputs and the response dynamics associated with the compartmentalized DSD reactions for each protocell are followed by confocal microscopy. **b**, Individual protocells can be configured to perform various tasks ranging from signal detection to Boolean logic operations. Individual modules can be combined to implement more complex population behaviors such as cascaded signaling, bidirectional communication and distributed computing.

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Poster No. 25

DNA Origami Directed 3D Nanoparticle Superlattice *via* Electrostatic Assembly

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Recent advances in the field of nanotechnology have given rise to a diverse collection of nanoscale objects with arbitrary shapes, sizes and material properties, but effective strategies for directing these objects into well-defined nanoscale arrangements are still needed [1]. Programmable and modular DNA nanostructures, such as DNA origami structures, have proven to be feasible templates for directing the higher-ordered arrangements of other compounds. Further, the DNA origami structures carry a high overall negative (surface) charge, which makes them suitable building blocks also in self-assemblies held together by electrostatic interactions.

Spatially well-ordered structures of metal nanoparticles have unique electronic, magnetic and optical properties, and hence these kinds of nanomaterials have aroused increasing interests [1]. Previously, DNA origami structures and other DNA-based motifs have successfully been used to control and direct the higher-order arrangements of metal nanoparticles [2]. However, an additional DNA functionalization of the particles is often required to link them to the DNA frames. We have recently demonstrated that highly ordered 3D metal nanoparticle superlattices could be formed also by plainly employing electrostatic interactions between the particles and the DNA nanostructures [3]. By utilizing the negatively charged DNA origami surface, we were able to assemble 6-helix bundle DNA origami and cationic gold nanoparticles (AuNPs) into well-ordered 3D tetragonal superlattices. Different combinations of DNA origami and AuNPs were studied, and the results reveal that shape and charge complementarity between the building blocks are crucial parameters for lattice formation. Our method is straightforward to use, takes place at room temperature in aqueous solution and is not limited to only AuNPs and the DNA origami shapes used in the work. Therefore, we believe that this modular approach could open up new opportunities for the construction of a variety of functional materials with high degree of translational order.

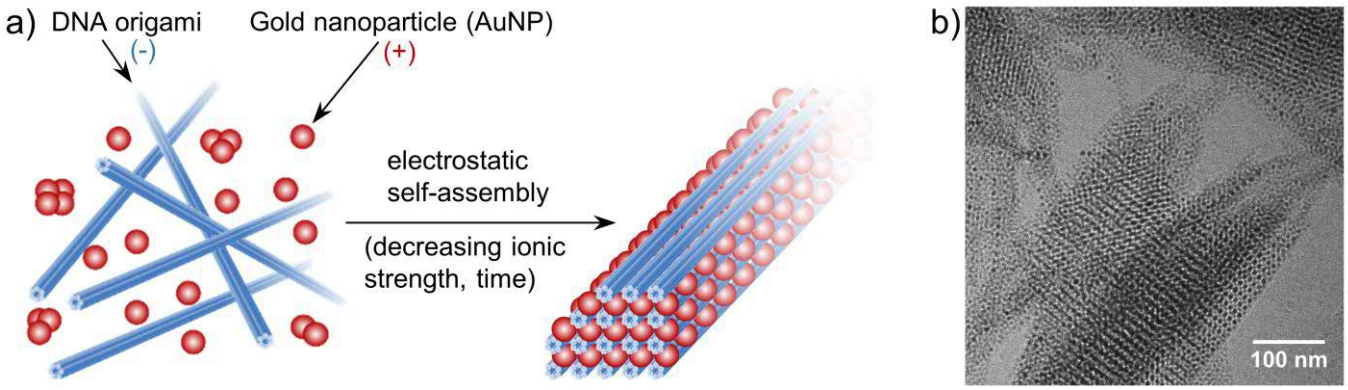

Figure 1: a) DNA origami-AuNP crystal structures are formed upon dialysis against gradually decreasing ionic strength. b) Cryo-TEM image of the formed superlattices.

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Poster No. 26

Computational Modelling of a Proximity Sensitive Assay for Superresolution Microscopy

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The complexity of cell behaviour is controlled by biomolecular interactions, incentivizing the development of strategies to image protein-protein pairs. The authors have developed Proximity Displacement PAINT (PD-PAINT) [1], an immunolabelling system where antibody-DNA conjugates reconfigure under proximity, allowing for superresolution imaging of the dimer using DNA PAINT.

To model the system numerically for *in silico* optimization of the DNA nanostructures, we explicitly extracted the configurational entropy of dimerization using Monte-Carlo molecular modelling. This estimate of the dimerization entropy can be combined with estimates of the in solution hybridization free energy of DNA nanostructures as evaluated by a Nearest-Neighbours model of DNA. This hybrid approach allows for the design space of similar DNA nanostructures to be explored which would otherwise be prohibitively computationally expensive.

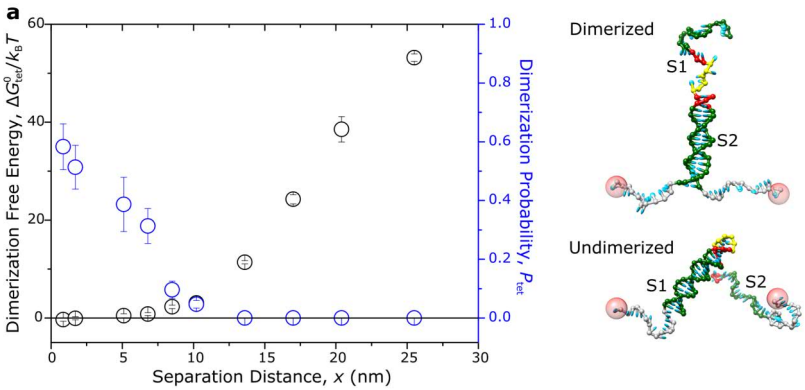

Figure 1: The free energy of probe hybridization as predicted by the oxDNA force field complemented by a Nearest-Neighbours model of DNA. As the probes approach they hybridize, until when the probes are adjacent, 60% of probes are in the open configuration.

References

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Poster No. 27

Toward making a database of design information of DNA nanostructure

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In the field of structural DNA nanotechnology, DNA double helix is used as a build block to assemble a nano-scale structure with complex geometry [1]. In the past few decades, various kind of DNA nanostructures has been demonstrated with a potential application in molecular-level robotics, smart drug delivery systems, and so on [2]. To rationally design such DNA nanostructures, a number of software has been developed such as caDNAno [3], Tiamat [4], SARSE [5], vHelix [6], and DAEDALUS [7]. Although those programs are available online, raw design information (e.g. caDNAno file) is not usually provided together with a publication. The situation causes an underlying problem that one has to pay a great effort to recycle DNA nanostructures of the others. Getting rid of the obstacle may enable us to engineer a new system on top of well-qualified DNA nanostructures.
In the field of molecular biology and synthetic biology, on the other hand, information of biomolecular structure and gene sequence is successfully stored in databases such as protein data bank (PDB) [8] and standardized gene parts library of BioBrick [9], respectively. Here, we propose a database that stores the design information of DNA nanostructures, especially targeting the caDNAno format (Fig. 1). In the current version that is developed offline, the input to the database is a caDNAno file which is converted to several useful formats such as PDB file, rendered images, and DNA sequence table. It is possible to add some information such as digital object identifier (DOI) to identify the publication about the structure. We also prepare some standard objects such as six helix bundles, rectangles, and cubes, which are listed in the database. From the web-based interface, users can access to the data for the purpose of lab experiment and/or computer simulations such as CanDo [10] and oxDNA [11]. In the presentation, we would like to discuss further requirements of the database for improving user experience. In the future, the database may serve as a catalog of DNA parts, which will accelerate the engineering process of complex DNA nanostructures.

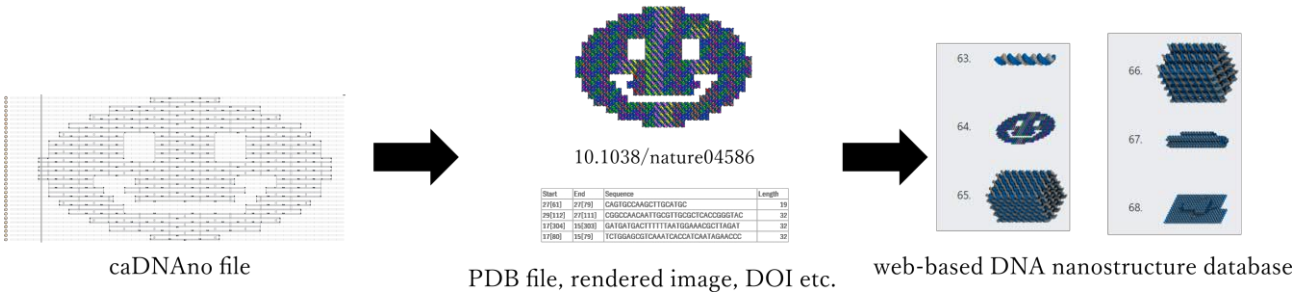

Figure 1: The concept of DNA nanostructure database. When the caDNAno file is uploaded, it is converted to several file formats, which will be listed in the database.

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Poster No. 28

Synthesis of magnetic nanoparticles using DNA molds

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A challenge for the bottom-up fabrication of nanoelectronic devices is the accurate material deposition on the nanometer scale. Concerning biomolecular materials, DNA nanotechnology meets this challenge by being highly precise at building DNA structures of nearly any desired form. We recently introduced the concept of a DNA origami mold-based particle synthesis that allows the synthesis of inorganic nanoparticles with programmable shape. Particularly, we demonstrated the fabrication of 40 nm long rod-like gold particles with quadratic cross-section [1] as uniform micrometer-long conductive gold nanowires of 20-30 nm diameter [2]. Direct conductance measurements demonstrated the metallic conductivity of these wires.
Here, we expand the mold-based fabrication method to nickel, being a ferromagnetic material. To allow a seeded-growth of nickel inside the origami molds, palladium nucleation centers needed to be introduced into the mold cavity. We therefore synthesized palladium nanoparticles and established an efficient functionalization protocol of the particles with single-stranded DNA. The functionalized particles were bound to complementary DNA strands inside the mold cavity from which a seeded nickel deposition was initiated. This provided rod-like nickel nanoparticles with an average diameter of 25 nm. The introduction of magnetic domains in the mold-based fabrication scheme provides a possible route to establish nanoscopic spin-valve structures.

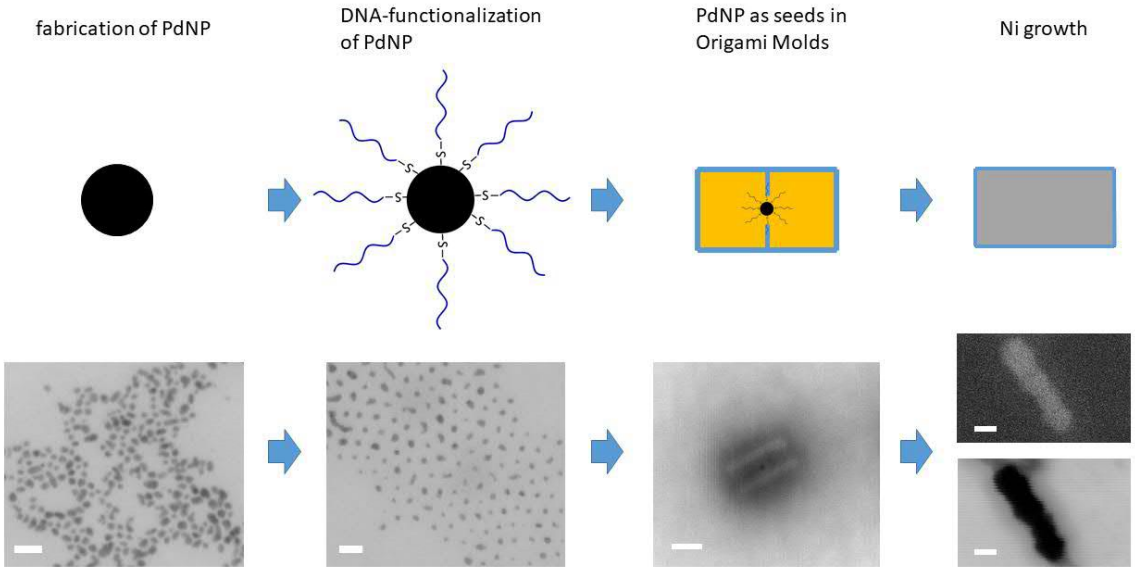

Figure 1: Scheme and tSEM images illustrating the mold-based synthesis procedure of nickel nanostructures. Palladium nanoparticles were synthesized, functionalized with single stranded DNA and placed inside DNA origami molds. These particles were used as a nucleation center for a seeded nickel growth while the origami shell confined the resulting structure. Scale bar: 20nm.

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Poster No. 29

Effects of staple age on DNA origami nanostructure assembly and stability

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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, as well as fundamental research at a single molecule level [3-5]. By hybridization of a given single stranded DNA scaffold with specially designed oligonucleotides one can achieve self-assembly of almost arbitrary structures. Furthermore, functional entities such as proteins can be arranged on a DNA origami with nanometer precision by chemical modification of selected staple strands which makes DNA origami nanostructures particularly useful as substrates for single molecule studies. Such applications critically rely on an intact DNA origami in order to obtain the desired molecular arrangements.

Thus, we here investigate the effects of staple age on the self-assembly and stability of DNA origami triangles and six-helix bundles (6HB). Our results show that the respective staple solution may be stored at -20°C for several years without significantly affecting DNA origami assembly. At the same time, however, staple age may have drastic effects on DNA origami stability under mildly denaturing conditions, as exemplified here for the washing of surface-immobilized DNA origami with water. Furthermore, these effects are found to depend on DNA origami shape and structure.

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SERS Enhancement Factors of Nanoparticle Dimers Self-Assembled on DNA Origami Scaffolds

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Surface enhanced Raman scattering (SERS) is a well-stablished analytical technique in which high intensity Raman signals originate in principle from the electromagnetic field enhancement at tight junctions or nanometer sized gaps between individual plasmonic nanostructures, such as gold or silver nanoparticles. Due to the strong plasmon resonance coupling of those nanoparticles, in this gap a so-called "hot-spot" is generated, which can be used not only for SERS studies, but also to drive chemical reactions and enable new ways of photocatalysis [1]. In this sense a precise control over the spacing of the individual nanoparticles is required to obtain the maximum SERS signal. DNA origami allows the precise control over the orthogonal organization of different entities and was already successfully applied in SERS and other plasmonic studies [2]. Herein we used nanoparticle dimers self-assembled using DNA origami nanotriangle scaffolds to determine enhancement factors (EF) of single nanostructures by correlation of AFM and SERS measurements. Obtaining the EF for a collection of randomly distributed molecules on a surface are usually easily experimentally obtained, however from a fundamental point of view and for comparison with theoretical calculations, it is important to understand how this average EF comes from the enhancement experienced by each molecule in the "hot-spot" [3]. Moreover, this EF is dependent not only on the obtained nanostructure, but also on the molecule structure and geometry, the position of the molecule, interparticle distance, etc., in this way obtaining the EF, with reliable statistics, can provide an indication of the maximum achievable EF by a determined system. Herein 80 nm gold nanoparticles modified with dyed-DNA strands were self-assembled into a dimeric structure using DNA origami nanotriangles, with separation between particles around ~3 to 4 nm. Using these assemblies, the EF can be determined with different Raman excitation wavelengths, which are correlated with the computationally calculated EF.

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Poster No. 31

Sites of high local frustration in DNA origami

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The self-assembly of a DNA origami structure, although mostly feasible, represents indeed a rather complex folding problem. Entropy-driven folding [1] and nucleation seeds formation [2] may provide possible solutions; however, until now, a unified view of the energetic factors in play is missing. By analyzing the self-assembly of origami domains with identical structure but different nucleobase composition, in function of variable design and experimental parameters, we identify the role played by sequence-dependent forces at the edges of the structure, where topological constraint is higher [3]. Our data show that the degree of mechanical stress experienced by these regions during initial folding reshapes the energy landscape profile, defining the ratio between two possible global conformations (Figure 1a and b). We here propose a dynamic model of DNA origami assembly (Figure 1c) that relies on the capability of the system to escape high structural frustration at nucleation sites, eventually resulting in the emergence of a more favorable but previously hidden state.

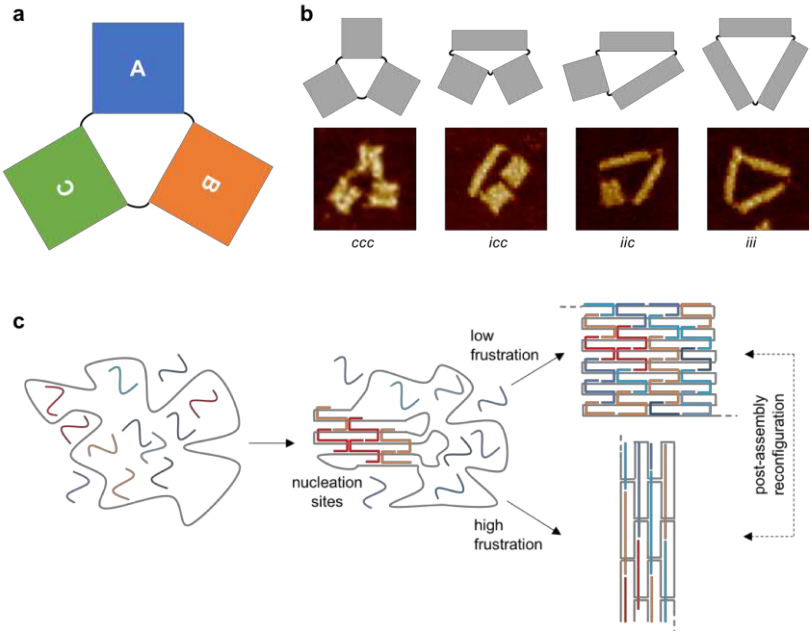

Figure 1. Topology- and sequence-dependent factors governing DNA origami folding. a) A DNA origami structure, composed of three quasi-independent domains (A, B and C) of identical shape but distinct sequence content, was observed to lead to four types of global isomers (**b**). In our proposed dynamic model of DNA origami folding, the final fate is dictated by the degree of topological stress experienced at the nucleation sites (**c**). Besides formation of the *iso* I state, favored by a low level of structural frustration, this mechanism enables to travel an alternative folding pathway leading to a typically less populated *iso* II state, which becomes competitive for structurally frustrated initiation seeds.

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Poster No. 32

A smart polymer for sequence-selective binding, pulldown and release of DNA targets

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Selective isolation of DNA is crucial for applications in biology, bionanotechnology, clinical diagnostics and forensics. Despite the diverse variety of approaches, current methods have critical shortcomings that require trade-offs between material cost, ease of use, versatility and performance. To address this challenge, we have developed a smart methanol-responsive polymer (MeRPy) that can be programmed to bind and separate single- as well as double-stranded DNA targets [1]. MeRPy's development was inspired by SNAPCAR, a recently reported method for scalable production of kilobase-long single-stranded DNA [2]. MeRPy acts as a ready-to-use macroligand for affinity precipitation. Captured targets are quickly isolated and released back into solution by denaturation (sequence-agnostic) or toehold-mediated strand displacement (sequence-selective). The latter mode allows 99.8% efficient removal of unwanted sequences and 79% recovery of highly pure target sequences. We applied MeRPy for depletion of insulin cDNA from clinical next-generation sequencing (NGS) libraries. This step improved data quality for low-abundance transcripts in expression profiles of pancreatic tissues. Its low cost, scalability, high stability and ease of use make MeRPy suitable for diverse applications in research and clinical laboratories, including enhancement of NGS libraries, extraction of DNA from biological samples, preparative-scale DNA isolations [3], and sorting of DNA-labeled non-nucleic acid targets.

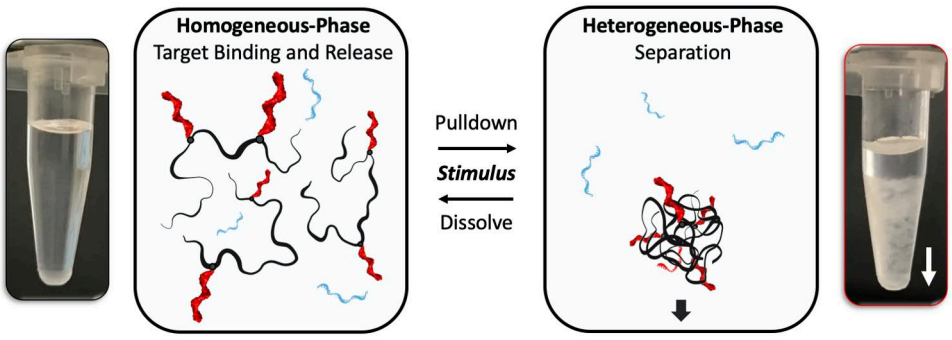

Figure 1: Methanol-responsive switching between homogeneous and heterogeneous phase enables MeRPy to rapidly bind, isolate and release DNA targets.

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DNA-Assembled Plasmonic Antennas

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Abstract
Artificially designed DNA structures can be used for the site-specific arrangement of functional heteroelements into optical or electronic nanodevices, e.g. for optical data processing and signal transmission. Here, we present the bottom-up assembly of optical antennas by arranging plasmonic nanostructures operating at optical frequencies with the help of DNA origami templates. As templates, we have used rectangular, two-dimensional origami structures with basic dimension of 40 nm x 150 nm. They specifically form dimers, so that DNA templates with sizes of either 40 nm x 300 nm or 80 nm x 150 nm were obtained. The incorporation of specific binding sites enables the local positioning of functional elements. For the construction of plasmonic active nanoantennas, complementary functionalized gold nanoparticles of different shape (spheres, rods, triangles), size (e.g. aspect ratio of the rods) and material (Au/Ag) were used. Depending on the design of the template structures and the size of the immobilized nanoelements, different designs of nanoantennas of about 100 nm up to 300 nm size have been synthesized.
We present results of the structural and optical characterization of these antenna structures. This includes bulk absorption measurements by UV-vis spectroscopy as well as characterization of individual antenna structures by low-loss electron energy loss spectroscopy (EELS) using monochromated scanning transmission electron microscopy (STEM) to gain insight into near-field coupling effects and the localization of bonding and antibonding plasmonic modes. Additional FEM simulations show that these experimental results are in good agreement with theory.

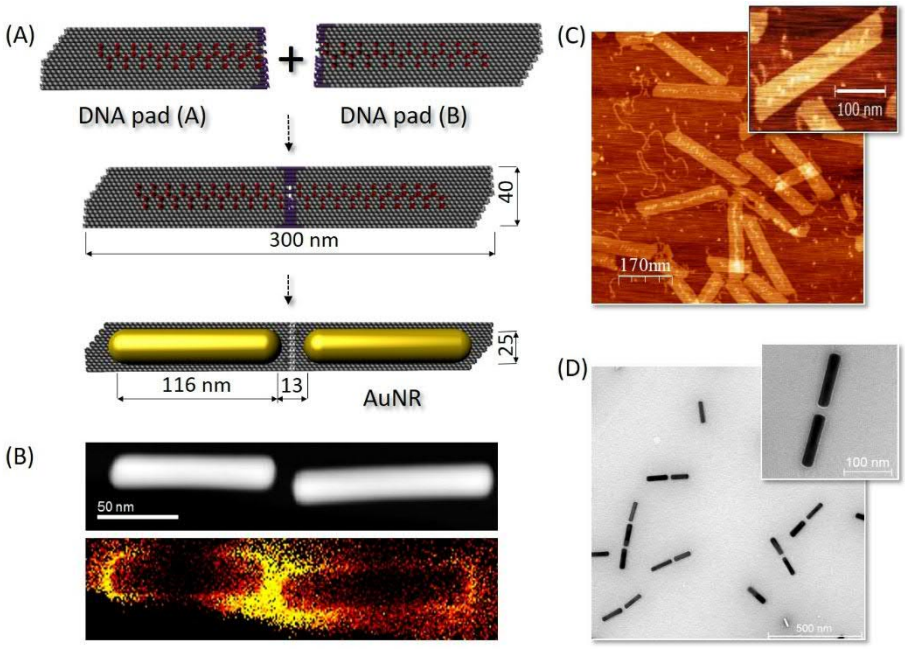

Figure 1: (A) Schematic illustration of the assembly of DNA based plasmonic nanoantennas; Characterization with (B) STEM and EELS, (C) AFM, (D) TEM

Poster No. 34

DNA Hybridization Chain Reaction (HCR) on Silica Nanoparticle Surface

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As one of the non-enzymatic amplification techniques, DNA hybridization chain reaction (HCR) has found wild application in the detection of various targets, like RNA and proteins.[1] Tradition HCR rate in solution dependents largely on the diffusion of DNA monomers hence the reaction time is greatly prolonged due to the totally random Brownian motion of molecules in solution. It was reported that confined DNA HCR monomer on a DNA template could greatly enhance the reaction rate and detection sensitivity.[2] In this project, DNA HCR monomers are going to be attached on silica nanoparticles. Mathematically speaking, a much bigger number of DNA chains could be confined and the HCR could not be blocked until all monomers react. Hence, we anticipate a rather fast and sensitive silica nanoparticle-based detection system.

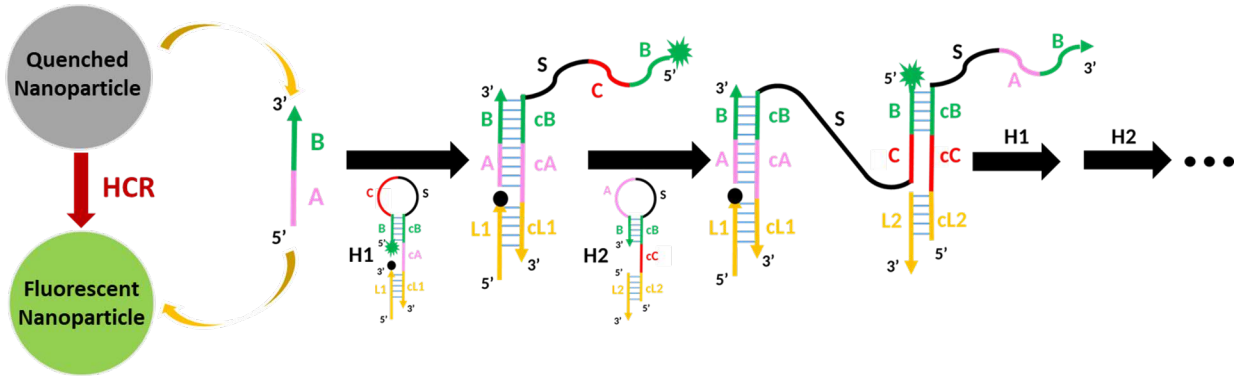

Figure 1: Schematic representation of HCR on Silica Nanoparticle.

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Poster No. 35

Interactive Visual Analysis for the Design of DNA Nanostructures

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DNA nanostructures are getting increasingly complex and their features of interest are often occluded by structural details. Although the caDNAno tool [1] provides a fast prototyping 2D interface for DNA origami structures [2], the modeling of arbitrary 3D shapes remains a challenge. In order to facilitate a flexible 3D CAD design of DNA nanostructures, an effective visualization pipeline is fundamental. Here, we present a system for the visual analysis of DNA nanostructures. Our interactive approach abstracts a DNA structure into a multitude of representations, enhancing the in silico design process. A continuous multi-scale approach depicts the structural details, from low-level atomic representation, to high-level nucleotide and single strand representations [3]. For DNA origami structures, we combine three-dimensional shape models, two-dimensional diagrammatic representations and one-dimensional linear alignments of the DNA sequences [4]. We developed transisted animations to smoothly transform between different scales and dimensions. Our approach provides the user with specialized visualizations to highlight the relevant structural details. Indications for structural stability, such as GC-content, melting temperature and crossovers can be visualized with our approach.

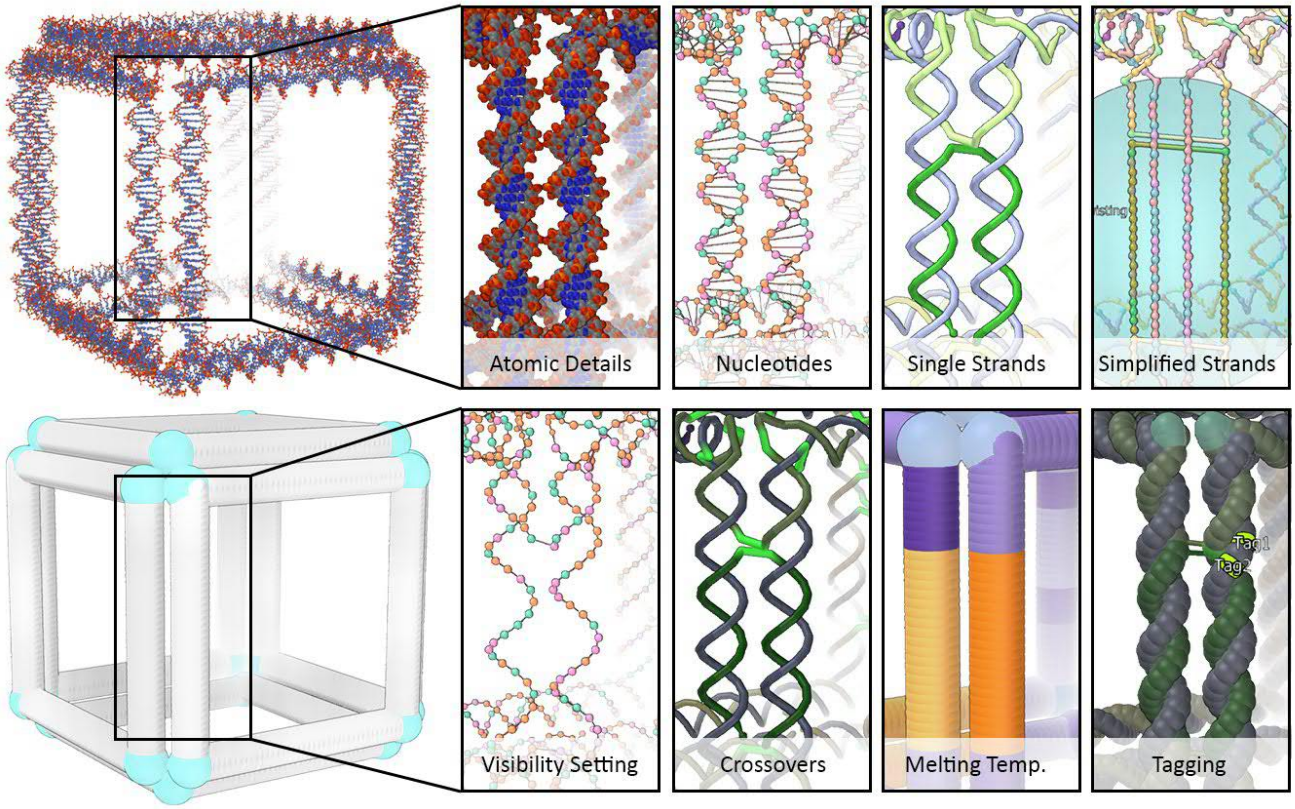

Figure 1: A multitude of interactive visualizations that enhances the design of DNA nanostructures.

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Poster No. 36

Automated Design of Scaffold-free Wireframe DNA Nanostructures

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DNA self-assembly, a process whereby a mixture of designed DNA strands self-assemble into custom nanoscale constructs, has emerged as a promising route for bottom-up synthesis of artificial nanostructures with potential applications in areas such as nanomedicine, biophysics and plasmonics. Recently, wireframe architectures offering a materially efficient scheme for designing physiologically stable DNA nanostructures have gained significant attention, especially due to new automated approaches [2, 3] for folding long, circular DNA origami scaffold strands into intricate polygonal mesh wireframes. However, scaffolded origami designs are inherently limited by the lengths of available scaffold strands and the topological complexity of routing such circular strands into more complicated target geometries.

Taking the limitations of scaffolded approaches into account, Wen et al. [1] introduced a general scaffold-free method for designing DNA wireframe nanostructures from short synthetic strands. In this work, we automate the design scheme of Wei et al. for meshes with an underlying space of an orientable topological surface. Such polygon meshes permit an antiparallel orientation of the polygon's bounding cycles, which in turn allows for a single-duplex renderings of the edges from the antiparallel double tracing performed by the cycles; see Figure 1. To convert the initial cyclic strands to shorter linear ones, the cyclic strands are nicked in every edge. We implemented the method using vhelix [2] as a backend, thus paving a route towards an integrated environment for designing scaffolded and scaffold-free 2D and 3D wireframe DNA nanostructures.

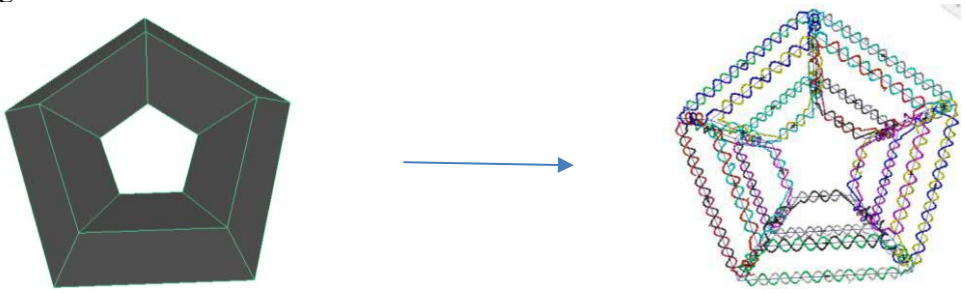

Figure 1: A pentagonal torus 3D model (left) rendered to a scaffold-free wireframe DNA (right).

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DNA Encapsulation of Particles

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Viral infections are one of medicine's biggest challenges, due to the rapid mutation of viruses, lack of targeting/inhibition mechanisms, and development of drug-resistance. This causes a huge social and economic burden worldwide. As an for example, Influenza virus infections result in 300,000 to 600,000 annual deaths and between 3 to 5 million cases of severe illness[1][2]. Since the traditional vaccines have become an insufficient way to combat viral infections, there is a demand for new methods that could target multiple viruses, are flexible, cheap and easy to produce.

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[3]. The specific base-pairing nature of DNA allows for rational design of self-assembled highly specific nano-structures[4]. One of the applications that could benefit from these properties is viral infection inhibition, by creating drug delivery structures, multivalent pathway for inhibitors or mechanical inhibition.

Aiming for mechanical inhibition of viral infections, a DNA structure could be formed that self-assembles in the presence of the virus or in a general particle. In this context, two techniques are considered. The first would employ a star-shaped DNA building unit, that self-assembles into a polyhedral that forms a shell around the particle[5]. The second technique uses three Y-shaped DNA structures connected together via a 4 arm DNA connector. Multiples of this hybrid structure would attach to the particle and the shell formation around the particle could be triggered by linking these structures via external stimuli like UV light [6]. Both techniques have the advantage of being tunable to target different particles.

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Poster No. 38

Nucleic acid nanoscaffold for targeted delivery of proteins across the blood brain barrier

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Recent advancements in the production of biologics including monoclonal antibodies (mAbs), recombinant enzymes, and nucleic acid based drugs have shown great promise for treatment of various disorders in the central nervous system. However, the delivery of biopharmaceuticals across the blood brain barrier (BBB) to active sites in the CNS remains one of the grand challenges in modern medicine. The presence of tight junctions, efflux transport systems, catabolic enzymes, and low levels of cytotoxic uptake at the BBB restricts more than 95% of all drug candidates from reaching targets in the brain. Passive diffusion through the capillary endothelium is restricted to highly lipophilic small molecules with molecular weights below 600 Da. One known strategy to overcome this limitation for large macromolecules is to exploit receptor-mediated transport (RMT) systems at the BBB which shuttle nutrients to the brain. To date this strategy has resulted in only 1-2% translocation efficiency in CNS delivery due to competition with endogenous ligands or high affinity mAbs remaining trapped in endothelial cells. In this project, a modular nucleic acid based nanoscaffold was employed to systematically test combinations of ligands targeting the BBB. A small library of targeting peptides capable of engaging RMT systems and adsorptive-mediated transcytosis were investigated to test if multiple weak interactions between targeting agents were capable of increasing uptake in brain capillary endothelial cells whilst hindering entrapment of the constructs. Efficient uptake in brain capillary endothelial cells was demonstrated in vitro for both multi-valent assemblies and multi-specific combinations of peptide targeting ligands. In a pilot study, increased uptake of assemblies containing multiple copies of a single ligand was observed using a primary cell co-culture model of the BBB. Future experiments will investigate intracellular trafficking and translocation efficiency of ligand combinations, as well as their capacity to ferry peptide and protein therapeutics. The most promising ligand combinations will subsequently be investigated in vivo for targeted delivery of therapeutic peptide drugs to the brain.

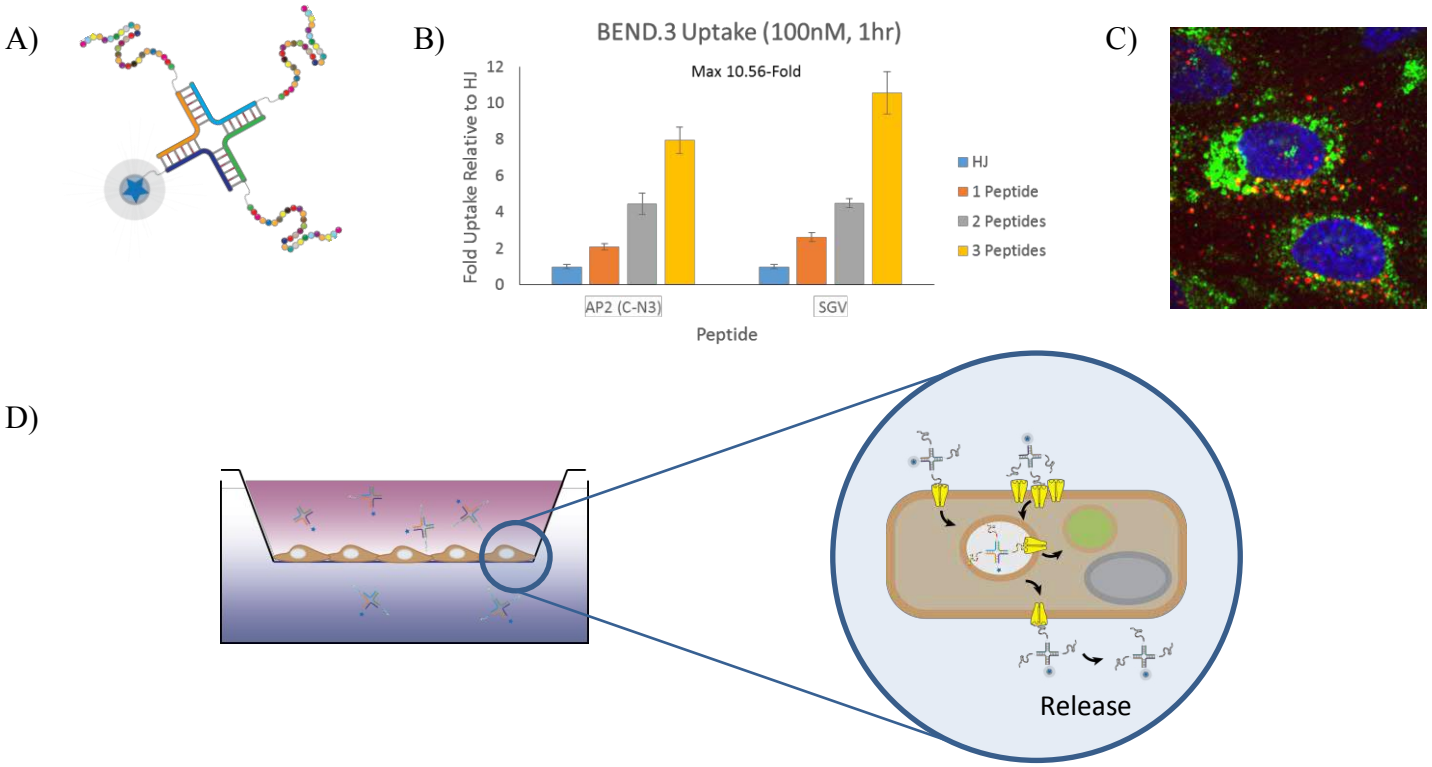

Figure 1: A) Multivalent Nanoscaffold containing 3 peptides and a fluorophore. B) Flow cytometry data of targeted uptake in brain capillary endothelial cells. C) Confocal microscopy image of internalized bivalent peptide targeted nanoscaffold (red). D) Schematic of *in vitro* intracellular trafficking assay.

Poster No. 39

Growth of Monodisperse DNA Origami-Silica Hybrid Nanostructures

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DNA origami technique has found attractions in numerous fields of fundamental and applied research, e.g., biosensors [1], bimolecular interactions [2], and drug delivery [3]. The technique provides a powerful method for fabrication of nanostructures of arbitrary shape with nanoscale precision. Furthermore, DNA structures can be used as nanotemplates for assembly of other materials. Silica-DNA composite is an attractive material, which provides highly chemical stability, biocompatibility and can be easily modified for advanced biomedical applications. However, the current silica biomineralization approaches maintain several limitations to control the silica shell growth on DNA origami, e.g., an incomplete silica coating due to the substrate-based reactions [4], and significant agglomeration and structural deformation of origami after silica coating [5]. Herein, we developed a novel method, which allows to control the growth of silica layers on DNA origami in solution. The roles of critical factors, i.e. type of coupling agents, environmental conditions, ratio of precursors, concentrations of Mg^{2+} ion and DNA origami, were determined via its influences on silica insulation process. Monodisperse DNA origami@SiO_2 nanostructures were achieved with uniform coating and ultrathin silica shells.

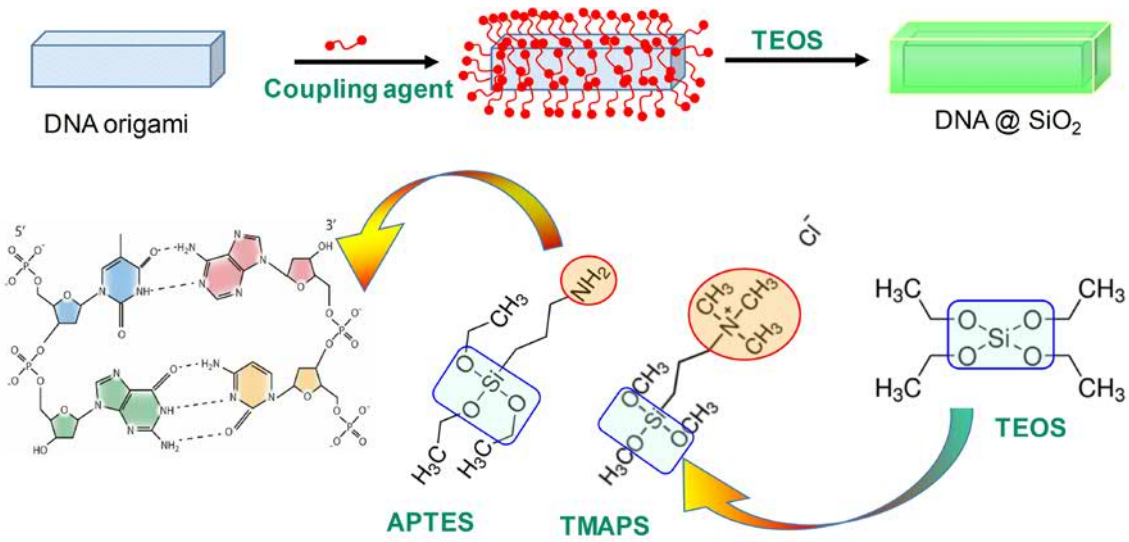

Figure 1: Schematic illustrations of the silica encapsulation of DNA origami. The reaction process of DNA origami with APTES, TMAPS and TEOS.

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Poster No. 40

Computer Controlled DNA Walker that Perform Several Steps a Minute

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Synthetic molecular machines are many orders of magnitude slower and less proccessive then biological molecular machines. Responding to this challenge, a major effort in our group is to develop fast and proccessive DNA based molecular machines. In recent years we have demonstrated a DNA bipedal motor that strides on a DNA origami track and operates by responding to 'fuel' and 'antifuel' DNA strands [1-4]. The strands are provided to the motor by a computer-controlled microfluidics device and motor progress is monitored by single molecule FRET. With this setup, we demonstrate the performance of 32 walking steps (64 consecutive chemical reactions with ~98.9% yield per reaction) which amount to 370 nanometers traveled by the walker [3].

However, initial motor designs were very slow; about an hour per step, and increased fuel concentration, necessary for faster walking, resulted in decreased processivity (reactions yields). Redesigning of the walking mechanisms, such that higher fuel concentration does not decrease the yield, resulted in two orders of magnitude increase in motor speed for the same yield, and three orders of magnitude (~10 s/step) for somewhat lower yields [5]. Altogether, we improved the motor performance (the product of speed and processivity) by about four orders of magnitude over eight years effort.

To further increase the motor performance, we are currently investigating various possible unwanted 'leakage' reactions. These includes unwanted strand replacement in the absence of toehold, influence of truncated strands (e.g., fuel strands) and nonspecific interaction of the walker system with the origami structure.

Our method enables fast and full control of the walker direction and speed and enables non-interrupting removal of excess reactants (as in solid-phase synthesis approach) which is necessary for proper operation of the motor. The method allows in situ monitoring of the motors state during operation which enables investigation of the motor dynamics and operation mechanisms. To the best of our knowledge, this is the first demonstration of computer controlled molecular machine and first demonstration of single molecule FRET analysis of the dynamics of biomolecules that are immobilized inside a microfluidics device.

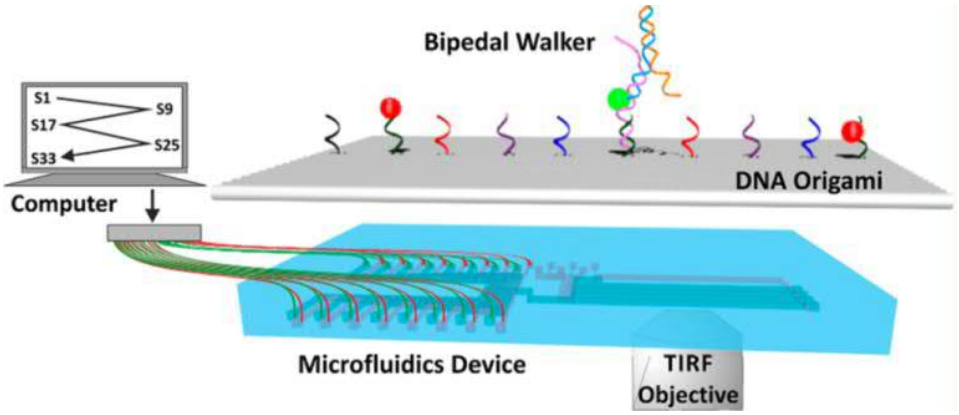

Figure 1: A schematic of computer-controlled microfluidics device positioned on a single-molecule fluorescence optical setup and of the bipedal walker striding on origami track.

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Poster No. 41

Towards Single-Molecule Voltage Imaging with DNA Origami

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Understanding the functioning of the brain has been in focus of research for decades. Since neuronal signals are transmitted through the cell via electrical signals, measuring the change in the electrical membrane potentials is a key aspect of neuronal research. Membrane potentials can be monitored with voltage-sensitive probes [1-3]. Commonly used voltage dyes often suffer from low photostability and low brightness making a single-molecule fluorescence read-out impossible. Further, many probes are invasive or need a transfected cell line [4] limiting the pool of addressable cells by nature.
Here, we present a DNA origami-based [5] alternative to common voltage sensors. By cholesterols bound to a DNA origami, the nanostructure is anchored onto a lipid bilayer. A voltage-sensitive unit on the DNA origami labelled with a dye changes its conformation due to the current electrical potential. Together with a second dye located on the nanostructure, Förster resonance energy transfer (FRET) can occur after excitation of the fluorophores. The energy transfer depends on the inter-dye distance and hence, correlates with different membrane potentials. As a voltage-sensitive unit a hydrophobic spacer is labelled with a charged dye which inserts into the lipid bilayer and alters its position depending on the potential. As this method is less invasive as well as transfection free and all components of this nano-device can be chosen modularly, it might me a promising tool to get insights into the complexity of the brain.

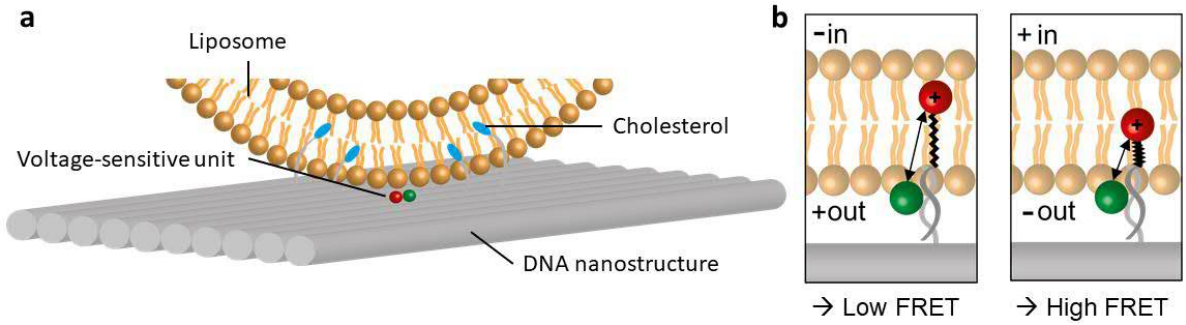

Figure 1: DNA nanostructure for voltage-sensing. a: A rectangular DNA origami (grey) is equipped with cholesterol strands (blue) to bind a liposome (orange). A voltage-sensitive unit is placed in the center of the nanostructure. b: A voltage-sensitive unit labelled with a charged dye inserts into the lipid membrane. With a change in the membrane potential the sensing unit changes its conformation and hence, a different FRET value can be read out.

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Poster No. 42

Designing switchable Cas12a guide RNAs

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Nucleic acid strand displacement is one of the most widely used processes in molecular computing owing to its well-understood nature and designability [1]. However, applications are limited by the availability of suitable interfaces to other functional systems. CRISPR-associated proteins are controlled by nucleic acid inputs and have proven to be powerful tools in genome editing, transcriptional regulation, and molecular diagnostics. Cas12a, specifically, has two features that differentiate it from the more commonly used Cas9: (i) It processes its own gRNA and (ii) has an unspecific ssDNase activity that can be used for sensitive DNA detection [2, 3].

Here, we use strand displacement to control the activity of Cas12a by designing extended guide RNAs (gRNAs) that are switchable via RNA strand displacement, called "strand displacement gRNAs" (SD gRNAs). By occluding the handle of the gRNA with a "switch" domain, we suppress binding of Cas12a (**Fig. 1A**). A single-stranded RNA "trigger" can displace this switch domain via a toehold, restoring the handle structure and promoting Cas12a binding. Cas12a then processes the SD gRNA, restoring a regular-length gRNA with full activity.

Using NUPACK's constrained multistate sequence design feature [4], this concept can be extended to orthogonal activation by different triggers (**Fig. 1B**), multi-input SD gRNAs similar to the work by Green *et al.* [5] (**Fig. 1C**), and sensing of natural RNA sequences such as mRNAs. It can also be adapted to *in vivo* transcriptional regulation using dCas12a, wherein the use of dCas12a's gRNA processing activity leads to repression efficiencies equivalent to regular gRNAs (**Fig. 1D**) [6].

Combining strand displacement with the abilities of Cas12a enables a range of new applications in sensing and synthetic biology, but also introduces a number of interesting design challenges that are uncommon for traditional strand displacement systems.

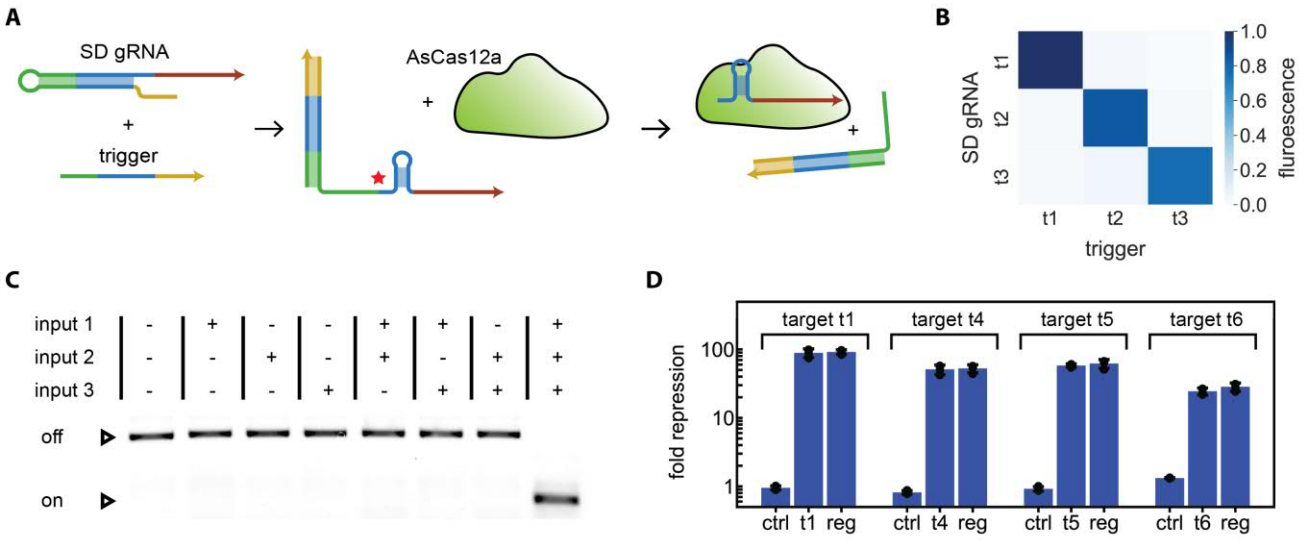

Figure 1: **A** The principle of strand displacement guide RNAs. The red star indicates the position at which Cas12a cleaves the SD gRNA. **B** Orthogonal activation of SD gRNAs by different trigger RNAs as measured by Cas12a's ssDNase activity. **C** A three-input SD gRNA AND gate. **D** Using SD gRNAs for transcriptional repression of mVenus for four different target sequences in *E. coli*. (ctrl: control trigger, t1-t6: cognate trigger, reg: regular gRNA)

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Poster No. 43

DNA Cubes with Membrane Protein-Mimicking Functions

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Cells use membrane proteins as gatekeepers to transport ions and molecules. DNA nanostructures with lipid anchors are promising as membrane protein mimics because of their highly tuneable assembly. Yet, the structural factors that determine their membrane function are still poorly understood. Here, we show that altering the pattern of cholesterol units on a cubic DNA scaffold dramatically changes its mode of interaction with lipid membranes. This results in simple design rules that allow a single DNA nanostructure to reproduce distinct membrane protein functions such peripheral anchoring and nanopore behaviour.

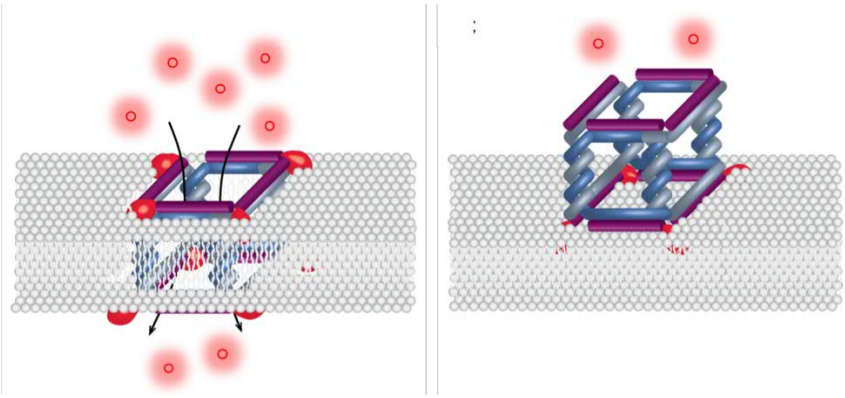

Figure 1: DNA cubes with cholesterol units on two opposing faces (left) cause fluorophore influx and likely span the membrane. DNA cubes with cholesterol units on only one face (right) do not show significant influx and are assumed to float on the membrane.

Strikingly, the DNA-cholesterol cubes constitute the first open-walled DNA nanopores, as only a quarter of their wall is made of DNA. This functional diversity can increase our fundamental understanding of membrane phenomena, and results in sensing, drug delivery and cell manipulation tools.

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Poster No. 44

A synthetic DNA-built enzyme flips 10^7 lipids per second in biological membranes

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DNA nanotechnology facilitates the rational design of nanodevices which can controllably interact with biological membranes via hydrophobic modifications and mimic the function of natural membrane proteins [1,2]. Here, we design a synthetic enzyme built from DNA that spontaneously inserts into lipid membranes by forming a toroidal pore [3]. This DNA-induced lipid pore connects the membrane's inner and outer leaflets and catalyzes spontaneous transport of lipid molecules between the bilayer leaflets, rapidly equilibrating the membrane's lipid composition (see Fig. 1). Through a combination of atomistic simulations and fluorescence microscopy we find the lipid transport rate catalyzed by the DNA nanostructure exceeds 10^7 molecules per second, orders of magnitude higher than the rate of lipid transport catalyzed by biological enzymes called lipid scramblases. We furthermore establish an easy-to-implement strategy to alleviate hydrophobically mediated aggregation, a common problem of DNA nanostructures designed to tether to lipid membranes. Reducing uncontrolled structural aggregation and the ability to alter a cell's membrane composition open new avenues for applications of membrane-interacting DNA systems in biophysical research and medicine.

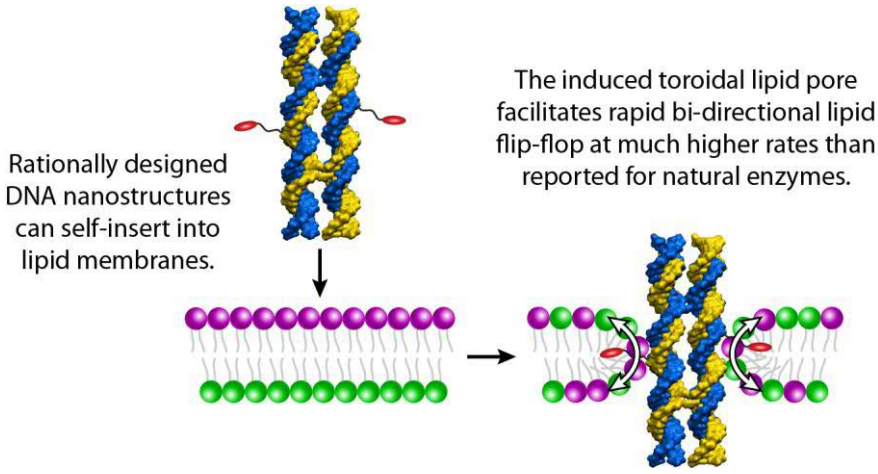

Figure 1: Schematic representation of DNA nanostructure membrane insertion and toroidal pore formation which equilibrates the membrane's lipid composition via rapid bi-directional lipid transfer.

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Poster No. 45

Interfacing cells with designer nucleic acid nanostructures to control cell uptake through multivalent interactions

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Cancer is one of the leading causes of mortality worldwide and the available treatment of this type of disease is often accompanied by adverse side effects and low efficacy. To circumvent this, much attention has been devoted to the discovery of molecular surface biomarkers characteristic of cancer cells. Many ligands have been developed for the targeted delivery of cytotoxic drugs and imaging agents within cancer therapy and diagnostics; including small molecules, peptides, monoclonal antibodies, and aptamers. A targeted drug delivery system relies on the binding specificity and affinity of the targeting moiety towards its cognate receptor. As we know, nature provides many examples where multivalent interactions between a receptor and its ligand enhances the overall binding affinity. Whereas affinity describes the strength of a single interaction between a receptor and its ligand, avidity refers to the accumulated strength of multiple binding interactions between a multivalent ligand and its receptors. Thus, the potential of many targeting moieties can be maximized by increasing the valency to improve the overall system-target interaction.

In this work, a modular, nucleic acid-based platform referred to as the holliday junction (HJ) has been used for the study of multivalent targeting moieties of different classes; such as small molecule, peptide, and aptamer. Using copper-free click chemistry, three of the HJ arms were conjugated with each ligand while the last arm carried a fluorophore to allow detection in different cell lines. The targeting efficiency of HJs bearing one, two, or three targeting ligands were evaluated by flow cytometry. Performing super-resolution microscopy using DNA-PAINT the mechanisms of nanostructure-target interactions will be studied for each ligand copy introduced using the HJ platform. A better understanding of the ligand-target interactions will aid in developing design principles for targeted drug delivery systems.

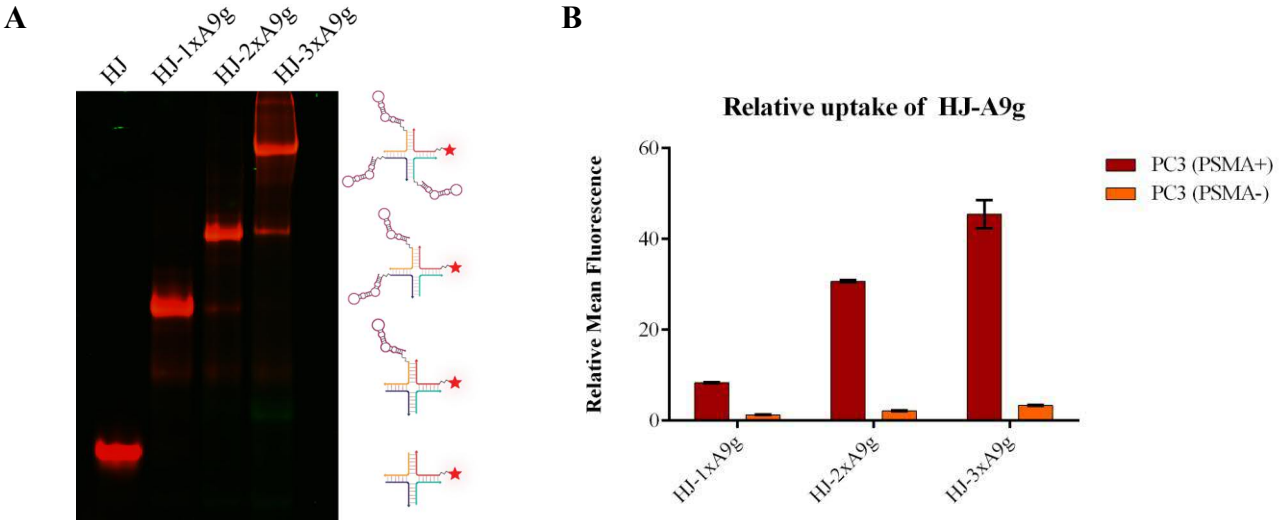

Figure 1: A) Native PAGE gel showing the assembly of HJ, and HJ bearing one, two, and three aptamers. B) Flow cytometry data of HJs with 1, 2, and 3 aptamers in prostate specific-membrane antigen positive and negative cell lines PC3.

Poster No. 46

Hierarchical assembly of biomimetic complexes

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Since the advent of structural DNA nanotechnology, scientists strive to realize artificial structures with growing complexity and functionality. DNA origami is a method of choice for the bottom-up assembly of biomimetic systems, as it allows the precise and programmable hierarchical self-assembly at the nanoscale, coupled with sub-nanometer spatial resolution [1]. In this work, the hierarchical self-assembly of different macromolecular structures is guided by programmable DNA interactions and used to construct homo- and heterooligomeric architectures of increasing complexity (Figure 1). The emulation of protein filaments is performed by designing and creating a highly modular DNA origami building block, which can undergo dimerization and multimerization reactions, depending on the addressed interfaces, eventually resulting in more than 15 filamentous structures with distinct ultrastructures and global elastic properties (Figure 1a-e). The synthetic DNA filaments were analyzed by single-particle techniques, showing the successful realization of artificial, biomimetic structures with persistence lengths similar to – or even larger than – those of natural protein filaments [2]. We are currently investigating the hierarchical assembly of a multi-compartment system starting from two types of building blocks. The structures are validated by single-particle and ensemble methods (Figure 1f-g).

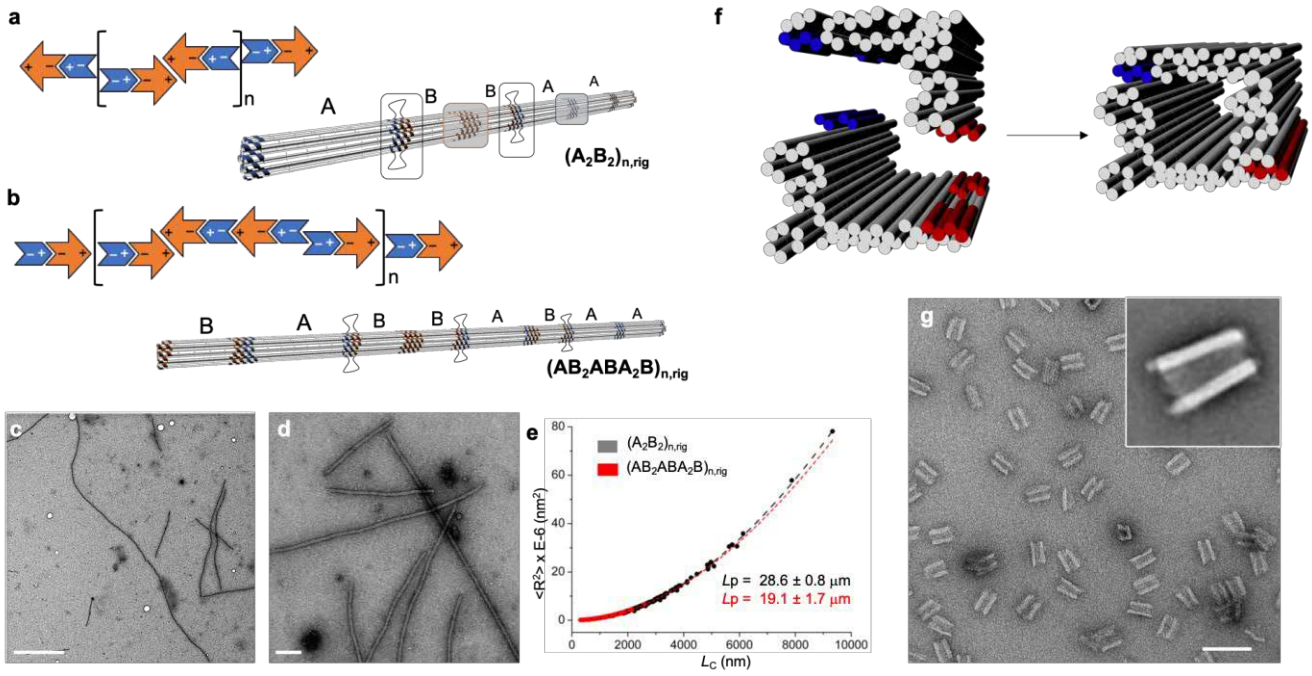

Figure 1. Hierarchical self-assembly of DNA-filaments and DNA-compartments. DNA origami filaments with programmable pattern are grown through hierarchical assembly of a unique building block (**a** and **b**). Representative EM images of $(A_2B_2)_n$ (**c**) and $(AB_2ABA_2B)_n$ (**d**) filaments obtained by a combination of base hybridization and base stacking interactions at selected AB interfaces. Scale bars are 1 µm. (**e**) Application of the worm-like chain model to the trajectories of the DNA filaments observed on the EM grid surfaces allowed to estimate their persistence length. (**f**) Hierarchical assembly of a square cross-sectional nanochamber built from two shape-complementary halves and (**g**) a representative EM micrograph of a purified sample. Scale bar is 100 nm.

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Poster No. 47

Lithographical nanopatterning with viruses and DNA

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Some nanoscale biological molecules such as DNA and viruses [1] are capable of forming programmable, self-assembling nanoparticles with excellent spatial accuracy and consistency. It has long been a goal of the DNA-based nanotechnology field to exploit those qualities in the making of equally accurate and controllable nanostructures from other materials [2-4]. Especially DNA origami [5], self-assembling fully tailored nanoscale macromolecules of DNA, have proven promising for this endeavor. Usually the origami have either been used as molds to be filled out with metal [6] or then the DNA has been directly metallized [7] to yield nanoparticles that mimic the DNA origami's shape. However, recently it became possible to use DNA origami in the manufacturing of metallic nanostructures by utilizing lithographical techniques in a process called DNA-assisted lithography (DALI). In DALI, DNA origami are used in the creation of a negative-pattern lithographical mask, which can subsequently be used to transfer the shape of the origami onto a substrate material as metallic nanostructures. After pattern transfer, the mask layers are removed with hydrofluoric acid (HF) etching, leaving only the metallic nanostructures on the substrate. [8, 9] The method has been proven capable of creating nanostructures with plasmonic functionality, such as surface-enhanced Raman spectroscopy (SERS) and plasmonic chiral dichroism (CD) [9].

A modification to the original DALI protocol is attempted here by adding a sacrificial layer of polymethylmethacrylate (PMMA) onto the substrate as the first step. As a result, the PMMA and all layers above it can be removed by using acetone, bypassing the need for more aggressive lift-off procedures such as HF etching. This has the potential to significantly expand the utility of DALI, as it will allow the process to be performed on substrates that are more common: for example silica-based glass instead of sapphire. It would also allow previously sensitive materials (Ag, Ti and Cu) to be used for the resulting nanostructures. Furthermore, patterning with other biological molecules than just DNA is demonstrated. A negative-tone mask patterned with Tobacco Mosaic Viruses (TMV) is grown using the same mechanism as with DNA, showing that DALI can also be easily expanded to patterning of virus particles, yielding possible applications in microfabrication and surface plasmon resonance (SPR) sensing. If successful, this improved protocol will be one step closer to the batch manufacturing of inexpensive metamaterials by providing a universal process for the pattern transfer of DNA- and virus-based nanostructures.

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Poster No. 48

Detection of Notch activation by rationally designed ligand-DNA nanopatterns

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Understanding the underlying signaling network of the Notch pathway is essential for the modulation and control of a diverse range of cellular processes during development, homeostasis, and disease. Many aspects regarding pathway activation remain unclear, and the present project aims to shed light on the effect of ligand spatial distribution on the activity of the receptor. We used DNA origami nanopatterns to place Jag1 ligands at well-defined positions, enabling us to simulate clustering of the ligand and assess its impact on the pathway at varying separation distances. We subjected breast cancer cells to stimulation with the nanopatterns using different combinations of anchoring sites and determined the activation by quantifying the release of the Notch intracellular domain (NICD) from the receptor. We observed that structures bearing clustered ligands corresponded to higher levels of activation as measured by *in situ* proximity ligation assay (PLA). These preliminary results are in agreement with a model of activation in which clustering of ligands amplifies receptor activation.

Poster No. 49

Nano-tailored biosensing interfaces for improved biomolecular interactions

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The interaction between bioreceptor and target is key during the development of sensitive, specific and robust diagnostic devices. Moreover, the performance of these devices is strongly related to the design of the biosensing interface at the nanolevel. Uncontrolled positioning of the bioreceptors on the surface of any biosensor with suboptimal inter-receptor distances and orientation will impede an optimal interaction between bioreceptor and target leading to a decreased biosensor performance[1,2].

In this work we present scaffold-based 3D DNA origami structures as a tool to nanostructure the surface of the disc-shaped microparticles in the microfluidic environment of the innovative Evalution™ platform. Starting from a 24-helix bundle[3], an antenna-like DNA origami structure was designed to precisely position thrombin-specific aptamers. An inter-receptor distance of 16.3 nm was found optimal, perfectly accommodating the aptamer-thrombin complex, covering a region of ± 7 nm diameter. Immobilization of the capturing aptamers through the assay-specific tailored origami structure enabled reproducible detection of fluorescent thrombin (CV: 4.1 %), but also revealed a 7.8-fold increased binding potential compared to directly-coupled aptamers (holding 5.3-fold more bioreceptors). In addition, optimal spacing of the bioreceptors through DNA origami resulted in an increase in the apparent reaction rate from 0.07 min^{-1} for the directly-coupled to 0.30 min^{-1} for origami-linked aptamers. Furthermore, we demonstrated that DNA origami nanostructured biosensing interfaces outperformed basic aptamer coupling with respect to limit of detection (LOD: 11 × improved) and signal-to-noise ratio (SNR: 2.5 × better) in a DNA-based sandwich bioassay.

In conclusion, our results highlight the potential for assay-specific DNA origami nano-tailored surfaces to improve biomolecular interactions at the sensing surface and hereby increasing the overall biosensor performance. The immobilization of bioreceptors using a well-designed DNA origami structure results in the formation of a less densely packed surface with reduced steric hindrance and a favored upward orientation which increases their accessibility leading to enhanced biomolecular interactions. The reported surface functionalization strategy provides a general approach that can be directly transferred for the detection of various target molecules. We believe that the obtained results will lead to better insight into the receptor-target interactions and improved sensing devices for diagnostics.

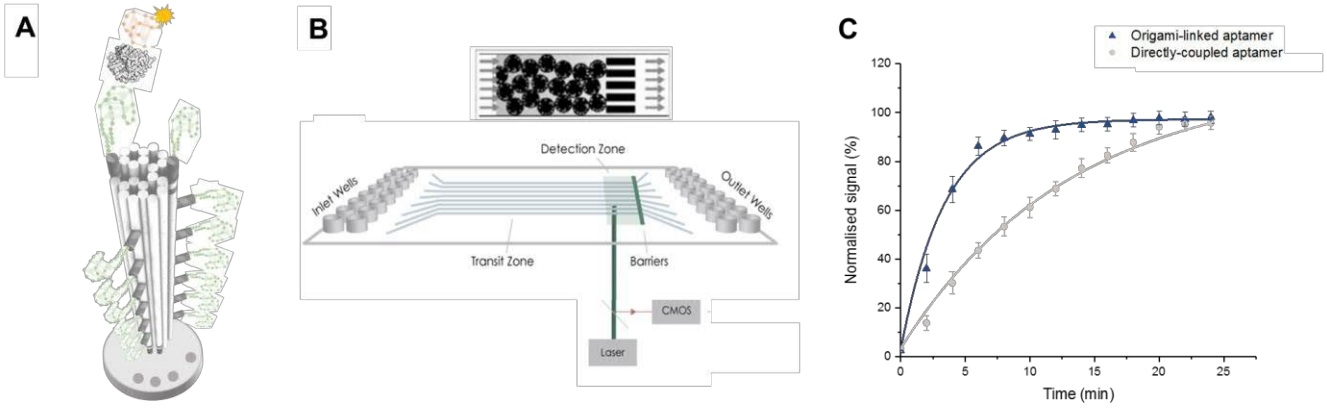

Figure 1: Schematic overview of the DNA-based microfluidic bioassay. A) Modified 3D DNA origami structure with strands for bioreceptor and biosensor surface coupling on the barcoded disks (not to scale). Thrombin is captured by an aptamer (TBA2) and detected through a fluorescent secondary aptamer (TBA1). B) Overview of the disposable microfluidic cartridge, confining the encoded microparticles, with a close-up of the detection zone. C) Reaction kinetics of thrombin (84 nM) with origami-linked and directly-coupled aptamer bioreceptors. Error bars represent one standard deviation (n = 3).

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Poster No. 50

DeRNAFold: Deep Learning-based RNA Secondary Structure Prediction

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Folding plays a vital role in synthetic biology applications. In fact, finding the native structure for an RNA sequence is an inevitable step for designing nano-scale structures, although it is an NP-hard problem (in general form with considering Pseudoknots) [1]. As a Computer Science problem, finding the Minimum Free Energy (MFE) structure *in-silico* is challenging so that attracts many researches.

NP-hardness of the structure prediction problem, brings the idea of using Machine Learning methods in mind. Although, there are some simple yet useful methods like Nussinov [2] and Zuker [3] algorithms are using Dynamic Programming, it is hard to extend them for pseudoknotted structures. Besides, recent breakthroughs of using Deep Learning in game playing and solving other problems would help. However, this work is a start due to modeling the problem as a supervised learning task spite of the fact that there is not much real data. Our methods has learnt general rules of folding which means it can predict elements in pseudoknot-free secondary structures like hairpin loops and stem.

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Poster No. 51

Temperature dependent charge transport on C-shape nanowires templated by DNA origami

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The fabrication and characterization of shape gold nanowires by means of a combination of the "top-down" and bottom-up" approaches are presented here. We analyze the electric transport of C-shape gold nanowires based on small DNA origami templates (90nm×70nm DNA origami nanosheets) having three functionalized sides holding a total of eight gold nanoparticles conforming one of the rings of a split-ring resonator (SSR) [1]. Electroless gold growth is applied to selectively grow the gold nanoparticles until they merge into nanowires [2]. Randomly distributed C-shape nanowires with a size below 150 nm laid down on SiO_2/Si substrates are contacted with gold electrodes by using electron beam lithography (BL). Charge transport measurements of the nanowires show hopping, thermionic and tunneling transports at different temperatures in the 4.2 K to 293 K range. This work demonstrates the conductive application of single, isolated and conductive nanowires with a C-shape being precisely contacted by metal using EBL to better understand the charge transport characteristics at different temperatures as a first step in the construction of split-ring resonators by DNA origami.

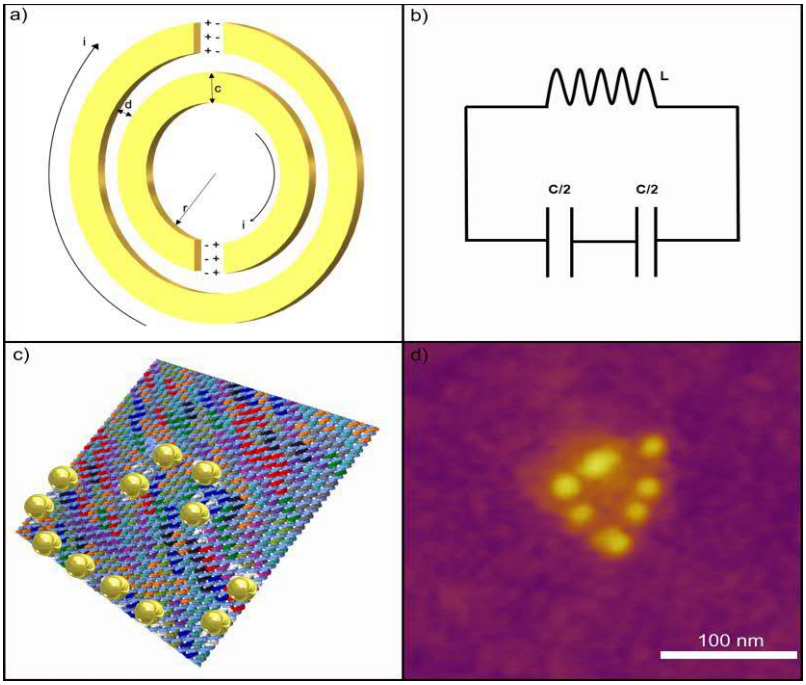

Figure 1: a) Draft of a split-ring resonator (SRR). b) Equivalent circuit of a SRR. c) Schematics of the DNA origami nanosheet:AuNPs conjugate shaping a SRR. d) AFM image of the nanostructure shown in c).

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Poster No. 52

Self-assembly and structural characterization of RNA Origami structures

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RNA folds as it is being transcribed from a single strand which confers RNA nanostructures ability to be synthesized and actuate *in vivo*, having a great potential to scaffold small molecules in spatially ordered assemblies, act as sensors and even response to different stimuli [1]. The design of RNA nanostructures takes inspiration from characterized motifs that are present in nature such as the kissing loop interaction [2], which is of particular interest to self-assemble RNA devices into multimers in a programmable manner thanks to the high stability and specificity of the interaction. In this work, we exploit the capacity of the kissing loop for assembling RNA structures to study the avidity effect of these interactions between RNA structures (6-helix bundle, fig1). We design and produce RNA 3D structures to study the strategies to assemble multiple RNA structures in an efficient and programmable manner using kissing loop interactions. We also establish a characterization pipeline for the structural determination of RNA 3D structures by purification after co-transcriptional folding and TEM and Cryo-EM imaging. Ultimately, these approaches provide useful structural insight into RNA Origami 3D structures.

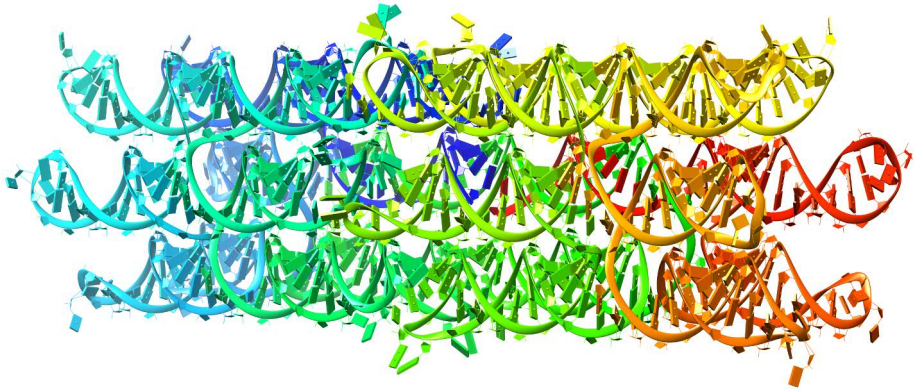

Figure 1: 3D Model of the 6-helix Bundle made with UCSF Chimera

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Poster No. 53

Coupling synthetic DNA-membrane receptors to lipid phase separation in artificial cells

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Biological cells sustain highly efficient and complex metabolic networks by compartmentalising them from their surrounding milieu with remarkably evolved semi-permeable membranes [1]. These bilayers are comprised of a mixture of lipid species, membrane-associated proteins, and cell surface receptors that in tandem impart capabilities for transporting matter and information inside and outside of the cell. Thus, it bestows cells with a means for adapting and responding to changes in their environment. Signal transduction, for instance, is hypothesised to be facilitated by the formation of lipid rafts [2]: clustering of membrane proteins through phase separation. This refers to the preferential rearrangement of lipids and sterols in multi-component bilayers into distinct phases with different degrees of order [3].

One of the main aims of bottom-up synthetic biology is the generation of artificial cells: micron-sized entities capable of replicating life-like behaviours by encapsulating biologically active material within a semi-permeable membrane [4]. These hold great potential that spans from the production of biomolecules to smart therapeutics. Nevertheless, the implementation of naturally-occurring sensing platforms – a greatly coveted trait from biological cells – has proven challenging, and has hindered the attempts of conferring artificial cells with means to respond and adapt to their surroundings. In that sense, the unprecedented programmability and control offered by DNA Nanotechnology for structures and their dynamics, coupled to the breadth of functionalisations available, renders the DNA molecule as an ideal candidate for biomimicry [5].

Aiming at the implementation of sensing functionalities in model membranes, hydrophobically functionalised DNA oligonucleotides were used to develop reactive DNA constructs, or "receptors". Through a rational sequence design, a modular receptor system is proposed as a membrane-bound DNA circuit that is coupled to lipid phase separation, as outlined in Figure 1. Consequently, a number of compositions of ternary and quaternary lipid mixtures have been studied, where phase separation behaviours were successfully replicated. In addition, low-demixing-temperature compositions, important for responsiveness to stimuli different than temperature, have been identified and characterised. Similarly, by using a fluorescent DNA receptor as a beacon, membrane-anchored DNA construct co-localisation in distinct domains, so-called partitioning [6], has been observed and measured upon phase separation. Finally, a two-component DNA receptor system was successfully designed and characterised in bulk. When interfaced with model bilayer membranes, investigations have been optimised to achieve no leakage, showing promise for a sharply triggered response to activation through co-localisation. These early findings speak for the feasibility of harnessing the synergy between the partitioning of amphiphilic DNA receptors and lipid phase separation in the quest of producing a rationally-designed synthetic platform of transducers for environmental sensing in artificial cells.

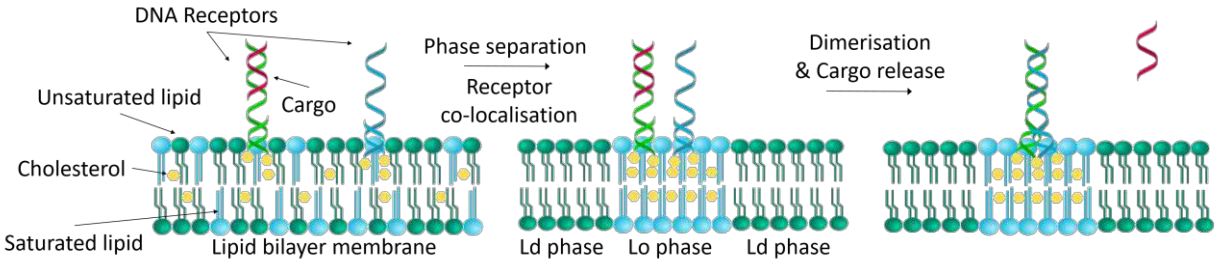

Figure 1: Co-localisation and activation of DNA-based receptors mediated by lipid phase separation and cholesterol partitioning.

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Poster No. 54

A DNA Origami and Layer-by-Layer Hybrid Carrier

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Functional DNA nanostructures are recently under extensive investigation for the protective delivery of active agents into living cells as they are biocompatible and sufficiently incorporated by cells [1]. Big attention was drawn to such DNA based drug delivery systems due to the availability of an extensive toolbox of multifunctional, stimuli-triggered switchable elements enabling a controlled release of an incorporated cargo.[2] Nevertheless, it remains challenging to determine the exact number of nanostructures internalized by a single cell and the related concentration of the delivered active agent. Even though the compactness of the structures protects them from digestion by DNAses, they bear generally low stability under physiological salt conditions and enzymatic environments. So far, many approaches (e.g. lipid encapsulation [3] or protein coating [4]) have been investigated to improve the stability. In this work we present a new way to have a defined number of DNA nanostructures delivered in a highly protected way into the cytoplasm of living cells.
Here, first results of the design of a hybrid carrier system containing DNA Origami nanostructures integrated into a super-ordinated polymeric Layer-by-Layer particle are shown.[5] This leads to an effective protection of a finely tunable number of adsorbed nanostructures to external factors on the endosomal uptake pathway and during the delivery process.
To this end we integrated DNA Origami nanostructures at different layer positions and showed their stability over the respective pH range on the endosomal uptake pathway, exposed to isolated lysosomal enzymes and in living cells. Furthermore, we achieved a more predictable uptake behavior compared to solely applied DNA nanostructures. Finally, a model agent was integrated into the nanostructures, which is intended to be subsequently released by use of toehold mediated strand displacement.
Thus, our hybrid systems represents a promising device for a more efficient investigation of DNA nanostructures inside the cytoplasm of cells and further acts as an advanced drug delivery system by specific targeting of single cell lines and intracellular compartments.

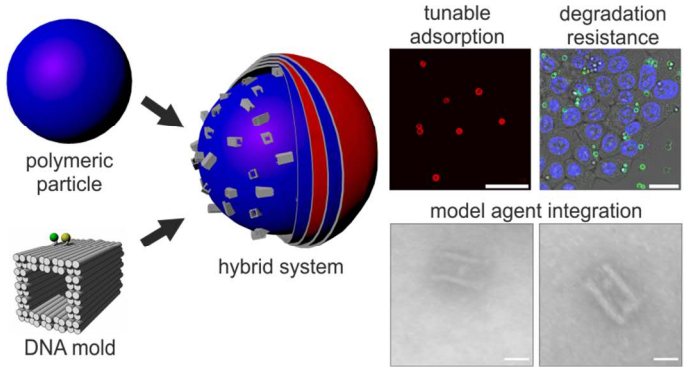

Figure 1: Design of the hybrid system with DNA Origami molds adsorbed to polymeric Layer-by-Layer particles and overview over the advanced properties of the hybrid system and tunable cargo integration.

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Poster No. 55

Designing functional transmembrane structures: towards full control over bilayer composition.

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The lipid bilayer is a barrier separating the cytoplasm from its surrounding environment, at the same time acting as an important signalling platform that is vital for the proper functioning of the cell. Transmembrane proteins embedded in the lipid bilayer are molecular machines responsible for transport across the membrane, as well as its physical and mechanical properties. Using DNA as a building material, we engineer structures that mimic the functionality of these proteins and also establish control over model membranes. [1-2]

We have developed a minimalist - but highly functional – nucleic acid construct, that self-assembles in solution and inserts into lipid membranes, combining several hydrophobic moieties. Taking into consideration the interactions between DNA, its hydrophobic modifications and lipids, we successfully changed the lipid symmetry of the bilayer, controlling both the rate and level of these changes. The constructs that we have engineered show how powerfully and efficiently a simple design can mimic naturally occurring molecules. Our DNA structures lay a solid foundation for further development of more complex and sophisticated platforms including active control.

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Tuning protein function using chemically-engineered DNA hosts

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Applying a host-guest recognition principle, we recently realized a ca. 6 MDa DNA-protein complex held in place exclusively by spatially programmed non-covalent interactions [1]. In our approach, the collective and convergent action of multiple peptide ligands pre-oriented towards the surface of the DegP protease is exploited to strengthen the binding affinity, circumventing the need for covalent protein tagging and favoring the formation of a 1:1 host-guest complex (Figure 1a-c). Preliminary results have shown the putative role of the DNA envelope in preventing protein degradation and enhancing its enzymatic activity towards a natural substrate. Our goal is to investigate this issue in greater detail, revealing and possibly quantifying the contribution given by the different parameters of the system, including the geometry of the cage, the type and spatial arrangement of the peptide ligands and the oligomerization state of the protein cargo. We are therefore currently performing point mutations at different amino acid residues of the DegP protein in order to monitor domain-associated conformational changes and understand the role of protein surface charge on the binding affinity to the DNA origami surface. We envisage that this method will enable to elucidate the mechanisms behind the allosteric regulation of DegP activity [2], and more in general, will open the way to a new approach to explore protein events at the molecular level.

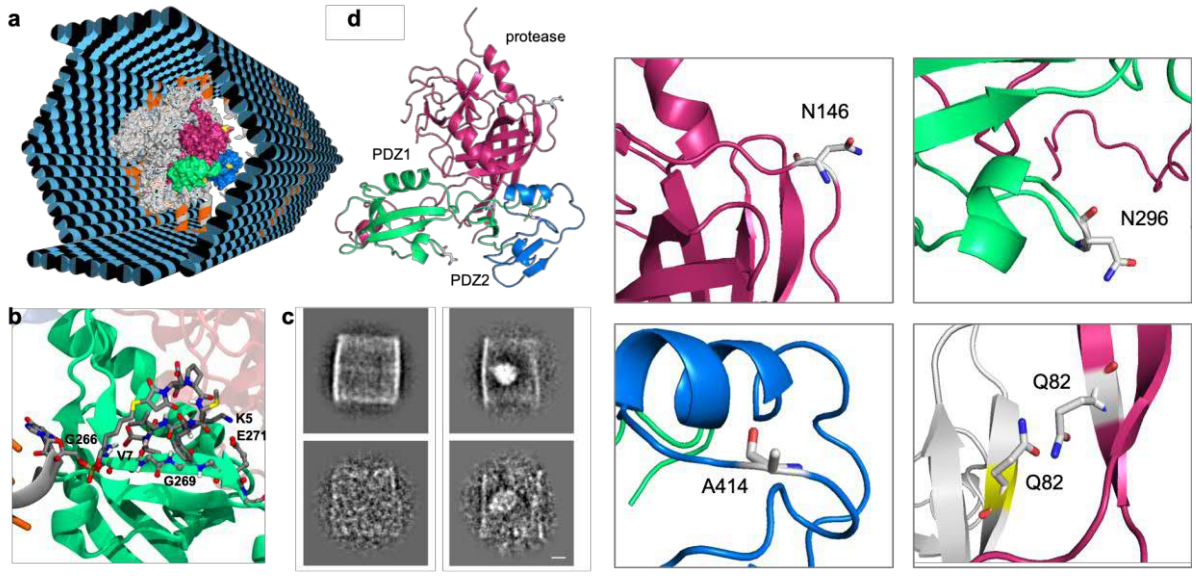

Figure 1. DNA-driven encapsulation of single protein molecules. (**a**) Molecular model of the DNA origami cage used for encapsulation of the DegP protease. (**b**) The protein is bound inside the cage through non-covalent interactions with the peptide motifs DPMFKLV. (**c**) EM characterization of the unbound and DegP24 bound cages. Scale bar is 10 nm. (**d**) Details of the three DegP domains: the protease (red), the PDZ1 (green) and PDZ2 (blue). Four point mutations (N156C, N296C, A414C and Q82A) are currently in course and will be introduced in different combinations within an active and inactive protein form, for a total of about ten mutants.

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Poster No. 57

Conformational transitions between metastable states of a DNA origami influence local susceptibility

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Inspired by the experiments of DNA origami cleaving through endonuclease enzymes [1], we performed molecular dynamic simulations to characterize the local and global fluctuations of triangle-shaped DNA origami. The simulations of about 20ms show that the first fluctuation mode presents two local minima, with an energy barrier that is lowered upon removing few staples from the structure. These changes in global fluctuations can alter the local accessibility of Hinp1I, suggesting that the introduction of a defect can alter the way DNA origami and enzymes can interact.
We show that the same principles of introducing defects in the staple arrangement can be applied to modify the global fluctuations of an infinite lattice made of DNA.

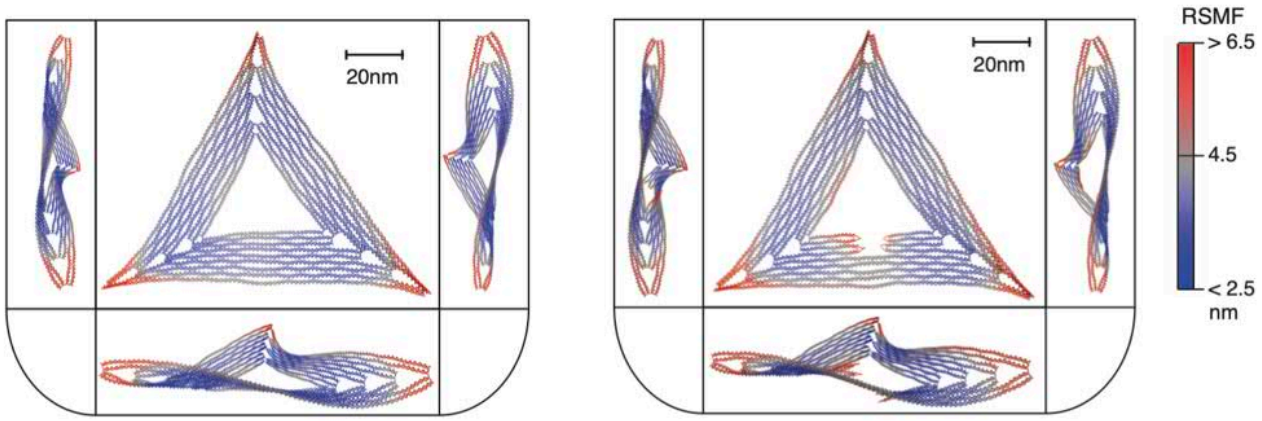

Figure 1: Average and root-mean-square-fluctuations of two DNA origami triangles presenting slightly different staple arrangements.

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Poster No. 58

Reversible deformation of a linear DNA origami structure through the cumulative actuation of tension-adjustable modules

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The natural muscle undergoes large deformations upon its contraction/relaxation through the cumulative action of the basic mechanical units. Inspired by this cumulative effect seen in biological systems, we herein designed a linear DNA origami structure that consists of repeats of a tension-adjustable module whose cumulative actuation results in a large deformation of the entire shape into an arched shape. We demonstrate that the degree of deformation is systematically controlled by replacing only a set of strands that is required for actuation of the module. Moreover, by employing tetraplex-forming sequences for the actuation, we realized stimuli-responsive reversible contraction and relaxation of the origami structure. The adjustability and expandability of our module-based design provide a versatile approach to design DNA nanostructures that exhibit a large deformation in response to external stimuli.

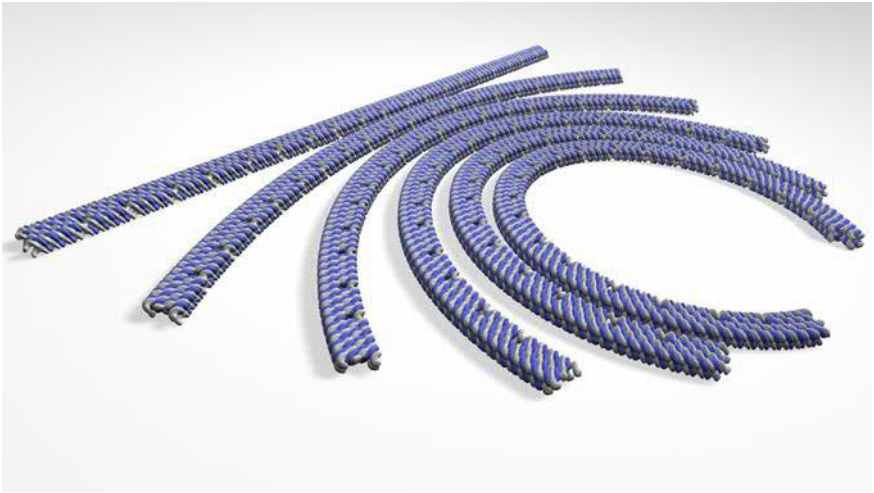

Figure 1: Schematic illustration of a series of arched DNA origami structures deformed from a linear shape.

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Poster No. 59

DNA Origami as Functional Nanodevices

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During the past half century, the nanotechnology has been one of the driving forces of the development, allowing breakthroughs in the fields of medical diagnostics, food industry and pharmaceutical industry [1,2]. In all of these fields, the structural DNA nanotechnology based innovations have shown potential to develop from proof-of-principle concepts into true applications - thanks to the superior properties of DNA such as robust self-assembly, simplicity of its preparation and versatile functionalization schemes [3].

In this context, DNA origami based structures have proven itself to be excellent building blocks for generation of hierarchical nanostructures. In the past few years, these structures have been successfully utilized as optical and electrical nanodevices [4-7] typically employing metallic nanoparticles and biomolecules. In addition, DNA origami based structures have been successfully utilized in label-free detection and multiplexing of several analytes using the principle of Surface Enhanced-Raman Spectroscopy (SERS). However, demand for newer innovations that allow production of universal, cost efficient, tunable and simple to use DNA nanodevices is still broad. Such innovations may include methods to precisely position molecules and nanoparticles to manifest new hybrid-structures or metamaterials with customizable biological, chemical, mechanical, electrical and optical properties.

Our overall aim is to develop DNA origami structures that can act as platforms for precise placement of arbitrary biomolecules and nanoparticles. These platforms could then be utilized in hot electron catalysis [8], chemical or biological sensing, SERS detection of sample at lower concentration down to single analyte level or fabrication of metamaterials with unique optical properties. The future goal would be to manufacture devices ready to use that could be further tailored for user-specified purposes.

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Poster No. 60

Switchable 2D/3D DNA origami nanodevices and light-up bio-orthogonal RNA nanoribbon

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We present switchable 2D and 3D DNA origami with a movable flap, whose opening movement was controlled by the hybridization between different DNA sensor probes and a variety of single stranded complementary targets, including natural RNA. The actuation mechanism has been demonstrated by atomic force microscopy (AFM), transmission electron microscopy (TEM), Förster resonance energy transfer (FRET), localized surface plasmon resonance (LSPR) tuning, colorimetric and chemiluminescent reactions [1,2]. More in detail, upon actuation the 3D DNA origami nanorobot moved the flap exposing a hemin/G-quadruplex horseradish peroxidase (HRP)-mimicking DNAzyme able to catalyze the H_2O_2-mediated oxidation of 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) or the oxidation of luminol by H_2O_2 to yield chemiluminescence. The intrinsic recognition abilities of the switchable 3D DNA nanorobot have been conjugated to an optical fiber transducer thus producing a new DNA origami genosensor. This organic-inorganic hybrid system can find potential applications in a wide range of fields, including point-of-care diagnostics or cellular *in vivo* biosensing when using ultrathin fiber optic probes [3].
Recently, we focused our research on the design of synthetic bio-adaptors that will act as standardised interface to the cell's essential biological processes. In this context, we considered RNA origami technique as a method for nanofabrication while the majority of RNA nanostructures are based on natural pre-folded RNA.
In our recent work [4], square DNA origami and triangle RNA-DNA hybrid origami were synthesized using 'bio-orthogonal' and uniquely addressable De Bruijn sequences (DBS) characterized by the lack of genetic information, restriction enzyme sites and repetitions larger than a predetermined design parameter. The same algorithm was applied to generate a short synthetic DBS scaffold folded into a RNA nanoribbon by seven staple strands at physiological temperature [5]. The RNA origami assembly has been verified by gel assay, AFM and using a new split Broccoli aptamer system able to bind the specific fluorophore only after the folding process. The Broccoli aptamer was divided into two nonfunctional sequences each of which is integrated into the 5' or 3' end of two staple strands complementary to the RNA scaffold. Using in-gel imaging and fluorescence measurements, we demonstrated that once the RNA origami assembly occurs, the split aptamer sequences were in close proximity to form the aptamer and turn on the fluorescence. Herein, we investigate and combine three different aspects: i) 'bio-orthogonality', ii) physiologically compatible folding at 37 °C and iii) assembly monitoring through a new split Broccoli RNA aptamer system. Our resulting RNA origami nanoribbon can open the way to new potential platform for future *in vivo* applications when genetically encoded and transcribed RNA are used.

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Poster No. 61

Fluorescence Sensing Assays Based On DNA-Origami Plasmonic Nanoantennas

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Specificity of Watson-Crick base pairing of DNA provides a unique opportunity to create multiple copies of well-defined self-assembled nanostructures *via* the DNA origami technique [1]. The great advantage of using DNA as building material is the possibility to precisely position molecules of interest and functional groups. Exploiting these features we designed 3D DNA origami nanostructure containing two plasmonic nanoparticles and a dye in between. Upon illumination with freely propagating light, the local electric field between nanoparticles increases (plasmonic hotspot), that enables enhancing of emission of the dye. We already have demonstrated the gain of several orders of magnitude of the fluorescence signal [2, 3]. In present work, we aim at making the plasmonic hotspot usable for molecular diagnostic and biophysics applications. To this end, we modified a hotspot with molecular recognition units to detect an enhanced signal only in the presence of a specific nucleic acid target [4]. Requirements to the design of recognition units, as well as the limitation of methods will be discussed. We provide the strategy of the integration of the designed sensing assays into low-tech devises such as smartphone-based microscopes [5] in order to develop inexpensive point of-care diagnostic platforms.

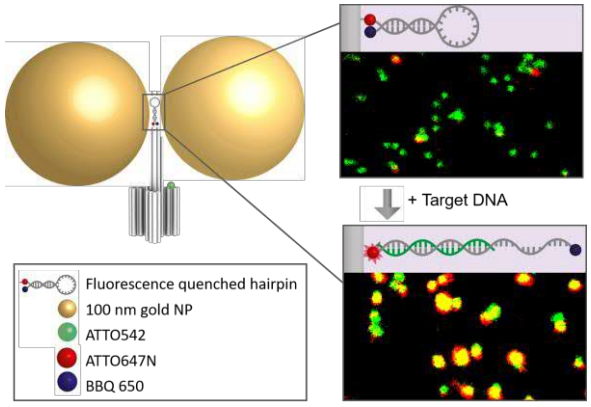

Figure 1: Schematic representation of nano-antenna: pillar-shaped DNA origami binds two gold nanoparticles (golden spheres) and a recognition unit for specific DNA sequence. After the target detection strongly enhanced fluorescence could be detected.

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Poster No. 62

Cotranscriptional Folding of RNA Using Stochastic Simulation

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This work aims to employ the prediction capability of the stochastic simulation technique [1] to study the cotranscriptional folding of RNA to form secondary structures. The influence of the cotranscriptional process to the formation of the final RNA structure has been shown to be significant in comparison with starting from a fully denatured sequence [2]. We start with an extension of the state-of-the-art stochastic simulation algorithm [3], which only supports the study of folding of RNA at the fully denatured state, to allow investigating the cotranscriptional folding of RNA. We then propose a new algorithm that is computationally efficient. Our new algorithm improves the simulation performance by reusing the computation, hence increasing the applicability of the approach.

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Poster No. 63

An optimized wireframe DNA origami for cell to cell engagement

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DNA, a biomolecule with molecular self-assembly properties, has emerged as a versatile nanomaterial to construct multifunctional platforms; DNA nanostructures can be modified with functional groups to improve their utilities as biosensors or drug carriers. Such applications have become possible with the advent of the scaffolded DNA origami method. The breakthrough technique−DNA origami−in structural DNA nanotechnology provides an easier and faster way to construct DNA nanostructures with various shapes. A DNA origami drug delivery system for cancer therapy with tunable release properties was prepared [1]. But the bio-stability of the structures was a big challenge, thus we just tested the system in vitro at that time. Our team recently has developed a new method to fabricate wireframe DNA assemblies with unprecedented 3D shape control at the nanoscale [2]. Innovatively and surprisingly, DNA origami produced via this new method are bio-stable. We are using this newly developed technology to fold aimed nanorobot.

In this project, we are optimizing DNA nanorobots that can engage T cells to malignant cells, improving the cell-to-cell interaction. The nanorobot itself has the following key properties: 1st, can maintain its stable monomer in physiological condition (in PBS, cell culture medium, blood.); 2nd, its configuration can change from locked state to open state. After loading antibodies, it has the following functions: 1st, inside the nanorobot, antibody1 is precisely patterned on one half of the nanorobot, while another half has antibody2. Thus, the open nanorobot can bind with both the malignant cells and T cells; 2nd, because of the bi-specific function, the nanorobot can improving the T cells-to-cancer cells interaction; 3rd, high specificity. The nanorobot can only be triggered once it meets the malignant cells; 4th, the nanorobot can stay outside the cells rather than being uptake by the cells, thus just working on the membrane.

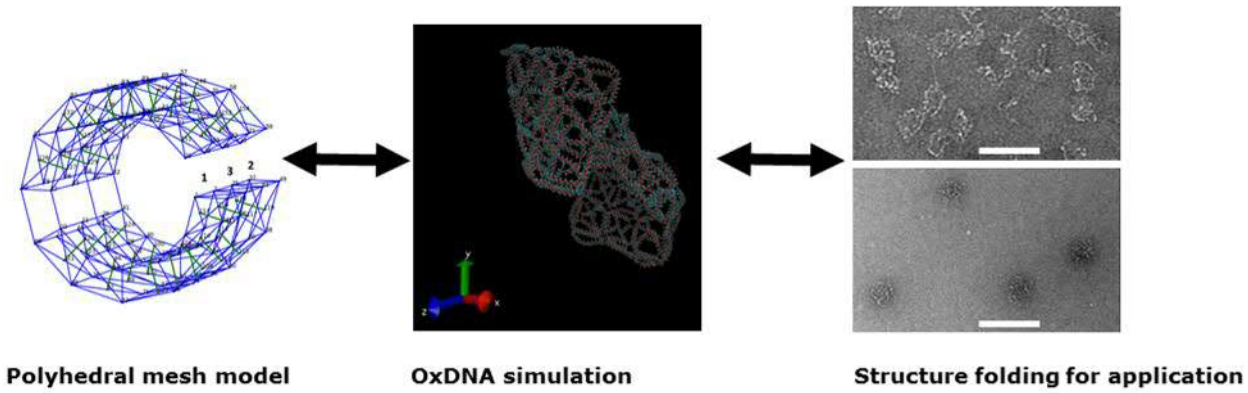

Figure 1: How we design and optimize the structure. The first step to design the wireframe mesh model for the structure. In this barrel-like wireframe structure, its mesh model includes two layers (the blue line), and the two layers are connected with each other through the internal lines (the green lines). Though this kind of design, the final simulated structure can maintain the curvature for its two half parts. The optimized structure then was finally folded and imaged by TEM. The bar is 100 nanometer.

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Poster No. 64

Durability analysis of the cryopreservation of DNA origami nanostructures

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DNA origami nanostructures as first presented by Rothemund [1] have demonstrated great potential as functional platforms for various biomedical applications. Due to their broad applicability in technology and science, stability analysis of DNA origami structures is of high importance [2]. While in the academic setting, experiments are typically performed using freshly prepared DNA origami samples, technological applications will require the storage of structurally intact DNA origami for extended periods of time. Among preservation processes, lyophilization has been discussed as a promising method for long term preservation of DNA origami structures [3] at ambient temperatures. One drawback of this method is that it requires temperatures of down to -80 °C which may not be reached using typical lab equipment. In another work, freezing has been shown to reduce the lifetime of DNA molecules under tension [4].

Here, we thus investigate the durability of triangular DNA origami structures under repeated freezing and thawing using single-molecule atomic force microscopy. The DNA origami triangles maintain their structural integrity over many freezing and thawing cycles, up until a certain threshold after which the fraction of intact DNA origami is rapidly decreasing. We also evaluate the possibility to further increase this threshold by addition of cryoprotectants.

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Poster No. 65

Nano-electronic components built from DNA templates

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On the nanoscale, fundamental properties and potential applications are greatly influenced by the size and shape of the material. "DNA Origami" takes advantage of base complementarity of individual short oligonucleotides, to fold a long "scaffold strand" into almost any continuous 2D or 3D shape.[1] We recently introduced a new concept of DNA mold-based particle synthesis that allows the synthesis of inorganic nanoparticles with programmable shape. We demonstrated the concept by fabricating a 40 nm long rod-like gold nanostructure with a quadratic cross-section.[2] We expanded the capabilities of the mold-based particle synthesis to demonstrate the synthesis of uniform conductive gold nanowires with 20-30 nm diameters.[3] With conductance characterization, metallic conducting wires were demonstrated. Here the concept is further expanded by designing mold monomers with different geometries and interfaces[4], and we can fabricate more complex 'mold-superstructure' in a unique and flexible way based on this modular DNA platform (see figure 1). We can also incorporate semi-conducting nano-rods into this mold-based system to fabricate single molecular transistor. [5]

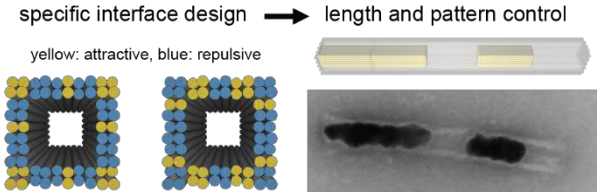

Figure 1: Sketch and tSEM images showing the modular DNA platform. With specific interface design by choosing different helix positions for attractive and repulsive reaction, the length and pattern of the metal structures can be controlled.

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