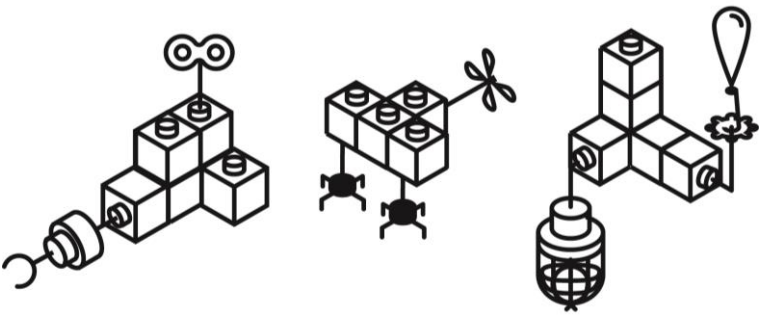
Functional DNA Nanotechnology



Rome, 6-8 June 2018

Book of Abstracts

Organizing Scientific Committee:

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Workshop program

6th June

10:30 - 12:00 Registration

12:00 -12:10 Opening

12:10 - 13:40 Session #1 - Chair: Hanadi Sleiman

12:10 -12:40	**Invited Lecture (IL) 1: Nadrian C. Seeman** (New York University): "DNA: Not Merely the Secret of Life"
12:40 -13:00	**O-1: Thomas Gerling** (Technical University of Munich): "High-Symmetry DNA Objects and Methods to Increase their Structural Stability"
13:00 -13:20	**O-2: Veikko Linko** (Aalto University): "DNA Origami for Biophysical Devices"
13:20 -13:40	**O-3: Lorenzo Di Michele** (University of Cambridge): "Highly porous responsive crystalline frameworks self-assembled from amphiphilic DNA nanostructures"

13:40 - 14:40 Refreshments + poster session

14:40 - 16:00 Session #2 - Chair: Alexander A. Green

14:40 -15:10	**IL-2: Ralf Jungmann** (Max Planck Institute and LMU): "Super-resolution with DNA-PAINT"
15:10 - 15:30	**O-4: Barbara Saccà** (University of Duisburg-Essen): "The role of the edges in the folding pathway of DNA origami"
15:30 - 15:50	**O-5: Alexis Vallée-Bélisle** (University of Montreal): "Thermodynamics and kinetics of DNA switches and DNA assembly"
15:50 - 16:00	**Flash presentations** (2 minutes each x 5)

16:00 - 16:30 Coffee Break + poster session

16:30 - 18:00 Session #3 - Chair: Yannick Rondelez

16:30 -17:00	**IL-3: Alexander A. Green** (Arizona State University): "RNA Nanodevices for Biocomputing and Diagnostics"
17:00 - 17:10	**O-6: Davide Mariottini** (University of Rome, Tor Vergata): "Making order of functionality through disorder"
17:10 - 17:20	**O-7: Andrew J. Lee** (University of Leeds): "Direct in situ observation of RecA mediated homologous recombination"
17:20 - 17:40	**O-8: Jonathan R. Burns** (University College London): "Determining the Orientation of DNA Nanostructures in Membranes"
17:40 - 18:00	**O-9:Jonathan Doye** (University of Oxford): "Mechanical properties of DNA nanostructures"

7th June

09:00-10:45 Session #4 - Chair: Andrew J. Turberfield

09:00-09:30	**IL-4: Hanadi Sleiman** (McGill University): "Amphiphilic DNA Nanostructures: Self-assembly and Biological Properties"
09:30 - 09:50	**O-10: Sébastien Bidault** (PSL Research University, Paris): "DNA-templated plasmonic nanostructures to enhance single molecule fluorescence emission"
09:50 - 10:10	**O-11: Eyal Nir** (Ben-Gurion University of the Negev): "Computer Controlled DNA Bipedal Walker that Perform Several Steps a Minute"
10:10 - 10:30	**O-12: Amelie Heuer-Jungemann** (Ludwig-Maximilians-Universität): "Silica encapsulation of DNA Origami"
10:30 - 10:45	**Flash presentations (2 minutes each x 5)**

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

11:40-13:00 Session #5 - Chair: Ralf Seidel

11:40 - 12:10	**IL-5: Tom de Greef** (Technische Universiteit Eindhoven): "Programmable DNA-based Communication in Populations of Artificial Cells"
12:10 - 12:20	**O-13: Christin Möser** (University of Potsdam): "Using DNA nanostructures to present and potentiate peptides in an oligovalent manner"
12:20 - 12:30	**O-14: Nayan Agarwal** (Technische Universität Dresden): "Structural transformation of wireframe DNA Origami via DNA polymerase assisted gap-filling"
12:30 - 12:40	**O-15: Emanuela Torelli** (Newcastle University): "Isothermal folding of a light-up bio-orthogonal RNA origami nanoribbon"
12:40 - 12:50	**O-16: Alexander Ohmann** (University of Cambridge): "A synthetic DNA-built enzyme flips 107 lipids per second in biological membranes"
12:50 - 13:00	**O-17: Erik Benson** (Karolinska Institutet): "Evolutionary refinement of DNA nanostructures using coarse-grained molecular dynamics simulations"

13:00 - 14:00 **Lunch**

14:00-15:10 Session #6 - Chair: Nadrian C. Seeman

14:00 - 14:30 **IL-6: Andrew J. Turberfield** (Oxford University): "Kinetic control of DNA hybridization reactions"

14:30 - 14:50 **O-18: Matteo Palma** (Queen Mary University of London): "DNA-Programmed Assembly of Nanohybrids for Single-Molecule Investigations: from Optoelectronics and Sensing to Cancer Cell Adhesion"

14:50 - 15:10 **O-19: Naama Lahav** (Weizmann Institute of Science): "Oligonucleotide–Small Molecule Conjugates as Tools for Programming Bacterial Behavior"

15:10-16:00 Session #7 Publishing presentations + discussion

Chiara Pastore (Associate Editor Nature Nanotechnology): "Publishing in Nature Nanotechnology"
Julia Echoff (Nature Communications):
"*Nature Communications* – The journal and its offers"

16:00 - 17:00 **Coffee Break + informal discussion**

17:00 - 20:00 **Social programme** (guided tour to the catacombs of San Callisto or bike tour of the Appian way)

20:00 **Social dinner + Award Ceremony**

8th June

09:00-11:00 Session #8 - Chair: Tom F. A. de Greef

09:00-09:30	**IL-7: Yannick Rondelez** (CNRS, Paris): "DNA-programmable dissipative communities"
09:30 - 9:50	**O-20: Irina Nesterova** (Northern Illinois University): "Analytical power of DNA i-motif: pH and beyond"
09:50 - 10-10	**O-21: Andreas Heerwig** (Technische Universität Dresden): "DNA origami-based nanostructures in motion"
10:10 - 10:20	**O-22: Elena Ambrosetti** (Karolinska Institutet): "Deciphering protein clusters at the cell membrane with DNA nanotechnology"
10:20 - 10:30	**O-23: Robert Oppenheimer** (University of Oxford): "Architectures for DNA-templated chemical synthesis"
10:30 - 10:40	**O-24: Francesca Garbarino** (Technical University of Denmark): "On-chip optomagnetic detection and discrimination of single base mutation in *Mycobacterium tuberculosis"*
10:40 - 10:50	**O-25: Andrea Idili** (University of California Santa Barbara): "Continuous, real-time measurement of a cancer chemotherapeutic in a living body using electrochemical aptamer-based sensors and a novel drift correction approach"
10:50 - 11:00	**O-26: Turkan Bayrak** (TU Dresden): "Functionalized DNA Origami Nanostructures for Molecular Electronics"

11:00 - 11:30 Coffee break

11:30-13:20 Session #9 - Chair: Ralf Jungmann

11:30- 12:00	**IL-8: Ralf Seidel** (Universität Leipzig): "DNA origami templated metal nanostructures"
12:00 - 12:20	**O-27: Adrian Keller** (Paderborn University): "Pharmacophore nanoarrays on DNA origami substrates as a single-molecule assay for fragment-based drug discovery"
12:20 - 12:40	**O-28: Alessandro Desideri** (University of Rome Tor Vergata): "Functionalized octahedral DNA nanocages for a targeted drug delivery"
12:40 - 13:00	**O-29: Marco Todisco** (University of Milan): "RNA supramolecular liquid-crystalline order catalyzes its own polymerization"
13:00 - 13:20	**O-30: Andrew Houlton** (Newcastle University): "A coordination chemistry approach to the assembly and functionalisation of DNA-based materials"

13:20 Refreshments + closing remarks

Invited Lectures

IL1 **Nadrian C. Seeman** (*Department of Chemistry, New York University*)
DNA: Not Merely the Secret of Life

IL-2 **Ralf Jungmann** (*Max Planck Institute of Biochemistry*)
Super-resolution with DNA-PAINT

IL-3 **Alexander A. Green** (*Arizona State University*)
RNA Nanodevices for Biocomputing and Diagnostics

IL-4 **Hanadi Sleiman** (*McGill University*)
Amphiphilic DNA Nanostructures: Self-assembly and Biological Properties

IL-5 **Tom F.A. de Greef** *(Eindhoven University of Technology)*
Programmable DNA-based Communication in Populations of Artificial Cells

IL-6 **Andrew J. Turberfield** *(University of Oxford)*
Kinetic control of DNA hybridization reactions

IL-7 **Yannick Rondelez** *(CNRS, Paris)*
DNA-programmable dissipative communities

IL-8 **Ralf Seidel** *(University of Leipzig)*
DNA origami templated metal nanostructures

Invited Oral Publishing

OP-1 **Chiara Pastore**
Publishing in Nature Nanotechnology

OP-2 **Julia Echoff**
Nature Communications – The journal and its offers

Posters

P-1 Spatially-confined DNA-peptide conjugates for biomarker detection
Abimbola .F. Adedeji, Miguel Soler, Giacinto Scoles, Matteo Castronovo, Sara Fortuna

P-2 Gene-therapy inspired polycation coating for protection of DNA origami nanostructures
Yasaman Ahmadi, Elisa De Llano, Ivan Barišić

P-3 Unravelling the properties of hybrid DNA-Supramolecular Polymers
Miguel Angel Aleman Garcia, Eva Magdalena Estirado, Lech G. Milroy, Luc Brunsveld

P-4 Electrochemical Surface Impedance Spectroscopy of Adhering Lipid Vesicles: A Sensing Technology for the Quantification of Ligands
Omar Amjad, Bortolo Mognetti, Pietro Cicuta, Lorenzo Di Michele

P-5 pH-controlled assembly and disassembly of DNA nanostructures
A. Amodio, L. Green, A. F. Adedeji, M. Castronovo, E. Franco, F. Ricci

P-6 Aptamer Functionalised Nanomaterial for Detection of antibiotic resistant *Acinetobacter baumannii*
Shahnawaz A Baba, Piyush Kalra, Naveen Kumar Navani

P-7 Kinetic study of CRISPR-Cas9 for dynamic DNA nanotechnology
Alexandre Baccouche, Teruo Fujii, Anthony Genot

P-8 Inkjet printing of DNA-based semiconducting nanowires
Tom Bamford, Andres Aldana, Atsinafe Oshido, Sarah Milsom, Andrew Pike, Andrew Houlton, Ben Horrocks

P-9 Spatial clusters in two species systems
Marianne Bauer, Erwin Frey

P-10 Network-forming DNA nanostars for the investigation of condensed matter physics
Giovanni Nava, Francesco Sciortino, Tommaso Bellini

P-11 Programmable DNA and RNA technologies for binding-responsive sensing of target biomolecules
Alessandro Bertucci, Alessandro Porchetta, Junling Guo, Agata Glab, Nicolas Oppmann, Frank Caruso, Francesca Cavalieri, Francesco Ricci

P-12 Flexibility defines structure in amphipilic DNA crystals
Ryan Brady, Nicholas J. Brooks, Vito Foderà, Pietro Cicuta, Lorenzo Di Michele

P-13 Structure beyond sequences: miRNAs a rich variety of conformations.
Alessandro D'Urso, C.M.A. Gangemi, S. Alaimo, A. Pulvirenti, D. Milardi, G. Oliviero, A. Ferro, C.M. Croce, R. Purrello

P-14 Adenita: A Software Toolkit for the Visualization and Modeling of DNA Nanostructures
Elisa De Llano, Haichao Miao, Tobias Isenberg, Eduard Groeller, Ivan Viola, Ivan Barisic

P-15 Dissipative DNA-based nanomachine for the release of molecular cargo in a time-controlled fashion
Erica Del Grosso, Alessia Amodio, Giulio Ragazzon, Leonard Prins, Francesco Ricci

P-16 DNA Secondary Structure Assisted Controlled Immobilization Strategy
Ankit Dodla, Bhaskar Datta

P-17 Toward *in vitro* implementation of dCas9-based regulatory networks
Emilien Dubuc, Pascal Pieters, Ardjan van der Linden, and Tom de Greef

P-18 A Hierarchical Carrier System Based on DNA Nanostructures and Layer-by-Layer Microcarriers
Florian Engert, Ralf Seidel, Uta Reibetanz

P-19 Colorimetric monitoring of nanoscale actuation in DNA-templated plasmonic nanostructures
Elise Y. Gayet, Laurent Lermusiaux, Gaëtan Bellot, Sébastien Bidault

P-20 Introducing reversible hydrophobic and magnetic properties to DNA nanostructures using proteins
Marisa A. Goetzfried, Cornelia Monzel, Nolan B. Holland, Maxime Dahan, Friedrich C. Simmel, Tobias Pirzer

P-21 Towards DNA-Templated Molecular Electronic Devices
Seham Helmi, Jonathan Bath, Arzhang Ardavan & Andrew J. Turberfield

P-22 DNA Nanostructures that Target and Rupture Bacterial Membranes
J.R. Burns, A.L.B. Pyne, I. Bennett, B. Lamarre, M.G. Ryadnov, S. Howorka

P-23 DNA origami for circular dichroism-based sensing
Yike Huang, Anton Kuzyk

P-24 Biosensing based on weak molecular interactions
S. Hwu, V. Gatterdam, J. Vörös

P-25 MD simulations capture the subtle structural features of a DNA origami nanovault
F. Iacovelli, G. Grossi, M. Falconi, E.S. Andersen, A. Desideri

P-26 Hybrid DNA origami – protein devices as sensors and cellular transport vehicles
H. Ijäs, B. Shen, V. Linko, J. A. Ihalainen

P-27 Programmable DNA-based Communication in Populations of Artificial Cells
Alex Joesaar, Shuo Yang, Bas Bögels, Ardjan van der Linden, Andrew Phillips, Pavan Bosukonda, Stephen Mann, Tom de Greef

P-28 DNA Origami-Directed 3D Nanoparticle Superlattice
S. Julin, V. Linko, M. A. Kostiainen

P-29 Heterochiral DNA Nanotechnology
Adam M. Kabza, Brian E. Young, Jonathan T. Sczepanski

P-30 Interfacing DNA Nanotech with Membranes to Optimize Detection
W. Kaufhold, R. Brady, J.Tuffnell, L Di Michele

P-31 Experimental and Theoretical Study of DNA Bipedal Motor Walking Dynamics and Origami-based Force-clamp System
Dinesh C. Khara, John S. Schreck, Philipp C. Nickels, Tommy E. Tomov, Yaron Berger, Thomas E. Ouldridge, Jonathan P. K. Doye, Tim Liedl, and Eyal Nir

P-32 Stability of DNA Origami Nanostructures in Low-Magnesium Buffers
Charlotte Kielar, Yang Xin, Boxuan Shen, Mauri A. Kostiainen, Guido Grundmeier, Veikko Linko, and Adrian Keller

P-33 Cryo Electron Microscopy of DNA Origami Nanostructures
Massimo Kube, Hendrik Dietz

P-34 The knowledge evolution of DNA Nanoscience and DNA Nanotechnology: similarities, complementarities and differences
Hanh Luong La, Rudi R.N.A. Bekkers

P-35 Use of multivalent interactions to achieve super-selective targeting in biological systems
R. Lanfranco, B. Matteo Mognetti, G. Bruylants, P. Cicuta, L. Di Michele

P-36 Thermodynamics and Kinetics of the Regulation and Self-Assembly of DNA Polymolecular Nanomachines
Dominic Lauzon, Alexis Vallée-Bélisle

P-37 α-$_L$-Threose Nucleic Acids as Biocompatible Antisense Oligonucleotides for Suppressing Gene Expression in Living Cells
Ling Sum Liu, Hoi Man Leung, Dick Yan Tam, Tsz Wan Lo, Sze Wing Wong, Pik Kwan Lo

P-38 Asymmetric DNA Scaffolds and their Application as Combinatorial Sensors and Molecular Security Systems
Omer Lustgarten, Raanan Carmieli, Leila Motiei, David Margulies

P-39 Sub-Ensemble Monitoring of DNA Strand Displacement Using Multiparameter Single-Molecule FRET
Laura E. Baltierra-Jasso, Michael J. Morten, and Steven W. Magennis

P-40 Thiol-free oligonucleotide surface modification of gold nanoparticles for nanostructure assembly
Anastasia Maslova, I-Ming Hsing

P-41 Catalyzed hairpin assembly of magnetic nanoclusters with single nucleotide discrimination
G.A.S. Minero, R.W. Baber, J. Fock, M.F. Hansen

P-42 DNA and DNA like polymer based self-assembled and hierarchical nanostructures for biosensing
Aboulfazl Mirzapoor, Ashutosh Tiwari, Anthony P.F. Turner, Bijan Ranjbar

P-43 Dual amplification strategy triggered by triple helix probe for the detection of microRNAs
Andrea Miti, Giampaolo Zuccheri

P-44 Design and development of DNA-based synthetic push-pull networks
Ismael Mullor-Ruiz, Guy-Bart V. Stan, Thomas E. Ouldridge

P-45 Modelling the Folding Pathway of DNA Origami
B. Najafi, K. G. Young, J. Bath, J. Doye, A. Louis, A. Turberfield

P-46 Quantitation without calibration: a new approach to nucleic acids' measurement
I.V. Nesterova, M. Debnath, J. Farace, K. Johnson

P-47 Photo-switchable artificial nucleosides for DNA origami machines
Fernanda A. Pereira, Thomas Gerling, Hendrik Dietz

P-48 Engineering Programmable Nucleic Acid Nanoswitches for the Rapid Detection of Antibodies in Bodily Fluids
Alessandro Porchetta, Bruna Marini, Rudy Ippodrino, Francesco Ricci

P-49 Rational control of the activity of a Cu^{2+}-dependent DNAzyme by re-engineering purely entropic disordered domains
Simona Ranallo, Daniela Sorrentino, Francesco Ricci

P-50 Allosterically Regulated DNA-based Switches for Controlled Release of a Molecular Cargo Activated by Biological Inputs
Marianna Rossetti, Simona. Ranallo, Andrea Idili, Giuseppe Palleschi, Alessandro Porchetta, Francesco Ricci

P-51 Photocontrol of DNA origamis melting and formation: towards light-controlled isothermal nanomachines
Caroline Rossi-Gendron, Sergii Rudiuk, Mathieu Morel, Damien Baigl

P-52 Resolving the Sequence of Events in the Folding of DNA Nanostructures
Fabian Schneider, Natalie Möritz, Hendrik Dietz

P-53 Plasmonic nanostructures through DNA-assisted lithography
Boxuan Shen, Veikko Linko, Kosti Tapio, Siim Pikker, Tibebe Lemma, Ashwin Gopinath, Kurt. V. Gothelf, Mauri A. Kostiainen, J. Jussi Toppari

P-54 DNA-Templated Assembly of the Bacterial Flagellar Motor's Cytoplasmic Ring
Joel Spratt, Samuel Tusk, Richard M. Berry, Andrew J. Turberfield

P-55 A Microsphere-Supported Lipid Bilayer Platform for DNA Reactions on a Fluid Surface
Aurora Fabry-Wood, Madalyn E. Fetrow, Carl W. Brown, III, Nicholas A. Baker, Nadiezda Fernandez Oropeza, Andrew P. Shreve, Gabriel A. Montaño, Darko Stefanovic, Matthew R. Lakin, Steven W. Graves

P-56 Complexing DNA origami frameworks through sequential self-assembly based on directed docking
Yuki Suzuki, Hiroshi Sugiyama, Masayuki Endo

P-57 Nucleic acid assembly mediated by the fluorous effect
Andrea Taladriz-Sender, Jamie M. Withers, Gabriella E. Flynn, Gerard Macias, Sarah L. Henry, Alasdair W. Clark, Glenn A. Burley

P-58 G-Quadruplex-Mediated Molecular Switching of Self-Assembled 3D DNA Nanocages
Dick Yan Tam, Hoi Man Leung, Miu Shan Chan, Pik Kwan Lo

P-59 All-Optical Imaging of Gold Nanoparticle Geometry Using Super-Resolution Microscopy
Adam Taylor, René Verhoef, Michael Beuwer, Yuyang Wang and Peter Zijlstra

P-60 Protein induced fluorescent enhancement based thrombin DNA aptasensor
Saurabh Umrao, Anusha, Vasundhara Jain, Banani Chakraborty, Rahul Roy

P-61 Strength and kinetics of DNA hybridization on a surface measured by Reflective Phantom Interface
L. Vanjur, T. Carzaniga, G. Zanchetta, M. Salina, T. Bellini, M. Buscaglia

P-62 Direct Single-Molecule Observation of Mode and Geometry of RecA-Mediated Homology Search
Andrew J. Lee, Masayuki Endo, Jamie K. Hobbs, A. Giles Davies, Christoph Wälti

P-63 DNA-origami mediated self-assembly of nanoelectronic circuits
R. Weichelt, J. Ye, Vladimir Lesnyak, Nikolai Gaponik, Ralf Seidel, Alexander Eychmüller

P-64 Nano-electronic components built from DNA templates
Jingjing Ye, Seham Helmi,Ralf Seidel

P-65 Surface Seeded Self-assembly of DNA Nanostructures
Tao Ye, Huan Cao, Qufei Gu, Warren Nanney

P-66 Quantifying specific and non-specific interactions between proteins and DNA via an optical label-free technique based on reflectivity
G. Zanchetta, T. Carzaniga, L. Casiraghi, G. Dieci, M. Buscaglia, T. Bellini

P-67 Kinetically Programmed, One-Pot DNA Reactions for Molecular Detection Directly in Whole Blood
Guichi Zhu, Carl Prévost-Tremblay, Dominic Lauzon, Marie-Élaine Bérubé, Alexis Vallée-Bélisle

IL-1

DNA: Not Merely the Secret of Life

Nadrian C. Seeman

Department of Chemistry, New York University, New York, NY 10003, USA

We build branched DNA species that can be joined using Watson-Crick base pairing to produce N-connected objects and lattices. We have used ligation to construct DNA topological targets, such as knots, polyhedral catenanes, Borromean rings and a Solomon's knot.

Nanorobotics is a key area of application. We have made robust 2-state and 3-state sequence-dependent programmable devices and bipedal walkers. We have constructed 2-dimensional DNA arrays with designed patterns from many different motifs. We have used DNA scaffolding to organize active DNA components. We have used pairs of 2-state devices to capture a variety of different DNA targets. We have constructed a molecular assembly line using a DNA origami layer and three 2-state devices, so that there are eight different states represented by their arrangements. We have demonstrated that all eight products can be built from this system. Recently, we connected the nanoscale with the microscale using DNA origami.

We have self-assembled a 3D crystalline array and reported its crystal structure to 4 Å resolution. We can use crystals with two molecules in the crystallographic repeat to control the color of the crystals. Rational design of intermolecular contacts has enabled us to improve crystal resolution to better than 3 Å. We can now do strand displacement in the crystals to change their color, thereby making a 3D-based molecular machine; we can visualize the presence of the machine by X-ray diffraction.

The use of DNA to organize other molecules is central to its utility. Earlier, we made 2D checkerboard arrays of metallic nanoparticles, and have now organized gold particles in 3D. Most recently, we have ordered triplex components and a semiconductor within the same lattice. Thus, structural DNA nanotechnology has fulfilled its initial goal of controlling the internal structure of macroscopic constructs in three dimensions. A new era in nanoscale control awaits us.

IL-2

Super-resolution with DNA-PAINT

Ralf Jungmann

Max Planck Institute of Biochemistry

Super-resolution fluorescence microscopy is a powerful tool for biological research. We use the transient binding of short fluorescently labeled oligonucleotides (DNA-PAINT) for simple and easy-to-implement multiplexed super- resolution imaging that achieves sub-10-nm spatial resolution.
We employ orthogonal DNA probes that allow sequential imaging of multiple targets using a single dye and a single laser source (Exchange-PAINT) and demonstrate whole cell imaging with DNA- and Exchange-PAINT using Spinning Disk Confocal microscopy, now allowing DNA-based super-resolution imaging deep inside cells, away from the glass coverslip.
We furthermore use DNA-PAINT to quantify both incorporation and accessibility of all individual strands in DNA origami with molecular resolution. We find that strand incorporation strongly correlates with the position in the structure, ranging from a minimum of 48% on the edges to a maximum of 95% in the center.
Finally, we present efficient ways to label a large variety of protein molecules in cells with DNA strands for DNA-PAINT.

IL-3

RNA Nanodevices for Biocomputing and Diagnostics

Alexander A. Green

Biodesign Center for Molecular Design and Biomimetics, The Biodesign Institute and School of Molecular Sciences, Arizona State University, AZ 85287, USA

Biological circuits enable cells and cellular systems to carry out artificial functions with applications in medicine, chemical production, and the environment. This talk will describe our work to develop RNA nanodevices for biological circuits that operate in living cells and low-cost diagnostics. Using RNA-based translational regulators as modular parts, we have implemented biological circuits that can execute multi-input logic operations in *Escherichia coli*. These ribocomputing circuits compute user-defined combinations of AND, OR, NAND, and NOR logic by harnessing programmable RNA-RNA interactions using cellular transcripts as inputs. To respond to the pressing global need for effective low-cost diagnostics, we have also developed RNA nanodevices that operate in inexpensive nucleic acid tests. These systems employ cell-free reactions that recapitulate the transcriptional and translational capabilities of cells, but can be preserved on paper at room temperature for over a year and reactivated simply by adding water. We will discuss how these portable cell-free systems can be used in tests requiring minimal equipment to detect infectious agents such as the Zika virus and dengue, and how they can provide specificity down to the single-nucleotide level to detect cancer-associated mutations and drug-resistant pathogens.

IL-4

Amphiphilic DNA Nanostructures: Self-assembly and Biological Properties

Hanadi Sleiman

Canada Research Chair in in DNA Nanoscience
Department of Chemistry, McGill University
E-mail: Hanadi.sleiman@mcgill.ca

DNA modification with hydrophobic moieties is an attractive approach to merge the programmability and anisotropy provided by DNA, with the hierarchical and long-range organization of hydrophobic interactions. This talk will first describe the investigation of amphiphilic DNA structures as mimics of the multiple functions displayed by membrane proteins. By tuning the position, orientation and flexibility of tethered cholesterol units, these structures can display peripheral membrane anchoring, surface clustering into organized patterns, or membrane nanopore behavior. We will also describe a new strategy to engineer amphiphilic DNA nanostructures for strong and specific binding to the protein human serum albumin (HSA), and the biological properties of these molecules. HSA is the most abundant protein in the blood and has been shown to accumulate inside cancer tissues and to protect structures from degradation and phagocytosis. Introducing protein-binding domains in DNA nanostructures represents a step forward to use them for in vivo applications, interfacing them effectively with biological entities. Finally, we will describe a method to 'print' DNA patterns onto other materials, such as nanoparticles and polymers, thus beginning to address the issue of scalability for DNA nanotechnology.

IL-5

Programmable DNA-based Communication in Populations of Artificial Cells

Alex Joesaar,[1] Shuo Yang,[1] Bas Bögels,[1] Ardjan van der Linden,[1] Andrew Phillips,[2] Pavan Bosukonda,[3] Stephen Mann[3] and Tom F. A. de Greef [1]

[1] Institute for Complex Molecular Systems, Eindhoven University of Technology, The Netherlands. [2] Microsoft Research, Cambridge CB1 2FB, UK. [3] Centre for Protolife Research and Centre for Organized Matter Chemistry, School of Chemistry, University of Bristol, UK

The quest to develop bottom-up constructed artificial cells is an important topic in synthetic biology and has led to the construction of different types of synthetic compartments that can act as simplified model systems for living cells. These synthetic cell-like compartments (protocells) have been configured to perform various biomimetic functions such as protein expression, responding to external stimuli, and predation. Here we use dynamic DNA nanotechnology to implement molecular communication in synthetic protocell populations. Protein-polymer microcapsules (proteinosomes)[1] act as synthetic protocells where chemical reaction networks based on DNA strand-displacement are selectively encapsulated. Short DNA oligonucleotides function as chemical messengers that protocells are able to sense and secrete. We demonstrate various network configurations, such as multi-stage signalling cascades, amplifiers, Boolean logic, and negative feedback. Our work shows that DNA based logic is a robust platform for engineering inter-protocellular communication, and that subjecting DNA circuits to selective compartmentalization allows them to exhibit functionality that is not possible under batch conditions.

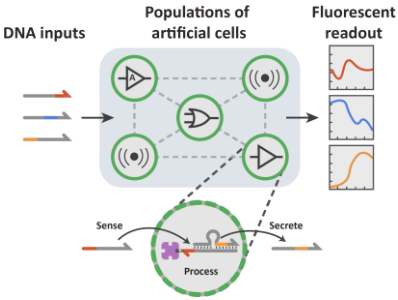

Figure 1 Cartoon showing the overall configuration of the system. Various DNA-based network motifs are selectively localized in artificial cells. The reactions are triggered by the introduction of short DNA oligonucleotides which can freely diffuse though the porous proteinosome membrane. The artificial cells can respond to the input stimulus by secreting other DNA oligonucleotides, which function as second messengers and can be sensed by cells from a different population.

References

[1] Huang, X. et al. Interfacial assembly of protein–polymer nano-conjugates into stimulus-responsive biomimetic protocells. Nat Commun 4, 2239 (2013).

IL-6

Kinetic control of DNA hybridization reactions

Natalie E. C. Haley[1], Thomas E. Ouldridge[2], Jonathan Bath[1] and Andrew J. Turberfield[1]

[1]University of Oxford, Department of Physics, Clarendon Laboratory, Parks Road, Oxford OX1 3PU United Kingdom
[2]Department of Bioengineering, Imperial College London, 180 Queens Road, London SW7 2AZ United Kingdom

DNA strand displacement [1,2,3] underpins the operation of DNA-based synthetic molecular machinery and systems for molecular computation. Autonomous systems generally operate out of equilibrium, making it important to control not only the free-energy changes that determine the direction of spontaneous change but also the rates of competing processes. I shall review strategies for kinetic control of DNA hybridization [3-7] and introduce a new method by which the rate of strand displacement is controlled by the placement of base-pairing defects.

References

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IL-7

DNA-programmable dissipative communities

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It is possible to craft synthetic analogs of genetic circuits that rely on DNA-base pairing rules to enforce network topology, and a few purified enzymes as machinery. These artificial and out-of-equilibrium reaction networks can implement precisely programmed non-linear dynamics, such a oscillation, excitability or bistability in well-mixed closed reactors.
We are now using the same molecular programming approach for the creation of synthetic communities, loosely inspired from the collective behavior of cooperating organisms or cells. This approach uses tethered DNA strands to program the local chemical behavior of microscopic solid agents distributed in a feeding solution. The DNA-programmed agents can sense the behavior of neighboring agents through multiple orthogonal chemical diffusion channels. In turn, they use these stimuli to decide their own actions. I will present collective behaviors involving thousands of agents of various types, for example retrieving information over long distances, or creating spatial patterns.

IL-8

DNA origami templated metal nanostructures

Ralf Seidel

Peter Debye Institute for Soft Matter Physics, University of Leipzig

Biological systems have developed a number of mechanisms how to assemble inorganic matter with complex shapes. For example, correspondingly formed biomolecular structures are used as templates for material deposition. DNA nanotechnology has recently provided a wealth of techniques to fold DNA into well-defined two- and three-dimensional structures in a programmable manner. Here we explore, how we can use such complex structures to synthesize inorganic materials with programmable shapes. To this end, we employ a previously established concept in which rigid three-dimensional DNA origami nanostructures are used as molds to dictate the final shape of metal particles that form by a seeded-growth procedure. We use individual molds as bricks to build extended and more complex mold superstructures, which support the formation of extended metal structures. Using this technique, we demonstrate the formation of linear conductive gold nanowires with high uniformity, the controlled formation of structures of different sizes, the incorporation of semiconductive materials as well as the modular incorporation of different functional mold elements which enables the formation of circular and branched mold geometries.

OP-1

Publishing in Nature Nanotechnology

Chiara Pastore

Associate Editor Nature Nanotechnology

In this presentation I will give an insider's view into publishing in the journals from the Springer Nature family. After giving an introduction on the journals' organisation, their scope and breadth of appeal, I will move to specifically describe Nature Nanotechnology. I will delve deeper into the editorial criteria that we apply at the journal and I will tell you about my daily life as an editor, which will give me the possibility to describe the editorial process in details. Finally I will briefly talk about our efforts to increase the discussion on the societal aspects linked to nanotechnology.

OP-2

Nature Communications – The journal and its offers

Julia Echoff

Nature Communications is the Nature Research flagship Open Access journal, publishing high quality and influential research. It features more than 80 editors with diverse scientific backgrounds, making it possible to process and accept submissions across the full spectrum of the natural sciences. The journal is committed to provide opportunities for authors that go beyond publishing their papers. This talk will inform you about Nature Communications and its unique features.

O-1

High-Symmetry DNA Objects and Methods to Increase their Structural Stability

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Laboratory for Biomolecular Design, Physics Department and Institute for Advanced Study, Technical University of Munich

In my presentation, I will talk about recent progress in some of the projects I'm working on. One important direction of research in structural DNA nanotechnology is the design and self-assembly of high-order assemblies. In biological objects, such as viruses, precursor objects with low symmetry often self-assemble into objects with higher symmetry, which in turn represent building blocks for higher-order assemblies. We aim to translate this principle into the arena of three-dimensional multilayer DNA origami objects. To this end, we designed corner-like objects with low symmetry that may assemble into a frame-like object with higher symmetry. Cubic assemblies can be formed from the frame-like building blocks in solution. In these building blocks, not only the shape of interacting interfaces are the same but also the sequences of participating basepairs.

In the second part of my talk, I will present two methods for enhancing the structural stability of DNA nanostructures. Typically, DNA nanostructures disassemble when exposing them to low ionic strength solutions, other organic solvents and to elevated temperatures, which renders applications in different fields as not readily accessible. By introducing cyano-vinyl-carbazole (^{cnv}K) moieties into DNA objects, one can create additional covalent linkages. More specifically, the ^{cnv}K moiety can be covalently linked by a [2+2] photo-cycloaddition with a pyrimidine base on the complementary duplex strand upon UV irradiation. Insertion of additional covalent bonds can be used to substantially increase the structural stability of DNA-based mechanisms and higher-order assemblies across binding interfaces.

O-2

DNA Origami for Biophysical Devices

Veikko Linko

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Customized DNA structures [1,2] can find a plethora of uses in molecular nanotechnology owing to their exceptional addressability. They can serve as templates for *e.g.* proteins [3,4], plasmonic nanoshapes [5,6], metal nanoparticles [7,8] and nanoscopic rulers [9]. Recently, an increasing effort has been put into applying DNA structures as smart drug-delivery vehicles, although it is well known that their transfection rates are rather poor and they are prone to degradation. To overcome these issues and to increase their biocompatibility we have coated the structures electrostatically using cationic polymers [10], virus capsid proteins [11] and protein-dendron conjugates [12]. Furthermore, we have studied their transfection properties [4,11,12], drug-loading efficiency [13] and stability in low-magnesium buffers [14,15]. Importantly, our inert protein coatings can attenuate the activation of immune response and protect the structures from endonuclease digestion [12].

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O-3

Highly porous responsive crystalline frameworks self-assembled from amphiphilic DNA nanostructures

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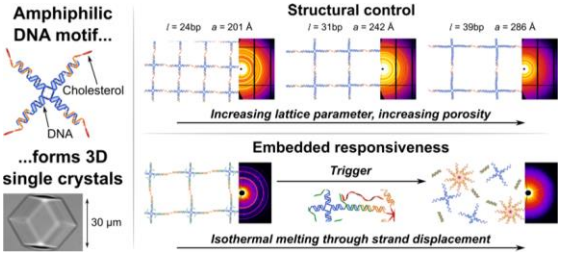
Several emerging technologies require the production of highly porous frameworks with a controlled nanoscale morphology. Due to the exquisite binding selectivity of nucleic acids, together with their facile synthesis and functionalization, DNA nanotechnology has emerged as a prime route for the production of programmable and functional nanoscale materials. Nonetheless, the preparation of 3D DNA frameworks which are, at the same time, crystalline and highly porous remains elusive. Indeed, branched motifs prepared with DNA origami or tiles can only support long-range order in 2D, while 3D crystallization has only been demonstrated with compact building blocks forming low-porosity networks.

We introduce a novel class of amphiphilic DNA building blocks that, combining the programmability of Watson-Crick base pairing with the robustness of hydrophobic forces, self-assemble into 3D macroscopic single crystals (Figure, left), in which the lattice parameter *a* can be tuned between 18.4 and 34.2 nm, and precisely prescribed by straightforward scaling of the DNA motifs (Figure, top right) [1,2]. We exploit the tunable porosity, reaching 85%, to control the partitioning of a broad range of molecular species, and create size-exclusion filters with a precise cutoff. The robustness and versatility of our approach enables the modification of the amphiphilic building blocks with responsive DNA motifs, which can be triggered to induce isothermal melting of the network (Figure, bottom right) [2].



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O-4

The role of the edges in the folding pathway of DNA origami

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The self-assembly of a DNA origami structure, although nowadays experimentally feasible in most cases, may represent indeed a rather complex folding problem. Recent studies on DNA origami assembly allowed to identify the important entropic role of the edges,[1] which act as initial folding seeds, driving the assembly along one or the other pathway through allosteric propagation to the entire structure.[2, 3] Despite these notable progresses, a unifying and quantitative view of origami assembly which takes into account both sequence-dependent and mechanically-induced conformational changes is still partial, particularly in light of the isomerization of the Holliday junction (HJ), the basic motif of every DNA origami structure.[4] In this work, we analyzed the folding and unfolding of a monolayer DNA origami composed of three quasi-independent domains, identical in size and crossover pattern but different in sequence content. Using a combination of AFM, temperature-dependent FRET-spectroscopy and single-molecule force-extension experiments, we monitored the assembly/disassembly of each domain in absence or presence of three types of edges, thus imposing different topological constraints on the same set of sequences and analyzing the effect of sequence content for identical mechanically-induced stresses. Our data show that the folding landscape of a “minimal origami domain” travels along two minima of energy re-conducible to the *iso I* and *iso II* conformers of the HJ. Although the assembly pathway is initially dictated by the difference in base-stacking energy between the two isomers, its final fate may be affected by mechanically-induced transformations due to unsustainable topological stress.

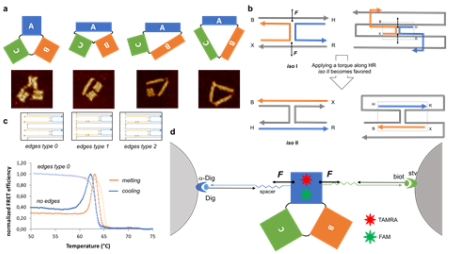

Figure. Quasi-independent domains of the same DNA origami structure, topologically identical but different in sequence-content, can undergo variable assembly fates simultaneously (a), depending on base-stacking energies the edges (c, d).

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O-5

Thermodynamics and kinetics of DNA switches and DNA assembly

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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 re-created several complex biochemical mechanisms (e.g. induced-fit mechanism, conformational selection mechanism, sequestration mechanism, allosteric cooperativity mechanism, complex cooperativity) 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 with applications in medicine and nanotechnology.

O-6

Making order of DNA Nanodevices through disorder

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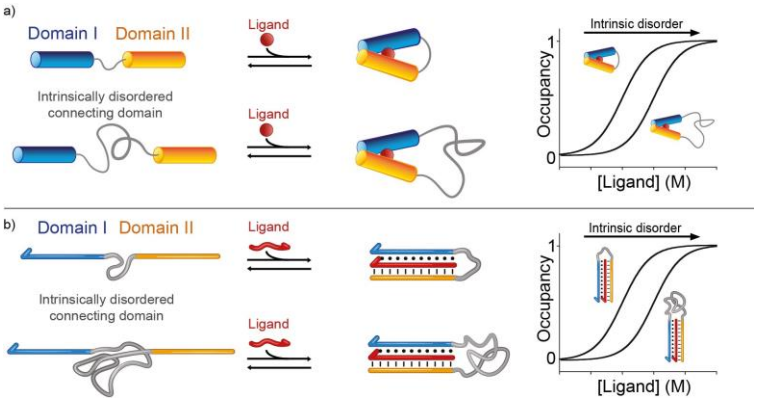
Proteins employ intrinsically disordered domains to gain control over their activity through purely entropic contribution. This discovery has recently challenged the original dogma that disorder plays against functional activity and that proteins require well-folded domains to function properly [1]. The possibility to mimic naturally occurring mechanisms to design and control synthetic molecular devices has led to tremendous advances in the fields of nanotechnology. In this vein, we report here a convenient and versatile approach to control the activity and response behaviour of synthetic nanodevices by rationally designing intrinsically disordered domains. We demonstrate that, similarly to intrinsically disordered proteins, such approach allows to finely modulate the affinity of a wide range of synthetic receptors through a purely entropic contribution in a highly versatile way without requiring any complex thermodynamic designing approach (Fig.1).



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O-7

Direct *in situ* observation of RecA mediated homologous recombination

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Homologous recombination – which exchanges identical or very similar DNA strands – is critical in maintaining genomic integrity. Central to this pathway is the ubiquitous protein RecA – and its homologues – which catalyse the alignment and exchange of DNA strands at regions of sequence homology. Not only is this protein invaluable in a biological context, its programmable specificity and fidelity has made it appealing for use in bionanotechnological applications.[1] The process of homologous recombination can broadly be broken up into three phases: the formation of an active nucleoprotein filament through the polymerisation of RecA monomers upon a ssDNA substrate; the location of a homologous dsDNA sequence and alignment of the filament with this sequence to form a triple-stranded complex; and strand exchange followed by disassembly of the complex. However, despite decades of research, to date the mechanism behind which RecA is able to orchestrate a search for homology and undertake a strand exchange remains illusive. DNA nanostructures are not only of great value for a range of bionanotechnology applications, but the ability of DNA to self-assemble into complex shapes – DNA origami – can be exploited to create DNA-based scaffolds on which functional protein components can be arranged in a determined spatial arrangement with spatial precisions on the molecular scale. Such DNA structures can provide an environment to host enzyme interactions which is both suitable for direct imaging, i.e. attached to a surface, as well as providing near-physiological and hence solution-like conditions for the enzymes to work. Here, we use such DNA origamis to observe directly and *in situ* Recombinase A (RecA)-driven homologous recombination on a single molecule level using high-speed atomic force microscopy (HS-AFM). We employ a DNA origami to display several DNA strands such that RecA is able to orchestrate a recombination reaction in a specified manner. The DNA origami not only provides the framework to hold the DNA strands, but also acts as a geometrical reference within which the orientation and relative positions of the DNA can be identified, enabling the unambiguous identification of the intermediates that occur during the homologous recombination process when the reaction is observed in real-time with HS-AFM. We present the direct observation of a RecA-orchestrated alignment of homologous DNA strands and the proceeding strand exchange to form a stable recombination product within a supporting DNA nano-structure. We demonstrate that the RecA filament is able to identify sequences of micro-homology as short as three nucleotides throughout the search for sequence homology, enabling pairing of seed sequences which are proceeded by subsequent sequence alignment where a match is located.

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O-8

Determining the Orientation of DNA Nanostructures in Membranes

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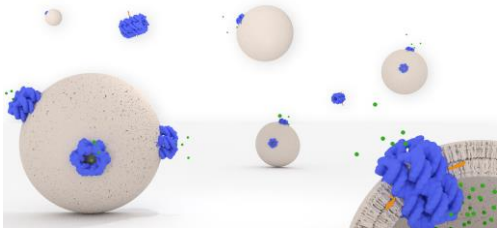
Identifying the interaction and topography of DNA nanostructures bound to cell membranes is a crucial next step in applied DNA nanotechnology. DNA-based nanopores are a new class of bilayer-puncturing nanodevices that can help advance biosensing, synthetic biology, and nanofluidics. We have recently engineered a range of compact DNA nanopores which can bind and span lipid bilayers to enable the controlled flux of molecules across the biophysical divide. However, several important questions remain unknown, including: what is the nanopore binding and insertion rate, how does the lipid composition and curvature influence insertion, and finally how do DNA nanopores insert into membranes? In this talk we present a new approach to identify these questions using a nuclease probe assay in combination with fluorescence spectroscopy and TIRF microscopy. Our strategy determines the molecular accessibility of DNA nanopores with a nuclease and can thus distinguish between the nanopores' membrane-adhering and membrane-spanning states. Our study can help advance the field of DNA nanotechnology by enabling scientists to identify membrane interactions in the development of more advanced DNA nanostructures.



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O-9

Mechanical properties of DNA nanostructures

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Here, we report results from simulations using oxDNA [1], a coarse-grained model of DNA at the nucleotide level, on a variety of mechanical properties of DNA nanostructures. Firstly, using the approach of Ref. [2], we extract the elastic moduli (bend, twist and twist-bend coupling) from simulations of a range of DNA nanotubes differing in the number of helices, internal twist and basic design (origami or single-stranded tiles), our results being in good agreement with available experimental values. Secondly, we use oxDNA to attempt to calibrate the forces being exerted in a DNA nanoscopic force-clamp [3], paying particular attention to the role of secondary structure in the single-stranded sections of the device. Thirdly, we explore the mechanical limits of DNA origamis that are subject to tension. For a two-dimensional Rothemund tile we observe a regular series of stick-slip features in the force-extension curve associated with the co-operative yielding of two rows of the origami, whereas for a three-dimensional origami we observe strain softening due to the less local staple connections. Fourthly, we hope to report on the mechanism of buckling for DNA origami nanotubes subject to extreme twists. Finally, we combine oxDNA with classical density-functional theory to compute the Frank elastic constants (and hence the cholesteric pitch) for liquid crystalline phases of twisted DNA origami nanotubes [4]. The handedness of the phases is opposite to what would one expect from simple arguments based on their twisted geometry. Our calculated values for the cholesteric pitch are in good agreement with the experimental results, but only when we include the effects of flexibility. The origin of the phase chirality is the chiral shape fluctuations of the nanotubes, which have an opposite effect to and dominate over the steric surface chirality due to the internal twist of the helices.

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O-10
DNA-templated plasmonic nanostructures to enhance single molecule fluorescence emission

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By providing intense optical fields that are confined at the nanoscale, plasmonic nanostructures can enhance both the excitation and decay rates of fluorescent emitters by several orders of magnitude. In order to couple efficiently and reproducibly quantum emitters to plasmonic antennas, DNA self-assembly is a particularly flexible and robust technique to introduce a single fluorescent molecule in the gap between gold particles with nanoscale precision (Fig. 1-a). Single molecule spectroscopy techniques have allowed us to demonstrate that DNA-templated gold particle dimers can enhance the spontaneous emission rates of dye molecules by more than two orders of magnitude (Fig. 1-b), while maintaining single photon emission properties [1]. Importantly, these plasmonic nanoantennas feature fluorescence lifetimes that can fall below 10 ps and quantum yields above 50% [2]. Furthermore, the versatility of nucleotide synthesis allows the introduction of multiple identical or different fluorescent dyes. In particular, the use of two spectrally-matched organic dyes enabled us to investigate the influence of plasmonic resonators on non-radiative energy transfer processes (such as FRET, Fig. 1-c) [3]. Importantly, by screening the electrostatic repulsion between negatively charged gold colloids, we can actively tune the interparticle distance in DNA-templated dimers and reach reproducibly gaps below 3 nm [4]. We will discuss our current work on the controlled introduction of multiple emitters in such a nanoscale gap to reach strong coupling regimes between the polarizable electron clouds of the fluorescent molecules and of the gold particles (Fig. 1-d) as recently discussed in the literature [5].

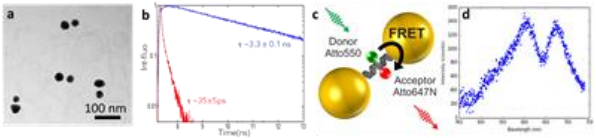

Figure 1: (a) Cryo-EM image of DNA templated gold nanoparticle dimers. (b) Fluorescence lifetime of a single molecule on DNA (blue) and in a gold particle dimer (red). (c) Scheme of a plasmonic nanostructure used to influence FRET. (d) Preliminary results of the strong coupling between 5 intercalating dye molecules and a 40 nm plasmonic dimer observed in scattering spectroscopy.

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O-11

Computer Controlled DNA Bipedal Walker that Perform Several Steps a Minute

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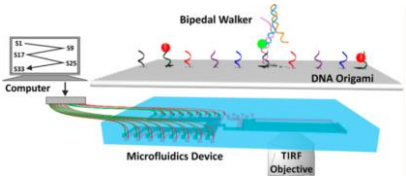
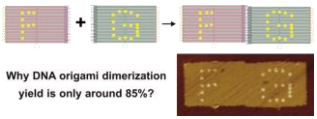
Synthetic molecular machines are orders of magnitude slower and less proccessive then biological molecular machines. A major effort in our group is to develop fast and proccessive DNA based molecular machines. In recent years we have demonstrated a DNA bipedal motor that strides on a DNA origami track and operates by responding to 'fuel' and 'antifuel' DNA strands[1]. The strands are provided to the motor by a computer controlled microfluidics device and motor progress is monitored by single molecule FRET. Importantly, the microfluidics allows the removal of excess strands and motors waste, crucial for proper operation of the motor. With this setup, we demonstrate the performance of 32 walking steps (64 consecutive chemical reactions with ~98.9% yield per reaction) which amount to 370 nanometers traveled by the walker. However, initial motor designs were very slow; about an hour per step, and increased fuel concentration, necessary for faster walking, resulted in decreased processivity (yield). Redesigning of the walking mechanisms, such that higher fuel concentration does not decrease yield, resulted in two orders of magnitude increase in motor speed for the same yield, and three orders of magnitude for somewhat lower yield. Altogether, we improved the motor speed-yield factor (SYfactor) by more than three and half orders of magnitude over eight years effort. Finally, our improved motor can walk over distance larger than the size of a single origami unit. However, current methods for sticky ends attachment of origami units to construct longer tracks allow origami dimerization with only about 85% yield. We have recognized the reason for the low yield, the formation of homo-dimers[2], and developed a method to overcome this problem. With this method we achieved origami dimerization with ~98-99% yield. The method is general and can significantly improve organization of different origami units into larger structures.





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O-12
Silica encapsulation of DNA Origami

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DNA nanotechnology allows for the bottom-up synthesis of nanometer-sized objects with high precision and selective addressability due to the programmable hybridization of complementary DNA strands. The introduction of DNA origami[1] has resulted in a plethora of objects of different shapes and sizes, many of which have been site-specifically modified with a variety of functional moieties such as proteins or nanoparticles. Potential fields of applications of these DNA nano-objects range from plasmonic metamaterials to nanomedicine.

Compared to many other biomolecules such as proteins or RNA, DNA displays a good chemical stability. Nevertheless, it is still highly susceptible to hydrolysis, depurination, depyrimidination, oxidation, alkylation and degradation by nucleases. Moreover, DNA origami structures often require high salt concentrations to maintain their structural integrity.[2] The mechanical stability of DNA structures is naturally limited by the bending and stretching behavior of the constituting DNA duplexes.

In order to overcome some of these shortcomings, different protection strategies have been employed such as coating with a lipid bilayer or a cationic poly(ethylene glycol)–polylysine block-copolymer.[3] Full mechanical resilience, e.g. to drying, has not been achieved yet.

In nature, DNA can be chemically and physically preserved for millenia as can be seen in fossils, where DNA is hermetically sealed by biomineralization. Here we show that DNA origami can be equally “fossilized” and protected by encapsulation in silica.[2, 4]

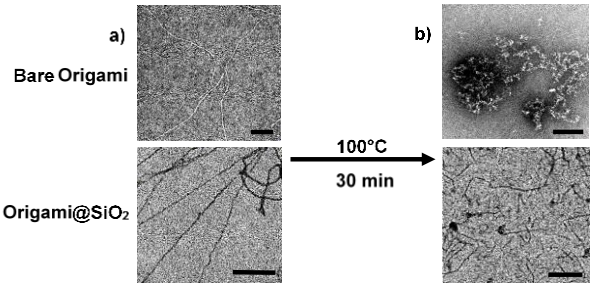

Fig. 1 TEM images of bare (top) and silica encapsulated (bottom) 14 helix bundles before (a) and after (b) heating to 100°C for 30 min. While bare structures completely disintegrate, silica-encapsulated structures remain intact after heating. Scale bars are 100 nm.

We demonstrate that different DNA origami structures can be silica-encapsulated, protecting them from degradation at e.g. high temperatures (**Fig. 1**) or in low salt environments. We anticipate that this approach will allow for the transformation of DNA origami structures from the solution to the solid phase, preserving their structural integrity. Especially with a view on more advances structures, such as 3D DNA

origami crystals[5], this technique will enable more detailed studies of structural morphologies and enable optical applications as features such as lattice spacings would be preserved and the structures are no longer subject to distortion or collapse due to drying effects.

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O-13

Using DNA nanostructures to present and potentiate peptides in an oligovalent manner

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DNA nanostructures enable the attachment of molecules (e.g. fluorophors, peptides, proteins etc.) to nearly any unique location on the structure. Due to the resolution provided by DNA-based nanostructures, spatial arrangements of several ligands suitable to their desired target can be created and closely controlled. In contrast to multivalent interactions, we aim to create directed oligovalent interactions between DNA nanostructures and naturally occurring counterparts. Therefore, bioactive peptides are attached to different DNA nanostructures (Figure 1) and their impact on biological systems like cancer cells, viruses and structural proteins are examined.
As an example, the efficacy of a peptide that binds to cancer-related EphrinA2 receptors was increased by attaching several of these peptides to DNA nanostructures and thus presenting them in an oligovalent manner. Not only activation of the downstream signalling pathways but also binding of the construct and phosphorylation of the receptors were investigated.
For another project DNA constructs were used to carry hemagglutinin-binding peptides. Hemagglutinin receptors are located on influenza A viruses and mediate binding to host cells and consequently viral infection. By blocking hemagglutinin receptors with peptide-conjugated DNA nanostructures, we aim to hinder viruses from binding to host cells. In a third project, synthetic actin crosslinkers made of DNA and actin-binding peptides were build and their effects on structural and dynamic behaviors of actin networks were characterized [1].

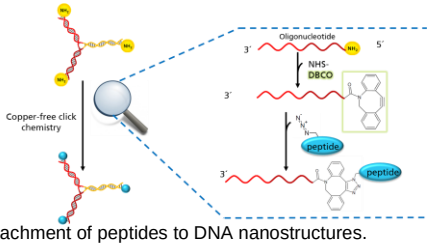

Figure 1. Schematic attachment of peptides to DNA nanostructures.

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O-14

Structural transformation of wireframe DNA Origami *via* DNA polymerase assisted gap-filling

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The programmability of DNA enables constructing nanostructures with almost any arbitrary shape, which can be decorated with many functional materials. Moreover, dynamic structures can be realized such as molecular motors and walkers. In this work, we have explored the possibility to synthesize the complementary sequences to single-stranded gap regions in the DNA origami scaffold cost effectively by a DNA polymerase rather than by a DNA synthesizer.[1] For this purpose, four different wireframe DNA origami structures similar to our previous work[2] were designed to have single-stranded gap regions. This reduced the number of staple strands needed to determine the shape and size of the final structure after gap filling. For this, several DNA polymerases and single-stranded binding (SSB) proteins were tested, with T4 DNA polymerase being the best fit. The structures could be folded in as little as 6 min, and the subsequent optimized gap-filling reaction was completed in less than 3 min. The introduction of flexible gap regions results in fully collapsed or partially bent structures due to entropic spring effects. Finally, we demonstrated structural transformations of such deformed wireframe DNA origami structures with DNA polymerases including the expansion of collapsed structures and the straightening of curved tubes (Figure 1). We anticipate that this approach will become a powerful tool to build DNA wireframe structures more material-efficiently, and to quickly prototype and test new wireframe designs that can be expanded, rigidified, or mechanically switched. Mechanical force generation and structural transitions will enable applications in structural DNA nanotechnology, plasmonics, or single-molecule biophysics.

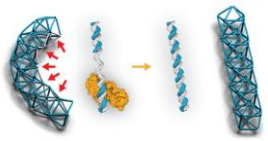

Figure 1. Structural transformation of the wireframe tubes using a DNA polymerase. Tube structures were folded with gap regions highlighted by red arrows. These gap regions act as entropic springs that force the entire structure to curve. After filling the gap regions with T4 DNA polymerase, tubes were straightened.[1]

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O-15

Isothermal folding of a light-up bio-orthogonal RNA origami nanoribbon

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A plethora of self-assembled DNA origami and hybrid RNA-DNA origami have been synthesized using the basic principle of Watson-Crick base pairing. Despite the RNA functional capacity, the synthesis of RNA nanostructures via 'scaffold' and 'staple' strands is underdeveloped and still lacking. While significant advances have been made in the DNA origami synthesis, the design and realization of RNA origami has been reported only recently[1]. Here we take into account the large gap between DNA origami and RNA origami development and inspired by a bottom-up origami toolkit, we present a light-up biologically inert (i.e. 'bio-orthogonal') RNA origami able to fold at constant temperature[2]. In our recent work[3], square DNA origami and triangle RNA-DNA hybrid origami were synthesized using 'bio-orthogonal' and uniquely addressable De Brujin 'scaffold' sequences (DBS). Unlike biologically derived 'scaffold', these DNA and RNA 'bio-orthogonal' scaffolds do not contain genetic information, restriction enzyme sites or ambiguity in the addressability making them candidates for *in vivo* applications. Here RNA 'staple' strands promoted the folding of a short 'bio-orthogonal' RNA 'scaffold' sequence into a nanoribbon: after an initial denaturation step, the self-assembly occurred at the physiological temperature (37 °C). The RNA origami assembly was verified by gel assay, atomic force microscopy (AFM) and using a new split Broccoli aptamer system able to bind the specific fluorophore only after the folding process. The Broccoli aptamer[4] is divided into two nonfunctional sequences each of which is integrated into the 5' or 3' end of two 'staple' strands complementary to the RNA scaffold. Using in-gel imaging and fluorescence measurements, we demonstrated that once the RNA origami assembly occurs, the split aptamer sequences are in close proximity to form the aptamer and turn on the fluorescence.
Herein, we investigate and combine three different aspects: i) 'bio-orthogonality', ii) physiologically compatible folding at 37 °C and iii) assembly monitoring through a new split Broccoli RNA aptamer system. Our resulting RNA origami nanoribbon can open the way to new potential platform for future *in vivo* applications when genetically encoded and transcribed RNA are used.

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O-16

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

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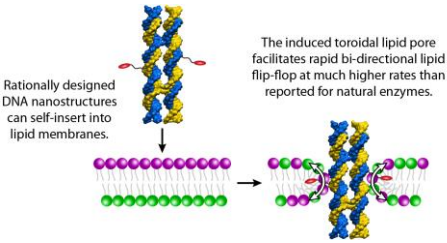
[†]These authors contributed equally to the work.

Mimicking enzyme function and increasing performance of naturally evolved proteins is one of the most challenging and intriguing aims of nanoscience. Here, we employ DNA nanotechnology to design a synthetic enzyme that substantially outperforms its biological archetypes [1]. Consisting of only eight strands, our DNA nanostructure spontaneously inserts into biological membranes by forming a toroidal pore that connects the membrane's inner and outer leaflets. The membrane insertion catalyzes spontaneous transport of lipid molecules between the bilayer leaflets, rapidly equilibrating the lipid composition. Through a combination of microscopic simulations and single-molecule experiments we find the lipid transport rate catalyzed by the DNA nanostructure to exceed 10^7 molecules per second, which is three orders of magnitude higher than the rate of lipid transport catalyzed by biological enzymes. Furthermore, we show that our DNA-based enzyme can control the composition of human cell membranes, which opens new avenues for applications of membrane-interacting DNA systems in medicine.



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O-17

Evolutionary refinement of DNA nanostructures using coarse-grained molecular dynamics simulations

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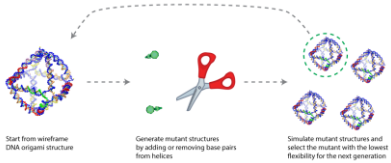
In the last decade, DNA nanostructures have made the leap from small assemblies of a handful of oligonucleotides to Megadalton objects assembled from hundreds of component DNA strands. Any design based on DNA is inherently constrained by the geometry of DNA, leading most design strategies to rely on lattice design rules for the placement of crossovers between DNA helices. In recent years, examples of lattice-free DNA designs have emerged, based on simple automatic optimizations of helix length in combination with unpaired bases to minimize strain in the structures[1]. These structures however suffer from non-optimal rigidity. In parallel with the development of DNA nanostructures, software packages for the simulation of nucleic acids have seen rapid development allowing for the simulation of the dynamics of full DNA nanostructure assemblies[2]. We implement a software based on the oxDNA package to simulate wireframe DNA origami structures and then score and rank the flexibility their helices. The software then automatically generates a number of mutant structures by adding or removing base pairs from one or several helices. These structures are simulated and evaluated, and the mutant structure with the highest rigidity is retained for the next iteration, creating an in-silico evolution towards more rigid DNA origami structures.



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O-18

DNA-Programmed Assembly of Nanohybrids for Single-Molecule Investigations: from Optoelectronics and Sensing to Cancer Cell Adhesion

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The controlled organization of individual molecules and nanostructures with nanoscale accuracy is of great importance in the investigation of single-molecule events in biological and chemical assays, as well as for the fabrication of the next generation optoelectronic devices. DNA has been shown to be an ideal structural material for this purpose, due to the specificity of its programmability and outstanding chemical flexibility. I will discuss the development of different DNA-functionalized nanohybrid platforms for single-molecule investigations in organic, inorganic and biological systems. In particular, I will present the controlled formation of reconfigurable single-molecule heterostructures where the spacing between a functional nanomoiety and an individual carbon nanoelectrode was controlled and dynamically tuned by a DNA spacer, employed as a molecular ruler. As a proof of concept, we assembled individual carbon nanotubes (CNTs) coupled to single colloidal semiconductor nanocrystals (Quantum Dots, QDs), chosen as model systems due to their tunable emissions and broad absorbances that further make them ideal candidates for novel light harvesting systems in photovoltaics and light emitting diodes. DNA interconnects of different length allowed us to modulate the electronic coupling between the two nanostructures with single-molecule control, as demonstrated via static and time-resolved photoluminescence investigations, as well as single-molecule measurements: this is an essential attribute for future device implementation. Additionally, stimuli-responsive CNT-QD nanohybrids were assembled, where the distance, and hence the electronic coupling, between an individual CNT and a single QD were dynamically modulated via the addition and removal of potassium (K^+) cations; the system was further found to be sensitive to K^+ concentrations from 1 pM to 25 mM. Finally, I will show how a facile strategy combining the programming ability of DNA as a scaffolding material with a one-step lithographic process can be employed to co-localize synergistic cell binding domains on surfaces with nanoscale precision. I will discuss how this platform can be used for cancer cell adhesion investigations with single-molecule control. In particular, we designed multi-valent DNA nanoplatforms and chemisorbed them on a Focused Ion Beam nanopatterned surface so to co-localize an $\alpha v\beta 6$-integring specific peptide and the epidermal growth factor (EGF) within 60 nm (optimal integrin spacing) in array configurations on the same substrate. We will demonstrate how the biochip developed allowed us to study, with single-molecule control, integrin-dependent responses and their cooperation with EGF in the adhesion of epidermal cancer cells. (As cells interact with the extracellular matrix via transmembrane proteins integrins, and integrin-mediated interactions play a

central role in many functional processes, including metastasis, it is of paramount importance to study the molecular mechanism of integrin-dependent biological responses and their cooperation with different cell-binding domains in cancer cell adhesion). To the best of our knowledge, our is the first example of a platform that combines multivalence capability and nanoscale spatial control for cell adhesion investigations with single-molecule resolution.

O-19

Oligonucleotide–Small Molecule Conjugates as Tools for Programming Bacterial Behavior

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Cell surface receptors constitute a wide range of membrane proteins that mediate the response of cells to changes in their environment. In recent years, much effort has been devoted to genetically modifying bacterial membranes with heterologous protein 'receptors' in order to provide them with new properties[1]. However, these methods cannot be used to incorporate synthetic molecules such as fluorescent probes, drugs and affinity tags on the bacterial surface.

Herein we present a method for modifying the bacterial membrane with a short oligonucleotide (ODN) that serves as a template for binding complementary ODNs (Figure 1). This approach allows us to decorate the membrane with different functionalities through high-affinity Ni(II)-NTA interactions and consequently, program bacterial behavior and response using different ODN-small molecule conjugates.

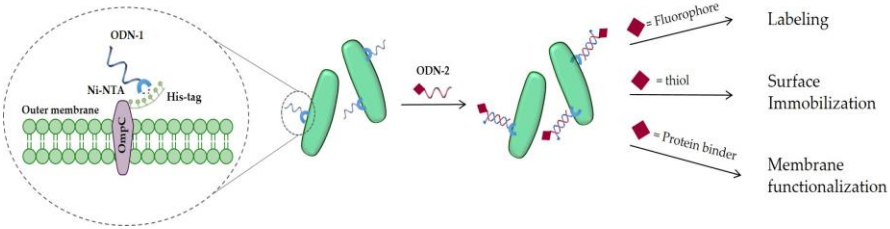

Figure 1. Design of a system able to detect and program bacterial behavior using ODN inputs.

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O-20

Analytical power of DNA i-motif: pH and beyond

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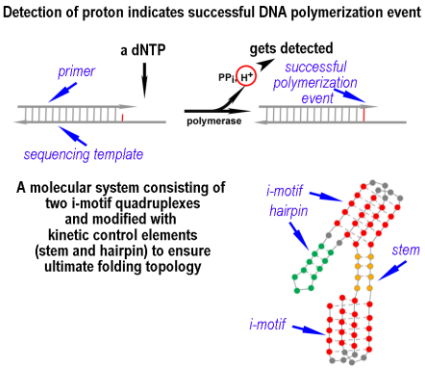
Over the past decade, DNA i-motif as a structure possessing high sensitivity towards protons, has emerged in a variety of analytical applications.[1] Those include our work on strategies for control over response range and response sensitivity in DNA i-motifs.[2-4] The strategies involve structural tools for tuning response range as well as elements for kinetic control over folding topology in i-motifs. Overall, the developments allow designing sensing systems with precisely defined operational parameters. Currently, we focus our efforts on establishing a new platform for measuring very small changes in proton concentrations over high and uncertain backgrounds. Particularly, we develop an i-motif based sensing system for detection of nanomolar changes in concentration of protons released during DNA polymerization. The capability is crucial for advancing next generation sequencing platforms based on sequencing-by-synthesis.[5] A proton is a by-product of nucleotide incorporation during DNA polymerization; therefore, its detection is an indirect indicator of a successful polymerization event (upper image). In the sequencing-by-synthesis protocol, a dNTP of one type is added to a sequencing template in the presence of polymerase. If added dNTP is complementary to a next base on the template, the nucleotide gets incorporated. The detection of a successful polymerization event reveals identity of a base in unknown sequence.

To detect very small (i.e. nanomolar) changes in H^+ concentration, two criteria must be met: the sensing system should possess high response sensitivity and the conditions must be buffer-less. To achieve ultimate sensitivity, we develop a molecular system consisting of multiple quadruplexes. We equip the system with structural elements to control its folding topology (lower image). Elimination of buffer introduces background's uncertainty. The tunable nature of nucleic acid scaffold allows tuning response range to an actual background. As a result, we are able to detect polymerization events at the levels 3-4 orders of magnitude below current technologies.



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O-21

DNA origami-based nanostructures in motion

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We demonstrate how origami nanolevers [1] on an electrode can be manipulated (switched) at high frequency by alternating voltages [2]. The orientation switching is long-time stable and can be induced by applying low voltages of merely 200 mV. The mechanical response time of a 100 nm long origami lever to an applied voltage step is less than 100 µs, allowing for a highly dynamic control of the induced motion. Moreover, through voltage assisted capture, the origamis can be immobilized directly from folding solution without purification, even in the presence of a large excess of staple strands. The results establish a way for interfacing and controlling DNA origamis with standard electronics, and enable their use as moving parts in electro-mechanical nanodevices.

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O-22

Deciphering protein clusters at the cell membrane with DNA nanotechnology

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The interplay and clustering between proteins on the cell surface is a key component in regulating membrane protein function. Protein assemblies regulate biological activity and have emerged as being of therapeutic importance: the nanoscale spatial ordering and the dynamic distribution of proteins in the cell membrane fine tune signalling pathways critical to various physiological and pathological processes [1,2]. The study of protein nanoscale environments at cell surface will shed light on the spatial regulation of receptor signalling and of the complex molecular networks downstream of receptor activation. Therefore, there is a huge need to find high-throughput methods to study how proteins organize at molecular level and how this determines the cell phenotypes. In this work, we present the development of a non-microscopy based super-resolution method for unbiased analysis of protein nanoclusters at the cell membrane, studying the frequency in which specific types of protein appear in the proximity of the protein of interest. Moving toward this goal we setup a DNA nanotechnology-based approach, using DNA nanostructures, to decipher the position and the identity of proteins within the assembly (Fig. 1).

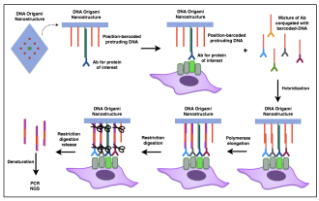

Fig.1 Schematic representation of the experimental design by which DNA nanostructure is used to decipher proteins assemblies on cell membrane.

STORM-TIRF super-resolution microscopy analysis and advanced biochemical assays are combined to validate the methodology and to gain a thorough understanding of the molecular portrait of protein clusters. As proof of concept we focused on clustering of the membrane receptor Her2. Although it has well known roles in driving tumour progression, the molecular mechanisms by which Her2 determines the different cell fates are still unclear [3]. Since Her2 forms homo- and hetero-dimers and oligomers, we aim to provide a precise portrait of the spatial organization of Her2-containing clusters and to understand how it correlates with cellular outcomes. Our approach has the potential to contribute to developing a new paradigm in targeting receptor signalling at the nanoscale, focusing not on the membrane receptors per se, but on their spatial organization.

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O-23

Architectures for DNA-templated chemical synthesis

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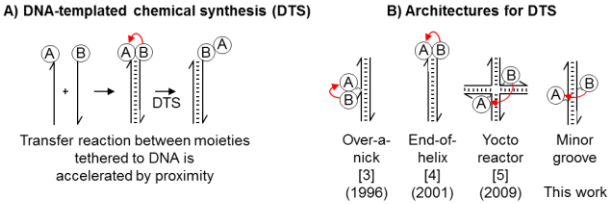
The ribosome is a nucleic acid catalyst conserved throughout Nature for its fundamental role in the translation of genes into proteins. The development of analogous synthetic molecular machinery, capable of genetically programmed synthesis, would permit the directed evolution of novel, functional polymers with biomimetic and completely non-biological backbones and side chains. Simple DNA-based artificial ribosomes that template the chemical synthesis of small molecules and sequence-controlled polymers have been demonstrated [1] (Figure A). There are currently a limited number of molecular architectures that enable DNA-templated chemical synthesis (DTS), each of which creates problems when used in the multi-step synthesis of a polymer. The 'end-of-helix' and 'over-a-nick' DTS architectures are asymmetric (Figure B), requiring the use of DNA adapters with reactants linked at 5' and 3' ends. Schemes for programmed synthesis based on a DNA walker and hybridization chain reaction result in the addition of monomers to the *furthest* end of the growing polymer, progressively increasing the distance between reactants: this has been shown to decrease DTS yield [2]. In the 'yoctoreactor' architecture the distance between reactants increases with the number of strands that form a junction.

Here, we present the design, simulation and characterization of a new DTS architecture in which reactants are brought together on either side of the minor groove of a DNA helix. We demonstrate increased yield during DTS using over-the-groove transfer. We discuss the application of this architecture in the design of artificial ribosomes.



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O-24
On-chip optomagnetic detection and discrimination of single base mutation in *Mycobacterium tuberculosis*

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We demonstrate the ability of an on-chip optomagnetic (OM) detection scheme based on the rotation response of magnetic nanoparticles (MNPs) to detect synthetic *Mycobacterium tuberculosis* DNA targets and to discriminate between the wild-type (WT) variant and the single point mutation mutant-type (MT) variant responsible for resistance against the first-line antibiotic, rifampicin. The assay is based on rolling circle amplification (RCA) with MT- and WT-specific padlock probes (PLPs). Upon addition of ligase, PLPs on matching targets are enzymatically joined to form circles, whereas those on mismatching targets remain open. In a subsequent amplification using phi29 polymerase, targets on circular PLPs are extended to form a long concatemer of the sequence complementary to the PLP, whereas non-circular PLPs are not extended (Fig. 1a).[1] The resulting rolling circle products (RCPs) form ~0.5 µm coils that are detected using 100 nm MNPs functionalized with a detection probe with a sequence overlapping part of the PLP backbone. Upon mixing of an MNP suspension with the RCPs, MNPs are bound to the RCPs and thus experience a significant increase of their hydrodynamic size; the fraction of these MNPs is quantified using OM measurements. In these, a magnetic field alternating at a frequency *f* is applied and the modulation of the light transmitted through the suspension is measured as function of *f*. The MNPs have a linked magnetic moment and an optical anisotropy. In the alternating magnetic field they periodically try to reorient themselves to align along the magnetic field causing a change in the transmitted light intensity. The timescale of particle reorientation depends on their hydrodynamic size.[2] We detect the depletion of free MNPs as the reduction of the OM signal at high frequency as function of the time after mixing of the MNPs and RCPs. The assay was based on previously published PLP sequence.[3] The PLP-target hybridization, ligation and RCA were performed sequentially in test tubes after which the RCP-containing solutions were mixed with the MNP suspension in plastic chips, which were quickly mounted for readout in the previously described OM setup capable of performing four parallel measurements.[4] All four combinations of WT and MT targets and WT and MT PLPs were studied simultaneously for a target concentration of 100 pM. Fig. 1b shows the signal from free MNPs vs. time after mixing. A depletion of free MNPs of more than 80% was observed for the matching target-PLP combinations, whereas the mismatching combinations showed a signal decrease of about 4%, which is comparable to the observation for the no target control sample. This demonstrates the ability of the assay with on-chip OM readout to detect M. tuberculosis and the mutation responsible for rifampicin antibiotic resistance with high specificity.

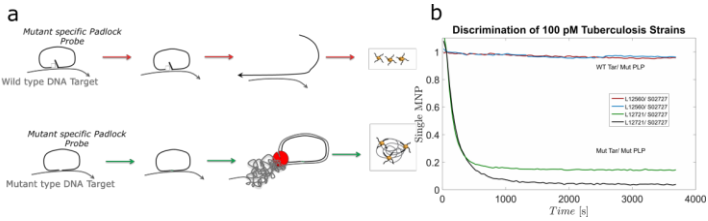

Figure 1 (a) Multistep assay (PLP hybridization, ligation and RCA) for detection of target with single nucleotide specificity. A matching PLP-target pair produces an RCA product whereas a mismatching pair does not. The RCPs are detected via the binding (depletion) of MNPs functionalized with probes targeting a sequence complementary to a part of the backbone of the PLPs. (b) Signal from free MNPs vs. time after mixing with the post-RCA sample.

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O-25

Continuous, real-time measurement of a cancer chemotherapeutic in a living body using electrochemical aptamer-based sensors and a novel drift correction approach

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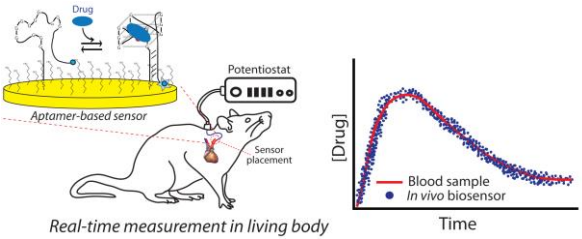
The development of sensors able to measure drugs in-situ in the body could revolutionize health care. Real-time monitoring of drug levels in blood, for example, would support the high-precision measurements of patient-specific pharmacokinetics and, ultimately, even closed-loop feedback-controlled drug delivery. Such personalisation of drug dosing would maximize drug efficacy while minimizing side effects. In response, we have developed electrochemical aptamer-based (E-AB) sensors, a modular sensing platform able to measure continuously and in real-time in the circulatory system of a living animal. Specifically, in this paper we rationally designed a new E-AB sensor against the chemotherapeutic drug camptothecin (CPT) and its derivatives. As the first step in this process we used an optical read-out to guide the re-engineering of the aptamer such that it undergoes a large conformational change upon target binding. We next converted this into an electrochemical read-out by attaching the re-engineered aptamer to an electrode and modifying it with a redox reporter. To ensure that E-AB sensors work in vivo in the veins of live animals we gave historically used a drift correction scheme termed “Kinetic Differential Measurements” (KDM) based on the different square wave frequency dependence of E-AB signaling. To exploit KDM approach, however, requires that the sensor exhibits a strong square-wave frequency response, which our re-engineered aptamer fails to do. To fix this we coupled the aptamer with a short, linear strand DNA that, together, generate the necessary frequency-dependence. Using this approach to KDM drift correction we are able to monitor in real-time the concentration of the camptothecin drug irinotecan in a living animal.



O-26

Functionalized DNA Origami Nanostructures for Molecular Electronics

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The DNA origami method provides a programmable bottom up approach for creating nanostructures of any desired shape, which can be used as scaffolds for nano-electronics and nano-photonics device fabrications. This technique enables the precise positioning of metallic and semiconducting nanoparticles along the DNA nanostructures. In this study, DNA origami nanomolds[1,2] and nanosheets are used for the fabrication of nano-electronic devices. To this end, electroless gold deposition is used to grow the AuNPs within the DNA origami nanomolds and nanosheets create eventually continues nanowires. In order to contact the fabricated nanostructues electrically, a method using electron-beam lithography was developed. The DNA origami nanomold and nanosheet based metallic wires were electrically characterized from room temperature down to 4.2K.
Temperature-dependent characterizations for four wires exhibiting different conductance at RT were performed in order to understand the dominant conductance mechanisms from RT to 4.2K. Two of these nanowires based on nanomold structure showed metallic conductance.[1] The other wires deviated from pure metallic behavior and they showed thermionic, hopping and tunneling charge transport mechanism.

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O-27

Pharmacophore nanoarrays on DNA origami substrates as a single-molecule assay for fragment-based drug discovery

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The identification of small molecules that can selectively interact with protein targets of biomedical importance represents a central problem in chemical biology and drug discovery [1]. The rational combination of experimental techniques from the fields of nanotechnology, single molecule detection, and lead discovery in drug development could provide elegant solutions to enhance the throughput of drug screening. In this work, we have synthesized nanoarrays of small pharmacophores on DNA origami substrates [2] that are displayed either as individual ligands or as fragment pairs. Atomic force microscopy-based single-molecule detection allowed us to distinguish potent protein-ligand interactions from weak binders, either as individual ligands or through bidentate interactions. Several independent binding events, *i.e.*, strong binding, weak binding, symmetric bidentate binding, and asymmetric bidentate binding are directly visualized and evaluated using this approach (see figure 1). Finally, we apply this method to the discovery of bidentate trypsin binders based on benzamidine paired with different aromatic fragments. We find that the combination of benzamidine with certain fluorescent dyes results in up to tenfold enhancement of the trypsin binding yield compared to benzamidine alone. These proof-of-principle experiments demonstrate the applicability of this DNA origami-based single-molecule binding assay to quantitatively study protein-pharmacophore interactions. Given that no modifications or immobilization of the target proteins are required, we can detect pharmacophore-binding in their native forms. DNA origami substrates provide a powerful platform to display single or multiple DNA-modified ligands with nanoscale precision and thus represent a versatile tool for fragment-based lead discovery research.

Fig. 1: Schematic representation and AFM image of different streptavidin binding events observed on a single DNA origami substrate: strong monodentate binding to biotin (central position), weak monodentate binding to iminobiotin (left position), and symmetric bidentate binding to iminobiotin (right position).

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O-28

Functionalized octahedral DNA nanocages for a targeted drug delivery

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In the last years, our group has been deeply involved in the design, assembly and structural-dynamical characterization of a truncated octahedral DNA nanocage, identifying the parameters that can modulate the yield of assembly and their mechanical– dynamical properties. The cage is composed by twelve double helices, each one connected to four single stranded linkers having different length and made by different bases. A peculiar property of this type of DNA structure is that oligonucleotides at the corners of the nanocage are covalently bound, making the system highly stable. Conformational variability of the cages are dependent on the number of bases composing the single stranded linkers and it is independent of the linker base composition. Functionalization of octahedral DNA cages with two, three or four hairpin forming DNA strands inserted in one corner of the structure permits a temperature controlled reversible encapsulation of a cargo enzyme, opening the road for a reversible payload encapsulation procedure. By a semiautomatic procedure, we can define the number of oligonucleotides and the sequences for building complex atomistic covalently-linked DNA nanocages with different geometry and sizes. Here we will describe how, depending on specific chemical functionalization, the cages can be targeted toward selected receptors, triggering specific cell uptake pathways that localize the cages into different cell compartments and confer a different stability. These data demonstrate that the selection of the cellular receptor is crucial for targeting specific sub-cellular compartments and for modulating the DNA nanocage intracellular half-life.

O-29

RNA supramolecular liquid-crystalline order catalyzes its own polymerization

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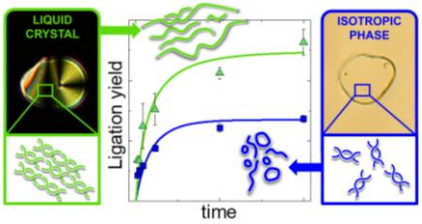
Supramolecular systems and their emergent properties are of great interest for a range of scientific fields such as biology, materials science and systems chemistry. Particularly, the study of self-catalytic supramolecular assemblies is growing in importance, also due to their possible key role in the appearance of Life on the prebiotic Earth[1]. To test whether prebiotic molecules of critical importance for the origin of the first living system could produce a self-synthesizing material, we studied the effect of a condensing agent (EDC) in a mixture of short (down to 6nt) double helices of RNA. When the concentration of these oligonucleotides is large enough, these solutions are known to develop long-range liquid-crystalline positional and rotational order[2]. Through an in-depth analysis of the reaction products and kinetics we show that the liquid-crystalline microreactors indeed exhibit catalytic activity, (i) enhancing their own ligation rate up to 6 times and (ii) driving the formation of long linear polymers that improve the stability of the assembly, instead of circular molecules that are the prevalent products of a reaction occurring in the isotropic phase and a dead end on the path to Life. Moreover, we have shown that the capability to self-assemble and assuming supramolecular ordering is retained even by mixtures of complementary single nucleotides (NTPs). These results demonstrate that RNA liquid-crystals are at full right self-synthesizing supramolecular assemblies and highlight the possible role of this class of materials in the origin of the first living systems.



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O-30

A coordination chemistry approach to the assembly and functionalisation of DNA-based materials

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Bottom-up materials design has been significantly advanced through DNA-based approaches that utilise the structure-building rules of the genetic code for programmable assembly.[1,2] However, for a range of potentially interesting applications the opto-electronic properties of DNA is rather limited. As a consequence, methods have been developed to the incorporation of technologically useful properties, such as long-range electrical conductivity. This has been achieved with incorporating pre-synthesised components, such as nanoparticles or by the deposition of metals and inorganics onto the DNA to form templated nanowires.[3,4] In our recent work, we have identified a self-assembly reaction based on Au(I) ions and a modified nucleoside which yields a coordination polymer with a one-dimensional helical structure (Figure).[5] Intriguingly, this luminescent gold-thiolate is a unique example that can be switched to an electrically conducting form. Furthermore, due to the polymer being structurally analogous to duplex DNA and assembled through highly selective gold-sulfur bonding its formation is compatible with regular DNA duplex. This is demonstrated with modified oligonucleotide duplex by the formation of luminescent concatameric hybrid structures.

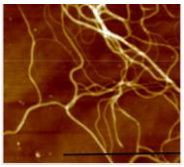

Figure. AFM image revealing the one-dimensional structure of the gold-based coordination polymer.

This new, coordination-polymer, approach expands the field of DNA-materials towards further complexity by allowing the incorporation of semiconducting properties *via* an alternative route and makes possible new types of construction protocols, compositional architectures and material combinations to be developed.

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P-1

Spatially-confined DNA-peptide conjugates for biomarker detection

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Our aim is to self-assemble DNA-peptide conjugates over ultra-flat gold surfaces, to form spatially-confined and bio-active nanosensors, which screen biomarkers that are over-expressed in ovarian cancer [1], including Beta-2-microglobulin (β2m), in a label-free fashion. In this poster, I will present our results as we combined the synergy of DNA-directed immobilization, nanografting, and atomic force topographic height measurements, to immobilize, construct spatially oriented DNA-peptide arrays, that recognise β2m and detect the binding interaction through the AFM topographic height measurements. We designed three distinct short cyclic peptides, optmised to bind to two different binding site on the β2m, and each was conjugated with unique 22-bps long DNA sequences, using NHS-ester-primary amine/lysine conjugation chemistry [2]. Using the aforesaid approaches, we constructed DNA-peptide nanoarrays of different surface coverage, and by measuring the topographic height before and after the β2m recognition, we obtained the dependency of the β2m recognition on the density of the DNA-peptide arrays. Also, our results show that each DNA-peptide array of constant surface coverage, binds to β2m in standard solution condition, with micro-molar affinity [3]. Distinctively, one out of the three DNA-peptide conjugates has higher sensitivity, approximately 7 μM affinity, as compared to others in the higher micro-molar range. Our findings put forward an approach to characterize the affinity of different DNA-based binders that are promising for biosensing and drug delivery applications [4, 5].

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P-2

Gene-therapy inspired polycation coating for protection of DNA origami nanostructures

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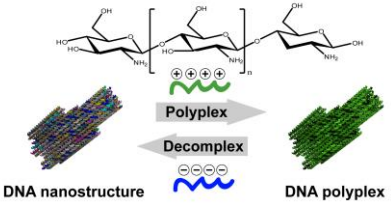
DNA nanostructures hold an immense potential to be used for biological and medical applications.[1] However, they are susceptible towards salt depletion and nucleases, the common condition in physiological fluids. Inspired by gene therapy advancements,[2] we overcame the structural instability of DNA origami nanostructures by coating them with the low-cost natural cationic polysaccharide chitosan and the synthetic linear polyethyleneimine. The complex of DNA with the cationic polymer known as polyplex was shown to be efficient for long-term protection of encapsulated DNA origami in salt-depleted media containing DNase I. Interestingly, we showed that the addressability of enzyme- and aptamer-functionalized DNA origami nanostructures is preserved upon coating with polycations. In addition, we showed that despite being highly vulnerable to salt depletion and enzymatic digestion, self-assembled DNA origami nanostructures are stable in cell culture media up to a week. This was contrary to unassembled DNA scaffolds that degraded in one hour. The stability of naked DNA origami nanostructures was revealed to be mediated by growth media, which compromise of inorganic salts, vitamins and free amino acids. DNA origami nanostructures remained not only structurally intact but also fully functional after exposure to cell media, as validated by the hybridization assay. The structural integrity and functionality of DNA origami nanostructures in cell culture media open the door for their short-term biological applications in which the structural details of DNA nanodevices are functionally crucial.



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P-3

Unravelling the properties of hybrid DNA-Supramolecular Polymers

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Previously our group has presented the self-assembly of auto-fluorescent C3-symmetrical amphiphilic molecules into supramolecular wires in aqueous media. These supramolecular assemblies work as 1-D scaffolds for the incorporation of monovalent building blocks to yield a multivalent supramolecular polymer. The intrinsic dynamic nature of the system allows the modular synthesis of multifunctional structures that can interact with biological components. For instance, it was shown that when the discotic monomers are decorated with mannose, the supramolecular wires can bind strongly to bacteria due to the multivalency of the system. We also demonstrated that discotic monomers can be functionalized with small molecules for the integration of proteins into the supramolecular columns.
Motivated by our previous work on the integration of proteins into supramolecular wires, we were keen to explore the potential of combining oligonucleotides (ODNs) with the same supramolecular wires. In this present work, we report the synthesis and properties of hybrid supramolecular wires made of short ODNs attached to monomeric discotic molecules.

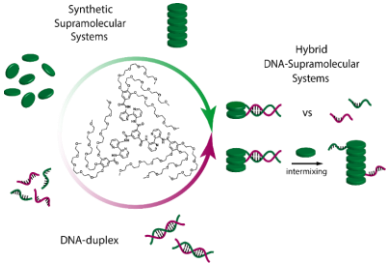

Figure 1. Schematic representation and chemical structure of BiPy Disc Self-assembly of DNA hybrid discs in water.

References

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P-4

Electrochemical Surface Impedance Spectroscopy of Adhering Lipid Vesicles: A Sensing Technology for the Quantification of Ligands

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We have recently demonstrated, using confocal microscopy, how the proportion of adhering multivalent receptor-functionalised lipid vesicles to a similarly functionalised supported lipid bilayer (SLB) is dependent on the concentration of a bridging ligand [1]. This suggests the suitability of such a system as a general mechanism for the sensing of ligands. For practical application, vesicle adhesion needs to be performed using an inexpensive and less-time consuming method, which can give an electrical readout. A potentially suitable technique is Electrochemical Surface Impedance Spectroscopy (EIS), where the measured impedance of an electrochemical cell is sensitive to changes at the surface of an electrode. EIS has long been used in the biological community as label-free method to analyse cell activities and, of specific interest to us, has been shown to be able to measure substrate coverage by cells [2]. Here, we apply the principle of EIS to measure the adhesion of lipid vesicles to a SLB mediated by ligands, in this case DNA oligonucleotides, in order to quantify ligand concentration and demonstrate the suitability of adhering lipid vesicles as a platform technology for the detection of (bio)molecules.

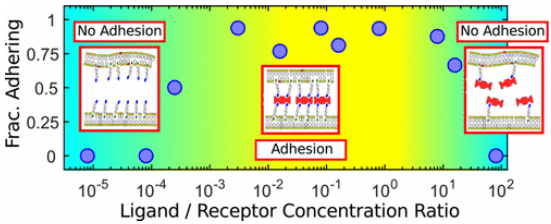

Figure 1: Vesicle Adhesion Dependency on Ligand Concentration

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P-5

pH-controlled assembly and disassembly of DNA nanostructures

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Inspired by cytoskeletal scaffolds, that sense and respond dynamically to environmental changes and chemical inputs with a unique capacity for reconfiguration, we propose two different strategies to trigger, control and reverse the self-assembly of DNA nanostructures.
The first strategy allows to trigger and finely control the assembly of supramolecular DNA nanostructures with pH. Control is achieved via a rationally designed strand displacement circuit that responds to pH, and activates a downstream DNA tile self-assembly process. We observe that the DNA structures form under neutral/basic conditions, while the self-assembly process is suppressed under acidic conditions.[1]
The second strategy allows dynamic and reversible control of the growth and breakage of micronscale synthetic DNA structures upon pH changes. We do so by rationally designing a pH-responsive system composed of synthetic DNA strands that act as pH sensors, regulators, and structural elements. Sensor strands can dynamically respond to pH changes and route regulatory strands to direct the capacity of structural elements to self-assemble into tubular structures.[2]
The strategies presented here demonstrates a modular approach towards building systems capable of processing biochemical inputs and finely controlling the assembly and disassembly of DNA-based nanostructures under isothermal conditions. The capacity to control and/or reversibly modulate nanostructure size may promote the development of smart devices for catalysis or drug delivery applications.

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P-6

Aptamer Functionalised Nanomaterial for Detection of antibiotic resistant *Acinetobacter baumannii*

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Nucleic acid aptamers are emerging as a new class of probes which have high affinity and specificity to their cognate targets. Aptamers are developed against cells, proteins, small molecules toxins, pesticides. Aptamers benefit from their ease of generation, low production cost, low batch-to-batch variability, reversible folding properties and low immunogenicity. Aptamers can be integrated into sensors, actuators and other point of care devices. We used Cell-SELEX technique to generate DNA aptamers targeting one of the most clinically significant pathogen *Acinetobacter baumannii*. *A. baumannii* has attained notoriety because of multiple antibiotic resistance and formation of biofilms on the surfaces of hospital equipment due to which the pathogen is responsible of a wide variety of clinical syndromes, most prominently respiratory tract infections, bacteraemia, skin and soft tissue infections, urinary tract infections, osteomyelitis, and intracranial infections and other hospital acquired infections. Due to the limited options for multidrug-resistant *Acinetobacter* infections, there is an urgent need to develop diagnostic methods for early detection of *A. baumannii*. So that suitable therapies can be deployed for its mitigation. We generated DNA aptamer against *A. baumannii* AYE after 19th round of SELEX. The progress of selection was monitored by performing Fluorescent Activated Cell Sorting (FACS). Putative aptamer population were cloned in PTZ57R/A vector. Number of putative aptamers were screened by amplifying the DNA cloned in the vector using fluorescence labelled forward primer and was followed by FACS. Finally 72 clones were sequenced and categorised based upon sequence similarity. One member of each group was synthesised commercially with fluorescent tag in order to further validate binding to the *A. baumanni*i AYE cells. Eight such aptamers were identified which showed high affinity and specificity for *A. Baumannii* cells. We carried out sequence-activity relationship study by truncation of the full length aptamers. These truncated variants were analysed using FACS and fluorescence microscopy on *A. baumannii* cells. We shall present the data of truncation results and also discuss the possible application of shortened *A. baumannii* aptamers on infection and adhesion of *A. baumannii* AYE on cell lines. We shall present our data on gold nanoparticle based aggregation method for the optical detection of *A. baumannii* AYE. Present efforts to develop a sensitive detection method with very low detection limit based on nanomaterial and peroxidase activity of DNA quadraplex will also be discussed.

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P-7

Kinetic study of CRISPR-Cas9 for dynamic DNA nanotechnology

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In the past 5 years, the CRISPR/Cas9 system has become the core engine of gene editing. The CRISPR system is an acquired immunity system found in prokaryotic. It records past attack from viruses by storing snippets of their DNA in the genome of the prokaryote (CRISPR array). Transcription and maturation of this array yield RNA guides that are loaded by Cas9 - a RNA-guided endonuclease. The loaded Cas9 then scans and cleaves DNA sequences that hold the guide sequence near a PAM sequence (NGG in Cas9)[1].

From a biotechnological point of view, the appeal of Cas9 lies in its simplicity and programmability: arbitrary sequences of DNA can be targeted by changing the spacer sequence of the RNA guide. Cas9 (and variants like dCas9 which target but do not cleave complementary DNA strands) have been put to use not only to edit genome, but also to orchestrate RNA transcription or engineer DNA looping in chromosomes[2]. Cas9 has also been used to trace the lineage of differentiating cells (by mutating a DNA ledger that is inherited by daughter cells)[3].

In spite of its taking over biotechnology, seldom has Cas9 been studied as a tool for DNA nanotechnology. Yet it holds a great potential due to its ability to detect and manipulate arbitrary DNA strands. For example, in dynamic DNA nanotechnology, the concentration of surrogate species are typically followed the fluorescence of tagged species, (occasionally gel electrophoresis), which is limited to 5-6 species. We envision that Cas9 may serve as a tool to temporally record the concentration levels of hundreds -if not thousands- of DNA species in DNA ledgers.

Here we present preliminary investigation of the kinetics of CRISPR Cas9 in view of integrating it in DNA dynamic circuits. Building on a published fluorogenic cleavage assay[4], we tested the dependence of Cas9 on the concentration of salts, RNA guides or substrate. We have also tested the compatibility of Cas9 with droplet microfluidics - which may open the door to high-throughput characterisation of its kinetics.

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P-8

Inkjet printing of DNA-based semiconducting nanowires

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DNA-based nanowires can be prepared by nucleating the growth of semiconducting material on DNA molecules.[1] The role of DNA within these systems is to provide a long, thin, chemically-robust template to direct the growth of materials as nanowires. With the diameter of its duplex structure being just over 2 nm, the thicknesses of wires can be confined to only a few nanometers. The poster introduces a wet chemical method for the synthesis of metal sulphides on DNA, with nanowires characterised by microscopy techniques (AFM, TEM, EFM and fluorescence) as well as current/voltage measurements. Particular focus is made on using cadmium sulphide (CdS), a binary II-VI semiconductor with a wide band gap of 2.42 eV. Alteration of band gap size is demonstrated through the formation of metal alloy sulphides, cadmium lead sulphide ($Cd_xPb_{1-x}S$) and cadmium zinc sulphide ($Cd_xZn_{1-x}S$), and identified by UV-VIS and fluorescence spectroscopy. The tuning of optical and fluorescent properties of nanowires suggests that they could be suitable for use in labelling technologies. The CdS/DNA nanowires are also shown to be potential candidates for the sensing of volatile organic compounds, such as ethanol, acetone and chloroform, with changes in material conductance upon exposure to vapours reported. The synthesis of nanowires within aqueous media allows for the easy formulation of inks.[1] Printing has been identified as a promising technique for device fabrication, being relatively cheap and adaptable and able to achieve high resolution. Work on tailoring CdS/DNA dispersions for use with a Dimatix 2800 inkjet system is explored, with printing of both highly resolved droplet arrays and tracks demonstrated on various substrates and over interdigitated electrodes. Subsequent characterisation of CdS/DNA morphology, fluorescence and electrical behaviour prove this to be a successful technique for processing nanowires towards labelling and gas sensing applications.

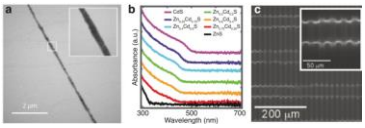

Figure 1. a.) TEM image of combed CdS/DNA nanowires. b.) UV-VIS spectra for $Cd_xZn_{1-x}S$/DNA alloy dispersions. c.) Fluorescence microscopy image of printed CdS/DNA tracks over interdigitated electrode.

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P-9

Spatial clusters in two species systems

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Well-known population dynamics models, such as Fisher Waves or Predator-Prey models, are becoming accessible with nucleic acids [1,2]. We are interested in simple two-species models that can lead to surprising spatial phase separation between two different types of players. In this context, we have studied a model inspired by the prisoner's dilemma, where one species always dominates, with memory and different mobilities for different players. By memory, we mean that there is a delay with which species change their fitness or replication speed. Different mobilities refer to diffusion or mixing rates between different locally well-mixed spatial sub-environments, which depend on the individual agent. These two modifications mean that even when one species survives in the long run, it survives via transient, strongly clustered states. We analyse the structures of these states, and would be interested to know if such states can be implemented with nucleic acids, given potential applications of such a phase separation.

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P-10

Network-forming DNA nanostars for the investigation of condensed matter physics

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The production of thermodynamically relevant numbers of identical self-assembled DNA nanoparticles enables new investigations of thermal, kinetic, and rheological key phenomena in condensed matter physics. Our studies are based on simple DNA nanoparticles shaped as either 3 or 4-arms stars, the arms being formed by 20 paired bases, and the tips by 6-base-long self-complementary overhangs. While at larger temperature (T) the solutions are dispersions of independent DNA nanostars, as T is lowered below 40 degrees, the tip-to-tip pairing leads to the formation of transient networks in which nanostars act as nodes having valence 3 or 4.
The phase diagram of these systems includes a consolution curve separating a vapor-like and a liquid-like phases that terminates in a critical point featuring critical susceptibility and slowing down[1]. The amplitude of the consolution curve and the location of the critical points depend on the valence of the nanostars[1]. At concentrations larger than the consolution region, the system displays a continuous equilibrium transition to a kinetically arrested state, which has all the features of the glass transition of the so called “strong glasses”[2].
The restructuring dynamics of transient networks has been reported to exhibit an intriguing lengh-scale independence. The access to a wide range of binding energies and lifetimes enabled by the DNA nanostar network has enabled understanding this dynamic phenomenon as a general phenomenon to be expected in network with fluctuating local elasticity [3]. The transient character of the network is also key to understanding its viscous behavior, which exhibits a strong shear-thinning transition, as probed by active microrheology. We show that the knowledge of topology, energies and lifetimes afforded by this DNA construct enables a full quantitative understanding of the Newtonian and non-Newtonian regimes [4].

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P-11

Programmable DNA and RNA technologies for binding-responsive sensing of target biomolecules

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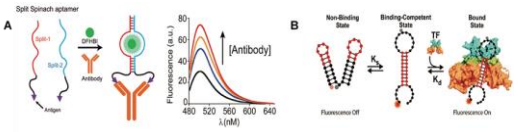
Nature harnesses dynamic networks of non-covalent interactions to store, process, and disseminate information. Inspired by this, nucleic acid nanostructures can be engineered into dynamic systems able to process information through programmable molecular motion and assembly. Nucleic acid actuators can be triggered by interactions with target biomolecules to generate binding-induced measurable outputs. Herein, we demonstrate that a proximity-based mechanism can control the assembly of a modular RNA system, performing detection of antibodies through a complementation assay. We used antibodies as bivalent substrates to template the assembly of a functional RNA structure and developed an assay in which a fluorescent Spinach aptamer, a synthetic RNA mimic of the Green Fluorescent Protein, is dynamically reconstituted from its split segments.[1] We employed antigen-tagged RNA strands that, upon binding to target antibodies, reassemble into the native conformation and yield a fluorescence output, showing high binding affinity, specificity for the target, and the ability to work in crude cellular extracts. Furthermore, we demonstrate that binding events can be detected by inducing a conformational change in a DNA molecular switch. We implemented the use of a DNA nanoswitch that enables probing of transcription factor binding activity directly in living cells, allowing for activity-based intracellular fluorescence imaging in real-time. Our strategy hinges on a DNA nanostructure that transduces, through a binding-responsive conformational change, the recognition of a specific transcription factor into a fluorescent signal.[2] We monitored intracellular trafficking using super resolution microscopy, performed live cell imaging of transcription factor binding activity, and achieved relative quantification of intracellular transcription factor expression. On the whole, we highlight the potential of dynamic nucleic acid-based systems as innovative tools for nanobiotechnology, bioimaging, and analytical assays.



A.Antibody-based assembly of a fluorescent split Spinach aptamer. **B.** A DNA nanoswitch allows for detection of transcription factor binding activity through induced conformational motion.

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P-12

Flexibility defines structure in amphipilic DNA crystals

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Periodically structured nanoscale frameworks are crucial for many emerging technologies including plasmonics, molecular filtration, and sensing. We recently reported on a new method for the production of such crystalline materials using amphiphilic cholesterol-functionalised DNA nanostars, *C-stars*, as building blocks which combines the design freedom and nanoscale structural control offered by DNA nanotechnology with the robustness of hydrophobic interactions (**Figure 1**).[1] In contrast to approaches reliant on Watson-Crick interactions alone, here long-range order emerges from the frustrated phase separation between the hydrophobic cholesterol and hydrophilic DNA. This mechanism is controlled uniquely by the topology and symmetry of the flexible nanostar motif, which makes C-star self-assembly robust against substantial design changes that preserve these features, enabling us to predictably tune material properties such as lattice parameter and porosity.[2]

Developing a greater understanding of the physical properties of the amphiphilic building blocks is critical for designing more complex architectures and predicting material properties. Here we reveal the role that geometry and flexibility in the C-star core play in the formation of ordered phases. Through a combination of small-angle x-ray scattering and molecular dynamics simulations, we demonstrate how the geometry of non-interacting nanostars, lacking cholesterol modifications, depends on the number of unpaired core bases and solvent conditions. Furthermore, these differences lead to substantial changes in the properties of crystalline frameworks assembled from cholesterol functionalised nanostars, with more flexible C-star variants showing a higher stability over a range of solvent conditions.

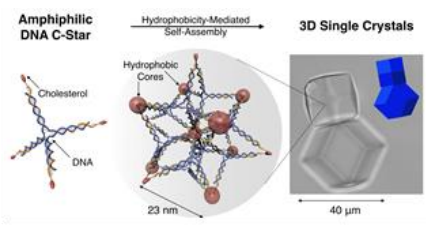

Figure 1: Amphiphilic DNA C-stars self-assemble into single crystals through Watson-Crick and hydrophobic interactions

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P-13

Structure beyond sequences: miRNAs a rich variety of conformations.

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Mature microRNAs (miRNAs) are a class of evolutionally conserved, single-stranded, small (approximately 19−23 nucleotides), endogenously expressed, and non-protein-coding RNAs that act as post-transcriptional regulators of gene expression in a broad range of animals, plants, and viruses.[1,2] The biogenesis of miRNAs is a multiple step process, which complete with the incorporation of the mature miRNA into RNA-induced silencing complex.[3] The RISC complex functions by perfectly or imperfectly matching with its complementary target mRNA, and induces target mRNA degradation or translational inhibition. Thus, alterative expression of miRNAs has been associated with a number of diseases, genetic disorders and tumors progression.[3] We think that the knowledge of the miRNA structure may give a new insight into miRNA-dependent gene regulation mechanism and be a step forward in the understanding their function and involvement in cancerogenesis. With this aim we characterized the conformation and structures adopted by several artificial miRNA in physiological conditions. Preliminary data obtained by CD melting experiments, using synthetic miRNA,[4] highlighted the important role played by the structures adopted by miRNA. Indeed the sequences showed a strong high ordered structures (characterized by CD, DSC, NMR and PAGE) induced a significant inhibition of the luciferase activity for two of the most prominent genes associated to lung cancer, c-MET and Epidermal Growth Factor Receptor (EGFR).

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P-14

Adenita: A Software Toolkit for the Visualization and Modeling of DNA Nanostructures

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We present Adenita, an open-source software that aims to provide an integrated in silico design toolkit for DNA Nanostructures. It facilitates the modular assembly of pre-existing and de novo designs, regardless of the used approach. Adenita is being developed in the context of the MARA project [1], a highly ambitious project aiming to produce a DNA nanorobot capable of targeted cell lyses. Currently, the existing design and visualization tools are insufficient to solve the specific challenges in our project. We aim to overcome these limitations with Adenita, a new semi-automated approach that we are developing as the MARA project advances. Adenita integrates visualizations [2] with user interactions and algorithms in a semi-manual approach. At the core, we use a hierarchical data model that enables us to combine both a top-down (DAEDALUS [3]) and a bottom-up (caDNAno [4]) design approach of the DNA Nanostructure. From the DNA data model, we create smooth visualizations that depict the structure in multiple scales from its atomic details to a high-level geometric representation of the target shape. In addition, we employ different layouts for the same structure [5]: 3D structural representations, 2D caDNAno-style diagrams, and 1D display of the linear sequences. Creators enable the parametrized generation of structural motifs, while Manipulators facilitate the advanced modifications of the structural properties, such as connecting components and adding bridging strands. Analysis of the designs is still in a preliminary phase, but it already enables the straightforward estimation of distances and the melting temperatures [6] of binding regions. The first DNA nanostructures that we designed with the new tool are now under experimental evaluation.

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P-15

Dissipative DNA-based nanomachine for the release of molecular cargo in a time-controlled fashion

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Supramolecular chemistry is moving into a direction where the composition of a chemical equilibrium is no longer determined by thermodynamics, but by the efficiency at which kinetic states can be populated by energy consuming processes.[1] Mastering this process in synthetic systems is essential for endowing materials, catalysts, drug delivery systems, with life-like properties such as adaptation, motility, and evolution.[2] Here, we show that DNA is ideally suited for programming chemically-fueled dissipative self-assembly processes.[3] Advantages include a perfect control over the activation site for the chemical fuel in terms of selectivity and affinity, highly selective fuel consumption which occurs exclusively in the activated complex, and a high tolerance of the systems for the presence of waste products. Finally, it is shown that chemical fuels can be used to selectively activate different functions in a system of higher complexity embedded with multiple response pathways.

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P-16

DNA Secondary Structure Assisted Controlled Immobilization Strategy

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The precise alignment of nucleic acids via Watson-Crick base-pairing has been widely recognized and applied as an analytical tool. Combining the base-complementarity design principle of DNA that can self-assemble into various structures with the nanoscale structure of DNA double helix has allowed impressive advances in DNA nanotechnology. Nucleic acid secondary structure have gained prominence as structural scaffolds that permit facile functionalization and controllable stability.

DNA secondary structures such as junctions which possess multiple arms serve as elementary units for engineering different DNA architectures and assemblies. The arms of the structure can act as robotic arms which can perform various tasks such as grasping the variety of molecules. Three arms of junctions have been utilized to make a triple biosensor that can detect three different metabolites depending on the stimuli received [1]. Binding induced junction formation using double ligands bound to two arms and fluorescent reporter attached to third arm have been used for detecting protein[2].

We wish to exploit nucleic acid secondary structures such as junctions as a controlled immobilization strategy. Secondary structures such as hairpins, quadruplexes and junctions could assist with the immobilization of various ligands. Such a novel strategy for controlled multiple immobilization will have a wide range of clinical applications.

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P-17

Toward *in vitro* implementation of dCas9-based regulatory networks

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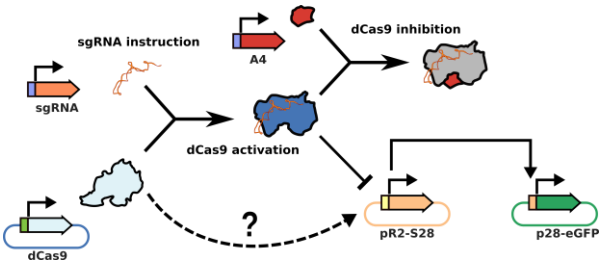
Synthetic biology utilizes genetic networks in order to induce a rationally designed behaviour in engineered organisms. dCas9 is a protein capable of binding DNA at any given sequence upon instruction by a single guiding RNA (sgRNA)[1]. This flexibility in sequence recognition allows the orthogonal repression of virtually any gene by dCas9, which can be used for the design of new regulatory circuits. Repression response provided by dCas9 lacks nonlinearity, which can be limiting for the development of complex networks such as multistable switches or oscillators[2]. Furthermore, new reports suggest unexplored non-RNA mediated interaction between dCas9 and DNA, yielding up- and downregulation of genes both *in vitro* [3] and in bacteria [4]. In order to better characterize interaction mechanisms between dCas9, DNA, and RNA, we reconstitute a genetic system displaying the key elements of dCas9-RNA-DNA interactions in a cell-free transcription-translation system. We further explore the use of dCas9 regulation in a context of transcriptional cascades. Finally, we utilize a small viral protein, AcrIIA4, in order to inhibit dCas9 repressive activity and drive non-linear, dCas9-mediated, changes in gene activity.



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P-18

A Hierarchical Carrier System Based on DNA Nanostructures and Layer-by-Layer Microcarriers

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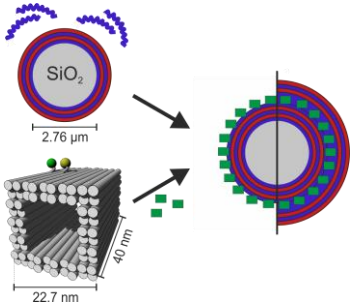
Compact nanostructures such as DNA origami provide a new possibility for the protected delivery of active agents into the cytoplasm. Such structures have been shown to be sufficiently incorporated by cells within 6 h post transfection without further treatment.[1] Furthermore, switchable lids allow a stimuli-triggered opening of the structures and thus a controlled exposure of incorporated agents to the targeted cell.[2] However, the limited stability under physiological conditions represents a crucial restriction when using DNA origami in drug delivery applications.[3]

To overcome this challenge, we integrated DNA origami into modularly built Layer-by-Layer (LbL) microcarriers forming a hybrid carrier system. The alternating adsorption of any kind of biopolymers on a solid spherical template building up a multilayer promises a high protection of an integrated cargo against external factors. To this end, DNA origami tubes were adsorbed at different positions of an LbL biopolymer multilayer. We could demonstrate that the DNA nanostructures form a single dense layer at any position while their structure remained unaltered. When integrated into deeper layer positions the DNA structures exhibited a remarkable stability over a broad pH range, in presence of isolated lysosomal enzymes, and even after incubation with cultured cells. Subsequently, BSA as a model cargo was loaded into the origami tubes and thus integrated into the carrier system providing comparable assembly characteristics to unloaded structures. Altogether, our hybrid system provides superior stability of DNA nanostructures during transport and uptake into cells. It thus represents a new and promising approach to develop advanced drug delivery systems that can target specific intracellular compartments.



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Colorimetric monitoring of nanoscale actuation in DNA-templated plasmonic nanostructures

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The programmed assembly of gold nanoparticles on DNA templates allows the design of nanostructures with optical properties that directly depend on the morphology of a biochemical scaffold. Indeed, the nanometer-scale sensitivity of plasmon coupling allows the translation of minute conformational changes into macroscopic optical signals.[1] In particular, we showed that it is possible to monitor, on a simple color camera, the conformational changes of a single DNA-templated gold particle dimer as its gap is reduced from 20 nm to 1 nm.[1] This spectroscopic approach allows us to analyze the influence of the gold surface chemistry[2] or of the local temperature[3] on the conformation of hybrid DNA-gold nanostructures; but also to observe stochastic dynamic fluctuations from metastable DNA scaffolds leading to blinking plasmonic resonances. We will also discuss recent results on the use of 3D DNA origamis as scaffolds for the assembly of plasmonic nanostructures (Figure 1-a-c). Indeed, DNA origamis are a flexible platform to produce organic nano-objects that shift their morphology when interacting with specific target biomolecules, such as DNA/RNA strands or proteins, as well as specific cations.[4] Using single-nanostructure scattering spectroscopy, we demonstrate that the conformation of the origami can be correlated to single nanostructure spectroscopy measurements (see figure 1-d). The flexibility of these biochemical templates opens exciting perspectives for the optical sensing of specific physicochemical stimuli that actively modify their 3D conformation.

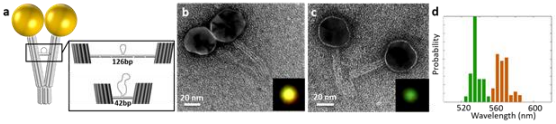

Figure 1. 40 nm gold nanoparticle dimers assembled on 3D DNA origami scaffolds: schematic representation (a) and TEM images when the origami arms are separated by 42 bp (b) and 126bp DNA spacer (c) (insets: typical darkfield images). (d) Distributions of plasmon resonance wavelengths of dimers with 42 bp (orange) and 126 bp (green) spacers.

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P-20

Introducing reversible hydrophobic and magnetic properties to DNA nanostructures using proteins

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Over the last years, a lot of progress has been made in engineering dynamic DNA nanostructures. While DNA is an excellent scaffold for the construction of different shapes, its chemical properties are limited. Here, we show two proteins, which enhance the functionality of DNA nanostructures to various stimuli. Initially, we want to introduce elastin-like peptides (ELP), which are derived from their natural analogue elastin, a common component of the extracellular matrix in both humans and animals. It consists of repetitive sequences of GXGVP, where X can be any amino acid except proline. Upon heating ELP undergo a phase transition from a soluble state into a non-soluble hydrophobic state; this hydrophobic collapse is fully reversible. The specific transition temperature depends on the amino acid sequence, contour length, and the salt conditions of the aqueous solvent. In this work, we used variations of this protein to reversibly assemble different origami structures by this switchable hydrophobic interaction.[1] The second protein is ferritin, which is a globular protein complex consisting of 24 subunits and can enclose iron crystals in an octahedral cage. The resulting semisynthetic magnetic nanoparticle is highly monodisperse and exhibit a diameter of around 8 nm.[2] By equipping structures such as dynamic DNA devices with these particles, they could be responsive to external magnetic stimuli. Both proteins are functionalized by a bifunctional NHS-linker and are bound to ssDNA using either copper-assisted or copper-free click chemistry. The ssDNA hybridizes to an extended staple in the targeted DNA nanostructure. By choosing different DNA-sequences, different binding sites and multiple modifications can be achieved.

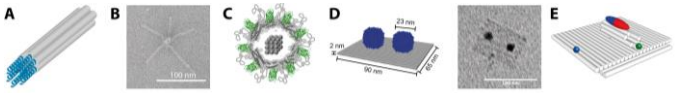

Figure: A) Illustration of a single-stranded tile tube modified with six ELP (blue). **B)** Tubes represented in A) assembled by a hydrophobic switch caused by 3 M NaCl. **C)** Scheme of a magnetic ferritin nanoparticle, consisting of the protein cage with a magnetite core, and functionalized with mEGFP and PEG. **D)** Left: Schematic illustration of two ferritin nanoparticles bound to a rectangular DNA structure. Right: TEM image of ferritin nanoparticles on DNA origami. **E)** Illustration of the self-assembled DNA nanostructure with nanoscale robotic arm, as published by Kopperger et al.[3]

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P-21

Towards DNA-Templated Molecular Electronic Devices

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The ultimate miniaturization of active electronic components requires the construction of electronic devices from single molecules [1]. Molecular electronics would provide advantages in device architecture, power consumption and functionality. However, building a single-molecule device is challenging as it requires absolute control over the fabrication process including the integration of heterogeneous components, unrelated to materials currently used in semiconductor fabrication, and their positioning with near-atomic precision. Here we present a new method for the templated conjugation of marginally water-soluble organic molecules to DNA-adapters, with the aim to provide close control over the position and orientation of the molecule within self-assembled single-molecule electronic devices. This method provides a way to conjugate different DNA sequences to the same molecule without the need to provide orthogonal chemistries on the target molecule. It also allows purification of the intended product and screening for the correctly assembled device. It thus provides a promising new tool for the programmable fabrication of molecular electronics.

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P-22

DNA Nanostructures that Target and Rupture Bacterial Membranes

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The generation of functional DNA nanostructures is of high important. In one route to new biologically relevant function [1], DNA nanostructures are equipped with membrane anchors. The chemically modified nanostructures can interact with lipid bilayers, for example, by puncturing them via hollow nanobarrels [2]. But the biological activity of DNA nanomaterials against membranes of bacteria has not been explored.

Here we report that DNA nanostructures target bacterial membranes to cause microbial cell death. The DNA nanostructures carry cholesterol lipid anchors and bind preferentially to cholesterol-free bacterial membranes than to cholesterol-rich, stiffer eukaryotic membranes. The highly negatively charged DNA nanostructures cause bacterial cell death by rupturing membranes, likely via electrostatic destabilization. Molecular modelling [3] will provide insight into the detailed physico-chemical mechanism. The DNA nanomaterials can inspire the development of polymeric or small-molecule antibacterial agents that mimic the principles of selective binding and rupturing to help combat antimicrobial resistance.

The antimicrobial DNA nanostructures complement previous engineering on membrane-binding DNA nanopores either to make them cytotoxic for eukaryotic cells [4] or to use them in synthetic biology to mimic key biological function of ion channels. This includes artificial channels that reversibly close in response to voltage [5] or specifically bind a ligand to open up and transport molecular cargo with high charge selectivity [6]. The latter constructs may be used for controlled drug release or the building of cell-like networks [7].

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P-23

DNA origami for circular dichroism-based sensing

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Nanosensors have great promise in medical applications. By taking advantages of the target recognition ability of aptamers, the programmability of the DNA origami, and the plasmonic property of the gold nanorod (AuNR), we developed a circular dichroism (CD) nanosensor based on switchable chiral assemblies, which specifically responded to analytes. In this work, we used the adenosine molecule as a model analyte. The origami-AuNR was demonstrated to generate CD signals corresponding to its states of chiral conformation in the previous work [1]. Here, we designed two molecular locks (ds-lock and sp-lock) to control the conformation of the origami-AuNR construct. In the ds-lock system, the presence of adenosine separated the hybridization of the aptamer (apt)-complementary strand (cs), unlocked the DNA origami bundles, led to the open state of the AuNR-origami construct, and decreased the CD signal. In contrast, for the sp-lock system, adenosine induced the complex formation between the two split aptamers to lock the bundles, to drive the construct to the closed state, and to enhance the CD signal. The nanosensor with ds-lock or sp-lock showed distinct sensitivities towards different adenosine concentration ranges (Figure 1).

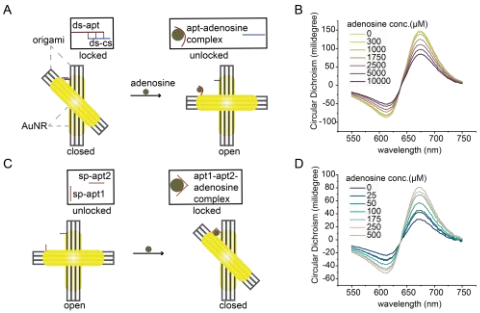

Figure 1. DNA origami-based nanosutures for CD sensing. (A) The principles of the aptamer-complementary strand (ds)-lock based nanosensor. (B) The CD spectra of the ds-nanosensor in various adenosine concentration. (C) The principles of the split-aptamer (sp)-lock based nanosensor. (D) The CD spectra of the sp-nanosensor in various adenosine concentration.

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P-24

Biosensing based on weak molecular interactions

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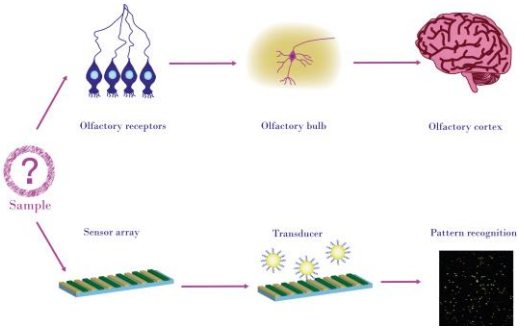
Inspired by the mammalian olfactory system which uses cross-reactive receptors for multianalyte detection, my project focuses on building a biosensor based on weak interactions rather than the traditional, highly specific “key-lock” configuration. By working with a multitude of weak interactions, a surface with various functional groups can differentiate analytes with distinct physical and chemical characteristics (for example, size, charge, ligands, etc.), as the weak, nonspecific interactions map out a particular fingerprint profile. With the long term goal of building a sensor based on non-specific interactions, as a first step, we demonstrated the concept using DNA-tagged Au NPs and micropatterned surfaces.Gold nanoparticles (Au NPs) have become one of the most interesting sensing materials because of their favorable optical properties. Au NPs decorated with DNA have been demonstrated for selective and sensitive detection of analytes. Since the proper choice of surface functionalization of the glass surface and Au NPs determines their interactions, we have chosen to use a photoprotective polymer for surface patterning. Activated polymer revels primary amines, where we then use NHS biotin for functionalization of the surface - a common technique for crosslinking or labeling peptides and proteins. The motivation behind the choice of DNA is that its sequence is easily tailored, and we can tune its binding strength with the complimentary DNA on the Au NPs. Once the system is established with DNA, it gives an easier transition to test with other molecules (aptamers, antibodies, peptide, etc.) seeing NHS biotin leaves us many options for functionalizing the surface. The readout system uses dark-field microscope, which is capable of providing single-molecule resolution, to track individual Au NP. By tracking the Au NPs, a fingerprint signal can be established.



P-25

MD simulations capture the subtle structural features of a DNA origami nanovault

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With the recent advancement of DNA nanotechnology, it has become possible to rationally design a wide range of dynamic structures, able to change conformation or exert a specific function in response to external stimuli [1-2]. Among these, DNA-based nano-containers are attracting interest in order to achieve a strategy for the precise control of the enzyme–substrate interaction. The Andersen group has designed and experimentally characterized a clever DNA origami [3], which can be used as a nanoscale vault to control the enzymatic reaction catalysed by an encapsulated protease. The presented structure, however, is not able to completely shield the entangled enzyme, maybe due to the formation of large pores on the nanocontainer surface. Molecular dynamics (MD) simulation can be a valuable tool to identify these kind of structural issues that cannot be predicted without an atomistic analysis of the systems. Here we present the results of a 200 nanoseconds classical MD simulation of the nanovault atomistic model (Fig. 1). Analysis of the trajectories shows that the system samples several conformations that provide an explanation for the experimental data. In detail, several large-sized pores can be detected on the surface, identifying regions that may be re-designed to reduce the porosity of the device and improve its sheltering capabilities.

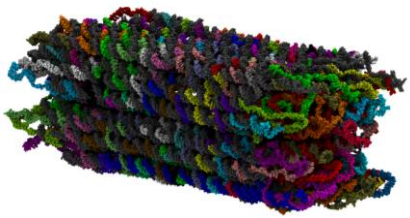

Fig.1. A snapshot of the atomistic model of the DNA nanovault. The different colours identify the different staple strands used to fold the structure. The picture has been obtained through the VMD program.

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P-26

Hybrid DNA origami – protein devices as sensors and cellular transport vehicles

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Rapid development of structural DNA nanotechnology has led to emergence of diverse DNA-based nanoscale applications in different fields of research [1]. A compelling way for expanding the functionality of such applications is by combining DNA nanostructures produced by the scaffolded DNA origami method with other biomolecules. Here we present two different approaches to create functional hybrid structures by fusing DNA origamis with proteins. We specifically focus on DNA-protein systems that exhibit controllable dynamic properties.

In the first project, we combine DNA origamis with photoactive proteins, phytochromes. The photosensory unit of *Deinococcus radiodurans* bacteriophytochrome has been observed to undergo large conformational changes upon red/far-red light illumination [2]. In this project, we study and amplify this motion generated by the conformational change by attaching the photosensory unit to DNA origamis.

The core of our second system is a DNA origami capsule sensitive to changes in its environment. It has recently been shown that coating DNA origami structures with bovine serum albumin (BSA) enhances their immunocompatibility and level of cellular uptake [3]. By applying the previously described methodology to our system, we aim to develop a DNA origami-based vehicle for controlled and smart cellular delivery.

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P-27

Programmable DNA-based Communication in Populations of Artificial Cells

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The quest to develop bottom-up constructed artificial cells is an important topic in synthetic biology and has led to the construction of different types of synthetic compartments that can act as simplified model systems for living cells. These synthetic cell-like compartments (protocells) have been configured to perform various biomimetic functions such as protein expression, responding to external stimuli, and predation. Here we use dynamic DNA nanotechnology to implement molecular communication in synthetic protocell populations. Protein-polymer microcapsules (proteinosomes)[1] act as synthetic protocells where chemical reaction networks based on DNA strand-displacement are selectively encapsulated. Short DNA oligonucleotides function as chemical messengers that protocells are able to sense and secrete. We demonstrate various network configurations, such as multi-stage signalling cascades, amplifiers, Boolean logic, and negative feedback. Our work shows that DNA based logic is a robust platform for engineering inter-protocellular communication, and that subjecting DNA circuits to selective compartmentalization allows them to exhibit functionality that is not possible under batch conditions.

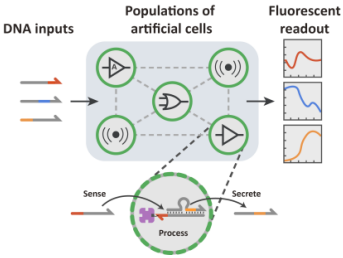

Figure 1 A scheme showing the overall configuration of the system. Various DNA-based network motifs are selectively localized into artificial cells. The reactions are triggered by the introduction of short DNA oligonucleotides which can freely diffuse though the porous proteinosome membrane. The artificial cells can respond to the input stimulus by secreting other DNA oligonucleotides, which function as second messengers and can be sensed by cells from a different population.

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DNA Origami-Directed 3D Nanoparticle Superlattice

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Spatially well-ordered structures of metal nanoparticles have unique electronic, magnetic and optical properties, and hence there is ever-increasing interest towards these kinds of nanomaterials [1]. DNA and DNA nanostructures have successfully been used to direct the higher-ordered arrangements of nanoparticles, but the programmable arrangement of them into, larger well-defined structures is still challenging [2].

Here we demonstrate a novel method based on electrostatic interactions in which DNA origami nanostructures are used to guide the higher-ordered arrangement of cationic gold nanoparticles (AuNPs) [3]. The DNA origami-AuNP assemblies are formed during dialysis against decreasing ionic strength. Further, characterization of the formed assemblies (by small-angle X-ray scattering, transmission electron microscopy and cryogenic electron tomography) indicates that the shape of the DNA origami structure is crucial for the formed structure type. We have successfully fabricated 3D AuNP superlattices using this method, which strongly suggest that electrostatic self-assembly guided by DNA origami structures is a promising tool for construction of novel, well-ordered structures with unique properties, such as lattice geometry, designed specifically for the chosen application.

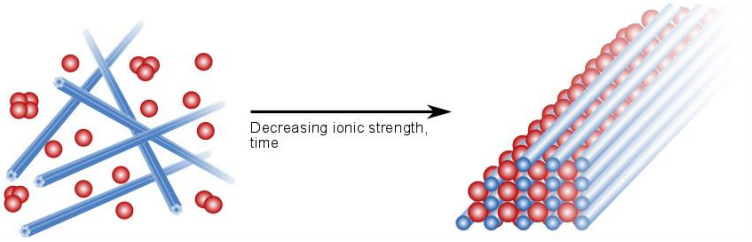

Figure 2 – DNA origami – AuNP crystal structures are formed during dialysis against decreasing ionic strength.

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P-29

Heterochiral DNA Nanotechnology

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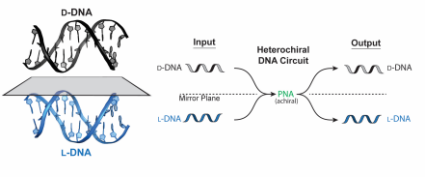

Contemporary DNA nanotechnologies are comprised of sophisticated systems capable of molecular computation, nanomaterial assembly, and functional automata.[1,2,3] The programmability of Watson-Crick basepairing facilitates straightforward designs of specialized hybridization based nucleic acid technologies. Despite the immense versatility of DNA as an engineering tool, natural D-nucleic acids suffer serious drawbacks in the context of living systems.[4] The native polymer is susceptible to enzyme mediated degradation as well as off-target interactions with native cellular components. L-DNA, the enantiomer of D-DNA, circumvents these issues that plague natural D-DNA. Unfortunately, L-DNA has identical physical properties to D-DNA, yet it is incapable of forming contiguous W-C base-pairs with D-DNA. Consequently, it has been impossible to integrate L-nucleic acid components with current hybridization based technologies which utilize exclusively the native enantiomer of DNA to operate. In this presentation I will introduce the idea of heterochiral nucleic acid technologies, nucleic acid systems capable of communicating sequence specific information between both enantiomers of DNA through hybridization based strand displacement reactions.[5] We utilize an achiral nucleic acid analogue, peptide nucleic acid (PNA), which is capable of hybridizing to either enantiomer of DNA with identical affinity and undergoing classical strand displacement reactions. This fundamental discovery opens the door to creating countless strange, new, and improved nucleic acid nanotechnologies.

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P-30

Interfacing DNA Nanotech with Membranes to Optimize Detection

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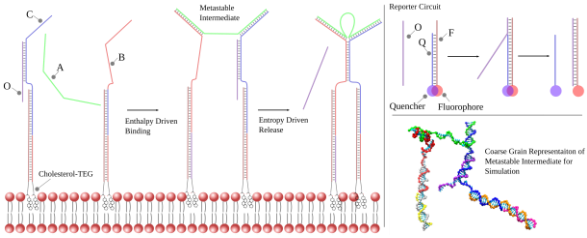
DNA circuits have been able to respond dynamically a wide variety of molecules. One key way to implement this interface is via binding-induced DNA strand displacement [1][2]. Here, two DNA nanostructures are linked by a ligand, allowing them to undergo a toeholdless strand displacement. However, a key draw back of this process is that as the size of the ligand increases, the rate of displacement decreases substantially.

We have alleviated this difficultly by confining the binding-induced DNA strand displacement nanostructures to the membrane of a Large Unilamellar Vesicle (LUV). This creates a synthetic cell-like structure which can respond to the presence of a ligand by the displacement of a DNA strand. Through detailed kinetic study, we find that these 'synthetic cells' are capable of responding to the presence of a ligand at faster rates than the same circuit in the bulk if the ligand is sufficiently large.

Furthermore, we have used coarse grain simulations [3] to elucidate precisely why membrane bound binding-induced DNA strand displacement is so effective when concerned with larger ligands. These developments will be key to detecting a more diverse range of molecules for creating DNA based diagnostic devices. They will also allow the interface of membrane accessorized DNA circuits with external sensing capability, allowing molecule sensing, logic, and signal amplification to all occur on the surface of a single vesicle.



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P-31

Experimental and Theoretical Study of DNA Bipedal Motor Walking Dynamics and Origami-based Force-clamp System

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Realization of bioinspired synthetic molecular machines that perform complex operations effectively requires an intricate understanding of the dynamics and mechanisms involved in the operation. We conducted a detailed experimental and theoretical study of the walking dynamics of a DNA bipedal motor and of the dependency of the walking efficiency on step size.[1] Step sizes were varied by placing the DNA foothold strands, on which the walker strides, at different positions on a rectangular DNA origami. The yield of the key step that primarily determines walker efficiency, namely the leg-placing reaction, was measured using single-molecule fluorescence. Coarse-grained simulations of the motor system were able to reproduce and explain three different experimental observations. First, by sampling the ensemble of transition state geometries for the leg-placing reaction, the simulations accurately capture the overall dependence of the stepping probability on the foothold separation and predict the maximum possible motor step size (~40 nm). Second, unexpected differences between experimental leg-placing yields for motors walking along the long and the short axes of the rectangular origami were explained by the preferential bending of the origami along the short axis. Third, simulations suggest that the somewhat lower leg-placing yields for small steps (~5 nm) compared to longer steps (~12 nm) is due to secondary structure in the single-stranded sections of the walker. The success of the coarse-grained simulations in describing the motor dynamics and explaining the experimental results demonstrates the utility of this approach for designing complex DNA machines. In a separate project, we have redesign a previously published DNA origami-based force clamp system that exert tunable pulling force on a DNA Holliday junction(HJ).[2] Our redesigned system enables pulling the HJ molecule with flexible poly-T strands with tunable lengths (instead of origami scaffold which contains secondary structure). This allows exact control of the pulling forces and accurate coarse-grained modeling of these forces. Furthermore, by using diffusion-based single molecule fluorescence method, instead of immobilized-based methods, and by using modified Hidden Markov Model (H2MM) for photons analysis we extend the dynamic range to several microseconds allowing measurement of the Holliday junction dynamics over the entire Mg^{2+} relevant concentrations. Our improved system can now be used to accurately measure the dynamic of host biomolecules in the low piconewton forces range.

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P-32

Stability of DNA Origami Nanostructures in Low-Magnesium Buffers

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DNA origami nanostructures as first presented by Rothemund [1] have demonstrated great potential as functional platforms for various biomedical applications. Many of these applications, however, are incompatible with the high Mg^{2+} concentrations required for DNA origami assembly. Here, we thus investigate DNA origami stability in a selection of low-Mg^{2+} buffers using the buffer exchange protocol of Linko et al. [2] (see figure 1). DNA origami stability is found to crucially depend on the availability of residual Mg^{2+} ions for binding to the phosphates in the DNA backbone. The presence of EDTA and monovalent cations may thus facilitate DNA origami denaturation by displacing Mg^{2+} ions from the backbone. Most remarkably, these buffer dependencies are also affected by DNA origami superstructure, presumably reflecting differences in the charge density distribution and the geometric arrangements of ion-binding sites. Superstructure dependence has been studied using the Rothemund triangle [1], a 24-helix bundle (24HB) [3], and a six-helix bundle (6HB) [4]. By employing rationally selected buffers and taking superstructure-dependent effects into account, the structural integrity of a given DNA origami nanostructure can be maintained even at Mg^{2+} concentrations in the low-µM range for extended periods of time. We further demonstrate that high Mg^{2+} concentrations are by no means a prerequisite for maintaining DNA origami stability. The obtained results qualify DNA origami nanostructures for a broad spectrum of biophysical and biomedical applications incompatible with high Mg^{2+} concentrations.

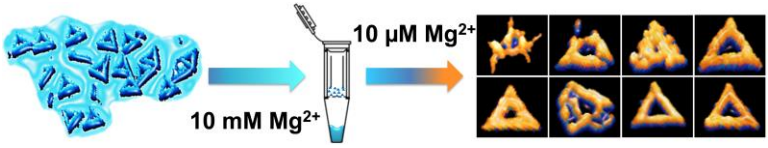

Fig. 1: Approach for investigating the stability of DNA origami nanostructures in low-Mg^{2+} buffers.

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P-33

Cryo Electron Microscopy of DNA Origami Nanostructures

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DNA nanotechnology holds potential for providing the means to construct advanced nano machines for drug delivery tasks or examination of biological processes. The precise positioning of structural elements is essential to achieve these tasks. We established methods that allow routinely employing single-particle cryo electron microscopy to determine solution structures of DNA origami objects. Cryo EM maps of a variety of DNA origami objects thus obtained revealed previously unobserved structural properties such as an global twist in honeycomb structures. Using masking techniques we were able in some cases to reconstruct details up to a resolution that allows discerning details of the backbone of the DNA double helix. Guided by the structural data, we iteratively refined the design of several exemplary DNA origami objects to match their desired global shape. Further adaption of the imaging and reconstruction methods for the specific requirements of DNA origami samples may allow solving structures with near-atomic resolution, which would provide a powerful tool for designers to rationally design structures with more complex functionalities.

P-34

The knowledge evolution of DNA Nanoscience and DNA Nanotechnology: similarities, complementarities and differences

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What drives the frontier of new knowledge development: scientific research, technological development, or the interaction between both? And how can firms, knowledge institutes and policy makers optimize knowledge development and their own contribution? These are among the questions frequently addressed by scholars in innovation sciences, but there are still considerable gaps in that knowledge. Using the field of DNA Nanoscience and DNA Nanotechnology as a case study, this paper examines the above questions be investigating scientific progress, reflected by academic papers, and technological progress, reflected by patents. Using a novel method called the concept approach, we built databases and networks for both domains. After analyzing the overall knowledge structure with main thrusts in each domain and we found that most of the sub-domains, including 'Functionalization', but excepting 'DNA computing', demonstrate strong signals of co-evolution.

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P-35

Use of multivalent interactions to achieve super-selective targeting in biological systems

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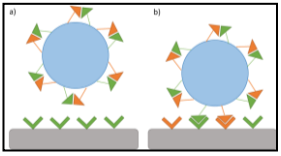
The selective targeting of few specific cells, as diseased ones, among vast and extremely diverse populations is a key step in the development of effective nanocarriers for drug delivery [1]. The simplest approach to selective targeting consists of functionalizing the carriers with a single ligand that binds strongly to a single receptor only found on target cells [2]. However, due to the large variety of cell types in the human body and the fact that each cell can express many different surface receptors, it becomes extremely difficult to identify a receptor that is only present on a specific cell. A better design choice consists of taking advantage of multivalency, i.e. the functionalization of the carriers with a large number of ligands weakly binding the receptors, which enables the targeting of only those cells expressing the receptors above a certain concentration threshold [3]. This approach is however very sensitive to poorly controlled thermodynamic parameters and fails in over-expression cases. In this work, we present a multivalent strategy capable of achieving selective targeting by binding only to surfaces displaying a specific combination of multiple receptors. Our synthetic model system is made of functionalized liposomes that mimic cells, and functionalized particles that serve as probes. Receptors on liposomes and ligands on particles are mimicked by complementary DNA strands. The multivalent scheme is achieved by controlling the competition between intra-probe bonds (loops) and bridges formed with target lipids vesicles. In our scheme, the free energy penalty of breaking a stable loop complex, featuring for instance two ligands (a), can only be compensated if all of the ligands in the complex find their partner on the vesicle, (b).This approach enables to accurately select towards the simultaneous presence of multiple receptors and can be extended to more than 2 target molecules by designing higher order loop complexes [4].



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P-36

Thermodynamics and Kinetics of the Regulation and Self-Assembly of DNA Polymolecular Nanomachines

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Bionanotechnology has become one of the most exciting fields of research in past decades. One of the domains of high activity is the development of nanomachines. Nanomachines are nanometric assemblies of molecules (e.g. DNA) that produce a quasi-mechanical movement (output) in presence of the specific stimuli (input).[1] These nanomachines can be used to deliver drug inside the body, to trigger downstream events for biocomputing systems, or used as a recognition element for biosensing. However, the yield of self-assembly of many DNA polymolecular nanomachines is not well controlled. They need to be annealed to yield a small fraction of the desired nanomachine that can then be purified by any separation techniques (e.g. HPLC, SEC, PAGE). Low yield can be avoided by understanding the thermodynamic and the kinetic of self-assembly. To overcome this problem, we used numerical simulations to explore the different thermodynamic and kinetic scenarios. Then we validated those simulations by using simple DNA nanomachines (**Fig. 1A**) that can be followed by fluorescence spectroscopy or gel electrophoresis. Our results show that increasing the number of DNA strand involved in the nanomachines enables a more sensitive self-assembly (**Fig. 1B**). Also, the concentration of each strand of the nanomachines can alter the sensitivity and the concentration at which the nanomachine will be formed (**Fig. 1C**). Binding constants between each strand need to be well defined to enable the total formation of the nanomachine and avoid the trapping into non-desirable conformation. Finally, kinetic studies of self-assembly reveal that metastable states can be observed when the wrong pathway of self-assembly is taken. This leads to kinetic trap that can be viewed as a global minimum in the energy landscape. In summary, we think that this proof-of-concept with a simple DNA nanomachine will open the door to more controlled self-assembled nanomachines.

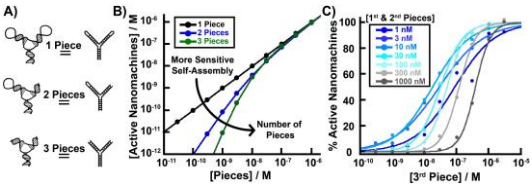

Figure 1. A) Schematic representation of all 3Way junction DNA nanomachines. B) A higher number of pieces in the nanomachines enables better sensitivity of self-assembly. C) A trimeric nanomachine can have different self-assembly sensitivity and K_{obs} depending on the fixed concentration of the 1st and 2nd pieces.

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P-37

α-$_L$-Threose Nucleic Acids as Biocompatible Antisense Oligonucleotides for Suppressing Gene Expression in Living Cells

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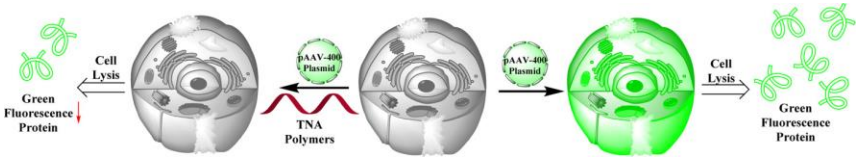
We investigated the biological properties and examined the potency of α-$_L$-Threose nucleic acid (TNA) polymers to suppress gene expression in living cells. We discovered that the TNA polymers exhibit strong affinity and specificity towards the complementary DNA and RNA targets, are highly biocompatible and non-toxic in living cell system, and readily enter a number of cell lines without using transfecting agents. Particularly, TNA exhibit better stability toward fetal bovine serum or human serum as compared to traditional antisense oligonucleotide which mean that the intrinsic structure of TNA is thoroughly resistant to biological degradation. Importantly, the efficacy of TNA polymer with GFP target sequence as antisense agents was firstly demonstrated in cellular conditions in which these polymers revealed high antisense activity in terms of the degree of inhibition of GFP gene expression. The GFP inhibition studies testified the TNA as a functional biomaterial and a valuable alternative to traditional antisense oligonucleotide such as PNAs, MOs and LNAs for a wide range of applications in drug discovery and life science research.



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P-38

Asymmetric DNA Scaffolds and their Application as Combinatorial Sensors and Molecular Security Systems

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Conventional methods for creating synthetic receptors are generally limited by the need to use water-soluble building blocks and performing multistep organic synthesis, which complicate using such receptors as biomimetics and sensors. To address this problem, we have developed a simple, versatile and robust method for preparing sets of water-soluble synthetic receptors through the self-assembly of oligonucleotides (ODNs) into asymmetric DNA G-quadruplex structures. By modifying these ODNs with supramolecular recognition elements and distinct fluorescent reporters (Figure 1), we have demonstrated the possibility of creating a novel type of pattern-generating fluorescent sensors that can discriminate among multiple different analytes, such as drugs of abuse, in a high-throughput manner. We have also shown that these sensors can function as molecular-scale security systems[1] that can authorize four different users simultaneously.

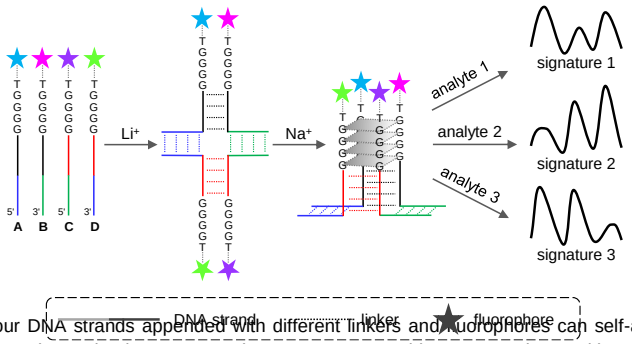

Figure 1. Four DNA strands appended with different linkers and fluorophores can self-assemble into a unique asymmetric quadruplex structure via a two-step assembly process. The resulting analytical device can generate a wide range of distinct emission signatures upon binding to different analytes.

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P-39

Sub-Ensemble Monitoring of DNA Strand Displacement Using Multiparameter Single-Molecule FRET

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Non- enzymatic DNA strand displacement is an important mechanism in dynamic DNA nanoscience. Here, we describe the use of multiparameter single- molecule Förster resonance energy transfer (FRET) spectroscopy as a sensitive tool for monitoring strand displacement. We monitored the displacement from double- stranded DNA (dsDNA) by single- stranded DNA (ssDNA) at 37 °C; the data were modelled as a second- order reaction approaching equilibrium, with a rate constant of $10\,m^{-1}s^{-1}$. We also followed the displacement from a DNA three- way junction (3WJ) by ssDNA. The presence of three internal mismatched bases in the middle of the invading strand did not prevent displacement from the 3WJ, but reduced the second- order rate constant by about 50 %. We attribute strand exchange in the dsDNA and 3WJ to a zero- toehold pathway from the blunt- ended duplex arms. The large parameter space allows the simultaneous monitoring of multiple reactants and products, and we anticipate that the single- molecule approach demonstrated here will be useful for studying complex DNA networks.

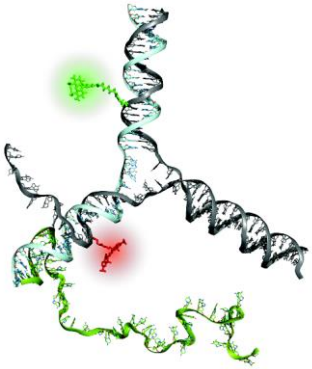

Fig. 1. Zero-toehold strand displacement monitored using single-molecule FRET

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P-40

Thiol-free oligonucleotide surface modification of gold nanoparticles for nanostructure assembly

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Gold nanoparticles (AuNPs) decorated with thiol-modified DNA strands (HS-DNA) are a convenient, easily adjustable, and highly controllable material for constructing plasmonic nanostructures with various shapes and functions. However, only few reproducible and robust methods involving DNA template as a key reagent are available for obtaining nanoparticle assemblies. One of the major challenges in nanostructure design by conventional methods is low controllability of the number of DNA strands on the AuNP surface. Here, we introduce an efficient approach for surface functionalization of AuNPs without support from origami structures, using unmodified DNA oligonucleotides by building DNA cages that trap nanoparticles. It allows to vary the process of nanostructure assembly, and create anisotropic nanoparticles that are necessary for directed structure construction. The developed method reduces production time in comparison with conventional HS-DNA modification protocols and helps to control the density of functional DNA strands designed for further hybridization with other AuNP conjugates. This approach was successfully coupled with DNA circuit based on hybridization chain reaction and can be used in nucleic acid detection.

P-41

Catalyzed hairpin assembly of magnetic nanoclusters with single nucleotide discrimination

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We demonstrate detection and single nucleotide discrimination of DNA targets based on hairpin assembly (HA) combined with an optomagnetic (OM) readout detecting the clustering of magnetic nanoparticles (MNPs). The HA assay[1] is based on two initially 'locked' hairpin stems *H1* and *H2* that are grafted onto two separate populations of 100 nm MNPs (Fig. 1a). A fully matching DNA target *C1* 'unlocks' the stem of *H1* via toehold strand displacement. The unlocked *H1* probe subsequently unlocks the stem of *H2* and displaces the *C1* target to form a stable *H1-H2* hybrid linking two MNPs. The clustering state of the MNPs is detected using the OM technique, which probes the rotational dynamics of MNPs in response to an applied oscillating magnetic field at frequency *f*.[2] The MNPs have a magnetic moment and a linked optical anisotropy and the magnetic field results in a modulation of the intensity of light transmitted through the MNP suspension (Fig. 1a). Depending on the hydrodynamic volume V_h, the MNP response shows a phase lag φ with respect to the field excitation. Clustering increases V_h and a phase lag is observed at lower frequencies.[2] The response of single MNPs was mainly detected at $f > 40$ Hz, whereas that from MNP clusters was detected at $f < 40$ Hz. As the HA signal, we take the average phase at $f < 40$ Hz after 50 min of HA reaction at 40°C. We studied the dose-response curve and limit of detection (LOD) for MNP hairpin probe densities of 100% and 40% and the specificity of the assay by comparing *C1* and *Mut* targets, where *Mut* had a single base mismatch in the toehold-recognizing part of *H1*. Figs. 1b and c show the average phase lag for $f < 40$ Hz vs. concentration of *C1* and *Mut* targets for the two probe densities. For 100% probe density, we found *C1* and *Mut* LODs to fall within the range of 1-10 nM and 10-100 nM, respectively, and 16-fold discrimination of *C1* from *Mut* at 10 nM. For 40% probe density (Fig. 1c), the *C1* LOD was improved to 0.1 nM, but the specificity was reduced. Less accessible toehold regions of highly 'crowded' *H1* and *H2* give better discrimination of the single base mutation in the DNA target.

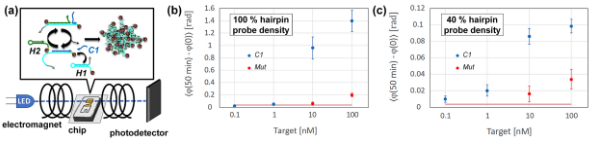

Fig. 1: **OM-HA detection.** (a) Schematic of assay and OM setup. Measurements were performed using plastic chips sandwiched between two heaters at 40°C. (b)-(c) Dose-response analysis of the HA triggered by *C1* as well *Mut* targets at the indicated hairpin densities.

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P-42

DNA and DNA like polymer based self-assembled and hierarchical nanostructures for biosensing

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In this study, a new effective and DNA and DNA like polymer based self-assembled and hierarchical nanostructures was designed that can be used for biosensing applications. SWNT-DNA hybrid Nano structure fabricates by region selective covalent combined between DNA and SWNT Nano particles These hybrid Nano structures have different electrochemical properties compare of alone SWNT and DNA single strand. Electrochemical behavior of SWNT-DNA hybrid Nano structures are studied by cyclic voltammetry and impedance spectroscopy technique. Due to wire shape SWNT-DNA hybrid Nano structures have favorable electrical properties could be used in fabrication of genomic biosensor which was detected mismatch and abnormality in ds-DNA. In other attempt, a three-dimensional (3D) hierarchical architecture for aerographite bioelectrodes was fabricated by template-directed self-assembly. The superlight aerographite was functionalised with guanine (G) rich polymer loaded with glucose oxidase (GOx) thorough π-π interactions of electron pairs of the guanine ring and the electron cloud of aerographite. These superlight and hierarchical graphene based nanostructure detected glucose in solution with satisfy sensitivity.

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P-43

Dual amplification strategy triggered by triple helix probe for the detection of microRNAs

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The triple helix is an alternative structure adopted by nucleic acids based on Watson–Crick and Hoogsteen base pairings involving three strands in the correct orientation.
Since the stability of the triple helix stem can be higher even compared to longer duplexes, triple stranded DNA has been used in biosensing to build aptasensors or sensors for nucleic acids detection [1-4] and good sensitivity and selectivity have been generally observed. Moreover, different recognition elements specific for different targets can be included in the nucleic acid probe on a same single-stranded sequence, allowing in principle a universal assay strategy [5]. In the literature, the use of only one of the involved oligonucleotides for signal amplification and transduction is generally reported. We herein propose the use of both the triple helix forming strands in this process as this would give an advantage, such as the chance to combine different transduction approaches leading to a stronger and more reliable detection method. For this reason, we designed triple helix DNA probes specific for different microRNAs, in which both the sequences hidden in the stem would be available for subsequent signal amplification and transduction after target recognition (Fig. 1). To simply test the feasibility of our system to induce a double response triggered by the target, we designed hybridization chain reactions (HCR) [6] started by both the oligonucleotides involved in the triple helix. Furthermore, in our peculiar design, the self-assembled nanostructures resulting from a single recognition event can interact with each other forming bigger nanostructures, thus enhancing the recognition signal. After characterizing this strategy in solution (see Fig. 2), we are now in the process of implementing it in a biosensor platform.

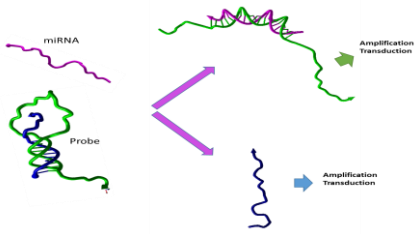

Fig. 1. Scheme of triple helix probe and splitting of the signal transduction-amplification.

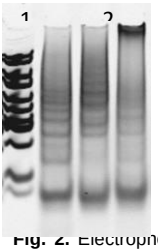

Fig. 2. Electrophoresis showing HCR products after individual triggering for triple helix probes (Lane 2 and 3), and the higher MW product from double triggering (Lane 4).

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P-44

Design and development of DNA-based synthetic push-pull networks

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Push-pull networks are an out-of-equilibrium biochemical network motif consisting of a substrate that can be switched between active and inactive states by two catalysts. These networks are prominent in protein regulation and ubiquitous in cellular signaling, which features multistep cascades [1]. In these cascades, the push-pull networks propagate and process signals related to the relative abundance of the catalysts. Push-pull networks are able to act both as noise filters and signal amplifiers working near the Wiener-Kolmogorov optimal regime [2]. Due to these features, artificial push-pull networks are an area of interest for synthetic biology as programmable and controllable biomolecular systems.

In our work, we aim to develop nucleic acid-based synthetic push-pull networks using 4-way strand displacement reactions as the fundamental functional motif [3]. The proposed design allows us to rationally design systems at the base-pair level, resulting in a high degree of control of the free energy landscape and kinetics of the reaction network.

In the present poster, we present results pertaining to the characterization of the push-pull network elements, from the deep profiling of the 4-way branch migration process at the free energy and kinetics level to the experimental evidence that our designed DNA-based network allows to achieve desired catalytic functions. These designed catalytic functions, along with the specificity allowed by design of DNA domains allows the coexistence of different catalysts and fuels through which fine tuning of the network dynamics can be achieved.

Potential applications for our devised system range from the exploration of the fundamental thermodynamic costs and tradeoffs in signal transduction to the construction of orthogonal, low-burden, programmable control systems for nucleic-acid-based synthetic biology.

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P-45

Modelling the Folding Pathway of DNA Origami

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DNA origami has proved to be a reliable technique, even though current design principles are largely based on empirical rules. To examine the kinetics of self-assembly, we extend a previous description of DNA origami[1] in which contiguous regions of designed staple-scaffold hybridisation are represented as single domains. We calculate domain interaction energies using nearest-neighbour thermodynamics, adding coaxial stacking terms for bound domains. Additionally, we use a freely-jointed chain model to describe the entropic changes in the scaffold strand that lead to higher local concentrations when binding subsequent domains of partly-bound staples. Interactions between the staple and scaffold strands are described as a continuous-time Markov chain and simulated through a kinetic Monte Carlo scheme. The model has been used to demonstrate the importance of kinetics in origami formation[2]. We present a systematic study of the mechanisms of cooperativity by comparing the relative effects of coaxial stacking and scaffold entropy, quantifying the contributions of individual staples to cooperativity and hysteresis. The systems under study use the same scaffold strand but differ both in the type of staples and their connections. This provides a convenient set of experimental results to explore the effect of key quantities such as the number of scaffold turns and the lengths of scaffold loops spanned by staple crossovers. We show that the number of coaxial stacking interactions has a larger effect on cooperativity, whereas hysteresis is strongly coupled to the total span of crossovers. Staples that bring together distant regions of the scaffold play a more important role in both cooperativity and hysteresis than those closing short scaffold loops. These “seam” staples tend to bind at the initial stages of annealing to facilitate folding, but unbind in the final stages of melting. The role of nucleation in origami assembly has long been a subject of debate, with parallels to both DNA brick assembly and protein folding. On the one hand, we expect the increased stabilisation of nuclei due to scaffold loops and coaxial stacking of domains to facilitate growth. On the other hand, the rate of nucleation is not expected to be limited by an initial activation barrier so there is no clear critical nucleus. We track clusters of connected domains and find that folding proceeds through growth and merging of multiple nuclei. To construct free energy landscapes, we extend the model to gather statistics during isothermal simulations. Using umbrella sampling to enhance rare transitions, we explore the free energy barriers during folding to study the relative contributions of coaxial stacking and scaffold entropy to the heights of these barriers.

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P-46

Quantitation without calibration: a new approach to nucleic acids' measurement

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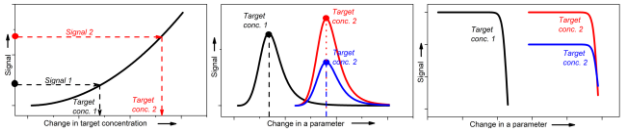
Nucleic acids are essential biomarkers in variety of contexts from monitoring food quality to medical diagnostics to applications in life-science research. Surprisingly, their *quantitation* is not as widely addressed as *detection*. While there are some established protocols (i.e. quantitative PCR and/or microarrays), they are, typically, very time consuming, expensive, and require professional personnel. The reason behind such complexity is that in order to quantify a target, a signal magnitude generated by the unknown sample needs to be correlated to some kind of calibration (left graph). However, any calibration comes with an increased cost and limitations such as availability of a pure quantitatively characterized standard of a target (or its proxy), or matching the calibration conditions/environment to the target assay to mitigate matrix effects. Furthermore, reliable calibration cannot be done in some situations, such as microarrays where density of probes, effects of their surface concentration on fluorophores and other factors make establishing absolute calibration impossible. As a result, many quantitation approaches are more relevant for comparing differences between samples rather than in measuring absolute quantities.[1]

The goal of our project is to develop a new general concept for quantitation of nucleic targets that will obviate the need for a standard. Specifically, we develop the approach to measurement based on a target's response profile features rather than on the absolute value of a signal. As illustrated in the middle graph, if the position of a target's response profile as a function of a condition depends on its absolute amount, then we can use it for the quantitative assessment of the target. This approach results in eliminating reliance on the signal's magnitude because it makes possible to define response profile features and position even if experimental factors affect the signal value. For example, for target at concentration 2 in the middle graph, even if experimental factors affect the signal magnitude, the absolute quantity can be determined from the position of the corresponding response profile. In order to develop a quantitation approach based on the position of a response profile, we establish a binding mechanism for the target - ligand interaction based on negative cooperativity with limited receptor concentration.[2] The binding model yields a response profile with position of inflection point (right graph) uniquely defining the target concentration over a wide concentration range.



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P-47

Photo-switchable artificial nucleosides for DNA origami machines

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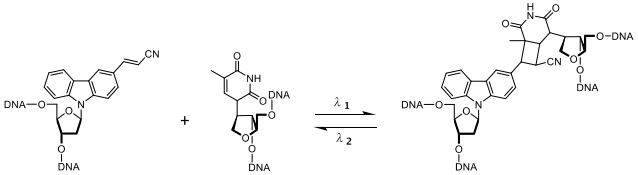
The DNA origami methodology introduced by Rothemund[1] enables the bottom-up self-assembly of rationally designed DNA nanostructures in which every nucleobase can be addressed and functionalized with chemical moieties. Accordingly modified DNA nanostructures may show more complex machine-like behavior with potential applications in bionanotechnology and material sciences[2]. One example of a DNA modification that holds potential for achieving actuation in DNA nanostructures is the cyano-vinyl-carbazole (^{cnv}K) moiety. ^{CNV}K can be covalently linked by a [2+2] photo-cycloaddition with a pyrimidine base on a complementary duplex strand upon UV irradiation. The covalent bond can be broken again with UV light of shorter wavelengths [3]. The incorporation of artificial nucleotides, such as the ^{cnv}K compound, through DNA solid phase synthesis (SPS) is a promising approach to functionalize DNA nanostructures [4]. Organic compounds which can undergo E/Z-isomerisation through photo-switching also offer a route to achieve actuation in DNA nanostructures. Diazene molecules, and in particular azobenzene groups, can be switched between cis- and trans- isomers upon light irradiation, which can be exploited to remotely control the conformational state of DNA nanostructures [5]. We present possible modifications of single-stranded DNA (ssDNA) with artificial nucleosides through SPS. One system is based on the ^{cnv}K and derivatives to reversibly form and break covalent bonds in DNA origami nanostructures. We also present strategies to incorporate ligands based on the diazene backbone into ssDNA to explore the potential of switching between the isomeric forms in DNA nanostructures.



Scheme for [2+2] cycloaddition between ^{cnv}K nucleotide and thymine.

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P-48

Engineering Programmable Nucleic Acid Nanoswitches for the Rapid Detection of Antibodies in Bodily Fluids

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Antibody detection is crucial for the diagnosis of many human disorders including infectious, autoimmune, and oncological diseases. Moreover, since immunotherapy represents nowadays the frontier to fight cancer, the detection of monoclonal antibodies becomes of paramount importance to correctly monitor the progression and efficacy of such therapies. To allow early diagnosis, prompt therapeutic actions and efficient immune-based therapy antibodies detection methods should be sensitive, quantitative and specific but also rapid and easy to use. Unfortunately, however, current methods routinely used for this purpose in clinical settings either require reagent-intensive laboratory-based techniques, multiple time-consuming incubation steps and/or sophisticated equipment.
Here we show a nucleic acid nanoswitch platform able to measure Immunoglobulins of type G and E (IgG and IgE) levels directly in blood serum and other bodily fluids in few minutes and without washing steps.[1] The system couples the advantages of target-binding induced co-localization and nucleic acid conformational-change nanoswitches. This novel sensing programmable platform can be adapted to the detection of any antibody for which the recognition element that can be coupled to the nucleic acid anchoring strand. We also demonstrate a clinically relevant application of our method monitoring the immune response of HIV-positive patient enrolled in a medical trial and treated with a peptide based (AT20 peptide) phase-I therapeutic vaccine. Specifically, we are able to discriminate between AT20-positive subjects (vaccinated patients) and controls (unvaccinated patients). This system can be used as a powerful diagnostic platform both for POC applications and large-scale analysis. Moreover, it is a useful tool to assess prophylactic vaccine population coverage and to verify the efficacy and the duration of the response.

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P-49

Rational control of the activity of a Cu^{2+}-dependent DNAzyme by re-engineering purely entropic disordered domains

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Here we modulate the catalytic activity of a Cu^{2+}-dependent self-cleavable DNAzyme by rationally introducing different intrinsically disordered regions that, through a purely entropic contribution, control the folding, and thus activity, of the DNAzyme.
To do this, we have designed a triplex-forming DNA sequence that, by recognizing a 11-base DNA strand through the formation of a clamp-like structure, folds into the highly conserved catalytic core of the Cu^{2+}-dependent self-cleavable DNAzyme. The affinity with which the triplex-forming DNA sequence binds to the 11-base DNA strand can be modulated by varying the length, and thus the entropy, of the loop that connects its two recognition portions (Figure 1). This allows to modulate the catalytic activity of the Cu^{2+}-dependent self-cleavable DNAzyme through a simple modulation of the entropy associated to its folding in a very versatile and precise way.

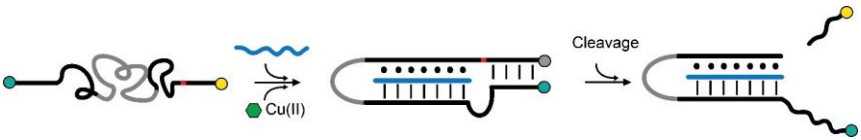

Figure 1. Triplex-forming Cu^{2+}-dependent self-cleavable DNAzyme.

P-50

Allosterically Regulated DNA-based Switches for Controlled Release of a Molecular Cargo Activated by Biological Inputs

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Here we demonstrate the rational design of a new class of DNA-based switches that are allosterically regulated by specific biological targets, antibodies and transcription factors, and are able to load and release a molecular cargo (i.e. doxorubicin) in a controlled fashion.[1] In our first model system we rationally designed a stem-loop DNA-switch that adopts two mutually exclusive conformations: a “Load” conformation containing a doxorubicin-intercalating domain and a “Release” conformation containing a duplex portion recognized by a specific transcription-factor (here Tata Binding Protein). The binding of the transcription factor pushes this conformational equilibrium towards the “Release” state thus leading to doxorubicin release from the switch. In our second model system we designed a similar stem-loop DNA-switch for which conformational change and subsequent doxorubicin release can be triggered by a specific antibody. Our approach augments the current tool kit of smart drug release mechanisms regulated by different biological inputs.

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P-51

Photocontrol of DNA origamis melting and formation: towards light-controlled isothermal nanomachines

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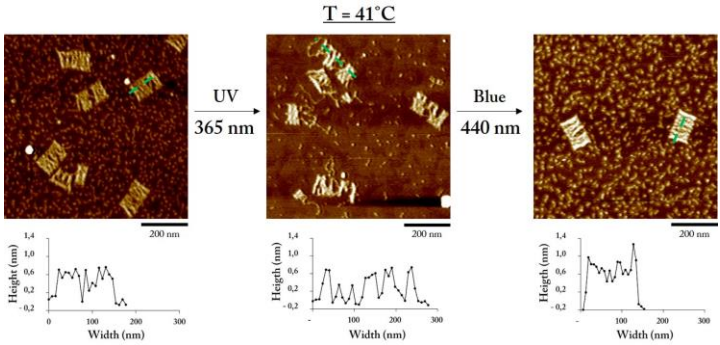
DNA origami[1] is a very elegant technique that has proven to be an efficient way to create highly complex 2D and 3D nanostructures with arbitrary programmable shapes. The resulting folded supramolecular objects are however static unless addition of a competitive oligonucleotide or submitted to a temperature variation. Our objective is to render DNA origamis dynamic at constant temperature and without covalent modification of the DNA through a photoreversible process by using a photosensitive intercalator, AzoDiGua,[2] recently developed by our group. AzoDiGua has the unique property to intercalate DNA only in its *trans*-form, thus stabilizing the double-helix and inducing a marked increase in its melting temperature (*Tm*). Upon UV irradiation, AzoDiGua isomerizes to its *cis*-form, which is ejected from the double helix, resulting in a decrease in *Tm* that can be recovered upon blue irradiation. In this presentation, I will describe a new method used to prepare origamis incorporating AzodiGua, as well as the study of the melting behaviour as a function of temperature. I will present conditions where origamis are fully formed at 41°C in the presence of *trans*-AzoDiGua and whose shapes were drastically changed upon UV irradiation. Interestingly, I will show that the original origami shape can be recovered at constant temperature upon blue irradiation.



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P-52

Resolving the Sequence of Events in the Folding of DNA Nanostructures

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DNA origami nanostructures are formed by self-assembly of a long circular single-strand (called "scaffold") and a set of hundreds of short oligonucleotides. A wide variety of successful designs illustrates the robustness of DNA origami technique. We and others have previously found that DNA origami fold highly cooperatively in narrow temperature intervals, and with optimized structure specific thermal ramps we could improve folding yield above 90 %.[1] Here, we focus on the question whether the formation of a structure follows a particular pathway, i.e whether there is a defined sequence of events by which DNA strands assemble and fold the scaffold molecule. If there were a defined sequence of events, it would be highly beneficial to reveal the connection between the pathway and the particular details of a DNA origami (sequences, topology of strand connectivity)

To investigate this question we relied on two complementary fluorometric assays to track the binding of single oligonucleotides and their segments to the scaffold. In one assay, we follow intra strand conformational dynamics via time-resolved fluorescent quenching, and in the other assay we study the proximity of staple strand termini via time-resolved FRET. In total, 3200 such pairwise reactions were measured. The data obtained with both assays clearly showed that there is a defined sequence of binding events, where a small subset of strands form the folding nucleus, followed by integration of the bulk of staple strands, and strands that come last. In a second part of this study, we attempted to reveal the physical origin of this sequence of events. According to our data, the topology of strand connectivity plays a major role, meaning that circular sequence permutations of the same strand diagram fold along similar pathways, whereas the local sequences of DNA strands play a minor role. There is no simple, discernible rule that would allow to extract e.g. the identity of the strands that nucleate folding. Our extensive data set provides ample constraints to develop a predictive model of DNA origami folding.

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P-53

Plasmonic nanostructures through DNA-assisted lithography

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Programmable self-assembly of nucleic acids enables the fabrication of custom, precise objects with nanoscale dimensions [1]. These structures can be further harnessed as templates to build novel materials such as metallic nanostructures, which are widely used and explored because of their unique optical properties and their potency to serve as components of novel metamaterials [2,3]. However, approaches to transfer the complicated spatial information of DNA constructions to metal nanostructures remain a challenge. We report a DNA-assisted lithography (DALI) method that combines the structural versatility of DNA origami [4] with conventional lithography techniques to create discrete, well-defined, and entirely metallic nanostructures with designed plasmonic properties [5]. DALI is a parallel, high-throughput fabrication method compatible with transparent substrates, thus providing an additional advantage for optical measurements, and yields structures with a feature size of ~10 nm. We demonstrate its feasibility by producing metal nanostructures with a chiral plasmonic response and bowtie-shaped nanoantennas for surface-enhanced Raman spectroscopy. In addition, different metals (Au, Ag, Cu) can be used to enrich the options of material for plasmonic applications [6]. We envisage that DALI can be generalized to large substrates by novel deposition techniques, e.g., spray-coating [7], which would subsequently enable scale-up production of diverse metallic nanostructures with tailored plasmonic features.

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P-54

DNA-Templated Assembly of the Bacterial Flagellar Motor's Cytoplasmic Ring

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The bacterial flagellar motor is one of the most complex protein machines found in nature. How it self-assembles and produces force are very much open questions. We have taken a novel approach to study the interactions and assembly pathway of the motor's cytoplasmic ring by building up the structure *in vitro* using the tools provided by DNA nanotechnology. The key constituent of this ring, a protein called FliG, was conjugated to a DNA strand allowing it to be precisely positioned on DNA scaffolds which replace its natural template composed *in vivo* of the protein FliF. Although clusters of FliG can be formed on these synthetic scaffolds we see no evidence of cooperative assembly. However, recent work has shown that the binding of FliG to FliF may induce a conformational change in the proteins which is crucial during the assembly process[1] and missing in our experiments involving DNA nanostructures templating FliG alone. To investigate the significance of the FliF-FliG interaction, a peptide corresponding to the cytoplasmic domain of FliF was conjugated to a DNA strand, and these peptide-DNA conjugates were organized on the same DNA scaffolds that clustered FliG alone without cooperativity. FliG binds stably to these peptide-DNA templates and preliminary results indicate that binding of FliG to the structures is facilitated by clustering of the peptides, i.e., that FliG assembles cooperatively on templates bearing the interacting domain of FliF. With this *in vitro* system now in place, many experiments, impossible to do in a live cell, can now be undertaken, allowing for new insights into the assembly pathway and inner workings of this remarkable molecular machine.

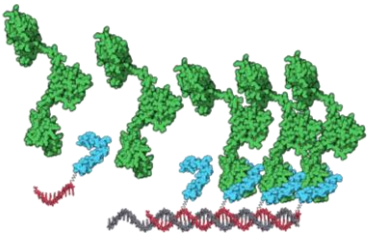

Figure. FliG (●) is assembled onto DNA scaffolds via interaction with a DNA-conjugated peptide (●) corresponding to the C-terminal domain of FliF.

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P-55

A Microsphere-Supported Lipid Bilayer Platform for DNA Reactions on a Fluid Surface

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A long-term goal of our research into molecular computing is to create large-scale circuits using nucleic acid chemistry as the computational substrate. Development of DNA computing devices in solution phase poses challenges of scale in the design, because every instance of a computational element must be mapped to a unique set of interacting strands, as well as challenges in the device operation, in the form of undesirable cross-talk between the elements. To enable modular design of larger circuits, we borrow from both electronics and cell biology, and wish to construct compartments in which to isolate computational elements. To make an effective compartmentalized system, we need to be able to (1) isolate reactions to surfaces and propagate reactions on surfaces, and (2) transmit signals across compartment boundaries using reactions that span surfaces. The present work concerns the first of these requirements.

We developed a multiplexed DNA-based system in which solution-phase single-stranded oligonucleotides bind surface-based DNA receptors, using toehold-mediated strand displacement and DNAzyme reactions. Receptors are covalently linked to a silica-microparticle-supported lipid bilayer, which provides a fluid surface. The physical and optical properties of the bilayer are tuned by adjusting its lipid composition, and we incorporate fluorescent lipids in varying concentrations to produce distinct spectral addresses that distinguish microsphere populations in a multiplexed solution. This microsphere-based system is stable for extended periods of time, and can be analyzed via flow cytometry.

We then optimized the experimental conditions to allow DNAzyme reactions, which require divalent cations, to be run on the phospholipid bilayer surface. Using these conditions, we ran reactions with surface-bound substrate molecules and solution-phase DNAzymes on the supported lipid bilayer.

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P-56

Complexing DNA origami frameworks through sequential self-assembly based on directed docking

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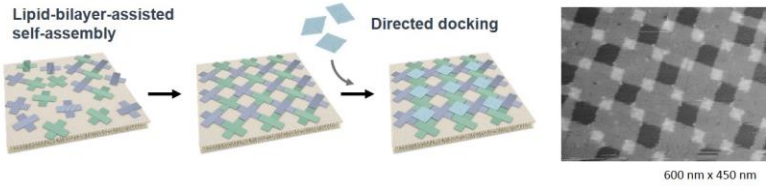
Ordered DNA origami arrays have the potential to compartmentalize space into distinct periodic domains that can incorporate a variety of nanoscale objects. Here, we aimed to use the internal cavities of a preassembled two-dimensional (2D) DNA origami framework to incorporate supplemental square-shaped DNA origamis (SQ-origamis) with shapes that were complementary to those of the cavities. In our approach, the framework was first self-assembled on a mica-supported lipid bilayer membrane from cross-shaped DNA origamis (CR-origamis), and subsequently exposed to the SQ-origamis. Using high-speed atomic force microscopy, we revealed that our SQ-origamis exhibited a dynamic adsorption/desorption behavior, which resulted in continuous changing of their arrangements in the framework. These dynamic SQ-origamis were trapped in the cavities by increasing Mg^{2+} concentration or by introducing sticky-ended cohesions between extended staples, both from the SQ- and CR-origamis, which enabled the directed placement of the SQ-origamis. Based on these features of our system, the framework that was self-assembled from two different CR-origami components was complexed into a checkerboard-like pattern through sequential self-assembly. Our study offers a platform to create supramolecular structures or systems consisting of multiple DNA origami components.



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P-57

Nucleic acid assembly mediated by the fluorous effect

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Controlling the nanoscale structure of functional interfaces is essential to engineer rationally designed nanoarchitectures. DNA nanostructures provide a high level of programmability, being excellent scaffolds to arrange multiple components at nanometer scale to construct interactive biomolecular complexes and networks. However, spatial and orientation positioning control of functional DNA nanostructures at the micro-range still remains as a challenge. Our approach is to combine lithographically patterned fluorous surfaces with DNA self-assembly, incorporating short-range organisational elements (i.e. Watson-Crick base pairing) with stronger, longer-range fluorous-fluorous recognition elements. We have recently shown how the fluorous effect[1-2] can be used as an effective tool to direct the immobilization of fluorous-tagged oligonucleotides (RF-ODNs) within fluorous-patterned surfaces (Figure 1).[3] Remarkably, in contrast to conventional covalent methods, fluorous-directed immobilization is fully reversible; enabling directed surface patterning, regeneration, and re-patterning of surfaces without any associated degradation of immobilization efficiency or disruption of Watson-Crick base-pairing. This presentation will describe our latest efforts to fully explore the potential and versatility of the fluorous-fluorous interaction on more complex DNA nanostructures systems.

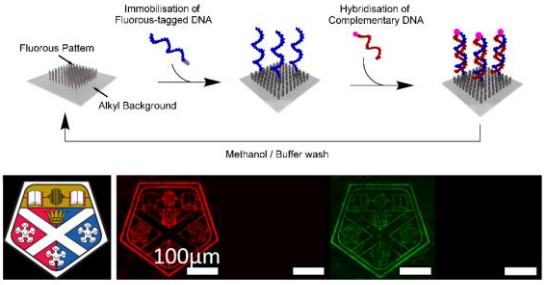

Figure 3 (A) Directed immobilisation of RF-ODNs onto a fluorous-patterned surface, hybridisation of complementary DNA, and surface regeneration. (B) Fluorescence images showing two cycles of this process.

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P-58

G-Quadruplex-Mediated Molecular Switching of Self-Assembled 3D DNA Nanocages

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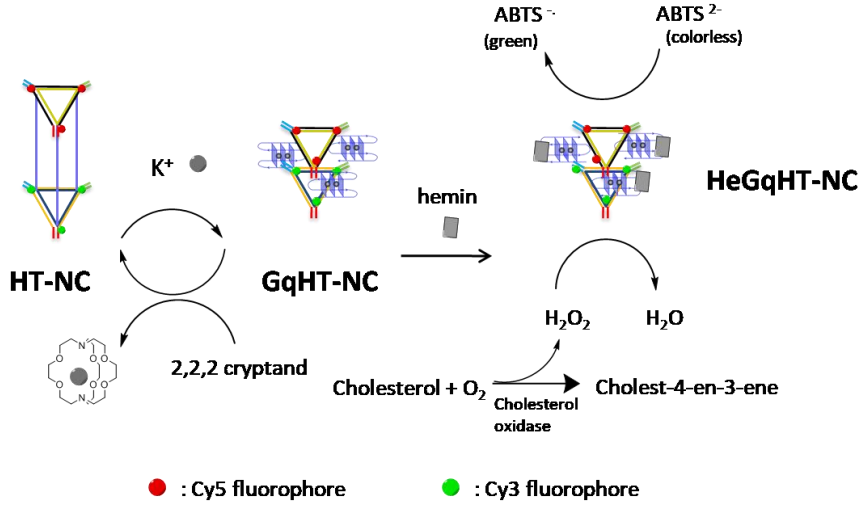
G-quadruplex is a G-rich DNA sequence forming specific structure in present of cations which naturally present in cells. This structure can be deformed by high temperature, lowering the cation concentration and ion-clelating ligands. Nowadays, researchers have applied this property to make various devices, eg. logic gate, switch, sensor, etc.. However, most of them are DNA in the form of single strand or origami. Therefore, we would like to explore the reversibility, catalytic activity as well as the biological properties in a self-assembled DNA nanostructure integrated with G-quadruplex sequence which diameter is only a few nanometers.



Scheme 1 Schematic representation of the formation of reversible HT-NC and its working principle as a colorimetric sensor for cholesterol.

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P-59

All-Optical Imaging of Gold Nanoparticle Geometry Using Super-Resolution Microscopy

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We demonstrate the all-optical reconstruction of gold nanoparticle geometry using super-resolution microscopy[1]. We employ DNA-PAINT to get exquisite control over the (un)binding kinetics by the number of complementary bases and salt concentration, leading to localization accuracies of ~5 nm. We employ a dye with an emission spectrum strongly blue-shifted from the plasmon resonance to minimize mislocalization due to plasmon-fluorophore coupling. We correlate the all-optical reconstructions with atomic force microscopy images and find that reconstructed dimensions deviate by no more than ~10%. Figure 1 illustrates an atomic force microscopy image of a single nanorod, overlaid with the geometry reconstructed from the localization events, illustrating the accuracy of the reconstruction. Numerical modelling shows that this deviation is determined by the number of events per particle, and the signal-to-background ratio in our measurement. We further find good agreement between the reconstructed orientation and aspect ratio of the particles and single-particle scattering spectroscopy. This method may provide an approach to all-optically image the geometry of single particles in confined spaces such as microfluidic circuits and biological cells, where access with electron beams or tip-based probes is prohibited.

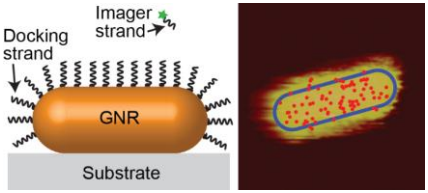

Figure 4 (left) Schematic illustrating gold nanorods functionalized with DNA docking sites, proving binding sites for fluorescent imager strands. (right) AFM image of a single gold nanorod, with red dots indicating super-resolved binding events, and the blue outline the reconstructed nanorod geometry.

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P-60

Protein induced fluorescent enhancement based thrombin DNA aptasensor

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Every day the need to detect biologically relevant molecules such as small molecules, proteins, cancer markers, etc is increasing. This challenge has led the researchers around the globe to develop of biosensors, which employ biomolecules to recognize the cellular target and utilize output elements to translate the bio recognition event into electrical, optical or mass-sensitive signals. One such strategy employs DNA Aptamers, the single/ double stranded DNA molecules that bind their target with high specificity. Our current work focuses on the thrombin binding DNA aptamer1 (TBA) that binds with thrombin with dissociation constant (Kd) below 50 nM. We employ a protein-induced fluorescence enhancement2 (PIFE) technique to measure thrombin binding with the TBA which is a simple 'turn-on' mechanism with a single dye linked to the aptamer. We further validated our PIFE results using Förster resonance energy transfer3 (FRET) and found no change in the sensitivity of the aptamer-based detection. Unlike FRET, PIFE does not rely on DNA conformational changes, which helps it to report protein binding directly and hence it is unaffected by salt based stabilization of the DNA conformations. The DNA aptamer assay also displayed high specificity even in the presence of 'natural' backgrounds. Therefore, we propose that PIFE based aptamer sensing of protein ligands can be simple but effective method.

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P-61

Strength and kinetics of DNA hybridization on a surface measured by Reflective Phantom Interface

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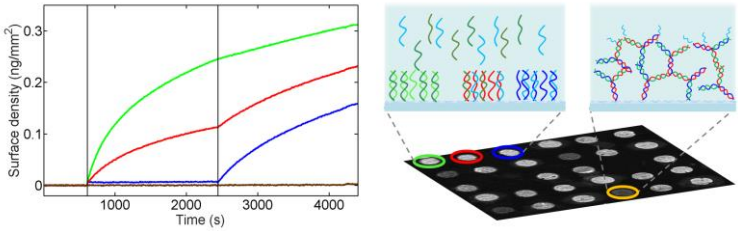

The use of DNA-based molecular probes on a biosensing surface enables unprecedented designs, with finely tuned structures and responsiveness [1]. However, despite the deep knowledge of DNA interactions in bulk solution, the modeling and optimization of DNA-DNA interactions on a surface are still challenging and perceived as strongly system-dependent. This incomplete knowledge hampers the design and the widespread use of more complex functional DNA structures integrated on the surface of biosensors. Here, we exploit the Reflective Phantom Interface (RPI), an optical, multiplexed, label-free technique [2,3,4], to unravel the key parameters controlling the interaction strength and kinetics of DNA-DNA hybridization on a surface. We investigated immobilized DNA probes with various sequences, lengths, structures and attachment chemistry, targeting simple complementary strands or DNA nanostructures. Electrostatic screening, probe-probe cross-interactions and probe-surface adhesion are all found to play a role in the hybridization kinetics, which in turns yields to different affinities. Overall, simple kinetic models accounting for all these effects on surface DNA hybridization are expected to guide the design of more complex functional structures on a biosensing surface.

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P-62

Direct Single-Molecule Observation of Mode and Geometry of RecA-Mediated Homology Search

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DNA origami provide a robust yet flexible framework to host a variety of enzymatic interactions enabling their study with high speed atomic force microscopy (HS-AFM). Not only do these architectures provide a support substrate for the reaction of interest but they allow multiple positional and orientational markers to be presented *in situ*, along with in-built controls. Hence, this method enables the mode and geometry of dynamic enzymatic interactions to be revealed at the single molecule level, which are unobtainable by any other approach. Here, we apply this methodology to study the early stages of homologous recombination as mediated by Recombinase A (RecA). The efficient repair of DNA via homologous recombination is critical in maintain genomic integrity throughout all cellular organisms. For this process the ubiquitous RecA, and its homologues such as the human Rad51, are of central importance, able to align and exchange homologous sequences within single-stranded and double-stranded DNA in order to swap out defective regions. However, to date the mechanism by which RecA is able to locate regions of sequence homology remains widely debated. We directly show using HS-AFM that nucleoprotein filaments formed from RecA and ssDNA undergo short regions of facilitated diffusion – or slides – along target dsDNA substrates interspersed within a primary mode of longer-distance random moves – or hops.[1] Our observations directly confirm the occurrence of facilitated diffusion within the homology location mechanism and suggest that it serves to check sequence registration surrounding regions of partial sequence homology. Moreover, we directly observe the characteristic dynamic interaction geometry and find it to be in line with that proposed by the MD docking simulations of others. These observations further suggest that the dsDNA interacts specifically with the nucleoprotein filament through its helical groove – residing transiently within the purported secondary DNA binding-site.[1,2] This work demonstrates that tailored DNA origami, in conjunction with HS-AFM, can be employed to reveal directly conformational and geometrical information of dynamic protein–DNA interactions, providing experimental validation for crystallographic and molecular dynamics studies at the single molecule level.

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P-63

DNA-origami mediated self-assembly of nanoelectronic circuits

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Driven by Moore´s Law, performance and density of integrated circuits have dramatically increased over the past fifty years. But conventional top-down lithography fabrication methods are losing their appeal due to rising costs, high energy consumption, as well as physical and technical limitations. Alternatives need to be developed, to overcome a cost explosion and to set a new standard for today's smart technology world. One promising way is the introduction of bottom-up techniques for the fabrication of nanoelectronic circuits. In particular, smart building blocks can be self-assembled by the means of DNA-Nanotechnology. A common strategy is DNA origami that serves as a molecularly defined "breadboard", to organize various nanomaterials into hybrid systems.[1] Having a self-assembled, programmable framework allows for the development of nanomaterial constructions with control over dimensions, stoichiometry, orientation, shape and composition. Based on the idea of using DNA-origami molds for the seeded growth of Gold nanoparticles (Au NPs)[2] our work introduces the self-assembly of semiconducting nanomaterials, e.g. Cadmiumsulfide nanorods (CdS NRs) with 40 nm length and 4 nm thickness (see Figure 1). We also show the possibility to assemble two different materials, namely CdS NRs and Au NPs into one DNA-origami mold structure by the means of different oligonucleotide sequences. In addition, we demonstrate the subsequent assembly of our smart building blocks for the build-up of more complex systems, followed by a seeded-growth procedure that allows for electrical contacting using electron beam lithography. We think that our strategy provides an elegant way towards the development of unprecedented nanoelectronic structures.

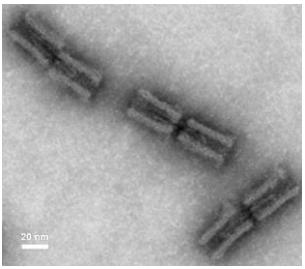

Figure 1. TEM images of self-assembled smart building blocks, containing CdS NRs attached into DNA-origami molds.

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P-64

Nano-electronic components built from DNA templates

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

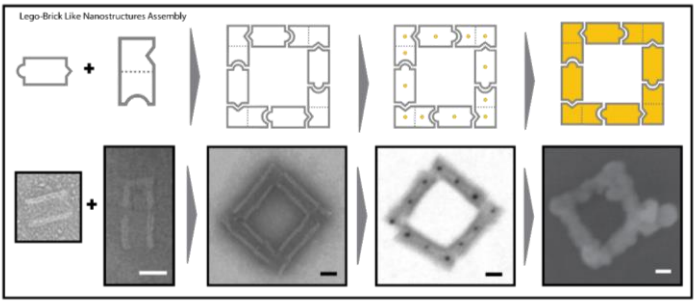

Figure 1. Sketch and TEM (SEM) images showing the Lego-Brick like nanostructure assembly of nano-circuit. By controlling the interface for the junction and mold structures, nano-circuit can form by just mixing the two monomers. With 5 nm gold seed decoration, the gold nano-circuit can be achieved with the same gold growth process. Scale bar: 20 nm.

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P-65

Surface Seeded Self-assembly of DNA Nanostructures

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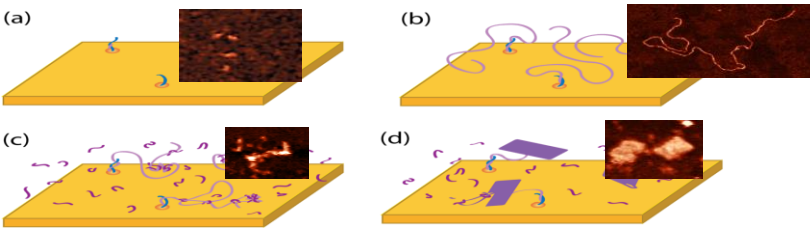
To date, the self-assembly of DNA nanostructures has mostly been carried out in the solution phase. As these structures need to be purified and deposited onto a solid support for microscopic characterization well as device applications. Supramolecular self-assembly on surfaces is appealing since it simplifies purification and circumvents deposition steps that are not only difficult to perform in a site-specific manner, but also are damaging to large, fragile structures. However, there exist numerous barriers toward such a surface-based DNA architecture, including the lack of means to encode instructions for self-assembly, non-specific adsorption of DNA structures, and difficulties in characterizing the structures.
Here we introduce surface seeded folding of DNA origami, which uses sophisticated means to manage the interactions between DNA and the surface, and elucidate the impact of these interactions on the self-assembly process. This novel approach successfully addressed the contradictory requirements of surface confinement and facile self-assembly and allowed specific chemical interactions on the surface to site specifically seed the formation of DNA origami of specific size and shape. The surface mediated self-assembly opens up new opportunities in complex DNA nanoarchitectures. In particular, it would be ideal for multistep assembly processes that are limited by the low yield, damaging purification steps for solution formed structures.



P-66

Quantifying specific and non-specific interactions between proteins and DNA via an optical label-free technique based on reflectivity

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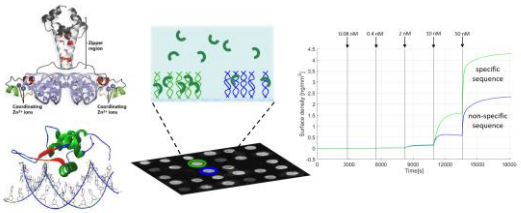

Protein-DNA interactions regulate a myriad of key biological functions and may even represent a novel tool for DNA nanotechnology [1]. However, understanding the hierarchy and the interplay between sequence-specific interactions and non-specific ones, not to say quantifying them, is typically challenging [2,3]. Here, we exploit an optical, label-free technique based on reflectivity (Reflective Phantom Interface, RPI), which has been applied to protein-protein [4] and DNA-DNA interactions [5]. We study the DNA-binding properties of two transcription factors, the gene regulator GAL4 [6] and the pre-rRNA-processing FHL1 [7]. We functionalize the surface of the biosensor with DNA double strands of various sequences, lengths and structures, and we measure binding equilibrium constants and kinetic rates for the two proteins. The difference we find between specific and non-specific binding for both proteins is to be mainly attributed to a higher detachment rate k_{off} from non-specific sequences. Moreover, by changing the ionic strength *I* of the solution, we can modulate the range of the non-specific electrostatic interaction. We find that, at low *I*, proteins bind more to DNA, but the specificity is weakened, while, at high *I*, binding is almost suppressed; perhaps unsurprisingly, the most effective compromise between affinity and specificity occurs at physiological *I*. Overall, our results are consistent with the picture that a purely electrostatic interaction between the residues and the DNA backbone precedes the formation of Hydrogen-bonds in the specific binding mode [3].

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P-67

Kinetically Programmed, One-Pot DNA Reactions for Molecular Detection Directly in Whole Blood

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Human designed chemistry typically relies on complex multiple steps reactions necessitating various cycle of purification and reaction conditions. In contrast, Nature has developed a wide variety of one-pot reactions where multiple reactions are sequentially programmed to achieve complex synthesis within a single location and condition. In this paper we report a versatile DNA-based one-pot, three reactions, that can be used to detect any specific molecules directly in complex biological samples. We show that careful programming of the kinetic of these different reactions enable to significantly increase the gain of the sensing mechanism and decrease the reaction time. Using this strategy in an electrochemical format, we demonstrate the multiplexed, quantitative, one-step detection of two models molecules in the low nanomolar range, in less than 10 minutes directly in whole blood. We discuss the potential applications of this one-pot assay in the field of point-of-care diagnostic sensors and, more generally, the importance of programming the kinetic of one-pot reactions in order to achieve high efficiency in a timely manner.

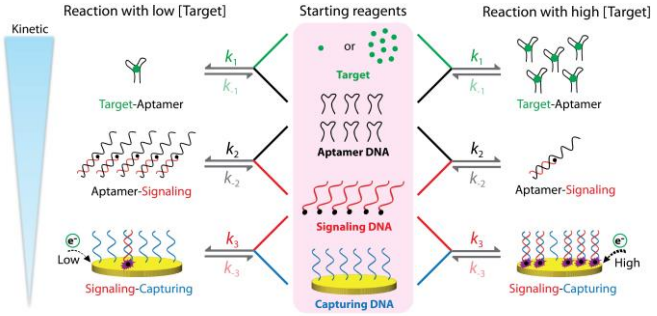

Figure 1. Schematic representation of a kinetically programmed, one-pot reactions for molecular electrochemical detection.