

FNANO 2021: 18th Annual Conference Foundations of Nanoscience

12 - 15 April 2021

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Programme (UTC Time Zone)

Monday 12 April

Session 1 - Nanophotonics

Chair: Ralf Jungmann

13:50	Introduction
14:00	Invited: Super-resolution with and without deep learning Christophe Zimmer, Institut Pasteur, France
14:25	Correlative DNA-PAINT/AFM Microscopy of DNA Nanostructures and Characterization of Addressable Sites Christopher Green, U.S. Naval Research Laboratory, USA
14:40	DNA Origami for magnetic plasmonics: design, assembly, and optical properties Ji-Hyeok Huh, Korea University, South Korea
14:55	DNA-Templated Programmable Excitonic Wire for Micron-Scale Exciton Transport Xu Zhou, Arizona State University, USA
15:20	<u>Posters 1</u>

Session 2 - Biomedical Nanotechnology

Chair: Thom LaBean

16:40	Invited: Virus trapping with DNA origami shells Jessica Kretzmann, Technical University of Munich, Germany
17:05	Real-time detection of dopamine using a microfabricated biosensor Ji-Won Seo, Stanford University, USA
17:20	DNA Nanoswitch Barcodes for Multiplexed Biomarker Profiling Arun Richard Chandrasekaran, University at Albany, USA
17:35	Invited: Applying Nanotechnology to Health in the Workplace John Sadowski, U.S. National Institute for Occupational Safety and Health, USA
18:00	<u>Posters 2</u>

Session 3 - Molecular Machinery

Chair: Andrew Turberfield

19:20	Keynote: Molecular Motors for Responsive Materials Ben Feringa, University of Groningen, Netherlands
20:00	Conformational Rearrangement of a Selected Clamping RNA Polymerase Ribozyme Enables Promoter Recognition, Self-Templated Priming and Processive Polymerization Razvan Cojocaru, Simon Fraser University, Canada
20:15	A nanoscale reciprocating rotary mechanism with allosteric mobility control Eva Bertosin, Technical University of Munich, Germany

Session 4

20:50 **ISNSCE Meeting**

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**Tuesday 13 April****Session 1 - DNA Nanostructures: Semantomorphic Fabrication****Co chairs: Ned Seeman and Hao Yan**

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|-------|--|
| 14:00 | Keynote: Building biomimetic structures with DNA nanotechnology
Chenxiang Lin, Yale University, USA |
| 14:40 | Mechanics and Design Principles of 2D Auxetic DNA Nanostructures
Ruixin Li, Purdue University, USA |
| 14:55 | Invited: Programmable icosahedral shell system based on the principle of virus capsids
Christian Sigl, Technical University of Munich, Germany |
| 15:20 | <u>Posters 3</u> |

Session 2 - DNA Nanostructures: Semantomorphic Fabrication**Co chairs: Ned Seeman and Hao Yan**

- | | |
|-------|--|
| 16:40 | Invited: Controlling the Transformations of DNA Origami by Modular Reconfigurable Units
Yonggang Ke, Emory University, USA |
| 17:05 | Hybrid protein-DNA and peptide-DNA nanostructures
Nicholas Stephanopoulos, Arizona State University, USA |

Session 2 - Principles and Theory of Self-Assembly**Co chair: Rebecca Schulman**

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|-------|--|
| 17:20 | Invited: Towards programmable assemblies through geometric frustration: Understanding the limits of self-limitation
Gregory Grason, University of Massachusetts, USA |
| 17:45 | Principles and mechanisms to control length and sequence distributions in autonomous templated copolymerisation processes
Jordan Juritz, Imperial College London, UK |
| 18:00 | <u>Posters 4</u> |

Session 3 - Nucleic Acid Nanostructures In Vivo**Chair: Yamuna Krishnan**

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|-------|--|
| 19:20 | Invited: Therapeutic modulation of tumor-associated macrophages with nanodevices
Lev Becker, The University of Chicago, USA |
| 19:45 | DNA Origami Signposts as Tags for Electron Cryotomography
Emma Silvester, University of Oxford, UK |
| 20:00 | Sequence Controlled DNA-Polymer Conjugates and Their Applications in Drug Delivery
Hassan Fakih, McGill University, Canada |
| 20:15 | The Effects of Overhang Placement and Multivalency on Cell Labeling by DNA Origami
Ying Liu, Carnegie Mellon University, USA |

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Session 4

- 20:50 **Robert Dirks Prize**
Chair: Niles Pierce
 2020 Prize – Dr. Zibo Chen, California Institute of Technology
 2021 Prize – to be announced

Wednesday 14 April

Session 1- DNA Nanosystems: Programmed Function

Chair: Fritz Simmel

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|-------|--|
| 14:00 | Invited: Reconfigurable DNA origami domino array-based (DODA) system
Jie Song, Shanghai Jiao Tong University & Institute of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, China |
| 14:25 | Exploring sequence space to design controllable G-quadruplex topology switches
Jean-Louis Mergny, École Polytechnique, France |
| 14:40 | Digital Nucleic Acid Memory: A New Approach to DNA-based Data Storage
George Dickinson, Boise State University, USA |
| 14:55 | Invited: DNA Origami Tools for Signal Amplification and Self-Repair at the Nanoscale
Viktorija Glemboczyte, Ludwig Maximilian University of Munich, Germany |
| 15:20 | <u>Posters 5</u> |

Session 2 - DNA Nanosystems: Programmed Function

Chair: Fritz Simmel

- 16:40 **DNA Self-Assembly in Constructing Hydrogel Composites with Nanocircuits**
Ming Gao, North Carolina State University, USA

Session 2 Protein and Viral Nanostructures

Chair: Nicole Steinmetz

- | | |
|-------|---|
| 16:55 | Invited: A virus nanoparticle as a resealable container
Adam Zlotnick, Indiana University, USA |
| 17:20 | Invited: Engineering the Vault Nanoparticle for Enzyme Stabilization
Leonard Rome, University of California, Los Angeles, USA |
| 17:45 | <u>Posters 6</u> |

Session 3 - Integrated Synthetic Systems

Chair: Jeremiah Gassensmith

- | | |
|-------|---|
| 19:20 | Invited: Self-assembled lipid nanoparticles for RNA delivery: SARS-CoV-2 vaccines, chemistry, and beyond
Kathryn Whitehead, Carnegie Mellon University, USA |
| 19:45 | Reprogramming DNA assembly pathways with small molecules and out-of-equilibrium systems
Felix Rizzuto, McGill University, Canada |

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- 20:00 **Stimuli Responsive DNA/Small-Molecule Hydrogels**
Christophe Lachance-Brais, McGill University, Canada
- 20:15 **Invited: Selective Organ Targeting (SORT) Nanoparticles for Tissue-specific mRNA Delivery and CRISPR-Cas Gene Editing**
Qiang Cheng, University of Texas, USA

Session 4

- 20:50 **ISNSCE Nanoscience Prize**
Chair: Hanadi Sleiman
Professor Samuel Stupp, Northwestern University

Thursday 15 April**Session 1 - Computational Tools****Chair: William Shih**

- 14:00 **Invited: Toward parameter-free, rapid prediction of DNA origami shape and mechanical properties through multiscale analysis framework**
Do-Nyun Kim, Seoul National University, Republic of Korea
- 14:25 **Toward a 3D Product Model for CAD and VR Nanoengineering**
Paul Sorensen, Paragon NanoLabs, USA
- 14:40 **A formal approach for automated generation of DNA origami designs**
Bolotito Babatunde, Carnegie Mellon University, USA
- 14:55 **Invited: Design of arbitrary freeform DNA origami structures**
Chao-Min Huang, Duke University, USA
- 15:20 **Posters 7**

Session 2 - Synthetic Biology**Chair: Alex Deiters**

- 16:40 **Invited: Stimulus-responsive self-assembly of protein-based fractals by computational design**
Sagar Khare, Rutgers University, USA
- 17:05 **Kinetic characterization and intracellular applications of heterochiral strand displacement reactions**
Nandini Kundu, Texas A&M University, USA
- 17:20 **Proton gradients from light-harvesting *E. coli* trigger DNA cortex formation for synthetic cells**
Kevin Jahnke, Max Planck Institute for Medical Research, Germany
- 17:35 **Invited: Quantitative biology with droplet microfluidics**
Adam Abate, University of California, USA
- 18:00 **Conference Close**



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Monday 12 April 2021

(Invited) Super-resolution with and without deep learning

J Bai, B Lelandais, M Lelek, A Aristov, X Hao, W Ouyang and C Zimmer

Institut Pasteur, France

Single molecule localization microscopy (SMLM) has matured into a powerful and widely used super-resolution imaging method. This talk will highlight recent and ongoing developments of our lab to address three challenges of SMLM by computation.

One challenge is 3D super-resolution imaging of entire cells. We present ZOLA-3D, a combined optical and computational method that enables versatile 3D super-resolution imaging over up to roughly 5 micrometer depth [1] and was recently adapted to dual objective microscopy.

A second challenge is to visualize cells at high resolution and with high throughput. SMLM delivers exquisite spatial resolution, but at the price of very low throughput. We present ANNA-PALM, a technique based on deep learning that after adequate training can reconstruct high resolution views from strongly under-sampled SMLM data, enabling considerable speed-ups without compromising spatial resolution [2].

A third challenge is to use and reuse SMLM data obtained by the community. We will highlight shareloc, an online platform designed to facilitate the sharing and reanalysis of SMLM data and illustrate its benefit by increased robustness of ANNA-PALM.

Finally, time permitting, we will also present Imjoy [3], a computational platform designed to facilitate the uptake of deep learning methods in the community.

- [1] A. Aristov, B. Lelandais, E. Rensen, and C. Zimmer, "ZOLA-3D allows flexible 3D localization microscopy over an adjustable axial range," *Nat. Commun.*, vol. 9, no. 1, p. 2409, Jun. 2018, doi: 10.1038/s41467-018-04709-4.
- [2] W. Ouyang, A. Aristov, M. Lelek, X. Hao, and C. Zimmer, "Deep learning massively accelerates super-resolution localization microscopy," *Nat. Biotechnol.*, vol. 36, no. 5, pp. 460–468, May 2018, doi: 10.1038/nbt.4106.
- [3] W. Ouyang, F. Mueller, M. Hjelmare, E. Lundberg, and C. Zimmer, "ImJoy: an opensource computational platform for the deep learning era," *Nat. Methods*, vol. 16, no. 12, pp. 1199–1200, Dec. 2019, doi: 10.1038/s41592-019-0627-0.

Correlative DNA-PAINT/AFM Microscopy of DNA Nanostructures and Characterization of Addressable Sites

Christopher M. Green^{1,2}, William L. Hughes³, Elton Graugnard³ and Wan Kuang³

¹U.S. Naval Research Laboratory, USA, ²National Research Council, USA; ³Boise State University, USA

To bring real-world applications of DNA nanotechnology to fruition, new and powerful microscopy techniques are needed to elucidate factors that limit the availability of addressable sites on DNA nanostructures. Correlative microscopy, the combination of two or more microscopy techniques, is an approach to improve upon individual



microscopies, though its application in DNA nanotechnology has been limited due to incompatible substrates or imaging conditions. For example, DNA-PAINT super-resolution microscopy[1] and atomic force microscopy (AFM) are powerful and complementary tools for characterizing DNA nanostructures, though a cross-compatible substrate that combines transparency, favorable DNA nanostructure adsorption, low affinity for single-stranded (ss) DNA imager strands, and near atomic-level flatness was previously lacking. We have developed an accessible strategy for high resolution, correlative DNA-PAINT/AFM imaging of DNA nanostructures, enabled by a simple and robust method to selectively bind DNA origami, and not ssDNA, to cover glass. With a simple stepwise imaging procedure (Fig. 1), we were able to achieve spatial correlation of 5 ± 3 nm between optical and topographic images, validating its use for single-strand defect metrology. With this technique, we examined addressable “docking” sites on DNA origami to distinguish between two defect scenarios – (1) structurally incorporated but inactive docking sites, and (2) unincorporated docking sites[2]. We found that over 75% of defective docking sites were incorporated but inactive, suggesting that strand incorporation played a minor role in limiting the availability of addressable sites. We further explored the effects of strand purification, UV irradiation, and photooxidation on availability, providing insight on potential sources of defects and pathways towards improving the fidelity of DNA nanostructures.

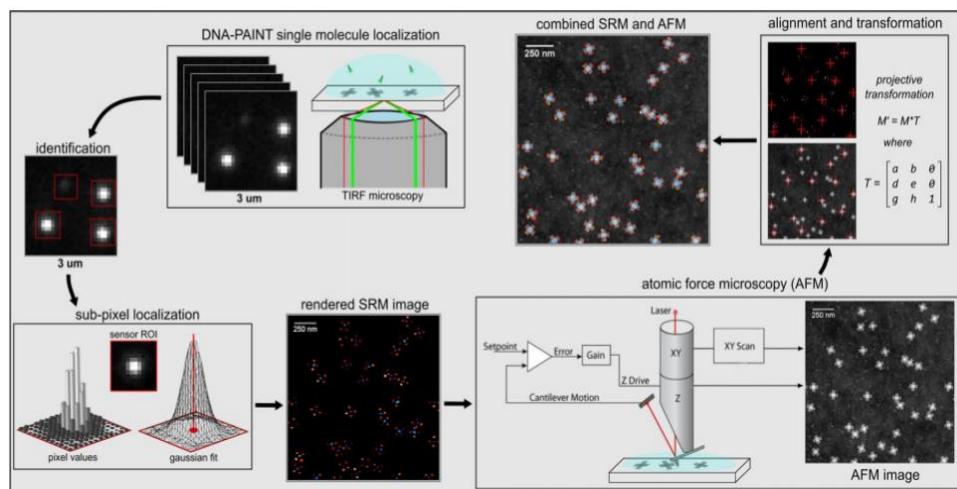


Fig. 1. Diagram depicting correlative DNA-PAINT and AFM imaging of DNA origami cross-tiles.

- [1] R. Jungmann, C. Steinhauer, M. Scheible, A. Kuzyk, P. Tinnefeld and F. C. Simmel, *Nano Lett.*, **10**, 4756 (2010).
- [2] M. T. Strauss, F. Schueder, D. Haas, P. C. Nickels and R. Jungmann, *Nat. Commun.*, **9**, 1600 (2018).

DNA Origami for magnetic plasmonics: design, assembly, and optical properties

Ji-Hyeok Huh¹, Pengfei Wang², Yonggang Ke³ and Seungwoo Lee¹,

¹Korea University, Republic of Korea, ²Shanghai Jiao Tong University School of Medicine, China, ³Georgia Institute of Technology and Emory University, USA

The creation of artificial magnetic response at optical frequency has been a pivotal goal of metamaterials society, because of its fascinating potential to enabling a variety of outstanding optical phenomena such as directional scattering, low or negative refractive index, and chiral metamaterials[1-3]. The magnetic metamaterials (or



metamolecules) have readily materialized in the relatively low frequency regimes (e.g., GHz and THz), as their required structural scales and complexity can be well addressed by conventional lithography (i.e., photolithography). However, the development of metamaterials working at the higher optical frequencies (visible to infrared) have been hindered by the lack of an appropriate fabrication methods, because it requires the manipulating the building blocks with the scale of sub-10 nm resolution. To tackle this challenge, we suggest a DNA-origami template-based strategy and provide the new designed platform for forming complex optical magnetic nanoring structures exhibiting an emergent optical magnetism.

As depicted in Fig. 1a. the basic building block, a ring cluster consisting of six AuNP seeds (~ 10 nm), is anchored onto a hexagonal DNA origami frame with nanometer precision and further grown to a bigger silver NPs. Taking advantage of the programmable intermolecular interaction, the highly intricate networks of metallic NP rings can be fabricated. Hierarchical assembly of the AuNP rings leads to the formation of higher-order networks of clusters and polymeric chains (see Fig 1b.). These sophisticated networks of NP rings can induce strong emergent plasmonic properties including anti-ferromagnetism, purely magnetic-based Fano resonances, and magnetic surface plasmon polaritons at visible regime. We envision that molecular self-assembly route may lead to the manufacturing of optical metamaterials with unprecedented nanostructural complexity and associated electromagnetic properties.

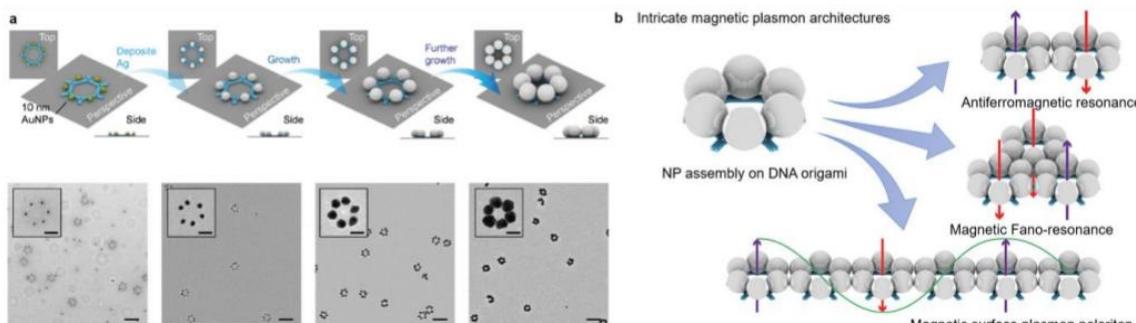


Fig. 1. Magnetic plasmon metamolecules and complex NP ring networks assembled on DNA templates.

- (a) Schematics and TEM images of magnetic rings, which were constructed via stepwise growth of AgNPs. #
(b) Programmable assembly of DNA origami leads to intricate magnetic plasmon architectures.

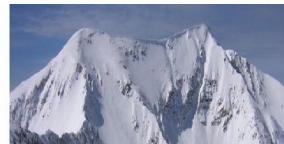
- [1] Y. H. Fu, et. al., Nat. Commun. **4**, 1527 (2013).
[2] J. Valentine, et. al., Nature **455**, 376 (2008). [3] H. Z. Zhiyuan Fan, et. al., J. Phys. Chem. C **117**, 14770 (2013)

DNA-Templated Programmable Excitonic Wire for Micron-Scale Exciton Transport

Xu Zhou, Hao Liu, Franky Djutanta Shuoxing Jiang, Xiaodong Qi, Lu Yu, Su Lin, Rizal F. Hariadi, Yan Liu, Neal Woodbury and Hao Yan

Arizona State University, USA

The efficient photon capture and excitation transport are essential for the development of photonic devices. Inspired by natural light-harvesting systems where the protein scaffolds are incorporated to organize densely packed chromophores with well-controlled position, orientation, dynamics and environment for efficient energy capture and transfer, [1] synthetic DNA scaffolds have been used as template to assemble cyanine dyes to form J-like aggregate for efficient energy transfer along defined path in nanoscale. [2][3] Here, we expand the DNA-templated excitonic system to sub-micron/micron scale with programmability and scalability. The four-helix-bundle DNA origami with



length of ~ 600 nm was employed as a template to arrange the cyanine dye K21 to form J-like aggregates with excitonic features as an efficient excitonic wire. The sub-micron exciton transfer along the excitonic wire was demonstrated using multiple spectroscopic techniques and Monte Carlo simulation. A directional energy transfer from donor to acceptor mediated by this excitonic wire over a distance of 400 nm was successfully achieved. Besides, the excitonic wire can serve as the scalable building block to form dimeric structure for micron-scale excitation energy distribution. The design of structural DNA template has also been expanded to more complex geometries, including L-shape, zigzag, ring and T-junction. Those excitonic systems exhibit robust energy transfer capabilities and have been used as modular building blocks for fabrication of higher order excitonic architectures. This study reveals the excitonic features of the sub-micron DNA-templated dye aggregates and offers a powerful toolset for rational design and feasible fabrication of excitonic circuits and photonic devices.

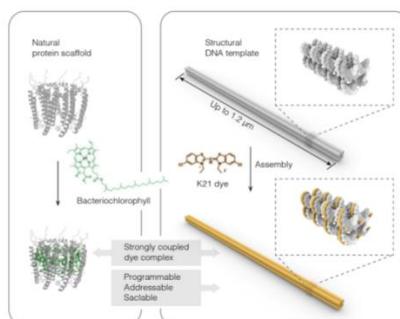


Fig. 1. Bio-inspired excitonic architectures that employ the structural DNA templates mimicking the natural protein scaffold (light harvesting complex 2 from purple bacteria) to arrange cyanine dye K21 to form strongly coupled chromophore complex for submicron or micron exciton transfer with programmability, addressability, and scalability.

- [1] T. Mirkovic, *et al.*, *Chem. Rev.* **117**, 249 (2017).
- [2] E. Boulais, *et al.*, *Nat. Mater.* **17**, 159 (2018).
- [3] X. Zhou, *et al.*, *J. Am. Chem. Soc.* **141**, 8473 (2019).

(Invited) Virus trapping with DNA origami shells

Christian Sigi¹, Elena M. Willner¹, Wouter Engelen¹, Jessica A. Kretzmann¹, Ken Sachenbacher¹, Anna Liedl¹, Fenna Kolbe^{1,2}, Florian Wilsch^{1,2}, S. Ali Aghvami³, Ulrike Protzer^{1,2}, Michael F. Hagan³, Seth Fraden³ and Hendrik Dietz¹

¹Technical University of Munich, Germany, ²Helmholtz Zentrum München and German Center for Infection Research (DZIF), Germany, ³Brandeis University, USA

DNA origami has enabled the construction of DNA nanostructures with unprecedented structural control over size, shape and surface functionality. Importantly, DNA origami objects are proving to be useful nanotools in an array of applications such as sensing, nanoplasmatics, nanophotonics and drug delivery.[1] In this work, we present DNA origami shells which can be functionalized to ‘trap’ viruses within the shell interior. We envisioned that by trapping viruses in shells, the viruses will be prevented from undergoing interactions with host cells, and therefore, infection. These shells are formed from user-defined triangular DNA origami building blocks which assemble in a one-step reaction with up to 95% yield. Using adeno-associated viruses (AAVs) as a proof-of-concept model, we demonstrate that this approach leads to effective virus neutralization in physiological conditions, without any adverse effects on cell viability.

- [1] P. Wang, *et al.*, *Chem* **2**, 359–382 (2017)



Real-time detection of dopamine using a microfabricated biosensor

Ji-Won Seo, Kaiyu Fu, and H. Tom Soh

Stanford University, USA

Continuous detection of biomarkers *in vivo* could transform molecular diagnostics, as it would provide real-time information about the physiologic state of a subject. To this end, our lab and others have demonstrated “real-time biosensors” that can continuously measure small molecule drugs in a live animal using an aptamer-based electrochemical sensor (Fig 1a) [1,2]. An important next step is to continuously measure biomarkers that are endogenously released by the body, in response to external stimuli or environment. Unfortunately, this has been exceptionally challenging because the majority of endogenous biomarkers exist at much lower concentrations in comparison to drugs (with a few exceptions such as glucose). Thus, there is an urgent need for real-time biosensors with much higher sensitivity.

To this end, we report a microfabricated, aptamer-based real-time biosensor that offers significantly higher sensitivity (Fig 1b). Our biosensor utilizes gold-nanoporous electrodes that provide significant improvement in sensitivity (up to 60-fold higher) compared to conventional planar electrodes (Fig 1c). Importantly, our biosensor is fabricated on a flexible substrate to minimize tissue damage and inflammation upon insertion into tissues.

As a model, we sought to continuously detect the neurotransmitter dopamine (DA) in artificial cerebrospinal fluid (aCSF) *in vitro*. When compared to planar electrodes, we observe a significant increase in electrochemical current upon DA recognition (Fig 1d). Importantly, our sensor is highly specific to DA, and we observe minimal signal from other chemically similar molecules including other neurotransmitters (Fig 1e). Finally, we achieved continuous detection of DA in aCSF *in vitro*, and we observe a clear response to changing DA concentration as a function of time (Fig 1f). With further improvements in sensitivity, our biosensor holds the promise of continuously detecting DA (and potentially other neuromodulators) in live subjects. This would greatly advance our understanding of neural circuitry by elucidating how neuromodulators affect behavior.

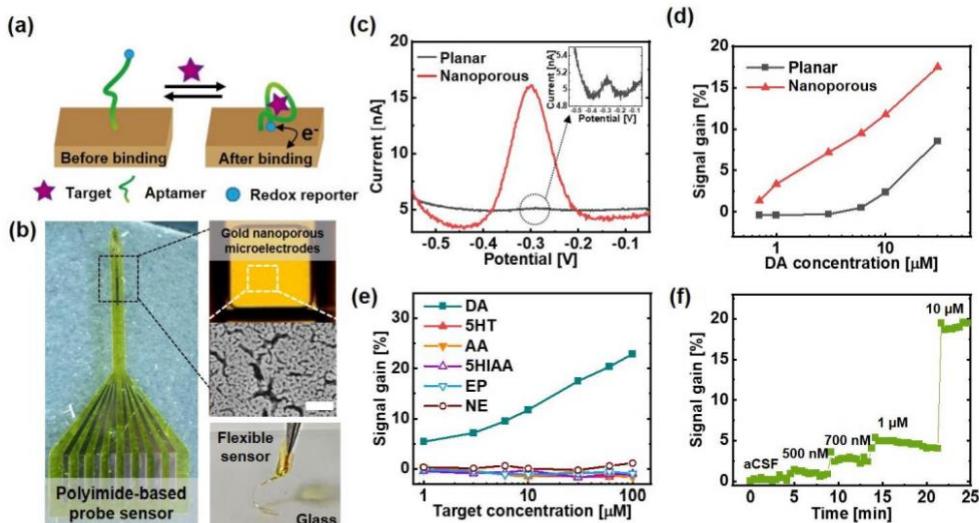




Figure 1. (a) Working mechanism of aptamer-based sensor. (b) Optical micrograph of the gold nanoporous-based biosensor. Recording site is composed of $100 \times 100 \mu\text{m}^2$ nanoporous electrode. Right upper image is optical and SEM micrograph of the nanoporous electrode. Scale bar, 300 nm. Right bottom picture shows the mechanical flexibility of the device. (c) Square-wave voltammogram of planar and nanoporous microelectrodes and (d) signal gain as changing DA concentration. (e) Signal gain as changing the target concentration. Measured targets were DA, serotonin (5HT), ascorbic acid (AA), hydroxyindoleacetic acid (5HIAA), epinephrine (EP), and norepinephrine (NE). (f) Real-time monitoring of DA at different concentrations.

- [1] Ferguson et al., Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals. *Sci. Transl. Med.* 5, 213ra165 (2013).
- [2] Arroyo-Curran et al., Real-time measurement of small molecules directly in awake, ambulatory animals. *PNAS* 114, 645-650 (2017).

DNA Nanoswitch Barcodes for Multiplexed Biomarker Profiling

Arun Richard Chandrasekaran¹, Molly MacIsaac², Javier Vilcapoma¹, Clinton H. Hansen², Darren Yang², Wesley Wong² and Ken Halvorsen¹

¹State University of New York, USA, ²Harvard University, USA

Molecular biomarkers play a key role in the clinic, aiding in diagnostics and prognostics, and in the research laboratory, contributing to our basic understanding of diseases. Detecting multiple and diverse molecular biomarkers within a single accessible assay would have great utility, providing a more comprehensive picture for clinical evaluation and research, but is a challenge with standard methods. We have developed DNA-based nanoswitches that overcome these difficulties, enabling detection and analysis of a wide range of biological molecules with a highly sensitive yet simple and low-cost method that can be used in any lab.¹ Using DNA nanoswitches, we demonstrate multiplexed detection of up to 6 biomarkers at once with each combination of biomarkers producing a unique barcode signature among 64 possibilities. As a defining feature of our method, we show “mixed multiplexing” for simultaneous barcoded detection of different types of biomolecules, for example, DNA, RNA, antibody, and protein in a single assay. To demonstrate clinical potential, we show multiplexed detection of a prostate cancer biomarker panel in serum that includes two microRNA sequences and prostate specific antigen. The barcoded assay enables all-at-once detection of biomarker panels, potentially reducing the number of steps and consequently the cost, time, effort, and opportunity for error. Such a system can gather information from multiple types of biomarkers to create a single barcode that can more accurately diagnose a disease, as compared to using only a single biomarker. Furthermore, our method provides direct detection without amplification, which makes absolute quantification more straightforward. The multiplexing also allows flexibility to include built in controls or references. Due to the programmability afforded by this DNA nanotechnology approach, our assay can act as a nearly universal biosensor, able to detect everything from microRNAs,² viral RNAs,³ enzymes⁴ and proteins.⁵

- [1] AR Chandrasekaran, M MacIsaac, J Vilcapoma, CH Hansen, D Yang, WP Wong & K Halvorsen, DNA nanoswitch barcodes for multiplexed biomarker profiling. *Nano Lett.* 21: 469 (2021).
- [2] AR Chandrasekaran, M MacIsaac, P Dey, O Levchenko, L Zhou, M Andres, BK Dey & K Halvorsen, Cellular microRNA detection with miRacles: microRNA activated conditional looping of engineered switches. *Sci. Adv.* 5: eaau9443 (2019).
- [3] L Zhou, AR Chandrasekaran, JA Punnoose, G Bonenfant, S Charles, O Levchenko, P Badu, C Cavalieri, CT Pager & K Halvorsen, Programmable low-cost DNA-based platform for viral RNA detection. *Sci. Adv.* 6: eabc6246 (2020).



- [4] AR Chandrasekaran, R Trivedi & K Halvorsen, Ribonuclease-responsive DNA nanoswitches. *Cell Reports Phys. Sci.* 1: 100117 (2020).
- [5] CH Hansen, D Yang, MA Koussa & WP Wong, Nanoswitch-linked immunosorbent assay (NLISA) for fast, sensitive, and specific protein detection. *Proc. Natl. Acad. Sci. USA* 114: 10367 (2017).

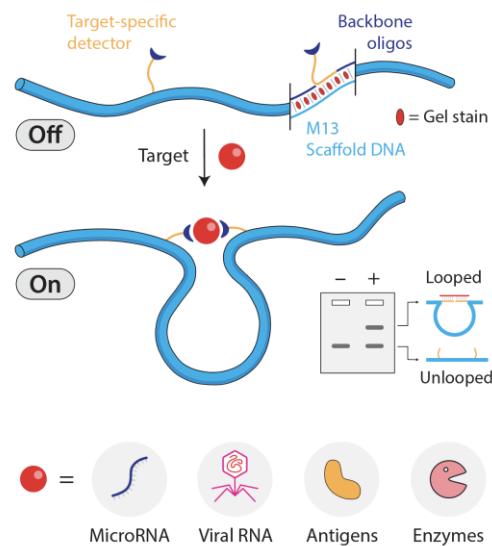


Figure caption: DNA nanoswitch is assembled from an M13 scaffold (7249 nt) and short complementary backbone oligonucleotides. Two of the backbone oligonucleotides are modified to contain recognition elements specific to the target to be detected (nucleic acids, antigens, enzymes). On target recognition and binding, the nanoswitch is reconfigured from the linear "off" state to the looped "on" state. The two states of the nanoswitch are easily identified on an agarose gel, with the presence of the on band indicating presence of the target biomarker in the sample.

(Invited) Applying Nanotechnology to Health in the Workplace

John P. Sadowski

National Institute for Occupational Safety and Health, USA

Translating laboratory innovations to actual usable applications has always been a challenge. This presentation proposes a framework for how nanotechnology researchers may focus their work on applications in occupational safety and health (OSH). It will include a discussion of technological needs in OSH, and proposes a process by which nanotechnology innovations may be matched to them and developed into applications.

OSH is health and safety in the workplace. As a field, its main activities are to identify workplace hazards, and develop and deploy hazard controls to prevent them. In order to characterize these in the laboratory and in actual workplaces, OSH heavily relies on technological tools, especially sensors of various kinds. OSH uses realtime sensors that are used both fixed to hazard controls (e.g., for ventilation systems, personal protective equipment, or alarms) or used actively by industrial hygienists as exposure assessment tools (e.g., handheld or mobile direct readout instruments used during field studies). These tools are most often used to sense airborne toxic substances,

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which may be gases or particulates [1]. In addition, OSH involves intensive laboratory work, especially in toxicology.

While nanotechnology research is very widespread and diverse, little of it is currently directed towards OSH applications. However, OSH applications may be attractive to researchers as being fast-to-market, as they lack the requirement for lengthy clinical trials needed for human medicine applications, and as a stepping stone to other uses that do require regulatory approval. Nanotechnology-enabled tools may offer improved sensitivity, cost, size, weight, speed, and biocompatibility for OSH applications, although challenges include manufacturability, durability, calibration, and toxicological properties of the nanomaterials [2].

In the case of particulates and bioaerosols, there is a need to sense not only their presence, but also their physical properties such as chemical or biological identity, size distribution, and agglomeration state, in real time. Currently, their analysis often requires samples to be sent to a laboratory, which is slow and costly. There has been increasing focus on developing and deploying wearable sensors, which require advancements in size and cost [3]. In some cases, disposable devices may be desirable. Nanosensors, including those incorporating biomolecules, have unique properties that make them candidates for fulfilling these needs.

To enable these advances, innovators need to be aware of OSH applications and have access to OSH-specific domain expertise. NIOSH, part of the U.S. Centers for Disease Control and Prevention, is the nation's research agency for the study of worker safety and health, and empowering employers and workers to create safe and healthy workplaces. Some examples of technology development supported by NIOSH include a continuous personal coal dust monitor based on a tapered element oscillating microbalance, direct-reading welding fume instruments based on laser-induced breakdown spectroscopy, and a lab-on-a-chip immunoassay for silica exposure [3]. Applications of nanotechnology for OSH is one of the 10 Critical Research Areas for NIOSH's Nanotechnology Research Center [4–5]. This presentation is intended to begin a dialogue to lead to substantive recommendations on specific technologies that may form the basis of nano-enabled OSH tools.

- [1] U.S. Occupational Safety and Health Administration. "Direct-reading instrumentation for air contaminants", OSHA Technical Manual II.3.III (2014). URL: https://www.osha.gov/dts/osta/otm/otm_ii/otm_ii_3.html#DirectReading
- [2] U.S. National Nanotechnology Initiative. "Nanotechnology-enabled sensing" (2009). URL: <https://www.nano.gov/node/100>
- [3] S. Ernest, A. Echt, E. Garza, J. Snawder, and R. Rinehart. "Wearable technologies for improved safety and health on construction sites" (2019). URL: <https://blogs.cdc.gov/niosh-scienceblog/2019/11/18/wearables-construction/>
- [4] U.S. National Institute for Occupational Safety and Health. "Nanotechnology: 10 critical topic areas" (2018). URL: <https://www.cdc.gov/niosh/topics/nanotech/critical.html> [5] U.S. National Institute for Occupational Safety and Health. "Continuing to protect the nanotechnology workforce: NIOSH nanotechnology research plan for 2018–2025" (2019). doi:10.26616/NIOSH_PUB2019116

(Keynote) Molecular Motors for Responsive Materials

Ben Feringa

University of Groningen, Netherlands

The fascinating molecular motors and machines that sustain life offer a great source of inspiration to the molecular explorer at the nanoscale. Among the major challenges ahead in the design of complex artificial molecular systems and is the control over dynamic properties and responsive far-from-equilibrium behavior. Chemical systems and

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adaptive materials ultimately require integration of structure, organization and function of multi-component dynamic molecular assemblies at different hierarchical levels. A major goal is to achieve and exploit translational and rotary motion.

In this presentation the focus is on the dynamics of functional molecular systems as well as triggering and assembly processes. We design motors in which molecular motion is coupled to specific functions. Responsive behavior will be illustrated in self-assembly and responsive materials with a focus on cooperative action, amplification along multiple length scales and 2D and 3D organized systems. The design, synthesis and functioning of rotary molecular motors and machines will also be presented with a prospect toward future dynamic molecular systems and responsive materials

Information on <http://www.benferinga.com>

- Molecular Machines: *Nature*, September 2015
- Molecular Switches: *Chemistry World*, June 2016
- *Vision statement ‘Materials in Motion’*: Adv. Mater. 2020

Conformational Rearrangement of a Selected Clamping RNA Polymerase Ribozyme Enables Promoter Recognition, Self-Templated Priming and Processive Polymerization

Razvan Cojocaru and Peter J. Unrau

Simon Fraser University, Canada

The hypothesized “RNA World” proposes that early in evolution RNA could have served both as the carrier of genetic information and as a catalyst. Later in evolution, these functions were gradually replaced by DNA and enzymatic proteins resulting in modern cellular biology. Arguably, the greatest limitation to exploring this hypothesis is the lack of a true RNA replicase: a molecular machine capable of processive and general replication of RNA. Where the most current evolved variants of the class I ligase RNA polymerase ribozymes achieve processivity by direct hybridization to RNA templates, we have recently implemented a complex selective strategy, that screened $\sim 10^{13}$ pool variants, to isolate a processive polymerase ribozyme that uses an RNA clamp to manifest many of the mechanisms of modern protein DNA-dependent polymerases.

This clamping domain takes advantage of hybridization mechanics, to first partially hybridize to a sigma factor-like specificity-primer. This creates an ‘open’ clamp complex (Fig. 1A). This ‘open’ clamp form is then able to search for and recognize a specific single-stranded RNA promoter. When found, the specificity-primer is displaced from the clamp onto the template (Fig. 1B), triggering a structural rearrangement to a ‘closed’ clamp form and simultaneously enclosing the template in a topological association. This allows the ribozyme to processively incorporate many NTPs without falling off the template (Fig. 1C).

When correctly assembled, the ‘closed’ clamp complex results in more than one order of magnitude increase in extension, synthesizing duplexes of 50-107 bp in size. The selected polymerase ribozyme has one additional functionality. It can synthesize part of its own specificity-primer using its own sequence as a template. This allows the polymerase to, in principle, evolve a promoter-based sense of ‘self’, where promoters are naturally related to the polymerases transcribing them. The demonstration that promoter recognition and processivity are mechanistically related is strikingly similar to modern DNA-dependent polymerases and shows how easily early RNA replicases could have preferentially recognized their own genomes and replicated specific gene targets in a primordial “RNA World”.

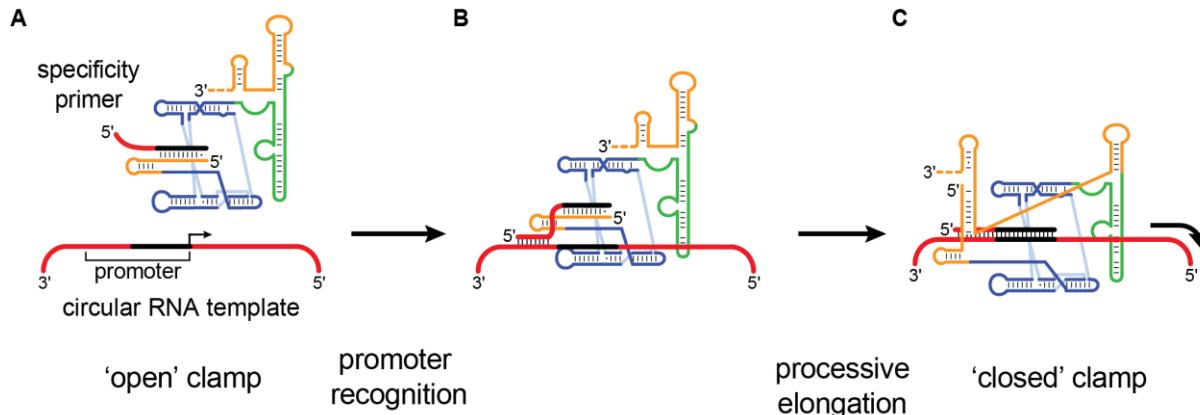


Fig. 1. Transcriptional initiation of the clamping RNA polymerase Ribozyme. (A) RNA specificity-primer activated 'open' clamp form. (B) Promoter recognition strips the sigma-like primer from the polymerase and triggers a conformational rearrangement to a 'closed' state. (C) The 'closed' topologically entrained form processively extends the template.

A nanoscale reciprocating rotary mechanism with allosteric mobility control

Eva Bertosin¹, Christopher Maffeo², Thomas Drexler¹, Aleksei Aksimentiev² and Hendrik Dietz¹

¹Technische Universität München, Germany, ²University of Illinois at Urbana-Champaign, Illinois, USA

Enzymes, molecular motors and other natural biomolecular complexes form by polymerization of different subunits. The functions of these molecular machineries are often encoded in the interaction among the individual components that form such assemblies. An example is the ATP synthase, a molecular machine present in mitochondria that produces ATP from ADP and inorganic phosphate. Here a proton flow induces the rotation of a central rotor which is coupled to conformational changes of the surrounding stator, where the ATP production is catalyzed in three distinct steps.

Inspired by the properties of such natural machines, we designed an artificial nanomachine that has the ability to adapt and change conformation induced by the coupled interactions among its subunits (Fig. 1).

Here, we show that by transferring these principles of natural assemblies to DNA origami we could produce complex structures with sub-nanometric precision. We designed, built and analyzed a tetrameric tight-fitting rotary complex. The individual components were designed such that they could adapt upon the rotary motion of a central DNA origami structure. We evaluated the individual parts and the whole tetrameric apparatus via cryo electron microscopy. The 3D maps, as well as single molecule analysis via total internal reflection fluorescence microscopy, indicate the rotation of the central rotor, which can undertake three different preferred positions inside the stator.

Moreover, we showed that changing the interaction strength of the stator components has clear influence on the rotation of the rotor, thus suggesting a coupled mechanism between the central rotor and the surrounding stator. The coupled interactions among the complex building blocks shown in this work represent a step towards the implementation of specific and biological-like functionalities into artificially designed nanostructures.

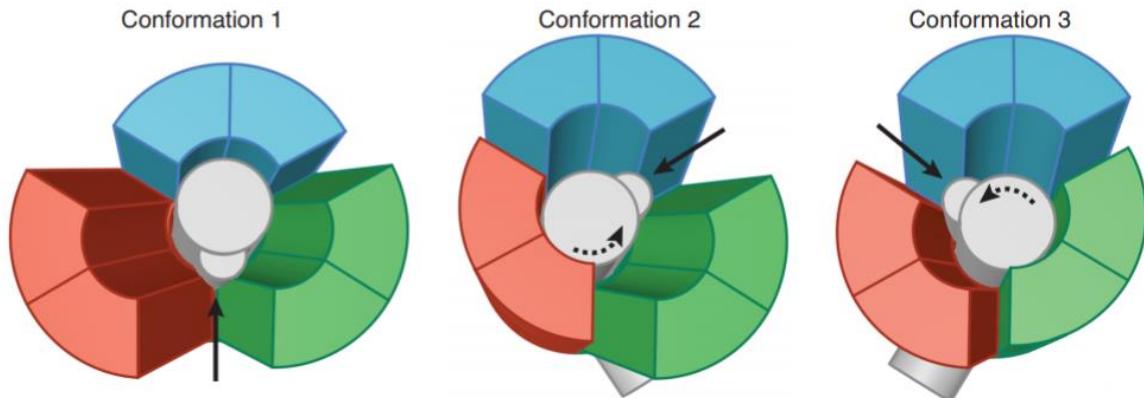


Fig. 1. Sketch of the system showing the rotation of the central rotor coupled to conformational changes of the surrounding stator.

(Keynote) Building biomimetic structures with DNA nanotechnology

Chenxiang Lin

Yale University, USA

In eukaryotic cells, a myriad of evolutionarily conserved biomolecule machineries control the formation of membrane-bound compartments and the molecular transport amongst them. However, evolution hands us these beautiful end products without a user manual, meaning such sophisticated systems can be difficult to dissect or re-engineer. Our research seeks to unlock mechanistic details of cellular organization and dynamics at molecular level by establishing cell-free platforms that robustly recapitulate the native environment of membranous compartments and protein complexes. Specifically, we utilize DNA nanotechnology, an emerging technique that programs supramolecular assembly in three dimensions, to build various biomimetic constructs with precisely controlled geometry and molecular placement. Here, I will share our progress on building a versatile nanoscale toolkit for high-precision membrane engineering [1-5] and an adaptable framework for building nuclear pore mimics [6-8]. I will also discuss how we tackle some of the long-standing questions about biomolecular interactions using such a "DNA-guided" engineering approach [4-8].

- [1] Y. Yang, et al., Nat. Chem. 8, 476–483 (2016).
- [2] Z. Zhang, et al., Nat. Chem. 9, 653–659 (2017).
- [3] M. W. Grome, et al., Angew. Chem. Int. Ed. 57, 5330–5334 (2018).
- [4] X. Bian, et al., Nat. Chem. Biol. 15, 830–837 (2019)
- [5] Y. Yang, et al., Nat. Chem. 13, DOI: 10.1038/s41557-021-00667-5 (2021).
- [6] P. D. E. Fisher, et al., ACS Nano. 12, 1508–1518 (2018).
- [7] Q. Shen, et al., bioRxiv. DOI: 10.1101/2020.08.10.245522 (2020).
- [8] Q. Shen, et al., bioRxiv. DOI: 10.1101/2021.02.07.430177 (2021).



Mechanics and Design Principles of 2D Auxetic DNA Nanostructures

Ruixin Li, Haorong Chen, and Jong Hyun Choi

Purdue University, USA

Architected mechanical metamaterials are artificial systems engineered for unconventional mechanical properties. Their mechanical behaviors are believed to emerge from their periodic cellular structures, while the constituent materials are not supposed to contribute to the properties. Their auxetic characteristics differ from those of regular materials. This uniqueness may be quantified by the Poisson's ratio which measures relative changes in two orthogonal directions of a material under elastic stretch.

Auxetic structures have negative Poisson's ratios, which is why they are often termed NPR materials. In contrast, regular materials show positive values. In other words, auxetic materials will expand horizontally, when they are stretched vertically. Similarly, they will shrink horizontally, if compressed vertically. These unique behaviors offer significantly improved indentation resistance, greater shear modulus, and enhanced fracture toughness compared with regular materials. The NPR materials may be used for impact absorption mechanisms and adaptive reconfigurable materials.

Here we demonstrated architected 2D NPR materials using DNA origami.¹ We constructed multiple wireframe configurations including re-entrant hexagon, re-entrant triangle, and rotating square as shown in Figure 1. We characterized their auxetic properties and Poisson's ratios, and investigated relevant mechanics. Given the nanoscale dimensions, we achieved their auxetic deformations via two-step DNA reactions (strand displacement and reannealing). Coarse-grained molecular dynamics (MD) simulations were also performed to study mechanics and deformation behaviors upon external mechanical loading, from which we extracted structural properties such as Young's modulus, flexure, and joint stretch. We found that (i) structural behaviors via DNA reactions and mechanical loads are similar and qualitatively consistent and that (ii) the auxetic properties are largely defined by geometrical designs, yet the DNA properties also play a role and must be taken into account. Finally, we developed an elastic model that accounts for edge rigidity and joint flexibility and provides a set of guiding principles for auxetic DNA materials.

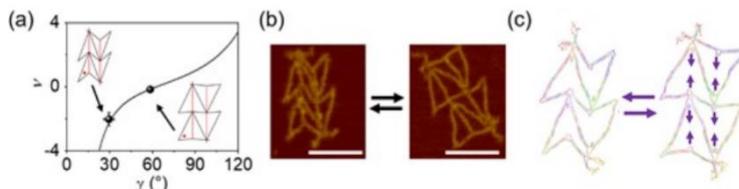


Figure 1. (a) Theoretical Poisson's ratio versus angle γ (indicated by red dot). (b) AFM images of a re-entrant triangle at two different conformations (angles). The structural transformation is completed by two-step DNA reactions. (c) Coarse-grained MD calculations of a re-entrant triangle under mechanical loads, simulating auxetic deformations in (b).

1 Li, R., Chen, H. & Choi, J. H. Auxetic Two-Dimensional Nanostructures from DNA. *Angewandte Chemie*, doi:10.1002/anie.202014729 (2021).

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(b). 1 Li, R., Chen, H. & Choi, J. H. Auxetic Two-Dimensional Nanostructures from DNA. *Angewandte Chemie*, doi:10.1002/anie.202014729 (2021).

[1] Li, R., Chen, H. & Choi, J. H. Auxetic Two-Dimensional Nanostructures from DNA. *Angewandte Chemie*, doi:10.1002/anie.202014729 (2021).

(Invited) Programmable icosahedral shell system based on the principle of virus capsids

Christian Sigl¹, Elena M. Willner¹, Wouter Engelen¹, Jessica A. Kretzmann¹, Ken Sachenbacher¹, Anna Liedl¹, Fenna Kolbe^{1,2}, Florian Wilsch^{1,2}, S. Ali Aghvami³, Ulrike Protzer^{1,2}, Michael F. Hagan³, Seth Fraden³, Hendrik Dietz¹

¹Technical University of Munich, Germany, ²Helmholtz Zentrum München and German Center for Infection Research (DZIF), Germany, ³ Brandeis University, USA

Viruses encapsulate their genome with a protein-capsid to protect it from the extracellular environment. A great number of these capsids have icosahedral symmetry and follow the principle of quasi-equivalence [1]. Here, we describe a programmable icosahedral canvas that is based on the principle of virus capsids and allows self-assembly of a family of icosahedral shells. The canvas is formed from triangular building blocks constructed from DNA. Various user-defined objects can be sculpted on the canvas, including full shells, half shells, and shells with user-defined apertures. We experimentally created shells with molecular masses ranging from 43 to 925 Megadaltons (8 to 180 subunits) and with internal cavity diameters ranging up to 280 nm. The shells form in one-step reactions with few defects and at high yields up to 95%. We used cryo-electron microscopy to validate the structure of all shells. The shells can easily be functionalized with other molecules and provide the basis for a variety of applications.

[1] D. L. Caspar, A. Klug, Physical principles in the construction of regular viruses. *Cold Spring Harbor symposia on quantitative biology* **27**, 1-24 (1962).

(Invited) Controlling the Transformations of DNA Origami by Modular Reconfigurable Units

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¹Emory University, USA, ²Wuhan University of Science and Technology, China, ³Duke University, USA, ⁴Shanghai Jiao Tong, China

The DNA origami technique is a robust method for the design of DNA nanostructures with prescribed shapes, including complex curved geometries. In addition to static structures, dynamic DNA origami has been employed for generating a rich repository of molecular nanomachines that are capable of sensing various cues and changing their conformations accordingly. The common design principle of the existing DNA origami nanomachines is that each dynamic DNA origami is programmed to transform in a specific manner, and the nanomachine needs to be redesigned to achieve a different form of transformation. Here we report a modular design method to programmatically tune the shapes of DNA origami nanomachines. The DNA origami consists of small, modular DNA units, and the length of each unit can be selectively changed by toehold-mediated strand displacement. By use of different combinations of trigger DNA strands, modular DNA units can be selectively transformed, leading to the programmable reconfiguration of the overall dimensions and curvatures of DNA origami. The modular design of programmable shape transformation of DNA origami can find potential applications in more sophisticated molecular nanorobots and smart drug delivery nanocarriers.

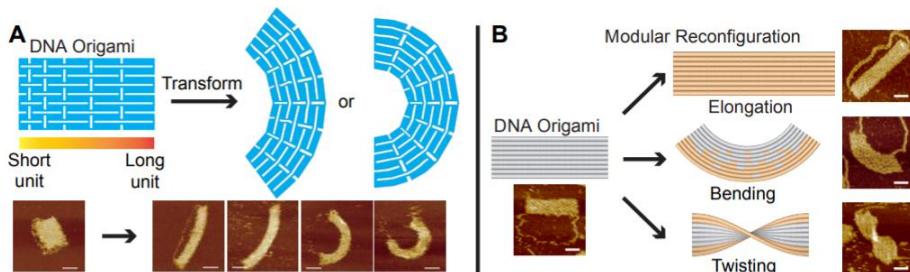


Figure. Controlling origami transformation with two modular design strategies. (A) Reconfigurable DNA nanoarrays constructed on the basis of dynamic DNA domino origami. The structures can transform between conformations with different degrees of curvatures. (B) Reconfigurable DNA origami consisting of individually operable, modular motifs. By selective expansion of DNA units in the DNA origami array, the shape of DNA origami, such as length, curvature, and twist, can be tuned programmatically. Scale bars: 50nm.

- [1] Wang, D.; Yu, L.; Ji, B.; Chang, S.; Song, J.; Ke, Y., Programming the Curvatures in Reconfigurable DNA Domino Origami by Using Asymmetric Units. *Nano Letters* 2020, 20 (11), 8236-8241.
- [2] Wang, D.; Yu, L.; Huang, C.-M.; Arya, G.; Chang, S.; Ke, Y., Programmable transformations of DNA origami made of small modular dynamic units. *Journal of the American Chemical Society* 2021, 143 (5), 2256-2263.

Hybrid protein-DNA and peptide-DNA nanostructures

Nicholas Stephanopoulos

Arizona State University, USA

Two of the most commonly used molecules for self-assembling nanotechnology are DNA and peptides/proteins. DNA nanotechnology has enabled the construction of complex structures with unparalleled addressability and anisotropy thanks to the predictable base-pairing rules and the wellunderstood physical properties of the double helix. This versatility, however, comes at the expense of chemical heterogeneity and limited functionality. By contrast, peptides and proteins have the advantage of expanded chemical diversity, diverse structural motifs, and biological relevance. However, most peptide/protein-based nanostructures are highly symmetric and not precisely addressable. The ability to merge the programmability of DNA nanotechnology with the chemical, structural, and functional diversity of proteins and peptides represents a powerful opportunity in bio-nanotechnology. Herein, we describe our work merging self-assembling protein and peptide motifs with DNA nanostructures through the use of site-specific protein/peptide-DNA conjugates (Fig. 1). We report two systems: (1) tetrahedral cages comprised of a heterotrimeric protein linked to a triangular wireframe DNA base (Fig. 1A); and (2) a supramolecular polymer of DNA origami cuboids linked by coiled-coil peptides (Fig. 1B). In both cases, the protein-DNA or peptide-DNA conjugates are synthesized through chemical conjugation with unique reactive amino acids, and the resulting hybrids are used as addressable building blocks for subsequent, hierarchical assembly with DNA structures bearing complementary handles. The tetrahedral protein-DNA cages are comprised of a highly stable homotrimeric aldolase protein modified with ssDNA handles.¹ We demonstrate the synthesis of cages comprised of both three and four helical turns of DNA (10 and 14 nm edge lengths, respectively), and demonstrate the three-dimensional structure using both AFM and indirect chemical characterization methods. We also demonstrate the use of two different site-specific

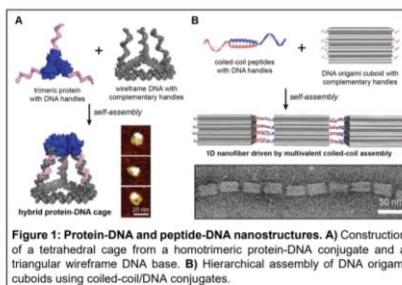
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coupling reactions, as well as the effect of modification site on cage assembly yield. The DNA components provide for unparalleled addressability of the cage, whereas the protein can provide novel structural, catalytic, or scaffolding properties (e.g. fusion with targeting protein/peptides). For the coiled-coil/DNA system, we modify each pair of a heterodimeric coiled-coil pair with unique ssDNA handles.² We demonstrate coiled-coil formation and hierarchical assembly of DNA tiles and origami cuboid structures using the conjugates. We probe several different assembly protocols, including: one-pot formation of origami fibers, hierarchical formation of dimers/trimmers/alternating copolymers, and polymerization of purified origami with a pre-formed coiled-coil/DNA building block in a second step. Importantly, the coiled-coil provides a novel self-assembly motif and molecular scaffold that is orthogonal to DNA hybridization, and will enable the incorporation of novel functionality. We also use bioactive fibronectin proteins modified with two orthogonal coils as “staples” that can assemble DNA structures without the need to chemically modify the protein with DNA.

[1] ACS Nano (2019) 13:3545 2. JACS (2020) 142:1406



(Invited) Towards programmable assemblies through geometric frustration: Understanding the limits of self-limitation

Gregory Grason

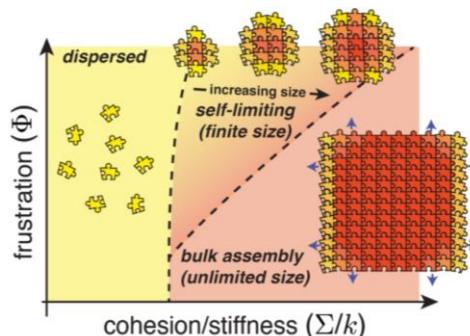
University of Massachusetts, USA

In hard materials, geometric frustration (GF) is most often associated with the disruption of long-range order in the bulk and proliferation of defects in the ground state. Soft and self-assembled materials, on the other hand, are composed of intrinsically flexible building blocks held together by deformable and non-covalent forces. As such, soft assemblies systems are able to tolerate some measure of local misfit due to frustration, allowing imperfect order to extend over at least some finite range. This talk will overview an emerging paradigm for self-organized soft materials, geometrically-frustrated assemblies (GFAs), where interactions between self-assembling elements (e.g. particles, macromolecules, proteins) favor local packing motifs that are incompatible with uniform global order in the assembly. This classification applies to a broad range of material assemblies including self-twisting protein filament bundles, amyloid fibers, chiral smectics and membranes, particle-coated droplets, curved protein shells and phase-separated lipid vesicles. In assemblies, GF leads to a host of anomalous structural and thermodynamic properties, perhaps most significant, the existence of self-limiting equilibrium states which terminate assembly at finite multi-block dimensions. The possibility to engineer self-terminating material architectures whose finite dimensions can be programmed via frustration is especially attractive for a range of functional material applications. But, at present, the connection between particle-scale properties (i.e. their shape, interactions and deformability) to emergent assembly behavior (i.e. the aggregation thermodynamics and assembly size and morphologies) is still poorly



understood. In this talk, I will highlight the some basic principles and common outcomes GFA uncovered from existing, largely continuum based, theoretical models[1]. I will describe some of the known mechanisms of frustration escape, in which assemblies overcome the thermodynamic limits of finite assembly through reaching bulk states of imperfect order. Here, I describe a basic framework for analyzing when a frustrated assembly can continuously deform (or flatten) its ill-fitting building blocks into compatible packings which avoid the unlimited accumulation of stress with assembly size[2]. I will describe some examples of particle-based GFA models for which we link the escape size (i.e. the maximum self-limiting size possible) to features of the constituent particles. Finally, I will describe opportunities and challenges to exploit the scale-dependent thermodynamics of GFA to engineer new classes of intentionally ill-fitting assemblies that target equilibrium architectures with well-defined dimensions on length scales that extend far beyond the size of the building blocks or their interactions.

- [1] G. M. Grason, “Perspective: Geometrically Frustrated Assemblies”, *Journal of Chemical Physics*, **145**, 110901 (2016) DOI: 10.1063/1.4962629
- [2] M. F. Hagan and G. M. Grason, “Equilibrium mechanisms of self limiting assembly” (2021) arXiv:2007.01927



A schematic assembly phase diagram for a generic GFA, for fixed concentration and temperature, for variable frustration strength, inter-block cohesive and stiffness. Here, GFA is illustrated by aggregation of “ill-fitting jigsaw” particles in 2D, where color, yellow-to-red, depicts, respectively, low-to-high stress.

Principles and mechanisms to control length and sequence distributions in autonomous templated copolymerisation processes

Jordan Juritz¹, Jenny M. Poulton² and Thomas E. Ouldridge¹

¹Imperial College London, UK, ²University of London, UK.

Templated polymer copying is fundamental to the synthesis of the diverse and complex nucleic copolymers and proteins in biology, and is a promising framework for the synthesis of synthetic, sequence-controlled and length-controlled copolymers [1]. In biological copying processes, such as transcription, accuracy is generated by specific base-pair interactions between the copy monomers and the template. Unlike the guiding interactions in self-assembly, these specific interactions are transient as the copy and template must eventually separate. Biological systems excel at copying templates autonomously; the process is chemically driven, without systematic time-varying conditions. However, current synthetic approaches to length and sequence control in templated copying either rely on non-autonomous chemical protocols or sacrifice tight length and sequence control for autonomy.

Theoretical investigations into autonomous discrimination mechanisms for sequence control, that explicitly account



for the separation of copy and template, confirm that accurate copying is an intrinsically non-equilibrium process [2]. DNA nanotechnology holds great potential for the implementation of such non-equilibrium-processes. Recently, a novel DNA strand displacement reaction motif [3], capable of autonomously generating non-equilibrium product distributions, was used to template the completion of dimers. Despite recent advances in our theoretical and experimental understanding of sequence control in these processes, little attention has been directed toward achieving control over the length distribution of copolymers in an autonomous context.

We ask what kinds of monomer-template and monomer-monomer interactions grant sequence and length control in autonomous copolymer synthesis. We simulate the stochastic dynamics of whole and partial copolymers and monomers on a single, finite-length template, as shown in Fig 1. We directly address the conflict between autonomy and length control in a simple model. We resolve the conflict with the introduction of novel mechanisms which autonomously synthesise copolymers with very tightly controlled lengths and sequences, that spontaneously separate from arbitrarily long templates. These mechanisms could be implemented with DNA strand displacement reactions [3] and may be beneficial for granting control over other autonomous copolymerisation protocols. By identifying the engineering principles of control in autonomous template copying systems, we present a key step toward the creation of evolvable, self-replicating synthetic copolymers.

1. Model

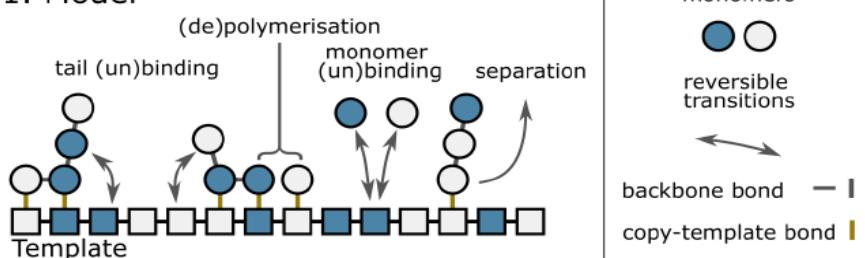


Fig 1: We simulate the vast state-space, stochastic dynamics of copolymer growth on a finite-length template. Monomers and the tails of copolymers can reversibly bind the template, and reversibly polymerise with neighbours. Copolymers are released into a dilute copolymer pool. We analyse the length and sequence distributions of the released copolymers in a series of models.

- [1] R. K. O'Reilly, *et al.*, Accounts of Chemical Research, 50(10), 2496–2509, (2017). <https://doi.org/10.1021/acs.accounts.7b00280>
- [2] J. M. Poulton, *et al.*, PNAS 116(6), 1946–1951, (2019). <https://doi.org/10.1073/pnas.1808775116>
- [3] J. Cabello-Garcia, *et al.*, ACS Nano, (2021). <https://doi.org/10.1021/acsnano.0c10068>



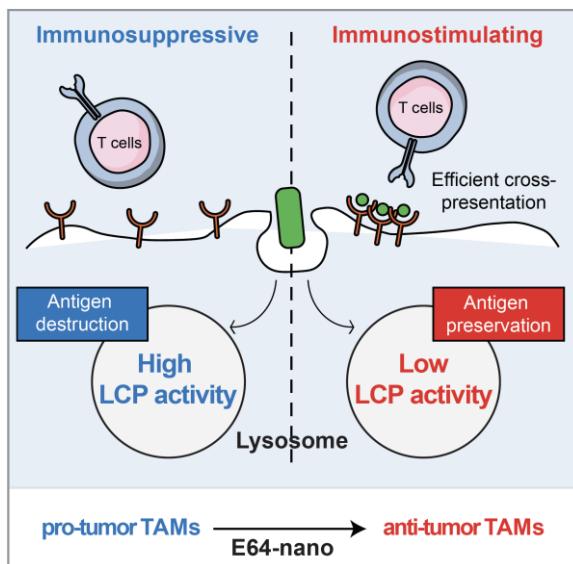
(Invited) Therapeutic modulation of tumor-associated macrophages with nanodevices

Lev Becker

The University of Chicago, USA

Activating CD8⁺ T cells by antigen cross-presentation is remarkably effective at eliminating tumors. Although this function is traditionally attributed to dendritic cells, tumor-associated macrophages (TAMs) can also cross-present antigens. TAMs are the most abundant tumor-infiltrating leukocyte. Yet, TAMs have not been leveraged to activate CD8⁺ T cells because mechanisms that modulate their ability to cross-present antigens are incompletely understood. Here we show that TAMs harbor hyperactive cysteine protease activity in their lysosomes which impedes antigen cross-presentation, thereby preventing CD8⁺ T cell activation. We developed a nanodevice (E64-nano) targeted to lysosomes of TAMs in mice. E64-nano inhibits the population of cysteine proteases present specifically inside lysosomes of TAMs, improves their ability to cross-present antigens, and attenuates tumor growth via CD8⁺ T cells. When combined with cyclophosphamide, E64-nano showed sustained tumor regression in a triple-negative-breast-cancer model. Our studies demonstrate that nanodevices can be targeted with organelle-level precision to reprogram macrophages and achieve immunomodulation *in vivo*.

Summary model



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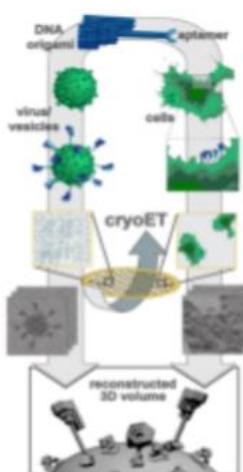
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DNA Origami Signposts as Tags for Electron Cryotomography

Emma Silvester^{1,3}, Ben Vollmer^{1,2}, Vojtěch Pražák^{1,3}, Daven Vasishtan^{1,3}, Emily A. Machala¹, Catherine Whittle¹, Susan Black¹, Jonathan Bath¹, Andrew J. Turberfield¹, Kay Grünewald^{1,2} and Lindsay A. Baker^{1,2}

¹University of Oxford, UK, ²Heinrich-Pette-Institut, Germany



Schematic of the signpost origami tagging approach. A DNA origami structure hybridized to an RNA aptamer targeting fluorescent fusion proteins is added to vesicles, viruses or cells. These tags identify specific proteins in tomograms of the sample enabling investigation of protein structure and arrangement in its native context.

Electron cryomicroscopy (cryoEM) directly visualises biological systems at nanometre resolution in a near native state. Electron cryotomography (cryoET) reveals molecular details of heterogeneous specimens including membranes, viruses and cells. With subvolume averaging, cryoET can produce sub-nanometre resolution structures of biomolecules in native environments. However tomograms of biological samples are crowded, with low contrast and signal-to-noise ratios. It is therefore difficult to identify specific proteins, unless they are identified by tagging. We demonstrate a method of tagging proteins for cryoET using DNA origami ‘signposts’ [ref]. This method does not depend on heavy metals (which produce artifacts in tomogram reconstruction) and causes minimal interference with the target system. The DNA origami nanostructure provides a high-contrast, asymmetric marker; specific tagging is achieved through incorporation of an RNA aptamer that targets GFP fusion proteins. We have characterised the structure and binding of our origami signpost tags, and successfully used them to tag GFP fusion proteins on native membrane vesicles, an enveloped virus, and the surface of intact mammalian cells. These tags provide a much-needed tool for the growing cryoET community, expanding potential applications of cryoET and enabling investigation of protein structure and arrangement in its native context. [ref] E. Silvester et al, title, Cell (2021), in press.



Sequence Controlled DNA-Polymer Conjugates and Their Applications in Drug Delivery

Hassan H. Fakih, Johans Fakhoury, Adam Katolik, Elise Malek-Adamian, Aurelie Lacroix, Danny Bousmail, Masad Damha, and Hanadi Sleiman

McGill University, Canada

Nucleic acid therapeutics, such as small interfering RNA (siRNA) and antisense oligonucleotides (AON), offer unique potential for gene therapy because of their effectiveness and directed silencing of the targeted gene of interest. However, significant challenges have impeded their translation into clinical applications, such as instability in biologically relevant media, off-target effects, and poor cellular uptake. An attractive solution is the use of drug delivery nanomaterials that protect and deliver the oligonucleotide drugs to their desired target site, such as liposomes and polymeric nanoparticles. However, the approval of these drug delivery materials has been slow due to many hurdles blocking their translation from lab prototypes to actual clinical applications. Most synthetic carriers are a mixture of polydisperse molecules that are not precisely controlled in size, shape, and composition. This leads to heterogeneity in properties, toxicity, and off target effects which are highly undesirable. DNA nanotechnology offers a very promising alternative, which utilizes nucleic acids as a material to build nanostructures that act as targeted drug carriers. This is due to the ease of manipulating DNA's structural parameters (size, shape, rigidity, functionalization) as well as chemical composition. These structures are monodisperse, bio-degradable, non-toxic, and can themselves be therapeutic. An especially powerful DNA functionalization is the attachment of hydrophobic polymers, which has led to the emergence of a new class of amphiphilic DNA block copolymers. We have developed a highly efficient solid-phase method to generate monodisperse and sequence-defined DNA-polymer conjugates.¹ Based on this method, we have designed a range of molecules and vehicles with various functionalities and applications in drug delivery, with fine control over their properties.^{2,3,4,5} In this work, we will be highlighting the importance of this sequence-controlled DNA-polymer conjugate method, which has allowed us to fabricate multiple systems for therapeutic applications. Specifically, we will be focusing on two systems developed from this method and their drug delivery applications: spherical nucleic acids (SNA) and albumin-binding Dendritic-DNA (DDNA) molecules. Studies such as characterization, stability, cell work, including gene silencing and conditional drug release, as well as in vivo investigations will be discussed.

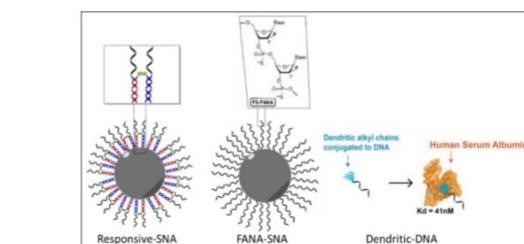


Figure 1 Representative scheme of the 3 particles developed and used for drug delivery (left to right): Responsive Spherical Nucleic Acid, FANA-modified Spherical Nucleic Acid, and Dendritic DNA-albumin complex.

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The Effects of Overhang Placement and Multivalency on Cell Labeling by DNA Origami

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Carnegie Mellon University, USA

DNA-based nanosensors and nanocarriers can be delivered to the surface of cells via attachment to hydrophobic cholesterol anchors. Universal approaches have been demonstrated for utilizing tethers to facilitate the binding of DNA origami to a wide range of cells. [1] In this work, we utilized the high level of nanoscale geometric control provided by structural DNA nanotechnology to systematically investigate the role of local shape and ligand placement on this targeted binding process. Inspired by previous receptor binding studies demonstrating that copy number, position, and spacing of ligands are critical to effective binding, [2-4] we investigated membrane labeling efficiency of three distinct DNA origami nanostructures (nanotile, nanorod and nanosphere) as a function of the placement and multivalency of binding overhangs.

Using endothelial cells as a model of adherent cell type, we found that the DNA origami labeling efficiency increases with the increasing number of binding overhangs, consistent with a model wherein the binding probability for each overhang is independent. Further investigations revealed that the placement of overhangs within DNA origami is also critical. For 2D nanotiles, overhangs on edges provided higher labeling efficiency than overhangs on the face. For nanorods, overhangs near the “pointy” end provided higher efficiency labeling than interior-located overhangs. Similarly, for nanotiles, we demonstrated that the cell labeling efficiency increases with proximity to the edge of the nanotile. The binding efficiency can therefore be modulated in both nanotiles and nanorods by moving the binding overhangs towards the edges and vertices, respectively. Finally, we demonstrated that the labeling efficiency of tether-attached nanotiles is also dependent on the proximity of bridge tethers to the edges or vertices of the nanostructures. The findings from this study facilitate a comprehensive understanding of factors that affect DNA origami membrane attachment via cholesterol anchors, thus providing guidelines for the rational design of future functional DNA nanostructures.

(Invited) Reconfigurable DNA origami domino array-based (DODA) system

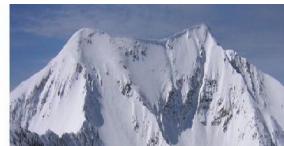
Jie Song

Shanghai Jiao Tong University & Institute of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, China

Information relay is the general phenomenon to assist and maintain diverse activities of the whole the matter in nature. One key challenge is how to imitate the information relay process at molecular level. Recent researches has revealed the information relay pattern from static pattern such as self-assembly, seeding growth, to dynamic pattern including reconfigurable structure transformation. As for dynamic pattern, Our Lab first proposed the design of Reconfigurable DNA molecular arrays (DODA) to realize the information relay behaviour on DNA origami structure and manipulated the transmission path through the structure design¹. The DODA system by assembling the interconnected modular antijunction units provide dynamic platforms to construct dynamic molecular systems to fully imitate complex dynamic behaviors in controllable systems. The antijunction unit can transform from one stable conformation to another conformation with the addition of a trigger strand. The dynamic behavior of a local antijunction unit could transfer to neighbor's conformation. Interestingly, this effect would be propagated and eventually lead to global structural change (Fig 1A).

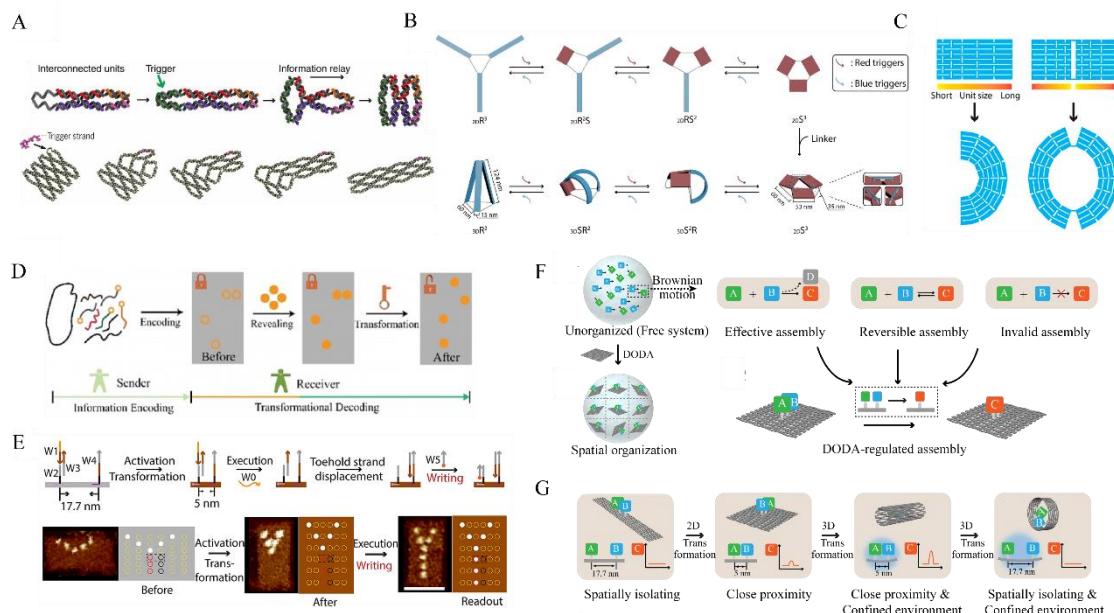
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Our Lab then extend the structure transformation to spatial transformation through the addition of a specific strand to limit the spatial domain, realizing the transformation from 2D to 3D² (Fig 1B). Furthermore, by tuning the sizes of DNA units within the DODA, the DODA can transform between a noncurved conformation and curved conformation³ (Fig 1C). Based on the conformation transformation of DODA, we develop a molecular information coding system for information security⁴ (Fig 1D). On this basis, we further develop a reconfigurable DODA-based dynamic pattern operation system to perform proximity induced molecular control for complex pattern operations⁵ (Fig. 1E).

Molecular self-assembly in biological system is regulated by spatially confined molecular mechanisms. One key challenge is to reconstitute and reprogram to fully imitate their complex dynamic behaviors to achieve similar complexity in the laboratory. We use the reconfigurable DODA system to control and regulate self-assembly reactions. The DODA-based system performs controllable and regulable self-assembly process for the assembling reactants compared to the free system (Fig. 1F). The platform of reconfigurable DODA endows a spontaneous cascade of stacking conformational transformation from “2D narrow (2D-N)” to “2D wide (2D-W)”, then to “3D narrow (3D-N)”, final to “3D wide (3D-W)” conformations by distinct “trigger” DNA strands. The conformational transformation not only regulates the assembling reactants into close proximity to undergo self-assembly reactions, but also spatially isolates the reactants to disassemble (Fig. 1G).



Summary Fig 1. Reconfigurable DNA origami domino array-based (DODA) system. (A) Transformation of the reconfigurable DNA origami domino array¹. (B) Orientation transformation can be realized from the 2DR3 to 3DR3 conformation by different triggers and linkers. The blue R conformation can transform to the red S conformation by red triggers, while the red S conformation can transform to the blue R conformation by blue triggers. The linker strand locks the adjoining two modules at another side to add spatial limitation and helps the structure transform from a two-dimensional structure to a three-dimensional structure with the addition of triggers². (C) Architecture design of curvature control with DNA domino origami³. (D) Information Coding in the DODA⁴. (E) Proximity-Induced Pattern Operations (Writing operation) in reconfigurable DODA⁵. (F-G). Controlling the self-assembly of biocatalytic architectures with reconfigurable DODA. Unpublished data.

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Exploring sequence space to design controllable G-quadruplex topology switches

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As non-classical nucleic acid structures, G-quadruplexes (G4s) not only play an important role in gene regulation and stability maintenance, but are also widely used in nanotechnology. Structural diversity is one of the main factors explaining the popularity of G-quadruplexes, but a comprehensive and integrated study of different factors determining G4 structural versatility is currently lacking. Herein, starting from a common G4 sequence as the parent chain, and then taking advantage of G4 versatility, we design a variety of strategies to control G4 structure, based on the regulation of loop length and flanking sequences, cation (type and concentration), and molecular crowding. These strategies allow to convert the G4 topology from parallel to hybrid, antiparallel, and then back to parallel. Such structural diversity reveals the coding regulation ability of G4 structures, with potential applications in nanotechnology.

Digital Nucleic Acid Memory: A New Approach to DNA-based Data Storage

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¹Boise State University, USA, ²U.S. Naval Research Laboratory, USA, ³North Carolina A&T State University, USA

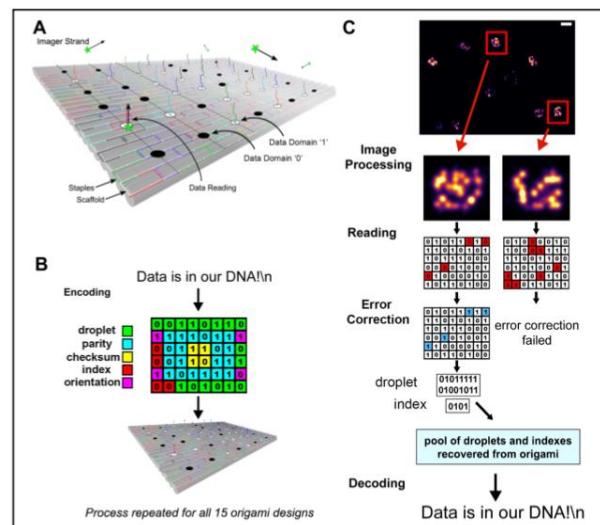
Digital Nucleic Acid Memory (dNAM) is an alternative approach to DNA-based data storage for applications requiring a limited amount of data to have high information density and redundancy. In dNAM, binary data is encoded by selecting single-stranded DNA with (1) or without (0) docking sites from a library of premade oligonucleotides. When combined with scaffold DNA, these staple strands form DNA-origami optical breadboards, presenting data that can be ‘read’ by monitoring binding of fluorescent imager probes using DNA-PAINT super-resolution microscopy. To ensure data integrity, a multi-layer error-correction scheme was created combining fountain and bi-level parity codes. As a demonstration, 15 origami were encoded with the message ‘Data is in our DNA!\n’: each origami encoding a unique data droplet, an index, error-correction and orientation information. Our error-correction algorithms ensured successful message recovery even when signals from individual docking sites, or entire origami, were lost. Unlike other approaches to DNA-based data storage, reading dNAM does not require DNA-sequencing. As such, it offers a new path for exploring the advantages of DNA as an emerging memory material.

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Fig. 1. (A) Illustration of a binary dNAM origami, a DNA nanostructure with specific sequences used to localize data strands to programmable sites within the DNA origami. (B) To enable reading of our test message, dNAM origami were synthesized based on designs generated during encoding: data droplet (green), parity check (blue), checksum (yellow), index (red), and orientation markers (magenta). (C) To ‘read’ the message a mixture of DNAorigami was imaged using DNA-PAINT (top panel). The decoding algorithm performed error correction where possible, and successfully retrieved the entire message when sufficient data droplets and indexes were recovered. Scale bar, 100 nm.



(Invited) DNA Origami Tools for Signal Amplification and Self-Repair at the Nanoscale

Viktorija Glembickyt, Michael Scheckenbach, Kateryna Trofymchuk, Lennart Grabenhorst, Florian Steiner, Martina Pfeiffer, Cindy Close, Florian Selbach, Renukka Yaadav, Tom Schubert, Carsten Forthmann, Jonas Zähringer and Philip Tinnefeld

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DNA nanotechnology and, in particular, advances in the DNA origami technique have enabled facile design and synthesis of complex and functional nanostructures. 1 For example, the unprecedented addressability of DNA origami can be used to arrange plasmonic nanostructures and emissive molecules to create antennas for light on the nanoscale capable of enhancing fluorescence signals up to several hundred fold. In this contribution, I will share our latest progress on the development of dimer DNA nanoantennas with plasmonic hotspots cleared for the placement of biomolecular assays.2 The high signal amplification provided by the dimer 100 nm silver nanoantennas (Fig. 1a) enabled us to detect single DNA molecules specific to gene carrying the antibiotic resistance on a portable, battery-driven and hand-held smartphone device. While a number of exciting applications emerge that utilize DNA nanostructures as nanomachines, biosensors, drug carrier vehicles or even nanorobots, one of the challenges in using them in complex chemical and biological environments remains their limited stability and fast degradation. In this talk, I will also share our efforts to utilize the self-assembly nature of the DNA origami approach to establish self-regeneration and self-repair strategies for DNA origami nanostructures.3 We use simple approaches that rely on the exchange of damaged building blocks with the intact parts in solution to prepare self-regenerating fluorescence labels and selfhealing nanorulers (Fig. 1b).

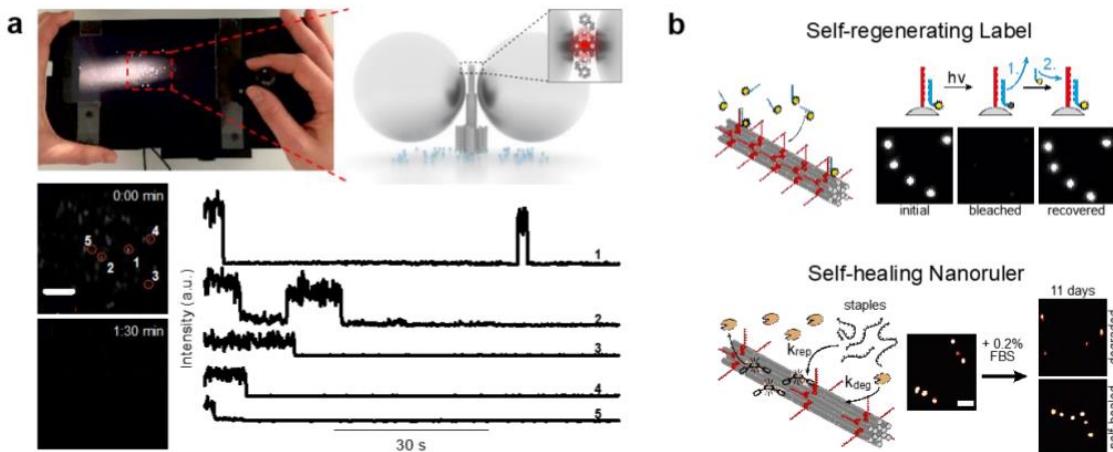


Fig. 1. (a) DNA origami nanoantennas² with cleared plasmonic hotspots used for detection of single molecules (top) and corresponding single-molecule transients acquired on the smartphone camera (bottom); (b) Self-repair strategies³ in DNA origami nanostructures used for the development of selfregenerating fluorescence brightness labels (top) and self-healing superresolution DNA nanorulers (bottom).

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DNA Self-Assembly in Constructing Hydrogel Composites with Nanocircuits

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Self-assembling materials have many impactful capabilities in design and fabrication of specific architectures and assembly processes. Thus, DNA self-assembly has been extensively used as an effective fabrication method to build complex materials. One such type of material, DNA hydrogels, are known for their tunable properties and diverse potential application areas. Other nanomaterials can also be integrated into such systems to make DNA-based hydrogel composites.

In our study, we have designed, built, and characterized hydrogels with linear DNA spacers, DNA tile crosslinkers, as well as crosslinkers that are DNA/nanomaterial conjugates with carbon nanotubes and gold nanoparticles. We have demonstrated the ability of fundamental DNA self-assembly to construct three-dimensional percolating networks using conducting nanoparticles and nanowires that are incorporated into the hydrogel composites. These composites showed interesting nonlinear electrical properties and adjustable mechanical strength. We are able to tune the viscoelastic properties of the hydrogels by applying different types of crosslinkers and spacers of different length. We have especially examined solid-like hydrogel composites with the use of interlocking DNA tiles and DNA wrapped carbon nanotube crosslinkers. Our research has explored potential applications of DNA technology in nanoelectronics and artificial computer hardware, which may achieve computing capabilities beyond architectures constructed with traditional lithography techniques in certain operations.

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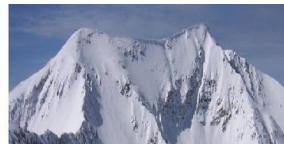


(Invited) A virus nanoparticle as a resealable container

Zhongchao Zhao,^{1,2} Caleb Starr,¹ Tariq Hussain,¹ Che-Yen Joseph Wang,³ Adam Zlotnick¹

¹Indiana University, USA, ²University of California San Diego, USA, ³Penn State University College of Medicine, USA

In life, a virus is a responsive material that binds various receptors, adsorbs to surfaces, trafficks in a cell, and packages and releases a cargo. Self-assembled virus-like particles (VLPs) usually have a limited subset of those activities. Our first goal was to create a modified subunit that would (i) form an isolatable complex, (ii) where the complex could nucleate capsid assembly, (iii) create an asymmetric capsid, and (iv) could be removed to release contents or to allow cargo to be packed into a pre-formed structure. To enable these goals, we have used a library of modified Hepatitis B Virus core proteins. The 149-residue assembly domain of wildtype core protein (CP149) is a homodimer that assembles into 120-dimer T=4 icosahedral capsid; *in vitro* assembly is driven by high ionic strength. To build a unique nucleating complex we designed a heterodimer where one half-dimer had a C-terminal His-tag so it could assemble in response to Ni⁺⁺ and the other half-dimer had a Y132A mutation that inhibits assembly (Cp149_{His}Cp149_{Y132A}). Heterodimer was produced with a bicistronic plasmid expression plasmid. When treated with low concentrations of Ni⁺⁺, Cp149_{His}Cp149_{Y132A} assembled into discrete hexamers, visualized by electron microscopy. Hexamers effectively nucleated capsid assembly. To build a capsid where the nucleus could be extracted, we used a Cp149 variant with a C-terminal cysteine (Cp150) that enables formation of C150-C150 disulfides to stabilize the capsid. From these capsids, the nucleating Cp149_{His}Cp149_{Y132A} could be extracted using EDTA to chelate Ni⁺⁺ and urea, resulting in holey capsids, visualized by cryo-EM image reconstruction. These holey capsids could be surface refilled with fresh Cp, demonstrating our ability open and close this container. By utilizing hetero- and homo-dimeric forms of Cp, we also have the opportunity to differentially address Cp to different receptors and package different cargos; it has occurred to us that heterodimers will also allow display of ligands on the capsid exterior without the crowding that would be caused by display on a homodimer. Thus, the library of HBV core protein variants supports hierarchical assembly of complex, asymmetric structures.



(Invited) Engineering the Vault Nanoparticle for Enzyme Stabilization

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¹UCLA David Geffen School of Medicine, USA, ²University of California, USA, ³University of California, USA, ⁴Vault Nano Inc, USA

Vaults are the largest ribonucleoprotein particle found in most eukaryotic cells, measuring 41 x 41 x 72.5 nm. Natural vaults are composed of 3 proteins and 1 or more untranslated RNAs. The Major Vault Protein (MVP) is the only structural component of the particle and when MVP mRNA is translated in cells lacking vaults, empty vault

particles are assembled on polyribosomes by an orchestrated process that faithfully produces empty vault shells from 78 copies of MVP. Recombinant vaults have an internal cavity ($40 \times 10^3 \text{ nm}^3$) that is large enough to contain hundreds of proteins (Figure). A 147 amino acid vault packaging domain has been identified that binds with high affinity to an MVP binding site on the inside of the assembled vault particle. This interaction domain, referred to as the INT-domain, acts similar to a “zip code” directing the fusion proteins containing this domain into the vault. We have used this strategy to package vaults with a wide variety of payloads including peptides, enzymes, chemokines and antigens. One application for the particle has been to stabilize enzymes for use in environmental remediation. We initiated these studies with manganese peroxidase (MnP) derived from the white-rot fungus *Phanerochaete chrysosporium*. The enzyme was fused to the INT domain and packaged into recombinant vaults. The catalytic efficiency of natural manganese peroxidase

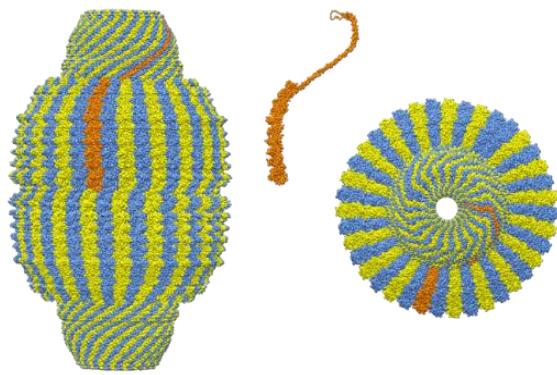


Figure: The vault particle X-ray structure, side image, single MVP and an end view. One MVP protein is colored red and the other chains blue and yellow. From *ACS Nano* 7: 889–902 (2013).

(nMnP), INT fused recombinant manganese peroxidase (MnP-INT), and vault encapsulated manganese peroxidase (vMnP) was examined for the degradation of phenol. MnP fused to INT and vaults packaged with the MnP-INT fusion protein maintained peroxidase activity. Furthermore, MnP-INT packaged in vaults displayed stability significantly higher than that of free MnP-INT, with slightly increased Km value. Additionally, vault-packaged MnP-INT exhibited 3 times higher phenol biodegradation in 24 h than did unpackaged MnP- INT. These results indicate that the packaging of MnP enzymes in vault nanoparticles extends their stability without compromising catalytic activity (*ACS Nano* 9: 10931–10940 (2015)). vMnP rapidly catalyzed the biotransformation of endocrine-disrupting compounds, including bisphenol A (BPA), bisphenol F (BPF), and bisphenol AP (BPAP). The vault-encapsulated MnP (vMnP) treatment removed 80–95% of each of the tested bisphenols (BPs) at lower enzyme dosage than free MnP which only resulted in a 19–36% removal, over a 24-h period. The toxicity of vMnP- treated samples, as measured in the model organism, *Caenorhabditis elegans*, was dramatically reduced for all three BPs, including the reproductive indicators of BPA exposure such as reduced fertility and increased germ cell death (*ACS Sustainable Chem. Eng.* 7: 5808–5817 (2019)). Strategies to further stabilize the vault-enzyme complex are under study. Our results indicate that the vault particle shows promise as an efficient approach for enzyme stabilization and should be applicable for treating other targeted organic compounds in contaminated waters.

**(Invited) Self-assembled lipid nanoparticles for RNA delivery: SARS-CoV-2 vaccines, chemistry, and beyond**Kathryn Whitehead

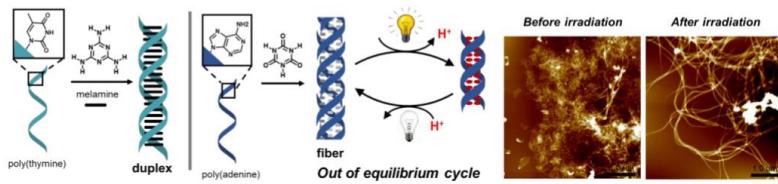
Carnegie Mellon University, USA

Messenger RNA (mRNA) therapeutics have been thrust into the limelight, thanks to the early, positive clinical trial news on a SARS-CoV2 vaccine from Pfizer/BioNTech and Moderna. These vaccines were made possible by a herculean effort to overcome the most significant barriers that have hindered translational efforts. Arguably, the largest challenge has been that RNA molecules do not readily enter their cellular targets within the body. This is because they are large (10^4 – 10^6 g/mol) and negatively charged; they do not have favorable biodistribution properties nor an ability to cross the cellular membrane of target cells. In response to these issues, industrial and academic laboratories, including my own, have created lipid nanoparticles that spontaneously package RNA and deliver the RNA to key cellular targets *in vivo*.

Here, I will describe biodegradable, ionizable lipid-like materials called ‘lipidoids’ that my lab has used to create RNA-loaded lipid nanoparticles that induce protein expression in mice. Lipidoids efficiently manipulate gene expression in a variety of biological systems, including the liver, the lungs, and immune cells. This talk will focus, specifically, on the development of a potent mRNA lipid nanoparticle delivery system and the cell-free prediction of lipidoid efficacy in mice. I will also briefly describe how our work relates to the lipid nanoparticles used in the BioNTech/Pfizer and Moderna SARS-CoV-2 vaccines.

Reprogramming DNA assembly pathways with small molecules and out-of-equilibrium systemsFelix J. Rizzuto¹, Casey M. Platnick¹, Xin Luo¹, Yao Shen¹, Michael D. Dore¹, Chengde Mao², Hanbin Mao³, Alba Guarné¹, Gonzalo Cosa¹ and Hanadi F. Sleiman¹¹McGill University, Canada, ²Purdue University, USA, ³Kent State University, USA

The programmability of DNA and its compatibility with oscillatory biological systems makes it an ideal material for generating out-of-equilibrium systems. However, the interactions and functionality of DNA are limited to the binary recognition of four nucleic acids. Expanding upon this DNA alphabet (to include metal ions, small molecules and artificial nucleobases) diversifies the types of chemistries that can be performed with DNA, and its ability to form new structural motifs for nanotechnology applications. Here, we will present two methods to reprogram the self-assembly pathways of single-stranded DNA: poly(adenine) recognises cyanuric acid to generate a hexamer that polymerises into micron-length fibres, while poly(thymine) recognises melamine to generate an antiparallel, right-handed DNA homoduplex. As new tools for DNA nanotechnology, our constructs are orthogonal to native DNA base-pairing, can be built into two- and three-dimensional structures, and can undergo strand displacement without the need for overhangs. When coupled with a system of proton release and dissipation, we can directly observe a switch in the kinetic trajectory of self-assembly at the single-molecule level. This dissipative pathway heals gaps within our nanostructures, converting highly branched, interwoven networks into nanorope superstructures. Distinct from thermal annealing, our new method provides an alternate chemical route for ‘error-checking’ that improves the morphologies and properties of DNA nanomaterials using out-of-equilibrium systems.



Small molecules can reprogram the morphologies of DNA nanostructures.

Stimuli Responsive DNA/Small-Molecule Hydrogels

Christophe Lachance-Brais, Mostafa Rammal, Christopher Hennecker, Xin Luo, Monica Taing, Violeta Toader, Anthony Mittermaier, Matthew Harrington and Hanadi F. Sleiman

McGill University, Canada

The programmability and the stimuli-responsiveness of nucleic acids make them excellent candidates for smart, biologically compatible hydrogels. DNA hydrogels have been made using Franklin-Watson-Crick base pairing, the i-motif and the G-quadruplex^[1]. Here we report the first use of a small molecule to guide the formation of a DNA hydrogel. Our group has recently discovered that cyanuric acid, a molecule with three thymine-like faces, can coax polyadenine to assemble into long supramolecular fibers.^[2] We develop the polyadenine/cyanuric acid motif into hybrid DNA hydrogels, thus joining the programmability of DNA with the functionality of small-molecule chemistry. The meeting of these two chemical spaces is especially useful, because it introduces functionality without affecting the scalability of DNA. By exploiting the DNA portion of the copolymer, we have designed complementary overhangs which crosslink the multimicron fibers into strong hydrogels. To our surprise, rheological measurements show that the storage modulus can reach upwards of 30 kPa at less than 1.5w%, an unusually high value for a DNA hydrogel. Additionally, we demonstrate that these gels possess stimuli responsiveness to melamine, polythymine as well as pH. We also investigate the combination of DNA structures such as the i-motif with these polymer hydrogels. Orthogonally to the control over the DNA portion, the copolymer can be modulated through the cyanuric acid (CA) moiety, which has a non-binding backside amenable to functionalization. We show through circular dichroism and AFM that it is possible to incorporate modified cyanuric acid into this assembly, opening the door to supramolecular functionalization of the hydrogels. We further characterize the thermodynamics of the copolymer's assembly as a function of the side chain for a library of cyanuric acid derivatives. We finally examine the effect of some of the cyanuric acid sidechains on the rheological properties of the hydrogels.

This hybrid stimuli responsive copolymer extends the chemical space of DNA hydrogels by adding a new supramolecular building block. It holds promise as a bridge between functional small-molecules and DNA hydrogels and already presents remarkable storage modulus.

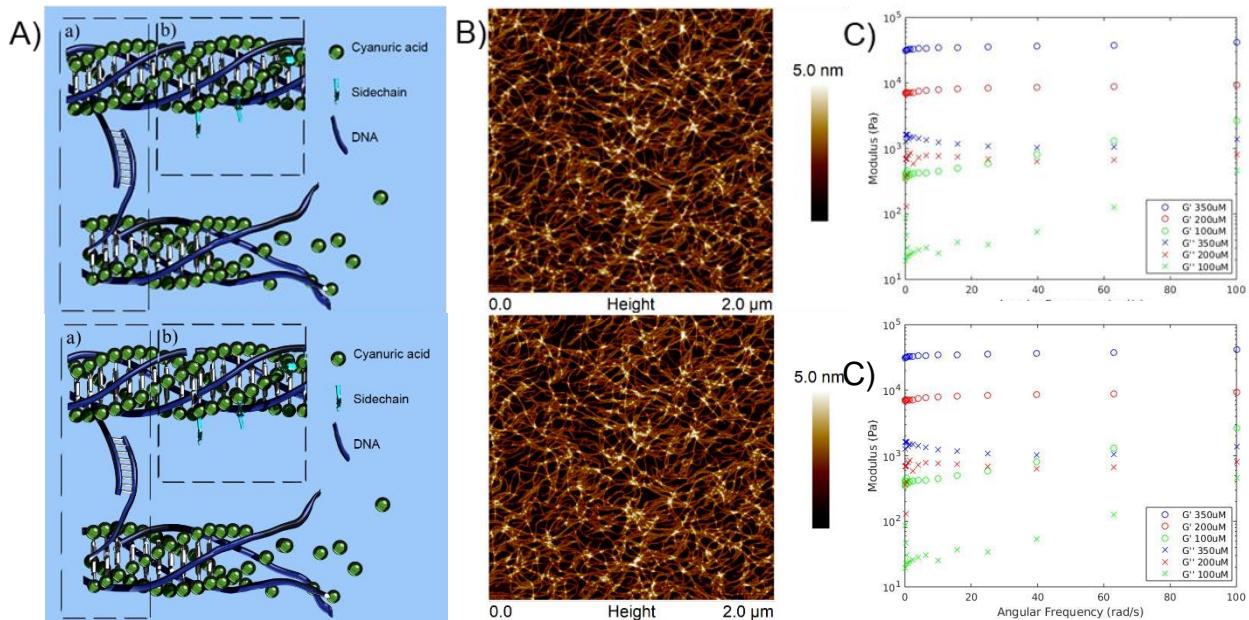


Figure 1: A) Representation of the polyadenine/CA assembly with a) dsDNA crosslinks and b) CA sidechains. B) AFM image of the entangled network a the polyadenine/CA ethylamine derivative. C) Rheological measurments of storage and loss moduli on polyadenine/CA hydrogels at various angular frequencies and DNA concentrations.

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(Invited) Selective ORgan Targeting (SORT) Nanoparticles for Tissue-specific mRNA Delivery and CRISPR-Cas Gene Editing

Qiang Cheng, Tuo Wei, Lukas Farbiak, Lindsay T. Johnson, Sean A. Dilliard and Daniel J. Siegwart

The University of Texas Southwestern Medical Center, USA

Lipid Nanoparticles (LNPs) represent one of the best strategies for mRNA effective delivery, especially have attracted great attention in recent months due to their use (EUA) in COVID-19 vaccines^[1]. However, mRNA-based protein replacement therapy and CRISPR/Cas9 gene editing in deep tissues which is required by intravenous (IV) administration, are still facing big challenges. We previously reported that liver-targeted mRNA LNPs could be rapidly developed by reengineering established liver-targeted siRNA LNPs, providing a general clue to achieve mRNA delivery into liver^[2]. Hereafter, we focused on targeted LNPs delivery outside of liver and recently reported a rational methodology, named Selective ORgan Targeting (SORT), to reach the goal^[3]. SORT allowed to predictably and systematically reengineer established LNPs, such as dendrimer-based LNPs (DLNPs), FDA-approved DLin-MC3-DMA LNPs (Onpattro SNALPs), and C12-200 lipidoid LNPs (LLNPs), to exclusively edit cells in the lungs, spleen, or liver via addition of a supplemental SORT lipid component. In particular, permanently cationic SORT lipids redirected existing liver-targeted LNPs to the lungs, anionic SORT lipids mediated selective delivery to the spleen, and ionizable cationic SORT lipids further enhanced liver efficacy. Lung-, spleen-, and liver-targeted SORT LNPs selectively edited high percentages of therapeutically relevant cells including epithelial cells, endothelial cells, B

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cells, T cells, and hepatocytes by delivering mRNA or Cas9 mRNA/sgRNA complexes. Following a single dose of 0.3 mg/kg Cre-recombinase mRNA in *tdTomato* mice, liver SORT LNPs edited ~95% of all liver hepatocytes; spleen SORT LNPs edited ~13% of all B cells and ~10% of all T cells; and lung SORT LNPs edited ~40% of all epithelial cells and ~65% of endothelial cells. Moreover, endogenous PTEN was edited effectually in specific tissues, and liver SORT LNPs enabled ~100% PCSK9 knockout in liver tissue and serum by Cas9 mRNA/sgRNAs co-delivery. Because the described SORT methodology allows generalizable (re)formulation and improvement of both new and existing LNPs with broad chemical diversity, it is envisioned that application of this technology will dramatically accelerate LNP development for protein replacement and gene correction therapeutics.

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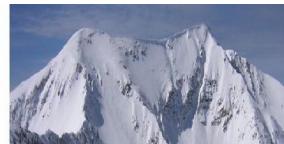
(Invited) Toward parameter-free, rapid prediction of DNA origami shape and mechanical properties through multiscale analysis framework

Jae Young Lee, Jae Gyung Lee, and Do-Nyun Kim

Seoul National University, Republic of Korea

In this presentation, we introduce a multiscale modeling approach to predicting the shape and mechanical properties of DNA origami nanostructures¹⁻⁷ from the constituent base sequences and their connectivity information. Unknown geometric parameters and stiffness values of various structural motifs including normal/nicked dinucleotide steps and crosslinking Holliday junctions are characterized from the trajectories of molecular dynamics simulation at the molecular resolution. They are then fed into corotational beam elements to construct the finite element model of a DNA nanostructure and predict quickly its overall shape and structural properties. In addition, the entropic effect of single-stranded DNAs and the electrostatic repulsive forces between helices are incorporated efficiently into the model. The capability and efficiency of the proposed method are demonstrated by solving various DNA nanostructure designs reported previously. Due to the multiscale nature of the proposed method, the predicted shape and properties can be obtained at the molecular resolution in minutes. As it estimates the model parameters from finer-scale simulations, we ultimately aim to circumvent any arbitrary parameterization in the model specific to certain designs. Its implementation as a standalone program SNUPI (Structured NUCleic acids Programming Interface) will be briefly introduced as well. This research was supported by the Basic Science Research Program, the National Convergence Research of Scientific Challenges, and the Creative Materials Discovery Program through the National Research Foundation of Korea (NRF) funded by Ministry of Science and ICT (NRF-2019R1A2C4069541, NRF-2020M3F7A1094299, and NRF2017M34D1A1039422).

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Toward a 3D Product Model for CAD and VR Nanoengineering

Paul F Sorensen, Curt R Larock, James P O'Connor, William L Patterson, III, Masudur Rahman, David L Danley, Michael L Norton and Steven L Armentrout

Paragon NanoLabs, Inc, USA

Just as computer-aided design (CAD) systems for engineering evolved from 2D drawings to boundary representation (B-Rep) parametric solid models composed of free-form curves and surfaces, DNA nanostructure design software has steadily evolved to support creation of constructs of increasing complexity. This progression has been characterized generationally¹, with first-generation (G1) packages supporting the design of origami structures, albeit with manual scaffold routing and crossover creation (e.g., caDNAno2), and second-generation (G2) tools that automate some of these steps (e.g., vHelix3). ATHENA4 and Adentia5 have recently emerged as third-generation design applications, offering most G1 and G2 capabilities plus user friendly interfaces and greater design versatility.

Our inSéquoTM Design Studio has followed this same progression, starting as a gridcentric tool for representing DNA nanostructures and evolving into a full featured design application that supports freeform 3D drawing and editing of functionalized nanostructures. Our ongoing development of inSéquo aims to advance the field through support of a robust 3D CAD product model with non-manifold B-Rep solid modeling, feature-based modeling, generative design, and laboratory automation. Toward this goal, we have developed a cross-platform product model and high performance graphics engine in modern C++ to support smooth user interactions with very large structures in both 3D desktop and virtual reality (VR) environments (Figure 1).



Fig. 1. A user manipulating a nanostructure design in inSéquoVR with graphical overlay to illustrate the experience.

The product model maintains topological integrity across multiscale representations of nucleic and amino acid strands and motifs and allows placement of biomolecules from the Protein Data Bank. Our experience creating complex designs motivated the development of a Python API for the product model, which has become a strong productivity enhancement, enabling designers to create parametric motifs algorithmically. inSéquo also supports



strand sequence optimization, along with coarse grain (e.g., OxDNA6) and all-atom molecular dynamics simulations via GPU accelerated containerized services that can be hosted locally or in the cloud. By preparing, running, monitoring, retrieving, and visualizing analyses using an integrated graphical interface, changes to the design may be selectively applied from analysis results. We endeavor to make these design and analysis tools more approachable to a broader audience of emerging nanostructure designers.

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A formal approach for automated generation of DNA origami designs

Bolutito Babatunde, D. Sebastian Arias, Jonathan Cagan and Rebecca Taylor

Carnegie Mellon University, USA

Over recent years, structural DNA nanotechnology has witnessed the unveiling of transformative tools that have advanced the design of DNA origami nanostructures. Currently, most structures are manually designed from the bottom-up which starts at the base scale to design large DNA assemblies. However, the complexity of the manual process often limits designs to simple structures and the slow design process limits the number of feasible designs for comparison. Such limitations spurred the creation of automated tools that utilize an approach that is fundamentally top-down by starting with an outline of the desired configuration and walking backwards to define the DNA base sequence to form the large DNA assembly. However, these automated tools also limit the design space by requiring the designer to fully conceptualize the design before implementation. A formal approach to generate designs from scratch would greatly advance the design of DNA origami structures. We introduce such a formal approach that utilizes generative optimization strategies to design DNA origami nanostructures through automation. This is achieved through shape annealing [1], which couples simulated annealing [2], a stochastic optimization method, with shape grammars [3], which use a formal rule-based method to define the generation of geometric shapes. Shape annealing optimally directs the generation of shapes by controlling the selection and implementation of transformative shape rules. This approach addresses the limitations previously stated by computing optimally directed scaffold routing patterns only with a set constraints and desired properties. During the annealing schedule, scaffold segments are selected in order to optimally grow the scaffold length for a desired function. As an initial demonstration of the method's capability to generate optimally driven scaffold routing patterns, we use three distinct polyhedral meshes as a constraint for a filling and coating application. By utilizing constraints to control the generation of the scaffold, this approach is fundamentally a hybrid top-down and bottom up design process. The results in Figure 1 illustrate the potential of shape annealing to code desired properties into DNA origami designs.

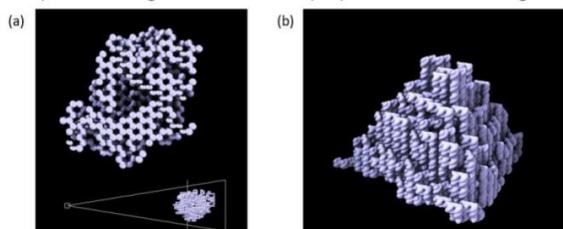


Fig. 1. Shapes generated with shape annealing algorithm for the filling and coating applications: (a) Cavity containing shape generated with tetrahedron mesh as the inner bounds of the design space with a total length of 12,901 bp; (b) Shape generated with tetrahedron mesh as the outer bounds of the design space with a total length of 7,728 bp.

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(Invited) Design of arbitrary freeform DNA origami structures

Chao-Min Huang^{1,2}, Wolfgang Pfeifer², Carlos Castro², Gaurav Arya¹

¹Duke University, USA, ²The Ohio State University, USA

DNA origami leverages Watson-Crick base pairing between single-stranded DNA scaffold and staple strands to construct nanostructures with precise geometries. Recently, the Su and Castro groups developed a new DNA origami design software MagicDNA [1] that integrates a GUI-based design tool and a built-in scaffold routing algorithm with an iterative design optimization framework through feedback from coarse-grained simulations [2] to achieve robust design of complex multicomponent DNA devices. However, designing complex freeform geometries in MagicDNA remains a tedious task due to the difficulty in specifying edge gradients and bundle orientations of the connected components. Here, we expand the design capability of MagicDNA for curved structures by creating a new graphical interface for sketching multiple splines of 2D or 3D free-form geometry and automatically converting these mathematical splines into nanoscale objects made out of DNA bundles with custom crosssection. We implement two different strategies for achieving this: 1) approximating the splines with straight segments and edge gradients; 2) calculating the cylinder length of each segment by extruding the cross-section along the path (Fig. 1). Followed by an assembly step and an automatic routing algorithm, we are able to design curved structures of arbitrary shapes while also allowing user input to tune the final design. Furthermore, we used coarse-grained oxDNA simulations to validate a wide range of 3D curved DNA origami structures, and we experimentally validated a subset of the designed structures using transmission electron microscopy and atomic force microscopy.

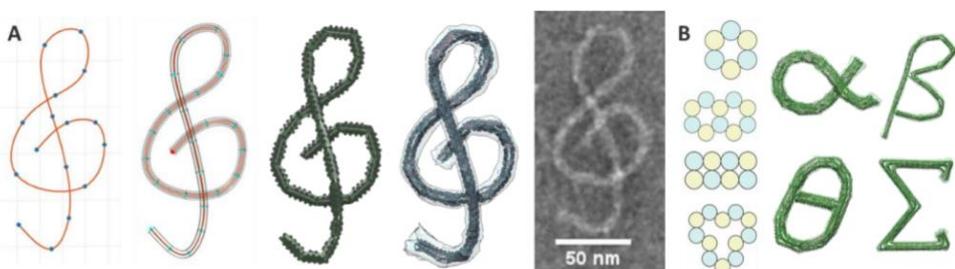




Fig. 1. (A) Starting with a built-in free-form spline sketch tool, the overall geometry is obtained by extruding cross-sections along the sketched path and discretizing it into DNA bundles, followed by coarse-grained simulations and experimental validation like TEM imaging. (B) The combination of various possible cross-sections and splines provides intuitive user inputs to design nanostructures made of multiple interconnected components.

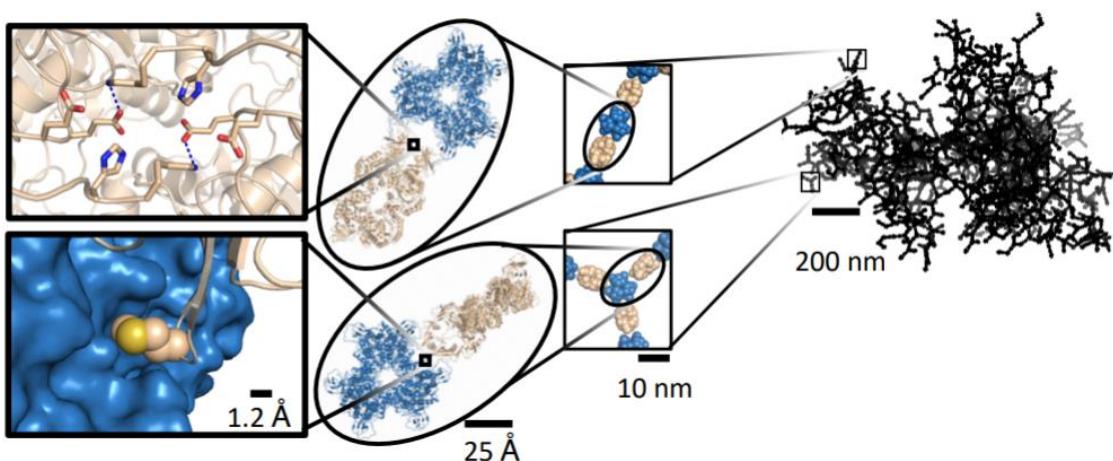
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(Invited) Stimulus-responsive self-assembly of protein-based fractals by computational design

Nancy E. Hernández, William A. Hansen, Denzel Zhu, Maria E. Shea, Marium Khalid, Viacheslav Manichev, Matthew Putnins, Muyuan Chen, Anthony G. Dodge, Lu Yang, Ileana Marrero-Berrios, Melissa Banal, Phillip Rechani, Torgny Gustafsson, Leonard C. Feldman, Sang-Hyuk Lee, Lawrence P. Wackett, Wei Dai and Sagar D. Khare

Rutgers University, USA

Fractal topologies, which are statistically self-similar over multiple length scales, are pervasive in nature. The recurrence of patterns in fractal-shaped branched objects, such as trees, lungs and sponges, results in a high surface area to volume ratio, which provides key functional advantages including molecular trapping and exchange. Mimicking these topologies in designed protein-based assemblies could provide access to functional biomaterials. Here we describe a computational design approach for the reversible self-assembly of proteins into tunable supramolecular fractal-like topologies in response to phosphorylation. Guided by atomic-resolution models, we develop fusions of Src homology 2 (SH2) domain or a phosphorylatable SH2-binding peptide, respectively, to two symmetric, homo-oligomeric proteins. Mixing the two designed components resulted in a variety of dendritic, hyperbranched and sponge-like topologies that are phosphorylation-dependent and self-similar over three decades ($\sim 10 \text{ nm}-10 \mu\text{m}$) of length scale, in agreement with models from multiscale computational simulations. Designed assemblies perform efficient phosphorylation-dependent capture and release of cargo proteins.





Kinetic characterization and intracellular applications of heterochiral strand displacement reactions

Nandini Kundu¹, Brian E. Young² and Jonathan T. Szczepanski¹.

¹Texas A&M University, USA, ²Stanford University, USA

Development of molecular devices capable of detecting and manipulating endogenous nucleic acids remain a widely pursued research area due to promising application in medicine and synthetic biology. In particular, DNA strand displacement reactions can be rationally programmed to interact with native biology using the rules of canonical Watson-Crick base-pairing and have shown significant potential in this regard. This technology, even with the use of chemical modifications, is however, often undermined by poor biostability, immunogenicity and off-target effects, limiting their utility in biological environments (1). An ideal alternative is the use of L-DNA/RNA, the enantiomeric form of native nucleic acids, which are exceptionally resistant to nuclease degradation and are non-immunogenic (2). However, while L-DNA/RNA still follow canonical WC hybridization rules with each other, they are incapable of forming contiguous base-pairs with native nucleic acids, a caveat that has until recently precluded their use in application at the interface with biology.

To overcome this limitation, we developed “heterochiral” DNA strand displacement reactions that exploit an achiral peptide nucleic acid (PNA) to exchange sequence information between chiral D-DNA and L-DNA (3), providing a route to interface biostable L-DNA-based nanodevices with endogenous nucleic acids. Indeed, we have already used this approach to interface microRNAs with an L-RNA-based fluorescence biosensor in living mammalian cells (4). In order to facilitate the rational design and optimization of nanodevices operating on the principles of heterochiral displacement, we now present a detailed kinetic characterization of this reaction. We show that the rate of heterochiral strand displacement can be predictably tuned across several orders of magnitude based on several common design parameters, including toehold length, mismatches, and nucleic acid type (DNA or RNA). We also provide insight into the biophysical mechanisms of heterochiral strand displacement, and reveal a unique stereochemical kinetic control that will add versatility to future designs. In addition to characterization studies, we show for the first time that heterochiral strand displacement circuits can be used to regulate gene expression in living mammalian cells. Overall, this work greatly improves the understanding of heterochiral strand displacement reactions, which we anticipate will broaden the scope and applicability of L-DNA/RNA nanodevices.

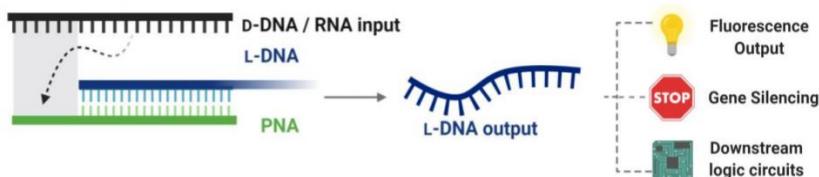


Figure 1: A heterochiral strand displacement reaction occurring from a PNA-L-DNA heteroduplex, initiated by endogenous nucleic acids. The L-DNA output can generate either a fluorescent readout or can be fed into similar downstream gates.

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Proton gradients from light-harvesting E. coli trigger DNA cortex formation for synthetic cells

Kevin Jahnke^{1,2}, Noah Ritzmann³, Julius Fichtler^{1,2}, Tobias Abele^{1,2}, Rasmus Schröder⁴, Joachim P. Spatz¹
Daniel Müller³ and Kerstin Göpfrich^{1,2}

¹Max Planck Institute for Medical Research, Germany, ²Department of Physics and Astronomy, University of Heidelberg, Germany, ³Eidgenössische Technische Hochschule (ETH) Zürich, Switzerland, ⁴Centre for Advanced Materials, Heidelberg, Germany

Bottom-up and top-down approaches to synthetic biology each employ distinct methodologies with the common aim to harness new types of living systems. Both approaches, however, face their own challenges towards biotechnological and biomedical applications. Here, we realize a strategic merger to convert light into proton gradients for the actuation of synthetic cellular systems[1]. We genetically engineer *E. coli* to overexpress the light driven inward-directed proton pump xenorhodopsin and encapsulate them as organelle mimics in artificial cell-sized compartments. Exposing the compartments to light dark cycles, we can reversibly switch the pH by almost one pH unit and employ these pH gradients to trigger the attachment of DNA structures to the compartment periphery. For this purpose, a DNA triplex motif[2] serves as a nanomechanical switch responding to the pH-trigger of the *E. coli*. By attaching a polymerized DNA origami plate to the DNA triplex motif, we obtain a cytoskeleton mimic that considerably deforms lipid vesicles in a pH-responsive manner. We foresee that the combination of bottom-up and top down approaches is an efficient way to engineer synthetic cells and functional DNA-based cytoskeletons.

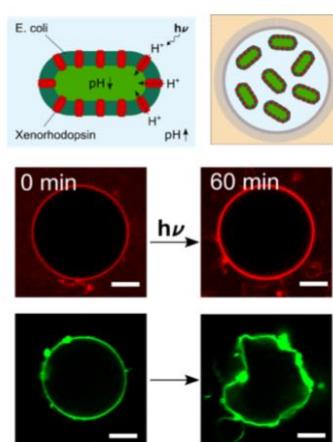


Fig. 1. Top - Genetically engineered xenorhodopsin-expressing *E. coli* generate a pH gradient upon illumination with white light. Middle - They can stimulate the attachment of pH-sensitive DNA to the periphery within or around synthetic cells upon light illumination. Bottom - The use of pH-sensitive DNA can be expanded with a DNA origami that deforms giant unilamellar vesicles as synthetic cell models upon a pH stimulus.

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- [1] Jahnke et al., preprint, ``Actuation of synthetic cells with proton gradients generated by light-harvesting E. coli'', DOI: 10.21203/rs.3.rs-82114/v1
- [2] Jahnke et al., preprint, ``Choice of fluorophore affects dynamic DNA nanostructures'', DOI: 10.1101/2020.12.12.422444

(Invited) Quantitative biology with droplet microfluidics

Adam Abate

University of California, USA

Many questions at the forefront of biology depend on the individual properties and interactions of millions of single cells. My lab develops methods for analyzing, sorting, and engineering single cells using droplet-based microfluidics. I will describe methods in which we are using this to detect rare cells in a population and evolve new cells and enzymes with enhanced properties.



Poster Programme

Poster Session 1 - Monday 12 April, 15:20 - 16:40 UTC

Track on Nanophotonics and Superresolution

- P1.01 Peptide Nucleic Acid Conjugation of Quantum Dots to DNA Nanostructures
Christopher Green

- P1.02 Can a DNA Nanostructure Constrain the Position and Orientation of an Attached Dye Molecule?
Divita Mathur

- P1.03 DNA Origami Superstructures for Photonic/Phononic Crystals
Sung Hun Park

- P1.04 Introducing graphene-on-glass coverslips for single-molecule biosensing
Renukka Yaadav

- P1.05 Poster Withdrawn

- P1.06 Bottom-up construction of complex standalone metal nanostructures in solution using DNA as a chaperone
Xin Luo

- P1.07 Substituent Effects on the Solubility and Electronic Properties of Dyes: Density Functional and Time-Dependent Density Functional Theory Calculations
Austin Biaggne

- P1.08 Rotaxane rings promote oblique packing and extended lifetimes in DNA-templated molecular dye aggregates
Matthew Barclay

- P1.09 Excited-State Dynamics in DNA-Templated Molecular Dye Aggregates
Jonathan Huff

Track on Biomedical Nanotechnology

- P1.10 Bioinspired Peptide-Nucleotide Nanofibers
Simone Hendrikse

Track on Molecular Machinery

- P1.11 Single-molecule mechanical detection of an artificial track-walking molecular motor beyond bridge-burning design
Zhisong Wang

**Poster Session 2, Monday 12 April, 18:00 - 19:20 UTC****Track on Biomedical Nanotechnology**

- P2.01 **DNA origami biosensor for the detection of cancer biomarkers**

Ivana Domljanovic, University of Fribourg, Switzerland

- P2.02 **Programmable low-cost DNA-based platform for viral RNA detection**

Lifeng Zhou

- P2.03 **Programmable surface grafting of DNA origami via TdT polymerization**

Yonggang Ke

- P2.04 **Using 3D-cell Culture to Determine Efficacy of DNA-Based Drug Delivery Vehicles**

Jathavan Asohan

- P2.05 **The combination of flattening, length-dependent rigidity and mechanochemistry accounts for microtubule breaking**

Juan Rodriguez

- P2.06 **Amine Structure Modulates the Immune Response of Lipid Nanoparticles**

Namit Chaudhary

Track on Molecular Machinery

- P2.07 **Modelling of Migration Dynamics with DNA Walkers on 2D Surfaces**

Yancheng Du

- P2.08 **Robotic end-to-end fusion of microtubules powered by kinesin**

Gadiel Saper

- P2.09 **High Performance Computer Controlled Fast and Processive Bipedal Motor that Travels Long Distances**

Samrat Basak

- P2.10 **Enzyme-free catalytic production of DNA complexes through far-from-equilibrium templating**

Javier Cabello Garcia

- P2.11 **Solving EXACT COVER problem using Network-based BIOCOMPUTATION**

Pradheebha Surendiran

**Poster Session 3, Tuesday 13 April, 15:20 - 16:40 UTC****Track on DNA Nanostructures: Semantomorphic Science**

- P3.01 DNA-nanostructure-directed precise silica mineralization in solution
Minh-Kha Nguyen

- P3.02 Origami dimerization mediated by the fluorous effect
Jiajia Zou

- P3.03 Modulation of DNA Crystalline Triangle Lattices by the Extension and Insertion of Double Helical Segments
Karol Woloszyn

- P3.04 Annealing of DNA Origami-Templated Gold and Tellurium Nanorods Coated with a High Heat Resistance Polymer to Fabricate Metal-Semiconductor Junctions
Basu Aryal

- P3.05 A Hoberman Flight Ring Made of DNA Origami
Ruixin Li

- P3.06 Computational Studies of DNA Origami Tile Cyclization
Hyeongwoon Lee

- P3.07 3D Arrangement of DNA Tensegrity Triangles Within a Hexagonal Lattice Using Mismatched Sticky Ends
Brandon Lu

- P3.08 Self-assembly of a DNA Ring Motif with Programmable Flexibility and Connectivity
Shiyun Liu

- P3.09 Automated solid phase synthesis of DNA nanostructures
Casey Platnich

- P3.10 Metal-Mediated Self-Assembling 3D DNA Crystals
Simon Vecchioni

- P3.11 Minimalist Strategies for Size Defined 2D and 3D DNA Nanostructures
Daniel Saliba

Poster Session 4, Tuesday 13 April, 18:00 - 19:20 UTC**Track on Principles and Theory of Self-Assembly**

- P4.01 Thermodynamics of DNA looping for origami folding
Jacob Majikes

- P4.02 Stability of the d(CGA) Motif: A Building Block for the Rational Design of pH Sensitive DNA Nanostructures
Emily Luteran



- P4.03 **Self-Regeneration and Self-Healing in DNA Origami Nanostructures**
Michael Scheckenbach
- P4.04 **Thermosetting supramolecular polymerisation of compartmentalised DNA fibres with stereo-sequence and length control**
Michael Dore
- P4.05 **Elucidation of a new complex pathway in 2D supramolecular polymerization of DNA-oligomer block copolymer amphiphiles**
Muhammad Ghufran Rafique
- P4.06 **Nano-sandwich composite by kinetic-trapping assembly from protein and nucleic acid**
Shi Chen
- P4.07 **Designing Polycube Assembly Rules using SAT**
Joakim Bohlin
- P4.08 **Arbitrary Placement of Heterogeneous Species on a Single Substrate Enabled via Engineered Steric Brush Interactions**
Marcello DeLuca

Track on Nucleic Acid Nanostructures in Vivo

- P4.09 **Sequence-programmed DNA crosslinking hotspots create ultra-stable nanomaterials for use as a biological platform**
Tyler Brown

Poster Session 5, Wednesday 14 April, 15:20 - 16:40 UTC

Track on DNA Nanosystems: Programmed Function

- P5.01 **Chemically controlled DNA Origami dynamic self-assembly**
Alba Monferrer i Sureda
- P5.02 **Light-Responsive Dynamic DNA-Origami-Based Plasmonic Assemblies**
Joonas Ryssy
- P5.03 **DNA origami-based goniometers for DNA bending proteins**
Ashwin Karthick Natarajan
- P5.04 **Controllable Protocell Aggregation Induced by DNA Signals**
Yancheng Du
- P5.05 **Responsive core-shell DNA particles destabilise lipid bilayers and trap bacteria**
Michał Walczak

**P5.06 DNA origami based biosensing**

Lennart Gräbenhorst

P5.07 "Printing" DNA Strand Patterns on Small Molecules

Fangzhou Zhao

P5.08 One-Pot Molecular Assembly of DNA and Semi-Artificial Glycopeptides to Give Multicomponent Supramolecular Materials

Sayuri Higashi

P5.09 Cascaded pattern formation in DNA based Reaction-diffusion system

Keita Abe

P5.10 A Curvature Ruler Enabled by DNA Nanotech

Ece Büber

P5.11 Large, square-shaped, DNA origami nanopore with gating function on giant vesicle membrane

Shoji Iwabuchi

P5.12 Seeding, Plating and Electrical Characterization of Gold Nanowires Formed on Self-Assembled DNA Nanotubes

Dulashani R Ranasinghe

Poster Session 6, Wednesday 14 April, 17:45 - 19:20 UTC**Track on DNA Nanosystems: Programmed Function****P6.01 Helicase Expedites a Catalytic DNA Reaction by Selectively Removing the Product from the Catalyst**

Pepijn Moerman

P6.02 Exciton Delocalization in Squaraine versus Cyanine Dye Aggregates Templated by DNA Holliday Junction

Olga Mass

P6.03 Poster withdrawn**P6.04 A Modular and Customizable Aptamer Transducer Designed for Feed-Forward Coupling with Catalytic Amplifiers**

Tim Hachigian

P6.05 Robust heterochiral strand displacement using leakless translators

Tracy Mallette

P6.06 Poster withdrawn**Track on Protein & Viral Nanostructures**



- P6.07 Ethanol-Mediated Assembly of Tobacco Mosaic Virus Coat Protein
Ismael Abu-Baker
- P6.08 Poster withdrawn
- P6.09 CRISPR-guided programmable self-assembly of artificial virus-like nucleocapsids
Carlos Calcines-Cruz
- P6.10 Functionalization and assembly of coiled-coil protein origami nanostructures using SpyCatcher/SpyTag system
Žiga Strmšek
- P6.11 Engineering a molecular trigger for HBV VLP disassembly
Caleb Starr

Track on Integrated Chemical Systems

- P6.12 DNA Cubes as a “Printing Press”; Printing DNA Patterns onto Polymer Nanoparticles
Sean Laxton

Track on Computational Tools for Self-Assembly

- P6.13 A Python library for structural DNA nanotechnology
Jorge Guerrero
- P6.14 CADAxISDNA: Automated Design of Smooth and Continuous 3D Surface Contours with DNA Origami
Daniel Fu

Poster Session 7, Thursday 15 April, 15:20 - 16:40 UTC

Track on Computational Tools for Self-Assembly

- P7.01 Simulation & Design of DNA Amphiphiles: Towards Photo-Responsive Micelles
Shayne Gracious
- P7.02 On Shape Counting Methods for DNA Tile Self-Assemblies
Siwei Liu
- P7.03 Making the most of DNA melt curves: Data collapse with affine transformations and consequences of experimental design
Jacob Majikes
- P7.04 How to Design Free-form DNA Nanostructures Online
Joakim Bohlin
- P7.05 Coarse-grained nucleic acid - protein model for hybrid nanotechnology
Jonah Procyk



- P7.06 Virtual DNA Lab – A computational simulation platform for DNA multi-strand dynamics**
Frankie Spencer

Track on Synthetic Biology

- P7.07 Reconstitution of ultrawide DNA origami pores in liposomes for transmembrane transport of macromolecules**
Nicola De Franceschi
- P7.08 A modular, dynamic, DNA-based platform for regulating cargo distribution and transport between lipid domains**
Roger Rubio Sanchez
- P7.09 In situ generation of RNA complexes for synthetic molecular strand displacement circuits in autonomous systems**
Wooli Bae
- P7.10 Mechanisms for the Controlled Division of Giant Unilamellar Lipid Vesicles**
Yannik Dreher
- P7.11 Poster withdrawn**
- P7.12 Amplified Self-Immulative Release of Small Molecules by Spatial Isolation of Reactive Groups on DNA-Minimal Architectures**
Alex Prinzen
- P7.13 Cation-regulated binding of DNA with phospholipid membranes**
Himanshu Joshi



Poster Session 1 - Monday 12 April, 15:10 - 16:40 UTC

Track on Nanophotonics and Superresolution

P1.01 Peptide Nucleic Acid Conjugation of Quantum Dots to DNA Nanostructures

Christopher M. Green^{1,2}, David A. Hastman^{1,3}, Divita Mathur^{1,4}, Kimihiro Susumu^{1,5}, Eunkeu Oh¹, Igor L. Medintz¹, Sebastián A. Díaz¹

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⁴George Mason University, USA, ⁵Jacobs Corporation, USA

DNA nanostructures have broad functionality as scaffolds for coordinating the arrangement and interactions of nanomaterials with desirable physical properties. While some nanomaterials, such as gold nanoparticles (AuNPs), can be easily conjugated with singlestranded (ss) DNA, many nanomaterials cannot be easily conjugated, requiring costly chemical modifications or the use of bulky ligands which reduce the precision of placement offered by DNA nanostructures. For example, the most common strategy to label semiconductor quantum dots (QDs) with ssDNA is biotin-avidin binding between biotinylated ssDNA and streptavidin conjugated to the QD surface[1]. While this strategy is efficient, it is limited by the size of the streptavidin linker and a lack of control over DNA orientation due to tetravalent binding of biotin to streptavidin[2, 3]. Here, we show that peptide nucleic acids (PNA) can be utilized as an efficient and economical alternative to DNA-DNA binding for conjugating nanomaterials to DNA nanostructures. Unlike DNA, PNA can be synthesized as a peptide-PNA hybrid by solid-phase peptide synthesis, providing access to the vast chemical functionality of peptides for conjugating various nanomaterials. To demonstrate this method on QDs, we utilized peptide-PNAs containing a six-histidine tag, which binds to ZnS shelled QDs through metal affinity coordination to Zn²⁺ ions on the QD surface[4]. With this technique, we achieved greater than 90% QD capture efficiency on a DNA origami while maintaining site specificity and multiple capture sites per origami. We additionally explored the effects of peptide charge on the efficacy of QD conjugation and found that peptide-PNAs could be uniquely altered to improve binding for different QDs. This methodology provides an alternative to DNA-conjugation of nanomaterials and could be adapted to other nanomaterials by modification of the peptide.

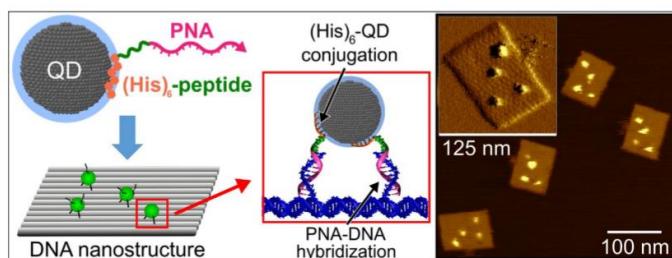


Fig. 1. Peptide-PNA conjugation of QDs to DNA origami.

- [1] H. Bui, C. Onodera, C. Kidwell, Y. Tan, E. Graugnard, W. Kuang, J. Lee, W. B. Knowlton, B. Yurke and W. L. Hughes, *Nano Lett.*, **10**, 3367 (2010).
- [2] M. Madsen and K. V. Gothelf, *Chem. Rev.*, **119**, 6384 (2019).
- [3] G. V. Dubacheva, C. Araya-Callis, A. Geert Volbeda, M. Fairhead, J. Codee, M. Howarth and R. P. Richter, *J. Am. Chem. Soc.*, **139**, 4157 (2017).



- [4] K. E. Sapsford, W. R. Algar, L. Berti, K. B. Gemmill, B. J. Casey, E. Oh, M. H. Stewart and I. L. Medintz, *Chem. Rev.*, **113**, 1904 (2013).

P1.02 Can a DNA Nanostructure Constrain the Position and Orientation of an Attached Dye Molecule?

Divita Mathur^{1,2}, Young C. Kim³, Sebastián A. Díaz², Paul D. Cunningham⁴, Brian S. Rolczynski^{4,5}, Mario G. Ancona⁴, Igor L. Medintz² and Joseph S. Melinger⁴

¹George Mason University, USA, ²Center for Bio/Molecular Science and Engineering, USA, ³Materials Science and Technology, USA, ⁴U.S. Naval Research Laboratory, USA

DNA-organized dye networks used for various applications rely on having efficient energy— and/or electron transport processes which, in turn, depend sensitively on the relative distance and orientation of the dye molecules [1]. In using DNA as a scaffold, a crucial question is to what extent can it control the dye position and orientation? The ability of DNA nanostructures to dictate the position is reasonably well addressed, but much less is known about the potential for controlling the orientation and its dependences on the dye attachment chemistry and on other aspects of the local microenvironments of the dye. We here employ a Cy3 probe dye and use its measured fluorescence properties and numerical simulations to compare the degree to which a DNA duplex and a 30-helix DNA origami bundle can provide the desired control over the dye (Figure 1) [2]. Experimentally, we find that the excited state lifetimes and rotational anisotropy decays are longer for the bundle than for the duplex indicating that the Cy3 is more constrained in the former. Atomistic molecular dynamics (MD) simulations are found to be in good agreement, and they further provide insight into how the microenvironment of the bundle is limiting the dye motion. Moreover, Förster resonance energy transfer simulations based on the MD data indicate that the constraints imposed by the DNA bundle can be sufficient to substantially raise the transfer efficiency (over the duplex) if the siting of the donor and acceptor is well chosen. And lastly, we explore strong excitonic coupling between two Cy3 dyes when positioned at the crossover site within the DNA bundle in comparison to a simple linear duplex. Overall, the results of this work should be useful for improving the performance of DNA-scaffolded dye networks.

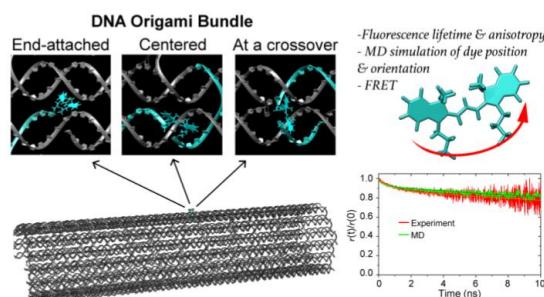


Figure 1: A DNA origami bundle on which a Cy3 dye is immobilized using different conjugation strategies (end vs internal) at different locations. Fluorescence properties are measured experimentally and via MD to determine control over dye position and orientation.

- [1] S. A. Diaz, S. M. Oliver, D. A. Hastman, I. L. Medintz and P. M. Vora, *J Phys Chem Lett*, **9**, 3654 (2018).
[2] D. Mathur, Y. C. Kim, S. A. Díaz, P. D. Cunningham, B. S. Rolczynski, M. G. Ancona, I. L. Medintz and J. S. Melinger, *J Phys Chem C*, **125**, 1509 (2021).



P1.03 DNA Origami Superstructures for Photonic/Phononic Crystals

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Korea University, South Korea

For DNA nanotechnology, arbitrary shaping the building blocks and nanoscopic recognition between building blocks offer the formation of hierarchical assembled macroscopic materials with high accuracy. [1-3] In this way, the superstructures entirely based on DNA origami have been presented with various motifs such as tensegrity and simple cubic lattices. [2,3] Interestingly, some 3D crystal motifs have fascinating physical property such totally reflection of the incident wave in specific wavelength. [4,5] For example, the crystal based on diamond lattices shows omnidirectional inhibition of electromagnetic wave propagation. Additionally, in case of elastic wave, the wave propagation can be prohibited by simple cubic lattices' crystal. In generally, the phenomena preventing the propagation of electromagnetic and elastic wave in crystals are called photonic and phononic bandgap, respectively. In this context, we demonstrate that DNA-based assembled crystal can be exerted as (i) photonic and (ii) phononic crystals. Based on FEM (finite element method) simulations, we show (i) the DNA-based crystals that are treated by a post-molding process can serve as photonic crystals and (ii) DNA-based crystals with silica cladding can work as phononic crystals.

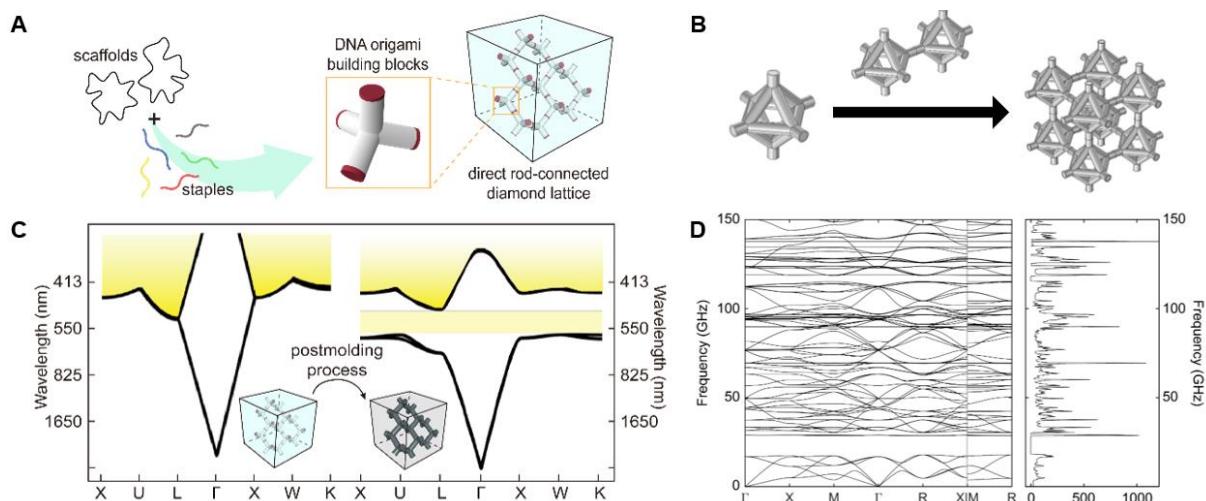


Fig. 1.

(A, B) schematic representation of the assembly of (A) photonic and (B) phononic crystal. (C) the photonic band structure for bare DNA-based crystal and its post-molded crystal. (D) the phononic band structure and phonon density of state for DNA/silica crystal.

- [1] N. C. Seeman, *J. Theor. Biol.* **99**, 237-247 (1982)
- [2] Y. Tian, *et al.*, *Nat. Mater.* **19**, 789-796 (2019)
- [3] T. Zhang, *et al.*, *Adv. Mater.* **30**, 1800273 (2018)
- [4] K. M. Ho, *et al.*, *Phys. Rev. Lett.* **65**, 3152-3155 (1990)
- [5] M. Maldovan, *Nat. Mater.* **14**, 667-674 (2015)



P1.04 Introducing graphene-on-glass coverslips for single-molecule biosensing

Renukka Yaadav¹, Izabela Kaminska², Karolina Zielonka² and Philip Tinnefeld¹

¹Ludwig-Maximilians-Universität München, Germany, ²Institute of Physical Chemistry of the Polish Academy of Sciences Warsaw, Poland

Graphene, a two-dimensional allotrope of carbon has many distinctive properties that have been the centre of research for almost two decades now. Our team is especially smitten by the fluorescence quenching ability of graphene. An excited fluorophore placed in the vicinity (up to ~ 40 nm) of graphene experiences a non-radiative energy transfer to graphene, leading to shortened intensity and fluorescence lifetime [1]. This near-field phenomenon follows the d^{-4} scaling law and can thus be used to sensitively report on distance changes. Furthermore, graphene is almost transparent with 2.3 % of light absorption, independent of wavelength in the visible and near infrared [2].

Graphene can thus be regarded as a label-free, broadband and unbleachable energy transfer acceptor. Combined with the addressability offered by DNA origami nanopositioners, new avenues for graphene-based biosensing, biophysics and superresolution microscopy can be explored, especially for applications in the field of life science.

Here, we introduce graphene-on-glass coverslips [3] for single-molecule biosensing. We show an assay in a novel unquenching format that uses graphene as a quencher to detect a single DNA molecule [4]. In line with this, we use pyrene-labeled DNA origami nanopositioners to chemically access the graphene surface. We further modify the nanostructure to place a capture strand labeled with a fluorophore, a closing strand, a biasing strand and a reference fluorophore (See Figure 1). The capture strand initially hybridizes to the closing strand, bringing the fluorophore close to graphene, resulting in a strongly quenched fluorescence lifetime. Upon target incubation, the capture strand hybridizes to the target strand to move freely, sampling different fluorophore-to-graphene distances. A short biasing strand is thus implemented to hybridize to the target strand in order to secure the fluorophore away from graphene restoring complete fluorescence. Overall, our approach allows a step-wise increase in signal contrast upon detection, indicating the potential of designing assays with DNA origami nanopositioners on graphene.

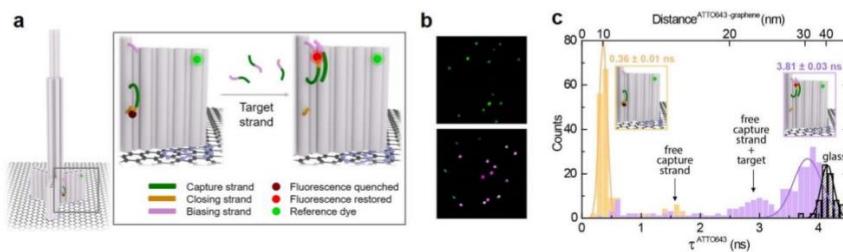


Fig. 1. Graphene-on-glass coverslips for biosensing. a) Sketch of the DNA origami nanopositioner with the assay. Inset shows the working of the assay. b) Single-molecule fluorescence intensity maps ($10 \times 10 \mu\text{m}$) before (top) and after (bottom) target recognition. c) Histogram of the fluorescence lifetime distributions fitted with Gaussian functions (the mean value and standard error obtained from the fit) of the assay before (orange) and after (lilac) target detection; assay measured on glass (black).

- [1] C. Zhu et al., 2D Materials. 2: 32004. (2015).
- [2] R. R. Nair et al., Science. 10.1126/science.1156965. (2008).
- [3] S. Krause et al., ACS Nano (n.d.) [4] I. Kamińska et al., (submitted 2021)

**P1.05 Poster withdrawn****P1.06 Bottom-up construction of complex standalone metal nanostructures in solution using DNA as a chaperone**

Xin Luo, Christophe Lachance-Brais, Amy Bantle and Hanadi F. Sleiman

McGill University, Canada

The ability to construct metallic nanostructures with arbitrary morphologies and pre-designed complexity has been an essential goal in nanoscience and technology. DNA nanotechnology has enabled the fabrication of increasingly complex DNA nanostructures with unprecedented specificity, programmability and sub-nanometer precision, which makes it an ideal chaperone to rationally organize and construct hierarchical metallic nanostructures. We have developed an Assemble, Grow, Lift-Off (AGLO) strategy (Fig. 1a) to construct robust complex pre-designed gold nanostructures in solution using only a simple 2D DNA origami sheet as a versatile transient template [1]. AuNP seeds are firstly assembled onto the pre-designed binding sites of the DNA origami template and then additional gold is slowly deposited to the AuNP seeds. The growing seed surface will eventually merge with adjacent seeds to generate one standalone gold nanostructure in a pre-designed shape. The gold nanostructures produced by the AGLO strategy consist of completely merged AuNPs and thus are robust and stable after removing the DNA origami template. Various 2D arrangements of AuNP seeds on the simple DNA origami template can be successfully converted into complex gold nanostructures with the target structure transformation rate over 80%.

Interestingly, growing silver on DNA origami-templated AuNP seeds in the same system yields completely distinct results. We discovered that when a growing silver surface of the Au@Ag core-shell nanoparticle developed into contact with a neighboring silver shell on the origami template, one Au@AgNP continued to grow while the other one was etched (Fig. 1b). Furthermore, this heterogeneous deposition/etching of silver can be blocked by incorporating an iron oxide nanoparticle in between the two AuNP seeds. We propose a contact-dependent same-metal Galvanic reaction mechanism to explain this intriguing heterogenous phenomenon.

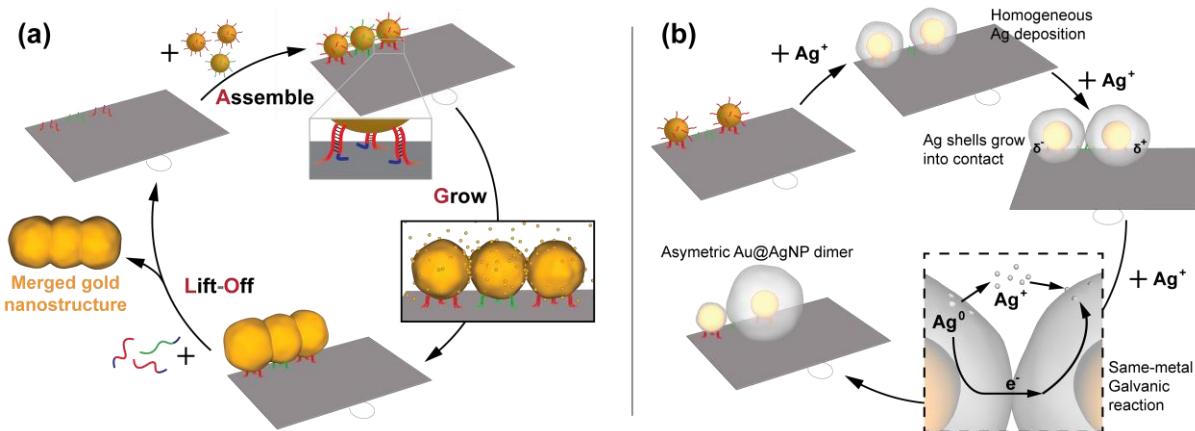


Fig. 1. (a) the Assemble, Grow, Lift-Off (AGLO) strategy to construct robust complex customizable gold nanostructures. (b) the heterogenous deposition of silver shells on origami-templated AuNP dimers.

[1] X. Luo, C. Lachance-Brais, A. Bantle and H. F. Sleiman, Chem. Sci. **11**, 4911-4921 (2020).



P1.07 Substituent Effects on the Solubility and Electronic Properties of Dyes: Density Functional and Time Dependent Density Functional Theory Calculations

Austin Biaggne¹, German Barcenas¹, Jeunghoon Lee¹, William B. Knowlton¹, Bernard Yurke¹, and Lan Li^{1,2}

¹Boise State University, USA, ²Center for Advanced Energy Studies, USA

DNA nanotechnology enables the construction of nanodevices for excitonic applications by enabling the positional manipulation of dyes covalently attached to the DNA. Recently, dye aggregates templated with DNA scaffolds have been studied by a number of groups for exciton delocalization resulting from the dye's dipole-dipole interactions. The dynamics of excitons in an aggregate depend on the difference between the dye monomer's ground and excited state dipole moments (Δd), the monomer's transition dipole moment (μ), and the inter-dye distances [1]. To improve the viability of dye aggregates for excitonic applications, the enhancement of electronic properties (i.e. larger Δd and μ) and improvement of aggregation (i.e. reduction in inter-dye distances by altering solubility) is necessary. The addition of functional group substituents on dyes has been demonstrated to alter their photophysical, electronic, and aggregation properties. To quantify the effects of substituents on dyes, density functional theory (DFT) and time-dependent (TD-) DFT can be used to determine optimized structures and electronic properties of dye monomers [2].

To determine how electron donating (D) and electron withdrawing (W) substituents impact the solubility and electronic properties of dyes such as Cy5 (shown in Fig. 1), DFT and TD-DFT methods were employed for dyes solvated in water, pyridine, quinoline, and isoquinoline [3]. Pyridine, quinoline, and isoquinoline solvents, which are structurally similar to DNA bases, were used to roughly estimate how substituents affect dye intercalation into DNA structures. The solvation energy (ΔG_{solv}), which is correlated with solubility, Δd , and μ of the substituted dyes were calculated. It was found that ΔG_{solv} generally decreases (i.e. the dyes become more soluble) upon substitution, and dyes with pairs of W substituents have the most negative ΔG_{solv} . The values of μ are relatively unaffected upon substitution relative to the unsubstituted dye. However, the values of Δd generally increase upon substitution and asymmetry of the dyes. Pairs of weak and strong W substituents or pairs of D and W substituents yield relatively larger Δd values than pairs of only D substituents. Our computational results advance the understanding of the effects substituents have on key dye attributes. The results of this study can help guide dye synthesis for tailoring the dipole properties of DNA-templated dyes for excitonic applications.

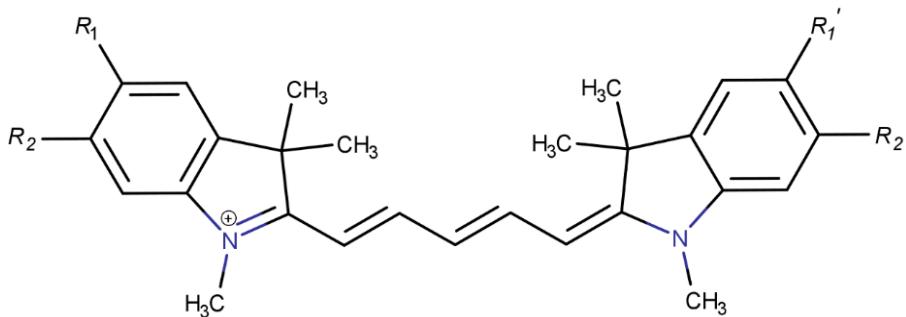


Fig. 1. Molecular structure of a Cy5 dye. Hydrogens at R positions were replaced with substituents.

- [1] D. Abramavicius, B. Palmieri, S. Mukamel, Chem. Phys. **357**, 79-84 (2009).
- [2] J. Fothergill, et al., J. Phys. Chem. A **122**, 8989-8997 (2018).
- [3] A. Biaggne, et al., Molecules **26**, 524 (2021).

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P1.08 Rotaxane rings promote oblique packing and extended lifetimes in DNA-templated molecular dye aggregates

Matthew S. Barclay¹, Simon K. Roy¹, Jonathan S. Huff¹, Olga A. Mass¹, Daniel B. Turner¹, Christopher K. Wilson¹, Donald L. Kellis¹, Ewald A. Terpetschnig², Jeunghoon Lee¹, Paul H. Davis¹, Bernard Yurke¹, William B. Knowlton¹ and Ryan D. Pensack¹

¹ Boise State University, USA, ²SETA BioMedicals, USA

DNA is a highly versatile material that can be used to template the assembly of molecular dye aggregates, which are of interest in a variety of fields including light harvesting and nanoscale computing. While the sub-nanometer (i.e., base pair level) precision of templating molecular aggregates enables the formation of molecular excitons (i.e., excited states delocalized over more than one dye), finer control over the relative orientations of the molecules is needed to further tailor the properties of these materials for specific applications. Here, we demonstrate sub-Angstrom control over the packing orientations of dye aggregates by templating rotaxane-encapsulated squaraine dyes onto a DNA Holliday junction nanostructure. We use experimental and theoretical analysis to show that, in contrast to a dimer aggregate of squaraine dyes that adopts a more common face-to-face, or H-aggregate, packing, the squaraine:rotaxane dimer exhibits an elusive oblique packing arrangement with near-equal intensity excitonically split absorption bands, which may potentially be useful for panchromatic light harvesting and excitonic entanglement. These results imply that it may be possible to combine DNA templating with rotaxane ring encapsulation to promote oblique packing in molecular dye aggregates generally by templating dyes with DNA and encapsulating them with a rotaxane ring.

P1.09 Excited-State Dynamics in DNA-Templated Molecular Dye Aggregates

Jonathan S. Huff, Daniel B. Turner, Olga A. Mass, Matthew S. Barclay, Bernard Yurke, William B. Knowlton, Paul H. Davis and Ryan D. Pensack

Boise State University, USA

Molecular dye aggregates are of interest to a broad variety of fields including light harvesting, computing, and sensing. DNA templating is a novel means in which to position and assemble dye aggregates with high precision, and with further developments it may be possible to construct dye aggregates with tailored properties. While considerable progress has been made in controlling the electronic structure of DNA-templated dye aggregates, recent work has shown that these materials commonly exhibit reduced fluorescence due to shortened excited state lifetimes, the causes of which are not yet well understood. Accordingly, we used time-resolved spectroscopy techniques to study the excited state dynamics of a number of DNA-templated cyanine aggregates. All of the aggregates we studied, each of which consists of two or more Cyanine 5 (Cy5) dyes attached to either a DNA duplex or Holliday junction, exhibit excited state lifetimes that are up to 100-fold shorter than the Cy5 monomer. We find that the reduced lifetimes are consistent with enhanced nonradiative decay introduced upon aggregation. Developing a better understanding of ultrafast nonradiative decay will facilitate the use of DNA-templated dye aggregates in a diverse set of applications.



Track on Biomedical Nanotechnology

P1.10 Bioinspired Peptide-Nucleotide Nanofibers

Simone I S Hendrikse^{1,2}, Chelsea R Forest,² Luke Marshall,² Pall Thordarson², Albert C Fahrenbach², and Amanda V Ellis¹ ¹ School of Chemical and Biomedical Engineering, The University of Melbourne, Australia ² School of Chemistry, University of New South Wales, Australia

In nature, fibrous assemblies continuously assemble and disassemble to fulfill a variety of functions, including providing structural support and driving cell motility. Inspired by the hierarchically complex structures they form that are necessary to perform these essential tasks, both natural and synthetic mimics have been developed.[1,2] By creating synthetic mimics, control over structure, and hence, function can be achieved. Moreover, new capabilities, not achievable in nature, can be realized. For example, the Tobacco Mosaic Virus (TMV) forms helical cylindrical fibrous assemblies with very high persistence lengths.[3,4] The highly infectious RNA of the virus is coated by so-called coat proteins, that protect the virus from enzymatic degradation, and keeps the virus stable for several years within infected tobacco leaf products, e.g., infected cigars. The assembly of virion TMV includes synergistic effects of both the RNA and already aggregated coat proteins (i.e., disks or small helices). In the absence of RNA, protein modules are intrinsically disordered, whereas upon RNA binding, a highly organized hybrid structure is obtained with extensive intermolecular interactions both laterally and axially, including salt-bridges.

Fascinated by the hybrid self-assembled structure of RNA with coat proteins in virion TMV, we developed peptide-nucleotide hybrid polymers that also form rigid fibers (Figure 1). Our peptide-nucleotide fibers are formed as a consequence of lateral non-covalent interactions between the peptide sequences, while having pendant mononucleotides, altogether forming rigid rods in aqueous solutions. Despite having similar peptide sequences, installing distinct mononucleotides at the periphery of the polymers induced different structure morphology; adenine mononucleotide functionalized polymers formed micrometers long tape-like structures, whereas thymine formed short straight rods (Figure 1B). The ability to facilitate complementary binding with its artificial complementary mimic and canonical RNA and DNA is currently being investigated. These polymers will serve as templates for the non-enzymatic synthesis of DNA and RNA.

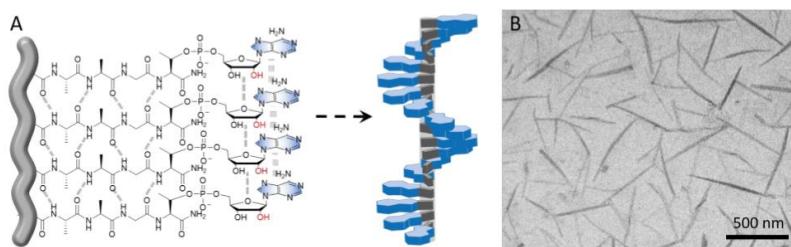


Fig. 1. (A) Suggested self-assembly of adenine functionalized artificial polymers, (B) TEM of thymine functionalized artificial polymers.

- [1] A. C. Mendes, E. T. Baran, R. L. Reis and H. S. Azevedo, WIREs Nanomedicine and Nanobiotechnology, **5**, 582 (2013).
- [2] O. J. G. M. Goor, S. I. S. Hendrikse, P. Y. W. Dankers and E. W. Meijer, Chem. Soc. Rev. **46**, 6621 (2017)
- [3] J. N. Culver, Annual Review of Phytopathology, **40**, 287 (2002)
- [4] D. Wu, et al., Nature, **574**, 658 (2019).
- [4] D. Wu, et al., Nature, **574**, 658 (2019)



Track on Molecular Machinery

P1.11 Single-molecule mechanical detection of an artificial track-walking molecular motor beyond bridge-burning design

Zhisong Wang

National University of Singapore, Singapore

A defining capability of molecular motors is self-sustained force generation by a single motor copy. Direct experimental evidence for force generation at single-motor level, though long established for biological molecular motors, is still missing for man-made counterparts. Here we report single-molecule mechanical detection of an artificial track-walking molecular motor [1] using magnetic tweezers. A single motor plus its track (both made of deoxyribonucleic acids or DNA) is assembled *in situ* under a tweezers-controlled bead, followed by a bead shift for subsequent detection of the motor's autonomous chemically fuelled motion against a constant force. The motor shows self-directed on-track walking by ~ 16 nm steps up to a distance of 120 nm (covering the entire track), yielding a stall force of ~ 2 pN. This force-generating capacity reaches the level of biomotors powering muscle's contraction, and paves the way towards similar force-demanding applications by artificial counterparts. This single-motor study also reveals fast subsecond individual steps, implying a big room to improve speed of DNA motors. Besides, the established single-molecule mechanical setup is generally applicable to other reported DNA motors for force measurement.

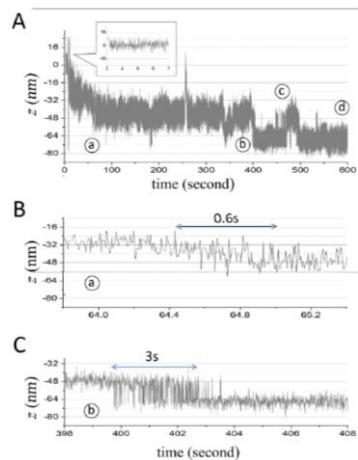


Fig. 1. A typical trajectory of the motor under an opposing force of 1.5 pN and a fuel concentration of 0.5 μ M. The top panel shows the whole trajectory, i.e., vertical position of the motor-dragged bead along the straightened track versus time (starting segment amplified in inset). The time zero is set at a time shortly after fuel/nicking enzyme addition and subsequent bead stabilization (within seconds). The displacement to positions with negative values indicates the motor's downward motion along a double-stranded DNA track. Other two panels show details of the motor's individual stepping events (a, b segments; time scale indicated).

[1] M. H. Liu, J. Cheng, S. R. Tee, S. Sreelatha, I. Y. Loh, Z. S. Wang, ACS Nano 10, 5882 (2016).



Poster Session 2, Monday 12 April, 18:00 - 19:20 UTC

Track on Biomedical Nanotechnology

P2.01 DNA origami biosensor for the detection of cancer biomarkers

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University of Fribourg, Switzerland

The main reason for cancer-related death is due to cancer cell spreading and the formation of metastasis [1]. Recently, it has been shown that blood circulating microRNAs (miRNAs) can be used as biomarkers to detect cancer and monitor progression [2]. However, miRNA detection in clinical diagnostics still remains a challenge. The development of DNA nanotechnology provides a promising approach to generate DNA origami biosensors to detect cancer biomarkers including miRNA [3]. In this study, we designed and characterized an optical DNA origami biosensor. This biosensor consists of three rectangular layers of DNA helices. The upper layer is connected to the middle layer with a hinge from the center that allows the opening of the layer at both sides (Figure 1). Also, they are connected with four locks of partially complementary DNA duplex on both sides to keep the device in a closed state. The binding of fully complementary miRNA causes the opening of the layers through toehold mediated strand displacement. To sense the binding of miRNAs, arrays of fluorophores were precisely positioned on the top and middle layers. For fluorescence-based detection, we are exploring two mechanisms: FRET and quenching. The transformation from the closed to open state increases the distance between donor and acceptor fluorophores resulting in a change in fluorescence signal. As a proof of concept, we are testing two breast cancer-related miRNAs expressed in HER2+ and triple-negative breast cancer subtypes. We determined the optimal Mg²⁺-concentrations during self-assembly and purification steps, and optimized the output signal. Our results confirmed that our biosensor has a high output signal resulting from a high number of incorporated fluorophores. In addition, our results showed the ability of the biosensor to detect two different miRNAs with both sensing approaches (FRET and quenching) in the bulk study and on the single-molecule level as well. We are now defining the limit of sensitivity on the single-molecule level. Based on these results, we foresee that our DNA origami biosensor will offer an effective strategy for specific and sensitive detection of miRNAs in clinical samples.

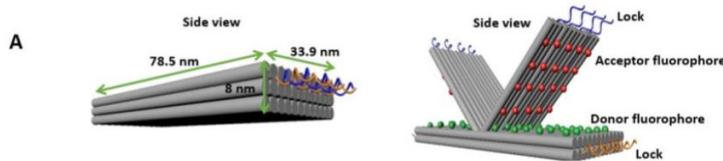
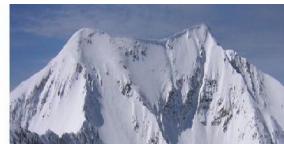


Fig. 1. (A) Schematic representation of closed and open DNA origami biosensor.

- [1] AW. Lambert, DR. Pattabiraman, RA. Weinberg, *Cell*. **168**, 670-691 (2017).
- [2] S. Kurozumi, et al., *J. Hum. Genet.* **62**, 15-24 (2017). [3] D. Selnihhin, et al., *ACS Nano*. **12**, 5699-5708 (2018).

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P2.02 Programmable low-cost DNA-based platform for viral RNA detection

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*Equal contributions

¹The RNA Institute, University at Albany, State University of New York, Albany, NY 12222, USA. ²Department of Biology, University at Albany, State University of New York, Albany, NY 12222, USA.

Detection of viruses is critical for controlling disease spread. Recent emerging viral threats, including Zika virus, Ebola virus, and SARS-CoV-2 (responsible for COVID-19), highlight the cost and difficulty in responding rapidly. To address these challenges, we develop a platform for low-cost and rapid detection of viral RNA with DNA nanoswitches that mechanically reconfigure in response to specific viruses (Fig. 1). Using Zika virus as a model system, we show non-enzymatic detection of viral RNA, with selective and multiplexed detection between related viruses and viral strains. For clinical-level sensitivity in biological fluids, we coupled with sample preparation using either RNA extraction or isothermal pre-amplification. Our assay can be performed with minimal lab infrastructure and is adaptable to other viruses, as demonstrated by quickly developing DNA nanoswitches to detect SARS-CoV-2 RNA in saliva [1].

Rapid detection is critical to slow down the spread of the COVID19 pandemic caused by SARS-CoV-2. We further develop the assay to detect SARS-CoV-2 RNA using DNA nanoswitches and isothermal nucleic acid amplification. As shown in our preliminary tests, this method can detect as low as 50 copies of SARS-CoV-2 RNA within one hour. Besides, the detection ability has been verified by using clinic RNA samples that had been confirmed by RT-qPCR. Besides, we reprogrammed our DNA nanoswitches so that they can detect influenza A, influenza B, and SARS-CoV-2 in a single reaction. Currently, each detection cost is less than three dollars, and the operation is simple, making it practical for point-ofcare tests. We expect further development and field implementation will improve our ability to detect emergent viral threats, such as SARS-CoV-2, and ultimately limit their impact.

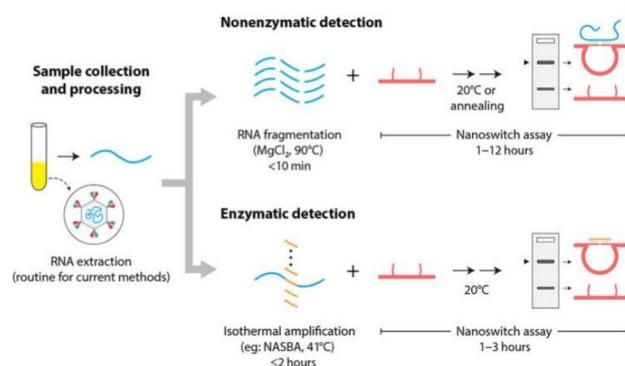


Fig. 1. Viral RNA detection by using DNA nanoswitches.

- [1] L. Zhou, A. R. Chandrasekaran, J. A. Punnoose, G. Bonenfant, S. Charles, O. Levchenko, P. Badu, C. Cavaliere, C. T. Pager, and K. Halvorsen, Sci. Adv. eabc6246 (2020).

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12 - 15 April 2021



P2.03 Programmable surface grafting of DNA origami via TdT polymerization

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Our research is motivated by the need for stable, nanoparticle-based drug delivery platforms that are composed of nucleotides or nucleotide analogues. Towards this goal, we designed and synthesized site-specifically functionalized DNA origami nanostructures (DONs) using surface-initiated TdT-catalyzed enzymatic polymerization (SI-TcEP). This synergistic combination of surface-initiated enzymatic polynucleotide brush synthesis with precisely engineered DNA origami presents an innovative pathway for the generation of stable, polynucleotide brush functionalized origami nanostructures. Our experimental results show that we have precise control over multiple parameters, including the shape of the origami core, location, height, and composition of the brush. Coarse-grained oxDNA simulation provides us with a powerful tool to design and predict the morphology of origami-brush nanostructures. In addition, we have shown that polynucleotide-functionalized DONs have significantly higher nuclease resistance compared to unprotected DNA origami. Furthermore, this stability can be spatially programmed by designing the positions of the decorating sites on the surface of DONs, potentially allowing the control of the degradation of drug delivery vehicles to facilitate cellular uptake. Together, these attributes will enable new applications of DNA nanotechnology, such as *in vivo* molecular sensors and smart nanoscale delivery systems.

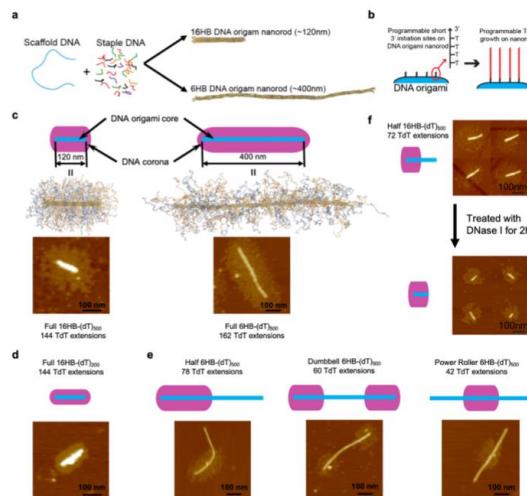


Figure. a) Schematic and simulation images of 16HB and 6HB DNA origami. b) 3' oligo(dT) extensions added to the surface of origami initiate TdT catalyzed nucleotide polymerization. c) Schematic, simulation, and AFM images of fully decorated 16HB and 6HB with poly(dT) corona. AFM imaging confirmed d) the controllable thickness of corona on 16HB and e) siteselective polymerization on 6HB. f) Polynucleotide brushes on half-decorated 16HB site-selectively protect DNA origami against nuclease degradation.



P2.04 Using 3D-cell Culture to Determine Efficacy of DNA-Based Drug Delivery Vehicles

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Cancer is a leading cause of death worldwide, and cancer rates are expected to increase as the population ages. Despite that, the approval of new drugs remains very low with an average likelihood of approval of approximately 10%.^[1] These low rates of approval come in part due to premature metabolism of drugs and toxicity due to off-target effects. Nanocarriers seek to improve delivery by protecting their payload while increasing site specific targeting. DNA nanocarriers in particular offer benefits including modifiability using non-canonical building blocks, bio-compatibility, predictable supramolecular interactions, and intrinsic therapeutic capability. Spherical nucleic acids (SNAs) offer additional benefits in comparison to other DNA nanocarriers as they possess a hydrophobic interior, are easy to synthesize and contain a single stranded DNA corona that can be used for therapeutic or targeting purposes.^[2-4] In addition, our SNAs demonstrate increased resistance to nucleases, and stability when exposed to changes in salt concentration and pH. To analyse the effects of SNA entry into cells we use 3D-cell culture to represent the tumor microenvironment. As opposed to traditional 2D-cell culture, 3D-cell culture better imitates tumor growth allowing for cell-cell interactions and growth in multiple dimensions. However, unlike *in vivo* studies 3D cell culture is mass producible and is high throughput. By looking at uptake of fluorescently labelled SNAs in both 2D and 3D cell culture, comparing entry into cells, and showcasing the stability and simplicity of our SNAs, we hope to better understand the mechanisms of uptake into cells and demonstrate that these carriers are a promising candidate for drug delivery.

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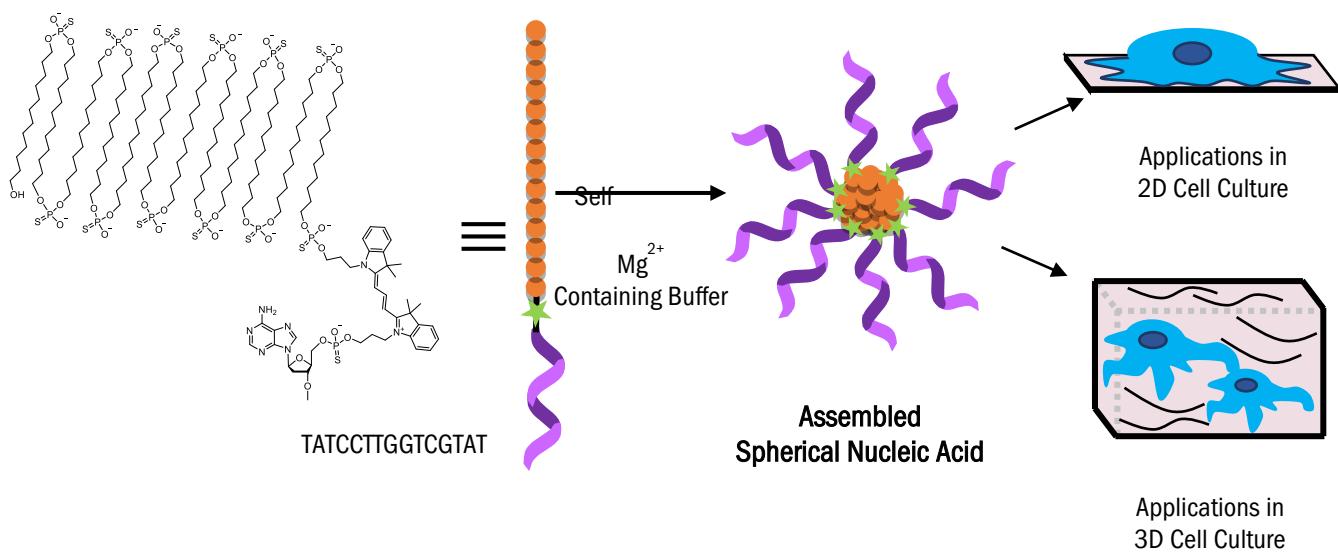




Figure 1. Using non-canonical amidites amphiphilic polymers can be made with hydrophilic DNA conjugated to a hydrophobic segment consisting of twelve hexaethylene repeats. These strands can self assemble in a magnesium containing buffer to form discrete spherical nucleic acids. These spherical nucleic acids have applications in drug delivery as determined by both 2D- and 3D-cell culture models.

P2.05 The combination of flattening, length-dependent rigidity and mechanochemistry accounts for microtubule breaking

Juan B. Rodriguez III, Stanislav Tsitkov, Neda M. Bassir Kazeruni, and Henry Hess

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Breaking is an important mechanical process of cytoskeletal filaments which has implications both on the activity of cellular processes and the dynamics of engineered active nano systems. The understanding of breaking has proven to be challenging due to the structural complexity of cytoskeletal filaments and has not been well characterized. Here we analyze the breaking dynamics of taxol-stabilized microtubules, as they are propelled by surface-adhered kinesin motors, by observing 129 individual breaking events across 300,000 images of individual microtubules. After analyzing the distributions of curvature for breaking and non-breaking microtubules, we found that for curvatures below $1 \mu\text{m}^{-1}$, the breaking rate increases exponentially as predicted by Bell's equation, but increases more slowly for curvatures above $1 \mu\text{m}^{-1}$. This threshold is similar to the threshold recently determined for the Brazier buckling of microtubules [1]. The reduction in the exponent is thus likely caused by cross sectional flattening in the microtubule, which leads to a reduction in the apparent stiffness of the filament when it is subjected to a curvature above a critical value. This demonstrates that the microtubule undergoes significant changes in its mechanical properties dependent on curvature and these mechanical changes need to be accounted for to fully describe the mechanical properties of cytoskeletal filaments. [1] Memet, E. et al., Microtubules Soften Due to Cross-Sectional Flattening. *eLife* 2018, 7, e34695.

P2.06 Amine Structure Modulates the Immune Response of Lipid Nanoparticles

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The vast majority of literature on RNA delivery vehicles focuses on efficacy [1]. The immune response of delivery vehicles is often not studied until they are tested in higher-order animal models. Nevertheless, the interaction of a drug with the immune system is just as important as efficacy in bringing it to the market. By understanding how delivery vehicles provoke the immune system, we can engineer well-tolerated vehicles during the initial phases of drug development. A thorough understanding of immune interactions will also allow us to choose the structures that are best suited for the nature of the therapy. For example, inflammatory delivery vehicles might be preferable for vaccines, whereas non-inflammatory delivery vehicles might be a better choice for protein replacement therapy [2]. This research intends to understand the effect of lipid nanoparticle chemistry on immunogenicity. We show that the amine group of the lipids regulates the magnitude of inflammatory immune response. Further, we identify the innate immune receptors that are triggered by lipid nanoparticles. Finally, we use molecular dynamics to identify the specific motifs in the lipids that enable immunostimulation. This work broadens our understanding of the impact of lipid structure on the immune response, and the structure-activity relationships can accelerate the design of potent and safe lipid nanoparticles.



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Track on Molecular Machinery

P2.07 Modelling of Migration Dynamics with DNA Walkers on 2D Surfaces

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DNA walkers are driven by various mechanisms including enzymatic reactions and strand displacement. Building DNA walkers with faster speed and better processivity has been a consistent goal in design. Effort has been made to understand the motion dynamics of walkers. Traditional models such as self-avoiding model may explain certain aspects of walker behaviors (e.g., velocity variations), but are not able to describe different motion types observed experimentally.

Here, we utilize an enzyme-powered DNA walker (shown in Fig. 1a) as a model system and adopt a random walk model to provide new insights on migration dynamics.[1] A variety of different migration modes were observed with the DNA walkers, including ballistic, Lévy, selfavoiding, and diffusive motions (Fig. 1b). To understand the walker motions, we performed a segmentation of walker trajectories and identified the distinct walking modes, each of which shows unique step time and velocity distributions. Statistics of experimental results demonstrated that mean squared displacement (MSD) scaling of the migration modes are related to step time and velocity distributions, and match well with the theoretical predictions as shown in Fig. 1c.

With a better understanding of the migration dynamics, we performed mechanistic studies on multiple factors that govern walker behaviors. We demonstrated that different types of cargoes carried by walkers have minimal influence on motions while the sizes of cargoes may affect migration modes due to different valencies. Sequence design of DNA walkers alters motions due to free energy and kinetic differences. Longer walker strands will have lower velocity and more stable movement. Presence of footholds results in more low MSD scaling motions observed in walkers. Environmental conditions including enzyme and Mg²⁺ concentrations also control the movements by changing the cleavage rate and binding strengths of walkers. Finally, a set of design principles were developed from the experimental results for tuning the behaviors of DNA walkers.

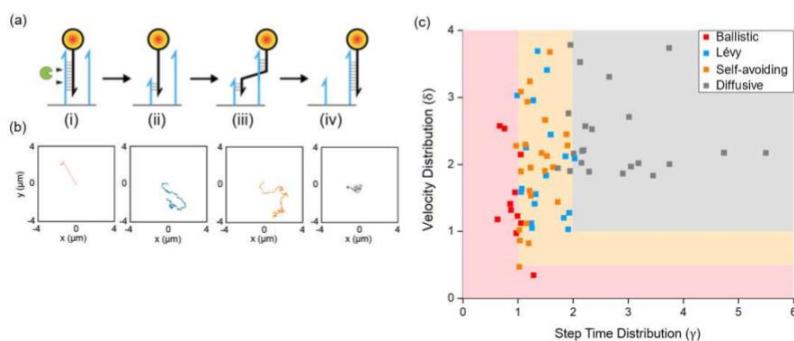


Figure 1. (a) DNA walker mechanism. (b) Four distinct walking modes: ballistic (red), Lévy (blue), selfavoiding (orange), and diffusive (gray). (c) Velocity distribution versus step time distribution from experimental and



theoretical results. The entire domain is painted with different colors to demonstrate theoretical predictions of ballistic (red), anomalous (orange) and diffusive (gray) motions.

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P2.08 Robotic end-to-end fusion of microtubules powered by kinesin

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The assembly of molecules by nanorobots has advanced significantly since “molecular manufacturing” was proposed. In contrast to a catalyst, which accelerates a reaction by smoothing the potential energy surface along the reaction coordinate, a nanorobot expends energy to accelerate a reaction relative to the baseline provided by thermal motion and forces. We designed a nanorobotic system to accelerate the assembly of end-to-end fusing of microtubules by confining the kinesin propelled microtubules in a chamber (diameter $d = 50 \mu\text{m}$, height $h = 0.5 \mu\text{m}$). This leads to a non-equilibrium distribution of microtubules induced by the work done by the kinesin motors, since the microtubules begin to follow the chamber boundary. The increased density and alignment of microtubules leads to a four-fold increase in the average length of the microtubules within 20 hours, and a shift in the length distribution towards longer microtubules. Moreover, the confinement leads to a ten-fold increase in fusion events between microtubules compared to unconfined microtubules gliding on a planar surface. In contrast to earlier nanorobots where the non-equilibrium distribution is built into the initial state and drives the process, our nanorobotic system creates and actively maintains the building blocks in the non-equilibrium state responsible for accelerated assembly.

P2.09 High Performance Computer Controlled Fast and Processive Bipedal Motor that Travels Long Distances

Samrat Basak, Ofir Perel, Maria Popov, Haggai Shapira, Breveruos Sheheade, Meitar Uralvitch and Eyal Nir

Ben Gurion University of the Negev, Israel

Biological molecular machines can perform hundreds of serial operations with remarkably high speed [1]. In comparison, synthetic molecular machines, including those made of DNA, perform orders of magnitude less operations and are orders of magnitude slower. In respond, a major effort in our group is to develop effective and fast DNA based molecular machines. In recent years we have demonstrated a DNA bipedal motor that strides on a DNA origami and operates by responding to 'fuel' and 'antifuel' DNA strands [2]. The strands are provided to the motor by a computer-controlled microfluidics device and motor progress is observed by single molecule fluorescence. With this setup, we demonstrate the performance of about 50 walking steps (100 consecutives chemical reactions with $\sim 98\%$ yield per reaction) which amount to 600 nanometers traveled by the walker [3].

However, motor dissociation rate-per-step remain at about $\sim 2\%$, limiting the number of steps and the overall distance the motor can travel. In addition, the motor stepping reaction is slowed by the presence of unwanted but unavoidable fuel/antifuel complex. Moreover, the motor was based on a track made of a single origami unit, limiting the length of the track to about 150 nm, and therefore, the motor was commanded to walk back-and-forth on the track.

Here we aim to identify the factors that leads for the $\sim 2\%$ motor dissociation per step, and by rational design of solutions, decreasing motor dissociation and increasing motor processivity. Furthermore, by optimization the fuel



and antifuel sequences, the unwanted fuel/antifuel complex expect to dissociate faster, increasing motor respond and therefore its speed. Finally, with a sticky end mediated [4] synthesized micrometer long track, made of several origami units connected, and labeling the walker using gold nanodot, will allow following the position of the walker walking unidirectional along the long track. Not like our current tracking method that use FRET based on organic fluorophores and that does not provide information on all the positions of the walkers along the track due to the photobleaching, the gold dot method will allow identification of the position of the walker at each step and allow investigate the motor performance along the entire long track using single molecule localization microscopy.

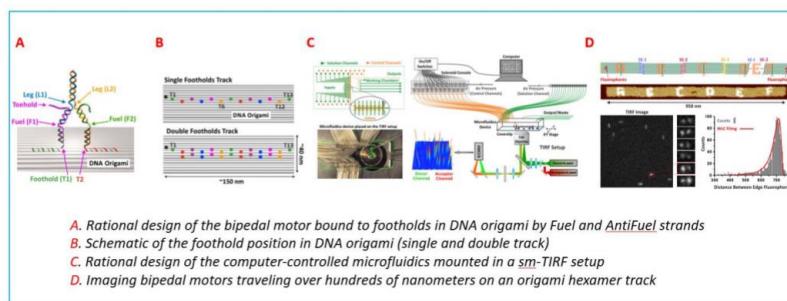


Fig. 1. Overview of the rational design of the motor, long track and the microfluidics based single molecule fluorescence setup.

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P2.10 Enzyme-free catalytic production of DNA complexes through far-from-equilibrium templating

Javier Cabello-Garcia, Wooli Bae, Guy-Bart V. Stan and Thomas E. Ouldridge

Imperial College London, UK

The use of templates is an established method to produce sequence-controlled assemblies, like polymers. Canonically, templating is depicted as a self-assembly process, where the formation of a specific product is favoured at equilibrium by template-product interactions. However, in a biological context, like protein formation, products must detach spontaneously from its template, which act as a catalyst (1). The catalytic nature of template-product interactions means that they are transient, so the template-complementary product cannot be favoured in equilibrium, necessarily pushing the templating process far-from-equilibrium (FFE) by fuel consumption (2). The result is a productsequence distribution peaked around the template-complementary sequence, even though, the equilibrium distribution of sequences is uniform due to the unspecific monomer interactions.

In this work, we build an only-DNA system capable of FFE-templating the catalytic production of sequence-specific dimers. The system is composed of two sets of complementary fuel-monomers, A and B, and a template, which



recognises and catalyses the polymerisation of a specific monomer from each subset, e.g. monomer A1 and monomer B3. The mechanism can autonomously regenerate its template thanks to handhold-mediated strand displacement, a novel displacement topology capable of the direct implementation of FFE templating (3). This displacement reaction increases the polymerisation rate of specific monomer with a DNA-DNA transient interaction, the handhold. After the dimer product is formed, the transient nature of the handhold ensures the autonomous detachment of the template, so it can keep catalysing the product formation. The result is a biased product distribution, without modifying the equilibrium distribution of equiprobable products. We measure and model the kinetics of the templating system, and we use this insight to catalyse, with a scarce amount of template, at least 20-fold of a specific dimer out of nine possible equally stable products.

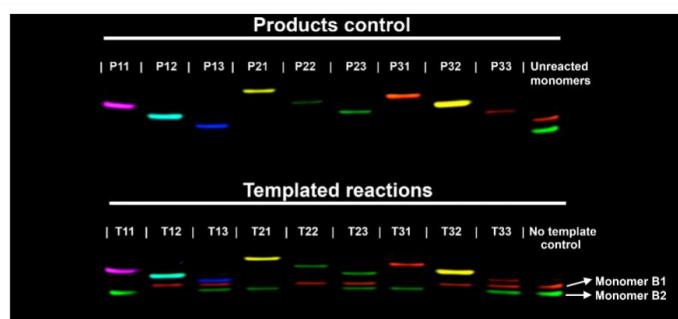


Fig. 1. 6-channel fluorescence image of PAGE demonstrating specific product formation. The system is composed of two sets of monomers, A and B. Each set has an alphabet of three different monomers, only differentiated by its fluorescence labelling and a domain not hybridised in the final product. Products control gel: Anneal of 100 nM of the specified pair of monomers, e.g. A1+B3=P13. Templated reactions gel: Fluorescence signal after 72 hours incubation of 100 nM of every monomer and 5 nM of a template capable of producing a specific product. FRET patterns in both gels are identical except for the excess of unreacted monomers in the templated reactions gel. Conditions: TAE 1x with 1M NaCl at 25°C.

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P2.11 Solving EXACT COVER problem using Network-based BIOCOMPUTATION

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¹Lund University, Lund, Sweden, ²Technische Universität Chemnitz, Germany, ³Linnaeus university, Sweden,
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Computational problems of combinatorial nature including non-deterministic polynomialtime (NP) complete problems currently require exponential time to explore the solution space as the problem size increases, making conventional serial computation intractable, and parallel computation a necessity. Network-based Biocomputation (NBC) was demonstrated to solve the subset sum problem (SSP) [1], by encoding it into a graphical network of



channels in a nanofabricated device, which was then explored by cytoskeletal filaments propelled by molecular motors to find all possible solutions. This approach of parallel computation promises to require orders of magnitude less energy than conventional computers due to high energy efficiency of the molecular motors. The current study focusses on using NBC to solve another problem, namely Exact Cover (ExCov). For an ExCov problem with a collection X of subsets with elements from a set Y , an **exact cover** is a subcollection X^* such that each element in Y is contained in exactly one subset in X^* . We demonstrate an algorithm to translate an ExCov problem with 64 potential solutions into an SSP network which is then solved by using a molecular motor system (actin-myosin II). The solution space in this problem is eight times larger than the SSP problem solved earlier. This work demonstrates that the NBC approach can be used for directly encoding more than one combinatorial problem, and also shows that our technology could be significantly scaled up. In future, our approach could be used in solving real world ExCov problems like designing of apartments and electric circuits and also solving puzzles like Sudoku and Rubik's cubes.

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Keywords: parallel computation, molecular motors, biocomputation, NP-complete

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Poster Session 3, Tuesday 13 April, 15:20 - 16:40 UTC

Track on DNA Nanostructures: Semantomorphic Science

P3.01 DNA-nanostructure-directed precise silica mineralization in solution

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DNA origami technique provides an efficient strategy for engineering of well-defined complex nanostructures with arbitrarily prescribed shapes and rich types of hierarchical organizations [1]. DNA structures have been proposed as templates, molds and masks for nanofabrication. However, the applicability of DNA origami-based assemblies and devices is often restricted by the limited compatibility with the biologically relevant fluids and common solvents employed in nanofabrication. Recently, silica mineralization has attracted significant interest as a protection strategy for the stability enhancement of DNA origami-based assemblies, and as an approach for transferring unprecedented control over the nanoscale morphology offered by the DNA origami technique into inorganic materials. Despite important advancements, achieving controlled silicification in solution with well-dispersed nondeformed silica coated DNA origami structures has remained challenging. Here, we demonstrate an approach that enables fabrication of DNA origami@SiO₂ nanostructures via well-established silica surface chemistry (Fig. 1). Our method combines tailored response to environmental changes and precursor concentrations with the growth of silica on 3D DNA origami structures. Well-dispersed DNA origami@SiO₂ structures in solution were achieved with uniform coating and ultrathin silica shells [2]. Furthermore, after the coating the DNA origami@SiO₂ structures were stable in water and in polar organic solvents and resisted nuclease-mediated degradation. We anticipate that our results will aid further advancement of DNA origami techniques as nanofabrication method.

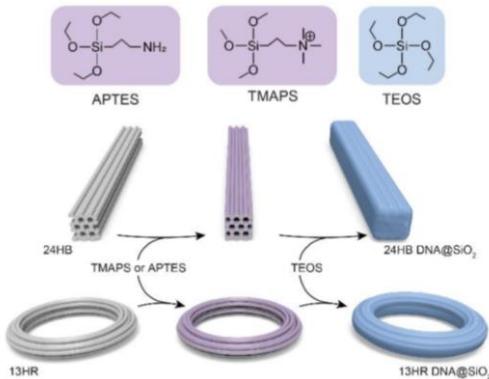


Fig. 1. Schematic illustration of the silica encapsulation of DNA origami structures.

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P3.02 Origami dimerization mediated by the fluorous effect

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The creation of fully addressable large-scale DNA origami ensembles is essential for opening up the complexity and utility of origami-based devices. Recent efforts to scale up the size of DNA origami constructs has focused on the use of longer scaffold strands, or with ssDNA overhangs (sticky-ends) joining origami subunits together in a multi-step fashion [1,2].

In this work we describe a new method to attach origami together. Staples along the edge of an asymmetric origami nanotile were modified to include perfluorinated compounds (fluorous ponytails). The interaction between fluorous ponytails, known as the fluorous effect [3], acts orthogonally to Watson-Crick base pairing, forming non-covalent linkages. When all edge staples were modified to contain fluorous ponytails on the 5' end, the majority of structures observed in Agarose gel and AFM appeared to be non-monomeric.

We envisage that the substitution of sticky-ends with fluorous ponytails can be used to reduce the number of unique sticky-ends required to form strong linkages, thereby reducing the chance of crosstalk to occur as well as simplifying the design process of dimer-formation. This work demonstrates that non-DNA methods can be used for not just high-efficient origami assembly, but also achieving reliable, specific hierarchical assembly with the combination of sticky-ends hybridization.

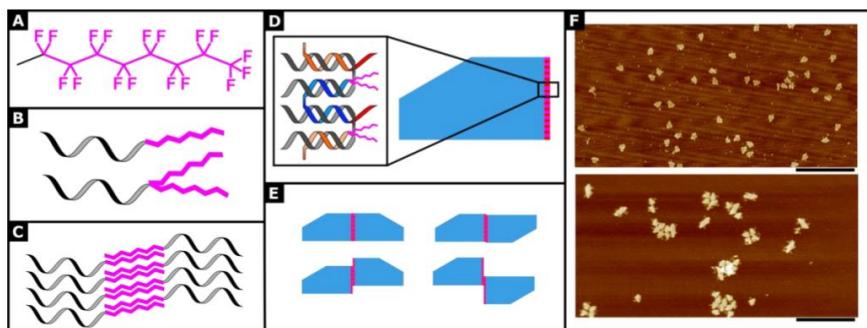


Fig. 1. (A) a C8F17 chain (fluorous ponytail); (B) Modified oligos containing single and branched fluorous ponytails; (C) Interactions between fluorous-modified oligos, demonstrating the interaction that occurs as a result of the fluorous effect; (D) Staples along the edge of origami were made to contain a branched fluorous ponytail on the 5' end, and four T's on the 3'; (E) Potential interactions and resulting homodimers that can arise from fluorous-modified origami; (F) AFM images of non-Rf origami (top) and branched-Rf origami (bottom). Scale bar is 800 nm.

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P3.03 Modulation of DNA Crystalline Triangle Lattices by the Extension and Insertion of Double Helical Segments

Karol Woloszyn¹, Yinglun Ma¹, Eric Zhu¹, Daniel Chernovolenko¹, Qiuyan Huang¹, Simon Vecchioni¹, Xinyu Wang¹, Yoel Ohayon¹, Jens J. Birktoft¹, Ruojie Sha¹, Chengde Mao² and Nadrian Seeman¹

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One of the main objectives of the field of DNA Nanotechnology is to design and form 3D DNA crystalline lattices to serve as hosts for various macromolecules.¹ Watson-Crick base pairing² implies that with careful sequence design considerations, synthetic DNA structures can be programmed to selfassemble in a designed fashion.¹ The basis of this work is a structure called the tensegrity triangle,³ a rigid DNA motif with pseudo-three-fold rotational symmetry, consisting of three double helices whose helical axes are linearly independent. These double helices are connected pairwise by Holliday-like junctions, and each helical terminus is capped with short single-stranded oligonucleotide segments, termed “sticky ends,” that allow the structure to propagate in 3D. These structures have been previously modified to include covalently fused triangles,⁴ torsionally-strained variants,⁵ and strand terminus modifications.⁶ Here, we expand the library of accessible motifs through symmetric (all triangle edges modified equally) and asymmetric extensions of inter-triangle regions, as well as linkages of adjacent triangles with double helical segments. Our modifications centered around a starting 2T7 tensegrity triangle; a motif with an edge length corresponding to two turns of DNA (one helical turn consists of 10.5 base pairs) with 7 base pairs for the inter-junction distance within a triangle. This inter-junction region was kept fixed at 7 base pairs while edge lengths were elongated through either extension or insertion; this design, albeit less thermodynamically stable, leads to larger cross-sectional areas and cavity sizes in their lattices – necessary for the harboring of larger macromolecules. We provide a design for 3 turn and 4 turn tensegrity triangles with 7 base pairs per inter-junction region through extension of inter-triangle regions (3T7 and 4T7, respectively), as well as analogous 3 turn and 4 turn triangles through sticky end insertion of 10 and 21 base pair duplexes, respectively, between adjacent 2T7 triangles (2T7+10 and 2T7+21). We also investigate asymmetric modifications: a 223-turn triangle, in which one edge



consists of 3 turns, a 224-turn triangle, in which one edge consists of 4 turns, and a 234-turn triangle, in which one turn consists of 2 turns, another 3 turns, and the last 4 turns. We report the successful formation of rhombohedral crystals that diffract to 7.5 Å for the 3T7, 7.9 Å for the 2T7+10, 11.1 Å for the 4T7, 11.9 Å for the 2T7+21, 5.6 Å for the 223, and 5.7 Å for the 224. Crystal formation and optimization is still ongoing for the 234 motif. We observe similar unit cell dimensions and volumes between the two analogous 3-turn (3T7 and 2T7+10) and 4-turn (4T7 and 2T7+21) triangle motifs, with the duplex-linked variants providing slightly larger cross-sectional areas and cavity sizes, while the asymmetric variants are in agreement with theoretical expectations. We are optimistic that this work will allow for the creation of larger and more complex 3D DNA crystal lattices to serve as the scaffolding for diverse guest molecules.

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P3.04 Annealing of DNA Origami-Templated Gold and Tellurium Nanorods Coated with a High Heat Resistance Polymer to Fabricate Metal-Semiconductor Junctions

Basu R. Aryal, a Dulashani R. Ranasinghe, a Chao Pang, a John N. Harb, b Robert C. Davis, c and Adam T. Woolley

Brigham Young University, USA

Over the past few years, metal and semiconductor nanorods have been synthesized and attached to DNA origami substrates in a controlled way to generate conductive heterostructures with an aim to fabricate functional DNA-mediated electronic nanodevices; an anticipated alternative bottom-up approach against the contemporary expensive and complicated top-down nanotechnology.¹ Semiconducting materials, a critical component of modern electronic devices, have been integrated progressively into the fabrication. To expand current DNA nanofabrication and provide a fundamental framework for the creation of electronic nanodevices, we have developed a unique annealing method² to connect gold and tellurium nanorods without electroplating, on a single DNA origami template by coating using a high heat resistance polymer.

Ribbon-shaped bar DNA origami templates (410 nm x 17 nm, Fig. 1A) were seeded with Au nanorods (45 nm x 9 nm) and Te nanorods (72 nm x 20 nm) site-specifically in an alternating fashion to make Au Te-Au structures (Fig. 1B). The DNA origami templated Au-Te-Au structures were coated with polymer and then annealed at different temperatures. At 170 °C, Au and Te nanorods appeared connected (Fig. 1C). Both Au and Te have melting points above 400 °C, but we expect that the junctions were established primarily due to atomic mobility of gold. Electrical characterization of Au-TeAu assemblies revealed nonlinear current-voltage (I-V) curves, and example of which is



provided in Fig 1D. These nanostructures formed simply through controlled seeding and annealing on DNA origami templates have potential to yield complex electrical nanodevices in the future.

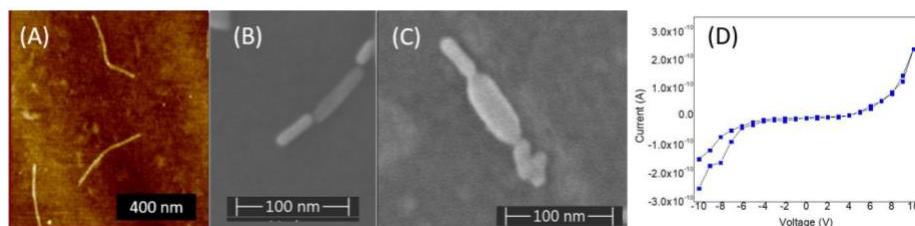


Fig. 1. Fabrication of Au-Te junctions: (A) bar DNA origami, (B) Au & Te nanorods on bar DNA, (C) connected Au & Te nanorods after annealing, and (D) I-V curve.

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- [2] Westover, T. R.; Aryal, B. R.; Ranasinghe, D. R.; Uprety, B.; Harb, J. N.; Woolley, A. T.; Davis, R. C., Impact of Polymer-Constrained Annealing on the Properties of DNA Origami Templatized Gold Nanowires. *Langmuir* **2020**, 36 (24), 6661-6667.

P3.05 A Hoberman Flight Ring Made of DNA Origami

Ruixin Li, Haorong Chen, and Jong Hyun Choi

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Deployable configurations are finite structures that preserve their overall shapes during expansion and contraction. Their structural transformation is unique with strong auxetic behaviors. This means that the Poisson's ratio for the reconfigurable structures is negative. There are multiple such examples. A Jitterbug transformer is one of them. Its eight rigid equilateral triangles are linked at vertices, and the triangles can rotate around the linkages. This mechanism enables deployable reconfigurations between octahedron and cuboctahedron. The Hoberman sphere is another example. It is a commercially available, popular toy for kids. The Hoberman sphere is made of rigid plastic edges with flexible linkages. It can shrink and expand by scissor-like actions at the joints, conserving the overall spherical shape. In nature, a particular type of virus (cowpea chlorotic mottle virus) is reported to be a deployable structure. In this work, we constructed a Hoberman flight ring using DNA origami and studied its properties.¹ The Hoberman flight ring is a simplified 2D version of Hoberman sphere and is a deployable structure.

For our Hoberman flight ring, we designed six equilateral triangles arranged in two layers as shown in Figure 1. The triangles are made of a topologically routed scaffold strand stabilized by a set of staple strands, together forming a trefoil knot. The nanoscale DNA flight ring can switch between two distinct states (open and closed) by sliding the triangles against each other via two-step DNA reactions (toehold-mediated strand displacement and reannealing). We monitored dynamic reconfigurations using AFM imaging and measured the relevant negative Poisson's ratios. The DNA flight ring shows the unique features of both Jitterbug transformers and Hoberman structures: (i) equilateral triangles as elements and flexible linkages at the vertices and (ii) two slidable layers.



This work provides detailed insights into the topological assembly and deployable reconfiguration of DNA structures. The deployable and auxetic nanostructures may serve as a versatile platform for topological studies and open new opportunities for bioengineering and biosensors.

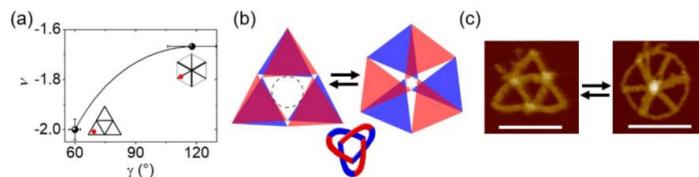


Figure 1. (a) Poisson's ratio versus angle γ (indicated by red dot). (b) Flight ring in open and closed states. The three red triangles are on top of the blue triangles. The geometrical representation (bottom) of the structures shows that the flight ring is a trefoil knot. (c) AFM images of a flight ring at two different conformations (angles). The structural reconfiguration is completed by two-step DNA reactions. Scale bar: 100 nm.

- [1] Li, R., Chen, H. & Choi, J. H. Topological Assembly of a Deployable Hoberman Flight Ring from DNA. *Small*, 17, 2007069 (2021).

P3.06 Computational Studies of DNA Origami Tile Cyclization

Ruixin Li, Haorong Chen, Hyeongwoon Lee and Jong Hyun Choi

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The DNA nanotechnology has emerged as a versatile method to synthesize complex nanostructures with high precision thanks to structural predictability and programmability. The method has been used for building static architectures, using DNA tiles and bricks. Dynamic structures, such as DNA tweezers, switches, and walkers, are also available based on toehold-mediated strand displacement reaction. DNA origami has been explored for both static and dynamic applications. While DNA origami has been a popular method, related mechanics are still under active investigation. For example, the mechanics of a DNA origami structure include the arrangement of double stranded DNA (dsDNA) bundles, the interconnection between the bundles by crossovers, and interaction between ds-regions within an origami structure. It is also interesting to determine the energy needed to move or deform a DNA origami construct.

Here we investigated the mechanical energy needed to cyclize a DNA origami tile. We used a simple, single-layer rectangle in square lattice as a model system. We intentionally designed the tile and arranged the crossovers such that it has an inherent twist in its equilibrium conformation. The square lattice tile serves as a platform to study the structural properties and energy needed to overcome the initial curvature and induce mechanical deformation (cyclization). In a previous report,[1] experiments were performed and theories were developed to determine the energy for cyclization of a tile. In this work we performed computational studies to elucidate related mechanical energy for cyclization with a quasiequilibrium deformation assumption. The computations were performed with a coarsegrained molecular dynamics (MD) model in oxDNA platform (Figure 1). We found that (i) the initial curvature may be overcome gradually from the initial to the last stage of cyclization and that (ii) the energy associated with the cyclization matches with experimental and theoretical results.

This work shed insights into the deformation details of a DNA origami structure and the necessary mechanical energy. A better understanding of quasi-equilibrium deformations could be useful for studying energy driven processes for adaptable, reconfigurable, selfhealing structures made of DNA origami.

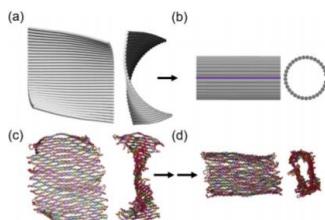


Figure 1. (a) CanDo simulation results of a DNA origami tile which is designed in square lattice (front and side views). Since the designed helicity (10.67 bp/turn) is slightly over the natural helicity (10.5 bp/turn) in the simulation, the tile shows a twisted curvature. (b) Schematics of the cyclized DNA origami tile in both views. The purple line indicates the linkers that seal the upper and lower boundaries. (c) Coarse-grained MD calculations of a DNA origami tile in front and side views. (d) MD simulation results of the cyclized DNA origami tile through a series of quasi-equilibrium deformations.

- [1] H. Chen, T. W. Weng, M. M. Riccitelli, Y. Cui, J. Irudayaraj, J. H. Choi, *Journal of the American Chemical Society* **2014**, 136, 6995-7005.

P3.07 3D Arrangement of DNA Tensegrity Triangles Within a Hexagonal Lattice Using Mismatched Sticky Ends

Brandon Lu¹, Simon Vecchioni¹, Yoel P. Ohayon¹, Ruojie Sha¹, Chengde Mao² and Nadrian C. Seeman¹

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The tensegrity triangle is a DNA nanostructure with helices that propagate in three dimensions, self-assembling to create a rhombohedral crystal lattice using complementary sticky end overhangs. Different combinations of complementary 2-, 3-, and 4-nucleotide sticky ends have formed crystals in the R3 rhombohedral space group. A noncomplementary 5'-AG and 5'-TC mismatches in the sticky end instead results in large, needle shaped crystals in the P6₃ hexagonal space group. Diffraction data were collected to 5.49 Å and solved using molecular replacement. We hypothesize that the hexagonal shape of the lattice is due to the DNA double helix bending at a crossover position, a departure from the linear double helices in the rhombohedral tensegrity triangle design. Rather than propagating in a straight line in its given dimension, each double helix twists in a left-handed meta-helix-like structure in that direction. The hexagonal lattice has an estimated volume of 900,000 Å³, which is triple the volume of the rhombohedral unit cell of 300,000 Å³. Individual base pair analysis indicates consistency in the base pairing within the R3 and P6₃ tensegrity triangles, with the main difference being the nonlinearity of the helices.

The small shift in sticky end sequence underscores the huge alteration in the phenotype of the crystal structure. A sequence change at the very end of the motif results in perturbation throughout the helix, highlighting the importance of the sticky end regions of self-assembling DNA structures. Based on conventional wisdom, the G-T and A-C mismatches should not allow for the continued propagation of the triangles in each direction. However, this pattern appears to be stable. Throughout multiple runs, solely hexagonal crystals formed, indicating the stability and predictability of this design. The ability for mismatched sticky ends to allow for cohesion between triangles is a novel discovery in a field where a single mismatch can disrupt structural formation due to the inability to hydrogen bond. This sheds light on the viability of predictable non-Watson-Crick interactions for designing more diverse structures.



The possibility of varying overall crystal phenotype simply by changing sticky end sequences further increases the flexibility of rationally designed DNA nanostructures in both structure and function. For example, the increased cavity size compared to its rhombohedral cousin allows for incorporation of larger guest molecules. Prior hexagonal DNA lattices have been used as solvent channels due to the stacked nature of the hexagons. Further research is currently underway on determining the sequence specificity of the mismatched sticky ends as well as testing the application of mismatched sticky ends to larger structures. In this way, we aim to investigate the usefulness of non-Watson-Crick pairing in controlling long-range architectures in topological self-assembly, and further create a library of mismatched sticky ends to increase the syntactic diversity of semantomorphic design.

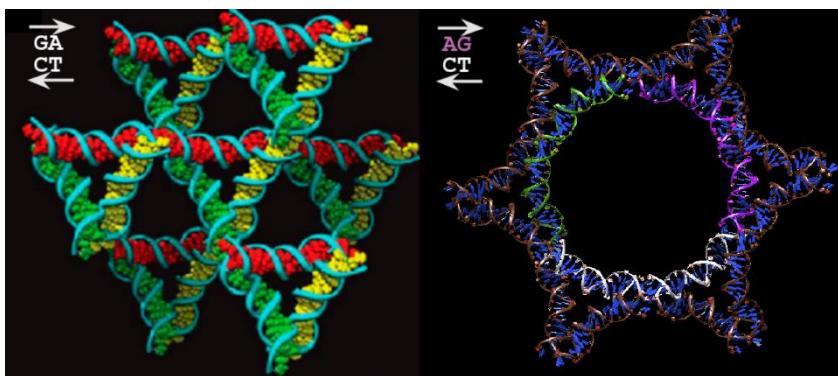


Fig. Comparison between R3 (left) and $P6_3$ tensegrity triangle (right) models. Notice the straight helices in the R3 model and the bent helices in the $P6_3$ model. Similarities in structure can be found in the center of each individual triangle. The only difference in design between the designs lies in the twomer sticky end mismatch.

P3.08 Self-assembly of a DNA Ring Motif with Programmable Flexibility and Connectivity

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¹Tohoku University, Japan, ²Ochanomizu University, Japan

Programmable self-assembly has become an important field in structural DNA nanotechnology. In recent years, a trend of using DNA origami as a motif for self-assembly has emerged. However, it is challenging to modulate the morphology of self-assembled structures. To build self-assembled structures with selectable morphology, we propose a self-assembling motif whose shape, connectivity, and flexibility are programmable [1]. The motif, which is fabricated using the DNA origami technique, is a closed-ring structure composed of multiple joints. The shape of the ring motif can be controlled by fixing the joints at different positions (Figure 1a). We observed the motif in the shape of heptagon and two different triangles by AFM (Figure 1b). The segments of the motif can be selected as connectable or unconnectable state. By turning the segments into connectable state at different locations, it is possible to obtain different self-assembled structures (Figure 1c). The flexible DNA ring motifs in different shapes can self-assemble into various structures. We observed the self-assembly of the motif with different combinations of shapes and connectivity by AFM (Figure 1d). Structures like dimers, linear and circular multimers were observed, indicating the versatility in self-assembly of the motif.

The flexible DNA ring motif provides a novel but simple principle to form self-assembled structures in a programmable manner. In the future, this method has a potential to realize self-assembly with higher complexity on a larger scale.

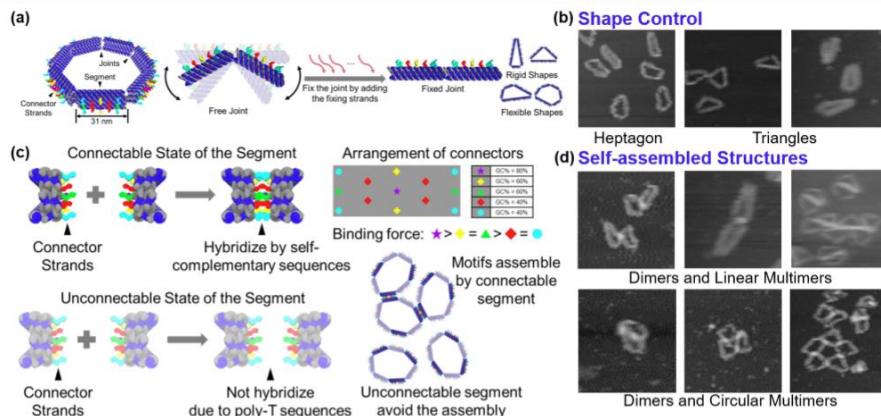


Figure 1. (a) Schematic of the deformation caused by the flexible joints of the DNA ring motif. The motif can take different shape by fixing the flexible joints at different positions; (b) AFM images of heptagonal motif and two kinds of triangular motifs. The shape of the motif can be adjusted by fixing the joint at different positions; (c) Schematic of the hybridization of the connectors on the segments of the DNA ring motif. The motifs can assemble with each other by the hybridization of the connectors; (d) AFM images of the self-assembled structures. Adjustment of the numbers and positions of flexible joints and joinable segments resulted in various self-assembly of the DNA ring motif.

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P3.09 Automated solid phase synthesis of DNA nanostructures

Casey M. Platnich¹, Yasser Gidi¹, Lorianne Ginot^{1,2}, Ryan Karimi^{1,3}, Gonzalo Cosa^{1*}, Hanadi F. Sleiman¹

¹ McGill University, Canada, ² Ecole Normale Supérieure de Lyon, France, ³ University of British Columbia, Canada

Automated DNA and peptide synthesis have revolutionized the field of chemical biology, with these solid-phase methodologies providing control over both the length and sequence of these complex biopolymers. In contrast, self-assembled systems are typically prepared in solution, with little control over the size dispersity and subunit stoichiometry. To generate new classes of supramolecular materials, new methods to control and modulate self-assembly are required. Inspired by the phosphoramidite synthesis of DNA, we have developed a fully automated platform for the synthesis of monodisperse DNA nanostructures. Using solid phase methods reminiscent of covalent strategies for making sequence-defined polymers, we incorporate the supramolecular subunits serially, allowing for complete control over the number and sequence of complementary building blocks.

We begin by grafting a “foundation rung” to a glass surface. Using a microfluidic pump system and purpose-built software, we then sequentially add the repeat units of the nanotube in a fully automated fashion, generating sequence-defined nanostructures built to the user’s specifications. By tagging each unit with a fluorescent label, single-molecule fluorescence methodologies can be used to characterize the nanotubes one at a time, allowing for the identification of heterogeneities in assembly and defect structures. Single-molecule approaches are also used to measure the kinetics of the different assembly steps, allowing for the optimization of our synthesis protocols. Overall, these methods enable the synthesis and detailed characterization of monodisperse, sequence-controlled nanostructures suitable for a variety of functions, including sensing applications.

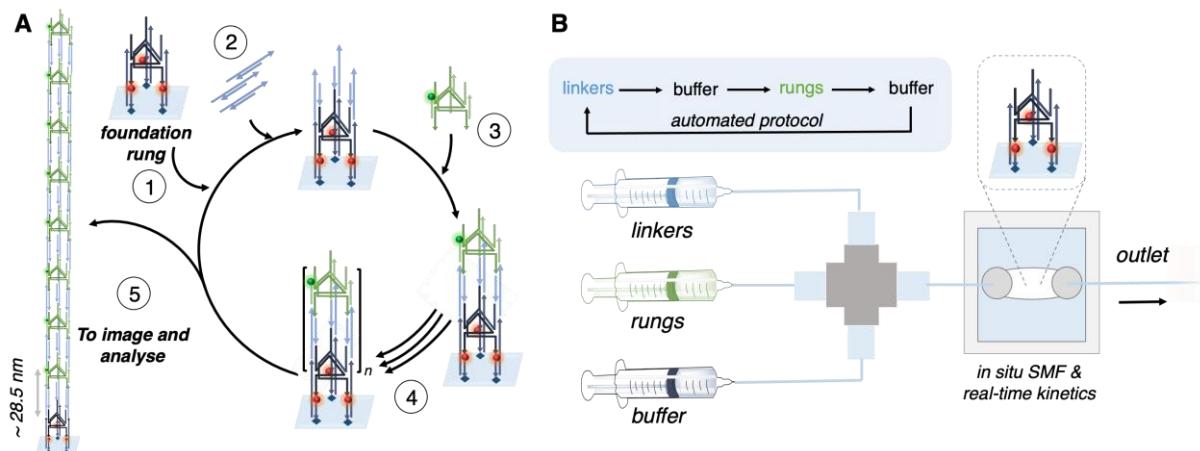
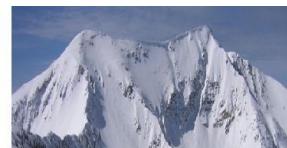


Figure 1. Summary of automated synthesis protocols. (A) Constructing a DNA NT step-by-step, with the number of cycles dictating the final NT length. (B) Schematic of prototype DNA NT synthesizer. Rungs, linkers, and buffer are pumped onto the glass coverslip in a time-programmed sequence, enabling control over length and sequence in the final assembly.

P3.10 Metal-Mediated Self-Assembling 3D DNA Crystals

Simon Vecchioni,¹ Ruojie Sha,¹ Brandon Lu,¹ Yoel Ohayon,¹ Lynn Rothschild,² Chengde Mao,³ Shalom Wind,⁴ Nadrian Seeman¹

¹New York University, USA; ²NASA Ames Research Center, USA; ³Purdue University, USA; ⁴Columbia University, USA

Since its inception, a primary goal of structural DNA nanotechnology has been to act as a templated scaffold for the crystallographic analysis of biomolecules. In this study, we utilize designer crystals to build and study a library of metal-mediated DNA (mmDNA) base pairs within a DNA lattice. By bridging sequence mismatches between six pyrimidine nucleobases using Ag⁺ and Hg²⁺ ions, and through the direct application of x-ray diffraction to these lattices, we establish the use of DNA nanotechnology as a means of elucidating fine-grain structural details in nucleic acid chemistry development. Using the well-studied tensegrity triangle (PDB: 3GBI, 5W6W), we introduce one mmDNA pair between junctions on each triangle edge (Fig. 1), and assemble crystals that diffract into space group R3 with resolutions between 2.95-4.65 Å.

These structures are phased and analyzed using single-wavelength anomalous dispersion from the designed metal ion sites. We identify and solve structures of over 20 base pairs using cytosine-like pyrimidines (deoxycytosine, 5-methyl-deoxycytosine, and 5-methyl-deoxy-isocytosine) and thymine-like bases (deoxythymine, deoxyuracil, 5-iodo-deoxyuracil). We investigate the effect of different pyrimidine functional groups on the success and subsequent shape of metal base pairing, and we describe the rotational effects of metal coordination inside the double helix. This study demonstrates the precise assembly of metallic species inside DNA architectures and is a step toward the topological self-assembly of electrically-active DNA nanomaterials.

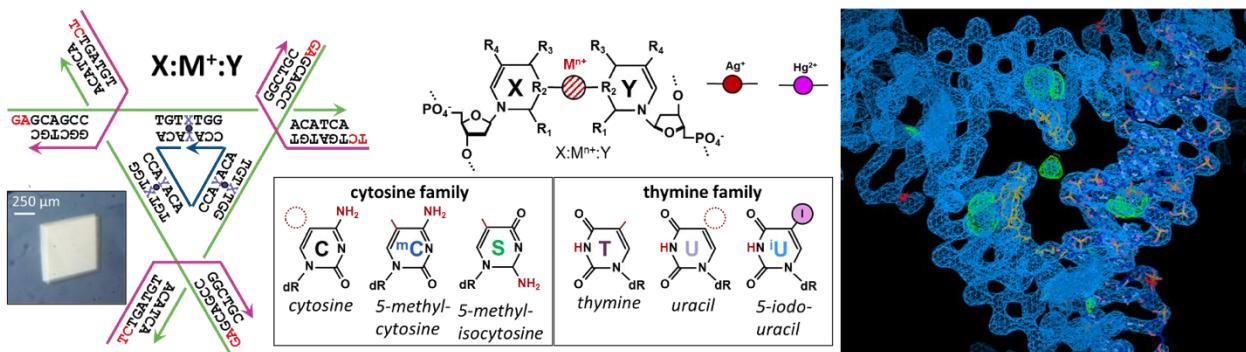


Figure 1: Scheme of the metalated DNA triangle. **A)** Modifications to 3GBI and 5W6W occur at the center of each triangle edge with a pyrimidine mismatch, X:Mⁿ⁺:Y. **B)** Design of the generalized metal base pair, the six pyrimidine nucleobases, and two metal ions utilized in this study are shown, along with **C)** electron density of C:Hg²⁺:T at 3.68 Å (crystal inset, A).

P3.11 Minimalist Strategies for Size Defined 2D and 3D DNA Nanostructures

Daniel Saliba, Tuan Trinh, Xin Luo, and Hanadi Sleiman

McGill University, Canada. H3A 0B8

Standard solid phase synthesis of DNA limits the length of DNA strands to ~ 150 bases. The Sleiman group has recently developed the process of sequential growth, which allows the facile preparation of extended DNA strands with full control over length (e.g., 2000 bases) and sequence^{1,2}. Using this strategy, we generated different monodisperse backbones with a range of sizes and with repetitive patterns of sequence. The repetitive domains offer simplicity, as only a few DNA strands are needed to assemble a relatively large structure, versus the hundreds of strands necessary for the assembly of DNA origami. These backbones have the potential of being mass produced, in a cost-efficient manner, using bacteriophage to generate single-stranded precursor DNA, containing the strand of interest incorporated within self-excising DNA domains³.

These backbones with repetitive domains are used in the asymmetric elongation of the unique arms of a “printed” branched DNA-small molecule motif, via polymerase chain reaction². The strategy allows full control over the addressability of our branched DNA nanostructure, offering deterministic positioning of different materials with control over their type, number, separation, and location. Also, the core of these branched units presents dynamic behavior that can induce the specific folding of these arms, via biotinstreptavidin interactions, into a potential 3D nanotube-like protein-DNA hybrid nanostructure.

Moreover, as sequential growth allows the easy preparation of extended DNA, an exploration of their use in producing more complex 3D nanostructures is examined, specifically in the assembly of 3D DNA nanotubes with length and sequence patterns that are pre-defined by the backbone sequence. It was found that different parameters affect the self-assembly and rigidity of the nanotubes including strength and flexibility of the connection points. Molecular dynamics simulations revealed a strong dependence of the nanotube’s rigidity on the sequence, length, and flexibility of the main building blocks of nanotube (Figure 1).

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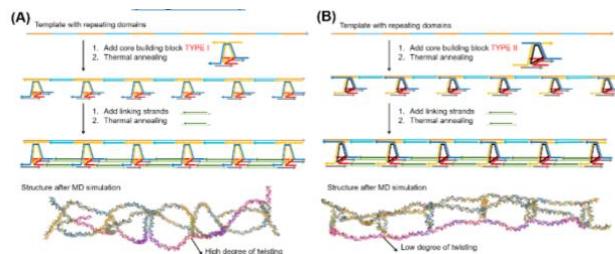


Figure 1. Schematic representation of nanotube design using a triangular rung unit having different designs, type I (A) and type II (B). Rung I resulted in a high twist on the rung corners while rung II showed enhanced rigidity.

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Poster Session 4, Tuesday 13 April, 18:00 - 19:20 UTC

Track on Principles and Theory of Self-Assembly

P4.01 Thermodynamics of DNA looping for origami folding

Jacob M. Majikes, Paul N. Patrone, Michael Zwolak, Anthony J. Kearsley and J. Alexander Liddle

National Institute of Standards and Technology, USA

An essential step to predicting the yield of DNA origami folding is a rigorous understanding of the thermodynamics of its unit process, the single fold, and the cooperative energetics that can influence that process. The entropic costs associated with large-scale looping play a key role in the folding of DNA origami, both at thermal equilibrium and when kinetics dominate folding. These costs can vary with changes in scaffold topology, and the dynamically evolving dsDNA content along the scaffold. By leveraging the high throughput of qPCR equipment, we examine the thermodynamics of these folds *via* van't Hoff analysis of melt curves.

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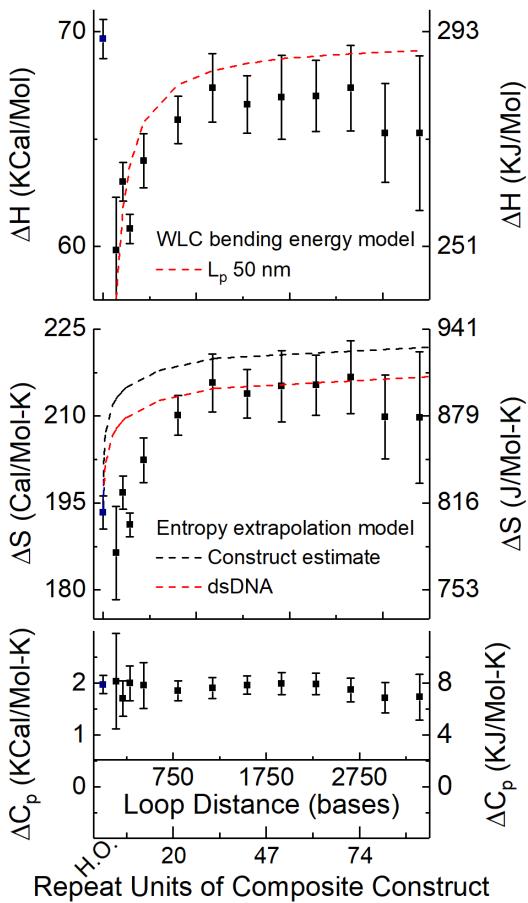


Figure 2. Single fold thermodynamics: Fitted ΔH , ΔS and ΔC_p . H.O. is a hybridization-only control sample.

We further examine the effect of staple excess and of molecular crowding agents, revealing unintuitive results in both cases. In contrast to whole origami, increasing staple excess for a single fold significantly reduces yield. Similarly, in contrast to expectations, molecular crowding conditions do not favor a folded state *via* a reduction in volume. We present these results and show how can inform an understanding of whole-origami systems.

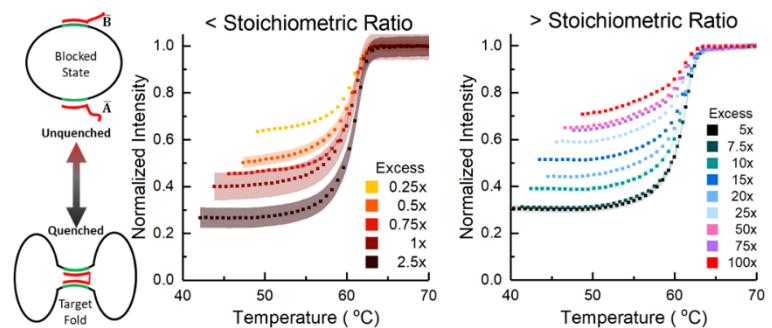


Figure 3. Single fold yield: Reporter intensity as a function of temperature and staple excess for a 3407 base fold. Scaffold folding reduces reporter intensity while side product formation does not.

P4.02 Stability of the d(CGA) Motif: A Building Block for the Rational Design of pH Sensitive DNA Nanostructures

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University of Maryland, USA

Non-canonical DNA structures that retain programmability and structural predictability are increasingly being used in DNA nanotechnology applications where they offer versatility beyond traditional Watson-Crick interactions¹. One potential advantage of non-canonical structures are their sensitivities to the local environment, including cations, salt concentration, or pH, which allow them to undergo predictable structural changes in response to environmental



perturbations^{2,3}. The d(CGA) triplet repeat motif is a structurally dynamic non-canonical motif that can transition from parallel-stranded homo-base paired duplex to anti-parallel unimolecular hairpin in a pH-dependent manner⁴ (Figure 1). Characterization of the stability of this motif will provide constraints for the incorporation of this motif into the design of DNA-based pH-sensitive nanodevices.

In our work, we evaluate the thermodynamic stability and nuclease sensitivity of the d(CGA) motif and several structurally related sequence variants at near-physiological temperature and salt concentration. Thermodynamic parameters obtained from UV absorbance melting curves show that the structural transition resulting from decreasing the pH is accompanied by a significant energetic stabilization as unimolecular hairpin structures are converted to parallel-stranded homobase paired duplexes. Further, the relative stability of the parallel-stranded duplex can be dramatically impacted by the frequency and identity of the 5'-nucleobase of each triplet. Increased nuclease resistance against double strand-specific nucleases in the parallel-stranded duplex form relative to the anti-parallel form suggest that the parallel motifs may offer unique advantages for the design, assembly, and delivery of nanostructures in cellular environments. The solution stability data presented in this work can be used to strategically design and optimize sequences for use in the rational design of DNA crystals or other DNA-based nanotechnology applications that could benefit from pH-triggered structural switching.

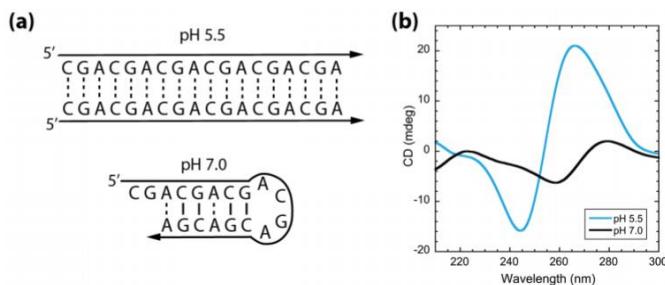


Figure 1. (a) d(CGA) triplet motif. (CGA)₆ is predicted to form a parallel-stranded homoduplex at pH 5.5 and anti-parallel hairpin at pH 7.0. Dashed lines represent non-canonical base pairing and solid lines represent canonical base pairing. (b) CD spectra of 10 μ M (CGA)₆ at pH 5.5 (blue) and pH 7.0 (black). At pH 5.5, the prominent positive band at 265 nm and negative band at 245 nm are consistent with parallel-stranded duplex formation. At pH 7.0, the positive band at 275 nm and negative band at 258 nm are characteristic of anti-parallel oriented strands.

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P4.03 Self-Regeneration and Self-Healing in DNA Origami Nanostructures

Michael Scheckenbach, Tom Schubert, Carsten Forthmann, Viktorija Glemboczyte, and Philip Tinnefeld

Ludwig-Maximilians-Universität München, Germany.

In the last years, DNA nanotechnology and DNA origami technique have enabled the facile design and high yield synthesis of complex and highly functional, self-assembling nanostructures.¹ Fundamentally though, such molecular devices are susceptible to rapid degradation and loss of their functionality due to the high proportion of surface atoms and molecules at the nanoscale.² Hence, autonomous or non-autonomous, *i.e.* externally triggered, self-



repair mechanisms are desirable. So far, approaches to stabilize DNA origami in challenging environments have mainly focused on static stabilization, *e.g.* by coating the structure or cross-linking the DNA backbone.³ In this contribution, we exploit the self-assembly nature and reconfigurability of DNA origami nanostructures to establish a route to dynamic self-repair mechanisms (Fig. 1A).⁴ By dynamically exchanging building units with intact analogues from solution, we were able to maintain the functions of brightness reference structures even after complete damage (Fig. 1B) and stabilize super-resolution nanorulers used for DNA PAINT over days (Fig. 1C). Based on the given examples, we illustrate the two possible self-repair mechanisms using this approach: the damage unspecific self-regeneration, which exchanges intact and defective building units, and the damage specific self-healing, *i.e.* the exchange of only defective building units. To investigate the properties of DNA origami at a single device level and to demonstrate the successful repair of the nanostructures we employ atomic force microscopy (AFM) and fluorescence super-resolution imaging methods.

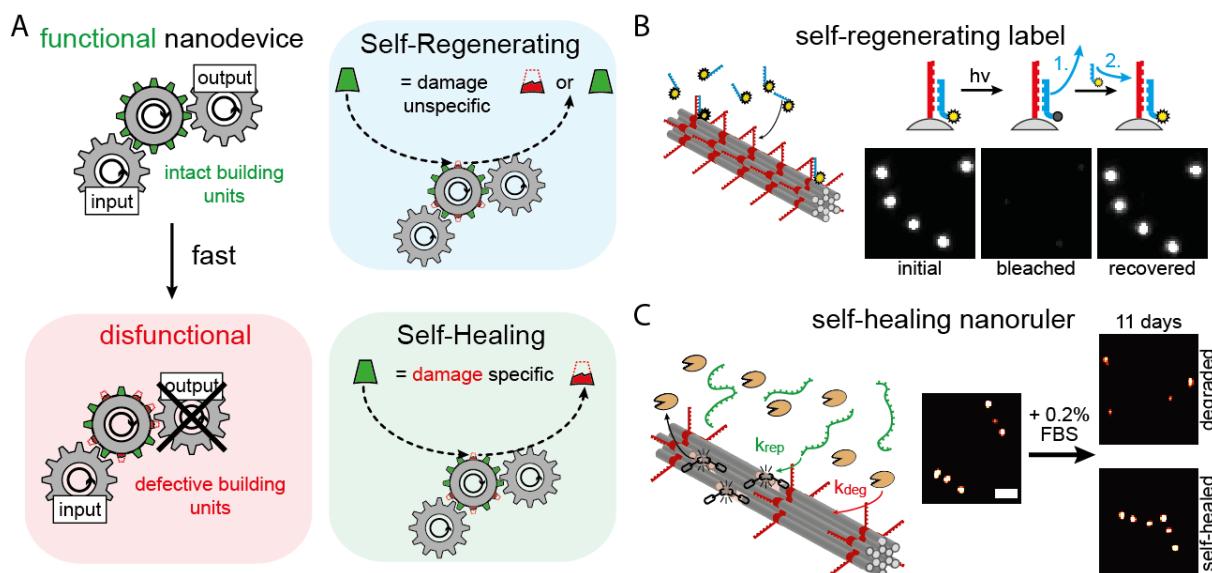


Fig. 1. (A) Schematic representation of a self-assembling molecular nanodevice as cogwheel with molecular building units represented by cogs, which are prone to fast degradation at the nanoscale. Steady-state exchange of random building units (intact and defective) with intact ones is defined as “self-regenerating”, while specific exchange of defective building units is defined as “self-healing”. (B) Unspecific exchange of brightness labels enables a self-regenerating brightness reference structure. (C) Exchange of defective staple strands in a degrading environment (fetal bovine serum) with intact staples from solution establishes a self-healing nanoruler with improved stability.

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P4.04 Thermosetting supramolecular polymerisation of compartmentalised DNA fibres with stereo-sequence and length control

Michael D. Dore¹, Tuan Trinh¹, Marlo Zorman², Donatien De Rochambeau¹, Pengfei Xu¹, Xin Luo¹, Jacob Remington², Violeta Toader¹, Jianing Li², and Hanadi F. Sleiman¹

¹McGill University, Canada, ²The University of Vermont , USA

DNA nanostructures are highly addressable and compatible with biological systems, but often require hundreds of unique strands for their assembly. On the other hand, nature can assemble complex structures from identical building blocks by compartmentalising the process: assembling molecules into sub-components and bringing these together across multiple length scales. Inspired by this process, we report DNA-polymer conjugates that assemble through a unique heat-driven hierarchical self-assembly mechanism to form fibres displaying blocks of different DNA sequences along their axis of polymerisation. Ions are manipulated to preassemble short cylindrical micelle ‘monomers’ that then non-covalently fuse at 90 °C. These one-dimensional ‘thermoset’ DNA-fibres retain the compartmentalisation programmed in the pre-assembled segments, allowing nanoscale organisation of gold nanoparticles. Length control over fibre segments is also achieved through gel purification of pre-assembled cylindrical micelles. Importantly, the DNA-polymers are sequence-defined, allowing us to show that stereochemical sequence of the hydrophobic core can be amplified into distinctive morphological traits in the DNA-fibres. Molecular dynamics simulations are used to model the structure of the supramolecular fibres and elucidate the microscopic mechanism of how stereochemical sequence dictates the folding of the DNA-polymers, manifesting in different fibre-fibre interactions.

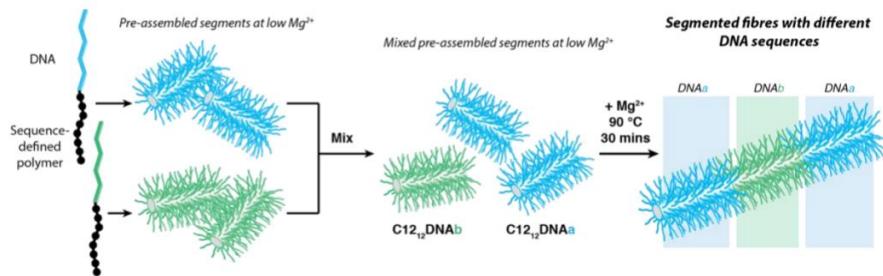


Fig. 1. The self-assembly of DNA-polymers is guided through a unique two-step process that allows stable cylindrical intermediates to be combined then subsequently fused together at high temperatures into long, segmented fibres. The cylindrical morphology is highly dependent on the DNA-polymer sequence due to the requirement for the polymer to assume a specific conformation.

- [1] Dore, M.D., Trinh, T., Zorman, M., De Rochambeau, D., Xu, P., Luo, X., Remington, J., Toader, V., Li, J., and Sleiman, H.F., Chem (currently under revision), (2021).



P4.05 Elucidation of a new complex pathway in 2D supramolecular polymerization of DNA-oligomer block copolymer amphiphiles

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McGill University, Canada

Synthetically accessible two-dimensional (2D) nanomaterials are of great interest due to their prospective applications in electronics, catalysis, and biotechnology, and as membrane materials. One method of accessing these topologically planar structures is through the supramolecular polymerization of amphiphilic block copolymers (BCPs) via self-assembly in a selective solvent. The use of DNA-oligomer amphiphiles as the BCPs for construction of supramolecular 2D nanoarchitectures offers the unique opportunity to both introduce functional cores in these structures as well as functionalize the surface of the 2D material via complementary DNA base pairing or blunt end interactions. These DNA-based 2D nanostructures have not been widely explored, and the few studied thus far have been limited by a lack of control over their shape and size.

Our group has previously demonstrated the morphological tunability of supramolecular architectures comprising self-assembled DNA-oligomer amphiphiles leading to nanospheres/micelles (0D) and nanorods (1D) [1,2]. Here we report a complex hierarchical growth mechanism of 2D nanosheets obtained through the self-assembly of synthetic amphiphilic DNA oligomers bearing π -conjugated chromophores in aqueous solution. We explore the effect of varying self-assembly parameters and amphiphile structure on the structural order and size of the nanosheets. The goal is to kinetically control the formation of the supramolecular architecture, allowing us to precisely tune the shape and size of the 2D assembly. This will enable the creation of synthetically accessible, well-defined, monolayered 2D DNA nanosheets – using a single type of block copolymer – that could potentially serve as economical alternatives to DNA-dense 2D DNA origami structures.

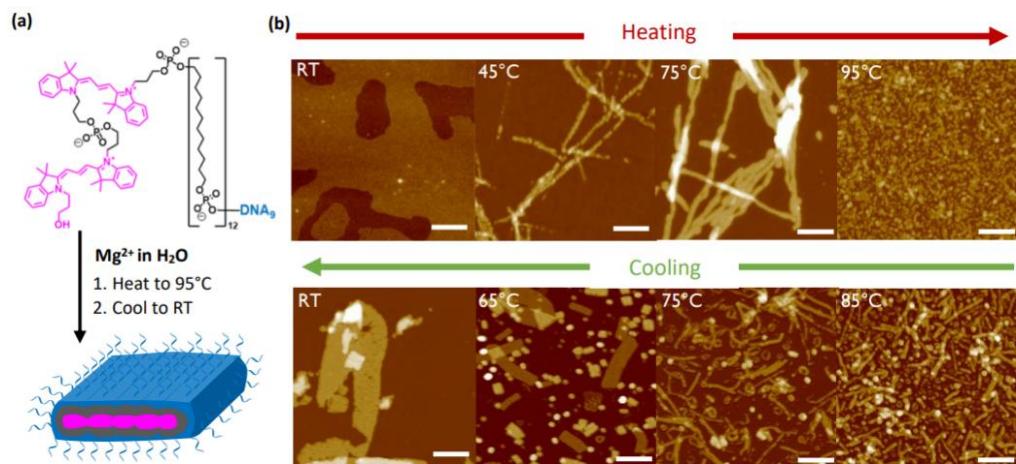


Fig. 1. (a) The block copolymer **Cy3₂-C12₁₂-DNA₉** ([2-Cy3]-[12-hexaethylene]-[9-mer DNA] sequence) undergoes cation-mediated self-assembly in aqueous solution upon annealing to form 2D nanosheets. (b) AFM images showing the kinetically trapped morphologies obtained during thermal annealing and cooling of **Cy3₂-C12₁₂-DNA₉** en route to the thermodynamic nanosheet structure. Scale bars: 250 nm.

- [1] T. G. W. Edwardson, et al., Angew. Chem. **126**, 4655–4659 (2014)
- [2] D. Bousmail, et al., J. Am. Chem. Soc. **140**, 9518–9530 (2018)



P4.06 Nano-sandwich composite by kinetic-trapping assembly from protein and nucleic acid

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Design and preparation of multi-layered composite materials have been a major challenge in the field of nucleic acid nanotechnology due to the limitations in geometry of interaction sites and the lack of efficient synthesis strategies. Here, we designed a homo-tetramer protein, streptavidin, as a core to precisely position co-parallel nucleic acid nanotriangles in a sandwich architecture. We developed a kinetically controlled multi-step synthesis protocol that facilitated the efficient formation of the first RNA-DNA-protein composite. The 3D architecture of the complex was confirmed by cryo-electron microscopy. The synthesis strategy for the nano-sandwich composite, which exploits kinetic trapping of the desired architecture through rapid self-assembly of the nanotriangle components, provides a general blueprint for controlled noncovalent assembly of complex supramolecular architectures from protein, DNA and RNA components. The design and synthesis approach described here expands the repertoire for bottom-up preparation of layered biomaterials and opens a door for the assembly of nucleic acid-protein composite extended materials.

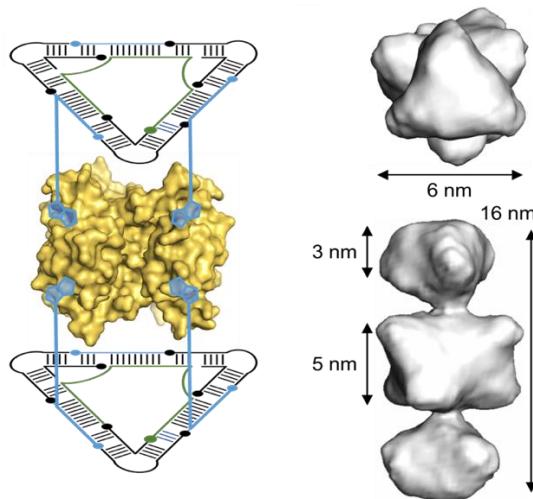


Figure 1. Design and cryo-em reconstruction of RNA-DNA-Protein composite sandwich.

P4.07 Designing Polycube Assembly Rules using SAT

Joakim Bohlin¹, Petr Šulc², Andrew J. Turberfield¹, and Ard. A. Louis¹

¹University of Oxford, UK, ²Arizona State University, USA

There is an increasing interest within the field of DNA nanotechnology to create finite-sized multicomponent objects. We present an interactive platform for designing a minimal set of components that will reliably assemble into a target 3D object.

When designing a self-assembling structure, there are usually many different assembly rules you can use. One bound would be the fully addressable assembly (Figure 1.d), where every building block and interface is unique.



The other extreme would be the minimal assembly rule (Figure 1.b), where you use as few interface and block types as possible.

The method presented here, derived from [1], determines if a provided polycube structure is satisfiable for a given number of cube types N_t and colours N_c , allowing the user to find the minimal assembly rule and compare it with alternative solutions.

The self-assembling structures are modelled as polycubes, clusters of cubes connected by torsional patches. An assembly rule consists of the set of available cube types, where each cube face can have a coloured patch. To assemble a rule, cubes from the input set are stochastically added wherever their patches have compatible colour and correct orientations, see Figure 1.a. Figure 1.b, 1.c and 1.d show three different rules assembling the same 2×2 polycube.

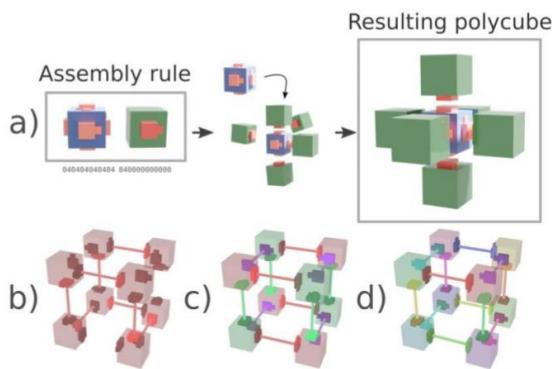


Fig. 1. (a) Polycube assembly process. An assembly rule with $N_c = 1$ and $N_t = 2$ assembled into a heptacube. No more cubes can be added after the sixth green cube, so the assembly terminates. (b) Minimal assembly rule, $N_c = 1$, $N_t = 1$, has correct shape mismatched connections. (c) Minimal assembly rule with proper connectivity $N_c = 3$, $N_t = 2$ (d) Fully addressable assembly $N_c = 12$, $N_t = 8$. The minimal assembly rule can be found by ruling out any smaller combination of N_c and N_t as unsatisfiable. Furthermore, since a satisfiable solution might still form additional structures, the rule is repeatedly assembled to confirm determinism. For example, while solutions can be found with a smaller N_c than in Figure 1.c, those solutions do not assemble deterministically. Finally, we present an online tool for designing and solving polycube structures, found at <https://akodiat.github.io/polycubes/solve>. While smaller polycubes can be solved directly in the web browser, we recommend using an external SAT solver for larger shapes.

[1] F. Romano, J. Russo, L Kroc, P. Šulc, Phys. Rev. Lett. 125, 118003 (2020).



P4.08 Arbitrary Placement of Heterogeneous Species on a Single Substrate Enabled via Engineered Steric Brush Interactions

Marcello DeLuca¹, Yunqi Yang¹, Ashwin Gopinath², Stefan Zauscher¹ and Gaurav Arya¹

¹Duke University, USA, ²Massachusetts Institute of Technology, USA

Most DNA origami (DO) devices float freely in solution, which limits their usefulness. For instance, in the area of biomolecule detection, spatially registered arrays of devices capable of detecting specific DNA sequences and proteins could enable simultaneous screening for many biomolecules with a single fluorescent signal type; this would require organizing these devices in space. Recent progress has allowed us to place one species of DNA nanodevice on a substrate with arbitrary orientation,^{1,2} but precisely placing multiple species on a single substrate is not currently possible. At the previous FNANO meeting, we proposed a technique that should allow for the precision placement of two species on a single substrate using lithographically defined binding sites. The technique involves designing the species to have a size discrepancy, adding repulsive brushes to the exterior of the smaller species to prevent them from “clogging” the larger binding sites, and introducing the smaller species first to prevent the larger species from occupying smaller binding sites. Using Brownian dynamics simulations and energy landscape analysis, we demonstrated the feasibility of this approach and developed the set of design conditions satisfying these requirements. However, this technique has not been experimentally validated and does not provide control over the rotational orientation of species. In this presentation, I will describe our recent experimental efforts to demonstrate this two-species placement concept. We fabricated circular DO tile devices and grew poly-thymine grafts from their exterior boundaries; these devices were populated onto a silica surface and imaged using AFM. The repulsive interactions of the brushes, one of the conditions for achieving two-species placement, were quantified using a combination of image analysis and Boltzmann inversion of the measured radial distribution function of the DO tiles. We also conducted Monte Carlo simulations of hard spheres to remove osmotic pressure effects, allowing us to derive the true brushrepulsion interactions. Finally, by carrying out coarse grained molecular dynamics simulations of these devices, we show that additional grafts on the top surface would further enhance the repulsive effects of the brushes. Finally, we have integrated the approach of Gopinath et al.,² wherein symmetry is broken using a “small moon” pattern for both species to allow them to be placed with absolute and arbitrary orientation on the surface.

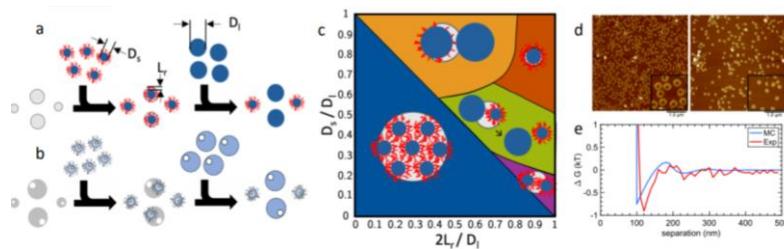
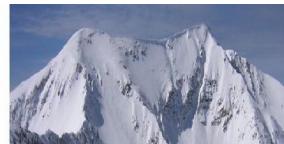


Figure 1: **a.** Two-species placement on a single substrate concept. **b.** Two species placement with rotational orientation control concept. **c.** Phase diagram of reaction products **d.** AFM images of DO tile devices with and without grafts. **e.** Boltzmann inversion of AFM-characterized un-grafted devices and Monte Carlo simulations of hard disks.

[1] ACS Nano 2014, **8**, 12, 12030-12040 2. arXiv 2018, 1808.04544



Track on Nucleic Acid Nanostructures in Vivo

P4.09 Sequence-programmed DNA crosslinking hotspots create ultra-stable nanomaterials for use as a biological platform

Tyler Brown, Daniel Saliba, Jathavan Asohan and Hanadi Sleiman

McGill University, Canada

Despite the promise shown by DNA nanomaterials in a biological scaffolding/therapeutic role, issues with stability and degradation in cells and during circulation have held them back from biological applications. Current strategies to combat this degradation have focused on introducing new, non-canonical nucleotides and/or linkages into a synthesized DNA strand. These new monomers and perturbations to the linkages between DNA bases are synthetically challenging to implement, and they may cause off-target effects and activation of the immune system.

Thymine, one of the four naturally occurring bases in DNA, has been well documented to undergo UV-promoted dimerization intra- or intermolecularly. The resulting materials are stable but have little control of the localization and degree of UV-promoted damage. This technique has previously been applied by the Dietz group¹ in DNA origami, linking the junctions between staple strands for increased stability. Herein we present a method to prepare DNA nanomaterials with improved control over the extent and localization of sequence-programmed crosslinked regions using transition metal catalysts which aid the 2+2 cycloaddition this crosslinking relies upon. These DNA “Gapmers”, prepared from canonical DNA nucleobases provide stability in a biological context, and will also provide a useful synthetic handle to create covalent crosslinks between DNA strands at will.

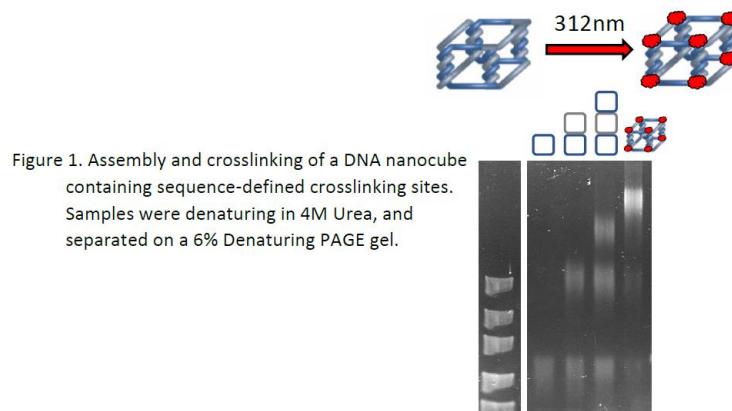


Figure 1. Assembly and crosslinking of a DNA nanocube containing sequence-defined crosslinking sites. Samples were denaturing in 4M Urea, and separated on a 6% Denaturing PAGE gel.

- [1] Gerling, T., Kube, M., Kick, B., & Dietz, H. (2018). Sequence-programmable covalent bonding of designed DNA assemblies. *Science Advances*, 4(8), 1-12.

**Poster Session 5, Wednesday 14 April, 15:20 - 16:40 UTC****Track on DNA Nanosystems: Programmed Function****P5.01 Chemically controlled DNA Origami dynamic self-assembly**

Alba Monferrer, Michele Stasi, Job Boekhoven & Hendrik Dietz

University of Munich, Germany.

Conformational states of dynamic DNA origami objects can be stabilized by weak, shortrange base stacking interactions that can engage between blunt-ended helices of shapecomplementary surfaces. The strength of these interactions sensitively depends on global solution parameters such as temperature or cation concentration, which enables fine tuning of the equilibrium of conformational states.[1]

In this work, we aim to develop a new strategy to control actuation of DNA nanotechnology by using a carbodiimide-based reaction network.[2] This reaction cycle consists of a chemical group which can be present in two states: negatively charged and neutral. This system works autonomously as long as there is fuel present, and drives it towards an out-of-equilibrium state. The system can spontaneously reverse back to the original state once the fuel is fully consumed.

This chemical modification, when placed at a terminal base of a stacking contact in a DNA origami object, changes the local charge density in the vicinity. We speculate that the additional negative charges introduced by the modification result in a stronger electrostatic repulsion and thus to a destabilization of the stacking contacts. By switching into the neutral state, this destabilizing effect is not present anymore and should trigger a conformational change of the DNA object. As a proof-of-concept study, we have developed two smaller DNA constructs to study the effect of this reaction cycle within stacked bases and double stranded DNA.

[1] Thomas Gerling, et al. *Science*. 347, 6229, 1446-1452 (2015) 2. Marta Tena-Solsona, et al. *Nat. Commun.* 8, 15895 (2017)

P5.02 Light-Responsive Dynamic DNA-Origami-Based Plasmonic Assemblies

Joonas Ryssy,¹ Ashwin K. Natarajan¹, Jinhua Wang², Arttu J. Lehtonen¹, Minh-Kha Nguyen¹, Rafal Klajn² and Anton Kuzyk¹.

¹Aalto University, Finland, ²Weizmann Institute of Science, Israel

DNA nanotechnology has enabled development of increasingly complex molecular switches [1]. DNA-based devices have been operated with different environmental stimuli such as light, temperature, electric fields, and pH [2]. Here we present a novel scheme for dynamic control of DNA self-assembly [3]. We used DNA origami technique to generate chiral plasmonic metamolecules (CPMs) with dynamic DNA triplex links. The CPMs were dispersed in photoresponsive medium containing a photoswitchable acid. Under the irradiation of visible light, the photoacid underwent photochromic reaction and released protons inducing self-assembly of the pH-responsive DNA triplex links which led to the change of spatial configuration of the CPMs. The CPMs displayed light-controlled dynamic behavior without the use of light-responsive modifications. The process was fast, reversible and did not exhibit any symptoms of fatigue. The ensemble of CPMs could express multiple stages and the strength of chiroptical responses could be controlled by the intensity of the external light stimulus. We anticipate that such dynamic remotely controlled system will aid the further advancement of DNA-based plasmonic nanomaterials.

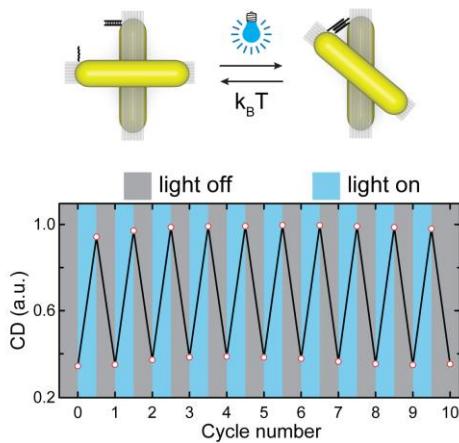


Fig 1. Dynamic light-responsive DNA-origami-based plasmonic metamolecules.

- [1] DeLuca, M. et al. 2020. Dynamic DNA Nanotechnology: Toward Functional Nanoscale Devices. *Nanoscale Horiz.* 5 (2); 182–201.
- [2] Krishnan, Y. et al. 2011. Nucleic Acid Based Molecular Devices. *Angewandte Chemie International Edition*. 50 (14); 3124–3156.
- [3] Ryssy, J. et al. 2020. Light-Responsive Dynamic DNA-Origami-Based Plasmonic Assemblies. *Angewandte Chemie International Edition*.

P5.03 DNA origami-based goniometers for DNA bending proteins

Ashwin karthick Natarajan, Joonas Ryssy and Anton Kuzyk

Aalto University School of Science, Finland

DNA origami offers precise positioning of nanoscale objects with profound accuracy. The positioning has facilitated the development of DNA origami-based functional nanomechanical devices enabling single particle studies of biomolecules [1,2]. Development of novel devices to study biomolecules bridges the knowledge gap existing in several biomolecular functions. Herein we expand DNA origami method to design a goniometer to study DNA bending proteins. We used TATA binding protein (TBP) as a case study to test our goniometer. Upon binding to the TATA box, TBP causes a bend of $\sim 90^\circ$ to DNA. Our device translates the bending of the TATA box by TBP into a visible angular change in the structure which could be observed with Transmission Electron Microscope (TEM). Further, we studied the role of transcription factor TF (II) A on bending of DNA by TBP. The utility of our device can be expanded to a wide range of DNA bending proteins with additional improvements.

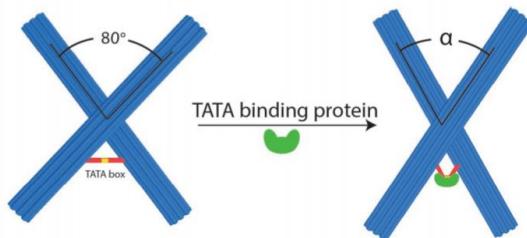


Figure 1: Schematic overview of DNA origami with TATA box in the bridge connecting two origami bundles and the structural change upon binding of TBP. References

- [1] P. C. Nickels, et al., **354**, 305–7, (2016).
- [2] J. J. Funke, H. Dietz., *Nature Nanotechnology* **11** 47–52 (201).

P5.04 Controllable Protocell Aggregation Induced by DNA Signals

Yancheng Du, Hengming Qiu, Feiran Li, and Jong Hyun Choi

Purdue University, USA

Protocells are simplified synthetic systems mimicking cell structures and functions.[1] They are used to replicate and understand various cell behaviors. In this context, it is important to develop protocells that mimic aggregation behaviors of biological cells. Cell aggregation is involved in cell viability, differentiation, and migration.[2] The key aspect in cell aggregation is the intracellular signals that control cell-cell interactions. Understanding such a signaling process is essential to study the cell aggregation and could contribute to tissue engineering research. However, a synthetic model system that can modulate dynamic processes of signal sensing, transducing, and responding has not been established. To develop such a synthetic system, we used DNA nanotechnology due to the excellent programmability.

In this work, we engineered lipid vesicles with DNA components and demonstrated their controllable aggregation behaviors. We designed two kinds of DNA-decorated vesicles, GUV (giant unilamellar vesicle) and SUV (small unilamellar vesicle), as shown in Fig. 1. The GUV has transmembrane channels made of DNA origami that are used to recognize and process intercellular signals. Our experiments showed that with introduction of DNA signals, the GUV recognized the external signals via origami pores and transduced them inside the GUV via enzymatic reactions. The intracellular reactions generated a new DNA signal that resulted in aggregation of SUVs on the GUV. This process was also made reversible by addition of oligonucleotides for ‘release’ signal. Dissociations of SUVs were observed, thus demonstrating the reversibility in the protocell aggregation behavior. Our study demonstrates synthetic protocells capable of chemical communication and coordination. These protocells could be used as model systems studying cell signaling processes or cell-to-cell interactions during cell culture.

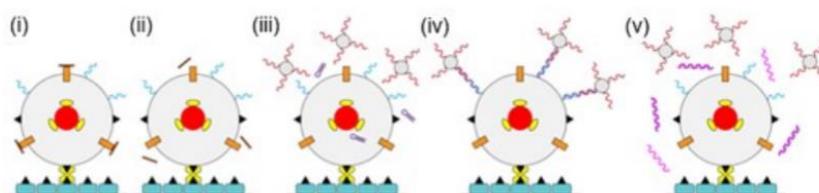




Figure 1. Scheme for reversible cell aggregation programmed by DNA signals. (i) A GUV with transmembrane channels (orange) and DNA strands (blue) is immobilized on the surface with biotinstreptavidin interaction. (ii) Introduction of a DNA signal results in dissociation of caps from DNA origami pores. (iii) Hairpin signals enter the GUV and are cleaved by an enzyme. Then, the fragment, a new DNA signal, is released from the GUV through the channels. (iv) SUVs respond to the DNA signals and aggregate onto the GUV. (v) Addition of releaser strands removes the binding strands via strand displacement, thus SUVs dissociate from the GUV.

- [1] I. A. Chen, P. Walde, *Cold Spring Harb Perspect Biol* 2010, 2, a002170.
- [2] T. Toyoda, S. Mae, H. Tanaka, Y. Kondo, M. Funato, Y. Hosokawa, T. Sudo, Y. Kawaguchi, K. Osafune, *Stem Cell Res* 2015, 14, 185-197.

P5.05 Responsive core-shell DNA particles destabilise lipid bilayers and trap bacteria

Michał Walczak¹, Ryan A. Brady², Leonardo Mancini¹, Claudia Contini³, Roger Rubio-Sánchez¹, William T. Kaufhold^{1,3}, Pietro Cicuta¹, and Lorenzo Di Michele^{3,1}

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Lipid bilayers play an important role in cellular biology as they act as the filter between the inner and outer environment of the cell. Their integrity, often associated with a proper functioning and vitality of the cells, can be compromised by a number of biological and synthetic agents, including antimicrobial peptides, amyloid aggregates, polymer particles and metal particles with charged coatings. Such agents, frequently considered to be toxic and highly undesirable, have a variety of beneficial applications such as the ability to control membrane leakage, which can be harnessed for biosensing and therapeutics. Here, we present a novel type of synthetic, DNA-based particles capable of disrupting lipid membranes.¹ The particles have a core-shell structure and self-assemble from cholesterol/DNA nanostructures, named C-stars,^{2,3,4} responsible for the formation of membrane adhesive core, and all-DNA nanoconstructs forming a protective hydrophilic corona around the core. These aggregates are stable in solution in the presence of liposomes and their size can be prescribed by changing the annealing protocol leading to self-assembly. The protective corona can be selectively displaced upon an addition of a trigger DNA oligonucleotide. The latter exposes the cholesterol-rich particle core and induces membrane disruption as caused by aggregation of the DNA-cholesterol complexes on the vesicle's surface, resulting in membrane rupture and cargo release. Furthermore, once activated, the core-shell particles assemble into a sticky DNA net capable of surrounding and immobilizing cell-like objects, as we exemplify with *E. coli* entrapment. This is reminiscent of the action of innate-immune cells, which can eject their genetic material to create a DNA net (Neutrophil Extracellular Trap) able to entrap pathogens. The design of the particles can be easily adapted to create antimicrobial or drug delivery systems.

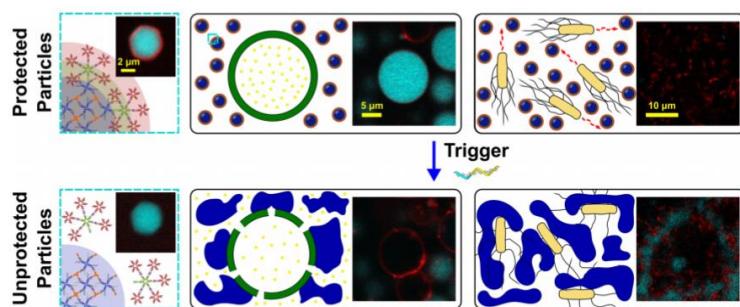




Fig. 1. Particle-induced membrane permeabilization and bacteria entrapment. As a result of addition of the trigger strand and subsequent displacement of the hydrophilic corona (left), cholesterol rich particle cores adhere to each other and start to aggregate on the surface of GUVs (middle). DNA aggregation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

- [1] Walczak M. et al., Under Review.
- [2] Brady R. A. et al., *Nano Lett.* **17(5)**, 3276-3281 (2017).
- [3] Brady R. A. et al., *J. Am. Chem. Soc.* **140(45)**, 15384-15392 (2018).
- [4] Brady R. A. et al., *J. Phys.: Cond. Matter.* **31**, 074003 (2019).

P5.06 DNA origami based biosensing

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A biosensor's response to an external stimulus, defined by the dissociation constant and the dynamic range, typically is governed by the strength of the receptor-ligand interaction. Strategies to influence this response have been developed, but usually involve changes to the binding interaction itself, e.g. by introducing point mutations in the protein sequence [1]. Here, the specificity of the interaction can be compromised. Only recently new ideas based on *de-novo* protein design have emerged [2]. DNA nanotechnology and especially the DNA origami technique presents itself as an excellent alternative for constructing complex biosensors. Its modular nature allows for precise arrangement of the recognition elements [3] and a plethora of potential candidates for such biosensors have been realized [4-6]. In this contribution, we present our progress on employing such nanostructures for advanced biosensing applications.

- [1] S. Joel, K. B. Turner and S. Daunert, *ACS Chem. Biol.* **9** (2014).
- [2] A. Quijano-Rubio *et al.*, *Nature asap* (2021).
- [3] A. Shaw *et al.*, *Nat. Nanotechnol.* **14** (2019).
- [4] A. E. Marras *et al.*, *PNAS* **112** (2015).
- [5] D. Selcuk *et al.*, *ACS Nano* **12** (2018).
- [6] H. Ijäs *et al.*, *ACS Nano* **13** (2019).

P5.07 "Printing" DNA Strand Patterns on Small Molecules

Fangzhou Zhao¹, Tuan Trinh², Mahla Poudineh³ and Hanadi Sleiman¹

¹McGill University, Canada, ²Stanford University, USA, ³University of Waterloo, Canada

Branched small molecule-DNA hybrids are composed of multiple DNA strands covalently attached to a small molecule core with a specific geometry. They can serve as building blocks for assembling nanostructures with a minimal number of DNA strands, thus greatly increasing DNA stability, assembly yield and cooperativity. Moreover, by simply changing the small molecule core, new geometries and functionalities can be added. These molecules can also serve as simple scaffolds for the organization of nanoparticles or biomolecules for applications in nanophotonics and catalysis.



However, these molecules have been challenging to synthesize, particularly when different DNA arms need to be attached onto a single synthetic molecule. We have developed a 'molecular printing' method to overcome these problems. The concept of DNA printing is the covalent transfer of specific patterns of DNA strands from a DNA nanostructure to another material. Tempered by a DNA scaffold junction, different DNA arms can be covalently linked to a small molecule core. Thus, each arm's length, sequence and directionality can be controlled.¹

To fulfill such DNA-small molecules applications in practice, large scale synthesis is required. Inspired by the ability of self-replication of DNA molecules in nature, this project involves the integration of this 'printing' template onto a microfluidic device. In this way, an immobilized 'mother' template can be easily separated from the 'printed' daughter product, thus allowing recycling for multiple rounds of reactions. The repetitive 'printing' steps occur with high coupling yields. As a result, small molecule-DNA hybrids can be obtained in large quantity with high efficiency and low cost, in an automated manner.

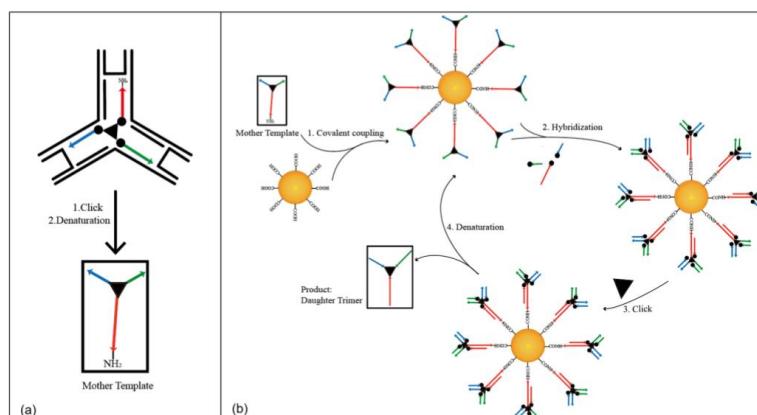


Fig. 1. (a) "Printing" mother trimer from a three-way junction; (b) Flow chart of the streamline process.

[1] Trinh, T.; Saliba, D.; Liao, C.; de Rochambeau, D.; Prinzen, A. L.; Li, J.; Sleiman, H. F., *Angew. Chem. Int. Ed.* **58** (10), 3042-3047 (2019).

P5.08 One-Pot Molecular Assembly of DNA and Semi-Artificial Glycopeptides to Give Multicomponent Supramolecular Materials

Sayuri Higashi¹, Koichiro M. Hirosawa¹, Kenichi G. N. Suzuki¹, Kazunori Matsuura^{2,3} and Masato Ikeda^{1,4}

¹Gifu University, Japan, ²Graduate school of Engineering, Japan, ³Tottori University, Japan, ⁴Nagoya University, Japan

Multicomponent supramolecular materials, which comprise plural supramolecular architectures exhibiting distinct self-sorting behaviors, have been receiving increasing attention because they can be implemented with sophisticated functions emerging from hierarchical structures such as living cells. Nevertheless, the application of supramolecular system design to engineer self-sorting behaviors among plural supramolecular architectures remains challenging. In this presentation, we show that the thermal annealing-induced one-pot assembly of multiple single-stranded DNAs and a single semi-artificial glycopeptide¹ (GP) results in the emergent formation of integrative self-sorted supramolecular nanostructures (ssSNs) consisting of a GP supramolecular nanoribbon



surrounded by DNA tile-nanotubes². Fluorescence imaging revealed the formation of each supramolecular nanostructure through orthogonal molecular assembling processes. Further, we revealed that the integrative ssSNs retain their biostimuli responsiveness so that each supramolecular nanostructure can be selectively degraded. Finally, we successfully constructed a complex soft nanomaterial composed of ternary supramolecular architectures (a GP supramolecular nanostructure, DNA tile-nanotube, and DNA microsphere) based on the present as well as previous findings^{3,4} (Fig. 1).

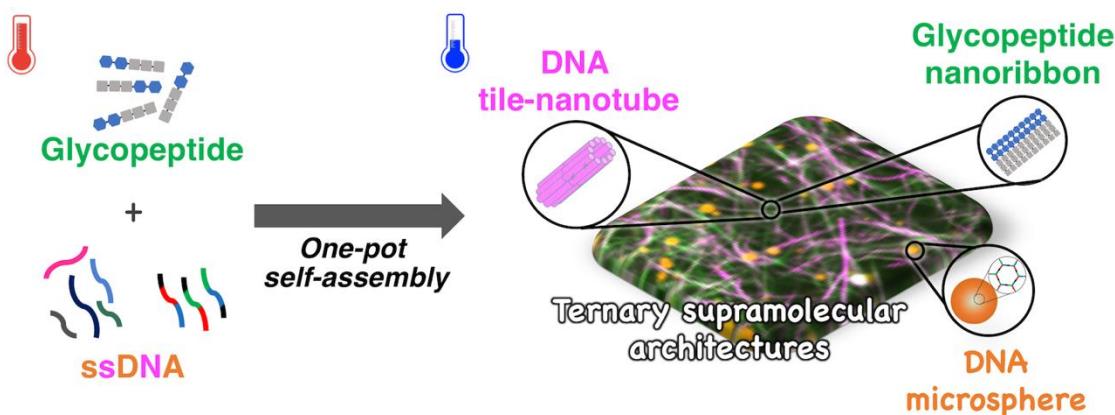


Fig. 1. Schematic representation of the ternary supramolecular materials comprising of self-sorted supramolecular architectures of DNA and glycopeptide

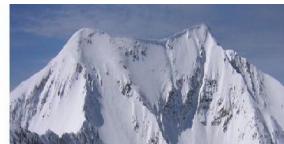
- [1] T. Tsuzuki, M. Kabumoto, H. Arakawa and M. Ikeda, *Org. Biomol. Chem.*, **2017**, 15, 4595–4600.
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P5.09 Cascaded pattern formation in DNA based Reaction-diffusion system

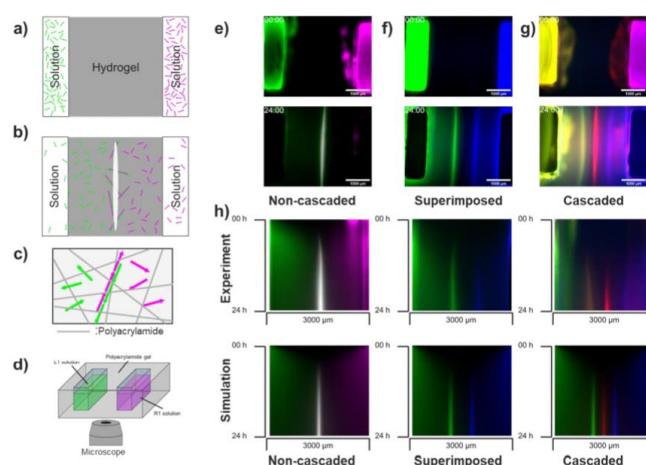
Keita Abe¹, Satoshi Murata¹ and Ibuki Kawamata^{1,2}

¹Tohoku University, Japan, ²Ochanomizu University, Japan

Pattern formation is an attractive mechanism for engineering, where regular patterns form spontaneously as designed. Based on numerical models such as reaction-diffusion systems, researches have been conducted to artificially form various patterns in both simulations and experiments. In recent years, synthetic DNA capable of designing chemical reaction network has been used to build several programmed reaction-diffusion systems [1]. For example, we have reported a method to modulate diffusion coefficient of synthetic DNA for programmed pattern formation [2]. These previous studies have concentrated on single-step pattern formation, however attention has been not paid on experimentally cascading pattern formations. In this study, we have demonstrated superimposed and cascaded pattern formations. First, we built a reaction-diffusion system in which DNAs diffuse from solutions of two pockets (Figure a), hybridize each other at the middle point between them, form polymers (Figure b), and are immobilized there (Figure c). In the experiment, we fabricated the system in a polyacrylamide hydrogel (Figure d),



and observed the line pattern formation of fluorophore-labeled DNAs using fluorescent microscopy (Figure e). Next, we employed an adjuster DNA to modulate diffusion speed of DNAs by changing the net molecular size, and showed that the position where the line appears can be shifted. We utilized orthogonal base sequence to run the reaction-diffusion in parallel and realized superimposed pattern formation (Figure f). Finally, by releasing new DNA strands during the superimposed pattern formation, we succeeded in the cascaded pattern formation in which a new line appears between the two lines (Figure g). In addition, reaction-diffusion simulation following partial differential equations well-agreed with the experimental results (Figure h).



Figures a) Initial state of the system. b) Immobilized DNA polymers in hydrogel. c) DNA strands and polymer in hydrogel. d) Configuration of experimental system. e) Line pattern formation. f) Superimposed pattern formation. g) Cascaded pattern formation. h) Kymographs of each pattern formation process in experiment and simulation

Our method used for cascaded pattern formation has a potential to provide various functionalities such as spontaneous structure materialization like the process of biological development.

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- [2] Keita Abe, Ibuki Kawamata, Shin-ichiro M. Nomura, Satoshi Murata "Programmable reactions and diffusion using DNA for pattern formation in hydrogel medium." *Molecular Systems Design & Engineering* 4.3 (2019): 639-643

P5.10 A Curvature Ruler Enabled by DNA Nanotech

Ece Büßer¹, Tim Schröder¹, Sarah Ochmann,¹ Henri Franquelim,² and Philip Tinnefeld¹

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Reliable and precise size measurement of particles is crucial as it has a direct effect on the physical features of materials. It is additionally important to develop cost-effective, rapid, and sufficiently precise systems that can measure the particle size on the nanoscale, since it is very difficult to track dynamic changes on submicron structures due to resolution limitations. The unique advantages of DNA nanotechnology allow us to make modular



designs where different functions can be optimized separately [1]. The ability of being membrane-active with modifications on the nanodevices makes it, for example, possible to sense cellular membrane curvature. Sensing the changes in the local curvature of membranes can help to understand the mechanisms of the cell membrane remodeling in intra- and extracellular processes [2].

In this work, we design dynamic and flexible DNA nanodevices that can adapt the curvature of the structure it is bound to. These nanodevices can thus be used as curvature rulers with the information of particle size. Based on our strong background in single molecule biophysics, we develop DNA origami curvature sensors with Fluorescence Resonance Energy Transfer (FRET) as transduction mechanism on the single-molecule level. FRET enables the measurement of distance-dependent conformational changes with nanoscale precision and high sensitivity [3].

As a model, segmented origami structures connected via DNA linkers incorporating a FRET pair are used. As the distance between the FRET pair differs with curvature, we provide a ratiometric FRET ruler to quantitatively measure the interaction of different curvatures with our flexible DNA origami sensor (Fig. 1.). To better control and calibrate the sensor properties, it is first tested on uniform spherical silica beads having different size, subsequently on alternative particles such as other DNA origami nanostructures and lipid vesicles. We will expand the use of these modular sensing devices to sensing on cellular membranes and livecell measurements.

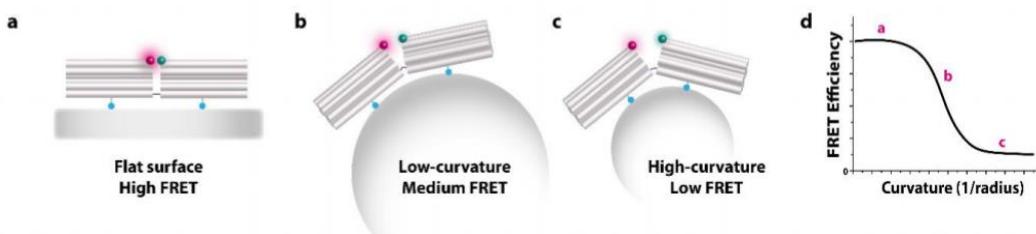


Fig. 1. Sketch of curvature-sensing DNA nanodevice

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P5.11 Large, square-shaped, DNA origami nanopore with gating function on giant vesicle membrane

Shoji Iwabuchi¹, Ibuki Kawamata^{1,2}, Satoshi Murata¹, and Shin-ichiro M. Nomura¹

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Intaking molecular information from the external environment is essential for the advanced functioning of closed lipid bilayer membrane systems, such as artificial cells[1,2] and molecular robots[3,4]. In this work, we report an artificial nanopore composed of DNA origami that penetrates the membrane of GUVs[5]. The structure has a pore with 10 nm in diameter, which is larger than conventional DNA origami nanopores[6]. By using additional DNA strands, we designed a molecular “lid” to realize size-dependent molecular transportation. The structure was confirmed by TEM and the passage of molecules through the GUV membrane and the blockade of the transport by lid ssDNA has been observed by confocal microscopy. We also succeeded in controlling size of transportable molecules by partially closing the lid by adding ssDNA strands. A controllable artificial nanopore would facilitate the communication between the vesicle components and their environment.

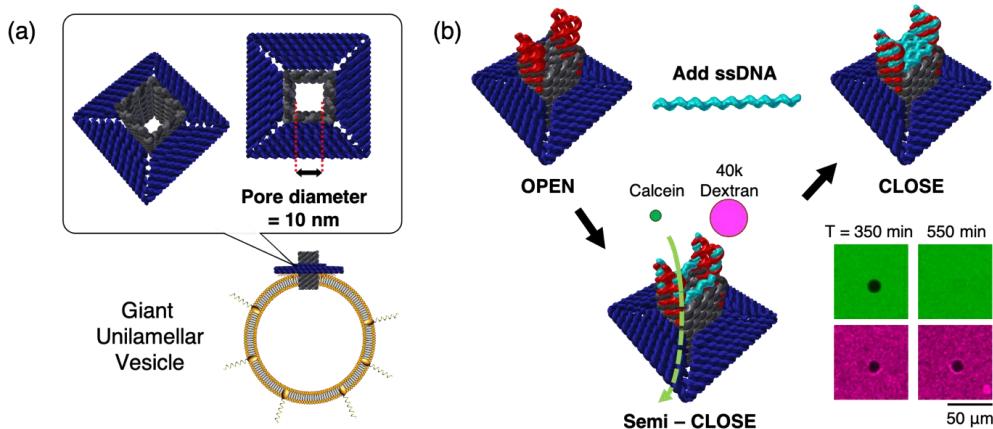


Fig. 1. (a) A schematic of the DNA origami nanopore and giant unilamellar vesicle(GUV).

(b) Schematic representation of the closure of the pore by the ssDNA. The lid, four ssDNAs that can bind to the staple DNA present on the upper part of the pore, allows the pore to be closed.

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P5.12 Seeding, Plating and Electrical Characterization of Gold Nanowires Formed on Self-Assembled DNA Nanotubes

Dulashani R. Ranasinghe¹, Basu R. Aryal¹, Tyler R. Westover¹, Sisi Jia¹, Robert C. Davis¹, John N. Harb, Rebecca Schulman² and Adam T. Woolley¹

¹Brigham Young University, USA, ²Johns Hopkins Institute ,USA

Self-assembled DNA nanostructures offer new opportunities in the field of nano-electronics. In this work, we fabricated gold (Au) nanowires using self-assembled DNA nanotubes. DNA nanotubes are not electrically conductive, so functionalization is necessary for creating conductive wires. Metallization of DNA structures typically involves binding of seed nanomaterials, followed by electrochemical growth. We used two approaches to seed DNA nanotubes: one involved Au nanorods and other one involved palladium (Pd) ionic seeding. Both methods used Au electroless plating to connect seeds. Au-seeded and plated structures had continuous lengths that ranged from 1 to 2 μ m with diameters of 35-60 nm. Pd-seeded and Au-plated structures had lengths of 1-1.5 μ m with diameters of 35-85 nm. We utilized electron beam induced deposition (EBID) to form conductive tungsten contacts and four-point probe techniques to study the electrical properties of the plated DNA nanotubes. The successfully measured resistance values for 15 nanowires (from a total of 41 structures where electrical characterization was attempted) were between 5 and 167 k Ω . Measured resistivity values for these 15 successfully studied wires were between 9.3×10^{-6} and $1.2 \times 10^{-3} \Omega\text{m}$. Nanowires formed on DNA nanotubes could be promising templates for use as self-assembling interconnects in future complex electronic circuitry.

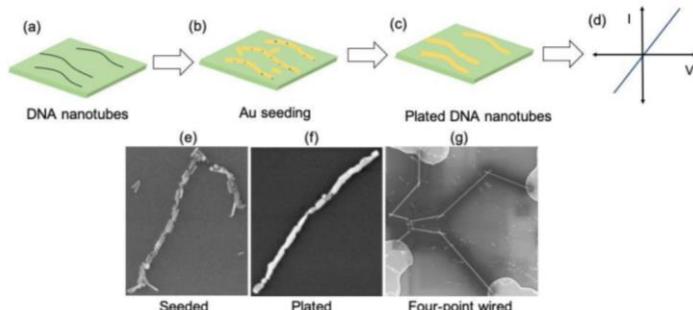


Fig. 1. Overview of fabrication of Au nanowires using DNA nanotubes and characterization. (a) DNA nanotubes on an oxidized Si wafer. (b) DNA nanotubes seeded with Au. (c) Plated DNA nanotubes. (d) I-V measurement. (e) SEM image of Au seeding. (f) SEM image of plated DNA nanotube. (g) SEM image of completed EBID pattern connecting to an Au-plated DNA nanotube seeded with Au nanorods to four Au pads.

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Poster Session 6, Wednesday 14 April, 17:45 - 19:20 UTC

Track on DNA Nanosystems: Programmed Function

P6.01 Helicase Expedites a Catalytic DNA Reaction by Selectively Removing the Product from the Catalyst

Pepijn Moerman, Momcilo Gavrilov, and Taekjip Ha, and Rebecca Schulman

Johns Hopkins, Baltimore, USA

The primer exchange reaction (PER) is a DNA-based isothermal catalytic reaction that appends a new domain with a user-specified sequence onto a single-stranded reactant [1]. PER has shown promise, among others, as a signal amplification for immunostaining in cells [2]. However, as for any catalytic reaction, its rate is limited by Sabatier's principle which requires that the binding strength between the reactant and the catalyst is strong enough for substantial binding but weak enough that the binding is reversible and the product doesn't poison the catalyst. Consequently, PER only works for primers that are approximately 10 nucleotides long at 37°C. Here we show that RepX, an ATP-dependent helicase that exclusively unwinds DNA duplexes with a single stranded 3' overhang, can increase the rate of PER by up to two orders of magnitude in the strong binding regime. This extends the range of applicability of PER to longer reactants and lower temperatures, where the reaction would otherwise be prohibitively slow. We show that RepX enhances the rate by selectively removing the product - and not the reactant - from the catalyst. Our findings suggest a general strategy of "dissipative catalysis" in which catalytic reactions are enhanced at the cost of energy consumption by actively removing product from the catalyst.

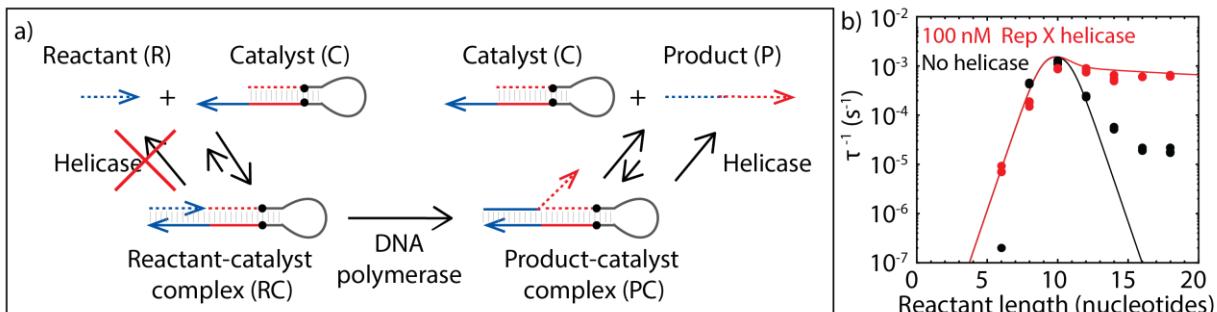


Fig. 1. (a) Overview of the three main steps of the PER process: First a reactant strand binds to the catalytic hairpin, then DNA polymerase appends a new domain onto the reactant by copying the hairpin sequence, and finally the product is removed from the catalyst. Rep X helicase speeds up this process because it can move the product - which has a single stranded 3' overhang - from the catalyst, but not the reactant. (b) Reaction rate as function of reactant length. The rate is expressed as the inverse time for the reaction to go to completion, τ . The reactant length controls the binding energy between reactant and catalyst; long reactants bind stronger to the catalyst. The dots represent measurements, and the lines are fits based on a reaction model.

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- [2] S. K. Saka, *et al.*, Nature Biotechnology. **37**, 1080-1090 (2019).

P6.02 Exciton Delocalization in Squaraine versus Cyanine Dye Aggregates Templated by DNA Holliday Junction

Olga A. Mass¹, Christopher K. Wilson¹, Simon K. Roy¹, Matthew S. Barclay¹, Lance K. Patten¹, Ewald A. Terpetschnig², Jeunghoon Lee¹, Ryan D. Pensack¹, Bernard Yurke¹, William B. Knowlton¹

¹Boise State University, USA. ²SETA BioMedicals, USA

DNA is a compelling template for organizing dye molecules into dye aggregates to promote excitonic coupling and exciton delocalization between the dyes, which is vital in such fields as light harvesting, solar energy conversion, organic optoelectronics, and nanoscale computing. A dsDNA template offers very precise control over the number and position of dyes within a molecular aggregate through a direct covalent tethering of dyes to DNA. An immobile 4-arm DNA Holliday Junction (HJ) template allows us to create dye aggregates not achievable with dsDNA. To deepen our understanding of exciton coupling and delocalization in molecular aggregates, we compared excitonic interactions in indolenine squaraine aggregates covalently templated by HJ with those of cyanine dye Cy5. This comparison was motivated by increased photostability and chemical diversity of squarinines versus Cy5 dye upon comparable and excellent spectral properties. Using a commercially available indolenine squaraine, we have created three different squaraine aggregate types: two dimers and a tetramer, all of which exhibited exciton delocalization. We applied molecular exciton theory, steady state optical characterization, and photophysical data analysis to quantitatively evaluate and directly compare the strength of excitonic hopping interactions between dyes that drive exciton delocalization. We found that the greatest excitonic hopping strength within indolenine squaraine dye aggregates is on par with that of analogous Cy5 dye aggregates. Using theory and experimental data, we extracted the geometries of dye aggregates. Indolenine squaraine dyes displayed primarily H-like stacking in all the aggregates examined while H-like and J-like stacking was observed in Cy5 dye aggregates. Our findings as well as



the superior photophysical properties of indolenine squaraine dyes over that of cyanine dyes make indolenine squaraine dye aggregates very attractive candidates for molecular exciton-based applications.

P6.03 Poster withdrawn

P6.04 A Modular and Customizable Aptamer Transducer Designed for Feed-Forward Coupling with Catalytic Amplifiers

Tim Hachigian, Drew Lysne, Elton Graugnard and Jeunghoon Lee

Boise State University, USA

Aptamer biosensing has attracted recent attention due to its potential for sensing applications in the medical, environmental, and food safety industries.¹ Additionally, aptasensors can be designed as point-of-care devices making them useful where lab equipment is less accessible.¹ Many studies have aimed to develop aptasensors with improved sensitivity and selectivity, while relatively few have focused on aptasensor modularity.¹ Here, we introduce a modular Aptamer Transducer (AT) that can be incorporated with a variety of DNA reaction networks by producing a customized output strand. Modularity of the AT was accomplished by separation of the aptamer input and output domains on a sensing substrate. Aptamer-ligand binding allows a structure switching element to be activated to initiate strand displacement with fuel strands and release the customized output ssDNA sequence. The well-known 23-mer adenosine binding DNA aptamer was incorporated into the AT design and shown to accommodate sequence designs for targeted reporter complexes, with the limit of detection down to 5 μ M.² Further, adenosine AT output was customized for two linear catalytic amplification reaction networks developed by Zhang et al. and Kotani et al. The ATs were able to trigger feed-forward signaling to these catalytic amplifiers without the need for amplifier reaction network redesign, further supporting modularity of the device.^{3,4} Future study of the AT will aim to improve the device sensitivity, reduce network leakage, and incorporate other aptamers. Through continued AT device improvement, future reaction networks could employ concurrent solution based multiplexed sensing using two or more aptamers.

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- [3] Zhang, D. Y., Turberfield, A. J., Yurke, B., & Winfree, E. (2007). Engineering entropy-driven reactions and networks catalyzed by DNA. *Science*. <https://doi.org/10.1126/science.1148532>
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P6.05 Robust heterochiral strand displacement using leakless translators

Tracy L. Mallette¹, Milan N. Stojanovic², Darko Stefanovic¹ and Matthew R. Lakin¹

¹University of New Mexico, USA, ²Columbia University Medical Center, USA



Molecular computing offers a powerful framework for *in situ* biosensing and signal processing at the nanoscale. However, for *in vivo* applications, the use of conventional DNA components can lead to false positive signals being generated due to degradation of circuit components by nuclease enzymes.¹ Our solution is to use L-nucleic acids, the chiral mirror images of naturally occurring Dnucleic acids, as alternative materials for constructing nucleic acid circuits. L-nucleic acids are not recognized by naturally occurring nuclease enzymes, which have evolved to target D-nucleic acids only,² and therefore should resist degradation in cells. Here, we use hybrid chiral molecules, consisting of both L- and D-nucleic acid domains, to implement leakless signal translators that enable D-nucleic acid signals to be detected by hybridization and then translated into a robust L-DNA signal for further analysis. We demonstrate the first cross-chiral toehold mediated strand displacement, translating chiral signals from L- to D- and vice versa. We show that our system is robust to false positive signals even if the D-DNA components are degraded by nucleases, thanks to circuit-level robustness. This work thus broadens the scope and applicability of DNA-based molecular computers for practical, *in vivo* applications.³

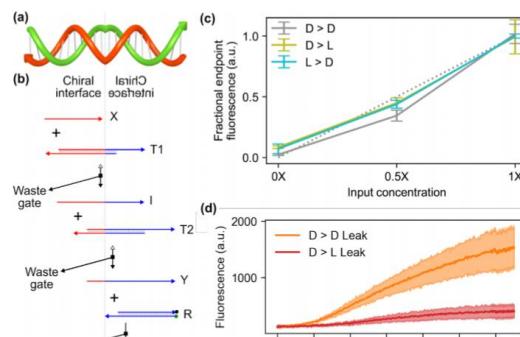


Fig. 1: Leakless heterochiral translator architecture and operation. (a) DNA chiral representation. (b) The two-step translation process in which an input oligonucleotide (X) is converted to an output oligonucleotide (Y) of the opposite chirality.⁴ (c) Performance of translation systems in buffer where the chiral translations are noted as “D” (right), “L” (left), and the direction as “>”. (d) Leak of full D-DNA system vs protected D- to L- architecture chimeras in 10% Fetal Bovine Serum.

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P6.06 DNA amphiphilic self assembly for therapeutic applications

Sepideh Kaviani, Hassan H. Fakih, Tyler Brown, Michael D. Dore, Jathavan Asohan and Hanadi F. Sleiman

McGill University, Canada

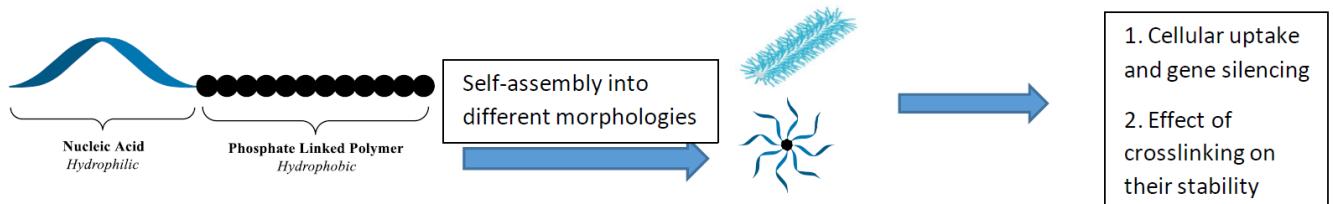
Self-assembly of DNA amphiphiles (in which DNA is covalently bound to synthetic hydrophobic moieties) can lead to the creation of DNA nanostructures, including vesicles, micelles, fibers, and helical nanoribbons. Their morphology could be changed by changing the polymer composition and stability (Crosslinking the core). The study and design of such structures has gained significant interest and growth with promising potential for medical applications, such as drug delivery and sensing.

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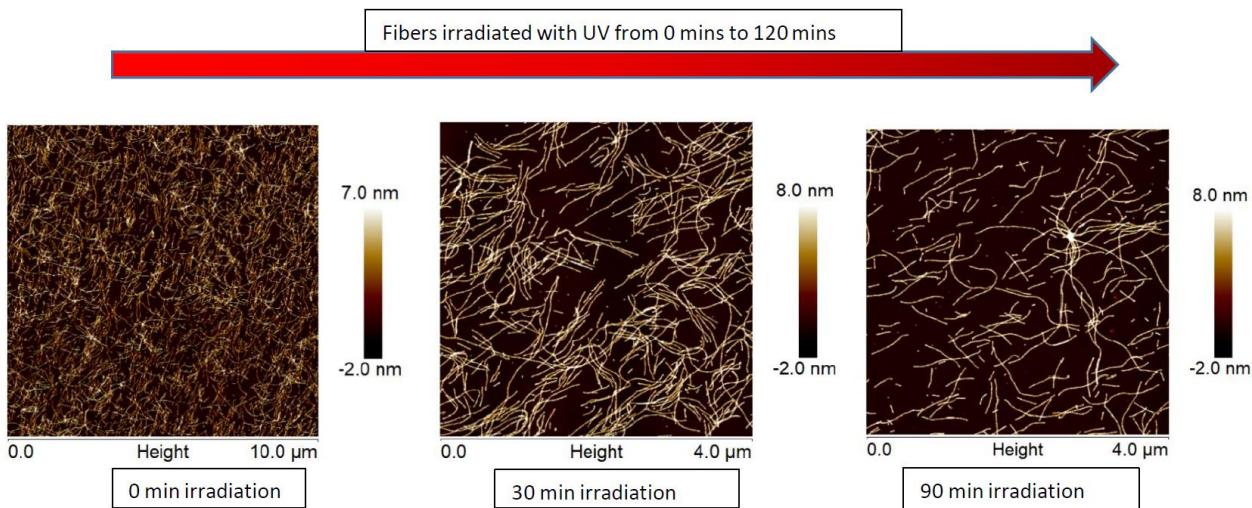
12 - 15 April 2021



Here we will present our studies on controlling the morphology, stability and biological properties of DNA amphiphilic assemblies using two strategies. First, by varying the sequences of two different hydrophobic monomers along the chain, we study morphological changes and their effect on biological activity of these nanostructures.



Second, by crosslinking the DNA-polymer interface of these amphiphiles, we attempt to increase the stability of these nanostructures *in vitro* or *in vivo*. We use UV based crosslinking of thymine bases on the interface. UV irradiation of cylindrical structures can increase their rigidity, and resistance to falling apart upon dilution. These studies are aimed at solving the problems confronting oligonucleotide therapeutics, such as stability, difficult cell entry and difficult extra-hepatic delivery.



Track on Protein & Viral Nanostructures

P6.07 Ethanol-Mediated Assembly of Tobacco Mosaic Virus Coat Protein

Ismael Abu-Baker and Amy Szuchmacher Blum

McGill University, Canada

The fabrication of materials with precisely controlled nanoscale features is a significant challenge in nanotechnology. Biological scaffolds, such as virus particles, present great promise as a platform for well controlled self-assembly at the nanoscale. Here we report on unusual changes in the assembly of wild-type tobacco mosaic virus coat protein (WT-TMV-CP) depending on the concentration of ethanol in solution. WTTMV-CP self assembles into either long rod or small disk virus-like particles (VLPs) depending on solution conditions. Disks are favoured at

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pH 7.0-6.5, and rods at lower pH. Assembly in the presence of ethanol inhibits formation of the larger VLP rods at low pH, making disks the major population.

Dynamic light scattering (DLS) and transmission electron microscopy (TEM) results show an initial inhibition of rod formation after the pH is dropped in the presence of ethanol. Samples without any ethanol form rods immediately, and samples containing ethanol remain in disk form. Preliminary results indicate that rods begin to assemble in the ethanol samples after several days. Samples with a low concentration of ethanol.

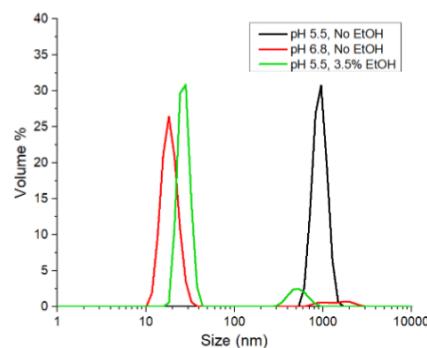


Fig. 1. DLS of WT-TMV-CP assembled in three different conditions

This phenomenon may be due to changes in solvent properties, such as molecular structure, dielectric constant, or diffusivity. Changes in the structure of water-ethanol mixtures have previously been reported, with an extensive hydrogen bonded network formed at around 7 mol% ethanol due to overlapping hydration spheres of ethanol molecules, and clusters of ethanol molecules forming in the hydrogen bonded network around 15 mol% ethanol.¹ While these structural transitions are close to apparent shifts in the behaviour of WT-TMV-CP in water-ethanol mixtures, it is not yet clear if solvent structure is the driving-force behind the modified assembly behaviour. This ethanol-mediated assembly allows the disk phase to be temporarily stabilized in previously inaccessible conditions for reactions that require lower pH. Many nanoparticle syntheses and organic coupling reactions require non-physiological conditions, so increasing the tolerance of biological scaffolds to a wider range of conditions is an important step towards templated self-assembly of nanomaterials.

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P6.08 - Poster withdrawn

P6.09 CRISPR-guided programmable self-assembly of artificial virus-like nucleocapsids

Carlos Calcines-Cruz¹, Ilya J. Finkelstein² and Armando Hernandez-Garcia¹

¹National Autonomous University of Mexico, USA, ²University of Texas at Austin, USA

De novo viromimetic proteins allow the fabrication of safe gene delivery systems and simple models to study viral self-assembly. We have previously reported virus like particles (VLPs) made of a synthetic triblock polypeptide C-S₁₀-B that protect encapsulated DNA from nucleases and can transfect cells [1]. However, the self-assembly of viromimetic proteins on specific nucleic acids—a crucial property that enables viral replication and success—has remained elusive. Inspired by viral packaging signals, we reasoned that using selective high affinity DNA binding proteins (such as CRISPR inactivated endonuclease dCas12a) that recruit C-S₁₀-B onto the DNA template might favor encapsulation of target DNA molecules.



First, we screened C-S₁₀-B concentrations and selected those that limit VLP self-assembly (≤ 50 nM) (Fig. 1a). At such low concentrations of C-S₁₀-B, we found that positioning up to 10 dCas12a on the DNA increased DNA encapsidation rates two- and three-fold with respect to the naked DNA. The same effect was found with nucleosomes, which suggests that arresting the linear diffusion of C-S₁₀-B promotes particle nucleation and growth. We then coupled the polymerization block (S₁₀) from C-S₁₀-B to dCas12a via the rapamycin-induced heterodimerization of FKBP and FRB, so that dCas12a-S₁₀ could recruit C-S₁₀-B at the target sites (Fig. 1b). This strategy increased VLP self-assembly rates two-fold relative to dCas12a_(FKBP) alone, and five-fold relative to the naked DNA. Furthermore, selective positioning of dCas12a-S₁₀ allowed discrimination of cognate versus non-cognate DNA in an equimolar DNA mixture (Fig. 1c). These CRISPR-guided VLPs could be a starting point for the creation of novel and programmable templated self-assemblies with multiple uses in bio(nano)technology.

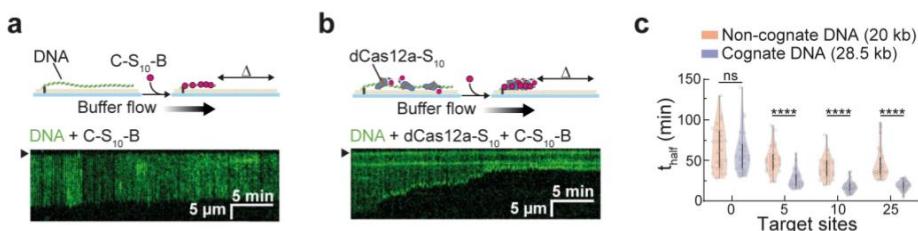


Fig. 1. (a) Representative kymograph showing condensation by the synthetic polypeptide C-S₁₀-B of a naked DNA strand (green, YOYO-1). (b) Representative kymograph showing condensation by C-S₁₀-B of a DNA strand previously decorated with dCas12a-S₁₀. (c) Decoration with dCas12a-S₁₀ triggers faster encapsidation of the cognate DNA strand in the presence of another competing non-cognate DNA.

[1] A. Hernandez-Garcia et al., Nat. Nanotechnol. **9**, 9 (2014)

P6.10 Functionalization and assembly of coiled-coil protein origami nanostructures using SpyCatcher/SpyTag system

Strmšek Žiga, Pavko Neža, Aupič Jana, Lapenta Fabio and Jerala Roman

National Institute of Chemistry, Ljubljana, Slovenia

Proteins are complex biopolymers that have the ability to fold into various nanostructures and perform diverse functions. With *de novo* design of protein structures, we are able to explore protein folds not present in the nature. Coiled-coil protein origami (CCPO) describes a type of modular *de novo* protein design based on specific arrangements of coiled-coil forming segments within the same polypeptide chain, designed to form polyhedral protein nano-cages¹⁻⁴. Our goal was to investigate the possibility of functionalizing nanoscale polyhedra, combining SpyCatcher/SpyTag system⁵ with Tet12SN, a tetrahedral polypeptide scaffold, thus gaining the ability to create bigger, more complex and functionalized nanostructures.

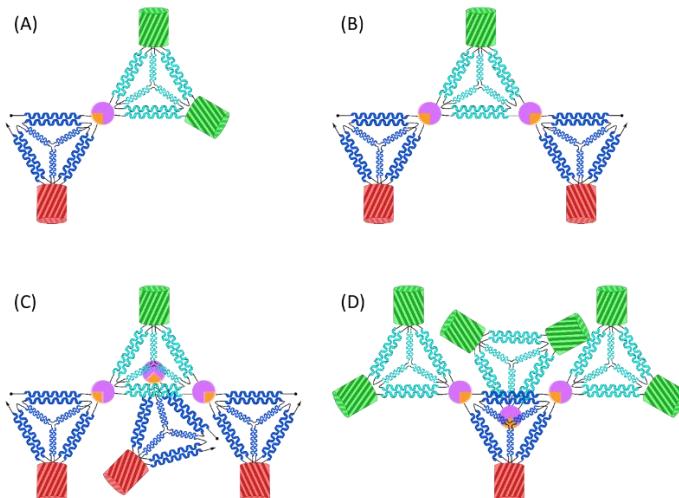


Figure 4: Scheme of the assembly of multichain CCPo protein cages using SpyTag/Catcher-mediated protein domain conjugation. SpyCatcher is shown in purple, SpyTag in orange, GFP in green, RFP in red and Tet12SN in blue.

SpyCatcher and SpyTag protein domains were successfully integrated into the vertices of Tet12SN aimed to construct protein complexes (Fig 1). The successful construction of protein complexes was confirmed by mobility shift on NATIVE-PAGE, SEC-MALS and SAXS. Moreover, the formation of most complex protein structure for all given examples was practically instantaneous and with high yield (>95%) at molar ratio of SpyCatcher:SpyTag of 1:1.

SpyCatcher/SpyTag system was successfully integrated into CCPo, thus expanding the CCPo toolkit. This strategy could be utilised for the design of even more complex protein structures, while coupled domains could be antigens, enzymes, chemical compounds, etc. thus utilising CCPo structures for diverse applications.

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P6.11 Engineering a molecular trigger for HBV VLP disassembly

Caleb Starr, Smita Nair, and Adam Zlotnick

The Hepatitis B Virus (HBV) capsid is a self-assembling nanostructure; our goal is to add function. HBV virus-like particles (VLPs) made from core protein (Cp) can be modified externally and internally to carry exogenous epitopes and proteins of interest or to package drugs and proteins. Cp is 183 amino acids long consisting of an assembly domain (1-149) and a C-terminal nucleic acid binding domain (150-183). The assembly domain construct, purified



as a homodimer, Cp149, assembles into 120-dimer complexes which have steep kinetic barrier to dissociation. Thus, the HBV VLP can be loaded with cargo, but cannot release that cargo in a biological milieu.

We have previously shown that HBV VLPs can be induced to fall apart when small molecules (e.g. maleimidyl BoDIPY-FL) react with a cysteine that was introduced at the interface between dimers, Cp149-V124C. We have now applied these findings to engineer redox-responsive capsid dissociation. Cp150 is a Cp construct with all native cysteines mutated to alanine and a cysteine appended to the C-terminus (C150). The new mutant, Cp150-V124C, has two cysteines: C150 allows capsid crosslinking. C124 can be modified to destabilize the capsid.

Cp150-V124C VLPs were assembled and the C150s oxidized to yield a capsid-wide network of C150 disulfide bonds. In a physiological ionic strength, the resulting capsids could only be dissociated by exposure to reducing agent and 3M urea. To induce metastability, C150-C150 crosslinked capsids were labeled with BoDIPY FL maleimide at C124. In oxidizing conditions, the VLPs did not readily dissociate. Upon treatment with reducing agents, the VLPs fell apart. DTT resulted in rapid dissociation. Reduction by βME (mimicking the physiological redox agent glutathione) resulted in a slower dissociation on the time scale of minutes to hours. Dissociation could also be attenuated by strengthening dimer-dimer association energy.

We also find that Cp150V124CBo is a feasible delivery vehicle to cells. While the bloodstream is an oxidizing environment, cytoplasm is reducing. To test if cytoplasmic reducing potential was sufficient to induce VLP dissociation, we transfected VLPs into HuH7-H1 cells. We found that C124-modified capsids readily dissociated in cells while unlabeled V124C VLPs did not dissociate. The combination of *in vitro* and *in vivo* data demonstrates that the VLPs show promise as a metastable delivery system.

Track on Integrated Chemical Systems

P6.12 DNA Cubes as a “Printing Press”; Printing DNA Patterns onto Polymer Nanoparticles

Sean Laxton and Hanadi Sleiman

McGill University, Canada

Due to the use of non-directional interactions, block copolymer self-assemblies are highly symmetric. Microphase separation of block copolymers can be tuned by varying the ratio of the two blocks, generating spherical, cylindrical, and lamellar morphologies; however, asymmetric assemblies are more difficult to accomplish. Attempts to make lower symmetry polymer assemblies have been envisioned through Janus and patchy particles. Conversely, DNA nanotechnology remains as one of the most specific and programmable self-assembly methods at the nanoscale. The assembly language of the four DNA bases through Watson-Crick base pairing has generated the most complex and asymmetric three-dimensional architectures. Combining DNA nanotechnology with polymer self-assembly harnesses the controlled assembly of DNA with the materials properties of polymers.

Here, we show that DNA nano-cubes can act as a scaffold to spatially orient DNA strands, which are then printed with specific DNA strand patterns on polymer particle surfaces. The scaffold shape can be tuned to print a specific pattern of DNA strands, each with a unique sequence, on the particle surface. Printing of different patterns can be done on the same polymer particle with any composition or shape. Particle composition, shape, and assembly is central to engineering a material’s properties; DNA printing onto polymer particles gives an unparalleled control of the assembled morphology of polymer particles through controlled hybridization of the printed strands, as well as the placement of a particle of a given composition and shape at a given position. DNA printed polymer particles will

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provide access to new discrete assemblies that can act as smart nanorobots, drug delivery tools-as well as access to continuous hierarchical assemblies to engineer new functional materials.

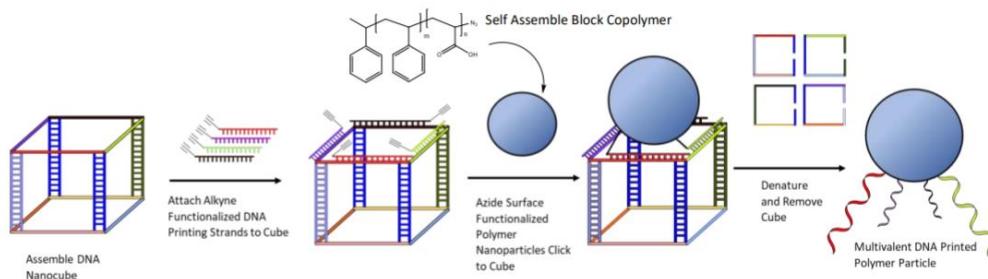


Fig. 1. Overview of DNA Cube Printing Process onto Polymer Particle

Track on Computational Tools for Self-Assembly

P6.13 A Python library for structural DNA nanotechnology

Jorge Eduardo Guerrero, Reza Zadegan

North Carolina A&T State University, USA

Structural DNA nanotechnology is a powerful technique for bottom-up self-assembly of nanoscale structures. Potential applications are vast and only limited by the researchers' imagination. For large and complex structures, the manual or semi-automatic designing process is time-consuming and requires a detailed inspection of the model, leading to user error. We introduce MENDEL, a software library that allows the automatic, extensive, and parametric DNA nanostructures design in this work. MENDEL contains a set of commands that automate the designing process, allow the abstraction of turning sites, compute staples, and parametrize scaling and repetitive features; thus, reducing user error, design complications, and time-to-complete. Running MENDEL through Blender renders a 3D representation of the model. Also, for community convenience, MENDEL generates caDNAno/CanDo compatible files. MENDEL is available as open-source software at [https://github.com/SBMLAB/MENDEL](https://github.com/SBMI-LAB/MENDEL). a. b. c.

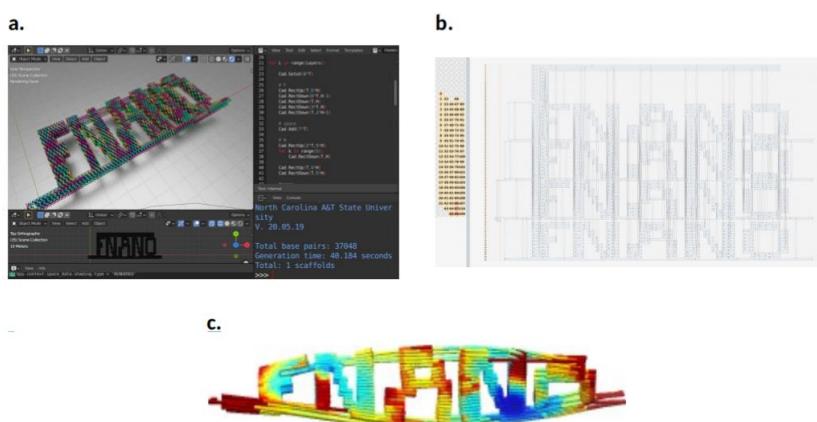




Fig. 1. In under 41 seconds, MENDEL generated a four-layered origami consisting of 37,048 base pairs. (a) Blender interface with scripting window and MENDEL commands, (b) CaDNAno file generated, (c) CANDO geometry prediction.

Acknowledgment This project was supported in part by NSF Grant No. MCB 2027738 and North Carolina Biotechnology Center Grant No.2020-FLG-3887.

P6.14 CADAxISDNA: Automated Design of Smooth and Continuous 3D Surface Contours with DNA Origami

Daniel Fu¹, Raghu N Pradeep², Fei Zhang³, Dewight Williams², John Schreck⁴, Hao Yan², John Reif¹

¹Duke University, USA, ²Arizona State University, USA, ³Rutgers University, USA, ⁴Columbia University, USA

The design and assembly of shaped, smooth-surface, closed shell 3D DNA origami nanostructures ("DNA Capsules") is a unique challenge in nanoscience. Applications of DNA origami nanostructures such as casting metallic nanoparticles using a DNA origami mold to produce plasmonic nanostructures or nanoelectric devices [1][2], capturing and confining single molecules within DNA origami nanoreactors to perform single-molecule chemistry [3], or passive modulation of circulation and uptake with shaped, DNA origami drug delivery vehicles [4] all beckon improvements in designing well-sealed, rigid, shaped, enclosing nanostructures to improve their own functionality. However, the modulation of shape, mechanical rigidity, surface permeability, and accessibility of complex DNA capsule shapes still faces limitations imposed by current design constraints such as single DNA helix wall thicknesses, circular symmetry geometries, and a lack of rapid automated design methodologies. Here, we preview the capabilities of CADAxISDNA, a new software package that automates the design for novel DNA capsules. We present an overview of input formats, implemented approximation and heuristic algorithms for handling crossover placement and strand routing in freeform, non-algorithmic shapes, such as mushrooms, clovers, and Klein bottles, and strategies for modulating rigidity and asymmetric shape by selectively thickening structural walls in single-wall thickness designs into bundles using coplanar helices.

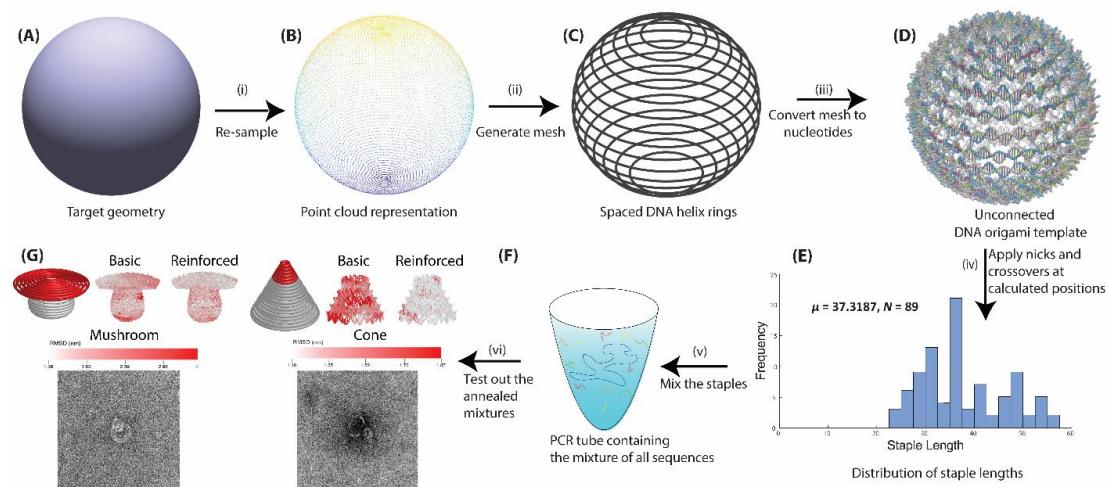


Figure 5 Example workflow of CADAxISDNA. (A) Draw the input. (B) CADAxISDNA samples and processes the input to (C) convert it to a set of contour lines where the (D) DNA helices will be placed. (E) The software then calculates crossover placement, scaffold routing, and nick placement, (F) outputs the staple sequences, which (G) synthesize DNA capsules.

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Poster Session 7, Thursday 15 April, 15:20 - 16:40 UTC

Track on Computational Tools for Self-Assembly

P7.01 Simulation & Design of DNA Amphiphiles: Towards Photo-Responsive Micelles

Shayne Gracious Hanadi F. Sleiman and Christopher J. Barrett

McGill University, Canada

Many groups have demonstrated the applicability of spherical nucleic acids (SNA's) and, in particular, their feasibility as drug delivery vehicles. Despite their widespread use, characteristics that contribute to their overall morphology are poorly understood. For example, our lab has previously demonstrated the formation of DNA nanofibers by the addition of Cy3 molecules to the 5' end of amphiphilic DNA molecules (Figure 1). Similarly, changes to the hydrophobic molecules used, DNA sequence, & ratio of DNA to hydrophobic region can also drastically affect the morphology. Using all-atomistic molecular dynamic (AA-MD) simulations, answers to these abnormal behavioural properties can be obtained. In this work, we first simulate single amphiphilic molecules to observe how these monomers fold on themselves in aqueous solution. We then gradually add more monomers to these systems, in an attempt to better understand their aggregation behaviours. Lastly, we use these results to study novel DNA amphiphiles that incorporate photo-responsive azobenzene motifs. It is hoped that the isomerization from *trans* to *cis* azobenzene can mimic the characteristic folding/aggregation properties of different monomers, thus enabling a reversible change in morphology through irradiation.

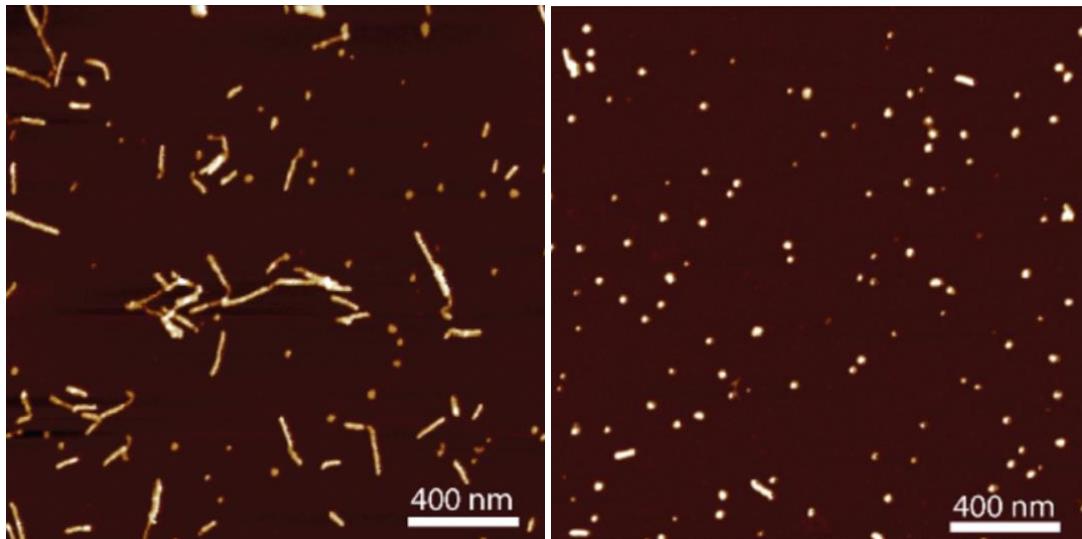


Figure 1. AFM images showing how the addition of Cy3 motifs (left) can alter the morphology of SNA's (right).

P7.02 On Shape Counting Methods for DNA Tile Self-Assemblies

Qing-Shan Jia, Siwei Liu, Hong Kang and Bryan We

Tsinghua University, China

The process of self-assembly of DNA tiles can compute simple binary arithmetic and logical operations. And the shapes of finite structures obtained through this process are important for understanding the capacity of DNA computing, especially when the set of DNA tiles is given. It is time consuming if not impossible to enumerate all these structures through experiments, especially for systems with a large number of DNA tiles. In this paper, we develop exact and approximate counting methods for the finite structures of DNA tiles self-assemblies. The exact method based on depth-first search can obtain the exact number of finite structures with time complexity $O(mn)$, where m is the number of DNA tiles in the largest finite structures, and n is the number of finite structures. With mathematical induction, we can prove that our exact method can obtain all potential finite structures. The sample-based method provides a faster estimation of the number of finite structures, which can sample all the finite structures at an exponential convergence to 1. We demonstrate the performance of the two proposed algorithms on rectangle DNA tiles with 4 sticky faces and with 8 pairs of binding relations as marking (we call them markers below) that may be placed on the sticky faces. Considering the structure of this problem, we used all 4 sticky faces with different combinations (but at least 4 types) of markers to create finite structures. There are 5984 combinations of markers, and we obtained 69 potential finite structure types through 14-hours chemical experiments, which we set as true value. The number of DNA tiles in the largest finite structure type is 6. The experiment settings are: 1) only consider the deterministic binding relationship which captures the steady-state; 2) there is a sufficient number of tiles for self-assembly. While in a real experiment, the density of particular tiles in the solution would affect the success rate of the final structure obtained from self-assembly; 3) markers will connect with sticky faces in pair first, but do not influent the shape of structures for their smaller shapes. Our exact method obtained the accurate number of the finite structures in 0.86 hours. And the sampling-based algorithm found estimations within 5% accuracy error within 5.97 seconds on average (8500 sample times), and converges to the actual number within 10.39 seconds on average (14000 sample times). The estimation number of the sampling-



based method with different sampling times is showed in Figure 1(a), and the time consuming is showed in Figure 1(b). Here we must point out that there will be $5985 \times 4^{20} = 6.58 \times 10^{15}$ potential combinatorial finite structures with 6 DNA tiles without considering the relationship of these sticky faces. If we consider structures with less than 6 DNA tiles, there will be more.

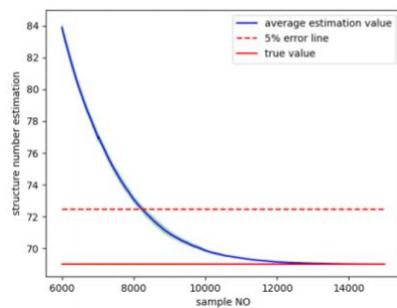


Fig. 1. (a) estimation of structure number of sampling-based method

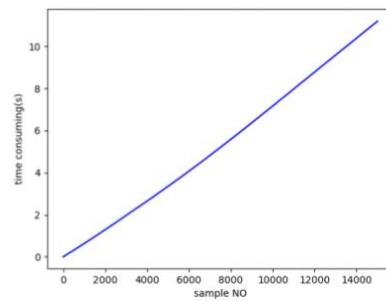


Fig. 1. (b) time consuming of sampling-based method

P7.03 Making the most of DNA melt curves: Data collapse with affine transformations and consequences of experimental design

Jacob Majikes, Paul N. Patrone, Robert F. DeJaco, Anthony J. Kearsley and J. Alexander Liddle

National Institute of Standards and Technology

Traditional calorimetry can be difficult to apply to nucleic acid nanofabrication systems. Often, these systems contain more components than can be resolved independently and have enough mass to make preparation of high molarity samples difficult. While van't Hoff analysis of nucleic acid melting data is straightforward, ensuring that it yields precise and accurate thermodynamic parameters which are consistent with other measurements and useful for prediction requires considerable care.

Here we present several useful tools and methodologies for extracting high quality thermodynamic information possible from typical melt curves.

We show how an affine (linear) transformation can be used to map replicate melt curve measurements onto one universal melt curve and minimize the associated uncertainties when combined with baseline subtraction. We also present the results of Monte Carlo simulations showing how uncertainty in melt curves can propagate through van't Hoff analysis, and derive insights that reveal how modest improvements in experimental design can significantly improve thermodynamic parameter extraction.

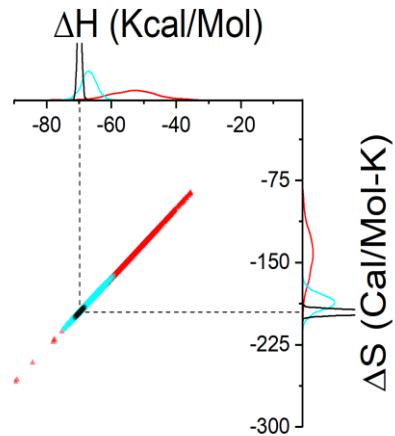
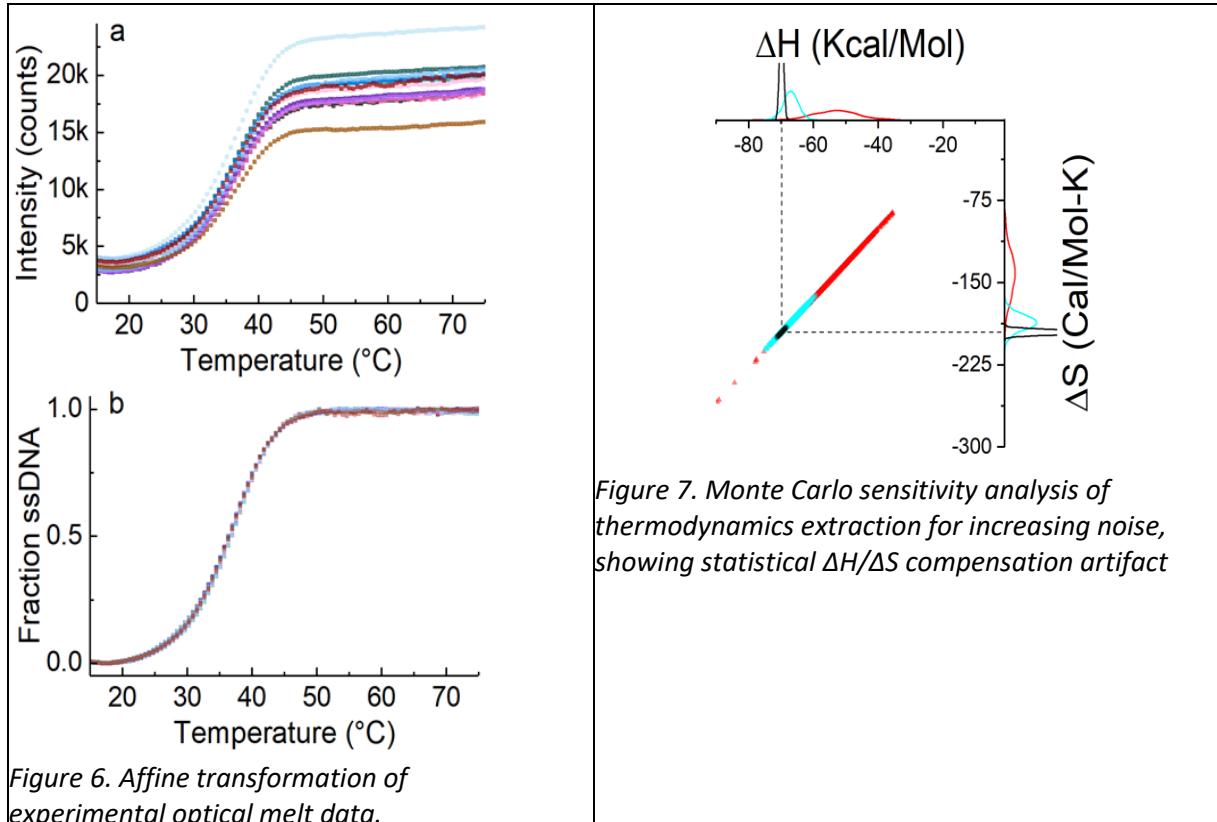


Figure 7. Monte Carlo sensitivity analysis of thermodynamics extraction for increasing noise, showing statistical $\Delta\text{H}/\Delta\text{S}$ compensation artifact

P7.04 How to Design Free-form DNA Nanostructures Online

Joakim Bohlin¹, Erik Poppleton², Michael Matthies², Aatmik Mallya² and Petr Šulc²

¹University of Oxford, UK, ²Arizona State University, USA

The online tool oxView [1] was originally created to facilitate visualization of oxDNA structures. While it had some limited editing features when published, those were for making changes to existing structures before simulation. In contrast, the updated software we present here can be used as an independent tool to design structures from scratch. The underlying software model is now separate from the oxDNA format, featuring the option to save your work into a JSON file with additional information about base-pairing, colouring and more. Designs can still be exported into oxDNA simulation files, a list of sequences, images, videos, or files for 3D printing and rendering.

To start drawing a structure, input a sequence of a strand and click create. A complementary strand can be automatically created by enabling duplex mode. See Figure 1.b for the complete toolset. Figure 1.a shows an example workflow, where the DNA tetrahedron from [2] is recreated. An initial duplex is created, duplicated and positioned to form the basic structure. The separate strands are then joined using the ligation tool, before the clustered helices (for simplicity drawn on a plane) are relaxed into the intended tetrahedral shape using rigid-body dynamics. Finally, the insertion tool can be used to insert additional nucleotides in the hinges.

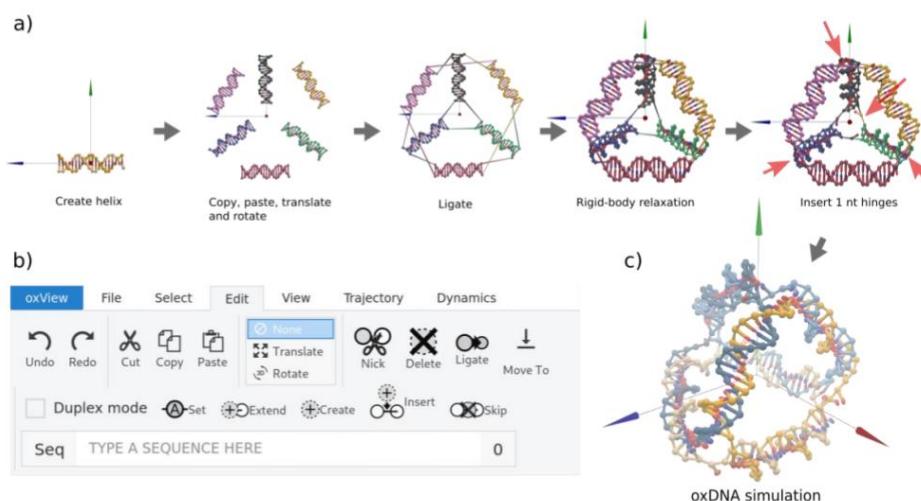


Fig. 1. (a) The steps required to create a DNA tetrahedron from scratch. (b) The structure-editing tools available in oxView, allowing nucleotides, strands and helices to be created, removed and manipulated. (c) The tetrahedron seen in (a) visualised during oxDNA simulation.

The design can easily be simulated from within the oxView interface, as seen in Figure 1.c, then modified and simulated further, depending on the simulation results. The presentation will be an interactive demo, but you can start designing already at: <https://sulcgroup.github.io/oxdna-viewer>.

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P7.05 Coarse-grained nucleic acid - protein model for hybrid nanotechnology

Jonah Procyk, Erik Poppleton and Petr Šulc

Arizona State University, USA

Hybrid DNA – protein nanotechnology offers an extended suite of molecular tools for design of novel materials with potential applications ranging from therapeutics to nanoscale machines. Combination of DNA's programmability with the diverse functionality of proteins limits the scale and complexity of hybrid designs only to that of our imaginations. The previously developed coarse-grained oxDNA/oxRNA models [1] of DNA and RNA have been popular tools for simulating nucleic acid nanotechnology designs. However, their applications to hybrid nanotechnology is limited as they do not offer representation of proteins. To aid in the design and in silico analysis of such hybrid nanostructures, we introduce here a coarse-grained DNA/RNA-protein model that extends the oxDNA/oxRNA models of DNA/RNA with a coarse-grained model of proteins based on an anisotropic network model representation [3]. Fully equipped with analysis scripts and visualization, our model aims to facilitate both hybrid nanomaterial design as well as biological studies. Our design software oxView[2] now allows for seamless editing of DNA, RNA, and protein PDB structures making the design phase easier than ever. Examples of applications include



hybrid-material Cryo-EM structure model fitting, analysis of peptide-delivering DNA nanostructure designs targeting a protein, and design and simulations of DNA-protein hybrid nanocage (Fig 1 and [4]).

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Figure 1: A hybrid nanocage as represented in the ANM-oxDNA model

P7.06 Virtual DNA Lab – A computational simulation platform for DNA multi-strand dynamics

Frankie Spencer¹, Usman Sanwal¹ and Eugen Czeizler^{1,2}

¹Åbo Akademi University, Finland, ²National Institute for Research and Development of Biological Sciences, Romania

The dynamics of nucleic-acids dynamical systems is intrinsically based on local interaction. The major acting mechanisms are that of Watson-Crick complementarity on one-hand, generating binding events, and thermal energy on the other, generating random motion and un-binding. It is thus predictable that such systems can be successfully captured by computational modeling paradigms based on local interactions, such as the rule-based modeling methodology [1, 2]. The Virtual DNA Lab (VDNA-Lab) software provides an easy to use graphical interface for creating, running and visualizing synthetic simulations for DNA assembly systems, such as assembly of DNA nanostructures, strand displacement cascades systems, DNA-tile assembly etc. It uses the NFsim computational modeling engine [3] to run simulations and generate outputs. These outputs can be visualized using the VDNA-

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Lab's own visualization tool, which allows also for further analysis and filtering. VDNA-Lab features: The tool consists of three main functionalities, each reachable from the menu bar of the application: - "Create Test Tube" for creating new test tube input files, - "Run Experiment" for simulating the assembly process within a given input test tube file, - "Visualize Test Tube" for visualizing the content of a test tube at the beginning, middle, or end of an experiment. At the core of this model implementation lie 12 binding and un-binding local interaction rules, each with its own (user-adjustable) kinetic rate constant, and each implemented through one or several rule-based reactions. Using these reactions/rules, we capture the dynamics of the DNA-dynamical system, by modeling: the initial binding of short toeholds, the "breathing" dynamics in-between bounded ssDNAs, random un-binding events, as well as the un-binding of loosely connected ssDNAs. Different from other computational modelling frameworks for DNA strand assembly, we can modify some of the systems parameters mid-simulation, such as the current temperature of the system. Thus, we can model also an entire annealing process for the formation of a DNA structure. The software is freely available from: https://github.com/Frankie-Spencer/virtual_dna_lab

The figure displays three windows of the VDNA Lab application:

- Create Test Tube Window:** Shows a list of ssDNA sequences to be created. One sequence is shown in detail: ssDNA(s) ||| 50 ||| TATCCTCCTGACAATTAGACCCCATTCCTGATGGGA. Below it, a text input field contains the sequence TATCCTCCTGACAATACACCGCCATTCTGATGGGA, with an 'Amount' dropdown set to 50. Buttons for 'Edit', 'Delete', 'Reset all', 'Add', 'Import from Species file', and 'Submit' are visible.
- Run Experiment Window:** Shows a confirmation message: "Dump file --- reference_file_nf.0.19.dump --- processed successfully ✓". It includes fields for "test tube" (C:/Backup April 2018/Desktop/Bjorn/Tmp/test_DAE-E_tile.species) and "Save simulation outputs directory" (C:/Runs/Outputs). A "Run" button is present.
- Visualize Test Tube Window:** Shows a message "Program ready to run!". It has a "Source file" field (C:/Runs/Outputs/reference_file--19/reference_file_nf.0.38.dump), a "History" list containing several dump files, and a "Run visualization" button.

Figure 1: The three main windows of the VDNA-Lab application: Create Test Tube; Run Experiment; and Visualize Test Tube



Figure 2. Left: fraction of the generated output of a DNA dynamical system simulation consisting of 5 ssDNAs which assemble into a DAE-E tile; nucleotides that are bound by hydrogen bonds are colored similarly. Right: the design scheme and nucleotide sequences of the 5 strands assembling the DNA tile; taken from [4]. Figure 1: The three main windows of the VDNA-Lab application: Create Test Tube; Run Experiment; and Visualize Test Tube

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Track on Synthetic Biology

P7.07 Reconstitution of ultrawide DNA origami pores in liposomes for transmembrane transport of macromolecules

Nicola De Franceschi¹, Alessio Fragasso¹, Pierre Stömmer², Eli van der Sluis¹, Hendrik Dietz² and Cees Dekker¹

¹Delft University of Technology, The Netherlands, ²Technische Universität München, Germany

Molecular traffic across lipid membranes is a vital process in cell biology that involves specialized biological pores with a great variety of pore diameters. In the last decade, advancements in the field of DNA nanotechnology have enabled the fabrication of a number of DNA origami nanopores, featuring pore sizes up to 9 nm^{1,2}. Self-insertion of these pores into membranes relies on the presence of chemical modification (e.g. cholesterol), that anchor and stabilize the hydrophilic DNA nanopores³. However, the efficiency of self-insertion decreases exponentially as the size of the pore increases⁴. This represents the major factor limiting the size of the nanopore that can be inserted. Here⁵, we overcome this challenge by employing a continuous droplet interface crossing encapsulation (cDICE) technique⁶ to incorporate newly designed ultrawide (~30 nm inner diameter, ~55 nm outer diameter) DNA origami pores into the membrane of giant unilamellar vesicles (GUVs). To demonstrate that the pore is functionally reconstituted, we measured the influx of multiple fluorescent macromolecules up to 22 nm large, consistent with the 30 nm inner diameter of the pores. We estimate that up to hundreds of pores can be functionally reconstituted in a single liposome. These ultrawide pores represent an exciting tool for various scopes, from the mimicking of large biological pores such as the nuclear pore complex, to applications in synthetic biology and drug delivery.

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P7.08 A modular, dynamic, DNA-based platform for regulating cargo distribution and transport between lipid domains

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Cell membranes feature highly evolved proteo-lipid machinery that co-localises in lipid rafts, nanoscale assemblies thought to underpin processes such as signal transduction¹. Bottomup synthetic biology aspires to replicate the phenomenology associated to biological systems in artificial cells, minimal entities with applicability ranging from high-tech therapeutics to biosynthesis². Synthetic lipid bilayers, frequently used to compartmentalise artificial cellular platforms, often lack the complex functionalities displayed by their biological counterparts. DNA nanotechnology, increasingly popular for the construction of synthetic cellular systems, can readily couple bio-inspired DNA devices with model lipid bilayers via hydrophobic handles³. In turn, DNA amphiphiles also partition in lipid domains of model membranes⁴, reminiscent of proteins in raft microenvironments⁵.

Here, we statically and dynamically modulate the lateral distribution of DNA nanostructures in phase-separated membranes by harnessing the tendency of cholesterol and tocopherol motifs to respectively enrich liquid-ordered (Lo) and liquid-disordered (Ld) phases. By prescribing combinations of multiple anchors, changes to nanostructure size, and geometry, our DNA devices are programmed to achieve partitioning states spanning the energy landscape. The functionality of our platform is exemplified with a responsive biomimetic DNA architecture that undergoes ligand-induced reconfiguration and mediates cargo transport between lipid domains. Our approach⁶ paves the way for the development of next-generation biomimetic DNA-based membrane platforms, a stepping stone for achieving sensing and communication in artificial cells.

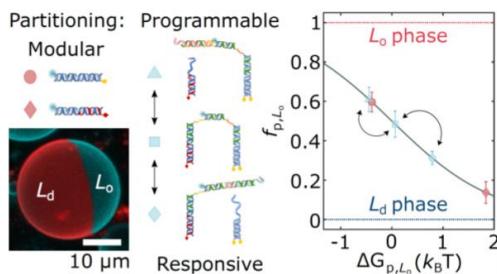


Figure 1. Modular, programmable and responsive partitioning of DNA nanostructures in lipid domains: Amphiphilic DNA nanostructures achieve static model membrane patterning (left) as well as reversible cargo transport between lipid phases, spanning the energy landscape (right).

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- [2] Oglecka, K., Rangamani, P., Liedberg, B. et al., Elife. 2014, 3, e03695.
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P7.09 In situ generation of RNA complexes for synthetic molecular strand displacement circuits in autonomous systems

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Synthetic molecular circuits implementing DNA or RNA strand-displacement reactions can be used to build complex systems such as molecular computers and feedback control systems[1]. Despite recent advances, application of nucleic acid-based circuits *in vivo* remains challenging due to a lack of efficient methods to produce their essential components – multi-stranded complexes known as “gates” – *in situ*, i.e. in living cells or other autonomous systems. Here, we propose the use of naturally occurring self-cleaving ribozymes to cut a single-stranded RNA transcript into a gate complex of shorter strands. We designed the ‘self-excising unit’ by connecting two hammerhead ribozymes that cut 5’ upstream and 3’ downstream (Figure 1a). After successful cleavages, the self-excising unit dissociates from the RNA transcript, leaving an RNA gate capable of performing strand-displacement reactions.

We first confirmed the functionality of each ribozyme in the self-excising unit by running the RNA transcript in a denaturing PAGE gel. We also showed that after self-cleavages, the self-excising unit dissociates from the gate in a native condition. Finally, we demonstrated real-time and *in-situ* strand displacement reactions using RNA inputs and gates produced via *in-vitro* transcription. Our circuit could discriminate input RNAs with different toeholds and can be assembled as a two-step cascade (Figure 1b). Our method opens a new possibility for operation RNA strand-displacement circuits via autonomous and continuous production of RNA strands in a stoichiometrically and structurally controlled way[2].

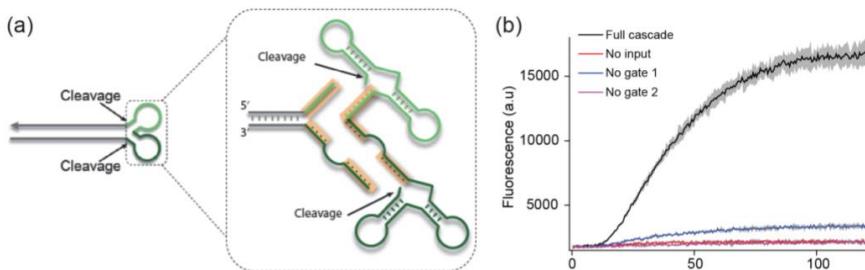


Fig. 1. (a) Scheme of the self-excising unit. (b) Real-time trace of two-cascade circuit with controls.

- [1] Simmel F.C. et al., Chem. Rev. 119(10):6326-69. (2019)
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P7.10 Mechanisms for the Controlled Division of Giant Unilamellar Lipid Vesicles

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Cell division is one of the hallmarks of life. Success in the bottom-up assembly of synthetic cells will, no doubt, depend on strategies for the division of protocellular compartments. Here, we demonstrate two mechanisms for the controlled division of giant unilamellar lipid vesicles (GUVs).

The first mechanism relies on the line tension of phase-separated vesicles and a surface-to-volume ratio increase via osmosis. We derive a conceptual model based on the vesicle geometry which makes four quantitative predictions that we verify experimentally (Fig. 1 (a)). We find that the osmolarity ratio required for division is $\sqrt{2}$, independent of the GUV size, while asymmetric division happens at lower osmolarity ratios.

Remarkably, we show that division can be achieved through distinctly different processes, including water evaporation, metabolic decomposition of sucrose and finally light-triggered uncaging of CMNB-fluorescein. The latter provides full spatio-temporal control of the osmolarity increase by local illumination of a chosen area, such that a target-GUV undergoes division whereas the surrounding GUVs remain unaffected [Dreher et al., Angewandte Chemie, 2020].

The second mechanism is based on osmotic deflation and an increase of the membrane spontaneous curvature. To controllably increase the spontaneous curvature, we use the photosensitizer Chlorine e6 which self-assembles into lipid bilayers and leads to lipid peroxidation upon illumination. Remarkably, we achieve full division of single-phased DNA-containing GUVs (Fig. 1 (b, c)). Overall, our work provides broadly applicable mechanisms for the division of synthetic cell compartments and adds to the strategic toolbox of bottom-up synthetic biology.

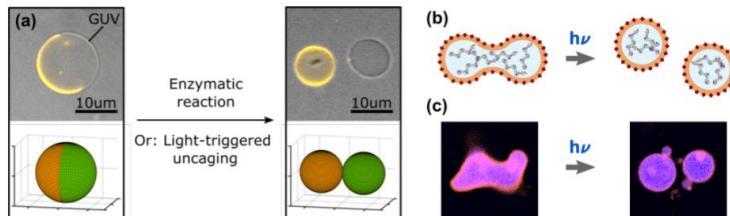


Fig. 1. Mechanisms for the division of giant unilamellar vesicles. (a) Confocal fluorescence images and theoretically predicted shapes of the division of phase-separated GUVs. (b) Illustration of the division of DNA-containing GUVs in the presence of Ce6. (c) Confocal fluorescence images of the division of DNA-containing GUVs in the presence of Ce6.

Fig. 1. Mechanisms for the division of giant unilamellar vesicles. (a) Confocal fluorescence images and theoretically predicted shapes of the division of phase-separated GUVs. (b) Illustration of the division of DNA-containing GUVs in the presence of Ce6. (c) Confocal fluorescence images of the division of DNA-containing GUVs in the presence of Ce6.

P7.11 Poster withdrawn

P7.12 Amplified Self-Immulative Release of Small Molecules by Spatial Isolation of Reactive Groups on DNA-Minimal Architectures

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Triggering the release of functional molecules in response to a particular stimulus has practical applications in a variety of different areas including smart therapeutics and bio imaging. Nucleic acids are a favorable stimulus for release due to their uniqueness and diversity across individuals. Due to their low concentration *in vitro* and *in vivo* it is important to amplify release from nucleic acid signals to give an appreciable response. Nucleic acid templated reactions tether reagents to short oligonucleotides, which bind to a nucleic acid template and react to release small molecules. Amplification in these systems is controlled by the reversibility of the short modified oligonucleotide sequences to bind and unbind the template. While this is favorable for amplification, these systems are limited to having multiple components to generate amplification and therefore their use *in vivo* is limited. Other isothermal amplification strategies such as the hybridization chain reaction (HCR), take advantage of toe-hold mediated strand exchange of metastable hairpins to generate amplification. Since the hairpins of HCR are meta-stable this allows us to design and build systems that operate as a single unit which is preferable for *in vivo* applications. Here, we report combining a nucleic acid templated reaction with HCR for the, amplified release of functional molecules, and then integrate these hairpins into single units as DNA tracks and spherical nucleic acids (Figure 1). [1]

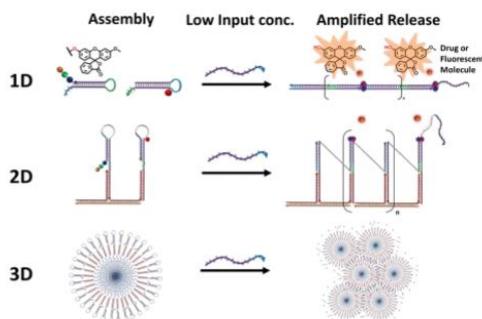


Figure 1. HCR resulting in a DNA templated disulfide exchange, releasing caged fluorescein on DNA-Minimal Tracks and spherical nucleic acids.

- [1] A. L. Prinzen, D. Saliba, C. Hennecker, T. Trinh, A. Mittermaier, H. F. Sleiman, *Angew. Chem. Int. Ed.* 2020, 59, 12900.

P7.13 Cation-regulated binding of DNA with phospholipid membranes

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The interplay between nucleic acids and lipids underpins several key processes in molecular biology, synthetic biotechnology, vaccine technology and nanomedicine. These interactions are often electrostatic in nature, and much of their rich phenomenology remains unexplored in view of the chemical diversity of lipids, the heterogeneity of their phases, and the broad range of relevant solvent conditions. Describing the ion-mediated interplay between nucleic acids and lipids is of great importance for biomimetic nanodevices, such as a DNA-based synthetic enzyme [1,2]. In a recent study, we synergistically combined our all-atom molecular dynamics (MD) simulations with the experiments performed by our collaborators to determine how divalent cations and the phase of the lipid bilayer membrane modulate the interactions between DNA and zwitterionic phospholipid membranes [3]. Confocal microscopy show that the DNA duplexes bind to the gel-phase lipid bilayer membrane in the presence of divalent cations, whereas no attachment was observed for liquid phase membranes.

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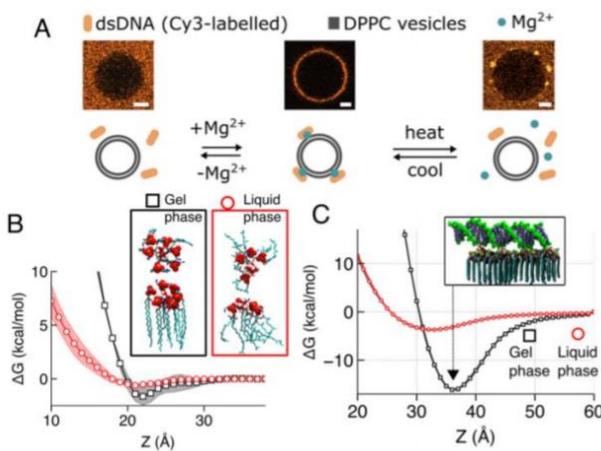


Figure 1: Cation induced binding of DNA with phospholipid membranes in gel phase. (A) The confocal micrographs images showing the cation mediated and phase dependent binding of DNA with phospholipid membrane. (B) The free energy profile of Mg^{2+} ion as a function of its distance to the midplane of the lipid membrane. Insets illustrate a representative coordination of the magnesium ions by lipid head groups at the minimum of free energy curves for gel and liquid phase membranes. (C) Free energy of the DNA fragment versus its distance to the membrane midplane. The inset image illustrates a representative configuration of Mg^{2+} ions near DNA at its free energy minimum in gel-phase.

Using enhanced sampling all-atom MD simulations, we found that the Mg^{2+} shows lower binding free energy with the phospholipid membranes in gel phase as compared to liquid phase. Our simulations further revealed that these Mg^{2+} ions on the surface of the membrane can bridge the interaction between the lipid headgroups and DNA backbone leading to their stronger binding in gel-phase phospholipid membranes. Finally, we showed that divalent cation induced binding mechanism can be utilized to control the degree of attachment of cholesterolmodified DNA nanostructures depending on their overall hydrophobicity and charge. Overall, our findings shed light on the electrostatic interaction governing the assembly of lipid and DNA which have profound implications in various area of biology, such as virus cell entry, RNA transport from the nucleus, as well as in the area of biomimetic nanodevices such as DNA nanostructures for intra-cellular delivery of drugs and genetic material.

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- [2] D. Sobota et al., Nano Lett., 20(6), 4306–4311, (2020).
- [3] D. Morzy et al. J. Am. Chem. Soc. Under review 2021

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