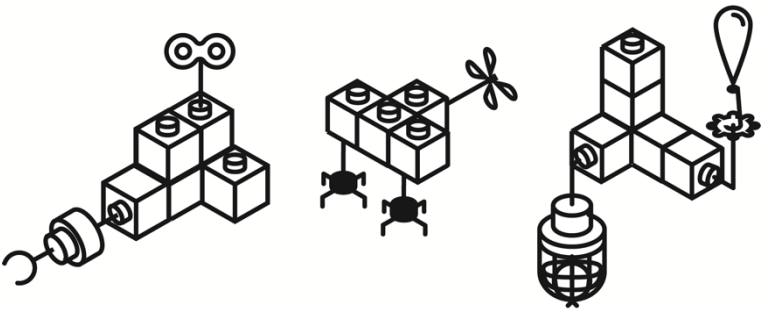
Functional DNA Nanotechnology



Rome, 25-27 May 2022

Book of Abstracts

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Workshop Programme

Wednesday 25th May

10:30- 12:00	**Registration**
12:00 - 12:10	**Opening**
12:10 - 12:40	**Invited Lecture (IL) 1: Hao Yan**, "Designer Nucleic Acid Architectures for Programmable Self-assembly" Arizona State University, USA
12:40 - 12:55	**O-1**: **Enzo Kopperger**, "Engineering Electrically Driven DNA-Based Mechanisms" Technical University of Munich, Germany
12:55 - 13:10	**O-2**: **Anton Kuzyk**, "DNA-origami-based plasmonic assemblies with tailored stimuli and optical responses", Aalto University, Finland
13:10 - 13:25	**O-3**: **Minke A.D. Nijenhuis**, "Folding dsDNA using Triplex Forming Oligonucleotides", Aarhus University, Denmark
13:25 - 14:25	**Refreshments + poster session**
14:25 - 14:55	**IL-2**: **Laura Na Liu**, "Dynamic plasmonic systems with controlled motion on the nanoscale" University of Stuttgart, Germany
14:55 - 15:10	**O-4**: **Haggai Shapira**, "Development of a High-performance DNA Origami Rotary Motor, Monitored by Defocused Imaging of Gold Nanorods", Ben Gurion University of the Negev, Israel
15:10 - 15:25	**O-5**: **Damien Baigl**, "Isothermal self-assembly of multicomponent and evolutive DNA nanostructures", Ecole Normale Supérieure (ENS), France
15:25- 15:40	**O-6**: **Iris Seitz,** "Optically responsive protein coating of DNA origami for antigen targeting", Aalto University, Finland
15:40 – 15:50	**Flash presentations** (2 minutes each x 5)
15:50 – 16:30	**Tea Break + poster session**
16:30 - 17:00	**IL-3**: **Maartje M.C. Bastings**, "Patterns in Biology: DNA-origami as nano-tool to control multivalent binding", École Polytechnique Fédérale de Lausanne (EPFL), Switzerland.

17:00 - 17:15 **O-7: Claudia Corti**, "Hybrid gold-DNA origami nanostructures for colorimetric sensing", Institut Langevin - ESPCI-PSL , France.

17:15 - 17:30 **O-8: Gregor Posnjak,** "DNA-origami based diamond type lattice with visible wavelength periodicity", LMU Munich, Germany.

17:30 - 17:45 **O-9**: **Casey M. Platnic,** "A dissipative pathway for the structural evolution of DNA fibres", University of Cambridge, UK.

17:45 - 18:00 **O-10**: **Alan Szalai,** "Orientation of dsDNA relative to graphene determined by single-molecule fluorescence lifetime microscopy", Ludwig-Maximilians-Universität München (LMU), Germany.

Thursday 26th May

9:00 - 9:30 **IL-4**: **Itamar Willner,** "Aptananozymes – A New Class of Aptamer-Modified Nanoparticles for Catalysis and Chemodynamic Medicine", The Hebrew University of Jerusalem, Israel

9:30 - 9:45 **O-11**: **Amelie Heuer-Jungemann,** "New insights into the DNA origami silicification reaction mechanism by in situ small angle X-ray scattering", Max Planck Institute of Biochemistry, Martinsried, Germany

9:45 - 10:00 **O-12: Joel Spratt,** "Tuning the insulin receptor signalling pathway response using insulin-DNA origami nanostructures", Karolinska Institute Stockholm, Sweden

10:00 - 10:15 **O-13**: **Felix J. Rizzuto**, "DNA sequence and length dictate the assembly of nucleic acid block copolymers", School of Chemistry, University of New South Wales, Australia

10:15 - 10:30 **O-14**: **Michael Pinner,** "A DNA-based artificial membrane budding system", Technical University of Munich, Germany

10:30 - 10:45 **Flash presentations** (2 minutes each x 5)

10:45 - 11:40 **Coffee break + poster session**

11:40 - 12:10 **IL-5**: **Lorenzo Di Michele,** "A three-agent communication pathway triggered by bacterial metabolism (that uses DNA nanotechnology)" Imperial College London, University of Cambridge, UK

12:10 - 12:25	**O-15: Yongzheng Xing,** "Designer DNA-based Membrane Nanopores for Portable Sensing of Diagnostic Proteins", University College London, UK
12:25 - 12:40	**O-16: Barbara Saccà,** "Thermodynamic and kinetic properties of DNA-confined enzymes", University Duisburg-Essen, Essen, Germany
12:40 - 12:55	**O-17: Adrian Leathers,** "Reaction-diffusion patterning of DNA-based artificial cells", University of Cambridge, UK
12:55 – 13:10	**O-18: Juliette Bucci,** "Temporal control of DNA-strand displacement reaction", University of Rome, Tor Vergata, Italy
13:10 – 14:10	**Lunch**
14:10 – 14:40	**IL-6**: **Elisa Franco, "**Dynamic control of DNA condensates via strand displacement", University of California, Los Angeles, USA,
14:40 - 14:55	**O-19: Alexander J. Speakman,** "Electrically Directed Gene Expression (EDGE): using switchable DNA triplexes and electrolysis to modulate transcription in a cell-free medium", University of Edinburgh, UK
14:55 - 15:10	**O-20: Aleksandra Adamczyk,** "Orienting single molecules in DNA origami constructs", University of Fribourg, Switzerland
15:10 - 15:25	**Flash presentations** (2 minutes each x 5)
15:25 - 15:40	**Irene Ponzo,** "switchSENSE and proFIRE - a DNA-based technology to discover molecular interactions and preparation of pure protein-DNA conjugates", Dynamic Biosensor, Germany
15:40 - 16:40	**Coffee Break + informal discussion**
16:40 - 20:00	**Social programme (tour to Nemi – lake and town)**
20:00	**Social dinner + Award Ceremony**

Friday 27th of May

9:00 - 9:30	**IL-7: Andreas Walther,** "Metabolic DNA Systems Inspired from Life:Protocells and Systems with Lifecycles", University of Mainz, Germany
9:30 - 9:45	**O-21**: **Matteo Castronovo,** "Enzymatic DNA ligation within two-dimensional DNA origami depends on nanostructure shape", University of Leeds, UK

9:45 – 10:00	**O-22**: **Alexis Vallée-Bélisle,** "Bio-inspired DNA switches for sensing and drug delivery applications", University of Montreal, Canada
10:00 - 10:15	**O-23: Giovanni Nava,** "Probing the conformational dynamics of long unstructured single stranded DNA chains", University of Milan, Italy
10:15 - 10:30	**O-24: Ioanna Smyrlaki,** "DNA Origami nano-patterns as a Precise Tool to study clustering of Notch receptor" Karolinska Institute, Sweden
10:30 - 10:45	**O-25: Guillaume Gines,** "DNA-enzyme neural networks enabling nonlinear concentration profile classification", Gulliver Laboratory,Université Paris Sciences et Lettres, France
10:45 - 11:30	**Coffee break**
11:30 - 11:45	**O-26**: **Christoph Wälti,** "Counting individual molecules: DNA nanostructures for diagnostic applications", University of Leeds, UK
11:45 - 12:00	**O-27**: **Adrian Keller,** "Hierarchical self-assembly of DNA origami lattices at solid-liquid interfaces", Paderborn University, Germany
12:00 - 12:15	**O-28**: **Rakesh Mukherjee,** "Kinetic proofreading in a DNA strand displacement network", Imperial College London, UK
12:15 - 12:45	**Award presentations + closing remarks**
13:00	**Light lunch**

Invited Lectures

IL-1 **Hao Yan** *(Arizona State University, USA),* Designer Nucleic Acid Architectures for Programmable Self-assembly.

IL-2 **Laura Na Liu** *(University of Stuttgart, Germany),* Dynamic plasmonic systems with controlled motion on the nanoscale.

IL-3 **Maartje M.C. Bastings** (*École Polytechnique Fédérale de Lausanne (EPFL), Switzerland*) Patterns in Biology: DNA-origami as nano-tool to control multivalent binding.

IL-4 **Itamar Willner** *(The Hebrew University of Jerusalem, Israel*), Aptananozymes – A New Class of Aptamer-Modified Nanoparticles for Catalysis and Chemodynamic Medicine.

IL-5 **Lorenzo Di Michele** *(Imperial College, UK),* A three-agent communication pathway triggered by bacterial metabolism (that uses DNA nanotechnology).

IL-6 **Elisa Franco** *(University of California, Los Angeles),* Dynamic control of DNA condensates via strand displacement.

IL-7 **Andreas Walther** *(University of Mainz),* Metabolic DNA Systems Inspired from Life: Protocells and Systems with Lifecycles.

Posters

P-1: **Adam Dorey**, University College London, "Synthetic protein-conductive membrane nanopores built with DNA".

P-2: **Aleksandra Bednarz,** Aarhus University, "Ion-dependent structural integrity and reconfigurability of DNA origami nanostructures.

P-3: **Alessandro Cecconello,** University of Padova, "Regulating in vitro transcription using RNA/DNA triplex-based biosynthetic machineries".

P-4: **Alexander M. Kloosterman,** Karolinska Institutet**, "**Spatial inference of barcoded transcripts from sequencing data".

P-5: **Alexia Rottensteiner,** University College London (UCL), "A Light-Actuated DNA Channel for Controlled Transport Across Membranes".

P-6: **Ali Khoshouei,** Technical University Munich, "CryoEM structure determination using DNA nanotechnology".

P-7: **Ana Martins,** University of Porto, "Neuronal targeting with functionalized tetrahedral DNA nanostructures".

P-8: **Andreas Peil**, University of Stuttgart; Max Planck Institute for Solid State Research, "DNA Assembly of Modular Components into a Rotary Nanodevice".

P-9: **Andrew Stannard**, Imperial College London, "Mechanically-modulated toehold mediated strand displacement ".

P-10: **Annelies Dillen,** University of Leuven, "Duplexed aptamers on fiber optic surface plasmon resonance sensors: a winning combination for continuous biosensing".

P-11: **Bhanu Kiran Pothineni,** Paderborn University, "Novel vancomycin-conjugated DNA origami-based nanoantibiotics".

P-12: **Chalmers Chau,** University of Leeds, "Single biomarker detection with affimer conjugated DNA origami through solid-state nanopore".

P-13: **Coline Kieffer**, Université Paris Sciences et Lettres, "Tunable-gain amplifier in DNA-enzyme reaction circuits and its applications in microRNA biosensing".

P-14: **Wooli Bae**, University of Surrey, "Building an RNA-Based Toggle Switch Using Inhibitory RNA Aptamers".

P-15: **Christoph Pauer**, Ludwig-Maximilians-Universität München (LMU), "Propulsion of magnetic beads asymmetrically covered with DNA Origami appendages".

P-16: **Volodymyr Mykhailiuk,** Technical University Munich, “DNAzymes for mass production of DNA oligonucleotides”.

P-17: **Christopher Frank,** Technical University Munich, “Cell surface-mediated conformational changes of DNA-Origami objects “.

P-18: **Diana Morzy,** EPFL, Switzerland, “Valency and entropic costs determine the cation-mediated DNA/lipid binding”.

P-19: **Elena-Marie Willner,** Technical University Munich, “Virus neutralization using icosahedral DNA origami shells”.

P-20: **Elija Feigl,** Technical University Munich, “WaffleCraft: Fully Automated Blocky DNA Origami Design Tool”.

P-21: **Fabian Kohler,** Technical University of Munich, “Precision Design and Characterization of DNA Origami Corner Motifs using Cryo-EM”.

P-22: **Farah El Fakih,** Ecole Normale Supérieure, “Reversible Supra-Folding of User-Programmed Functional DNA Nanostructures on Fuzzy Cationic Substrates”.

P-23: **Florian Rothfischer,** Technical University of Munich/ Ludwig-Maximilians-Universität München, “Control of enzyme activity by a DNA nanoscale robotic arm”.

P-24: **Francesca Smith,** Imperial College London, “Characterisation of RNA/DNA hybrid strand displacement kinetics”.

P-25: **Gerrit Wilkens,** Jagiellonian University, Malopolska Centre of Biotechnology, “Blowing “bubbles” with DNA origami”.

P-26: **Giacomo Fabrini**, Imperial College London, “Cation-Responsive and Photocleavable Hydrogels from Noncanonical Amphiphilic DNA Nanostructures”.

P-27: **Viktorija Kozina,** Technical University Munich, “Targeting antigen patterns with programmable T-cell engagers”.

P-28: **Igor Baars,** Karolinska Institutet, “Spatial reconstruction using barcoded DNA sequences”.

P-29: **Jacky Loo,** Aalto University, “Colorimetric Visualization with Visible Chirality”.

P-30: **Jing Huang,** CENIDE and ZMB, University of Duisburg-Essen, “A DNA-confined unfoldase/protease nanomachine”.

P-31: **Viktorija Glembockyte**, LMU Munich, “Self-regeneration and self-healing in DNA nanostructures”.

P-32: **Nada Farag,** University of Rome Tor Vergata, “Programmable decoration of DNA-based scaffold through dynamic exchange of structural motifs”.

P-33: **Kevin Jahnke,** Max Planck Institute for Medical Research; Heidelberg University, Rational engineering of DNA cytoskeletons for synthetic cells”.

P-34: **Lena Stenke**, University Duisburg-Essen, Germany, “Dynamics of DNA origami filaments growth from a ditopic monomer”.

P-35: **Lorena Baranda**, University of Rome Tor Vergata, “Protein-Templated Reactions Using DNA-Antibody Conjugates”.

P-36: **Ulrich Kemper,** University of Leipzig, “DNA mold-based fabrication of palladium nanostructures”.

P-37: **Ludwig Rotsen**, Univ. Grenoble Alpes, “Substrate-assisted self-assembly of DNA origamis for lithographic applications”.

P-38: **James Vesenka,** University of New England and Leibniz-IPHT Biophotonics, “AFM analysis of G-wire DNA structure and nanoparticle decoration”.

P-39: **Marcel Hanke**, Paderborn University, “Salting-out of DNA Origami Nanostructures by Ammonium Sulfate”.

P-40: **Tania Patino,** University of Rome Tor Vergata, “Bioengineering DNA-based enzyme-powered nanoswimmers”.

P-41: **Sara Bracaglia,** University of Rome Tor Vergata, “Programmable cell-free transcriptional switches for antibodies detection”.

P-42: **Marcus Fletcher**, University of Cambridge, “G-Quadruplex DNA based fluorescent sensing for quantification of potassium ion flux across giant proteoliposomes”.

P-43: **Matthew Aquilina**, University of Edinburgh, “Multiplexed Label-Free Biomarker Detection by Targeted Disassembly of Variable-Length DNA Payload Chains”.

P-44: **Maximilian Nicolas Honemann,** Technical University of Munich, “A novel lattice design for scaffolded DNA origami structures”.

P-45: **Michal Walczak**, University of Cambridge, “Stimuli-responsive DNA particles underpin three-agent signaling networks with live bacteria and synthetic cells”.

P-46: **Daniela Sorrentino,** University of Rome Tor Vergata, “Allosteric regulation of DNA-based nanodevices using in vitro transcription”.

P-47: **Nathanv Wu**, University of Edinburgh, “A DNA Nanotechnology Assay to Detect Double-Stranded DNA for Medical Applications”.

P-48: **Nico Alleva,** Max-Planck Institute for Polymer Research, “Diverse, highly efficient grafting to strategy for the patterning of DNA-origami”.

P-49: **Richard Kosinski**, University of Duisburg-Essen, “The role of DNA nanostructures in the catalytic properties of an allosterically regulated protease”.

P-50: **Roger Rubio Sanchez**, Imperial College London, “A modular, dynamic, DNA-based platform for regulating cargo distribution and transport between lipid domains”.

P-51: **Sabrina Gambietz**, University Duisburg-Essen, Germany, “Thermal and mechanical properties of topologically identical origami domains at the ensemble and single-molecule level”.

P-52: **Sayantan De,** University Duisburg-Essen, Essen (Germany), “A DNA logic gate to sense molecular distances”.

P-53: **Seppe Driesen**, University of Leuven, “Towards DNA-only digital biosensing with DNA nanosensors”.

P-54: **Sergii Rudiuk**, Ecole Normale Supérieure, “DNA-protein nanogels as transfectable multienzymatic nanoreactors“.

P-55: **Sofia Julin**, Aalto University, “pH-Responsive DNA Origami Lattice”.

P-56: **Steffan Møller Sønderskov**, Aarhus University, “High-resolution surface charge density visualization of DNA nanostructures”.

P-57: **Serena Gentile,** University of Rome Tor Vergata, “Spontaneous reorganization of DNA-based polymers in higher ordered structures fueled by RNA”.

P-58: **Teun Huijben,** Technical University of Denmark, “Fast and exact reduction of mislocalizations near spherical nanoparticles by a fully analytical PSF”.

P-59: **Miguel Paez-Perez,** Imperial College London, “Effect of lipid composition on the efficiency of fusogenic DNA nanostructures”.

P-60: **Ken Sachenbacher,** Technical University Munich, “Triple-stranded DNA as a structural element in DNA origami”.

P-61: **Marco Lolaico, Karolinska Institute,** “Enhanced stiffness of wireframe DNA nanostructures with square lattice edges”.

P-62: **Neda Bagheri**, University of Rome Tor Vergata, “Enhancement of CRISPR/Cas12a trans-cleavage Activity Using Hairpin DNA Reporters”.

IL-1

Designer Nucleic Acid Architectures for Programmable Self-assembly

Hao Yan

Center for Molecular Design and Biomimetics, Biodesign Institute
& School of Molecular Sciences
Arizona State University

DNA and RNA has emerged as an exceptional molecular building block for nano-construction due to its predictable conformation and programmable intra- and inter-molecular base pairing interactions. A variety of convenient design rules and reliable assembly methods have been developed to engineer DNA nanostructures of increasing complexity. The ability to create designer DNA architectures with accurate spatial control has allowed researchers to explore novel applications in many directions, such as directed material assembly, structural biology, biocatalysis, DNA computing, nano-robotics, disease diagnosis, and drug delivery. In this talk I will discuss some of our work in the field of structural nucleic acid nanotechnology, and present some of the challenges and opportunities that exist in DNA and RNA based molecular design and programming. Specifically, I will discuss some of the new designs for 3D DNA crystals and the use of the crystals as host to organize guest molecules and visualize their atomic level structures. I will discuss the use of DNA template to organize dye molecules for long range energy transfer over sub-micron distances for potential light harvesting applications. I will also discuss our progress in using DNA and RNA nanotechnology for biomedical applications.

IL-2

Dynamic plasmonic systems with controlled motion on the nanoscale

Laura Na Liu

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Max Planck Institute for Solid State Research, Heisenbergstrasse 1, 70569 Stuttgart, Germany

A fundamental design rule that nature has developed for biological machines is the intimate correlation between motion and function. One class of biological machines is molecular motors in living cells, which directly convert chemical energy into mechanical work. They coexist in living cells, but differ in their types of motion. Such natural structures offer inspiration and blueprints for constructing DNA-assembled artificial systems with controlled motion on the nanoscale. In this talk, I will present two artificial analogs of molecular linear and rotary motors with tailored optical functionalities built using DNA origami. I will also highlight the ongoing research directions and conclude that DNA origami has a bright future ahead.

IL-3

Patterns in Biology: DNA-origami as nano-tool to control multivalent binding

Maartje M.C. Bastings

Programmable Biomaterials Laboratory (PBL), Institute of Materials, School of Engineering, École Polytechnique Fédérale Lausanne (EPFL), Station 12, 1015 Lausanne, Switzerland

DNA-based nanostructures are actively gaining interest as tools for biomedical and therapeutic applications following recent developments of protective coating strategies, which prolong structural integrity in physiological conditions [1]. For tailored biological action, these nanostructures are often functionalized with targeting or imaging labels using DNA base pairing [2]. DNA nanotechnology provides a modular platform for precise ligand positioning [3], not unlike important receptor-targeting molecules found in nature. Numerous biological processes, often crucial for the organism's functioning, rely on the concept of multivalency [4]. Multivalency is based on interactions that are weak when singular but combined ensure stable connection: strength in numbers. Coupling of multiple receptors with one ligand implies that the spacing of bond-forming sites needs to spatially match. With geometrical requirements at nanoscale, multivalency is a technologically challenging concept to harness and understand. At the PBL, we aim at studying multivalency in biological systems, particularly with respect to its dependency on the receptor patterns and concentrations, which form the base of super-selective binding [5]. Crystallography and super-resolution microscopy data provide evidence of geometrical patterns and defined distances of active molecules as fundamental parameters to regulate receptor binding and cell signalling. We combine this information with the toolbox offered by DNA nanotechnology to build constructs of well-defined geometry and spatially controlled ligand presentation, tailored to the biological target structures. Here, we demonstrate the importance of controlled nano-spacing of immune-stimulatory molecules for the activation of immune cells [6]. We create DNA origami nanoparticles that present CpG-motifs in rationally designed spatial patterns to activate Toll-like Receptor 9 (TLR9). We show that stronger immune activation is achieved when active molecules are positioned at the distance of 7 nm, matching the active dimer crystal structure of the TLR9 receptor. Moreover, we show how the introduction of linkers between particle and ligand can influence the spatial tolerance of binding. These findings are fundamental for a fine-tuned manipulation of the immune system, considering the importance of spatially controlled presentation of therapeutics to increase efficacy and specificity of immune-modulating nanomaterials where multivalent binding interactions are involved.

References

[1] H. Bila et al. 2019. Biomaterials Science. 532-541
[2] A. Eklund et al. 2021. ACS Nano. 17668-17677
[3] S. Wickham et al. 2020. Nature Communications. 1-10
[4] D. Morzy et al. 2022. Angewandte Chemie. e202114167
[5] A. Comberlato et al. 2022. Nano Letters. ASAP

IL-4

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

Itamar Willner

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

The conjugation of DNAzymes or homogeneous catalysts to aptamer strands yields functional assemblies mimicking native enzymes by engineering active sites that concentrate the substrate in proximity of the catalytic site[1]-nucleoapzymes. Similarly, the conjugation of photocatalysts to an aptamer receptor that binds electron acceptor ligands yields organized assemblies emulating functions of the photosynthetic apparatus-photoaptazymes.[2] The concept of catalyst-aptamer catalytic conjugates is now extended to include catalytic nanoparticles functionalized with aptamer receptor binding sites-aptananozymes.[3] These include Cu^{2+}-modified C-dots functionalized with the dopamine/tyrosinamide aptamer, polyadenine-stabilized Au NPs functionalized with the dopamine aptamer and Ce^{4+}-modified C-dots functionalized with the dopamine aptamer or the tyrosinamide aptamer. Key results that will be addressed include:

- Enhanced catalytic oxidation of the dopamine to aminochrome by H_2O_2 using a series of structurally modified aptananozymes. Structure-function relationships controlling the catalytic transformations will be presented.
- Chiroselective oxidation of L-/D-DOPA by the aptananozymes will be introduced.
- Catalyzed H_2O_2-driven oxygen insertion into tyrosinamide to yield the catechol product is followed by the formation of amidodopachrome by the Ce^{4+}-ion-functionalized C-dots aptananozymes in the presence of H_2O_2/ascorbate.
- Aerobic oxidation of glucose to gluconic acid and H_2O_2 by the polyA Au NPs and the Ce^{4+}-C-dots aptananozymes. The dual catalytic activities of the aptananozymes will be applied to engineer bioreactors for the cascaded aerobic oxidation of dopamine/tyrosinamide to aminochrome/amidodopachrome by glucose.
- Mechanistic aspects related to the generation of reactive oxygen species (•OH or $O_2^{\bullet-}$) by the different aptananozymes.

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

References:

1. (a) E. Golub et al., *J. Am. Chem. Soc.*, **2016**, *138*, 164-172. (b) G.-F. Luo et al., *Adv. Funct. Mater.,* **2019,** *29*, 1901484. (c) Y. Biniuri et al., *ACS Catal.,* **2018**, *8*, 1802-1809.
2. G.-F. Luo et al., Angew. Chem. Int. Ed., **2020,** *59*, 9163-9170.
3. Y. Ouyang et al., *J. Am. Chem. Soc.,* **2021,** *143***,** 11510-11519.

IL-5

Lorenzo Di Michele

"A three-agent communication pathway triggered by bacterial metabolism (that uses DNA nanotechnology)"

Imperial College London, University of Cambridge, UK

Multiple life forms have evolved the ability to respond to the proliferation of microbes by activating pathways that limit their growth or spread. Notable examples include the immune systems of animals, but also antimicrobial responses in plants and fungi. The ability to recapitulate similar behaviours with synthetic nano systems could underpin valuable antimicrobial solutions in healthcare, agriculture, and food industry.
Here we demonstrate the bottom-up design of a basic antimicrobial pathway in which two synthetic agents operate in concert to limit the growth and arrest the motion of *E. coli* (Figure 1). The main actors of this three-agent system are amphiphilic DNA nanoparticles that, when experiencing a drop in pH caused by glucose metabolism in *E. coli*, form a sticky network capable of trapping the bacteria and arresting their motion [1,2]. In parallel, the (triggered) DNA particles also permeabilise lipid vesicles loaded with antibiotics, the release of which hinders bacterial growth.

[1] M. Walczak, R. Brady, L. Mancini, C. Contini, R. Rubio-Sànchez, W. Kaufhold, P. Cicuta and L. Di Michele, *Nature Comm*., 12:4743 (2021)
[2] M. Walczak, L. Mancini, J. Xu, F. Raguseo, J. Kotar, P. Cicuta and L. Di Michele, in preparation (2022)

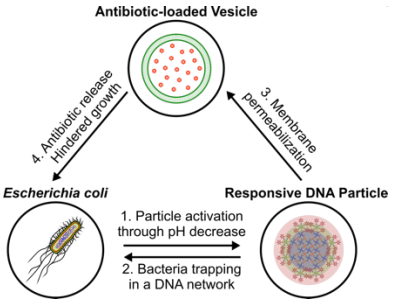

Figure 1: A three-agent signalling network involving amphiphilic DNA nanoparticles (bottom-left), antibiotic-loaded lipid vesicles (top) and E. coli (bottom-right). The bacteria decrease the pH of the solution as a result of their natural glucose metabolism (1). The decrease in pH "activates" the particles, which aggregate in a "sticky" material that traps the swimming bacteria, arresting their motion (2), and permeabilises the vesicles (3). The latter thus release their payload, hindering bacterial growth (4).

IL-6

Dynamic control of DNA condensates via strand displacement

Elisa Franco

University of California, Los Angeles

The toolkit of DNA nanotechnology has recently provided a framework to design and build motifs for phase separation of condensates. Using DNA nanotechnology we are exploring the mechanisms and design rules by which condensates can be controlled through chemical reactions. We focus on multivalent, star-shaped DNA motifs whose interactions can be designed by engineering single stranded domains (sticky ends). I will describe, by theory and experiment, how modification of the structure of DNA nanostars affects their macroscopic condensation kinetics. By changing the various domains of the DNA monomer we achieve a predictable growth profile of the condensates. Further, we obtain reversible dynamic control of condensate formation and dissolution by utilizing DNA strand displacement reactions that deactivate or reactivate the DNA monomer interaction domains. By changing DNA monomer design and sequence makeup, we explore the tunability of these reactions under different conditions. Our approach may be useful to build synthetic membraneless organelles whose formation can be temporally controlled via chemical reactions.

IL-7

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

Andreas Walther

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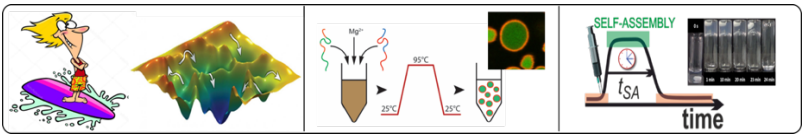
Living self-organizing systems operate far-from-equilibrium and display energy-dependent adaptive functionalities that are orchestrated through feedback loops and metabolic reaction networks to allow tailored response in complex sensory landscapes. These principles serve as an inspiration to promote complexity and life-like functions in soft matter systems, which include for instance to pre-organize temporal behavior or install mechanisms for complex adaptative behavior. The pre-organization of the temporal fate of systems requires new types of internal control mechanisms, such as kinetic control over opposing reactions (built-up/destruction), the integration of feedback mechanisms, or the use of energy dissipation to sustain structures only as long as a chemical fuel is available. Even higher complexity and new functions are in reach by essentially embedding multi-sensory metabolic reaction networks into these systems. In this talk, I will discuss two avenues towards autonomous and adaptive DNA active matter systems with simplistic metabolica reaction networks inside. On the one hand, I will discuss the formation of DNA-based protocell architectures with the ability to house abiotic catalysts driving downstream morphological adaptations. On the other hand, I will discuss the use of ATP as a chemical fuel to drive chemically fueled out-of-equilibrium systems using activation/deactivation networks. The latter allows to program self-assemblies and materials with lifetimes and programmable steady state dynamics.



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O-1

Engineering Electrically Driven DNA-Based Mechanisms

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DNA has been used as building material for constructing nanoscale machinery in an astounding variety. Examples range from hinges over sliders to rotor-stator complexes and combinations thereof [1-5]. Since such devices, unlike their macroscopic counterparts, operate on a similar scale to the molecules that compose them, it is increasingly challenging to achieve the desired movement characteristics. We aim to improve the accuracy by which we can actuate such nanomachinery and control its motion behaviour by systematic engineering. Our efforts are guided by single particle fluorescence tracking experiments and we pay particular attention to rotational mechanisms. For example, smooth movements of nanoscale bearings are difficult to realize due to interactions between their constituents sliding against each other in close proximity. We study the energy landscapes of these interactions for a variety of rotating mechanisms and relate them to their structural features. Understanding this relationship can guide the development of DNA mechanisms with tighter tolerances so that they operate closer to their intended motion behaviour. While the variety and degree of sophistication of movable mechanical components is steadily growing, the implementation of fast and efficient drive mechanisms remains challenging. In previous work [6] we demonstrated the use of external electric fields to drive a DNA robot arm with milliseconds response time. While the use of electrophoresis to move DNA appears superficially simple, its application to control substrate-bound nanoscale devices involves a combination of various electrokinetic effects. In ongoing work, we dig deeper into the physical processes to identify the critical parameters for efficient electrical control and characterize the influence of the substrate, buffer composition and structure geometry on the operation of the DNA robot arm.

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O-2

DNA-origami-based plasmonic assemblies with tailored stimuli and optical responses

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The DNA origami technique has emerged as one of the most versatile bottom-up nanofabrication methods. In this talk we will discuss our recent results related to application of DNA origami for fabrication of plasmonic systems with novel stimuli and optical responses. Specifically, we will present fabrication of *i*) light-responsive dynamic plasmonic assemblies with easily regulated steady out-of-equilibrium states1; *ii*) chiral plasmonic systems with visually detectable reconfigurable optical activity; *iii*) metal shells with tailored complex morphologies and optical responses within near-infrared window.

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O-3

Folding dsDNA using Triplex Forming Oligonucleotides

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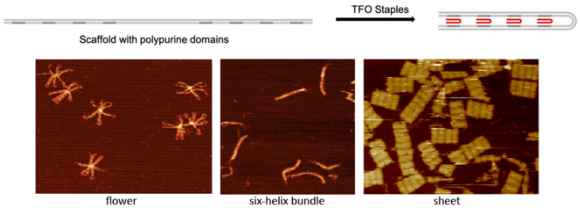
DNA-based nanostructures are typically assembled through the hybridization of single-stranded (ss) DNA. The DNA origami technique for instance folds a long ssDNA scaffold into defined shapes using numerous small staple strands.[1] Herein the Watson-Crick double helix and its base pairing bonds function as the the figurative brick and mortar, respectively, and they enable the self-assembly of a great diversity of nanoscale objects. DNA triplexes are formed when a triplex forming oligonucleotide (TFO) binds a stretch of purines in one of the strands of the dsDNA helix. This triplex formation is mediated by specific hydrogen bonds known as Hoogsteen (HS) or reverse Hoogsteen (rHS) base pairing, depending on the orientation of the TFO. In the context of structural DNA nanotechnology, DNA triplexes have commonly been exploited as functional elements; for instance to implement pH responsive modules within structures.[3] However, the vast potential of DNA triplexes and (r)HS base pairing as "bricks and mortar" structural elements remained relatively unexplored; this being despite the facts that the DNA triplex motif is geometrically robust and the (r)HS base pairing is sequence-specific. We present TFOrigami as a new strategy for folding dsDNA scaffolds into defined shapes using TFO staples. To this end, we have designed and expressed dsDNA scaffolds featuring stretches of polypurine domains, and have defined and tested design rules for the self-assembly of 2D and 3D TFOrigami structures with well-defined geometries. Structures folded include sheets, flowers, and honeycomb-lattice bundles, with more variety being tested. TFOrigami structures feature unique and beneficial properties, while largely retaining the programmability and addressability of conventional DNA origami. For instance, the inherent stiffness of dsDNA scaffolds allows the use of a smaller staple set for a similarly-sized structure. While most of our designs utilized HS base pairing and were thus assembled at acidic pH, their design rules are mostly interchangeable with rHS base pairing. TFOrigami can thus also be employed at neutral pH, making the method compatible with conventional DNA origami and general DNA nanotechnology.



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O-4

Development of a High-performance DNA Origami Rotary Motor, Monitored by Defocused Imaging of Gold Nanorods

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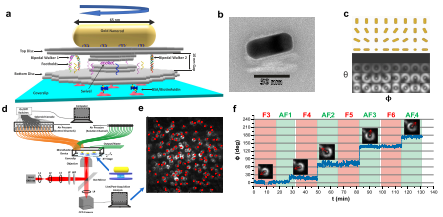
The biological molecular rotary motor ATPase performs an incredible number of rotations at high speed and resiliency, orders of magnitude faster and more rotations than synthetic molecular rotors. Here I present our development of a DNA origami rotary motor designed for robustness and high performance. Our rotor consists of a bottom stationary origami-disk and a top rotor disk that are connected by a ss-DNA swivel that allows free rotation but prevents rotor dissociation. The rotor is propelled by two sets of bipedal walkers, fed by DNA Fuels and Anti-fuels that are provided by a computer-controlled microfluidics device, in a mechanism developed previously by our group for linear motors.[1,2] In contrast to the linear motor which dissociates from the track in the event of an operational error, the top rotary disk is irreversibly locked to the bottom disk by the swivel element, in principle, solving the issue of processivity. Rotation is monitored by imaging defocused light scattered from gold nanorods attached to the rotors. The strong and stable flux of photons allows high spatiotemporal resolution analysis of individual rotors for long time periods.[3] Rotors lacking the propulsion mechanism show semi-free Brownian rotation with transient parking, and dozens of legs placing and legs lifting reactions, and four controlled directional steps were demonstrated for the complete rotors. With our design we expect to demonstrate dozens of complete controlled unidirectional rotations at high speed.



(a) Design of the DNA origami rotary motor. **(b)** TEM image of the rotor. **(c)** Gold nanorod orientations in 3D and their corresponding theoretical defocused images. **(d)** Experimental setup consisting of the microfluidics system and the defocusing optical setup. **(e)** Defocused image of dozens of angle-resolved rotors' nanorods. **(f)** Four rotation steps of the rotor powered by the microfluidics-based fuel before anti-fuel mechanism[2].

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O-5

Isothermal self-assembly of multicomponent and evolutive DNA nanostructures

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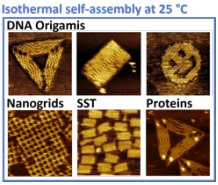
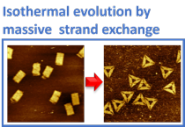


By exploiting the sequence-dependent base pairing principle between synthetic DNA single strands, DNA structural nanotechnology is a particularly powerful approach to program the assembly of hundreds of different components into elaborate functional superstructures. The flawless character of this assembly is usually ensured by a thermal annealing step where the DNA mixture is first heated above its melting temperature before being slowly cooled down to avoid kinetic traps and ensure proper sequence-specific DNA hybridization. Such a thermal treatment hinders any possibility for spontaneous nanostructure formation or evolution under fixed environmental conditions. It also leads to energetically highly stabilized structures for which the dynamic actuation and transformation is challenging and typically relies on supplemental action on preformed objects. In this presentation, I will show that the major methods of structural DNA nanotechnology can now be operated by the same generic isothermal DNA self-assembly principle, leading to a breadth of user-defined elaborate DNA nanostructures that can be spontaneously formed at room or body temperature, keeping intrinsic reconfigurability and a capability of complete morphological transformation never achieved so far.



Using a generic magnesium-free buffer containing NaCl, I will show that a complex cocktail of hundreds of different DNA strands can spontaneously assemble to form DNA origamis of any desired shape, DNA nanogrids of extended dimensions and well-formed SST assemblies, while allowing the concomitant isothermal functionalization of these nanoscaffolds by proteins (top figure). In situ AFM allows us to follow the self-assembly process in real time and demonstrate that this self-assembly proceeds through multiple folding pathways, the system escaping kinetic traps until it reaches its equilibrium target structure. Finally, I will present some unique characteristics of this thermodynamically controlled isothermal assembly method, ranging from shape selection in a highly-multicomponent mixture of competing DNA strands to the first complete shape shifting of DNA origamis spontaneously evolving at constant temperature from one morphology to a radically different one by the massive exchange of all its constitutive staple strands (bottom figure).

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O-6

Optically responsive protein coating of DNA origami for antigen targeting

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Over the past decade, the interest of using DNA nanotechnology for medical applications has been increasing [1]. Although being biocompatible, several challenges have been described for DNA nanostructures, amongst others facilitating targeted delivery and ensuring stability against degradation and/or in salt depleted conditions. Previously, a variety of targeting techniques, as well as coating strategies for increased stability have been presented [2]. In this work, we were able to electrostatically attach a single-chain variable antibody fragment (scFvC) and bovine serum albumin (BSA) to DNA origami by constructing highly charged protein-dendron conjugates. Using this approach, a combination of antibody display, and coating effect can be achieved, with BSA acting as a 'camouflaging' agent, which has already been shown to enhance transfection [3]. Due to a photosensitive group integrated into the dendron, BSA could be released by UV irradiation, which resulted in binding of the origami structure to the antigen in a specifically designed plate assay. Therefore, we believe that this approach can provide a powerful tool in DNA origami targeting [4]. Furthermore, we have recently investigated electrostatic interactions between cowpea chlorotic mottle virus (CCMV) and several DNA origami shapes [5].

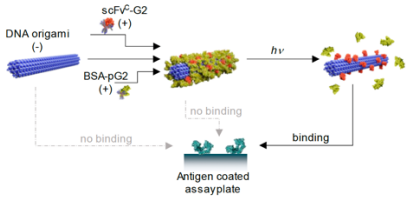

Figure 1: Protein-coated DNA origami structures are assembled by stepwise addition of positively charged protein-dendron conjugates (scFvC-G2, BSA-pG2). After UV irradiation, the 'camouflaging' agent BSA is released from the structure and binding to the antigen is enabled.

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O-7

Hybrid gold-DNA origami nanostructures for colorimetric sensing

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Colorimetric sensing based on plasmonic nanostructures allows the detection of target biomolecules using a simple optical readout [1] but requires ensemble measurements, limiting the overall sensitivity of this approach. To develop a colorimetric sensing platform compatible with single-molecule detection, we assemble gold nanosphere dimers on a 3D DNA origami that acts as a nanoscale actuator (Fig. 1-a). Indeed, DNA origamis are a flexible platform to produce nanostructures that shift their morphology when interacting with specific target molecules, such as DNA/RNA strands, proteins or specific cations [2]. To translate such conformational changes in colorimetric information, we exploit the nanoscale dependence of plasmon coupling between the two gold nanospheres. We recently demonstrated that darkfield microscopy allows the far-field monitoring of nanoscale distance changes in single gold dimers on a simple color camera [3]. The Y-shaped DNA origami scaffold features an active site with a conformation that can be tuned by hybridizing specific DNA single strands (Fig. 1-b). The overall morphology of the hybrid nanostructure is governed by the geometry of the DNA origami but also by steric and electrostatic repulsion between gold nanospheres. We observe that the conformation of the active site is only visible in the optical response of the nanostructures for high ionic strengths when these steric and electrostatic repulsions are reduced. Colorimetric sensing of DNA single strands is performed at high ionic strengths using a strand displacement reaction (Fig. 1-c). These measurements are performed both in single nanostructure scattering spectroscopy or by monitoring the hue of single dimers in darkfield images, providing similar statistical responses (Fig. 1-c) and opening exciting perspectives for the colorimetric sensing of individual DNA strands on a color camera.

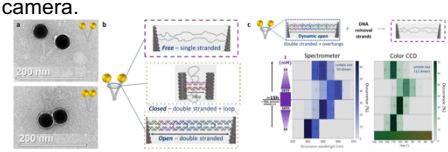

Figure 1: (a) EM images with negative staining of 40 nm gold particle dimers in open (top) and closed (bottom) conformations. (b) Schemes of the different conformations of the DNA origami active site and (c) strand displacement reaction monitored by single nanostructure spectroscopy (left) or colorimetric sensing (right).

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O-8

DNA-origami based diamond type lattice with visible wavelength periodicity

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Small DNA origami units can polymerize into crystalline lattices with periodicities on the scale of up to 100 nm [1-3]. These structures can serve as scaffolds for organization of particles or molecules [2,4], they can be modified by wet chemistry [5] or reductions [6] or could even be used as photonic materials if their periodicity could be expanded to length scales comparable with wavelength of light. The inverse diamond lattice is one of the crystal systems which can open the widest photonic band gaps and therefore a prime candidate for realizing DNA origami-based photonic crystals [7]. We have developed a tetrapod DNA origami which polymerizes into a directly rod-connected diamond lattice with a lattice constant of 160 nm. The tetrapod monomers co-crystallize with 24 helix bundle extensions into a lattice with a 400 nm unit cell. In both cases the crystallites grow to sizes on the order of several microns. We can dry the uncoated lattices or coat them with silica shells of various thicknesses to provide structural stability. We demonstrate that atomic layer deposition can cover the silica-coated lattice in a metal oxide.

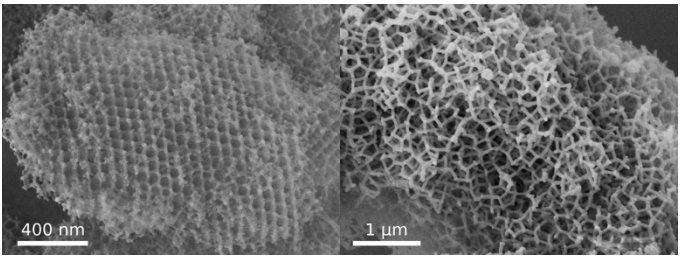

Figure 2 Left: Diamond lattice with 160 nm periodicity. Inset: model of the structure. Right: Co-crystalized lattice with 400nm unit cell.

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O-9

A dissipative pathway for the structural evolution of DNA fibres

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Biochemical networks interconnect, grow and evolve to express new properties through the continuous consumption and transformation of energy. Emulating this natural behaviour, we demonstrate herein that annealing *via* slow proton dissipation selects for otherwise inaccessible morphologies of fibres built from DNA and cyanuric acid. By developing new single-molecule fluorescence assays, we directly visualise and probe the elongation mechanisms of these fibres and conclude that proton dissipation (achieved using a reversible photoacid) changes the growth mechanism of supramolecular polymerisation. Just as the growth kinetics of natural fibres determine their structural attributes to modulate function, our system of photoacid-enabled de- and re-polymerisation selects for defect-free materials by healing gaps within our fibres, converting highly branched, interwoven networks into parallel nanocable superstructures. This work demonstrates the utility of out-of-equilibrium systems to steer the nanoscale assembly of materials, supporting new chemical methods of error-correction. The single-molecule methods established are easily adapted to study other supramolecular polymerizations and will enable an unprecedented understanding of these non-covalent assemblies.

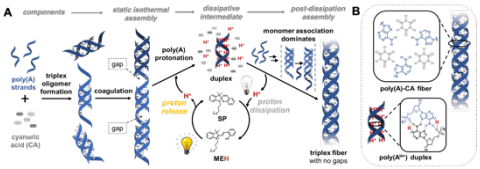

Figure 1. A light activated cycle of proton dissipation to regulate the assembly of fibre states. (A) Irradiation with white light releases protons from MEH that disassemble fibres to stabilize a dissipative self-assembled duplex. Upon light removal and proton dissipation, slow assembly to the fibre is observed. This structure has fewer gaps than the pre-irradiated state. (B) Legend showing the interior structure of each state.

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O-10

Orientation of dsDNA relative to graphene determined by single-molecule fluorescence lifetime microscopy

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Since its discovery in 2004, graphene has attracted the attention of the scientific community due to its unique electronic, optical and mechanical properties. While absorbing only 2.3% of visible light, graphene can act as a broadband, unbleachable acceptor system for non-radiative energy transfer from fluorescent dyes. The efficiency of this process follows a d^{-4} power law with a characteristic length scale of d_0 ~ 18 nm. Therefore, fluorescence lifetime or intensity measurements can be used to study the relative position of fluorophores with respect to the graphene surface.
Recently, our group has optimized a protocol to obtain high quality graphene-on-glass coverslips[2]. Moreover, we have used DNA origami nanostructures as chemical adapters to place fluorophores at predesigned distances on top of graphene coverslips and exploit the full potential of graphene energy transfer (GET)[1,2].
Here, we present our recent advances on studying the interaction between single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) with graphene through GET measurements. These interactions have raised great interest during the last decade, since the combination of graphene and DNA have been used for many applications in different research fields. Simulations have shown that dsDNA are expected to either stand perpendicular to the graphene surface, or in some cases lie on the carbon surface with its axis parallel to the graphene layer[3]. However, there is a lack of experimental evidence of these orientations, especially at the single-molecule level. Assisted by DNA origami structures, we immobilized hybrid ssDNA-dsDNA complex on graphene and observed by single-molecule fluorescence lifetime imaging that most of the dsDNA stand upright (Fig. 1). Inspired on this, we will exploit the perpendicular orientation of dsDNA with respect to graphene to develop DNA-based sensors, as well as to perform programmable movements on DNA origami structures.

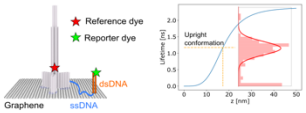

Figure 1. Left: Schematic of the experimental design. DNA origami structures assist the positioning of sparse ssDNA-dsDNA hybrid complex on top of graphene. Right: lifetime results from 119 single-molecule measurements, overlaid with the GET theoretical curve.

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O-11

New insights into the DNA origami silicification reaction mechanism by i*n situ* small angle X-ray scattering

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DNA origami allows for the formation of arbitrarily shaped nanostructures with nm precision control. However, many potential real-life applications have been hampered due to the instability of DNA origami in biological media, low salt buffers, in the presence of nucleases or at high temperatures. Silicification has been introduced as an excellent way of increasing the mechanical and thermal stability of DNA origami nanostructures as well as providing chemical protection [1-3]. However, so far, it remains unclear how silicification affects the internal structure of the DNA origami and whether the whole DNA framework is embedded or if silica just forms an outer shell. Especially for applications involving precise placement of guest molecules on/inside the DNA origami, this is essential knowledge. By using *in situ* small angle x-ray scattering (SAXS), we were able to show that silicification induces substantial condensation of the DNA origami at early reaction times (Fig. 1a). Contrast matching of the DNA double helix Lorentzian peak reveals that silica growth is not restricted to the outer origami surface, but also occurs on the inner surface, penetrating the whole structure. The observed condensation is partly caused by depletion forces as water is slowly displaced by silica within the origami structure. Towards the end of the reaction, the overall size of the silicified origami increases again, which is in accordance with increased shell thicknesses observed by TEM and AFM in previous studies [1]. Remarkably, we found that thermal stabilization of the origami up to 60°C could already be observed for sub-nm silica deposition in the highly condensed state (Fig. 1b). DNA origami objects with flat surfaces showed a greater tendency towards aggregation during silicification than origami with curved surfaces, likely also due to depletion forces. Our studies provide novel insights into the silicification reaction and allow for the formulation of optimized reaction protocols based on DNA origami shape and design.

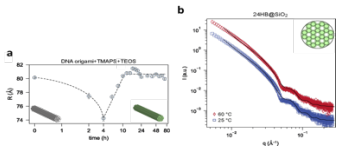

***Fig. 1 a**) the radius (R) of the overall cylindrical shape shows the extent of condensation over time during silicification. **b)** SAXS data show that even at sub-nm silica coating, structures remain stable when exposed to elevated temperatures for prolonged periods of time.*

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O-12

Tuning the insulin receptor signalling pathway response using insulin-DNA origami nanostructures

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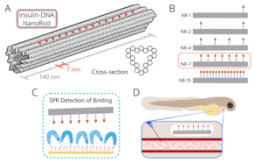
Insulin is a peptide hormone which promotes the uptake of glucose into cells through interaction with the insulin receptor[1] and impaired insulin signalling can contribute to the development of diseases such as diabetes[2] and cancer[3]. Many novel insulin-based therapies have been developed to remedy impaired insulin signalling yet, to date, none have factored into their design the nanoscale spatial organization of the insulin receptor on the cell membrane. Here we present insulin-DNA origami nanostructures which bind and activate the insulin receptor with varying strengths based on the designed spacing and stoichiometry of the insulin displayed (A). We found that binding and activation was optimal with insulin placed approximately 15 nanometres apart (B) and that the structures exhibit a strong avidity effect, with a dramatic increase in the residence time of the constructs as more insulin is incorporated on the structure (C). Furthermore, we found that this optimal insulin-DNA nanostructure strongly activated various transcriptional pathways associated with glucose metabolism and was able to decrease blood glucose levels in zebrafish (*Danio rerio*) lacking pancreatic beta cells (D). Our results thus indicate that ligand and hence receptor density is an important parameter for the activation of downstream signalling. Moving forward, our insulin-DNA nanostructures provide a useful tool for further interrogation of the insulin signalling pathway and can act as a starting point for the development of novel, targeted drugs for the treatment of diabetes.



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O-13

DNA sequence and length dictate the assembly of nucleic acid block copolymers

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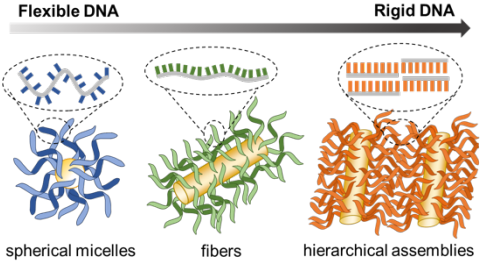
Attaching artificial oligomers to a DNA strand produces amphiphilic block copolymers. In water, the phase separation between hydrophobic and hydrophilic blocks produces diverse nanostructures. Here, we show that the sequence and length of single-stranded DNA directly influence the self-assembly of these sequence-defined DNA block copolymers. While increasing the length of DNA leads to predictable changes in self-assembly, changing only the sequence of DNA produced different structures, ranging from spherical micelles to fibres and networked superstructures. We rationalize these observations by showing that different single-stranded DNA sequences behave as different hydrophilic blocks – they have unique volumes, electronics, persistence lengths, and melting temperatures. These subtle variations translate into remarkable morphology and assembly mechanism differences, including a new pathway for the polymersation of DNA fibers, through a kinetically-trapped intermediate. DNA is often used as a programming code, driven by base complementary, that aids in nanostructure addressability and function. Here, we show that the inherent physical and chemical properties of single-stranded DNA sequences also make them an ideal material to direct self-assembled morphologies and select for new methods of supramolecular polymerization.



O-14

A DNA-based artificial membrane budding system

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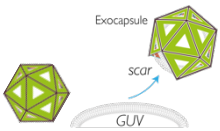
Scaffolded DNA Origami is the art of manufacturing well defined 3D-structures on the nanoscale using DNA as a building material1. A several kilobase long, single-stranded 'scaffold' strand of predominantly bacteriophage origin is mixed with an array of oligonucleotides of typically +/- 50 nucleotides in length and exposed to a thermal annealing ramp to drive the correct folding of the DNA origami structure. The programmability of Watson-Crick base pairing interactions has allowed the production of nanostructures ranging from simple brick structures1 to giga-Dalton sized multimeric assemblies2 by methodologically simple one-pot folding reactions. The relative ease of producing these nanostructures expands the experimental toolkit on the nanoscale and enables synthetic biology applications, among which we are particularly interested in means to manipulate lipid bilayers. Previous work demonstrated deformation of giant unilamellar lipid vesicles (GUVs) in response to Origami structures3,4. Using DNA origami triangles with the ability to self-assemble into icosahedral shells5, we aim to create a fully artificial lipid membrane budding system.Our triangular origami shares structural core properties with the cellular vesicle sculpting protein clathrin, namely the ability to form cage-like assemblies with a pre-defined curvature encoded within the structure itself. The programmability of DNA origami allowed us to easily functionalise our structure for lipid membrane tethering and fluorescence-based analysis. We show that self-assembly of membrane tethered triangles gives rise to DNA-lipid hybrid structures consisting of DNA exoskeletons enclosing vesicular cargo ('Exocapsules'). Both, GUVs and cells may serve as donors of lipid material and Exocapsules may thus contain lipid membranes of synthetic and natural origin. We found that a multitude of different membrane attachment moieties can be used to tether origami triangles to the lipid bilayer before assembly into Exocapsules. The size difference between lipid donors (> 1 µm) and Exocapsules (approx. 80-100 nm) suggests a budding-like mechanism to be involved in the assembly of Expcapsules. In support of this hypothesis, we occasionally spot holes ('scars') in the DNA exoskeleton which we believe mark the putative site of the bud neck that formed during the assembly process. We expect our platform to find applications as a tool for the sampling and study of biological membranes.



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O-15

Designer DNA-based Membrane Nanopores for Portable Sensing of Diagnostic Proteins

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Membrane nanopores are key for molecular transport in biology, portable DNA sequencing, label-free single-molecule analysis and nanomedicine.1 Transport traditionally relies on barrel-like channels of a few nanometres width but there is considerable scientific and technological interest for wider structures of tuneable shape. Yet, these nanopores do not exist in nature and are challenging to build using existing de novo routes for proteins.2

To address this challenge, herein we explore the designability and functionality of DNA nanotechnology and develop a modular strategy to align tunable DNA helix-bundled subunits to form confined polygonal shapes, and generate a series of nanopore structures with well-defined sizes and geometries. By taking advantage of the rational functionality of these DNA nanostructures, we can precisely place the recognition site of a protein analyte inside the nanopore lumens, and the protein analyte can be directly detected by specific binding events. Finally, by optimizing the working conditions of the DNA nanopores, we demonstrate the utility and potential of the custom-engineered pores by direct single-molecule sensing of 10 nm-sized proteins with widely used research and handheld analysis devices.

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O-16

Thermodynamic and kinetic properties of DNA-confined enzymes

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DNA-based nanocompartments have been realized for the spatial confinement of enzymes, thus emulating a key strategy used by the cell to control its metabolism. When internally modified with protein-specific ligands, these structures typically show an increased binding affinity and an enhanced rate of substrate processing when compared to the same amount of enzyme in freely diffusive conditions. These data suggest a key role played by the DNA microenvironment around the protein, the origin of which is not yet fully understood. We use DNA origami tools to target this issue in detail, facing both the thermodynamic and kinetic aspects of the problem. We herein present our achievements until now on this topic and how we envision the use of multicompartment DNA systems as minimal models of protein factories that emulate specific steps of a biological process or even combine them in a way not existent in nature.

O-17

Reaction-diffusion patterning of DNA-based artificial cells

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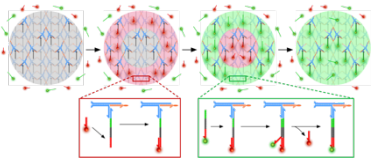
Developing compartmentalized functional molecular structures is a crucial step toward engineering artificial cellular systems. Here, we present our findings from using reaction-diffusion processes to pattern amphiphilic DNA condensates and show an example of application as a prototype membrane-less synthetic cell. We designed, produced and characterized micron-size functional units that are formed by self-assembly of amphiphilic building blocks consisting of DNA junctions with cholesterol tags. These DNA building blocks can host dynamic DNA circuits, which have been used to trigger disassembly, response cascades and interaction between different functional units [1,2,3]. In this project, we interface the condensates with a competitive DNA reaction-diffusion circuit, used to form up to 5 different compartments in a single DNA condensate. The components of the reaction-diffusion circuit competitively bind to single stranded DNA overhangs incorporated into the amphiphilic building blocks of the crystals. The pore size of the condensates allows shorter DNA strands (red strand in Fig.) to diffuse faster than longer ones allowing them to bind first to the overhangs (red box on Fig.). Longer DNA strands (green strand on Fig.) on the other hand diffuse slower but can displace shorter strands from the overhangs via toehold mediated strand displacement (green box on Fig.). The presence of diffusing strands of multiple lengths creates propagating concentric binding patterns in the DNA condensates (see Fig.). At any given time, these reaction-diffusion patterns may be stopped and maintained, by adding excess amounts of complementary DNA. Finally, we use this approach to create a core-shell patterning in DNA condensates where the core contains a DNA template for a fluorescent RNA aptamer and the shell contains binding sites for said aptamer. A T7 RNA Polymerase can enter and synthesize fluorescent RNA aptamers in the core, which then bind to the shell. Our approach can thus be used to create a highly simplified membrane-less synthetic cell.



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O-18

Temporal control of DNA strand displacement reaction

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Integrating DNA based systems with temporal controlled functionalities will expand our ability to develop biomolecular devices and materials with similar features to biological processes. Over the past decades, DNA has emerged as a powerful building block to construct multiple nanoscale structural and functional systems. In particular, toehold-mediated DNA strand displacement is one of the most used reaction to control functional DNA nanodevices.[1,2] Here, we propose an innovative strategy to temporally control the strand displacement reaction by rationally designing different blocker strands that prevent binding to the toehold domain. The blocker strands are also designed to be specifically degraded by an enzyme thus restoring the ability of the invader strand to induce a strand displacement reaction. This was achieved by employing two different classes of enzymes: an endonuclease (RNase H) and Base Excision Repair (BER) enzymes. RNase H is able to specifically degrade an RNA blocker strand hybridized to a DNA strand, while the repair enzymes (i.e. Uracil-DNA Glycosylase (UDG) or Formamidopyrimidine DNA Glycosylase (Fpg)) catalyse the release of DNA blocker strand by removing their specific mutated bases (deoxyuridine or 8-oxo-7,8-dihydroguanine). Our strategy is versatile, orthogonal and could be adapted to different systems. To show its potential we demonstrate the possibility to apply this approach for temporally control the activity of a downstream process like a cargo releasing receptor and a synthetic biochemical switching system.

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O-19

Electrically Directed Gene Expression (EDGE): using switchable DNA triplexes and electrolysis to modulate transcription in a cell-free medium

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The combination of DNA nanotechnology and cell-free gene expression has a wide variety of applications for biology and medicine, including novel therapeutics and diagnostics. We have developed a technique we call 'Electrically Directed Gene Expression (EDGE)', a method of using DNA triple helices as electrically controlled nanoswitches for regulation of transcription in a cell-free medium. Triple helices, or triplexes, are DNA nanostructures that are capable of the inhibition of gene expression [1], and can be designed to require specific pH conditions in order to form [2]. We induce electrolysis by applying a voltage to a cell-free transcription solution, which alters the pH, leading to a reversible conformational change in the triplex (Figure (a)). When the triplex is closed, transcription is inhibited, and we have been able to monitor this by measuring the fluorescence signal (Figure (b)) from the transcription product, a light-up RNA aptamer (iSpinach). Changing the design of the triplex enables us to tune the system to react as desired to electrical controls. By taking a modular approach, we have designed a combinatorial set of EDGE constructs, all of which will give different transcription dynamics.
EDGE is the subject of a patent application that was filed on 31st March 2022.

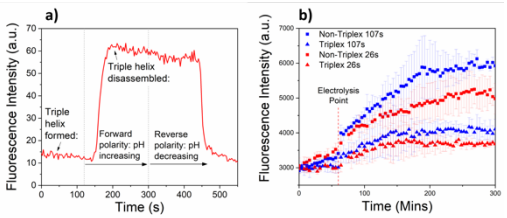

Figure a) Electrolytic control of triplex formation using Cy5/BHQ Labelled oligonucleotides. b) Electrolytic activation of transcription and triplex-mediated inhibition demonstrated using triplex forming EDGE constructs and non-triplex controls encoding the fluorescent aptamer iSpinach as a transcription product. Two different electrolysis durations are demonstrated (26s and 107s) to induce differing levels of pH shift.

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O-20

Orienting single molecules in DNA origami constructs

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

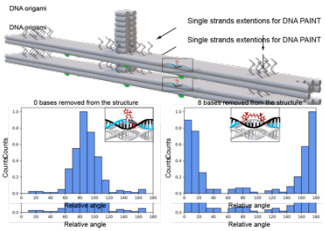

Fig 1. Different orientation of a fluorescent dye in respect to DNA origami 'host' structure obtained for the structures with a different number of removed adjacent nucleotides.

We present a method to both position and orient single photon emitters within DNA origami constructs. In particular, we exploit the ability of DNA origami to exert forces in order to "stretch" covalently incorporated dyes and deterministically align them with the orientation of double-stranded DNA helix they are located at (Fig 1.). We study the dye´s three dimensional orientation and wobbling using three independent techniques: polarization-resolved excitation measurement, point-spread function (PSF) analysis [3] and the four-polarization image splitting method [4] combined with a super-resolution (nanoscopy) measurement using the DNA-PAINT technique [5] to retrieve the orientation of the DNA origami "host" structure. Our results show that by simply removing a number of nucleotides adjacent to both ends of the doubly-linked fluorophore, the dye transitions from a non-predictable orientation given by a combination of external factors to an orientation aligned with the predesign direction of the host ds-DNA helix. We believe this work shows a simple way to deterministically orient dyes which constitute the last degree of freedom required to manipulate the interaction of single photon emitters and fully control the coupling to other species.

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O-21

Enzymatic DNA ligation within two-dimensional DNA origami depends on nanostructure shape

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DNA ligation is a workhorse of recombinant DNA technology being the enzymatic process allowing assembling long DNA sequences that encode for RNA and protein synthesis in microorganisms. Furthermore, it has been applied to stabilise DNA nanostructures, despite it being generally known to be less efficient in folded/ branched DNA architectures due to steric hindrance.

In this work, we investigated the mechanism of action of T4 DNA ligase within the sharp DNA triangle, a two-dimensional (2-D) DNA origami formed by three, identical trapezoids. We focussed on the ligation of three, consecutive DNA staples within a trapezoid and analysed ligation end-products by using fluorescence labelling and gel electrophoresis. By generating nanostructure variants comprising only one or two trapezoids, and a crop of a trapezoid, we demonstrate that T4 action strongly depends on the shape of the 2-D DNA surface and is greatly enhanced in the smallest variant. Furthermore, we varied the mechanical stiffness around the three ligation sites by introducing localised defects in the DNA triangle, demonstrating it has only minor effects on the action of T4.

Our findings isolate the different effects of (local) steric hindrance and (global) nanostructure shape on T4 ligation. To explain this new geometric contribution, we hypothesise that enzyme molecules bind weakly to the surface of the DNA nanostructure and reach the ligation sites by carrying out 1-D diffusion following folded, 2-D patterns within the DNA surface. These unprecedented results may find applications in the manufacturing of nucleic acid for pharmaceutics and DNA-based information storage devices.

O-22

Bio-inspired DNA switches for sensing and drug delivery applications

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Natural nanomachines rely on biomolecular switches, biomolecules that undergo binding-induced changes in conformation or oligomerization to transduce chemical information into specific biochemical outputs. In order to understand the design principles of these switches, we have developed a synthetic biochemistry approach, which consists in re-creating complex biochemical systems using a simpler, programmable polymer such as DNA. Using this strategy, we have recently re-created several complex biochemical mechanisms based on allostery, configurational cooperativity, and Le Chatelier's principle in order to understand their thermodynamics and design principles. In my talk, I will explain how a better understanding of these mechanisms significantly helps our efforts to build better switches and nanomachines (1) for applications ranging from sensing (1,2) to drug delivery (2,3).

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O-23

Probing the conformational dynamics of long unstructured single stranded DNA chains

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Despite the availability of accurate modeling tools for bio-molecular interactions at atomistic scale, a quantitative description of the heterogeneous conformational dynamics of long bio-polymers remains elusive. The characterizations of natively unfolded proteins and of the tertiary structure of very long single stranded DNA or RNA chains are common examples of this challenge [1]. Here we present a combined computational and experimental study aiming at identifying the most accessible regions of an unstructured long single stranded DNA chain with a small fraction of double stranded regions at room temperature. We exploited a multi-scale computational analysis on the conformational dynamics of the bacteriophage M13mp8, a single stranded sequence with a length of about 7000 nucleotides widely exploited as a scaffold for DNA origami structures [2]. We predicted the secondary structure topology through the calculation of the base pairing probability matrix. Then, for each single stranded region we simulated the molecular conformations by the nucleic acids dedicated software OxDNA [3]. We selected the M13mp8 regions with the largest conformational dynamics and designed 20-mer DNA probes complementary to these sequences in order to experimentally test their accessibility. We measured the ability of 12 probe strands to capture the M13mp8 in different conditions of temperature and ionic strand by a label-free optical biosensor [4][5]. The experiments confirmed the predictive capability of the computational study. Four of the selected probes emerged as strong binders of M13mp8 and one displayed exceptional binding capabilities. The observed behavior indicates a persistent order in the dynamical structure of the long sequence. These results pave the way to the design of optimal probes for the binding of mRNA or viral genome at room temperature without enzymatic amplification [6].

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O-24

DNA Origami nano-patterns as a Precise Tool to study clustering of Notch receptor

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Notch signaling pathway is a cell-cell communication system with fundamental roles in embryonic development and the nervous system, including neural stem cell proliferation and differentiation. Different Notch ligands can activate distinct target genes, which can further lead to different cell fates. It has recently been shown that DLL1 and DLL4 ligands activate their targets in a different manner that probably involves ligand receptor clustering. In our study we used DNA origami nano-patterns, to investigate the spatial organization of Jag1 ligands to the activation of Notch pathway. Site specific conjugation method was used to display proteins at well-defined positions on a 140nm nanotube made by DNA. Structural and functional characterization achieved by gel retardation assay, transmission electron microscopy (TEM), DNA paint and Surface Plasmon Resonance (SPR). In order to investigate the role of spatial regulation on Notch signaling, we treated iPSc-derived neuroepithelial stem-like (lt-NES) cells with Jag1 nano-patterns at various spatial configurations in solution. NES cells, that resemble early neuronal cells in the humans, were treated with equal amounts of dimers, tetramers, hexamers and octamers of Jag1 nanocalipers. The resulting canonical Notch signaling activity was analyzed by RNA sequencing and real-time qPCR. Our data indicate that higher orders Jag1 nano-patterns activate Notch pathway, suggesting that clustering can increase Notch activation. Hence, “DNA origami nano-patterns” can serve as a controllable tool to investigate microenvironment interactions in solution and enlighten signaling events crucial for the development and relative diseases.

O-25

DNA-enzyme neural networks enabling nonlinear concentration profile classification

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As the field of molecular diagnostics is maturing, the integration of multiple molecular clues is determinative to profile and stratify complex diseases such as cancer. One classical strategy is to use multiplex assays (e.g. biochips or NGS-based approaches) to measure accurately each of these markers independently and reconstruct the profile from data analysis. Another approach, explored here, consists in designing molecular circuits capable of concentration pattern recognition [1, 2]. These circuits, architecturally comparable to neural networks, take concentrations of many markers as input and process this information through a series of cascaded and interconnected reactions, yielding sample classification according to the network structure and parameters. We here present DNA-encoded enzymatic neurons with programmable weights and biases that emulate perceptrons, computing the weighted sum of input concentrations and returning an ON or OFF signal according to a build-in nonlinear activation function [3]. We built a majority voting algorithm that works on 10-bit input patterns, demonstrating accurate and straightforward weight equalization and bias adjustment. We finally assembled these neurons into multilayer architectures that perform pattern recognition over nonlinearly separable data.

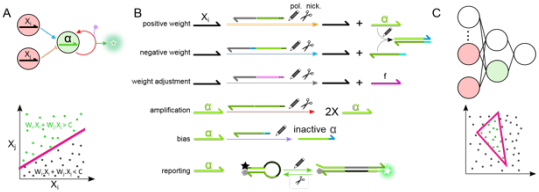

Figure. (A) A DNA-encoded enzymatic perceptron performs linear classification. (B) Biochemical implementation of each perceptron component. (C) Assembled into multilayer perceptrons, molecular neural networks allow nonlinear space partitioning.

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O-26

Counting individual molecules: DNA nanostructures for diagnostic applications

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Rapid and low-cost detection of disease biomarkers is becoming increasingly important in modern healthcare where a growing focus is placed on early diagnosis. The ability to detect small concentrations of biomarkers in patient samples is one of the cornerstones of modern healthcare. In current state-of-the-art biosensors for such clinical applications, an antibody or antibody mimetic is used to capture relevant biomarkers in the sample, and in general, each antibody–biomarker interaction contributes a small amount to the accumulated assay signal. However, the individual immuno-interactions cannot be identified anymore and only manifest themselves as part of this ensemble-averaged signal. Arguably, the ability to detect biomarkers with single entity resolution rather than via ensemble-averaging techniques would provide significant advantages for the detection of ultra-small biomarker concentrations. Of the many single molecule methods which have been developed over recent years, the use of nanopores, single molecules translocate through the nanoscale pore causing a momentary modulation in the otherwise steady ion current, is a promising approach. However, it is very difficult to detect specific molecules in complex mixtures as the signals resulting from different proteins are generally very similar and thus difficult to differentiate. Here, we present a biosensor platform using DNA origami featuring a central cavity with a target-specific DNA aptamer as carriers for translocation through nanopores, which enables individual biomarkers to be identified and counted to compile a sensing signal. We show that modulation of the ion current through the nanopore upon the DNA origami translocation strongly depends on the presence and in fact the size of a central cavity. While DNA origami without a central cavity cause a single peak in the ion current, DNA origami of the same dimensions but featuring a central cavity lead to double peaks in the ion current. We also show that the peak characteristics, in particular the peak amplitude and the dwell time, are different depending on the presence or absence of a central cavity. In this work, we exploit this to generate a biosensing platform capable of detecting human CRP in clinically relevant fluids.

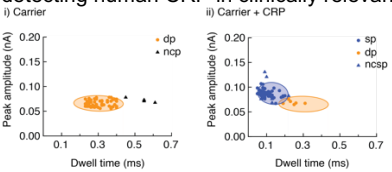

Figure: Scatter plot of translocation peaks for: (i) unoccupied carriers; (ii) carriers incubated with CRP. sp: single peak
dp: double peak
ncsp: not classified

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O-27

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

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

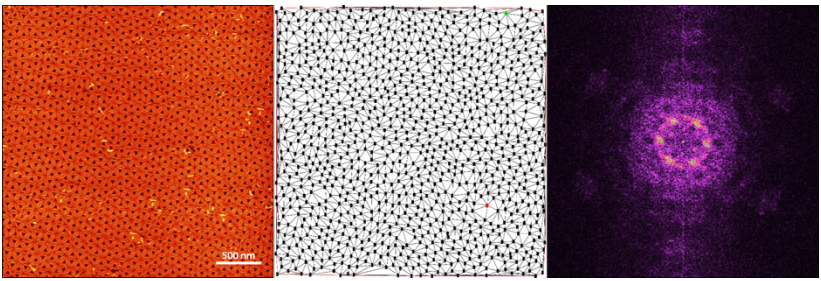

Fig. 1: Atomic force microscopy image (left), Delaunay triangulation (center), and Fast Fourier Transform (right) of a highly ordered DNA origami lattice on a mica surface.

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O-28

Kinetic proofreading in a DNA strand displacement network

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In many biosynthetic processes, the specificity of correct product formation is often orders of magnitude higher than that dictated by the free energy differences between correctly formed and incorrectly formed complexes. To describe this phenomena, Hopfield proposed a scheme called “kinetic proofreading”[1], where small free energy differences are utilised repeatedly over multiple steps in a reaction cycle to extract a large overall discrimination. A crucial component of the kinetic proofreading mechanism is the consumption of fuel to drive the reaction around the cycle; it is an inherently non-equilibrium (or kinetic) mechanism. Various aspects of synthetic nanotechnology like computational strand displacement cascades, tile assembly systems or diagnostics-based applications have tried to address the problem of recognition sites discrimination. Despite this, kinetic proofreading has not, to our knowledge, previously been demonstrated in synthetic systems. Here we introduce a non-enzymatic DNA-based kinetic proofreading method that efficiently discriminates between very similar recognition domains via a fuel-consuming cycle. We first characterise the synthetic proofreading mechanism in detail. We then demonstrate its application in a system in which sequence-specific dimers are catalytically produced from a pool of similar monomers with distinct recognition domains by molecular templates. The underlying templating reaction exploits a recently introduced topology called handhold mediated strand displacement (HMSD)[2]. Finally, we extend the system to the clinically relevant challenge of distinguishing between single nucleotide polymorphisms (SNPs). This highly desirable result arises because duplexes with mutated strands are converted into stable waste complexes by the fuel-consuming cycles inherent to kinetic proofreading.

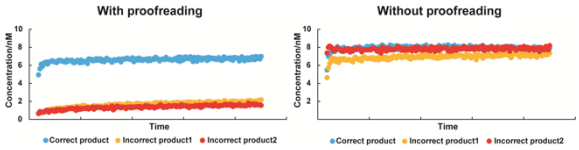

Fig. 1. An external reporter showing the formation of products with different monomers. When the proofreading fuel supply is present (left panel), only the monomer with a perfectly matching recognition domain is converted into the product in significant amounts, even in the long time limit. When there is no proofreading fuel (right panel), all the monomers form a similar amount of dimeric product.

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P-1

Synthetic protein-conductive membrane nanopores built with DNA

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University College London

Nanopores are key in portable sequencing and research given their ability to transport elongated DNA strands or small bioactive molecules through narrow transmembrane channels1-8. Transport of folded proteins or other big cargo could lead to similar technological benefits. Yet this has not been realized due to the shortage of wide and structurally defined natural pores. Here we report that a synthetic nanopore designed via DNA nanotechnology can accommodate folded proteins. Transport of fluorescent proteins through single pores is kinetically analyzed using massively parallel optical readout with transparent silicon-on-insulator pore-cavity chips as well as with electrical recordings to understand how diffusion vs electrophoresis influence transport in nanoconfinement. Pores allow a strikingly high diffusion flux of more than 66 protein molecules per second which can also be directed beyond equillbria when coupled with biomolecular recognition. The new pores may be exploited to sense diagnostically relevant proteins with portable analysis technology, to create molecular gates for drug delivery, or to build synthetic cells.

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P-2

Ion-dependent structural integrity and reconfigurability of DNA origami nanostructures

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DNA origami enables the self-assembly of custom-designed unique nanodevices and molecular breadboards. These DNA origami nanostructures offer the possibility of predictable arrangement on the nanoscale of DNA and other components, ranging from small fluorophores to large proteins. They are thus interesting platforms for a number of applications where spatial control is key, provided that their structure remains as designed and is not altered by the imaging solution or environment. Here, we studied the structural integrity and reconfigurability of DNA origami nanostructures triggered by a range of mono- and divalent cations. High-resolution techniques, i.e. AFM and FRET spectroscopy, were used to monitor both global and local structural responses of the DNA origami structures to the ionic environment. Our results show the performance of the origami structures in the presence of different ion types and concentrations and upon ion exchange. Moreover, we identified the existence of highly dynamic semi-folded states that exhibit self-healing properties. Our study aims at diversifying conditions where DNA origami structures serve as robust molecular platforms for enabling new applications.

P-3

Regulating in vitro transcription using RNA/DNA triplex-based biosynthetic machineries

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In vitro design of triplex nanostructures was successfully introduced in nucleic acid-based nanotechnology producing switchable mechanisms for sensing, smart drug delivery, and dynamic nanostructures control (1). For quite some time, triplex structures have been thought to play a role in vivo (2), and yet, a complete picture of their mechanism of action, including the identification of specific RNAs, is missing. Recently, the study of long non-coding RNAs (lncRNAs), a class of RNAs with several regulatory functions, revealed their involvement in triplex formation in vivo and its effect in gene expression (3). In this report, a biosynthetic transcriptional regulation system was developed, in which several bacterial promoters were designed to contain one or two sequence-specific targets for ribonucleic triplex forming oligonucleotides. The promoters were developed using as a template the general E. coli promoter architecture (4), resulting in a universal approach, potentially applicable to any promoter. The transcription rate was measured using an innovative method involving a transcription unit for the fluorescent RNA aptamer Broccoli (5). The artificial promoters were placed upstream of the Broccoli template and their modulation was operated by the formation of RNA-DNA triplex structures in different positions at the promoter site. The fluorescence emission associated with the formation of the RNA aptamer was used to monitor the transcription rates. This work is expected to shed light on the involvement of RNA/DNA triplexes in gene expression modulation while it provides a general approach for studying and testing triplex sequences in vitro.

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P-4

Spatial inference of barcoded transcripts from sequencing data

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The development of sequencing technologies in the past decades has allowed for more detailed analysis of biological tissue than ever using single-cell sequencing. The field of spatial transcriptomics aims to provide further resolution by not only determining what transcripts are present in a tissue, but exactly where they are located. Although the field has been rapidly developing, most methods rely on elaborate microscopy setups, which limits resolution, number of targets, and requires expert usage.
Here we propose DNA microscopy, a technique where only sequencing information is used to infer spatial organization [1, 2]. Through a series of PCR steps, DNA or RNA molecules in a sample are barcoded and amplified. The amplicons are concatemerized with one another using overlap-extension PCR. Sequencing these concatemers reveals the adjacency between them which can be represented in a graph. Depending on the experimental setup, different reconstruction strategies can be used to obtain a visual reconstruction based on this graph. In addition, changing the PCR conditions can change the properties of the resulting graph which will affect the reconstruction. The method has the potential to visualize the transcriptomes of tissue slides and cells without relying on microscopy.

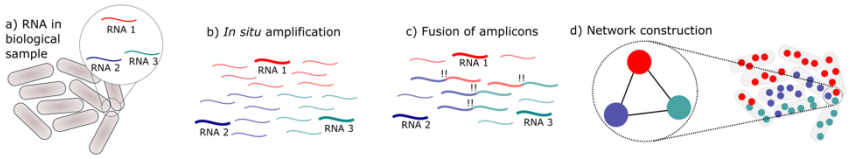

Figure 1. DNA microscopy reconstructs images of RNA distribution using only sequencing information. a) In a biological sample, three different RNA molecules are targeted. (b) Conversion of RNA to cDNA and their amplification *in situ* produces amplicons that diffuse around their origin. (c) These amplicons can overlap with one another, and form fused amplicons called concatemers. (d) Sequencing the fused concatemers will reveal which RNAs are adjacent to one another, as represented in a graph. (e) The graph is then used reconstruct an image of the RNA distribution.

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P-5

A Light-Actuated DNA Channel for Controlled Transport Across Membranes

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Nanopores represent the most relevant border crossings in biological cells.1 Fabricating advanced nanopores for controlling transport upon external triggers such as light are of considerable interest in basic science and technology as biological photo-gated channels have a fragile chemical nature and are difficult to engineer.2 Here, we use the powerful design tools of DNA nanotechnology3 to fabricate a synthetic nanochannel4 with a light-controllable molecular valve. The synthetic channel self-assembles from six DNA strands and a lid strand featuring light-switchable azobenzene moieties5,6 to allow wavelength-dependant reversible opening and closing of the gate. Our results showcase successful assembly, bilayer incorporation, and selective actuation of the channels for tunable cargo transport. Our light-triggered pore may be used in biosensing, synthetic biology, and targeted cell killing.

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P-6

CryoEM structure determination using DNA nanotechnology

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The signal-to-noise ratio is the fundamental limiting factor when performing single particle analysis. The particle's size ultimately confines it, and its atomic scattering factor values how much contrast the particle can produce. The images have a fundamental noise level resulting from the limited electron exposure before radiation damage becomes too severe. Based upon physical considerations, Richard Henderson estimated in 1995 that single-particle analysis would not resolve the structure of samples with molecular weights less than 38 kDa [3]. Furthermore, based on the performed experiments, it is complex and challenging to determine the structure of particles smaller than 100 kDa using cryoEM. Using an imaging scaffold, such as proteins, can overcome this problem, as Yuxi Liu and colleagues did in 2019 [1].
Protein production, purification, and adjusting buffer conditions such as pH, salt concentration, and temperature variations have become the bottleneck when we use it as a tool for increasing sample size [2].
It is possible to utilize DNA origami instead of protein scaffolding because DNA origami folding and purifying processes are fast, efficient, economical, resistant to a wide range of conditions. Therefore, it provides us flexibility in choosing the target for the structure determination.
We have designed a DNA origami stage with a molecular weight of around 800 kDa and use cryoEM to get insight and improve its design iteratively. The identical DNA origami stage was employed in our studies to image the DNA aptamer and DNAzyme under varied buffer conditions. Besides this project's ultimate goal, we can determine some noncanonical DNA conformations structures like G-quadruplex.

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P-7

Neuronal targeting with functionalized tetrahedral DNA nanostructures

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The development of therapeutics for neurological disorders faces many challenges, among which are ineffective brain entry, unspecific cell targeting and low cell uptake [1]. Using principles of DNA nanotechnology for seamless bottom-up self-assembly, we are engineering a novel multifunctional tetrahedral DNA nanostructure (TDN) aiming at promoting three outcomes: an effective brain delivery, an active neuronal targeting and neuroprotective therapeutic effect. In the present work, we have functionalized TDNs either with neurotrophin-mimetic aptamers or with peptides and evaluated their potential for neuronal cell targeting and uptake. TDNs were produced by self-assembly of four single-strand oligonucleotides. The functionalization with the ligands was accomplished through direct hybridization of TDN anchors with a reverse complementary DNA extension (added to the aptamers or conjugated to the peptides). Structural characterization was performed by polyacrylamide gel electrophoresis (PAGE), dynamic light scattering (DLS) and atomic force microscopy (AFM). Neuronal cell targeting potential of nanostructures was evaluated in both neuronal and non-neuronal cell lines, as well as primary neuronal cortical cells (NCC), by confocal microscopy and flow cytometry. TDNs were produced in high yields and precisely functionalized with three RNA aptamers or with three peptide-DNA conjugates. Aptamer-functionalized TDNs (TDN-Apt) showed an enhanced cell association in neuronal cell lines expressing a specific neurotrophin receptor, in comparison to TDNs or scrambled aptamer-functionalized TDNs (TDN-scrApt). Accordingly, low cell binding was observed when using non-neuronal cells. With peptide-functionalized TDNs, no differences in comparison to TDNs were obtained. In primary NCC, a similar tendency to neuronal cell lines was observed. The enhancement on neuronal cell uptake of TDN-Apt relative to TDN reveals potential to be further explored as a delivery vector for nucleic acid (NA) drugs. Work is on-going regarding TDN-Apt functionalization with NA drugs. The proposed system will be further characterized, aiming to translate it into a unique and efficient nanotechnology to promote brain delivery, neuronal targeting and neuroprotection.

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P-8

DNA Assembly of Modular Components into a Rotary Nanodevice

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Rotary motors are a crucial part of the molecular machinery of cells and support and sustain vital biological functions.1 The bacterial flagellar motor for instance is a rotary machine that self-assembles from intricate modular components. It resembles a two-cogwheel gear system that can perform bidirectional rotations to control the migration behavior of the bacterial cell.2-4 These and other examples of supramolecular rotation in nature provide inspiring insights into the creation of synthetic molecular devices, which exhibit biomimetic functions with the potential to go beyond limitations of natural systems. For instance, integration of non-biological modular components, i.e., nanocrystals, carbon nanotubes, arbitrarily shaped DNA origami structures, etc., can substantially enlarge the degrees of freedom to design artificial dynamic systems and small-scale robotics with tailored optical, magnetic, electrical, and other properties. In this work, we demonstrate a DNA-based, self-assembled planetary gearset nanodevice, comprising multiple rotary modules that are compactly linked together (Figure 1). Our rotary nanodevice consists of three modular components, small origami ring, large origami ring, and gold nanoparticles (AuNPs). They mimic the sun gear, ring gear, and planet gears in a planetary gearset accordingly. These rotary modules are tightly orchestrated to work cooperatively and yield programmable bidirectional rotations powered by DNA fuels. We demonstrate the rotation mechanism of the rotary nanodevice on a single-particle level by direct imaging using Transmission Electron Microscopy (TEM) and optically track the rotation dynamics of the devices using fluorescence spectroscopy in real time. The experimental results are further supported by theoretical calculations. This work will shed light on and advance the rational design of modularly assembled artificial nanomachinery for executing complex tasks on the nanoscale.

Figure 1. Schematic of the rotary nanodevice. Schematic of the DNA-assembled rotary structures and their modular components, which consist of small (dark gray) and large (light gray) DNA origami rings as well as AuNPs (brown and golden) and fluorophores (blue and green).

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P-9

Mechanically-modulated toehold-mediated strand displacement

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For over two decades, nucleic acid strand displacement reactions have been employed in a panoply of dynamic DNA nanotechnologies [1]. Ever more elaborate reaction topologies of toehold-mediated strand displacement (TMSD) have been developed to exquisitely control strand displacement rates for the purpose of realising specific functionalities/outputs (e.g. [2]). To date, however, the potential role of nanomechanical forces in moderating the process of TMSD has been unexplored. Here we show a novel approach to investigate mechanical modulation of TMSD in a simple reaction topology. By positioning the toehold domain of a substrate-incumbent complex inside a 'bubble' (see Figure), the adjacent non-complementary domain can be tailored (e.g., sequence, length, and hybridisation state) with the intention of inducing local tensile or compressive forces on the unhybridized substrate toehold domain. We explore whether the associated TMSD rate can be modulated by controlling these forces upon hybridisation with the complementary invader toehold domain, observing order of magnitude variations in line with mechanical reasoning. In addition, these bubbled toeholds exhibit a strong binding energy dependency (i.e., GC-content) on the TMSD/hybridisation rate.

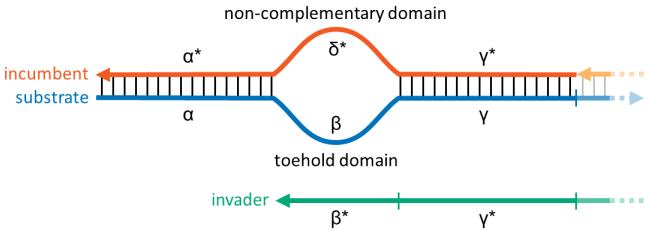

Figure. Tailoring the non-complementary domain adjacent to the toehold domain controls the latter's end-to-end distance, effectually inducing local longitudinal forces.

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P-10

Duplexed aptamers on fiber optic surface plasmon resonance sensors: a winning combination for continuous biosensing

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Continuous biosensing will revolutionize the field of diagnostics, as it enables real-time feedback, thereby overcoming the tedious and time-consuming steps that are currently required for biomarker monitoring [1]. To establish such a biosensor, several challenges need to be tackled, being that it (i) cannot rely on exogenous reagents or multistep processes, and should obtain (ii) a dynamic response and (iii) long-term stability [1-2]. In our previous work, we established one-step competitive assays for various molecular targets by implementing trans-duplexed aptamers (DAs) on fiber optic SPR (FO-SPR) sensors, that induced the release of gold nanoparticles (AuNPs) upon target binding [3], thereby overcoming the need for multistep processes. In our current work, we are implementing cis-DAs that induce the redistribution of AuNPs upon target (ATP) binding as presented in Figure 1A, to overcome the remaining need for exogenous reagents and thereby further improve our biosensor towards continuous measurements. Therefore, we first optimized the thickness of the gold fiber coating (currently 50 nm, optimized to maximize the sensitivity to refractive index changes), to enable coupling of the surface plasmons on the AuNPs and gold film, by matching their absorption spectra. This could be obtained by decreasing the gold thickness to 15 nm, as shown in Figure 1B. Notably, the enhanced signal generated by this phenomena decays with the distance between the AuNP and gold film on the nanometer scale, and therefore, a significant signal could be obtained due to the spatial redistribution of AuNPs on FO-SPR sensors. This can be seen in Figure 1C, which shows the real-time FO-SPR signal obtained during DA formation (binding between the aptamers and aptamer complementary elements), both for a fiber coated with a gold layer of 15 and 50 nm, respectively. Altogether, by applying cis-DAs to initiate the redistribution of AuNPs on an FO-SPR sensor, a real-time and significant signal could be obtained without external reagents or multistep processes, which renders our concept suitable for continuous measurements. In the future, we will characterize the response of our sensor to ATP binding, its reversibility and long-term stability.

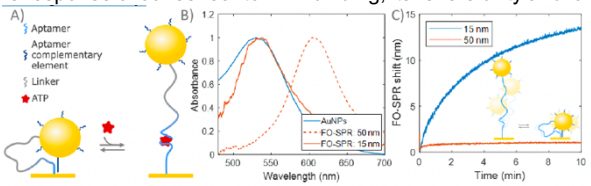

Figure 1. A) Schematic representation of the cis-DA that initiates the redistribution of AuNPs on an FO-SPR sensor in presence of ATP. B) Absorption spectra of the AuNPs (20 nm) and FO-SPR sensors coated with a gold layer of 50 or 15 nm, respectively. C) Real-time FO-SPR shift obtained during DA formation (binding of the aptamer and aptamer complementary element).

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P-11

Novel vancomycin-conjugated DNA origami-based nanoantibiotics

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Antimicrobial resistance has become a global burden because of the uncontrolled and increased use of antibiotics. Vancomycin is a cell wall-targeting glycopeptide antibiotic considered as a drug of last resort against various Gram-positive pathogens. However, since the 1980s, vancomycin resistance has spread around the globe and is now commonly observed in nosocomial infections [1,2]. Previous studies have shown that vancomycin susceptibility can be restored in such pathogens by the multivalent presentation of vancomycin on nanoparticle surfaces [3]. In this study, we use DNA origami nanostructures to display defined nanoarrays of vancomycin molecules and investigate the effect of geometric vancomycin arrangement and vancomycin density on antibacterial activity. To this end, azide-modified vancomycin was coupled to selected amine-modified staple strands via copper-free click chemistry and incorporated into 2D DNA origami triangles (Figure 1). Minimum inhibitory concentrations (MICs) against Bacillus subtilis were determined and show a clear effect of oligovalency, with larger numbers of vancomycin molecules per DNA origami resulting in lower MIC values compared to free vancomycin. DNA origami-based nanoantibiotics displaying optimized vancomycin nanoarrays thus have the potential to overcome vancomycin resistance in bacterial pathogens.

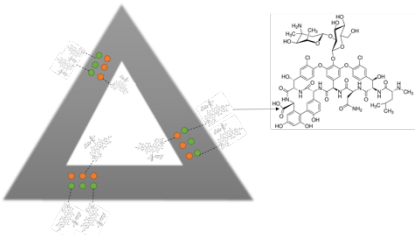

Figure 1: Schematic representation of the 2D DNA triangle origami presenting a vancomycin nanoarray.

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P-12

Single biomarker detection with affimer conjugated DNA origami through solid-state nanopore

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Biomarkers are molecules that can be used as the prognostic indicator of a disease, different biomarkers are associated with different diseases and can be easily obtained from patient's biological fluid such as blood. Various technologies have been and successfully used for the detection of these biomarkers, however, single molecule resolution detection of these biomarkers cannot be distinguished as they only manifest as part of the ensemble-averaged signal. To address this, we propose to use DNA origami functionalized with affimers to capture protein biomarkers of interest from fluid then followed by single molecule detection with solid-state nanopore. The high structural stability of DNA origami makes it ideal as a carrier and can be detected with solid-state nanopore [1], protein affimers are small proteins (<15 kDa) that are highly specific to protein targets at the nanomolar range and can be easily conjugated to the DNA origami via amine-reactive chemistry. Here, we show preliminary translocation results of these proteins target bounded affimers conjugated DNA origami. The translocation signals of these DNA origamis can be further enhanced with our unique polymer electrolyte bath system [2,3]. In the future, multiple DNA origamis with different affimers will be stitched together to form a DNA origami ribbon, this can potentially capture multiple different biomarkers in the same biological fluid and achieving multiplexed detection.

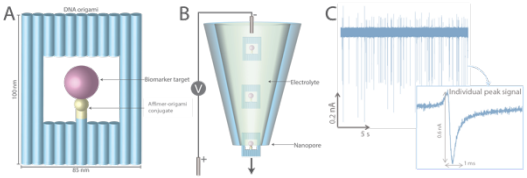

Figure. A) The design of the biomarker-affimer-DNA origami conjugate. B) The schematic of the nanopore experiment. C) The translocation data of the DNA origami.

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P-13

Tunable-gain amplifier in DNA-enzyme reaction circuits and its applications in microRNA biosensing

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Nucleic acid detection classically relies on an amplification reaction that exponentially replicates the target sequence or a signal sequence (e.g. PCR, LAMP or RPA, Figure A, left). Although exponential assays can achieve exquisite sensitivity, their quantitative version requires the monitoring of the amplification process in real-time, and uses expensive optical equipment. On the contrary, linear amplification reactions naturally preserve information on the initial target concentration, which can be extracted from the end-point signal only (Figure A, middle). However these systems (e.g. linear RCA) are poorly sensitive, which hampers their implementation in nucleic acid testing. Building on the PEN-DNA toolbox [1], we have recently reported a DNA-enzyme circuit for the sensitive detection of microRNA biomarkers [2], [3]. As for PCR, this isothermal amplification reaction is exponential, the target concentration being correlated to the amplification time in a real-time format. In this work, we leverage the modularity of these circuits and add a linear amplification module downstream of the exponential module (Figure B). This linear module converts the exponential amplification start times back to the concentration domain and allows for quantitative end-point measurements (Figure A, right). The amplification gain and saturation are easily tuned by modifying the concentration of the linear module (Figure C), and its associated fluorescence reporter, respectively. This additional feature allows for sensitive microRNA quantification using a simple end-point measurement (Figure D-E).

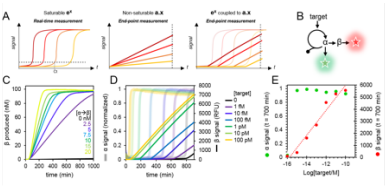

Figure. (A) The coupling of a sensitive exponential amplification (left) with a linear amplification (middle) allows to convert a real-time assay into an end-point readout (right). (B) Structure of the DNA-enzyme network implementing a tunable-gain linear amplifier. (C) Adjustment of the linear amplification gain by tuning the concentration of linear template α→β. (D) Real-time signal for various concentrations of a target microRNA (let-7a). (D) While the end-point readout of the exponentially amplified DNA strand is poorly informative of the target concentration, the linear amplifier allows to recover this information.

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P-14

Building an RNA-Based Toggle Switch Using Inhibitory RNA Aptamers

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Synthetic RNA systems offer unique advantages such as faster response, increased specificity, and programmability compared to conventional protein-based networks [1]. However, RNA systems also usually have limited functionality compared to proteins. Here, we use RNA aptamers capable of inhibiting the transcriptional activity of RNA polymerases to build an in vitro RNA-based toggle switch using [2,3.4]. In our toggle switch, a T7 promoter drives the expression of SP6 inhibitory aptamers, and an SP6 promoter expresses T7 inhibitory aptamers, creating the bistability. To monitor the activities of both polymerases simultaneously, we implemented Broccoli and malachite green light-up aptamer systems which have little overlap in their emission spectra. We show that the two distinct states originating from the mutual inhibition of aptamers can be flipped by adding short DNA oligonucleotides to sequester the RNA inhibitory aptamers. Finally, we assessed our RNA-based toggle switch in more cell-like setting by introducing controlled degradation of RNAs using a mix of RNases. Our results demonstrate that in vitro systems can be used to prototype novel biological circuits in more controlled environment.

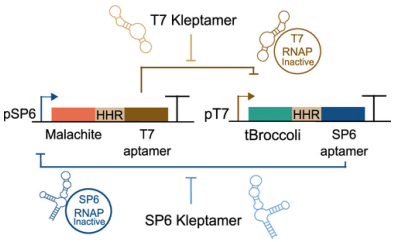

Figure 1. The schematic of the toggle switch. T7 inhibitory aptamers are transcribed by SP6 RNA polymerase while SP6 inhibitory aptamers are produced by T7 RNA polymerase. The mutual inhibition generates bistable states dominated by one of the aptamers. Short DNA oligonucleotides complementary to the RNA aptamers are added to sequester the RNA aptamers to flip the state of the toggle switch.

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P-15

Propulsion of magnetic beads asymmetrically covered with DNA Origami appendages

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Swimming at low Reynolds number requires a non-time symmetric stroke. [1] Two classic examples are provided by Nature: bacteria that move by rotating corkscrew-like appendages (flagella) and sperm propelled by whipping a flexible tail to propagate a sinusoidal wave. Using the latter example as inspiration, we fabricate magnetic microswimmers by combining magnetic beads and elongated flexible non-magnetic structures, in our case DNA origami six-helix bundles. We find that beads anistropically covered with bundles move ballistically when wagged by an external magnetic field whereas fully covered particles exhibit Brownian motion, to support the notion that motion is induced in a mechanism akin to sperm. This notion is further supported by the bead dynamics exhibited at a range of bundle coverages and driving frequencies in combination with the form of their velocity-frequency profiles. In addition, we find that when the easy axis of the magnetic beads is aligned with the origami coverage, the swimming direction follows the direction of the magnetic field. For future projects, combining magnetic particles with DNA origami could have application for in vivo/ex vivo targeted delivery.

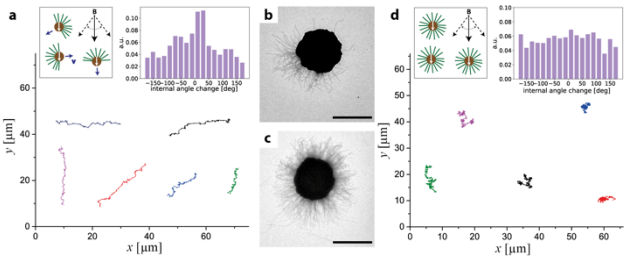

Figure: a Anisotropic covered particles wagged by an oscillating magnetic field display randomly directed ballistic motion shown by the internal angle change distribution and the raw tracks. b TEM image of an anisotropic covered particle used for the experiments in a. c TEM image of a homogeneously covered particle, used for the experiments in d. d Homogeneous coverage of magnetic beads results in Brownian motion, displayed by the flat angle distribution and the tracks showing random walks.

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P-16

DNAzymes for mass production of DNA oligonucleotides

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A recently developed phage-mediated production method of single-stranded precursor DNA allows for scalable fabrication of DNA nanostructures 1. In this approach a phagemid is constructed from DNA fragments of custom length and content interleaved by sequences of self-cleavable cassettes consisting of two class IIR3 DNAzymes2. After cleavage the resulting staples and scaffold strands can be purified and readily used for DNA nanostructure assembly. While this enables the generation of large quantities of separate DNA molecules at required ratios, each strand retains flanking scar sequences, i.e. overhangs with fixed sequences originating from the IR3 DNAzyme cassette. These “cleavage scars” present hurdles for making DNA origami with completely user-defined sequences. This issue could be solved by shifting the cleavage site of DNAzymes to their stem. DNAzyme are available that in principle would allow for cleavage with shorter scar fragments, for instance IIR1 2, but the large size of the catalytic core of these existing DNAzymes produces undesirable nucleic acid “waste” in mass-production methods. To address this problem here we developed an in vitro selection procedure to evolve self-cleaving DNAzymes from fully randomized space with selective pressure applied to the cleavage site shifted to the edge of predesigned stem. After multiple rounds of selection our pool contained DNA sequences that successfully perform the desired minimized-scar self-cleavage reaction in presence of Zn2+ under physiological conditions. The thus obtained DNAzymes can overcome issues caused by long scar carryover sequence in currently used method while having a smaller catalytic core compared to existing IIR1 DNAzyme.

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P-17

Cell surface-mediated conformational changes of DNA-Origami objects

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Targeted delivery of T-cells to cancer cells is of growing interest to the field of immunotherapy. While T-cell engaging therapies against cancer types expressing one cancer-specific surface antigen are highly effective, the number of potential single antigen targets is limited [1]. Additionally, nonspecific T-cell activation caused by the continuous availability of the T-cell binding site can increase side effects [1]. Detection of multiple surface antigens and site-directed, conditioned engagement of T-cells could help to expand the range of treatable cancer types. However suitable drug candidates capable of recognizing specific antigen patterns and conditioned T-cell engagement, are missing. Here we report the design of a DNA-Origami based pattern recognizing nanoswitch for the use as site-specific T-cell engager. We show specific binding of Fab-domain functionalized DNA-Origami objects to NALM-6 cells, investigate strategies to control cellular binding through the shape of DNA-Origami objects, and test a nanoswitch for its pattern recognizing capabilities using flow cytometry and FRET. Our results show that the DNA-Origami method can be used to build functional nanoswitches that can perform surface-induced conformational changes. We use this finding to design another nanostructure capable of encapsulating a T-Cell engaging antibody-fragment and performing conformational dependent binding to the CD3-antigen of JURKAT cells. Our findings pave the way for the design of highly specific surface antigen recognizing nanoswitches as a platform technology for usage in cancer immunotherapy.

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P-18

Valency and entropic costs determine the cation-mediated DNA/lipid binding

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Whether as a carrier of genetic information or a nanoengineered bioconstruct, the molecule of DNA performs its function in the presence of lipid membranes1. DNA activity is often tightly coupled with its interactions with lipids, and studying the fundamental principles of such intermolecular phenomena can provide a new landscape for nanoscale bioengineering and medical discoveries. We examined the electrostatics-based attraction between DNA and lipid bilayers, mediated by multivalent cations2. As DNA/lipid complexes target physiological conditions, where ions are an ever-present component of cellular media, electrostatic interactions are amongst the basic phenomena determining DNA activity3. We here describe the DNA/lipid ion-mediated attachment through the theory of multivalency, where the strength of interactions is dependent on the valency (number of phosphates of DNA) and the entropic costs (flexibility of DNA and mobility of lipid headgroups) of such binding. We developed a library of various DNA constructs and observed their behaviour in different environments, assessing the importance of the valency and dynamic parameters on the strength of cation-mediated attachment to model lipid bilayers. The results shed light on fundamental interactions between DNA and lipids, significant for synthetic DNA-based molecular machines, drug-delivery platforms and DNA-lipid emulsions, like vaccines, but also crucial for the investigation of Nature's principles guiding the biochemical machinery of all organisms.

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P-19

Virus neutralization using icosahedral DNA origami shells

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As the global SARS-Cov2 pandemic of 2019 has shown, viral infections can become a serious threat to global public health and economy and present a big challenge for modern society. Since new viruses can emerge and spread quickly, having readily adjustable antiviral medications applicable for a broad spectrum of viruses would present a great advantage. In this work we show a number of icosahedral half shells, built from modular subunits that can function as a broad antiviral platform. The platforms are built from DNA origami subunits and can be modified using virus binding moieties consisting of DNA-protein conjugates.

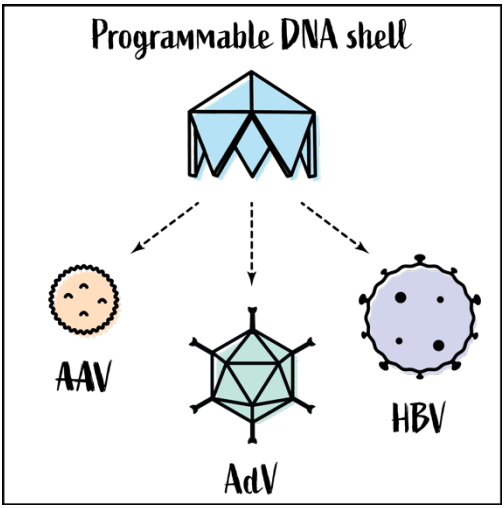

Fig. 3: Examples of viruses envisaged for capture

Using these components, the virus of interest can be identified and engulfed by the icosahedral DNA shell and is therefore inhibited to bind and enter its host cells.
These virus binding entities can be easily exchanged, and therefore the virus trapping shells adjusted to the virus of interest.
By stabilizing these constructs using UV irradiation and PEG-poly-lysin coating, the DNA origami shells can be further stabilized to withstand in vitro and in vivo conditions. Subsequently, the neutralization capacity could be demonstrated in vitro by neutralizing Hepatitis B core particles and in human HEK293T cells which were exposed to the Adeno-associated-virus 2 and hepatitis B.

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P-20

WaffleCraft: Fully Automated Blocky DNA Origami Design Tool

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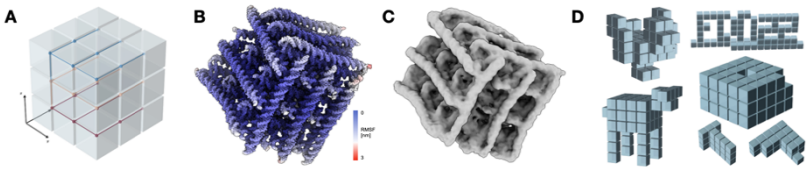
The topology of a DNA origami nanostructure has a strong influence on quality and yield of the final product [1]. Experts in the field are required to account for, among other parameters, staple-segment length distribution, sequence dependence, scaffold routing permutations, blunt-end passivation, and twist correction [2]. Crafting new high-quality nanoobject depends upon extensive knowledge and experience by the experimenter. We develop a new polydirectional design paradigm for DNA origami that enables encapsulation of both these aspects into the fully automated computational tool *WaffleCraft*. Our new lattice is based on a triangulated core cell composed of 105 scaffold bases. Helices of the core cell are oriented in three directions producing a compact and space-filling nanostructure. This polydirectionality allows the extension of the design in all three spatial directions, by attaching another core cell to one side. Importantly, the whole structure can be optimized by refining the core cell and defining a connectivity ruleset. This places the expertise for optimal quality and yield of the DNA nanostructure at the code provider rather than the user of the *WaffleCraft* tool. Our nanoobject drafting process is loosely based on the game principle of the videogame *MineCraft*, were individual blocks are placed next to each other to form larger structures. Here, a block corresponds to a core cell arranged on a cubic grid which in TEM images often resembles the appearance of a waffle. A matrix, designating the block placement and a scaffold strand sequence are the only required input. Within a couple of seconds, the software performs the scaffold routing, creates the topology and provides the optimal oligonucleotide sequences. Additionally, the tool provides suggestions about the optimal conditions for self-assembly and can generate initial configurations for a structure prediction run with the coarse-grained DNA model oxDNA [3]. *WaffleCraft* is distributed and developed as an open-source python application.



A) Cube representation of the input matrix of a 3x3x3 27 block design. Colored lines indicate the scaffold routing. **B)** OxDNA structure prediction colored by root mean squared fluctuation. **C)** Experimental cryogenic electron microscopy data at a resolution of around 10Å. **D)** Set of exemplary WaffleCraft DNA nanoobject designs.

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P-21

Precision Design and Characterization of DNA Origami Corner Motifs using Cryo-EM

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The methods of DNA nanotechnology enable the rational design of objects with user-defined shapes and functions for various applications. High-resolution cryogenic electron microscopy (cryo-EM) provides crucial experimental structural feedback to characterize and improve the de-novo created objects and general design rules to fully exploit the potential of the technology [1]. In the popular approach of multi-layer design, where helices are arranged parallelly to each other, corners are an important design motif to diversify the design space [2, 3]. Here, we present design strategies to build corners of various global geometric properties. We address the challenge of accurately designing the corner sites given the individual strand orientations of the helices. We characterize the implementations of the design strategies using the reconstructed electron density maps of multiple objects providing sub-nanometer structural details of motifs such as single-stranded loops and cross overs. In addition to the shape, we characterize the flexibility of corner designs using principal component analysis-based methods and show the effect of implementing angle braces as a method to customize the flexibility via rational design.

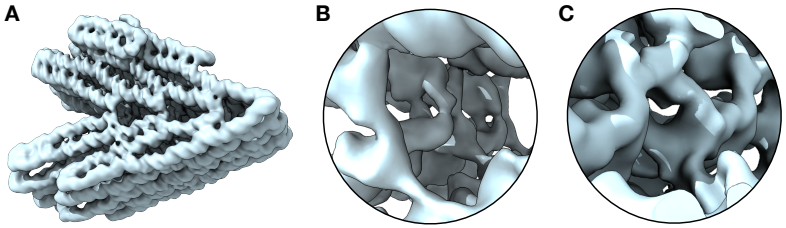

Figure 4 Strand orientation-specific corner design. (A) Cryo-EM density map of a 45° corner object. (B) and (C) Exemplary zoom-in views onto the corner sites of two of the four layers containing precisely designed loops and crossover design motifs.

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P-22

Reversible Supra-Folding of User-Programmed Functional DNA Nanostructures on Fuzzy Cationic Substrates

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Programmable DNA base pairing is a powerful way to build user-defined nanostructured assemblies, such as DNA origamis and DNA nanogrids. Although two-dimensional (2D) origamis of virtually any desired shape can now be quickly and easily produced, attaining further levels of organization, actuation or dynamics is still a desired challenge. Here we report that, upon adsorption on a soft cationic substrate prepared by layer-by-layer deposition of polyelectrolytes, both 2D origamis and DNA nanogrids undergo a rapid higher-order transition of folding into three-dimensional (3D) compact structures (origamis) or well-defined µm long ribbons (nanogrids), a process we refer to as suprafolding. We show that the suprafolding mechanism is mainly of electrostatic nature through onsurface inter-polyelectrolyte complexation, thus requiring enough fuzziness of the substrate to allow 3D reconfiguration instead of conventional flat adsorption. Interestingly the electrostatic nature of this actuation makes it reversible: once supra-folded, origamis can be switched back on the surface into their 2D original shape through addition of heparin, a highly charged anionic polyelectrolyte known as an efficient competitor of DNA polyelectrolyte compleaxation.

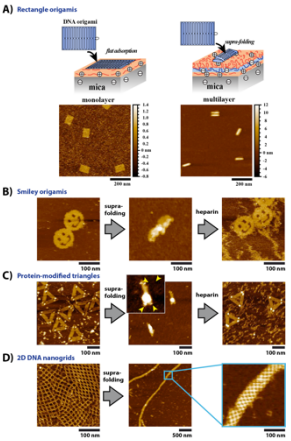

Figure 1. Reversible supra-folding of DNA nanostructures on cationic substrates.

Orthogonal to DNA base-pairing principles, this reversible structural reconfiguration is also versatile; we show in particular that i) it is compatible with various origami shapes, ii) it perfectly preserves fine structural details as well as sitespecific functionality, and iii) it can be applied to dynamically address the spatial distribution of origamitethered proteins.

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P-23

Control of enzyme activity by a DNA nanoscale robotic arm

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Nanomechanical DNA systems enable a high level of control to position and manipulate individual molecules [1]. These novel capabilities pave the way for precise and reversible control of enzymatic activity in contrast to typical biological enzyme regulation mechanisms. DNA nanorobotic systems were already employed to manipulate enzymatic activity by different enzymatic configurations on top of a DNA origami platform [2-4].
In a different manner, the use of split-enzymes [5] could provide the possibility of mechanical regulation of enzymes, as these enzymes are activated through the proximity of the enzymes subunits to reassemble an enzymatic active site. This would enable mechanical control of enzymes of interest at the interface of DNA nanotechnology and enzyme engineering. We provide a concept for the implementation of a reversibly switchable split-enzyme controlled by a DNA nanorobotic arm. We demonstrate a first proof of concept of the enzyme control by a DNA strand displacement reaction in a bulk reaction setup [6]. In the future we strive to employ different split-enzyme systems on top of this nanorobotic device and want to demonstrate mechanical control of an enzyme triggered by DNA strand displacement on a single molecule level. We thereby aim to study the enzymatic activity of individual devices using single molecule fluorescence microscopy using the enzyme activity as an active form of readout. The main challenge for our project lies in the identification of suitable enzyme candidates, which can fulfill the needs for single molecule analysis combined with the possibility for mechanical control. A suitable enzyme candidate for the verification of mechanical control combined with a single molecule capable readout of the enzymatic activity would ideally exhibit a high turnover rate combined with a fluorescent reaction product. In a final step we also want to show the ability of enzyme control in real-time using electric fields using our DNA nanorobot origami not only for the control of proximity but also real-time switching of the enzyme activity [7].

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P-24

Characterisation of RNA/DNA hybrid strand displacement kinetics

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Strand displacement reactions form the basis for most dynamic nucleic acid reaction networks. The kinetics of this core mechanism have been extensively and systematically characterised for DNA-based reactions [1]. Moreover, the kinetics of DNA/DNA strand displacement systems have been successfully captured in predictive models [2,3], facilitating the design and realisation of increasingly complex *de novo* reaction circuits which exploit this fundamental understanding [4].

While DNA remains a critical building material in nucleic acid nanotechnology, recent years have seen an increase in the number of designs exploiting RNA within reaction circuits [5]. The unique properties of RNA, including increased structural flexibility and catalysis, open up the field of dynamic nucleic acid nanotechnology to novel design functionalities [6]. Furthermore, the vital and fundamental intracellular roles of RNA, ranging from structural scaffolding to gene expression regulation, makes RNA an informative biomarker for many diseases and developmental disorders [7]. As such, an ability to effectively interface RNA within dynamic nucleic acid designs is becoming essential. Nevertheless, our current understanding of RNA/DNA hybrid strand displacement kinetics remains limited, with many reaction schemes relying on design principles derived from DNA/DNA strand displacement data.

Herein, we develop a continuous-time Markov chain model with the potential to predict the kinetics of RNA/DNA hybrid toehold exchange. We present experimental data of RNA/DNA hybrid toehold exchange kinetics across a range of common design parameters, including toehold length and branch migration domain length, which reveal subtle design trade-offs within such systems. We propose that our fully parameterised model has the potential to inform the rational design of RNA/DNA strand displacement systems and reveal more general design principles.

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P-25

Blowing "bubbles" with DNA origami

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Artificial lipid vesicles called liposomes are valuable tools for studying membrane proteins in native-like conditions and also have gained great utility as drug delivery systems, as in the case of mRNA vaccines against COVID-19 [1]. In vivo, lipid vesicles are in the size range of about 40 to 100 nm [2]. However, producing small controlled liposomes of precisely controlled size remains difficult. An intriguing method for size-controlled liposome production is the templated liposome formation on DNA origami scaffolds [3,4]. The DNA origami technique allows making nanoscale structures with defined shapes built from DNA [5] that can be modified with other biomolecules such as protein or lipid-molecules attached to specific position on a DNA origami structure [6]. Here we expended the original work [3] on templating liposome growth on DNA origami templates and show that lipid "bubbles" can be formed on a DNA origami that is directly bound on a streptavidin coated magnetic-bead based solid-support. This enables both purification of liposomes templated by DNA origami structures from excess of lipid-molecules as well as separation of lipid "bubbles" and DNA origami that can be released separately or combined by choosing appropriate strand displacement reaction.

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P-26

Cation-Responsive and Photocleavable Hydrogels from Noncanonical Amphiphilic DNA Nanostructures

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Thanks to its biocompatibility, versatility and programmable interactions, DNA has become increasingly popular as a building block for 2D and 3D nanoarchitectures and nanomaterials. Given the exploding interest in synthetic biology and nanomedicine, growing attention has been devoted to functional, stimuli-responsive DNA frameworks for applications in artificial cells, biosensing, tissue engineering and drug delivery, particularly in the form of DNA-based hydrogels [1, 2]. However, the applicability of such nanomaterials *in vivo* is still hampered by the inability of most DNA nanostructures to reversibly respond to physiological stimuli, such as cation concentration or pH. So far, indeed, most implementations rely on strand displacement, which use non biologically available, single-stranded DNA as input. Here, we present how combining cation-responsive DNA G-quadruplex (G4) structures with amphiphilic DNA constructs [3, 4] produces noncanonical nanostructures, termed "Quad-Stars" [5], capable of assembling isothermally into responsive hydrogel aggregates via a simple, enzyme-free, one-pot reaction. The embedded G4 structures allow us to reversibly trigger the assembly/disassembly by adding or removing K^+ ions. The hydrogel particles can further be photo-disassembled in a localised and irreversible way upon near-UV irradiation in the presence of a G4-binding porphyrin photosensitiser. The combined reversibility of assembly, cation-responsiveness, cargo-loading capabilities and biostability under model physiological conditions make Quad-Stars an excellent candidate for next-generation biosensors and responsive drug delivery carriers.

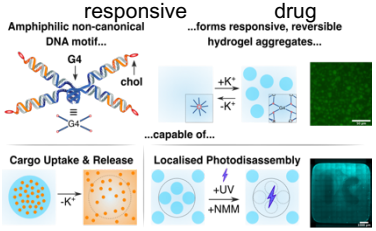

Fig. 1. Quad-Stars self-assemble in the presence of K^+ ions and disassemble upon their chelation thanks to the tetramolecular DNA G4 in their core. The amphiphilic hydrogel aggregates can uptake small hydrophobic molecules and release them upon disassembly. Near-UV exposure in the presence of a porphyrin photosensitiser further leads to guanine photo-oxidation and aggregate disassembly.

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P-27

Targeting antigen patterns with programmable T-cell engagers

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The development of the bispecific T-cell engaging antibody blinatumomab has been one of the big medical breakthroughs in cancer therapy in the last decade. This success has led to the development of many similar bispecific antibodies that are now in clinical research.[1] While highly promising, these approaches still possess significant drawbacks. For example, if the target antigen is not exclusively present only on cancer cells, on-target off-tumor lysis of healthy cells may be induced and result in strong sideeffects. [2,3] Additionally, addressing only one antigen limits the spectrum of potential molecular targets.[1] These issues may be addressed by targeting specific antigenpatterns instead of single antigens. Here we report the development of a DNA-Origamibased antibody platform that recognizes antigen patterns and cause directed T-cell mediated lysis of double-positive target cells. We investigated how design parameters and the antibody arrangement effects the on- and off-target lysis specificity using a variety of target antigens. Finally, with our current generation, we demonstrate more than a 15-fold decrease in lysis efficiency of single-positive bystander cells compared to double-positive target cells.

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P-28

Spatial reconstruction using barcoded DNA sequences

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Transcriptomics is a growing field that has formed a cornerstone in biological research. However, many transcriptomics methods with spatial information are limited to optics and can therefore only target a small selection of targets often in two-dimensional space. Inversely, sequencing techniques generate full transcriptomes do not contain spatial information. Here, we propose a method, in which a spatial information is generated *in situ* by DNA polymerization of modified barcoded strands. These amplified polymerase colonies (polonies) will be able to exchange information, encoding the spatial layout into DNA sequences[1][2]. This data is sequenced and could then be appended to transcriptomic sequencing data, allowing for full three-dimensional reconstruction of a sample including its transcriptomic information, without the drawbacks of optics.

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P-29
Colorimetric Visualization with Visible Chirality

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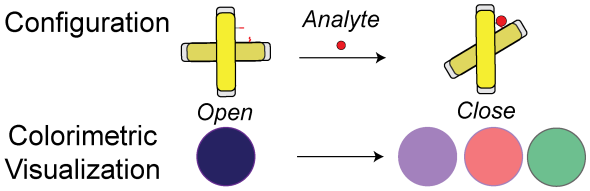
Chiral structures with pronounced optical activities could enable colorimetric readout and convenient naked-eye detection of chiroptical responses. DNA origami has been widely used to generate reconfigurable chiral plasmonic metamolecules (CPM) with dynamic chiral optical responses. However, reported optical activities, in terms of dis-symmetry factor, or *g*-factor, are typically below 2% and are usually detected using CD spectrometry, rather than colorimetric techniques. Although chiral nanoparticles could be fabricated to obtain a superior optical activity (*g*-factor of up to 20 %), it is currently limited to static structures.
In this work, I will present fabrication of CMP with a g-factor that is high, switchable (from 1 to 16 %), and tunable across the visible spectrum. Colorimetric visualization of CMPs with a high g-factor at sub-nM concentrations was achieved. Moreover, the dynamic change in the g-factor, which correlates with the special configuration of our CMP, was employed for constructing responsive plasmonic nanoswitches for molecular biosensing. Addition of target analytes brought a drastic color change that could be readily detected with the naked eye. The detection limit of a protein analyte was down to femtomole without additional signal amplification. We anticipate that our approach will advance the development of plasmonic sensing schemes and create opportunities for other applications based on reconfigurable plasmonics.



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P-30

A DNA-confined unfoldase/protease nanomachine

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Compartmentalization is a natural strategy of the cell to control biochemical processes in space and time.[1] Achieving high control over the spatial confinement and relative location of distinct molecular machines may offer a tool to engineer small protein factories that emulate specific steps of a biological process or even combine them in a way not existent in nature. In this project, the aim is to create a simplified model of a protein unfolding/degradation machinery that includes the ATPase p97 segregase[2] and one or more serine proteases confined into distinct DNA origami chambers linked in a row. We first show the high yield formation and full functionality of a ca. 10 MDa DNA-encaged p97 translocation machine that applies mechanical forces on a substrate molecule to segregate it from a protein complex and unfolds it by threading the peptide chain through the central pore of the p97 channel, aligned with the DNA cavity axis. Furthermore, we illustrate a systematic study of the impact of encapsulation and proximal polyanionic surfaces on the enzymatic activity of a chymotrypsin protease loaded into a second chamber.[3] The data show that chemical conjugation of the protein to the DNA strand, as well as the spatial confinement and local concentration of enzymes have an impact on the reaction rate. Activity assays at the bulk level demonstrated enhanced substrate turnover numbers for DNA nanocage-encapsulated chymotrypsin as compared with chymotrypsin in solution and offer a general platform to investigate protease activity regulation. Combination of the two DNA-encaged systems and control over the direction of substrate entry and exit is in course. We envisage that this study will lay the bases for the realization of semisynthetic nanomachines that, similarly to natural reaction cascades, may enable to perform distinct biochemical steps within spatially organized nano-compartments.

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P-31

Self-regeneration and self-healing in DNA nanostructures

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DNA nanotechnology and, in particular, advances in the DNA origami technique have enabled facile design and synthesis of complex and functional nanostructures.[1] These nanostructures - like all molecular devices - are, however, prone to rapid functional and structural degradation due to the high proportion of surface atoms at the nanoscale in complex chemical environments.[2] Besides stabilizing mechanisms, approaches for the self-repair of functional molecular devices are desirable. In this contribution, I will share our strategies to utilize the self-assembly nature of the DNA origami approach to establish self-regeneration and self-repair strategies for DNA nanostructures.[3] We use simple approaches that rely on the exchange of damaged building blocks with intact parts in solution to prepare self-regenerating fluorescence labels (Figure 1a) and self-healing nanorulers (Figure 1b). Finally, I will also discuss more recent results in applying these strategies to develop self-regenerating strategies to photostabilize DNA nanostructures used for super-resolution DNA-PAINT imaging.

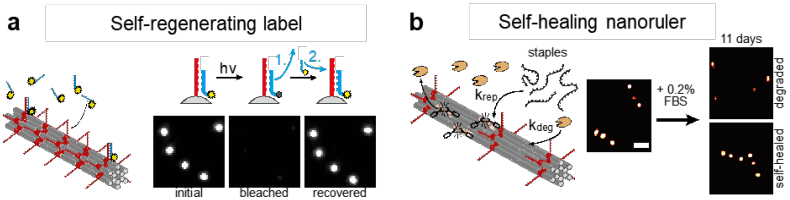

Figure 1. Self-assembly and reconfigurability of DNA origami is exploited to realize self-repairing functional nanodevices. a) self-regenerating brightness label is realized by constant exchange of labeling strands from solution. b) Structural self-healing of a DNA origami nanoruler is achieved by excess of staple strands that exchange with enzymatically or photo-induced damaged strands.

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P-32

Programmable decoration of DNA based scaffold through dynamic exchange of structural motifs

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DNA damage caused by different mutagens, leading to genomic instability and carcinogenesis, is counteracted by cellular protection multi-enzymatic mechanisms, such as base excision, mismatch and nucleotide excision repair.[1,2] Here, we demonstrate the possibility to use Base Excision Repair (BER) enzymes along with the endoribonuclease activity of RNase H enzyme as molecular cues to control the exchange reactions. An enzyme-responsive strand labelled with a certain tag would be degraded in the presence of the target enzyme and exchanged by an exchanging strand labelled with a different tag in a simple single-step enzymatic reaction.[3] To achieve this, we have enzyme-responsive strands made of RNA that respond to RNase H enzyme or containing mutated bases (2-deoxyuridine) that, in the presence of the target repair enzyme (i.e. RNase H or Uracil-DNA Glycosylase (UDG) is replaced by an exchanging DNA strand labelled with a different tag (Fluorophore or antigen). The two enzymes are orthogonal: they can be employed in the same solution to guide the decoration of a DNA scaffold without cross-talk.

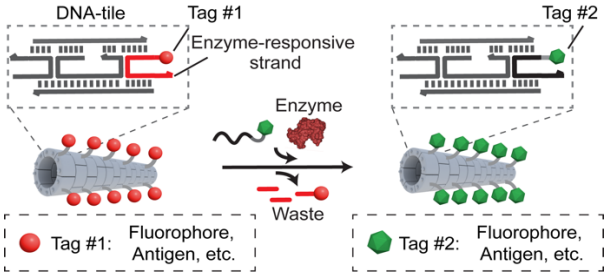

Figure 1. programmable decoration of DNA based scaffold through dynamic exchange of structural motifs. Decoration of DNA-based scaffolds could undergo dynamic exchange using enzymes as molecular cues to control the exchange reactions of the structural motifs forming the scaffold. An enzyme responsive strand labelled with a certain tag would be degraded in the presence of the target enzyme and replaced by an exchanging strand labelled with a different tag by the enzyme activity.

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P-33

Rational engineering of DNA cytoskeletons for synthetic cells

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The development and bottom-up assembly of synthetic cells with a functional cytoskeleton sets a major milestone to understand cell mechanics and to develop man-made cellular machines. However, the combination of multiple elements and functions remained elusive, which stimulates endeavors to explore entirely synthetic bio-inspired and rationally designed solutions towards engineering life. To this end, DNA nanotechnology represents one of the most promising routes, given the inherent sequence specificity, addressability, and programmability of DNA. Here, we demonstrate functional DNAbased cytoskeletons operating in microfluidic cell-sized compartments and lipid vesicles. The synthetic cytoskeletons consist of either stimuli-responsive DNA origami [1] or DNA tiles self-assembled into filament networks [2,3]. These synthetic cytoskeletons can be rationally designed and controlled to imitate features of natural cytoskeletons, including dynamic instability, ATP-triggered polymerization, morphology control and vesicle transport in cell-sized confinement. Also, they possess engineerable characteristics, including assembly and disassembly powered by DNA hybridization, light or aptamer-target interactions and autonomous transport of gold nanoparticles. This work underpins DNA nanotechnology as a key player in building synthetic cells from the bottom up.

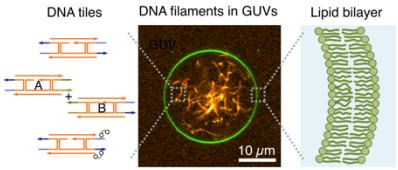

Figure: Giant unilamellar vesicle containing rationally engineered DNA cytoskeletons

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P-34

Dynamics of DNA origami filaments growth from a ditopic monomer

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Non-covalent polymerization and depolymerization of natural protein filaments, such as F-actin or microtubules, are tightly regulated cellular processes that enable the rapid localized growth and directed shrinkage of the cytoskeleton. Our approach to synthetic biology strives to mimic such dynamic behaviour using linear DNA origami polymers and the programmability of DNA association by nucleobase complementarity rules.[1] The first step towards this goal is a deep understanding of the inherent kinetic behaviour of hierarchical DNA origami assembly. This study[2] examines the thermodynamics and kinetics of self-association of a DNA origami unit with two distinct interfaces (A and B). Applying either base-hybridization or base-stacking interactions, the ditopic monomer can be triggered to grow into linear structures of random sequence or programmable periodicity (Fig.1). FRET experiments revealed the onset of the reaction and allowed to gain insights into energetic aspects of the polymerization process, while DLS and AFM were used to investigate the reaction mechanism. Our data evidence that the dynamics of DNA origami polymerization is regulated by the type of interaction used to link the monomers together, i.e. base hybridization or base-stacking. Whereas the former obeys a nucleation-and-growth mechanism typical of natural polymerization reactions, the latter follows an isodesmic model typical of synthetic $\pi-\pi$ conjugation systems.

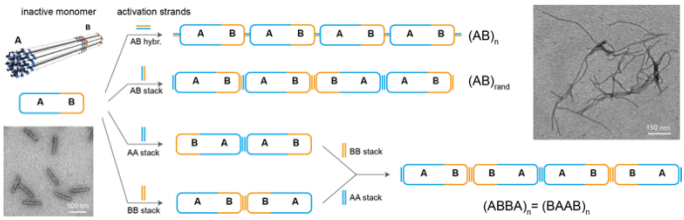

Figure 1. The inactive AB ditopic unit is a 24-helix bundle origami structure, whose tips can be activated by hybridization or stacking strands (indicated, respectively, by horizontal and vertical lines) to promote its self-association into linear oligomers of defined or random polarity (respectively indicated as $(AB)_n$ and $(AB)_{rand}$). Ordered stacked oligomers (indicated as $(ABBA)_n$ or $(BAAB)_n$) are attained upon step-wise association of pre-formed stacked dimers.

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P-35

Protein-Templated Reactions Using DNA-Antibody Conjugates

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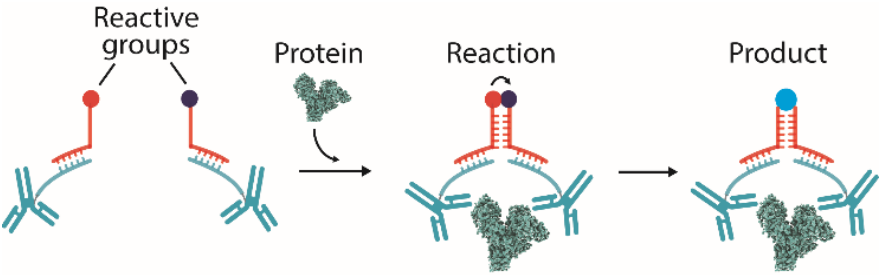
DNA-templated chemical reactions have found wide applications in drug discovery, programmed multistep synthesis, nucleic acid detection, and targeted drug delivery. The control of these reactions has, however, been limited to nucleic acid hybridization as a means to direct the proximity between reactants. In this work a system capable of translating protein–protein binding events into a DNA-templated reaction which leads to the covalent formation of a product is introduced. Protein-templated reactions by employing two DNA-antibody conjugates that are both able to recognize the same target protein and to colocalize a pair of reactant DNA strands able to undergo a click reaction are achieved. Two individual systems, each responsive to human serum albumin (HSA) and human IgG, are engineered and it is demonstrated that, while no reaction occurs in the absence of proteins, both protein-templated reactions can occur simultaneously in the same solution without any inter-system crosstalk.



P-36

DNA mold-based fabrication of palladium nanostructures

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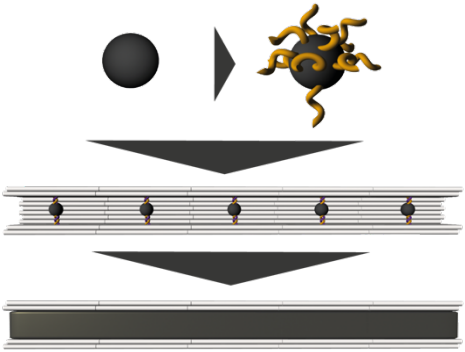

A challenge for the bottom-up fabrication of nanoelectronic devices is the accurate spacially-resolved material deposition on the nanometer scale. Concerning biomolecular materials, DNA nanotechnology meets this challenge by being highly precise at building DNA structures of nearly any desired form. To exploit this concept for other materials, we recently developed a DNA origami mold-based nanoparticle synthesis scheme that allows the fabrication of metallic nanoparticles with DNA-programmable shape. Particularly, we demonstrated the fabrication of gold nanostructures with aspect ratios of up to 7 grown from single seeds [1] as well as the fabrication of rolling-pin-, dumbbell-, loop- and T-shaped gold nanoparticles [2].

Here, we expand the mold-based fabrication method to palladium. To allow a seeded-growth of palladium inside the origami molds, 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 palladium deposition was initiated. This provided grainy rod-like palladium nanoparticles with an average diameter of 25 nm. Using an annealing procedure, homogeneous palladium nanowires were obtained. In combination with the seeded growth of gold these two metals can even be deposited within one DNA template structure.

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P-37

Substrate-assisted self-assembly of DNA origamis for lithographic applications

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To go beyond the resolution limits of the conventional lithography, alternative techniques, such as the directed self-assembly of organic materials, have emerged. The development of DNA nanotechnologies has allowed the creation of complex DNA origami shapes at nanoscale resolution. The high versatility of this method opens up opportunities for advanced lithography due to the addressability of DNA origami on thermal oxidized silicon substrates [1] and the transfer of their shape into the Si with a dry etching process [2] for nanoelectric applications. The objective of this work is to explore the substrate-assisted self-assembly of DNA origami 2D arrays on thermal SiO2 substrates (SiO2 on Si). Some studies, made on mica substrates, showed that it is possible to control the diffusion processes of the DNA origamis, based on the competition between monovalent and divalent cations on the surface of the oxide [3]. We developed a method to perform and characterized this substrate assisted self-assembly on the SiO2 surfaces (Figure 1). To form the 2D arrays, we designed and synthesized two shape-complementary square monomers that self-assembled together in 2D arrays in solution via shape-complementarity through π-π low energy interactions present in the DNA molecules [4].

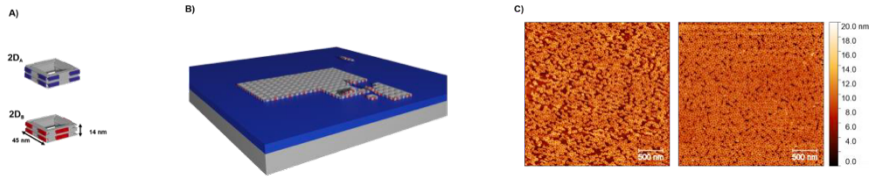

Figure 1: A) 3D model of the shape-complementary DNA origamis. B) 3D model of the substrate-assited self-assembly of the 2D arrays on a thermal SiO2 surface. C) Liquid AFM image of the 2D arrays formed onto a thermal SiO2 surface after two different times of incubation.

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P-38

AFM analysis of G-wire DNA structure and nanoparticle decoration

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

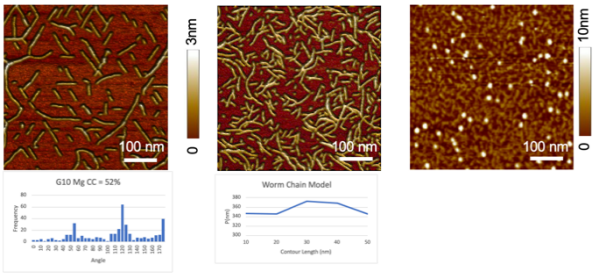

Fig. 1: G-wires equilibration adsorption on mica with autocorrelation function.
Fig. 2: G-wires kinetically adsorbed on amino acid treated mica with persistence length.
Fig. 3: Thiolated-G4T2G4 attached to 5nm gold nanoparticles.

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P-39

Salting-out of DNA Origami Nanostructures by Ammonium Sulfate

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

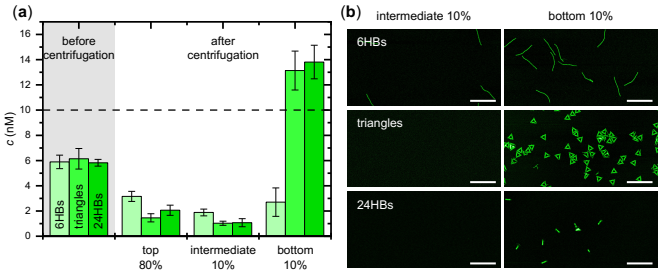

Figure 1. (a) Determined concentrations of DNA origami shapes before and after centrifugation in 3 M ammonium sulfate by UV absorption. (b) Representative AFM-images of DNA origami shapes in the intermediate 10 % and bottom 10 % fractions.

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P-40

Bioengineering DNA-based enzyme-powered nanoswimmers

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Inspired by nature, researchers have been exploring the rich multifunctionality of molecular motors, cells and other microorganisms to engineer artificial smart nanosystems able to move, interact with their environment and perform complex tasks at the nanoscale. Recently, artificial nanomachines that harness chemical energy from in situ chemical reactions and convert it into active motion have been developed. Particularly, enzymes are powerful biological catalysts that convert substrates into products.[1] By harvesting this chemical energy, enzymatic micro- and nanomachines are able to self-propel. These motor-fuel complexes hold a great potential towards nanomedicine thanks to their versatility, bioavailability and full biocompatibility. Although the field is still in its infancy, several milestones have been reached, such as enhanced anti-cancer drug delivery [2] specific targeting and penetration in 3D bladder cancer spheroids. [3] However, when biomedical applications are envisaged, several fundamental questions need to be resolved: what are the optimal design features of enzyme-powered nanomachines? How to integrate multiple and smart functionalities? How to achieve full biocompatibility? Here, we present the use of synthetic DNA as an innovative tool to build programmable DNA-based nanoswimmers which interact with the surrounding environment thanks to the use of pH responsive DNA nanoswitches [4]. Additionally, we use the unique spatial addressability of DNA to control enzyme functionalization for optimal swimming capabilities. The unique programmability and biocompatibility of DNA could pave the way towards overcoming current challenges on the development of synthetic self-propelled nanomachines by providing new tools to achieve full biocompatibility and design tunability.

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P-41

Programmable cell-free transcriptional switches for antibodies detection

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Antibody detection is important in several clinical settings because it informs on current and past infection and can provide information about clinical outcomes. There is a growing need to expand medical monitoring and diagnostics of human diseases, and from this perspective, synthetic biology devices would bring new capabilities to diagnostics methods by creating sensors with new functions, expanding the range of targets, and improving sensitivity and specificity. In recent years, cell-free biosensors for the detection of nucleic acid, small molecules, and proteins[1-2] have been developed with excellent sensitivities and specificities. Despite the above advances, the examples reported so far of cell-free biosensors have been developed for a limited number of targets. We demonstrate here a cell-free diagnostic platform for the detection of specific antibodies directly in blood serum based.[3] The approach we propose couples the advantageous features of responsive DNA-based conformational switching probes (i.e. programmability and specificity) with those of cell-free diagnostic methods (i.e. sensitivity). The antibody-responsive transcriptional switch is composed of two modules: the transcriptional switch module and the antibody-responsive module. The first is a double-stranded DNA switch designed to adopt a stem-loop hairpin conformation that prevents efficient transcription of an RNA light-up aptamer due to incomplete formation of the promoter sequence. The second module is composed of two antigen-conjugated DNA strands that, upon bivalent binding to a target antibody, are brought into close proximity and can hybridize to form a functional bimolecular complex. Such complex induces a conformational change on the switch and makes the promoter sequence accessible for transcription. The so-activated transcriptional switch can transcribe, in the presence of RNA polymerase and nucleotides, a reporter light-up RNA aptamer that signals the presence of the target antibody (Fig.1).

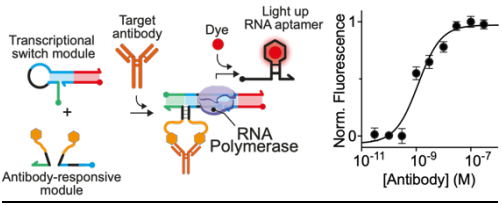

Figure 1. Programmable antibody-responsive transcriptional switches.

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P-42

G-Quadruplex DNA based fluorescent sensing for quantification of potassium ion flux across giant proteoliposomes

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Ion transport in cells involves many coupled pathways in a complex chemical environment, therefore interpreting transport measurements in native membranes is difficult. Artificial cell models based on Giant Unilamellar Vesicles (GUVs) are being increasingly used to develop sophisticated model cell membranes, whilst maintaining control over the membrane composition and environment. Yet, techniques to study ion transport across such GUV based artificial cells are limited in number. To that end, we present a novel fluorescence based technique for the study of potassium ion transport across model cell membranes by combining fluorescently modified G-quadruplex DNA and the microfluidic production of GUVs. We show that G-quadruplex based sensors can enable potassium ion detection at a broad range of wavelengths whilst maintaining sensitivity. Furthermore, by combining G-quadruplex ion sensing with microfluidic manipulation, we can quantify the flux of potassium ions across synthetic cell membranes at the single cell level. As a case study, we reconstitute a membrane protein into GUVs and provide an analysis framework that may be used to study the transport properties of proteo-GUVs. Whilst here we demonstrate potassium ion sensing, G-quadruplexes and other DNA nanostructures are responsive to several different ions and biomolecules, and so we anticipate our technique can be easily adapted to study transmembrane transport of many biologically relevant solutes.

P-43

Multiplexed Label-Free Biomarker Detection by Targeted Disassembly of Variable-Length DNA Payload Chains

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Simultaneously studying different types of biomarkers (DNA, RNA, proteins, metabolites) has the potential to significantly improve understanding and diagnosis for many complex diseases [1]. However, identifying biomarkers of different types involves using several technically complex or expensive methodologies, often requiring specialized laboratories and personnel [2]. Streamlining detection through the use of a single multiplexed assay would greatly facilitate the process of accessing and interpreting patient biomarker data. DNA nanotechnology-based diagnostic methods are versatile alternatives that could help address many of the challenges of biomarker detection. However, limitations such as scalability, regulations and cost [3] have prevented their widespread use outside research laboratories. In this work, we present a new technique [4] for scalable and multiplexable biomarker detection.

The detection principle is based on sets of variable-length DNA payload chains. By leveraging strand displacement or aptamer-binding events, long payload chains can be disassembled in the presence of a target biomarker, releasing shorter payload chains of different sizes that yield characteristic band patterns in gel/capillary electrophoresis (Figure A). This strategy has enabled us to detect with high sensitivity and specificity DNA sequences including BRCA1, an RNA sequence (miR-141) and the steroid aldosterone, both individually and in a multiplexed assay (Figure B). Furthermore, we show that our method suffers no loss of sensitivity when conducted in fetal bovine serum (Figure C) and can be applied using capillary electrophoresis, which may be more amenable to automation and integration in healthcare settings.

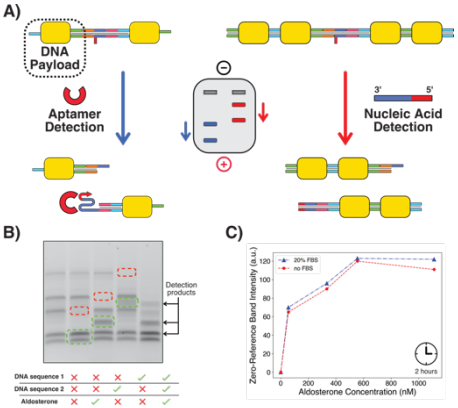

Figure A) Detection scheme. B) Multiplexed detection of two DNA sequences and aldosterone. C) Aldosterone detection in FBS.

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(Also submitted to journal)

P-44

A novel lattice design for scaffolded DNA origami structures

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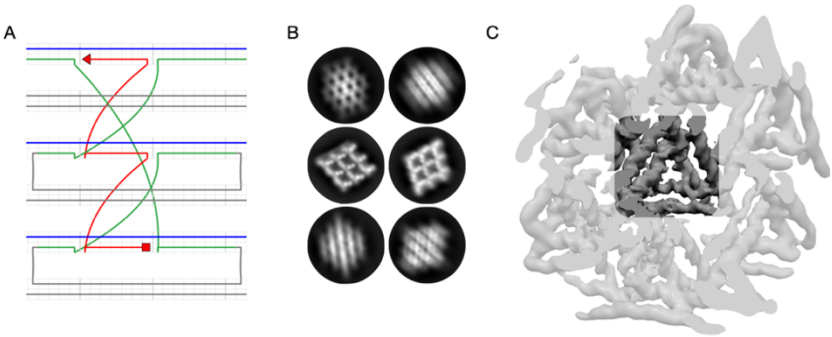
Here we present a novel approach to orient and connect helices in a densely packed scaffold-based DNA nanostructure. Structures are built by placing self-similar repetitive voxels. Each voxel contains six 21 basepair long DNA helices, that are pairwise oriented in all three spatial directions. The six DNA helices are held together by both staple-scaffold, as well as staple-staple interactions, with the core of each subunit mimicking the Seeman-Triangle[1]. The orientation and connectivity of the helices allow us to route the scaffold through each subunit and thereby connect the subunits using hamiltonian or branched pathways. We optimized the unit cell iteratively, resulting in a refined subunit, that allows for the direct assembly of structures in an automated design tool without prior knowledge or optimization by the designer. Key optimizations within the subunit were the placement of crossovers relative to each other and the removal of kinetic traps, which improved folding kinetics and final folding yield, as well as the stability of folded structures. Furthermore, we characterized the influence of scaffold routing and subunit connectivity within the structure and found optimal conditions for folding reactions. We built structures of different sizes, including multi-scaffold assemblies, to prove the generalizability of the method. The software tool is named *WaffleCraft* based on the typical TEM-patterns found in class averages. Lastly, we characterised our structure using Cryo-EM, to gain further insight into the assembly and connectivity of our novel *WaffleCraft* structures.



A) Cadnano representation of the subunit. In red, green, and blue the strands that mimick the Seeman Triangle. **B)** Exemplary 2D Class Averages of a 27 subunit *WaffleCraft* cube. **C)** Cutout from a Cryo-EM reconstruction at ~10 Å resolution of the same Cubic structure with one subunit highlighted.

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P-45

Stimuli-responsive DNA particles underpin three-agent signaling networks with live bacteria and synthetic cells

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Lipid bilayers play an important role in cellular biology as they act as an active filter between the inner and outer environment of the cell. Their integrity, often associated with proper functioning and vitality of the cells, can be compromised by a number of biological and synthetic agents, including antimicrobial peptides, amyloid aggregates, polymer particles and metal particles with charged coatings. Such agents, frequently considered to be toxic and highly undesirable, have a variety of beneficial applications underpinned by the ability to control membrane leakage, which can be harnessed for biosensing and therapeutics. Here, we present a novel type of synthetic, DNA-based particles capable of disrupting and permeabilizing lipid membranes in a controlled manner.[1] The particles have a core-shell structure and self-assemble from cholesterol-modified DNA nanostructures,[2,3,4] forming the membrane-adhesive core, and all-DNA nanoconstructs forming a protective hydrophilic "corona". If unperturbed, the particles are colloidally stable, and their size can be prescribed by changing the annealing protocol leading to self-assembly. The protective corona can be selectively displaced upon exposure to external stimuli (macromolecules, pH changes, light), exposing the hydrophobic core and inducing particle aggregation. If, at this stage, membranes are present, the sticky material will adhere to their surface destabilizing and permeabilizing them. Furthermore, the sticky "DNA net" formed upon particle activation is capable of capturing and immobilizing swimming cells as we tested with *E. coli*. This is reminiscent of the action of innate-immune cells, which can eject their genetic material to create an Extracellular Trap to combat pathogens. The ability to both permeabilize liposomes and to trap bacteria, as well as the pH responsiveness of particles can be utilized to create a synthetic three-agent signaling network, in which natural pH gradients from *E. coli* trigger the displacement of protective corona from particles, resulting in a simultaneous bacteria immobilization within a DNA net and cargo release from liposomes. Once released, the cargo molecules can interact with bacteria and force them to respond in a programmed manner.[5]

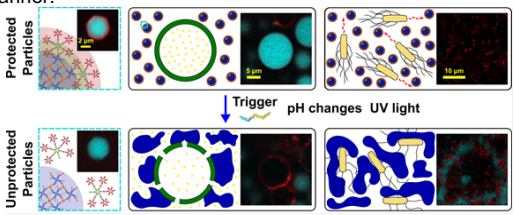

Fig. 1. Particle-induced membrane permeabilization and bacteria entrapment. As a result of exposure to external stimuli (macromolecules, pH changes, light) and subsequent displacement of the hydrophilic corona (left), cholesterol rich particle cores adhere to each other and start to aggregate on the surface of GUVs (middle). DNA aggregation leads to GUV rupture and/or cargo release. Furthermore, once activated, particles form a sticky DNA net, which in turn is able to trap and arrest *E. coli* (right).

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P-46

Allosteric regulation of DNA-based nanodevices using *in vitro* transcription

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Re-engineering biochemical reaction networks and mimicking cell functions is nowadays one of the main challenges of synthetic biology.[1] To precisely regulate the activity of biomolecular receptors, like proteins and enzymes, Nature exploits different mechanisms, including allostery. For example, the affinity of a receptor for its specific target can be shifted towards higher concentrations when an allosteric modulator (e.g., inhibitor) is bound to a site distal from the ligand's binding site.[2] By combining the principles of *in vitro* transcription with those of allostery, we engineered a nanodevice made of synthetic DNA designed to respond to the presence of specific RNA inputs (Figure 1).[3] We have selected as a model system a ligand-binding DNA-based nanodevice (molecular beacon) designed to adopt a stem-loop structure flanking two tails at the two ends of the stem (dark grey) and engineered to bind to the loop portion a single-stranded DNA (blue strand). An *in vitro* transcribed RNA strand (pink) acts as an allosteric modulator (e.g., inhibitor) binding the two tails of the molecular beacon mediating the release of the DNA target. Instead, the transcription of an RNA activator strand (green) causes the displacement of the RNA inhibitor bound to the receptor thus ultimately restoring the load state of the nanodevice. Coupling the versatility of DNA-based nanodevices with the programmability of synthetic genetic circuits can be conveniently handled for a wide range of biotechnological applications.

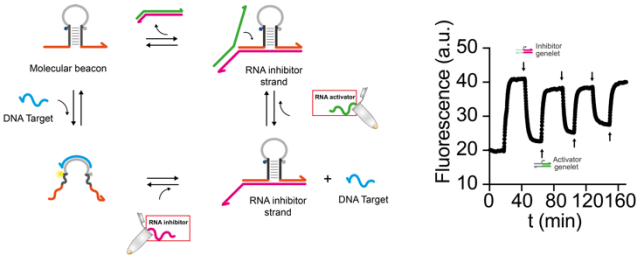

Figure 1. General representation of the allosteric mechanism.

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P-47

A DNA Nanotechnology Assay to Detect Double-Stranded DNA for Medical Applications

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DNA nanotechnology has the potential to provide novel methodologies for biomarker detection. One example biomarker is circulating tumour DNA (ctDNA), which is DNA released from cancerous cells into the bloodstream. The level of circulating tumour DNA in the bloodstream is indicative of tumour size [1]. Additionally, the mutations present in the ctDNA can provide valuable insights into disease progression [2]. However, current detection methods are expensive, complex, and often are not sensitive enough to detect low levels of ctDNA associated with the early stages of cancer or during recurrence. Unfortunately, ctDNA is double-stranded, so it is often incompatible with DNA nanotechnology assays. Here we show a toehold-mediated DNA strand displacement assay coupled with asymmetric PCR (aPCR) to identify specific double-stranded DNA sequences. We produced a single-stranded DNA fragment using asymmetric PCR from a double-stranded DNA of interest. This newly formed single-stranded DNA was used as a target strand to displace a DNA toehold coupled with a fluorophore and quencher. This provides a fluorescence readout that standard laboratory equipment can read, such as a fluorometer or plate reader. We found that aPCR can produce sufficient quantities of single-stranded DNA to be analysed downstream. Furthermore, the output signal depends on the concentration of single-stranded DNA produced (Figure). Our results demonstrate how DNA nanotechnology can report on double-stranded DNA biomarkers such as ctDNA. This assay could determine common mutations found in cancer via changes observed in strand displacement rate. Furthermore, by treating the DNA prior to aPCR, we believe that the assay could provide further insights into the DNA present, such as methylation status, providing more information to clinicians from a single DNA nanotechnology-based assay.

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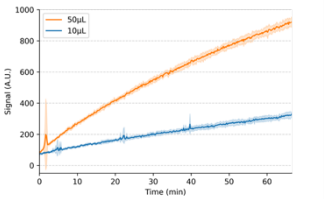

Figure: Strand Displacement Kinetics of 20nM toehold complex added to either 10µL or 50µL of aPCR reaction (N=8, Band=Standard Deviation).

P-48

Diverse, highly efficient *grafting to* strategy for the patterning of DNA-origami

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DNA-origami is a highly versatile platform and, in combination with the wide selection of polymers, facilitates the creation of multifunctional, yet precise hybrid nanomaterials. This also endows unprecented geometrical possibilities to generate polymeric architectures that are beyond conventional methods. We combine these unique features to create 3-D architectures and aim to introduce a simple way of constructing complex nanostructures to the synthetic polymer community. The unique advantage of DNA-origami relies on the possibility to functionalize each grid position independently. One strategy to introduce polymers to the DNA-origami surface is the polymerization from the DNA-origami via the *grafting from* strategy.[1,2] This limits the monomer scope and the polymerization technique due to the stringent conditions such as high ionic strength buffers, low reaction volumes and mild reaction conditions. Here we circumvent these limitations by creating a *grafting to* protocol to pre-synthesize different DNA-polymer conjugates and anneal theses conjugates in a programmable way to the DNA-origami surface. To create those DNA-polymer architectures, a *grafting to* protocol with high conversion (~70-90%) was developed to generate various DNA-polymer conjugates via NHS coupling. The polymer library contains homo and block copolymers of dimethyl acrylamide, oligoethylene glycol acrylate, N-isopropyl acrylamide, hydroxyethyl acrylate and diacetone acrylamide, which were polymerized prior coupling via RAFT. The obtained DNA-polymer conjugates were patterned successfully onto the surface of various origami architectures to accommodate patterns of different shapes and origami templates such as rings and triangles with different polymer combinations.[3] We envision that the methodology would foster and open new possibilities to create precision-based nanomaterials for material science and nanomedicine.

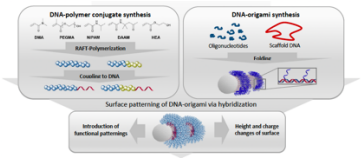

Figure 1: Project strategy of the *grafting to* method to create various DNA-polymer conjugates for DNA-origami.

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P-49

The role of DNA nanostructures in the catalytic properties of an allosterically regulated protease

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In nature, enzymatic reactions are often located within cellular compartments, thus favouring higher concentrations of substrates and catalysts as well as shielding the target species from unwanted side reactions. DNA nanotechnology is an ideal platform to mimic this principle, as it allows the selective binding and precise arrangement of guest molecules with nanometre-scale accuracy. Interestingly, many studies on enzyme cascades demonstrate that the close proximity of multiple enzymes immobilized onto DNA nanostructures often results in increased catalytic activities. Comparably fewer studies on single enzyme-DNA systems report the same phenomenon, thus raising the question whether multiple factors may contribute to the intriguing observations. To tackle this question, we used thrombin, an allosterically regulated serine protease, specifically bound *via* aptamers to DNA origami structures with a variable degree of confinement.[1] We compared the hydrolysis of three substrates that differ only in their net-charge due to a C-terminal amino acid residue that is likely involved in the allosteric regulation of the enzyme. Our data show that, for all substrates, the reaction speed increases with the level of DNA tethering but the extent of this effect is strongly dependent on DNA/substrate interactions. For substrates of opposite charge, enzyme confinement within a DNA origami cavity leads to completely different kinetic behaviours, turning the worst substrate into the best performing one. Applying the transition state theory and the kinetic linkage scheme for thrombin, we formulate a model wherein the negatively charged DNA environment nearby the enzyme is likely to affect local electrostatic features on the protein surface, thereby interfering with charge-dependent mechanisms of substrate recognition. This ultimately influences the catalytic performance of the system and may offer an alternative tool to regulate allosteric processes through spatial confinement.

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P-50

A modular, dynamic, DNA-based platform for regulating cargo distribution and transport between lipid domains

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Biological membranes facilitate numerous processes critical to life, ranging from signal transduction to motility and adhesion. Many of these functionalities are reliant on the tight spatio-temporal regulation of the distribution and interactions of membrane-bound machinery1. Artificial cells are entities built de-novo that replicate the rich phenomenology associated to biological systems2, and are frequently constructed with synthetic lipid bilayers. These artificial cellular membranes require purposefully designed pathways to exhibit the complex functionalities displayed by their biological counterparts. As a prime route to build architectures from the bottom-up3, DNA nanotechnology allows to engineer bio-inspired devices that mimic the structure and activity of membrane inclusions. To that end, amphiphilic oligonucleotides are often used to couple DNA nanostructures to lipid bilayer membranes4,5. Here, we harness the tendency of cholesterol and tocopherol motifs to enrich liquid-ordered (*L*o) and liquid-disordered (*L*d) domains, respectively, to statically and dynamically modulate the lateral distribution of DNA nano-devices on the surface of phase-separated membranes. By prescribing combinations of multiple anchors, changes to nanostructure size, and geometry, our DNA devices are programmed to achieve a wide range of partitioning states. We demonstrate the functionality of our platform with a reconfigurable biomimetic DNA architecture that achieves cargo transport between lipid domains across the membrane of cell-sized vesicles6.Our results highlight the potential that amphiphilic DNA nanostructures have for biomimicry, and pave the way for the development of DNA-based pathways that expand our arsenal to construct synthetic cells with advanced capabilities.

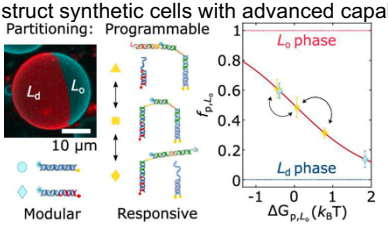

Figure 1. Modular, programmable and responsive partitioning of DNA nanostructures in lipid domains: Amphiphilic DNA nanostructures achieve static model membrane patterning (left) as well as reversible cargo transport between lipid phases, spanning the free-energy landscape (right).

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P-51

Thermal and mechanical properties of topologically identical origami domains at the ensemble and single-molecule level

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The self-assembly of a DNA origami structure, although mostly feasible, represents a rather complex folding problem that greatly depends on the thermal and mechanical properties of joined Holliday junctions, the fundamental units of every DNA origami domain. In a previous work,[1] we analyzed the assembly products of three origami domains with identical topology but different nucleobase composition and compared the results obtained for distinct extents of mechanical strain at the edges of the structure. Our data revealed that the folding pathway of a monolayer DNA origami structure has two minima of energy, corresponding to the two possible right-handed isomers of all constituent crossovers, resulting in the formation of a canonical (*can*) and an isomerized (*iso*) shape. In this work, we performed a systematic study of the thermal and mechanical properties of each origami domain in order to better understand the relationship between their sequence-content and their isomerization degree. Several FRET melting and cooling curves were recorded at different heating rates to observe how thermal hysteresis emerges. Various analytical approaches and theoretical methods were used to characterize the reaction orders and k_{on}/k_{off} rates.[2-4] Furthermore, UV melting profiles were recorded to analyse the global thermal behaviour of each origami domain. Correct folding was always confirmed by agarose gel electrophoresis and atomic force microscopy. Finally, single-molecule force spectroscopy studies were performed to characterize the mechanical response of the individual domains upon application of a constant force. Using a dual optical tweezer, origami domains were trapped between two beads and pulled apart at 10 nm/sec. Force-distance curves revealed a complex mechanical unfolding behaviour with isomerization forces ranging between 4 and 12 pN, and a distance change of about 30-40 nm, as expected for a total *can*-to-*iso* conformational transformation.

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P-52

A DNA logic gate to sense molecular distances

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The aim of this project is to design a device that is capable of sensing inter-molecular distances and can translate this information into a quantitative fluorescent signal. As a proof-of-principle, two input DNA strands, A and B, are located on top of a rigid and planar DNA origami surface at variable but predefined distances, thus providing distinct types of input information to be encoded by the logic gate. This latter is programmed to work as an AND logic gate, meaning that the output signal will be generated only if both A and B are present. Thus, given a pre-defined AB interspace, addition of the AND logic gate will initiate a cascade of single-strand displacement reactions[1] that terminates with the emission of a fluorescent signal, the intensity of which as well as its kinetic profile are expected to be dependent on the input AB distances. Our purpose is to generate a nanoruler for ensemble measurements that can sense and reliably quantify the nm-scale distance between two well defined DNA-tagged molecules, e.g. DNA-modified antibodies, peptides or small proteins. Such a nanoruler can find applications as a ready-to-use bioanalytical tool for targeting and imaging purposes.

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P-53

Towards DNA-only digital biosensing with DNA nanosensors

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High quality diagnostic tools are indispensable for prevention and early diagnosis of many diseases, as recently proven by the COVID pandemic. In this context, DNA nanotechnology has been broadly researched over the past years to develop more sensitive and robust biosensors for multiplex target detection[1]. Here we show the development of DNA nanosensors with incorporated target detection, signal generation, and signal amplification (Figure 1A). To achieve this, we first designed and characterized a 100 nm DNA origami disk, which was successfully assembled as shown by AFM imaging (Figure 1B). This DNA origami structure was then equipped with ssDNA staple extensions for functionalization with a DNAzyme and its fluorophore-quencher (F-Q)-labelled substrate. Thanks to the intrinsic properties of DNA origami, we can precisely control the desired number of functional strands and their interspacing with ~5 nm resolution. This was validated in a single molecule photo-bleaching study, using TIRF microscopy on a commercial super-resolution microscope (Figure 1C). Finally, we implemented a model system for nucleic acid (NA) detection by inhibiting the DNAzyme with a complementary sequence, resulting in a competitive DNAzyme-based bioassay[2]. Upon target addition, binding of the target to the complementary inhibitor sequence results in a concentration-dependent increase of the fluorescent signal, which was observed in bulk measurements using the spectrophotometer (Figure 1D). With this concept, we show for the first time, signal generation by a DNAzyme with co-immobilized substrates on DNA origami. Future integration with aptamers in an aptazyme-based[3] (i.e., aptamer and DNAzyme in 1 sequence) bioassay will allow multiplex detection of not only DNA but also protein targets. Moreover, by immobilizing these DNA nanosensors on a patterned surface implemented in (i)SIMPLE[4] microfluidics, bulk readout will be replaced by digital readout of individual DNA nanosensors, resulting in a point of care biosensing concept with unprecedented sensitivity.

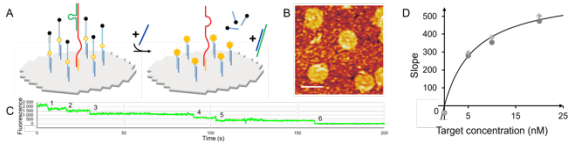

Figure 1: (A) Schematic overview of the DNA nanosensor concept and proof-of-concept bioassay for NA target detection and DNAzyme-based signal generation. The DNA nanosensor consists of a DNA origami nanostructure (grey), DNAzyme (red), 6 FQ-labelled DNAzyme substrates (light blue, F = yellow; Q = black). The addition of the NA target (dark blue) removes the inhibitor (green), thereby activating the DNAzyme. (B) AFM image showing circular DNA origami disks (scale bar = 100 nm). (C) Single molecule photobleaching of DNA origami with 6 fluorescent labels, proven by the 6-step fluorescent decay. (D) Demonstration of NA detection (0-20 nM) by DNA nanosensors, represented by the slope of the fluorescent signal increase in function of the target concentration as measured with the spectrophotometer (n = 2).

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P-54

DNA-protein nanogels as transfectable multienzymatic nanoreactors

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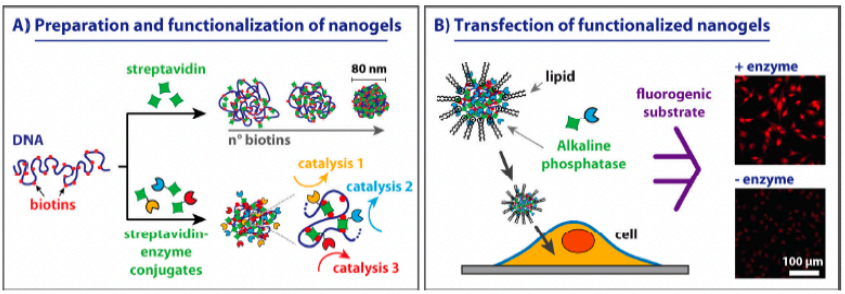
Structural DNA nanotechnology attracted recently much attention due to its capacity of bottom -up building of nanometric structures with unprecedented spatial resolution and programmability. Less organized entropic DNA nanotechnology, however, also employs DNA as main component for preparation of nanomaterials with the advantage of building softer and multi-functional nanostructures. In this talk I will describe the preparation of novel DNA nanogels based on noncovalent intramolecular statistic crosslinking of multibiotinylated DNA by streptavidin protein having 4 highly specific biotin binding sites.1 The resulting hybrid DNA-protein nanogels greatly combine the functionalizability of streptavidin with the capacity of DNA to be transfected through cell membranes. By using streptavidin conjugated to enzymes, they have been successfully functionalized either with individual enzymes, or with a mixture of three enzymes (i.e. horseradish peroxidase, alkaline phosphatase and β-galactosidase), without losing their respective catalytic activities (Fig. A).2 Then, liposome-based DNA transfection approach was used to transfer these enzymatic nanoreactors into the living cells. I demonstrate that efficient transfection occurred at lower lipid/DNA charge ratios in comparison with conventional transfection of plasmid DNA, indicating that full charge neutralisation is not a requisite for transfection of three-dimensional DNA nanostructures.3 After
transfecting cells with nanogels functionalized with alkaline phosphatase, strong catalytic activity was successfully detected inside the cells, thus demonstrating the possibility of using this nanogel platform as a cargo for simultaneous transfection of hundreds of active enzyme molecules (Fig. B).



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P-55

pH-Responsive DNA Origami Lattice

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During the recent years, the advances in the field of nanotechnology have been impressive, but for many applications, more complex and highly ordered nanomaterials in which the individual components interact with each other in a predefined way would be desirable [1]. Programmable and modular DNA-based nanostructures have proven to be feasible templates for controlling and directing the spatial arrangements of other components and therefore they have been used to for example assemble increasingly complex static nanoparticle lattices [2]. DNA-based nanostructures have also been used to produce a variety of small dynamic devices [3], but their use for the construction of larger dynamic assemblies whose structural properties are changed in response to environmental stimuli such as pH, salt concentration, light or temperature have been rather limited. In this work, we present a dynamic, two-dimensional (2D) DNA origami lattice that changes its lattice parameters in response to the pH of the surrounding solution [4]. Our DNA-based lattice is built by connecting pliers-like DNA origami units that can be switched between a narrow "X"-shape and a wider "+"-shape with the help of "pH locks" made of pH-sensitive helix-forming DNA sequences [5]. At low pH, the arms of the DNA origami are locked into the "X-shape", whereas the arms are released by increasing the pH. Moreover, this switchable DNA origami lattice can also be used as a template for the construction of pH-responsive arrangements of other compounds, which we here demonstrate by assembling a dynamic 2D nanoparticle lattice.

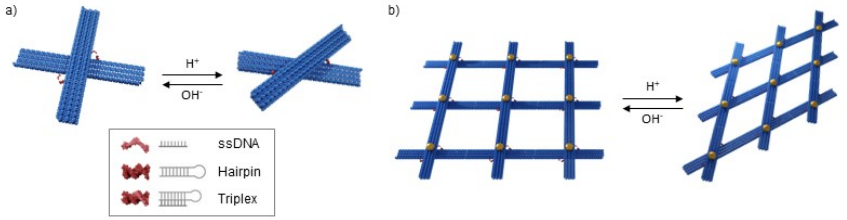

Figure. a) The DNA origami unit can be reversibly switched between a wide "+"-shape and a narrower "X"-shape with the help of pH-sensitive locks. At high pH, the locks are open (ssDNA and hairpin), whereas they are closed at low pH (triplex). b) A pH-responsive two-dimensional (2D) nanoparticle lattice is constructed by connecting the DNA origami units together using blunt-end interactions.

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P-56

High-resolution surface charge density visualization of DNA nanostructures

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DNA charge density affects strand hybridization and the interaction with biomolecules, ions and surfaces, thus being a major factor in determining its biological response. This important attribute depends on conditions such as pH and ionic concentration thereby leading to changes in the biophysical properties[1]. This has been demonstrated in the application of DNA nanopore devices[2] that are created based on structural DNA nanotechnology approaches such as DNA origami. Visualizing and quantifying the surface charge density of DNA nanostructures under various conditions is advantageous in the process of engineering DNA-based breadboards or delivery devices inside biological systems. To realize this, an experimental protocol has been established based on applying glass nanopipettes as high-resolution surface charge sensors under physiologically relevant conditions.[3] First, we examine ionic conditions that permit the adsorption of DNA nanostructures onto mica which is an appropriate substrate surface that is atomically flat. Next, under physiological conditions, we image DNA nanostructure topography in addition to visualizing the surface charge density distribution. These findings aid in the understanding of the interplay between DNA nanostructures and ions, which is important when introducing DNA-based devices into environments with complex ion distributions such as in biological systems. Our future studies aim at conjugating biomolecules to DNA nanostructure platforms to enable single-molecule surface charge mapping.

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P-57

Spontaneous reorganization of DNA-based polymers in higher ordered structures fueled by RNA

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One interesting feature of many living biomolecular systems is that their operation is governed by kinetics rather than thermodynamics.[1] A strong current interest in the field of supramolecular chemistry is aimed at developing synthetic materials and self-assembled polymers that, like their naturally occurring counterparts, are controlled by kinetic processes. Recently it has been shown that external conditions (i.e. pH, solvent, temperature) can be used to control supramolecular self-assembly pathways which permits identical building blocks to self-assemble in different kinetically stable assembly products. Yet, in these systems transition between different kinetic states can only occur in the direction of a kinetic state that is lower in energy and the systems are destined to eventually progress towards the thermodynamically most stable state.[2] Here, we demonstrate the design of DNA-based addressable building blocks (tiles) that self-assemble into polymer-like structures that can be autonomously reorganized exploiting RNA as chemical fuel in the presence of RNA-degrading enzyme (RNase H).[3] To do so, we have engineered a DNA polymeric system consisting of multiple assembling units (tiles) that can be deactivated by distinct RNA fuels (Fig. 1a). By simply modulating the kinetic of enzymatic degradations at which the individual components are reactivated, it is possible to control the supramolecular organization of the units into the final polymer. Our strategy allows a spontaneous and reversible switching between kinetic states of the assembly. Importantly, the demonstration that it is possible to rearrange a disordered DNA-polymer in a higher order block-copolymer shows that our strategy also permits transitions that are energetically uphill (Fig. 1b).

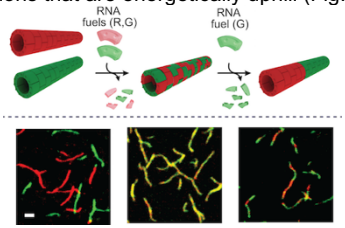

Figure 1. Spontaneous reorganization of DNA-based polymers triggered by RNA fuels.

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P-58

Fast and exact reduction of mislocalizations near spherical nanoparticles by a fully analytical PSF

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DNA origami-functionalized nanoparticles (NP) are ideal and highly versatile tools for developing (bio)sensors and optical antennas [1,2]; however, the optical properties of such systems are very sensitive to the position and orientation of the fluorescent molecule(s) relative to plasmonic nanoparticles. The benchmarking, calibration, and fluorescent readout of these nanophotonic systems is usually performed using localization microscopy, where the position and/or orientation of a single fluorescent molecule is determined with nanometer-precision based on the shape and position of the point spread function (PSF). However, the NP acts as a plasmonic scatterer near the fluorophore and consequently distorts and displaces the PSF, making it no longer Gaussian [3-5]. This leads to mislocalization of the fluorophore, when the PSF is analyzed with a two-dimensional Gaussian, which is common practice in the field. Here, we present the first fully analytical description of the PSF of a fluorophore near a spherical NP of any material and size (Fig. 1). This method allows for the reduction of mislocalizations by 1) serving as a predictive tool for optimal experimental design (e.g., NP material, size and fluorophore) and 2) as an exact PSF fitting algorithm. In contrast to popular numerical approaches, our method is exact, 3 orders of magnitude faster, open-source and does not require an expensive license to commercial numerical software. This method eliminates the need for numerical calculations and makes it possible to quickly (seconds instead of hours) and easily (open-source Python code) identify parameter regimes where the PSF is non-Gaussian or can be used in a fitting procedure to extract the true position of the fluorophore. We anticipate that this tool will be instrumental for future characterization of plasmonic (bio)sensors, NP near-fields [6], NP geometry [7] and surface functionalization [8].

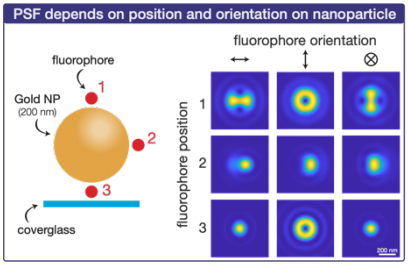

Fig 1. PSF depends on position and orientation of the fluorophore on a NP. Figure shows the analytical PSF for an emitter separated 10nm from 200nm gold spherical NP, for 3 positions and 3 orientations. Wavelength 675nm, scale bar applies to all.

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P-59

Effect of lipid composition on the efficiency of fusogenic DNA nanostructures

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Membrane fusion is one ubiquitous phenomenon linked to many biological processes and represents one crucial step in liposomal-based drug delivery strategies. In biology, the membrane fusion is tightly regulated, and often necessitates of specialized proteins to overcome the large energetic cost needed to disrupt the lipid bilayers and commence the fusion process. Over the past years, synthetic DNA nanostructures have been developed, which mimic the activity of fusogenic proteins and enable the implementation of rationally-designed fusion pathways. However, the influence of lipid composition on DNA-mediated membrane fusion has received limited attention, with most studies relying on highly fusogenic compositions which do not faithfully represent the lipid content of cellular membranes.1,2 Here, we systematically investigate the effect of membrane composition on the fusogenic ability of DNA nanostructures (Fig. 1a), as well as the on the morphological and biophysical properties of the fusion products. We determine that fusogenicity strongly correlates with the presence of conical PE lipids, (Fig. 1b) which we ascribe to their ability to stabilise non-bilayer phases in the de-hydration conditions induced by the DNA constructs.3 These findings could guide the development of next generation fusogenic nanostructures with improved performance. Secondly, we observed that DNA-mediated fusion of liposomes with different compositions, e.g. rich in saturated and un-saturated lipids, leads to the emergence of complex lipid architectures, such as nested or dumbbell morphologies (Fig. 1c), which was accompanied by alterations in the membrane's biophysical behaviour (Fig. 1d).3 These results highlight the potential of fusogenic DNA nanodevices as a means of programmably reshaping lipid assemblies and direct the behaviour of compartmentalised systems, a key requirement in the development of synthetic artificial cells.

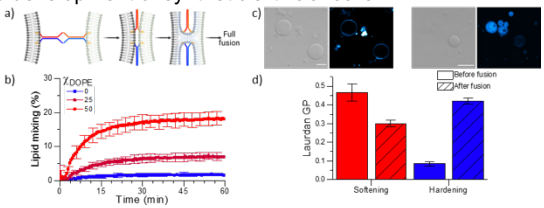

Fig. 1. (a) DNA zippers bring together opposing membranes allowing for spontaneous membrane fusion to take place. (b) Such membrane remodelling requires the presence of conical lipids (χ: %PE lipids compared to lamellar PC ones). (c) The fusion of highly ordered DPPC/DOPE/Chol membranes (left) with DOPC/DOPE/Chol liposomes leads to membrane remodelling. Scalebar: 30μm. (d) The addition of "softening" or "hardening" fusogenic vesicles is reflected by alterations in membrane fluidity, as reported by Laurdan.

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P-60

Triple-stranded DNA as a structural element in DNA origami

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Besides double-stranded DNA, there exists a multitude of other DNA secondary structures which form through non-canonical base pairing. One of these alternative structures is Triplex DNA.
In DNA origami, neighbouring double stranded DNA helices are bound together via swapping strands. This crossover formation can also be achieved when a single stranded extension of one helix forms a triplex in a neighbouring helix. The aim of this work is to increase the structural toolkit of DNA origami via a third strand. Using triplexes as a design element leads to an increase in crossover density, which may result in higher rigidity of our DNA origami structures and crossovers, induced by triplex formation, also lead to structures which fold via pH gradient.

P-61

Enhanced stiffness of wireframe DNA nanostructures with square lattice edges

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In recent years, interest in wireframe DNA origami has increased, mainly due to the more efficient scaffold usage and the possible applications in biomedicine [1,2]. One common problem with this type of structures is their lower mechanical stiffness compared to more classical closely packed structures [3,4]. Different approaches have been presented to improve on this particular issue, such as more than one helix per edge or making different design choices [4,5]. Here we present a way to enhance the stiffness of wireframe DNA nanostructures based on the previously published BSCOR-vHelix software package [3,6]. In short, our new approach is based on the addition of additional helices to the edges of the wireframe structure. The additional helices are organized in a square lattice, thus increasing the stiffness of the single edges. In addition, thanks to the presence of additional helices, the vertices of the structures can also be reinforced, further increasing the mechanical stability of the structure. The routing of the scaffold is based on the one from the BSCOR software, so these reinforced edges can be incorporated together with more flexible, single helix edges. We demonstrated the increased stiffness and the possibility of folding this new type of structures using a combination of computational (oxDNA[7] simulations) and experimental techniques (negative staining and Cryo electron microscopy, AFM). The development of this kind of structures could open the way for the design of nanostructures with parts with different flexibility or stiffness, custom-tailored for the specific task.

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P-62

Enhancement of CRISPR/Cas12a trans-cleavage Activity Using Hairpin DNA Reporters

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Beyond the remarkable editing ability of CRISPR-Cas (clustered regularly interspaced short palindromic repeat-CRISPR-associated) systems, the RNA programmed non-specific (trans) nuclease activity of CRISPR-Cas Type V and VI systems has opened a new era in the field of nucleic acid-based diagnostics [1, 2]. However, when using Cas enzymes without preamplification of the target, most CRISPR-based platforms shows relatively slow signal generation and limited sensitivity. Here, we report on the enhancement of trans-cleavage activity of Cas12a enzymes using hairpin DNA sequences as FRET-based reporters. We discover faster rate of trans-cleavage activity and improved affinity (Km) of Cas12a for hairpin DNA structures and provide mechanistic insights of our findings through Molecular Dynamics simulations. Through the rational design of hairpin DNA probes, we significantly enhance FRET-based signal transduction compared to the widely used linear single stranded DNA reporters. Our signal transduction enables faster detection of clinically relevant DNA targets with improved sensitivity and specificity either in the presence or in the absence of an upstream pre-amplification step.

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