Abstract Booklet

FNANO 2021: 18th Annual Conference Foundations of Nanoscience

12 - 15 April 2021

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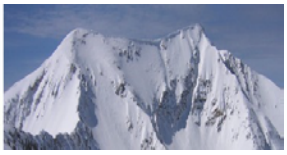

FNANO 2021: 18th Annual Conference Foundations of Nanoscience
Monday 12 April 2021

(Invited) Super-resolution with and without deep learning

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

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[1]U.S. Naval Research Laboratory, USA, [2]National Research Council, USA; [3]Boise 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.

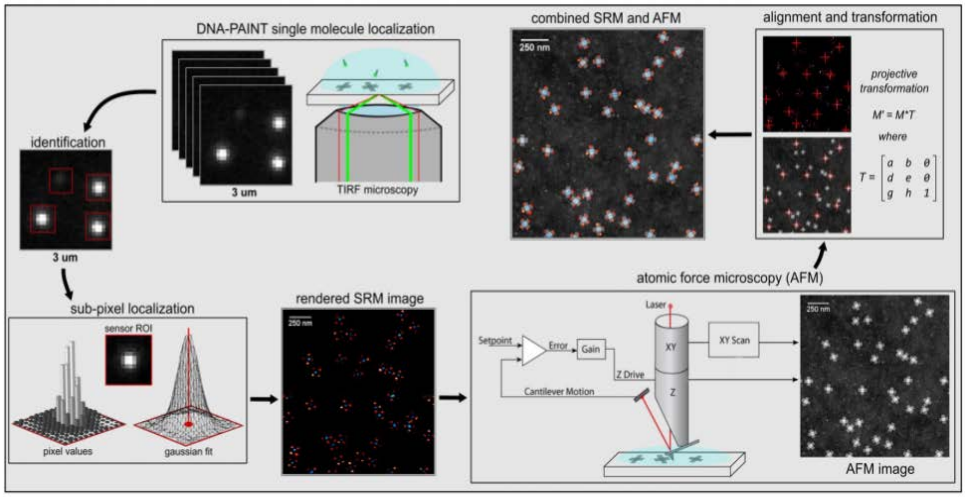

Fig. 1. Diagram depicting correlative DNA-PAINT and AFM imaging of DNA origami cross-tiles.

[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

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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.

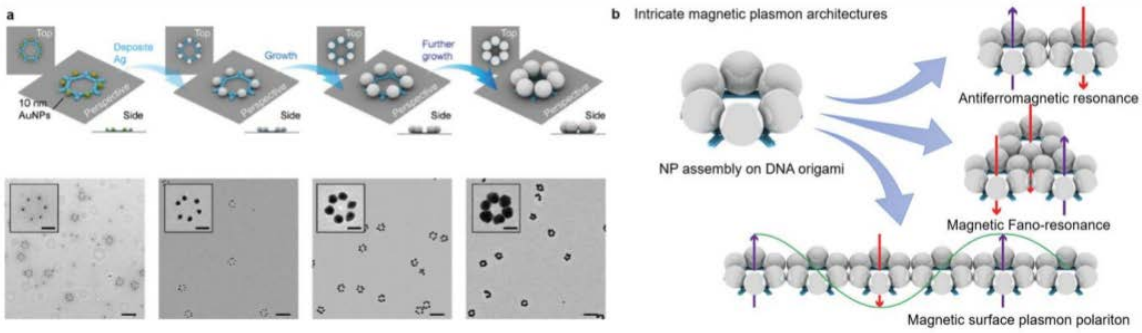

Fig. 1. Magnetic plasmon metamolecules and complex NP ring networks assembled on DNA templates.
(a) Schematics and TEM images of magnetic rings, which were constructed via stepwise growth of AgNPs. #
(b) Programmable assembly of DNA origami leads to intricate magnetic plasmon architectures.

[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.

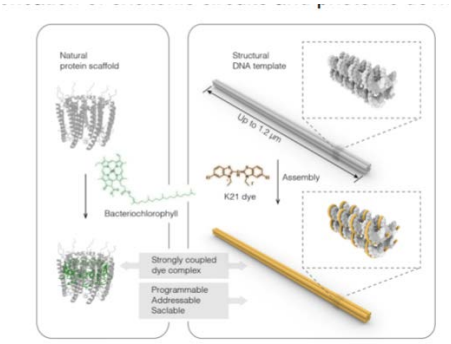

Fig. 1. Bio-inspired excitonic architectures that employ the structural DNA templates mimicking the natural protein scaffold (light harvesting complex 2 from purple bacteria) to arrange cyanine dye K21 to form strongly coupled chromophore complex for submicron or micron exciton transfer with programmability,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

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

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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-nanoporus 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.

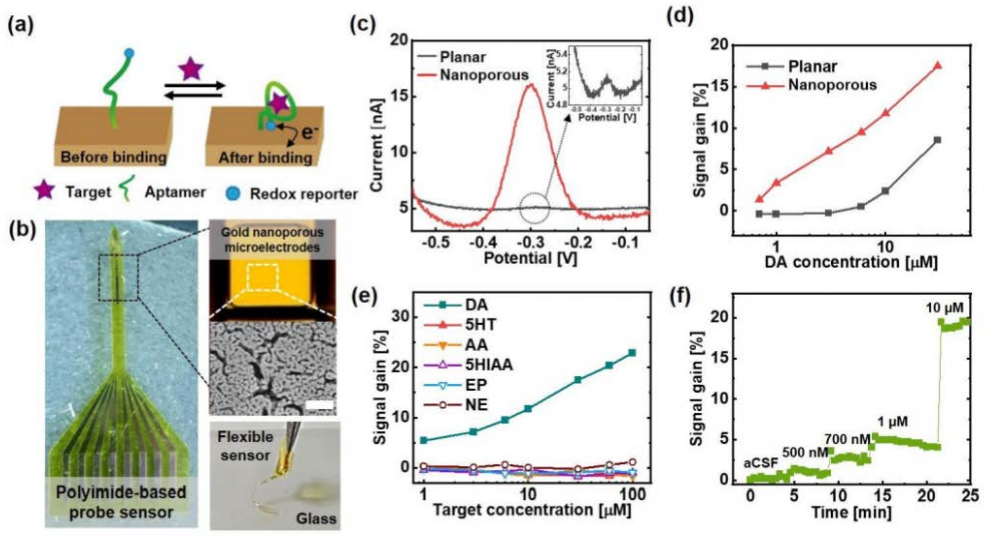


Figure 1. (a) Working mechanism of aptamer-based sensor. (b) Optical micrograph of the gold nanoporous-based biosensor. Recording site is composed of 100 × 100 μm^2 nanoporous electrode. Right upper image is optical and

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-Curras et al., Real-time measurement of small molecules directly in awake, ambulatory animals. PNAS 114, 645-650 (2017).

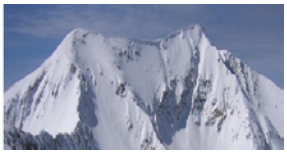
DNA Nanoswitch Barcodes for Multiplexed Biomarker Profiling

Arun Richard Chandrasekaran,[1] Molly MacIsaac,[2] Javier Vilcapoma,[1] Clinton H. Hansen,[2] Darren Yang,[2] Wesley Wong,[2] Ken Halvorsen[1]

[1]State University of New York, USA, [2]Harvard 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 consequentially 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] enzymes[4] 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 Cavaliere, 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 Phy. 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).

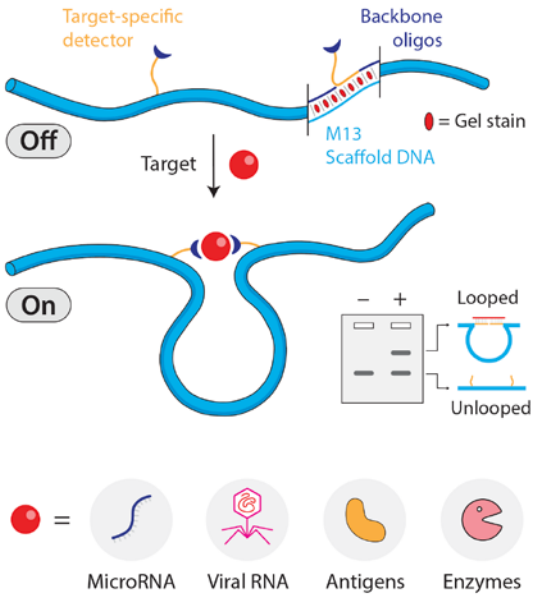


Figure caption: DNA nanoswitch is assembled from an M13 scaffold (7249 nt) and short complementary backbone oligonucleotides. Two of the backbone oligonucleotides are modified to contain recognition elements specific to the target to be detected (nucleic acids, antigens, enzymes). On target recognition 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, 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 needto 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.

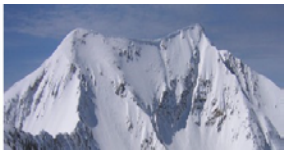
[1] U.S. Occupational Safety and Health Administration. "Direct-reading instrumentation for air contaminants", OSHA Technical Manual II.3.III (2014). URL: https://www.osha.gov/dts/osta/otm/otm_ii/otm_ii_3.html#DirectReading
[2] U.S. National Nanotechnology Initiative. "Nanotechnology-enabled sensing" (2009). URL: https://www.nano.gov/node/100
[3] S. Earnest, 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/NIOSHPUB2019116

(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 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 of 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

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[1]Technische Universität München, Germany, [2]University 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.

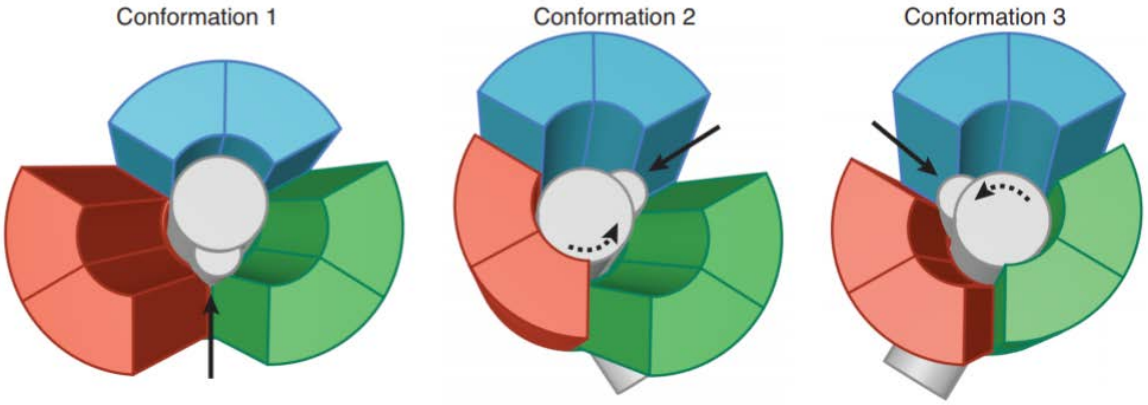

Fig. 1. Sketch of the system showing the rotation of the central rotor coupled to conformational changes of the surrounding stator.

(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].

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[4] X. Bian, et al., Nat. Chem. Biol. 15, 830–837 (2019)
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Mechanics and Design Principles of 2D Auxetic DNA Nanostructures

Ruixin Li, Haorong Chen, and Jong Hyun Choi

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Architectured 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 architectured 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.

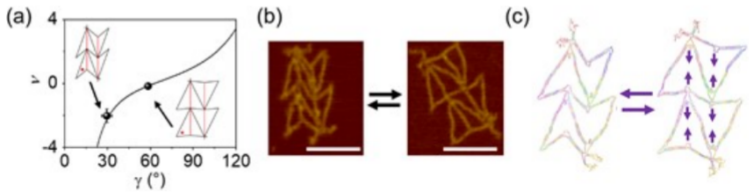

Figure 1. (a) Theoretical Poisson's ratio versus angle *γ* (indicated by red dot). (b) AFM images of a re-entrant triangle at two different conformations (angles). The structural transformation is completed by two-step DNA reactions. (c) Coarse-grained MD calculations of a re-entrant triangle under mechanical 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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[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 Sigl[1], Elena M. Willner[1], Wouter Engelen[1], Jessica A. Kretzmann[1], Ken Sachenbacher[1], Anna Liedl[1], Fenna Kolbe[1,2], Florian Wilsch[1,2], S. Ali Aghvami[3], Ulrike Protzer[1,2], Michael F. Hagan[3], Seth Fraden[3], Hendrik Dietz[1]

[1] Technical University of Munich, Germany, [2] Helmholtz 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

Dongfang Wang[1], Lei Yu[2], Chao-Min Huang[3], Bin Ji[4], Gaurav Arya[3], Jie Song[4], Shuai Chang[2], Yonggang Ke[1]

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

University 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 toeholdmediated 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.

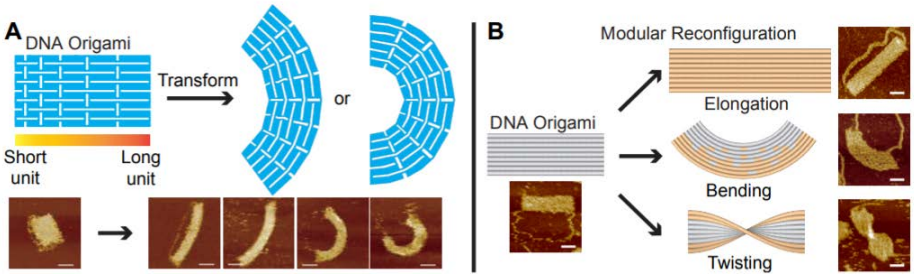

Figure. Controlling origami transformation with two modular design strategies. (A) Reconfigurable DNA nanoarrays constructed on the basis of dynamic DNA domino origami. The structures can transform between conformations with different degrees of curvatures. (B) Reconfigurable DNA origami consisting of 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.

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Hybrid protein-DNA and peptide-DNA nanostructures

Nicholas Stephanopoulos

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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

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/trimers/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

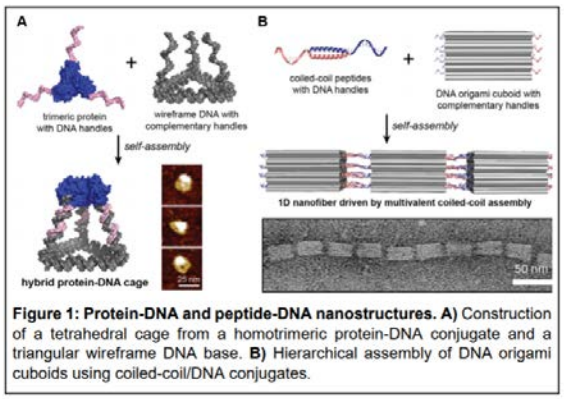

Figure 1: Protein-DNA and peptide-DNA nanostructures. A) Construction of a tetrahedral cage from a homotrimeric protein-DNA conjugate and a triangular wireframe DNA base. **B)** Hierarchical assembly of DNA origami cuboids using coiled-coil/DNA conjugates.

(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-termining 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.

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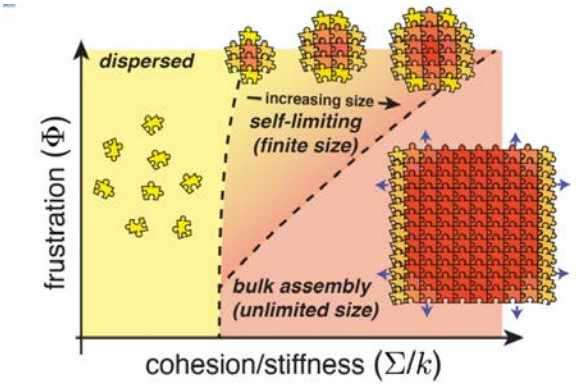
[2] M. F. Hagan and G. M. Grason, "Equilibrium mechanisms of self limiting assembly" (2021) arXiv:2007.01927



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

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[1]Imperial College London, UK, [2]University 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.

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.

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

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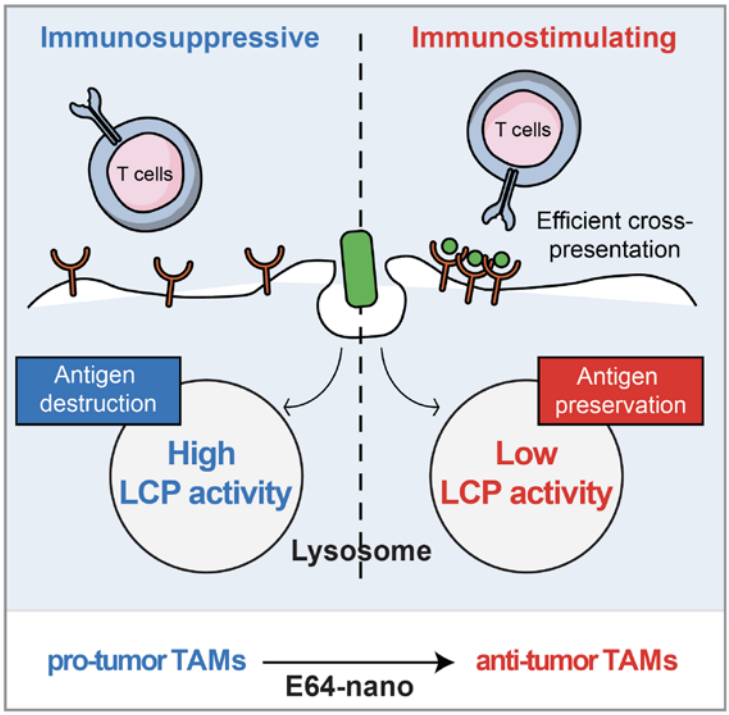
Activating $CD8^+$ T cells by antigen cross-presentation is remarkably effective at eliminating tumors. Although this function is traditionally attributed to dendritic cells, tumor-associated macrophages (TAMs) can also cross-present antigens. TAMs are the most abundant tumor-infiltrating leukocyte. 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*.

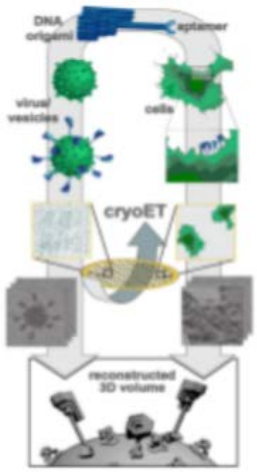
Summary model



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. Machala[1], Catherine Whittle[1], Susan Black[1], Jonathan Bath[1], Andrew J. Turberfield[1], Kay Grünewald[1,2] and Lindsay A. Baker[1,2]

[1]University of Oxford, UK, [2]Heinrich-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 heterogenous 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.

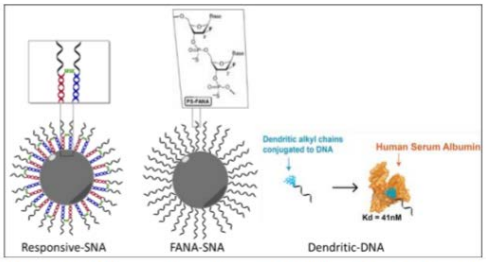


Figure 1 Representative scheme of the 3 particles developed and used for drug delivery (left to right): Responsive Spherical Nucleic Acid, FANA-modified Spherical Nucleic Acid, and Dendritic DNA-albumin complex.

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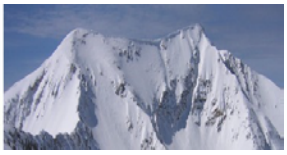

The Effects of Overhang Placement and Multivalency on Cell Labeling by DNA Origami

Ying Liu, Piyumi Wijesekara, Sriram Kumar, Weitao Wang, Xi Ren and Rebecca E. Taylor

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

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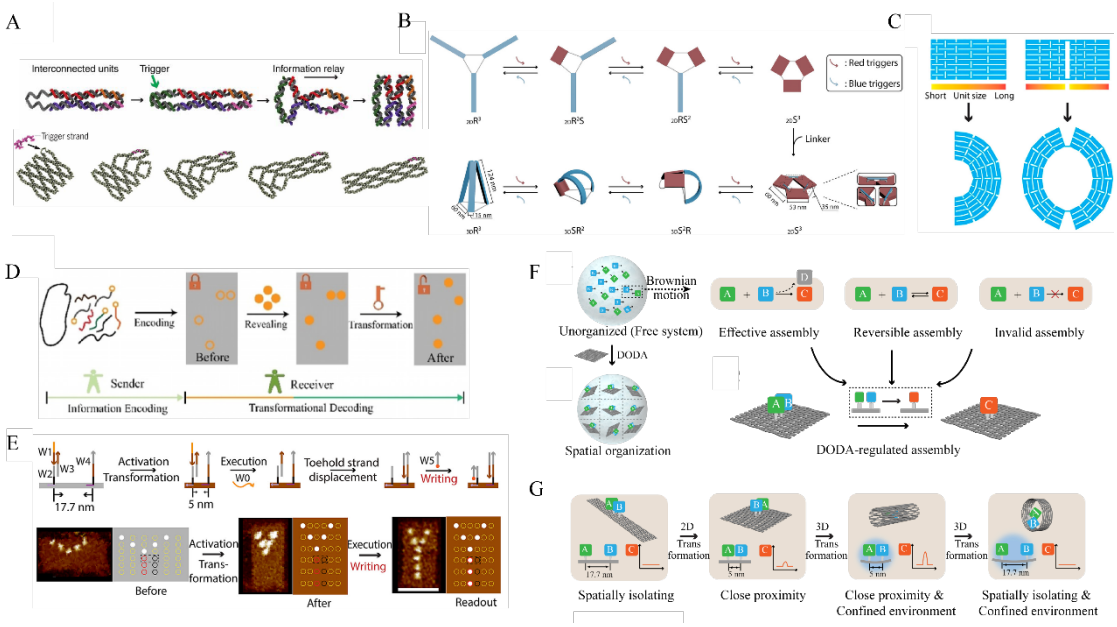
Information relay is the general phenomenon to assist and maintain diverse activities of the whole the matter in nature. One key challenge is how to imitate the information relay process at molecular level. Recent researches has revealed the information relay pattern from static pattern such as self-assembly, 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 design[1]. 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).

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 3D[2] (Fig 1B). Furthermore, by tuning the sizes of DNA units within the DODA, the DODA can transform between a noncurved conformation and curved conformation[3] (Fig 1C). Based on the conformation transformation of DODA, we develop a molecular information coding system for information security[4] (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 operations[5] (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 array[1]. (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 triggers[2]. (C) Architecture design of curvature control with DNA domino origami[3]. (D) Information Coding in the DODA[4]. (E) Proximity-Induced Pattern Operations (Writing operation) in reconfigurable DODA[5]. (F-G). Controlling the self-assembly of biocatalytic architectures with reconfigurable DODA. Unpublished data.

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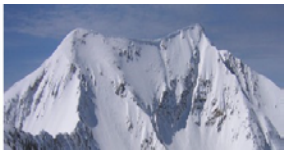

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Exploring sequence space to design controllable G-quadruplex topology switches

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† These two authors contributed equally to this work.

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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[1]Boise State University, USA, [2]U.S. Naval Research Laboratory, USA, [3]North 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 DNAbased 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.

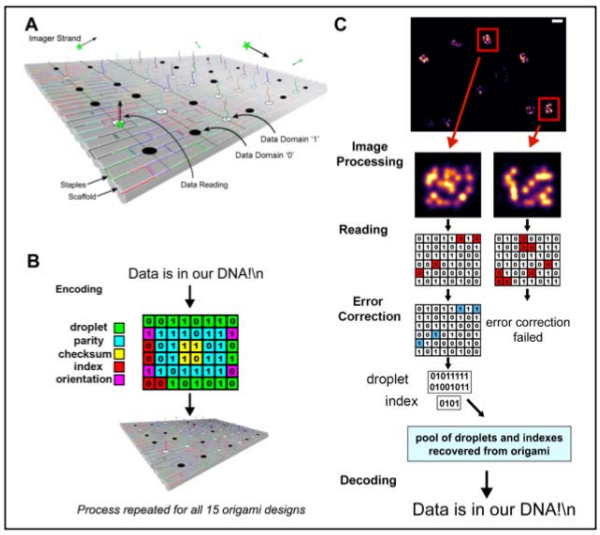

Fig. 1. (A) Illustration of a binary dNAM origami, a DNA nanostructure with specific sequences used to localize data strands to programmable sites within the DNA origami. (B) To enable reading of our test message, dNAM origami were synthesized based on designs generated during encoding: data droplet (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 Glembockyt, 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

Ludwig Maximilian University of Munich, Germany

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).

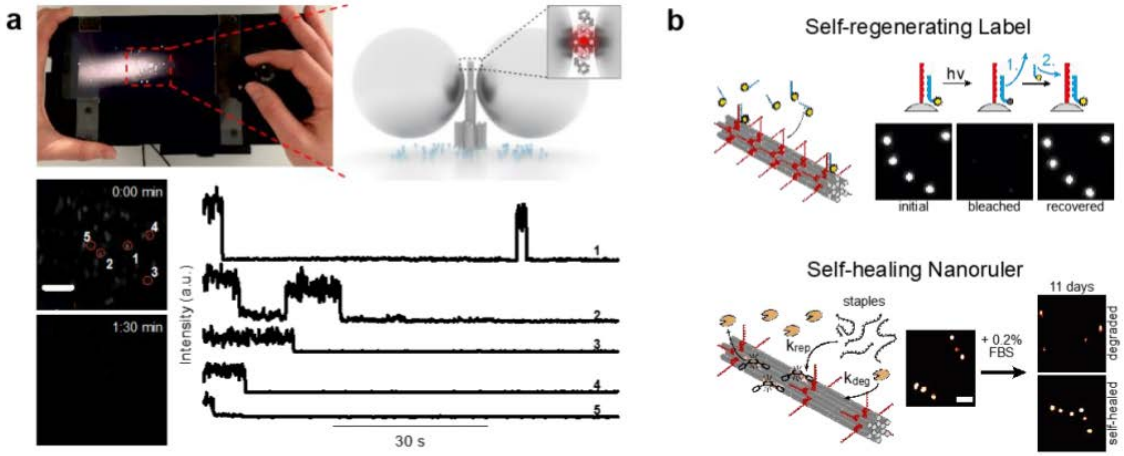

Fig. 1. (a) DNA origami nanoantennas2 with cleared plasmonic hotspots used for detection of single molecules (top) and corresponding single-molecule transients acquired on the smartphone camera (bottom); (b) Self-repair strategies3 in DNA origami nanostructures used for the development of selfregenerating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

(Invited) A virus nanoparticle as a resealable container

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[1]Indiana University, USA, [2]University of California San Diego, USA, [3]Penn 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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[a]UCLA David Geffen School of Medicine, USA, [b]University of California, USA, [c]University of California, USA, [d]Vault 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 x 10^3 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 (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.

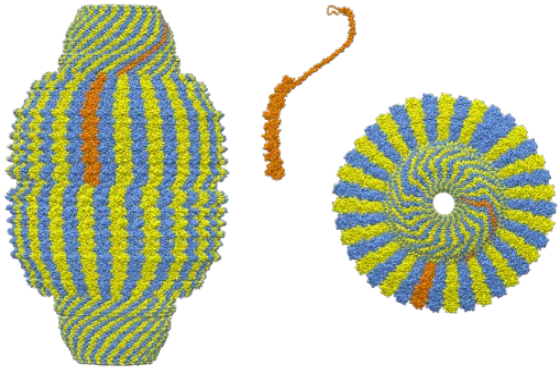

Figure: The vault particle X-ray structure, side image, single MVP and an end view. One MVP protein is colored red and the other chains blue and yellow. From *ACS Nano* 7: 889–902 (2013).

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

Kathryn Whitehead

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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 systems

Felix J. Rizzuto[1], Casey M. Platnich[1], Xin Luo[1], Yao Shen[1], Michael D. Dore[1], Chengde Mao[2], Hanbin Mao[3], Alba Guarné[1], Gonzalo Cosa[1] and Hanadi F. Sleiman[1]

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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.

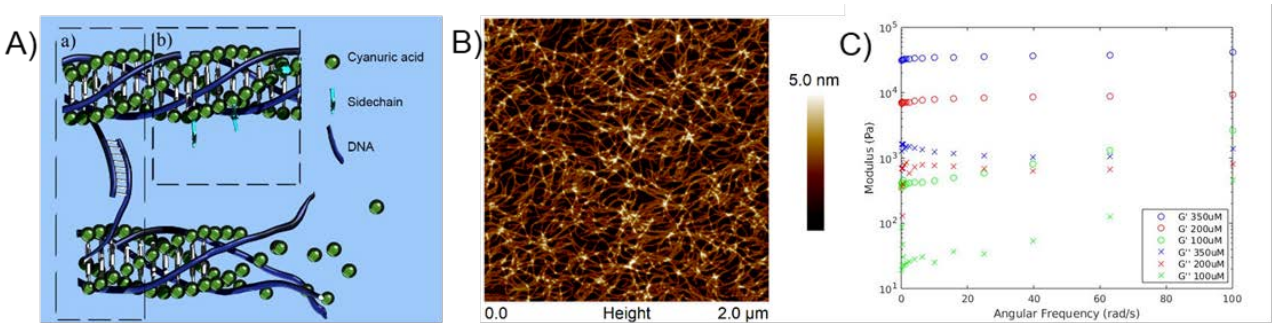
Stimuli Responsive DNA/Small-Molecule Hydrogels

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.



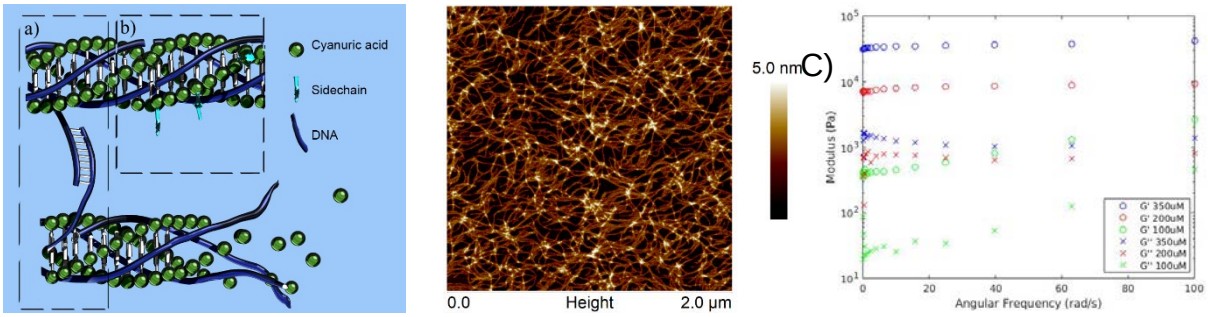


Figure 1: A) Representation of the polyadenine/CA assembly with a) dsDNA crosslinks and b) CA sidechains. B) AFM image of the entangled network a the polyadenine/CA ethylamine derivative. C) Rheological measurments of storage and loss moduli on polyadenine/CA hydrogels at various angular frequencies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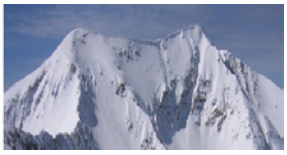

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(Invited) Toward parameter-free, rapid prediction of DNA origami shape and mechanical properties through multiscale analysis framework

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

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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

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Parabon 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 thirdgeneration design applications, offering most G1 and G2 capabilities plus user friendly interfaces and greater design versatility.

Our inSēquio™ 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 inSēquio 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 inSēquioVR 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. inSēquio 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

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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.

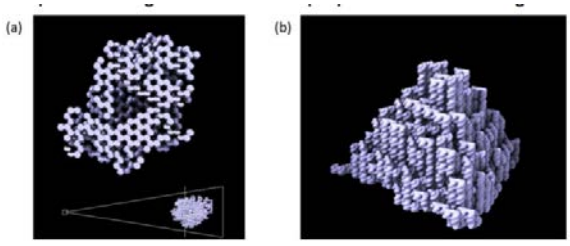

Fig. 1. Shapes generated with shape annealing algorithm for the filling and coating applications: (a) Cavity containing shape generated with tetrahedron mesh as the inner bounds of the design space with a total length of 12,901 bp; (b) Shape generated with tetrahedron mesh as the outer bounds of the 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 Pfeifer[2], Carlos Castro[2], Gaurav Arya[1]

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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.

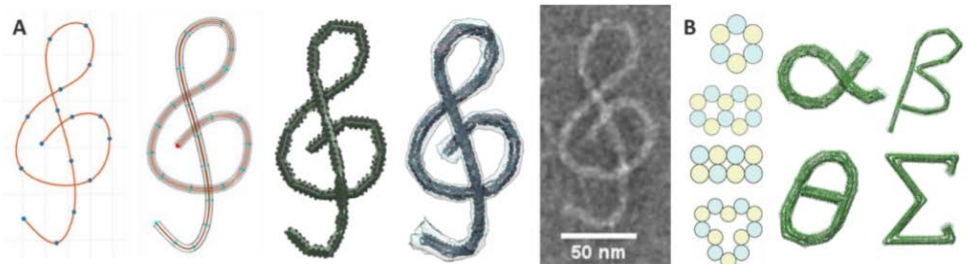

Fig. 1. (A) Starting with a built-in free-form spline sketch tool, the overall geometry is obtained by extruding cross-sections along the sketched path and discretizing it into DNA bundles, followed by coarse-grained simulations and experimental validation like TEM imaging. (B) The combination of various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

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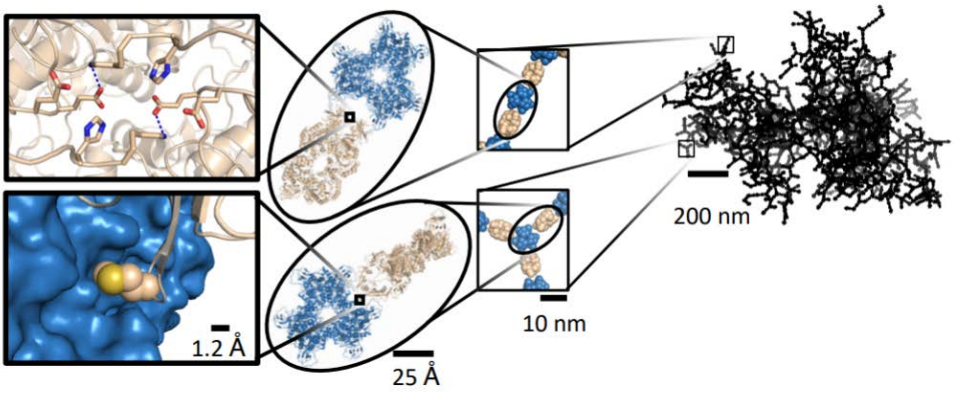
(Invited) Stimulus-responsive self-assembly of protein-based fractals by computational design

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-Berríos, 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 selfassembly of proteins into tunable supramolecular fractal-like topologies in response to phosphoryla- tion. 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 (~10 nm–10 μm) 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

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[1]Texas A&M University, USA, [2]Stanford 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.

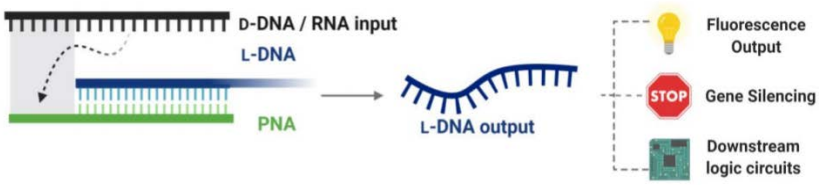

Figure 1: A heterochiral strand displacement reaction occurring from a PNA-L-DNA heteroduplex, initiated by endogenous nucleic acids. The L-DNA output can generate either a fluorescent readout or can be fed into similar downstream gates.

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

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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.

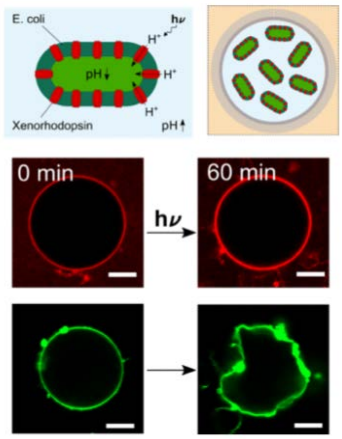

Fig. 1. Top - Genetically engineered xenorhodopsin-expressing E. coli generate a pH gradient upon illumination with white light. Middle – They can stimulate the attachment of pH-sensitive DNA to the periphery within or around synthetic cells upon light illumination. Bottom - The use of pH-sensitive DNA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

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[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.

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