Foundations of Nanoscience:

Self-Assembled Architectures and Devices



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Snowbird Cliff Lodge Snowbird, Utah April 22-25, 2024



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21st Annual Conference on Foundations of Nanoscience: Self-Assembled Architectures and Devices (FNANO24)

Snowbird Cliff Lodge, Snowbird, Utah Monday, April 22 – Thursday, April 25, 2024

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CONFERENCE OVERVIEW:

Foundations of Nanoscience is a yearly conference on the scientific underpinnings of nanoscience, with self-assembly as a central theme. Topics include self-assembled architectures and devices, at scales ranging from molecular to meso-scale. Methodologies include experimental as well as theoretical approaches. The conference spans many traditional disciplines including chemistry, biochemistry, physics, computer science, mathematics, engineering, molecular biology and molecular medicine.

Prior FNANO conferences have had a significant impact on the emerging fields of nanoscience and self-assembly, by bringing together leading researchers in a strongly interdisciplinary forum. This 21st Conference on Foundations of Nanoscience follows in this tradition, featuring invited talks by distinguished nanoscientists in a schedule with many contributed posters and open discussion periods to allow for scientific interaction.

THANKS

We express our sincere gratitude to all our sponsors including Parabon Nanolabs and Delong Instruments, and in particular to Laura Kienker, ONR Program Officer, Dr. Stephanie McElhinny, ARO Program Officer, and Mitra Basu, NSF Program Officer for their continued support of FNANO.

> Happy is he who gets to know the reasons for things. Virgil (70-19 BCE)

CONFERENCE MOTIVATION: The Challenge of Self-Assembly of Molecular Scale Structures

Construction at the molecular scale, in the 1 - 100 nanometer range, is one of the key challenges facing science and technology in the twenty-first century. This challenge is at the core of an emerging discipline of Nanoscience, which is at a critical stage of development. There have been some notable successes in the construction of individual molecular components (e.g., carbon nanotubes, and various molecular electronic devices), and the individual manipulation of molecules by scanning probe devices. However, a key challenge that remains largely unmet is the construction of complex devices out of large numbers of these components. We need methods to help us hold, shape, and assemble molecular components into complex structures and systems.

Top-down methods for nanofabrication, such as e-beam lithography, are well understood, and widely used in engineering and manufacturing processes but have inherent limitations in scale. Self-assembly is a much less well-understood construction process. Self-assembly is a bottom-up method of construction whereby substructures spontaneously self-order into superstructures driven by their selective mutual affinities. Chemists have for many decades used self-assembly methods, for example, for the self-assembly of lipid or polymer layers, but the resulting structures usually have limited complexity and are not readily programmable. Living cells also assemble by bottom-up methods and, by contrast, display complex and dynamic functional architectures. New synthetic methods, in particular those based on biomolecular self-assembly, are allowing the creation of synthetic systems with some of the same attributes. We hope that this Conference encourages the self-assembly of a community of scholars who will be able to provide insights into this critical topic in nanoscience and nanotechnology.

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Abstracts

Monday April 22nd, Morning Track on DNA Nanostructures: Semantomorphic Science

Track Chair

Hao Yan

Arizona State University

Design strategies for reconfigurable DNA origami

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In this presentation, I will introduce two design strategies that we have developed to construct reconfigurable DNA origami nanostructures. First, how we realized a paper-folding mechanism at the nanometer scale will be introduced [1]. The main idea is to build a wireframe 2D structure (we call DNA wireframe paper) whose edges follow a crease pattern in paper origami so that it can be reconfigured into various shapes. By optimizing the structural stiffness of folding lines, repeatable and reversible folding/ unfolding could be achieved with a high yield. It could be designed to be responsive to DNA/RNA strands and pH or light-source change. Furthermore, more complex paper folding could be realized by making a larger DNA wireframe paper via hierarchical assembly.

Second, I will introduce how we used chemo-mechanical deformation (unwinding of DNA by its binders) to induce shape change via DNA binding molecules. Topologically closed structures like rings exhibited an ultrasensitive threshold response as they were suddenly reconfigured when a critical concentration of DNA binders was reached while they maintained their initial configuration below the critical concentration due to twist-bending instability [2]. On the contrary, topologically open structures showed a gradual twist reconfiguration as the concentration of DNA binders increased. This gradual reconfiguration was useful in controlling the chiroptical responses of gold nanoparticles attached to a single DNA origami structure [3].

This work was supported by the National Convergence Research of Scientific Challenges (NRF-2020M3F7A1094299) through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT.

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[2] Young-Joo Kim, Junho Park, Jae Young Lee, and Do-Nyun Kim, "Programming ultrasensitive threshold response through chemomechanical instability", *Nature Communications*, 12: 5177 (2021)

[3] Taehwi Kim, Chanseok Lee, Jae Young Lee, and Do-Nyun Kim, "Controlling chiroptical responses via chemo-mechanical deformation of DNA origami structures", *ACS Nano*, published online (2024)

Engineering Assemblies and Dynamic Structures with DNA and RNA Fei Zhang Department of Chemistry, Rutgers University, Newark, NJ 07102, USA E-mails: fei.zhang@rutgers.edu

We explore the creative application of DNA and RNA motifs in developing a wide array of nanostructures and dynamic devices, showcasing the vast potential of structural nucleic acid nanotechnology. Our exploration includes using distinctive DNA tile-based systems, such as T-shaped¹ and parallel² crossover tiles, for assembling diverse structures like 2D arrays, nanotubes, and reconfigurable nanorings. The talk will also present our ongoing projects in dynamic DNA origami, including multi-state adaptable cages for protein capture, cascaded circular tweezers, and rotational nanoclocks. Furthermore, one of our significant focuses is on extending DNA design principles to de novo RNA design, yielding novel RNA arrays and assemblies integrated with sensing aptamers. Particularly, one of our ongoing projects introduces a co-transcriptional RNA nanostructure system based on paranemic cohesion, offering an innovative alternative for RNA single-chain folding approaches. These developments in DNA and RNA nanostructures aim to expand the toolkit available in nucleic acid nanotechnology, opening new avenues for application in biological systems.

Reference:

Yang, Q.; Chang, X.; Lee, J. Y.; Saji, M.; Zhang, F. DNA T-shaped crossover tiles for 2D tessellation and nanoring reconfiguration. *Nature Communications* **2023**, *14* (1).
 Lee, J. Y.; Yang, Q.; Chang, X.; Jeziorek, M.; Perumal, D.; Olivera, T. R.; Etchegaray, J. P.; Zhang, F. Self-assembly of DNA parallel double-crossover motifs. *Nanoscale* **2024**, *16* (4), 1685-1691.

Functional DNA nanotechnology for cell mimics

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A fundamental design rule that nature has developed for biological machines is the intimate correlation between motion and function. One class of biological machines is molecular motors in living cells, which directly convert chemical energy into mechanical work. They coexist in every eukaryotic cell, but differ in their types of motion, the filaments they bind to, the cargos they carry, as well as the work they perform. Such natural structures offer inspiration and blueprints for constructing DNA-assembled artificial systems, which mimic their functionality. In this talk, I will discuss DNA nanostructures with distinct motion and functions that interrogate synthetic cells. The interplay between the dynamic behavior of DNA nanostructures and synthetic cells gives rise to peculiar phenomena, which may rejuvenate the field of synthetic biology and greatly enhance the technological value of DNA nanotechnology.

Monday April 22nd, Poster session Track on DNA Nanostructures: Semantomorphic Science A

Track Chair **Hao Yan**

Arizona State University

Toward Three-dimensional DNA Industrial Nanorobots

Authors:

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Abstract:

Nanoscale industrial robots have potential as manufacturing platforms, capable of automatically performing repetitive tasks to handle and produce nanomaterials with consistent precision and accuracy. We demonstrate a DNA industrial nanorobot, that fabricates a three-dimensional (3D) optically active, chiral structure from optically inactive parts. By making use of externally controlled temperature and ultraviolet (UV) light, our programmable robot, ~ 100 nanometers in size, grabs different parts, positions and aligns them so that they can be "welded", releases the construct and returns to its original configuration ready for its next operation. Our robot can also self-replicate its 3D structure and functions, surpassing single-step templating (restricted to two-dimensional (2D)) by using folding to access the third dimension and more degrees of freedom. Our introduction of multiple-axis precise folding and positioning as a tool/technology for nano manufacturing will open the door to more complex and useful nano-micro devices.

Abstract Figure:

Fabrication, processing and chiral production. Schematics of (A) formation of threedimensional (3D) trimer robots by folding and ultraviolet (UV) crosslinking (purple star) of two-dimensional (2D) trimers through (B) photo-crosslinking with 3-cyanovinylcarazole (^{CNV}K) cycloaddition reaction in the DNA duplex and (C) folding DNA origamis to 90° by two double stranded DNA struts. (D) A non-denaturing agarose gel showing the mobility difference between the 3D robot trimer and 2D trimer. The gel was run at 48 °C. (E) In-air atomic force microscopy (AFM) of the 3D seed trimer robot. The scale bar is 50 nm. (F) Flowchart of the fabrication program for chiral optically active trimer constructs from achiral gold nanorod (AuNR) monomer plates and pictured preprogramming external control steps and purposes. Note: L AuNR refers to tiles that have gold nanorods attached perpendicular to the equal sign of the cross-tile origami. (G) Circular dichroism spectra showing different chirality obtained by rearranging the Au nanorods decoration on the plates or by using a differently programmed robot. The scale bars are 100 nm.



dsRNA Bricks: Complex Self-Assembled RNA Nanostructures of More than 100 Unique Components

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The programmed self-assembly of nucleic acids, particularly through DNA origami¹ and DNA bricks², has enabled the creation of fully addressable nanostructures with diverse functionalities. However, the structural complexity of RNA nanostructures constructed to date does not match that of their DNA counterparts, despite their potential for higher structural and functional diversity. Primary challenges of directly implementing DNA origami or DNA bricks technologies with RNA include (1) the economic production of numerous synthetic strands and (2) the tendency of single-stranded RNA (ssRNA) to break down in the conditions (such as prolonged annealing process under high concentrations of Mg²⁺) needed for assembling complex RNA nanostructures.

In nature, ribosomal RNA (rRNA) maturation involves the cleavage of a long polycistronic precursor transcript into individual mature rRNA components, which subsequently fold into stable secondary/tertiary structures and assemble with ribosomal proteins to form functional ribosomes³. Inspired by this natural process, we have developed a technique called double-stranded RNA (dsRNA) bricks to create RNA nanostructures with the structural complexity comparable to conventional DNA origami. We constructed a long polycistronic RNA transcript that encodes multiple brick sequences separated by repetitive linker sequences, which provide specific cleavage sites for the release of individual brick sequences. These brick sequences fold into tiles that are predominantly double-stranded (hence termed dsRNA bricks) and thereby are more stable compared to ssRNA. These dsRNA bricks contain minimal ssRNA loop regions that mediate the programmable branched kissing-loop (bKL) interactions⁴ (**Figure 1A**). This method allows us to construct various RNA nanostructures assembled from different dsRNA bricks (**Figure 1B**). To enhance the structural complexity, we developed an algorithm to select orthogonal bKL sequences. Based on this, we successfully realized finite-sized 2D and

3D nanostructures composed of up to over 100 distinct dsRNA bricks (**Figure 1C**), representing the most complex RNA nanostructures ever made⁵. Our research represents a significant breakthrough towards the scalable production of complex RNA nanostructures, with the potential of cellular production.



Figure 1. Complex RNA nanostructures realized by dsRNA bricks. (A) Workflow of dsRNA bricks preparation. (B) Designs and AFM results of dsRNA brick self-assembled tiles. (C) Scale of finite-sized dsRNA brick-based nanostructures. Upper and lower panels show 2D and 3D nanostructures, respectively. All images are 300 nm × 300 nm.

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Passivating blunt-ended helices to control monodispersity and multisubunit assembly of DNA origami structures

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DNA origami enables the synthesis of bespoke nanoscale structures suitable for diverse applications. Effective design requires preventing uncontrolled aggregation, while still facilitating directed multi-subunit assembly. Uncontrolled aggregation is often caused by base-stacking interactions between arrays of blunt-ended helices, a problem which is typically mitigated by incorporating disordered single-stranded DNA (like scaffold loops or poly-thymine brushes) at the end of double-stranded DNA helices. Such disordered regions are ubiquitous in DNA origami structures yet their optimal design requirements in various chemical environments remains unclear. Our study systematically investigates scaffold loops and poly-nucleotide brushes for aggregation prevention and control of multi-subunit assembly, examining length, sequence and MgCl₂ concentration dependencies. Additionally, we introduce a novel strategy using orthogonal doublestranded DNA helices to create a steric shield against base stacking. Our results reveal key considerations. Poly-thymine brushes excel in achieving monodispersity across diverse conditions whereas scaffold loops aid directed multi-subunit assembly. Orthogonal DNA helices remove the need for disordered regions altogether, preventing aggregation over a broad range of MgCl₂ concentrations and facilitating controlled multisubunit assembly. This study expands the toolbox for DNA origami design and enables a more informed approach for achieving control of monodispersity and multi-subunit assembly in DNA origami structures.



Figure 1 A) Various representations of DNA and DNA origami. **B)** DNA origami bundles often terminate with arrays of blunt-ends, which cause aggregation due to base-stacking. **C)** To prevent aggregation, single-stranded scaffold loops can be exposed by truncating or omitting staple strands at the ends of the DNA origami structure. **D)** A different strategy is the inclusion of poly-nucleotide (e.g. poly-thymine) brushes **E)** We present a novel method for passivation, in which double stranded DNA end-caps are formed orthogonal to the DNA in the bundle. **F)** Electron micrographs and 2D averages of four DNA origami designs with different strategies for passivation. Scale bars are 50 nm. **G)** Summary table with pros and cons of each strategy.

Controlled synthesis of DNA-GNR conjugates and guided assembly of GNR incorporated DNA nanostructures

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We have developed strategies to control on-resin coupling reactions to modify DNA during nucleoside oligomerization, while the incipient DNA molecule is still attached to the resin. We have explored inter- and intra- strand coupling in the context of Pd-catalyzed reactions. Our strategy employs a dilution method, which clearly differentiates intra- and inter-strand DNA crosslinking. These achievements in intra- and inter-strand couplings present a convenient and controllable approach for DNA functionalization and the fabrication of materials within DNA scaffolds, for example, graphene nanoribbons (GNRs). Despite extensive efforts towards discovering a reliable and effective strategy for the synthesis of graphene nanoribbons, progress in achieving precise preparation of GNRs and their assembly into electronic devices remains limited. Our work addresses the urgent need for a method that can reproducibly and precisely produce graphene nanoribbons by using DNA as a template. Specifically, we have obtained water-soluble DNA-GNR conjugates. We have also achieved self-assembly of the DNA-GNR conjugates into 2-dimensional DNA scaffolds (DNA origami). This new approach provides DNA-GNR nanostructures with atomic precision and unveils optical properties of GNRs.

2D self-assembly of shape-complementary DNA origamis for lithographic applications

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Both in biological and synthetic systems, molecular self-assembly is key to acquire specific properties such as a given shape, function, a high degree of organization, or biocompatibility. In nanotechnology in general and in semiconductor industry in particular, there is an ever-increasing need for smaller and more complex features at an ever-lower cost. To address the challenge of patterning at sub-10 nm scale, novel strategies must be envisioned. Among emerging technologies, directed self-assembly (DSA) of materials to create the lithography mask onto the substrate receives a lot of interest due to their theoretical high-resolution and uniformity. In these DSA lithographic techniques, blocks copolymers (BCP) are the most mature ones but have limited pattern morphologies. By virtue of its inherent small helix diameter (2 nm), DNA can be programmed to self-organize into various 1D, 2D and 3D morphologies at nano-scale resolution [1, 2]. Therefore, DNA is a promising masking material for bottom-up lithography techniques.

Although DNA origamis are limited in size (from tenths to a hundred nanometers), 2D and 3D patterning and high molecular weight objects can be obtained through binding of numerous origamis [3, 4]. The classic DNA self-assembly method relies on base-pairing [5], forming strong but irreversible connections which potentially result in defect accumulation (missing unit, broken connection...). Another way to do 2D assemblies is to use shape-complementarity: it allows the creation of reversible DNA nanostructures [6] similarly to how puzzle pieces are linked together. The reversible interactions based on π - π stacking have the potential to avoid or even correct defects in-situ. Here, by using them, we aim to reach high quality of 2D organization of DNA units.

We develop new shape-complementary origamis, e.g. squares of 49 by 49 nm in size with a 30 by 30 nm hole in their center, for 2D (see Figure 1) periodic patterns with controlled dimensions and high-resolution. The 2D patterns have been created on large scales (up to 10 microns), with a high degree of order and little to no defects by optimizing the experimental parameters. The dimensions of the origamis and the patterns have been measured by Aselta SIMPL software, which extracts contours of objects and performs measurements on them. Moreover, controlling the shape and the number of available connections of DNA monomers allows to craft complex geometrical architectures. Examples include Archimedean lattices, such as the "Ruby" lattice which is based on the self-assembly of squares and triangles.



Figure 1: TEM image of square DNA origamis self-assembling into a 2D square lattice. Bottom right inset is the Fast Fourier Transform (FFT) of the image. Scale bar is 500 nm.

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NanoSiTE: Characterization of Site-Occupancy in Functionalized Nanostructures through Combinatorial Barcoding

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DNA nanotechnology is a rapidly growing field within biomedical research, where DNA nanostructures are emerging as a platform for targeted drug delivery. DNAorigami structures are self-assembled nanostructures created by folding long singlestranded DNA into 2D and 3D structures. The possibility for precise and controlled functionalization of these nanostructures through immobilization of drugs and binders enable fine-tuned drug delivery to specific locations and environments. Since the spatial organization of the binders will dictate structure interaction with cells and tissues, it is vital to be able to characterize the site-occupancy of the functionalized DNA nanostructures. Currently, this characterization is done using Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), and super-resolution fluorescence microscopy, yielding high resolution and accurate images of the structures. However, when using these methods, it is only feasible to image a very small fraction of all structures present in a sample. Furthermore, different structures offer a variety of constraints that could limit the possibility to characterize the structures. This is especially true for complex and deformable structures.

To address these limitations, we developed NanoSiTE (Nanostructure Siteoccupancy characterization through Tag Extension), a sequencing-based characterization method that uses a split-pool approach for combinatorial barcoding of functionalized sites in individual nanostructures. An overview of NanoSiTE is presented in Figure 1.



Figure 1 Overview of NanoSiTE. DNA nanostructures are designed with protruding single-stranded DNA in sites of functionalization and a functionalizing binder is allowed to hybridize to these sites. Each hybridizing strand includes a position barcode and a binder barcode, which are elongated prior to digestion of the binder. A structure specific barcode is added through split-pool combinatorial barcoding. The strands are then digested from the nanostructure and sequenced. The structure barcode is used to deduce the structure origin of the strand, while the position and binder barcode are used to determine the position and identity of the functionalizing element, respectively. Illustration made in BioRender.com.

The DNA nanostructures are designed with protruding DNA strands which can hybridize to complementary DNA strands conjugated to the binders. These protruding strands contain barcode information of both the position of the protruding strand within the structure (position barcode) and the identity of the binder conjugated to the protruding strand (binder barcode). To label all the protruding strands in a single structure with the same barcode (structure barcode), we perform four sequential splitpool tag extensions, where DNA adapters are ligated to the protruding strands in a combinatorial matter. These protruding strands are then sequenced, where the position barcode, binder barcode, and structure barcode can then be used to yield information about site-occupancy of different binders at close to single-structure resolution.

We have applied the method to two DNA origami nanostructures with HER3 affibodies as functionalizing elements with different spatial organizations on the nanostructures. We observe a fully ligated structure barcode in approximately 65% of reads, yielding 90% ligation efficiency per ligation reaction. From the data, we were able to establish the overall yield of functionalization as well as characterize populations of nanostructures with varying degree of functionalization. In addition, by analyzing correlation between occupancy in different binding sites, we can establish potential hinderance in functionalization caused by other binders. Finally, we can also determine the average binder frequency for both the entire structure and each individual binder position. We are now using the method to characterize a variety of structures functionalized with different binders.

NanoSiTE allows for a rapid and simple characterization of site-occupancy distributions of functionalized DNA nanostructures, including complex and deformable structures. The method is easily implemented for different functionalized nanostructures. With NanoSiTE, accurate characterization of functionalized DNA nanostructures will become more readily available to biomedical researchers compared to current imaging techniques. Hence, this method could serve as a stepping-stone for further use and development of DNA nanotechnology for cell biology targeted drug delivery.

Controlling membrane tension by dilatable DNA nanorings

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Membrane tension, defined as force per unit length (or energy per unit area) acting on a membrane surface, is a crucial physical parameter that regulates many vital cellular processes such as cell migration and membrane trafficking.[1] Conventional approaches to control tension of model membranes include applying osmotic shock, pulling membrane tethers, and extracting lipid molecules from bilayers. However, it remains challenging to precisely control membrane tension at the nanometer scale while preserving the lipid composition and biochemical environment of the membrane. Dynamic DNA nanostructures that interact with lipid bilayers can drive membrane-remodeling events and lead to changes in membrane tension.[2] Herein, we present a DNA nanodevice that regulates membrane tension of small unilamellar vesicles (SUVs) in response to specific DNA signals. Specifically, we built reconfigurable membrane-templating DNA nanorings that change their conformations from a closed state to various predefined open states, applying incremental mechanical stress on the templated vesicles. We demonstrate nanoring dilation induced vesicle expansion by electron microscopy and membrane tension increase by Laurdan (a membrane-tension sensitive dye) fluorescence. We foresee that such devices will be useful for studying the membrane tension dependent behaviors of membrane proteins (e.g., ion channels) and reconstituting membrane tension driven processes such as bulk lipid flow in model lipid bilayers.



Fig.1 | A DNA nanodevice for controlling membrane tension. Schematics of DNA nanoring dilation (left) mediated liposome (blue) expansion (right). Negative-stain electron micrographs show the DNA nanorings (left) and their templated liposomes (right) in the closed and open states. Scale bars: 50 nm.

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FNANO 2024

Monday April 22nd, Poster session Track on Principles and Theory of Self-Assembly

Track Chair **Rebecca Schulman** Johns Hopkins University

Single-molecule nanopore sensing enables optimized assembly of DNA/RNA hybrid nanostructures

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Nucleic acid nanotechnology facilitates the design and assembly of complex nanostructures based on the programmable self-assembly of DNA and RNA. To date, the field has primarily focused on the folding of DNA, capitalizing on its chemical stability. The use of RNA motifs has been more limited due to the multitude of degradation pathways (both enzymatic and hydrolytic) specific to RNA. Compared to DNA, RNA performs a more diverse array of biological functions - including signaling, catalysis, and gene regulation - in addition to information storage. As such, integrating RNA into DNA nanostructures further expands the functionality of these architectures.

The biological function of RNA is governed by its secondary structure. While DNA primarily exists in the canonical double helix, RNA may sample a plethora of substructures, including hairpin loops, multibranch loops, and base pair stackings. To incorporate RNA into DNA nanostructures, these secondary structures must be disrupted to promote RNA/DNA base pairing. In DNA nanotechnology, thermal annealing is employed, wherein the RNA/DNA are heated and then slowly cooled enabling equilibration at each temperature. In doing so, the mixture is brought to the thermodynamic minimum corresponding to the desired design. This methodology is not compatible with RNA, which undergoes self-cleavage at elevated temperatures. As such, new low-temperature procedures to generate RNA/DNA hybrids while minimizing RNA degradation are required.

Here, we present a new protocol which uses chemical (as opposed to thermal) denaturation to promote the hybridization of DNA oligos to RNA sequences. By disrupting the self-complementary of RNA and capitalizing on the multivalency of incoming complementary DNA strands, a stable hybrid duplex is generated at room temperature. Using the same method, we can append DNA dumbbells to the RNA, producing a three-dimensional 'barcode'.¹ This barcode can then be read out using state-of-the-art solid-state nanopore measurements,² allowing us to probe the outputs of this self-assembly pathway at the single-molecule level. We find that by impeding the self-folding of RNA through chemical denaturation and subsequently hybridizing the DNA complements, self-cleavage is inhibited. An optimized method to create hybrid DNA/RNA architectures while ensuring the integrity of RNA sequences is thus realized.³



Figure 1. Overview of RNA-DNA nanostructure assembly and single-molecule analysis. (A) RNA with native secondary structure is denatured using chemical methods at room temperature. The addition of short, complementary DNA strands (including dumbbell structures) results in the formation of a hybrid duplex construct ('RNA ID'). (B) The RNA ID is analysed with solid-state nanopore sensing, resulting in a current signature specific to its three-dimensional shape. The idealized current signature is shown below the structure design. (C) A typical, real single-molecule translocation event, which matches the design of the RNA ID.

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Silver-mediated DNA base pairing: uncovering solution-phase selfassembly and nanomechanical properties

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Abstract

Silver-mediated DNA base pairing is a promising approach to expand the applications space of DNA nanotechnology. Ag⁺ can form coordinate bonds between natural nucleobases at neutral pH, forming base pairs that differs from the hydrogen bonding in Watson-Crick-Franklin (WCF) DNA. Ag+-DNA base pairs can mediate entire DNA duplexes to form 1D linear "wires" of Ag⁺ (Fig 1a).^{1,2} Experiments and simulations have shown that these Ag⁺-DNA duplexes can be far more thermally and chemically stable than WCF base pairing, making Ag⁺–DNA a potential tool for designing robust selfassembled DNA systems.^{1,3} Additionally, C–Ag⁺–C and G–Ag⁺–G duplexes have been found to have increased rigidity compared to their C-G WCF DNA counterpart in the gas phase with ion mobility spectrometry (IMS).⁴ However, there is still a lack of understanding of the formation mechanisms and nanomechanical stiffnesses of Ag⁺-mediated DNA duplexes, which are critical features for designing DNA nanostructures that incorporate such elements. We investigated the solution-phase formation and nanomechanical properties of 20-mer dG homobase Ag⁺–DNA duplexes using circular dichroism (CD) spectroscopy, electrospray ionization mass spectrometry (ESI-MS), atomic force microscopy (AFM), and surface forces apparatus (SFA) measurements. This research bridges the established theoretical and gas-phase experiments with the development of solution-phase nanoscale assembly of Aq⁺-mediated DNA nanostructures.

CD spectroscopy shows that mixtures of dG₂₀ and Ag⁺ form complexes that are dependent on stoichiometry of [Ag⁺]:[base]. Using ESI-MS, we find that a ratio of 0.5 [Ag⁺]:[base] is sufficient to form (dG₂₀)•[Ag⁺]₂₀ duplexes. Liquid phase AFM showed that at stoichiometries > 0.5 [Ag⁺]:[base], mixtures of dG₂₀ and Ag⁺ form longer fibers that have heights consistent with single duplexes, suggesting possible end-to-end assembly of (dG₂₀)•[Ag⁺]₂₀ duplexes (Fig. 1c). Using temperature-controlled CD, we show that at a stoichiometry of 0.5 [Ag⁺]:[base], heating to ca. 50°C is sufficient to disrupt intermolecularly folded G-quadruplexes (G4) and allow the formation of (dG₂₀)•[Ag⁺]₂₀ duplexes; heating appears essential for full disruption of the G4 structure. Finally, we used SFA to measure the nanomechanical stiffness of (dG₂₀)•[Ag⁺]₂₀ duplexes affixed to gold substrates. Ag⁺-mediated dG₂₀ duplexes were found to be ≥30% stiffer compared to WCF DNA. These findings demonstrate the necessary conditions for the formation of the

 (dG_{20}) • $[Ag^+]_{20}$ duplex and the resulting physical properties of the duplex, building the foundation for future nanostructure development using Ag⁺–DNA base pairing.



Figure 1: a) Structure of $G-Ag^+-G$ base pairs formation and Ag^+-DNA strand assembly schematic. **b)** CD spectra of homobase $dG_{20} Ag^+-DNA$ duplex with increasing [Ag⁺]:[base] to demonstrate change in conformation of structure in presence of excess silver. **c)** AFM topograph of $dG_{20} Ag^+-DNA$ duplex forming longer fiber-like structure when assembled with 1.0 [Ag⁺]:[base]. Height profile on the right shows the ~2.5 nm height of duplex consistent with height of a duplex.

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Hairygami Re-Visited, Overhangs and Intrinsic Curvature in Flat Origami

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DNA origami has been proposed as a useful tool to precisely add multiple functionalities on a single platform thanks to the programmable nature of DNA. The most common way of adding functionalities is through extending single stranded overhangs from staple strands. Among the commonly studied DNA origamis is the flat rectangular design, it has precisely placed nick points at ≈ 6 nm, produced at a high yield and robust annealing process and was utilized in numerous applications for computation, single molecule sensing and chemical libraries. However, the in depth understanding of how multiple overhangs can affect the architecture of DNA origami was hardly touched.

Recently, the Šulc lab presented a study about the effect of multiple overhangs on flat origami architecture which they called 'hairygami'. The study concluded that the increase in number of overhangs can introduce an entropically favored convex curvature to allow more 'conformational volume' for each strand. [1] (fig. 1a, outer overhangs) Based on this effect, the probability of folding the flat origami into a tube in enclosing the overhangs would be very low. One solution is to introduce some curvature in the flat origami though applying crossover shifts where their number and intensity would be dependent on the required curvature angle. [2] However, the question about whether curvature is still needed in case of using both inner and outer overhangs remains unanswered.

In our project, we seek to study the effect of introducing overhangs both on the inner and outer surface of a flat origami that would eventually fold into a tube. With that goal in mind, we simulate different overhang patterns and intrinsic curvature with oxDNA [3] (fig. 1b). The preliminary simulations agreed with the predictions that overhangs increasingly curve the flat origami in a convex manner. In case of adding overhangs on both surfaces of flat origami, no crossover shifts would be required if the inner overhangs were outnumbered by outer counterparts. More patterns are currently under study.



Figure 1. CaDNAno screenshots and oxDNA simulations of small flat DNA origami model a)without any cross-over shifts, b) with 2 right cross-over shifts, the flat model was designed on CaDNano 2.0 and the simulations were run on ox-view online platform, the design has 12 inner overhangs (extended from red nick points) and 24 outer overhangs (extended from the blue nick points shown on the caDNAno diagram.

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Complex donuts: subtle modifications to DNA sequence dictate pathway complexity in supramolecular DNA toroids

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Biological polymers, such as DNA, actin, and peroxiredoxin proteins may adopt toroidal (ring- or donut-like) supramolecular configurations under specific conditions. In the case of DNA, a toroidal morphology aids in the compact packing of the biopolymer in viruses and in sperm chromatin. On the other hand, arranging synthetic polymers into toroidal nanostructures is motivated by their prospective applications in separation and catalysis, and as nanoreactors. Moreover, owing to their low mononuclear phagocytic uptake and high tumor accumulation relative to other similarly sized morphologies, nano-toroids have great potential use as therapeutic agents and in drug delivery.

While stable DNA toroids may be obtained via the origami approach, the use of DNA amphiphiles allows for an assembly requiring a singular unique sequence. DNA amphiphiles augment the sequence definition of nucleic acids with a host of interactions not available to DNA, thus increasing the monomer scope and design space, enabling the formation of hierarchical and anisotropic supramolecular assemblies. These assemblies also exhibit polymorphism which is indicative of complex self-assembly pathways dependent on chain folding and amphiphile packing. Although tuning amphiphile structure and assembly conditions allows for the preferential selection of nano-structure morphology within the free energy landscape, obtaining toroidal assemblies from DNA amphiphiles remains challenging.

Herein we report the formation of room-temperature stable nano-toroids in aqueous solution using DNA amphiphiles incorporating a bent pi-stacking unit at the end of a flexible, foldable hydrophobic alkyl chain block. While toroid formation is dependent on extrinsic parameters such as annealing conditions, we show for the first time that subtle and precise changes to the DNA sequence affect amphiphile packing and intermolecular interactions, allowing us to tune the energy landscape and select for the toroid morphology. We elucidate a competitive self-assembly pathway, where kinetically trapped nanofibers form spontaneously in ambient conditions and undergo sequence-dependent disassembly upon annealing followed by assembly into toroids. The toroids are stabilized at ambient conditions using either a small molecular chaperone or through co-self-assembly with a secondary DNA amphiphile. To the best of our knowledge, these are the first reported toroidal DNA nanostructures employing a single unique DNA sequence.



Fig. 1. (a) Structure of the sequence-defined DNA-DBCO amphiphile: DBCO-C12₁₂-DNA₉ ([dibenzylcyclooctyne]-[12-hexaethylene]-[9-mer ssDNA). The DNA sequence selects for the self-assembly pathway which (b) favors or (c) disfavors nano-toroid formation. The supramolecular toroidal nanostructures as characterized via (d) AFM in fluid, (e) AFM in air, and (f) bright field TEM.

Mesoscale Assembly and Silicification of DNA Origami through Controlled Growth of Polynucleotide Brush Patches

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DNA origami nanostructures excel in selfassembly, offering precision in nanoscale design and functionalization across diverse sizes. While large-scale shapes and have assemblies been achieved by harnessing the molecular recognition capabilities of DNA, this molecularly engineered assembly process is slow. In contrast, colloidal systems are ubiquitous in industry, where various types of non-specific interactions such as van der Waals. electrostatic, depletion. and polymermediated steric forces are used to selfassemble colloidal particles into macroscopic materials. Here, we report on strategies we developed that allow not only for site-specific polymerization but also for temporal control and de-grafting (cleavage) of polynucleotide brushes but also for sequential control of these processes. Spatiotemporal control over brush growth allows us to program the surface chemistry of the building blocks to regulate their interactions with each other and achieve assembly (Fig. **1**).[1] Furthermore, we report how site-specifically placed polynucleotide brushes influence the silicification of DNA origami. We show that long DNA brushes suppress the aggregation of DNA origami during the silicification process and promote robust silica growth on DNA origami surfaces (Fig. 2).[2] Our experiments are supported and explained by coarse-grained molecular dynamics simulations. Together our work provides a powerful toolset for the development of novel DNA-based organic-inorganic nanomaterials.

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Figure 1. Top Self-assembly of DNA origami into micelles using site-specifically grown polynuceotide brushes. **Bottom** Self-assembly of brush-functionalized origami cubes into dimers and strings.



Figure 2. Brush-directed silicification of 6HB DNA origami and radial charge density distribution simulations. Double-stranded brushes dramatically increase silicification.

FNANO 2024

Monday April 22nd, Morning Track on Principles and Theory of Self-Assembly

Track Chair **Rebecca Schulman** Johns Hopkins University

Macromolecular assemblies: growth, form and dynamics

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I will describe our studies of: (i) the structural and kinetic aspects of dynamic instability in macromolecular assemblies, e.g. microtubules (ii) effective theories for the growth and form of filamentous assemblies, and (iii) the dynamics of contractile injection systems and other related rapid macromolecular spring-like engines. In all cases, I will show how simple aspects of geometry, kinetics and statistical and continuum mechanics allow us to explain biological observations in a minimal setting, and might serve as inspiration for artificial mimics of these remarkable nano-machines.

Core-shell architecture in DNA nanostar droplets

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DNA nanostars, formed from multi-arm junctions, demonstrate liquid-liquid phase separation.¹ Palindromic ssDNA sticky ends on each arm allows for interaction between stars, leading to the formation of phase separated liquid droplets. Droplets have been functionalised, achieving selective compartmentalisation of biomolecules, or triggerable fusion and separation.^{2,3} DNA nanostar droplets have potential in modelling internal cellular compartmentalisation and in creating artificial cells. Here, we have used our understanding of nanostar phase separation to create mixed multi-nanostar droplets with core-shell microscale architecture.



A: Two-star LLPS, from star formation to layering. **B**: Layered and patchy 2-star droplets. **C**: Melt curves of star combinations in B, showing ΔT_{PS} . **D**: Adding the outer 'membrane-like' phase to the droplets prevents fusion over time, a key feature required for stable compartments. **E**: Proteins can be programmed by DNA handles to partition in the core.

A range of nanostars (NSs) were designed, with different numbers and lengths of DNA strands, as well as different lengths and binding strengths of sticky ends (SEs). This provided insight into trends in physical characteristics and LLPS behaviour. Droplet size, viscosity, and density were each affected by various combinations of geometry, valency, and SE strength. Importantly, we found that melt curve analysis gives a precise indication of the phase separation temperature (T_{PS}) of each system. This demonstrated that increased valency dramatically increases the phase separation temperature above the predicted T_M of each SE. Combined, these analyses allow us to predict which phase will form first for mixed droplet systems.

We designed geometrically similar NSs with orthogonal sequences; when stars with different core and SE sequences are mixed they form non-interacting droplet populations. Selective replacement of SEs in one NS, giving limited complementarity to the other NS, was then used to induce interaction between the 2 droplet types. In systems with 2 stars with a similar phase separation temperature ($\Delta T_{PS} < 3 \, ^{\circ}C$), complementarity of 25% between NSs creates patchy droplets, as seen previously in the literature. If instead we increase $\Delta T_{PS} > 7 \, ^{\circ}C$, distinct core-shell droplets form. This is likely due to the initial formation and stabilisation of the higher T_{PS} droplet, which forms the core. Later phase separation of the lower T_{PS} NS, which has limited complementarity to the existing droplets, can nucleate on the core surface and form an outer layer. The degree of complementarity at which this occurred depended on the valency of the outer star; 16% for lower valency, 25% for higher. At 50% complementarity, regardless of NS or ΔT_{PS} , complete mixing of droplets would occur. Thus, through the design of nanostar phase separation behaviour, multi-phase NS systems can be achieved with either patchy, layered, or mixed phases.

Core-shell architectures allow for the creation of phase separated droplets with regions of distinct physical characteristics. T_{PS} is significantly determined by valency, while at room temperature viscosity is significantly affected by SE strength and somewhat by NS size. These considerations allow us to produce core-shell droplets with a liquid core and gel-like shell, or gel-like core and liquid shell. Importantly, we found that gel-like shells stabilised liquid cores over time. Under conditions where core-only liquid droplets are seen to fuse and mix their contents, liquid droplets encapsulated within a gel-like NS shell instead remain as distinct systems. When the properties are reversed, over time the liquid shells merge into large amorphous structures containing multiple gel-like cores. Both types of morphology provide new possibilities in the creation of DNA-based artificial cells. Similarly, microscale architectures allow for distinct chemical environments within the patterned droplet. Due to the unique base pair sequence in each orthogonal star, we show that selective partitioning of functionalised biomolecules is possible. This has potential uses in the creation of microreactors, with control over the concentration of reagents, catalysts, or antagonists in selected areas of the structure.

Our work provides a novel technique for the analysis of DNA nanostar LLPS, allowing for the design and creation of microscale patchy, layered, or mixed architectures in multi-star systems. In the future this will be used to create new cellular models and artificial cells.

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A topological mechanism for robust and targeted function in biological networks

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Living and active systems exhibit various emergent dynamics during system regulation, transcription, and growth. However, how robust dynamics arises from stochastic components remains unclear. Towards understanding this, I develop topological theories that support robust edge states, effectively reducing the system dynamics to a lower-dimensional subspace. I will introduce stochastic networks in molecular configuration space that model different systems from a circadian clock to gene transcription. This edge localization is robust to random perturbations and allows for targeted functions in biological networks. Further, we show that non-reciprocal transitions are strictly necessary for edge states and their strong localization. Our work indicates new pathways for the design and control of active networks.

FNANO 2024

Monday April 22nd, Afternoon Track on Chemical Tools for DNA Nanotechnology

Track Chair Andrew Ellington University of Texas at Austin

Building Synthetic Cells: From Actin Assembly to Membrane Fusion

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Building synthetic cells from the bottom-up from biological building blocks including nucleic acids, proteins, and lipids has garnered significant interest over the past decade. As mimicry of a biological cell, reconstituting biological behaviors in synthetic cells has the potential to uncover basic design principles of biological functions and how selfassembly could give rise to emergent cellular behaviors. My lab is broadly interested in the mechanobiology of biological membranes, with emphasis on how cells generate, sense, and respond to physical forces. Using synthetic cells as an experimental system, I will discuss our efforts in reconstituting actin networks with different architectures in synthetic cells (Figure a) and how this compares with DNA-based cytoskeleton which showcases the potential for DNA nanotechnology to mimic the diverse functions of a cytoskeleton. In the second part of the talk, I will describe our recent efforts in engineering calcium-triggered DNA-mediated membrane fusion (Figure b). By using a vesicle-invesicle system in synthetic cells harboring mechanosensitive ion channels, we have demonstrated a mechanically responsive synthetic exocytosis scheme. This platform could see applications in smart drug delivery, as well as uses in exploring communications between synthetic and natural cells.



a. (top) Continuous droplet interface crossing encapsulation to encapsulate actin and actin-binding proteins in cell-size vesicles as synthetic cells. (bottom) Confocal fluorescence image of a giant vesicle encapsulating 1.5 μ M α -actinin, 0.5 μ M fascin, and 5 μ M actin (merge image only shows labeled fascin and actin). Scale bar, 10 μ m. **b.** Schematic of DNA-mediated membrane fusion induced by calcium. Complementary single-strand DNA oligonucleotides form double helix bundles in a minimal model for membrane fusion. Without calcium, surface-bound PEG chains linked with a calpain cleavage site physically prevent DNA oligos from interacting with their complementary DNA oligos. In the presence of calcium, protease calpain cleaves off the PEG chains, allowing membrane fusion to occur.

Synthetic Communication Networks via Engineered Nanopores

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From computing to power transmission, network architectures have enabled processing and long-distance signal propagation that impact almost every facet of our daily lives. Most modern networks are built from inorganic materials, ensuring longevity and robustness but limiting malleability, responsiveness, and adaptability. Living cells also form large networks across tissues, such that selective signals are disseminated and detected by distinct cell populations, giving rise to coordinated physiological action. Derived from soft matter, living signal networks exhibit higher-order activity that goes beyond the bounds of more static inorganic structures to re-organize and evolve over time. Synthetic cells - bottom-up assembled, unilamellar structures - seek to emulate the advantageous properties of living cells, capitalizing on defined cellular building blocks and logic without the drawbacks of maintaining living systems. My lab is interested in implementing molecular technologies in synthetic cells to construct large, tissue-like structures with user-defined control over higher-order activity. Here, I discuss our recent advances in generating synthetic cell-based networks as a new communication platform. Our approach centers on self-assembled membrane nanopores and controlling their assembly in the membrane. I will describe our work engineering connexin protein nanopores - which transmit molecular signals through their nanopore structure - to be light sensitive. To do this, we make use of encapsulated liposomal structures that rupture in the presence of light, in turn releasing enzymes that act on connexins and lead to their membrane assembly. With these molecular tools in hand, we show that nanopore pairing across synthetic cells allows for direct signal transfer and communication between neighbors in a connexin-dependent manner. By combining multiple connexins and liposomes in the same synthetic cell assembly, we demonstrate responsive, orthogonal signal transfer in synthetic cells, an important step toward synthetic communication networks.

Photo-control of DNA assemblies through time and space

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ABSTRACT

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 programmable nanotechnology applications.

In this talk, I'll discuss how visible light, small molecules, and protons can be used synergically as stimuli to create out-of-equilibrium DNA architectures.¹⁻² By programming these nanomaterials in both time and space, these single-stranded and DNA origami systems are capable of life-like properties – they can grow, 'evolve' and repair themselves by accessing unique assembly pathways under kinetic control. This new method of annealing nanomaterials has recently led to the development of more sophisticated out-of-equilibrium systems, including new modes of organizing DNA origami units into hierarchical structures under spatiotemporal control. We can now form polymers and matrices of DNA origami away from equilibrium, enabling new possibilities in tissue scaffolding and catalysis.



Figure 1. Light can control the morphology of discrete (left) and polymer (right) architectures.

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Monday April 22nd, Poster session Track on Chemical Tools for DNA Nanotechnology

Track Chair Andrew Ellington University of Texas at Austin

Elucidating the structure-property relationships of chiral DNAstabilized silver nanoclusters

<u>Rweetuparna Guha</u>, Anna Gonzàlez-Rosell, Malak Rafik, Benjamin Katz, Stacy M. Copp* *Corresponding author: <u>stacy.copp@uci.edu</u> Department of Materials Science and Engineering, University of California, Irvine, USA.

DNA-stabilized silver nanoclusters (AqN-DNAs) are ultrasmall nanoparticles with sequence-tuned fluorescence and atomically precise sizes. Chromatographic purification together with mass spectrometry has shown that AgN-DNAs are composed of 10 - 30 silver atoms encapsulated by one to three single-stranded DNA oligomers (Fig. 1a). The templating DNA oligomer dictates the structure and size of the Ag_N core, thereby yielding sequence-tuned photophysical properties. Ag_N-DNAs display tunable emission wavelengths that span from visible to near-infrared (NIR) (Fig. 1b).¹ Moreover, the inherent chirality of the DNA template extends its influence on the nanocluster core, imparting chirality to the Agn-DNA structure. The tunable photophysical properties of these chiral Ag_N-DNAs are further influenced by external environmental conditions such as pH and salt concentration of the solution. This dynamic adaptability of photophysical characteristics positions Aq_N-DNAs as probing tools in bio-and/or chemical sensing and bioimaging applications. Notably, the NIR-emissive Ag_N-DNAs hold significant promise in fluorescence bioimaging at extended tissue depths since NIR light can penetrate tissues up to several centimeters.² Existing NIR organic fluorophores are dim and have poor photostability, while bright NIR quantum dots are toxic. NIR-emissive Aq_N-DNAs are biocompatible, bright, and photostable compared to existing NIR emitters, making Aq_N-DNAs an immensely promising bio-labels for deep tissue imaging.



Figure 1. **a**) The crystal structure of NIR emissive Ag_{16} -DNA⁷ demonstrating the folding of DNA oligomers (purple) around a rod-shaped cluster of 16 silver atoms., **b**) The DNA sequence-tuned fluorescence of Ag_N -DNAs produces a diverse palette of fluorophores,⁴ **c**) Far-red and NIR emissive Ag_N -DNAs containing 6 valence electrons ($N_0 = 6$) yield distinct UV and visible chiroptical signatures that depend on the ligand chemistry.⁵

Hundreds of NIR-emissive Ag_N-DNAs have been recently discovered by highthroughput experimentation,^{3,4} but their synthesis pathways and structure-property relationships remain poorly understood. To tune the spectral properties of these chiral emitters for bioimaging applications, it is important to understand how nanocluster composition and structure relate to Ag_N-DNA fluorescence properties. Here, we present chemical synthesis strategies for achieving high yields of NIR-emissive Ag_N-DNAs, together with a detailed analysis of the molecular compositions and circular dichroism (CD) signatures of 19 atomically precise far red and NIR Ag_N-DNAs.^{5,6} We find that increased storage temperature after chemical reduction and/or increased pH of the solution can foster the formation of larger NIR-emissive Ag_N-DNAs.⁶ High-resolution mass spectrometry of chromatographically isolated Ag_N-DNAs shows a variety of surface ligand chemistries for far red to NIR-emissive Aq_N-DNAs. Ligand chemistry strongly influences both Stokes shift and nanocluster structure (Fig. 1c). Moreover, the valence electron count of the Agn-DNA determines its optical absorption spectrum and the mechanism of emission: either nanosecond-lived fluorescence or microsecond-lived luminescence. CD spectroscopy shows that both ligand chemistry and Ag_N core structure, including valence electron count, yield distinct UV and visible chiroptical signatures. The visible CD signatures of NIR Ag_N-DNAs could be harnessed to probe the structural integrity of NIR Ag_N-DNAs when functionalized for tissue staining and to search for chlorido-stabilized Ag_N-DNAs that possess significantly enhanced stability in physiological salt concentrations. By advancing the fundamental chemical understanding of NIR Ag_N-DNAs, this study enables the design of novel biocompatible NIR Agn-DNAs as bioimaging agents and expands the applications of DNA technologies in targeted labelling for biomedical NIR fluorescence imaging.

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Isothermal self-assembly of DNA nanostructures in different cations

Authors: Arlin Rodriguez, Bharath Raj Madhanagopal, Johnsi Mathivanan, Arun Richard Chandrasekaran

Self-assembly of DNA nanostructures typically requires magnesium ions and a thermal annealing step which restricts their applications. In other solution conditions tested for isothermal assembly of DNA nanostructures, only a limited set of divalent and monovalent ions have been used so far (typically Mg²⁺ and Na⁺). Here, we demonstrate the isothermal assembly of the DNA nanostructures in a wide variety of ions using the double crossover motif as a representative structure and expand the strategy to other multi-helical-domain nanostructures. We show successful isothermal assembly of the structure in Ca²⁺, Mg²⁺, Sr²⁺, Na⁺, K⁺, Li⁺ and Ni⁺ and provide quantified assembly yields at different temperatures. We further show that the choice of metal ion has an effect on the optimal temperature for isothermal assembly. Our work presents new assembly conditions for a wide range of DNA motifs that will be useful for functionalizing DNA nanostructures for various applications.

DNA Origami Placement on Nanopatterned Self-assembled Monolayers

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DNA origami placement (DOP) is a technique to place DNA origamis onto a patterned substrate with site-specificity and alignment. Via nanolithography techniques, nanopatterns of binding sites with affinity for DNA are printed in a passivated background, so DNA origamis could be placed precisely following the designated position and orientation of the patterns. By integrating the nanometer resolution offered by bottom-up placement of DNA origami as a functionality breadboard into top-down nanofabricated binding sites with arbitrary positioning and long-range order, DOP has a broad range of potential applications, such as nanophotonics, sensor arrays, and nanoelectronics.

Despite the potential, current DOP methods remain inaccessible to many researchers for their cleanroom-based expensive and time-consuming processes of nanopattern production, such as electron beam lithography (EBL) or nanoimprinting. A few wetlab techniques like nanosphere lithography (NSL) could serve as an alternative for pattern production, but at the cost of lower yields and lack of arbitrary positioning. Currently DOP is mostly done with hydrophilic silanol binding sites in hydrophobic trimethylsilyl background on Si or SiO₂ substrate. To improve the deposition yield, high concentration of Mg²⁺ is required to increase the origami-pattern attraction, which however may lead to aggregation of the nanostructures. Some attempts have focused on keeping DOP working under specifically optimized conditions, like decreased Mg²⁺ concentration, but there lacks systematic research on more general aspects that would affect DOP, such as surface chemistry, surface topography and deposition solution composition.

Here we show that nanopatterned self-assembled monolayers (SAMs) supported on gold could be a viable alternative to the commonly used Si wafer for DNA origami placement (Figure 1). Our approach (1) can be performed in a standard wet lab at significantly lower costs and shorter turnaround times than traditional EBL based DOP, while maintaining high patterning resolution around 10 nm; (2) allows in situ microscopy throughout the patterning and deposition process; (3) has a broad range of customizable choices for pattern/background SAMs to change the surface chemistry and surface topography of 0.2 nm level precision.

With our new approach, we have elucidated how DOP is significantly affected by surface chemistry, topography, and deposition solution composition. (1) The attraction between DNA origami and binding sites depends on the surface charge density. Using binding site material with higher anionic charge density, which is more attractive, could improve deposition yield. Meanwhile, using background with high deposition resistance endgroups not only eliminates non-specific DNA origami adsorption, but also increases the energy penalty for misalignment (partial overlapping with the background), improving alignment accuracy. (2) Pattern with a higher depth energetically unfavors misalignment due to deformation of the deposited DNA origami, reducing multiple occupancy, and improving the alignment accuracy. (3) Mg²⁺ in the deposition solution increases the DNA-pattern attraction, but also limits the mobility for misaligned deposited DNA origami to re-align, and for unwanted deposited DNA species (e.g., staples) to be displaced. We find with optimal Na⁺ concentration introduced, DNA origami gains mobility to re-align or displace deposited staples, thus alignment accuracy and deposition yield could be improved. These findings help us achieve high yield and alignment accuracy for DOP.

In conclusion, our approach provides an alternative platform that does not require cleanroom access and allows us to tailor interfacial interactions to improve the yield and accuracy of DOP. It may also help build more complex hierarchical structures.



Figure 1. A brief procedure for DOP on nanopatterned SAM. (a) Hexadecanethiol SAM formation on gold substrate; (b,c) Nanografting of mercaptoundecanoic acid (MUDA) SAM patterns; (d,e) DNA origami deposition.



Figure 2. AFM images after pattern nanografting (A1, B1, C1) and after rectangular origami deposition (A2, B2, C2) on the substrate of MUDA pattern in an oligo(ethylene glycol)₆ (OEG-6) passivated background. The scale bars are 200 nm. At [Na⁺] = 25 mM, [Mg²⁺] = 12.5 mM, the deposition yield is 100% on over 100 patterns, with zero DNA origami adsorbed on the background.

FNANO 2024

Monday April 22nd, Poster session Track on Protein and Viral Nanostructures

Track Chair **Nicole Steinmetz** University of California San Diego

Title: Engineered control of virus-like particle structure and stability via intersubunit interface mutations

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Abstract: Virus-like particles (VLPs) from bacteriophage MS2 provide a platform to study protein self-assembly and create engineered systems for drug delivery. Here, we aim to understand the impact of intersubunit interface mutations on the local and global structure and function of MS2-based VLPs. In previous work, our lab identified locally supercharged [T71K/G73R] that concentrate positive charge at capsid pores, double mutants enhancing uptake into mammalian cells. To study the effects of particle size on cellular internalization, we combined these double mutants with a single point mutation [S37P] that is known to switch particle geometry from T=3 to T=1 icosahedral symmetry. These new variants retain their enhanced cellular uptake activity and are able to deliver smallmolecule drugs with efficacy levels similar to our first-generation capsids. Surprisingly, these engineered triple mutants exhibit increased thermostability and unexpected geometry, producing T=3 particles instead of the anticipated T=1. Transmission electron microscopy reveals various capsid assembly states, including wild-type (T=3), T=1, and rod-like particles, achieved with different combinations of these point mutations. Molecular dynamics experiments recapitulate the structural rationale in silico for the single point mutation [S37P] forming a T=1 virus-like particle and show that this assembly state is not favored when combined with mutations that favor rod-like architectures. Through this work, we were able to investigate how interdimer interface dynamics influence VLP size and morphology and how these properties affect particle function in applications such as drug delivery.

DNA origami directed virus capsid polymorphism

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Virus capsid proteins are responsible for encapsulating the genome of most known viruses and thus possess unique assembly properties. The assembly is based on both protein-protein and protein-nucleic acid interactions and results commonly in icosahedral or helical symmetries. Gaining absolute control over the size and shape of assemblies would be advantageous for the development of new delivery systems, however it remains challenging [1,2]. So far, directed assembly has been strictly limited to specific shapes and symmetries [3]. In this work, we exploit DNA origami structures to direct the assembly of single protein subunit capsids in a modular manner. To this end, the user-defined DNA origami nanostructures are utilized as binding and assembly platforms for single proteins, which allows us to achieve control over both size, shape and topology of the formed capsid protein assemblies. The assembly of capsid proteins from cowpea chlorotic mottle virus (CCMV) on such DNA origamis results in well-defined and highly ordered complexed structures. Further characterization using single-particle cryo-electron microscopy reconstruction shows that the capsid proteins efficiently encapsulate the DNA origami. Depending on the applied stoichiometry the capsid proteins are observed to assemble on the DNA origami in a single or double layer configuration in which the layers are preferentially formed by helical arrangements of hexameric capsomers. This approach of templating is highly versatile since it is neither shape-limited, nor exclusive to one type of capsid protein. Furthermore, the applied coating protects the DNA origami from nuclease degradation. Therefore, we believe that these findings might have a direct impact on DNA nanotechnology-based bioengineering, including applications in next-generation cargo protection and targeting strategies [4].



Figure 1:Formation of capsid-coated structures by isolation of capsid proteins from native cowpea chlorotic mottle virus (CCMV) and complexation with DNA origami structures. The dimensions of the assembly are defined by the origami template.

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Directed evolution of artificial repeat proteins for bionanomaterial science: Protein origami and nanocrystal growth directors.

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Precise recognition and high affinity are two intrinsic properties of natural proteins that can be exploited to sculpt bionanomaterials in 3D with extended chemical versatility and atomically-precise spatial organization. Indeed, the morphology of crystalline inorganic nanoparticles can be controlled by two ubiquitous strategies: (1) the growth inhibition of crystal facets by molecules with strong affinity for a subset of crystalline planes of the growing material and (2) the epitaxial growth on an ordered molecular template. In both cases, the chemical properties at the organic-inorganic interface are both essential and extremely complex to design. Proteins offer a wide range of chemical properties brought by the amino-acid side chains but also the potential to fold into extremely precise 3D superstructures able to further self-assemble into higher order architectures. Recent progress of in-silico protein design¹ has proven extremely efficient in uncovering de novo proteins able to recognize biomolecular targets and to assemble into virus-like² or fibril³ morphologies.

Here, we first demonstrate the in vitro design and directed evolution optimization of artificial proteins that spontaneously self-assembled into complex protein origami with a tunable 3D geometry.⁴ Our approach consists in the ab initio design of a library of billions of fully folded rigid and thermostable ankyrin-like artificial proteins (alpha-Repins) and the in vitro evolutionary selection of a subset of proteins showing high affinity and specific binding for a selected target.^{5,6} We will detail the selection of a brick protein (Fig. 1a) and a staple protein and their easy production. Upon mixing the two units, the staple stiches together two bricks (Fig. 1b) and trigger their spontaneous self-assembly into extremely large and perfectly ordered helical superstructures, within minutes and at room temperature (Fig. 1c). Their regioselective interactions are optimized to spontaneously form tubular protein origami helices as observed by X-ray scattering, cryoelectron microscopy and tomography (Figs. 1d,e). Our approach can be widely generalized and the rational redesign of the brick and/or the staple allows us to modify the protein origami geometrical features.⁷



Figure 1: Models of (a) a brick protein bearing two recognition half-domains (red and blue), (c) a staple protein (orange) binding two brick proteins in a preset orientation and (c) the resulting superhelical protein origami. (d, e) Cryoelecton micrographs of a crystal of aligned protein origami. (f) Gold icosahedrons (green), decahedrons (red) and 2D nanoplates (blue) obtained by a seed-mediated synthesis in the presence of Au(111)-binding alphaRep proteins.

Secondly, our protein engineering method allows to create proteins that exhibit a high affinity to specific atomic planes of inorganic materials therefore acting as crystal growth directors.⁸ For this, the evolutionary selection of a subset of proteins is conducted with a target composed of Au(111) crystal facets. The selected proteins display a marked nanomolar affinity for the chosen material. A seeded growth of gold nanocrystals in the presence of the selected alpha-Rep reveals the exclusive formation of Au(111)-terminated nanostructures - icosahedrons, decahedrons and 2D nanoplates - in high yield (Fig. 1f), demonstrating the morphosynthetic efficiency of the selected proteins.

The strategies proposed here can be generalized by exploiting the programmability of the protein sequence, the combinatorial power of phage display, the robust 3D folding of the alpha Rep family and the intrinsic chemical designability of amino acids. The demonstrated concepts pave the way to creating ordered protein templates with surface chemistry tailored to control the growth and morphology of technologically-relevant inorganic nanomaterials.

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Engineering Trojan Horse Nanoparticles for Targeted Endosomal Delivery of Viral Fusion Inhibitors

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The global distribution of flaviviruses poses a significant health risk, with ~390 million dengue virus (DENV) infections per year. Current DENV vaccination is limited to those who have been previously infected, as vaccination of naïve individuals can lead to antibody-dependent enhancement and exacerbation of disease. Furthermore, the development of direct-acting antivirals (DAAs) is hindered by drug resistance. DAAs targeting conserved proteins involved in viral entry may have increased barriers to resistance, as viral escape could compromise viral infection. However, DENV fusion occurs within a protected endosomal compartment, presenting a significant barrier for DAAs targeting this critical step, as any DAA must be present in this same compartment as the fusing virus.

Here, we address this hurdle by developing engineered nanoparticles (NPs) which bind virions prior to endocytosis and then deliver the DAA within the same compartment to block viral fusion. These molecular "Trojan horses" use a targeting moiety that binds to the surface-exposed glycoproteins, as well as a DAA payload, which can inhibit viral fusion by targeting a conserved site. We used DENV envelope (E) protein and anti-E-DIII antibodies as a proof of principle to characterize the functionality of the targeting moiety and the expression of a DAA payload respectively through biolayer interferometry (BLI) and ELISA; negative-stain electron microscopy was used to confirm NP assembly. Antiviral activity was assessed using both single-cycle reporter particles as well as authentic DENV. Overall, this approach provides a modular platform to engineer NPs for targeting viral fusion, ultimately demonstrating how to effectively target such conserved proteins within a protected environment. While our approach uses DENV, this platform is broadly applicable to other internal-compartment fusing viruses.
Co-delivery of Tumor Antigens from Cell Lysates with Adjuvant Cowpea Mosaic Virus for Ovarian Cancer Treatment

Authors:

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Abstract

Cancer immunotherapy has become a promising approach to treat ovarian cancer. To achieve specific anti-cancer immunity, tumor tissues collected through surgical debulking can be processed into tumor cell lysates (TCLs) as a source of neo- and tumor-specific-antigens. Cowpea mosaic virus (CPMV) is a plant virus with the ability to activate TLR2, 4, and 7 as an effective immunostimulatory adjuvant for cancer immunotherapy development. In the current work, we formulated an antigen-adjuvant co-delivered device as a cancer immunotherapy, where TCLs were packaged within liposome to serve as tumor specific antigens and CPMV was conjugated to the liposome surface to act as a triple-TLR agonist adjuvant. This complex device achieved co-delivery of both antigens and adjuvants into the same antigen presenting cells for antigen presentation, then led to the establishment of an adaptive and tumor-specific immunity for ovarian cancer treatment.

Nucleic acids meet plant viruses: from assembly to gene delivery

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Abstract

Nucleic acid therapeutics and gene delivery platforms have the potential to drastically alter how we treat disease. Recent advances in biotechnology have enabled the production of virtually any functional protein or peptide in the human body through the introduction of messenger RNA (mRNA) as a vaccine or therapy. To produce proteins in living systems mRNA needs safe, stable, and efficient delivery vehicles that protect the nucleic acid from degradation, promote cellular uptake, and facilitate mRNA payload release. Lipid nanoparticles (LNPs) have enjoyed recent clinical success for the delivery of mRNA to combat – especially against coronavirus disease 2019 (COVID-19) and, more recently, high-grade ductal breast carcinoma clinical trial patients – marking significant milestones for mRNA nanomedicines. We report a new gene delivery platform that demonstrates the feasibility of using purified coat proteins (CPs) from the plant viruses to form virus-like particles (VLPs) – or nucleoprotein complexes with designer mRNA through in vitro self-assembly. We observe that the transfection of these VLPs into eukaryotic cell lines allows for the disassembly of VLPs and the successful translation of the target proteins. Importantly, we also establish that the size of the resulting VLP can be precisely controlled as a function of the length of the mRNA resulting in designer nucleic acid delivery vehicles for tunable pharmacology.

FNANO 2024

Monday April 22nd, Poster session Track on DNA Nanostructures: Semantomorphic Science B

Track Chair

Hao Yan

Arizona State University

Solid phase, user-friendly assembly of micrometer hierarchical DNA origami structures

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Hierarchical self-assembly promises nanometer control across micrometer lengths and beyond DNA structural nanotechnology and bridges top-down (lithographic) and bottom-up (molecular self-assembly) fabrication techniques. This scale-up has the potential to scaffold previously inaccessible applications in fields such as cell biology and photonics, where single-molecule spatial coordination is required at micron scales. However, several practical limitations bar current hierarchical self-assembly techniques from these applications. First, hierarchical assembly is highly sensitive to subunit incorporation rates, making the production of complex structures a challenge. This in turn limits the size of assemblies. Second, automation is typically required to achieve the spatial addressability needed for functionalization with molecular payloads.

We present a solid phase hierarchical assembly method for the improved production of micron-scale crisscross DNA origami structures. Crisscross origami achieves complex hierarchical assembly (i.e. "megastructures") through cooperative association between fully programmable pre-folded origami subunits [1]. Megastructures are fully spatially addressable at a resolution up to 14 nm and can be structurally reinforced through stacked subunit layers to improve rigidity. In the context of single origamis, solid phase support can provide purification of folded nanostructures from unincorporated components [2]. In the context of crisscross origami, assisted assembly with magnetic beads can overcome size limitations through growth cycles that iteratively introduce subunits at high concentration. To eliminate the requirement for automation, we streamlined this process with enzymatic ligation for a workflow achievable by manual pipetting. We expect these advances improve the quality and accessibility of hierarchically assembled origami structures while also pushing the size limits of programmable self-assembly.

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Figure 1. Crisscross origami assembly with magnetic bead solid support. Origami subunits are initially folded and excess staples are depleted. The seeding origami (red) is first hybridized to magnetic beads. Sequential assembly steps follow in which subsets of 6-helix bundle subunits are added and attached to a growing structure. Finally, an invader strand is added to displace completed structures for collection. The iterative wash and incubating steps enabled by solid phase assembly allow synthesis of both cleaner and larger final structures. Scale bar is 200 nm.

A DNA molecular printer capable of printing on a glass surface

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Atomically precise manufacturing (APM) aims to create materials and devices through the meticulous physical manipulation of atomic or molecular elements, guiding chemical processes to achieve the precise arrangement of atoms or molecules¹. However, foundational technology is lacking. Scanning probe manipulation techniques such as atomic force microscopy and scanning tunnelling microscopy offer picometer-level positioning accuracy but are impractical for scalable nanofabrication. The DNA origami technique stands out as one of the most reliable methods for massively parallel, bottomup synthesis of complex nanostructures. A programmable DNA molecular printer, comprising sliding rails and a write head that positions a catalytic oligonucleotide, has been shown to be capable of modifying pixels on a two-dimensional DNA origami canvas with nanometre precision (Figure 1)².



Figure 1: A 2D molecular printer made from DNA origami². (A) The three printer components. (B) OxDNA simulation of the assembled printer. Molecular printers are imaged by (C) TEM and (D) DNA-PAINT. Scale bars: 100nm.

We present upgrades to the printer design (Figure 2) and preliminary demonstration of direct patterning of a glass surface using position-controlled click chemistry.



Figure 2: Improved printer design. (A) Canvas with reduced curvature. (B) A new frame incorporates stoppers made from DNA tiles to limit the motion of the slider and thereby increase the speed of write-head repositioning. (C) Closer attachment of the cross rail increases positioning accuracy. (D) A new printing mechanism designed to reduce uncatalysed deposits of ink on the canvas.

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DNA Based Nanodevices for Understanding Eukaryotic Epigenomes

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DNA nanotechnology is an emerging field that enables the fabrication of biologically compatible nanoscale devices with mechanodynamic functions. As part of a highly collaborative project, we are developing DNA-based nanodevices for probing eukaryotic epigenomes. The physical organization of eukaryotic epigenomes is evolutionarily conserved, where histone protein octamers repeatedly wrap genomic DNA into nanometer size nucleosome spools, which are the basic building block of chromosomes. Nucleosome organization controls the physical accessibility of the genome to gene regulatory factors, and plays a central role in cell function, development, and human disease. Here, we use hinge-like DNA origami nanocalipers, Nanoscale DNA Force Spectrometers (nDFS), with tunable mechanical properties to perform force spectroscopy experiments on tetranucleosomes, a model of nucleosome clutches. The nDFS can be used as a ruler to measure the end-to-end distance of individual tetranucleosomes, and we can therefore measure a continuous free energy landscape as a function of end-toend distance. Such a measurement has not been feasible using other methods such as optical traps. In addition, since we can also observe the structure of the tetranucleosomes in TEM images, we can relate qualitative compaction metrics to quantitative metrics such as forces and free energy. We find evidence supporting the notion that higher tensile forces are applied to more compact tetranucleosomes. These studies provide proof of concept for using nanocalipers as force spectrometers on tetranucleosome clutches.



Figure: TEM Images of hinge-like DNA origami nanostructures with tetranucleosomes attached.

Monday April 22nd, Afternoon Track on Protein and Viral Nanostructures

Track Chair **Nicole Steinmetz** University of California San Diego

Self-assembly, genome packaging, and structure-based discovery of RNA phages

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To spread from host to host, small spherical RNA viruses package their RNA genomes into protective protein shells called capsids. This packaging process involves two remarkable feats of nanoengineering: first, within the crowded cytoplasm of an infected host cell, the viral proteins must identify the viral RNA from a diverse pool of cellular RNA, including large numbers of ribosomal and messenger RNAs; second, the viral proteins must assemble around the viral RNA into a specific guaternary structure, forming a proper icosahedral capsid while avoiding numerous kinetic traps and malformed states. Our lab is working to experimentally measure and understand these remarkable feats of nanoengineering using a combination of sensitive in vitro experiments and guantitative in vivo assays. Working with bacteriophage MS2, a long-standing model system, we use interferometric scattering microscopy (iSCAT) to measure the self-assembly kinetics of individual capsids around individual strands of RNA. In addition, we perform live-cell packaging assays in which RNA molecules with different sequences and structures compete against cellular RNAs for packaging into capsids. Our experiments show that MS2 capsids assemble around RNA by a nucleation-and-growth mechanism, in which the nucleation kinetics are controlled by collective properties of the RNA molecule-namely, specific sets of multiple stem-loop structures. These results provide clues about how MS2 and related RNA viruses package their genomes with high selectivity. They also supply a new approach to discovering bacteriophage genomes within environmental sequencing datasets by detecting characteristic properties of their folded structures.

ON THE LINK BETWEEN MOLECULAR MECHANICS AND PHOTONICS IN SUPER-RADIANT VIRUS-LIKE PARTICLES

BOGDAN DRAGNEA

The problem of controlling the boundary between quantum and classical behavior is of great technological significance, and for the fundamental nature of the measurement process. Gaining access to quantum properties that do not have classical correspondent, such as entanglement, tunneling, and delocalization, requires suppression of quantum decoherence processes – whereby quantum states lose fundamental information via interaction with the environment. In quantum applications with molecular or atomic systems, including quantum computing and sensing, slowing down decoherence is achieved by lowering the temperature, working in vacuum, and exerting tight structural and compositional control at microscopic scales. However, away from quantum technology research labs, the survival of a number of biological species depends on sensitive geolocation, and efficient enzymatic activity and solar light harvesting. Unsurprisingly, in these instances, and perhaps others, natural evolution seems to have already tapped into "non-trivial" quantum behavior to enhance function. Since life on Earth, as we know it, depends on liquid water, it looks like Nature may have already found out how to overcome the influence of decoherence and harness quantum behavior in messy, aqueous environments, at high temperature, to increase the survival chances of migratory birds and photosynthetic organisms. How is this possible? In this talk, I will describe new phenomena occurring in luminescent virus-like particles that might shed some light on this matter.

1

Antibody Bottlebrush Conjugates (ABCs): A New Platform for Targeted Delivery

Bin Liu¹, Hung V.-T. Nguyen,¹ Jeremiah A. Johnson¹

¹Department of Chemistry, MIT, United States

Antibody drug conjugates (ADCs), which are composed of a monoclonal antibody for cell targeting linked to a cytotoxic payload, have emerged as a promising class of targeted chemotherapeutics. Despite their success in the clinic, ADCs suffer key drawbacks. For instance, the number and type of payload molecules conjugated to each ADC is limited due to the restricted number of conjugation sites as well as the deterioration of physical properties as the number of conjugated payloads increases. Moreover, extensive optimization of each drug payload and linker chemistry needs to be done to achieve a maximal therapeutic index for each ADC. To address these challenges, we have developed a new antibody-targeted prodrug platform, which we refer to as "antibody bottlebrush conjugates" or "ABCs." ABCs feature an antibody conjugated to bottlebrush polymers of similar size and shape. The latter carry inactivated "prodrugs" attached at each repeat unit along a bottlebrush backbone shielded by hydrophilic poly(ethylene glycol) (PEG) chains that protect the prodrugs from premature activation and release. The high density of PEGylation can improve the pharmacokinetics of ABCs, while the prodrug linker structure can be molecularly tuned to control the payload release rate and mechanism. Finally, different payloads and antibodies can be easily mixed-and-matched, enabling modular development of novel ABCs with predictable properties.

Fig. 1. Schematic of an antibody bottlebrush conjugate (ABC).

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Tuesday April 23rd, Morning Track on Synthetic Biology

Track Chair **Alex Deiters** University of Pittsburgh

Illuminating the RNA world

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Light has been used for centuries to interrogate biological systems. Because photons can be deployed and detected with high spatial and temporal resolution, optogenetic tools and genetically-encoded fluorescent reporters, which use pulses of light to regulate cellular activity or monitor the expression, location, and activity of various biological molecules, respectively, have transformed biomedical sciences. Lightactuated systems have largely been limited to membrane proteins, whereas fluorogenic reporters have found great utility in many areas of cell biology, but they suffer from a large autofluorescence background and their continual excitation leads to photodamage, limiting their applications in live animals. We have developed a synthetic photoriboswitch—a riboswitch that binds a photoisomerizable ligand. We showed that bacterial mRNA translation can be turned on or off with very short (~ms) pulses of light, triggering gene expression faster than most fundamental steps in transcription or translation. On the detection side, we have recently engineered an RNA-based bioluminescent system, which exhibits strong RNA-dependent photon turn-on in vitro and in vivo and paves a way towards RNA sensing in living animals. Latest applications and future directions of these molecular tools will be presented.

Next generation drug discovery: Induced proximity medicines

<u>P. Ryan Potts</u> Department of Discovery Technology Platforms, Amgen Research, USA. <u>Ryan.potts@amgen.com</u>

Induced proximity is a novel strategy for targeting the highly intractable proteins that are involved in many human diseases. By bringing together disease targets and biological effectors, such as enzymes, ubiquitin ligases, or transcription factors, proximity-based therapeutics can modulate the function, stability, or localization of the target proteins. This approach can overcome some of the limitations of conventional small molecules or biologics, such as poor selectivity, low potency, or unfavorable pharmacokinetics.

My talk will cover the difficulties we encounter in finding new drugs and the potential of using proximity biology to address challenging drug targets. I will explain the principles of proximity-based treatments and showcase some examples, such as targeted RNA degradation. I'll show how Amgen's Induced Proximity Platform is using basic biological knowledge of targets and effectors along with adaptable chemistry platforms to progress the new generation of drug discovery programs.

DTAC: DNA-based Proteolysis Targeting Chimera

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Approximately 85% of the proteome is considered undruggable by traditional, occupancy-driven pharmacology employing small molecular inhibitors. Proteolysis-targeting chimeras (PROTACs) and associated molecules that induce targeted protein degradation via the ubiquitin-proteasome system have emerged as a revolutionary strategy for addressing "undruggable" targets¹. The success of small molecule based-PROTAC largely depends on the optimal linker length²⁻⁴. However, it remains challenging to control the spatial orientation when using flexible chemical likers. The orientational control of ligands can influence protein facing with E3 ligase complex that may facilitate ubiquitination, as well as selective ubiquitination of a single protein in the case of a protein complex^{5,6}. Inspired by programmability and biocompatible nature of DNA/RNA biomolecules, we hypothesize to construct DNA based Proteolysis-Targeting Chimeras (DTACs) with spatial control of distance and orientation between E3 ligase inhibitor (E3-i) and protein of interest inhibitor (POI-i). As a starting test module, we constructed a library of 20 bp DNA duplex of DTAC against CDK4/6 protein complexes. As per our hypothesis, DTAC version with optimal spatial distance and angle showed efficient degradation of CDK4/6 complexes at 20 nanomolar concentration for 48 h when tested in U251 brain cancer cell line. Owing to programmability and simplicity, we believe this will accelerate the evaluation of further unknown protein targets, streamlining the design process and reducing the associated costs.



Figure: A) Overview of DNA templated targeted protein (POI) degradation mechanism of DTAC. **B)** Dose-dependent (Concentration in nanomolar) degradation of CDK4/CDK6 protein complexes. **C)** Time-dependent screening of CDK4/CDK6 protein complexes degradation with 50 nM DTAC.

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Controlled incorporation of non-canonical amino acids (ncAAs) into proteins in living cells has emerged as a powerful tool for biological research and biotechnology. Our group focuses on advancing the scope of this technology for both probing complex biological questions, as well as to create opportunities to develop next-generation biotherapeutics. Our research goals include: 1) Creating new platforms to genetically encode ncAAs with diverse chemical structures, 2) Developing new bioorthogonal conjugation reactions with new capabilities, 3) Creating next-generation biotherapeutics, such as precisely modified viral vectors for enhanced gene therapy, 4) Investigating the roles of various post-translational modifications of human proteins, and 5) Developing technology to monitor dynamic changes in the cellular proteome. This presentation will focus on our recent work on a novel strategy for the directed evolution of synthetic biology parts in live mammalian cells.

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Tuesday April 23rd, Poster session Track on Synthetic Biology

Track Chair **Alex Deiters** University of Pittsburgh

Synthetic capacitor-like RNA and protein biocondensate facilitate a variety of biotechnological and therapeutic applications

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The recent recognition of the importance that membraneless biocondensates play within cells to ensure proper cellular function has led to the emergence of a new field in synthetic biology that aims to not only develop tools for exploring these natural phenomena, but also for harnessing it towards a wide variety of nano-and biotechnological applications. Broadly speaking, biocondensates are phase separated aggregates of proteins or of proteins and RNA that encode some functional role that can only be triggered within the dense or condensate phase. Therefore, biocondensates are genetically encoded structures with emergent biological, chemical, physical, and material properties.

To explore the complexity of biocondensate structure, we opted to construct and study protein-RNA biocondensates that we term synthetic RNA-protein (sRNP) granules. Unlike natural biocondensates. our synthetic granules are composed of synthetic long-non-coding RNA molecules (slncRNA) and phage coat RNA-binding proteins. The sequences for the slncRNA molecules are computed using an AI-tool named "CARBP"¹, yielding non-repeat sequences that generate a cassette of functional hairpin structures. The protein component contains a phage-coat-protein moiety (i.e. PP7, MS2, Q β , and GA) that can bind the hairpins in a specific fashion. When mixed, the RNP protein component and the slncRNA molecules phase separate to form a dense gel-like phase, and a dilute low-density liquid-like phase².

We study the synthetic RNP granules both *in vivo* and *in vitro*. Our results indicate that the granules exhibit gel-like or glass-like characteristics depending on the type of granule binding protein and the number of hairpins encoded on the slncRNA. In particular, the gel-like phase both protects its components from degradation and equilibrates at a significantly slower rate than the dilute surrounding phase. Consequently, the sRNP granule act as a capacitor-like entity that stores its protein and RNA components in high density and dissolves over a time scale of hours, which is decoupled from the cell-cycle².

This characteristic facilitates several applications. First, we utilize granules to provide a boost for gene expression via its protection of both the RNA and protein components, thus increasing the protein-product titer that can be generated from a given cell. This capability is crucial for the biotechnology and food-tech sectors (Fig 1A-B). Second, granules can be functionalized to form broad-spectrum decoy antivirals. That is, self-assembled, genetically encoded, stable nanoparticles that present to invading virions false targets, thus compelling them to release their genetic cargo to an empty vessel. We demonstrate the efficacy of this approach against both SARS2 (Delta and Omicron BA.1 variants) and Influenza H1N1 viruses (Fig. 1C-D). Finally, granules can be used as a probe to quantitatively identify post-translation modifications on proteins, facilitating rapid protein characterization that goes beyond what's possible with current mass-spec and

sequencing approaches. We demonstrate quantitative detection of sialic acid, a form of naturally occurring glycosylation, on both synthetic and naturally occurring proteins.

In summary, synthetic biocondensates are a new field in synthetic biology, where we engineer emergent material properties into genetically encoded multi-molecular structures, which can be in turn be adapted for a variety of biotechnological and therapeutic applications.

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Figure 1: sRNP granules and their applications. (A) Hyper-expression via protein capacitors. Engineered generic granule binding proteins together with slncRNA form gellike particles that store proteins at high density in cell poles. (B) Protein hyper-expression: protein titers with (left falcon) no slncRNA, (middle falcon) slncRNA with 4 hairpins, and (right falcon) slncRNA with 24 hairpins. (C) Decoy antivirals. Engineered antiviral granule binding proteins together with slncRNA form gel-like antiviral decoy particles. (D) Antiviral decoy granules inhibit SARS2-delta and -omicron variant infection in Vero cells.

eScaf: an engineered Escherichia coli strain for scalable production of long single-stranded DNA

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Long single-stranded DNA (ssDNA) is a versatile molecular reagent with applications including RNA-guided genome engineering and DNA nanotechnology, yet its production is typically resource-intensive. We introduce a novel method utilizing an engineered *E. coli* "helper" strain named "eScaf" and phagemid system that simplifies long ssDNA generation to a straightforward transformation and purification procedure (Fig. 1). Our method obviates the need for helper plasmids and their associated contamination by integrating M13mp18 genes directly into the *E. coli* chromosome. We achieved ssDNA lengths ranging from 504 to 20,724 nucleotides with titers up to 250 µg/L following alkaline-lysis purification (Fig. 2). The efficacy of our system was confirmed through its application in primary T cell genome modifications and DNA origami folding. The reliability, scalability, and ease of our approach promises to unlock new experimental applications requiring large quantities of long ssDNA.



Fig. 1 Overview of ssDNA production using the eScaf helper strain. Our eScaf method streamlines conventional helper plasmid + phagemid ssDNA production systems by integrating the phage genes directly into the chromosome, establishing a 'helper strain' that requires only the phagemid for transformation. Like in standard helper plasmid or helper phage preps, phage-like particles containing ssDNA are released into the culture medium and can be isolated through PEG fractionation. The target ssDNA is subsequently extracted from these particles using alkaline lysis followed by ethanol precipitation for purification.



Fig. 2 ssDNA production by M13mp18 and M13KO7 helper strains. M13mp18 and M13KO7-based helper strains can robustly produce ssDNA within a size range of 504 to 10,080 nucleotides. The M13KO7-based strains exhibit a higher presence of off-target species and background than the M13mp18-based strains.

Robust Bio-Secure DNA Based Memory

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Information storage in synthetic DNA oligomers is attractive due to the inherent physical density, stability, and energy efficiency of nucleic acids. Information retention –during writing, storage, and retrieval processes– requires development of efficient encoding/decoding systems. Additionally, potential intrusion of artificial or organic malevolent biologically active molecular machines could potentially cause catastrophic biosecurity concerns. Here we present an improved information storage method that focuses on efficiency and biosecurity.

Herein this paper, we have developed and experimentally tested an algorithm to write data in pool of DNA strands by applying a fountain code (rateless erasure code), a Reed Solomon code, and an oligomer mapping code that ensures Bio-Security. We validated our method through wet-lab experiments and wrote, stored, and fully retrieved 105,360 bits of information. We validated the siosecurity aspects of our method through in-silico experimentation using a BLAST-run to compare our generated oligomers to existing genes documented in the public databases, a Plasmidhawk software analysis to determine our oligomers could not be artificially traced to have originated from another lab, and utilized an open-source software to determine whether our oligomers could have expressed any sequences that potentially originate or empower biologically meaningful functions.

This work is supported through NSF award # MCB 2027738.

Tuesday April 23rd, Poster session Track on DNA Nanosystems: Programmed Function A

Track Chair **Friedrich Simmel** Technical University, Munich

Engineering of sigmoidal enzyme reaction kinetics on DNA origami by the modulation of local substrate binding

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Abstract

In recent years, the field of DNA self-assembly has seen remarkable advancements, fostering the rapid development of nanoscale biomolecular structures like periodic DNA crystals and DNA curvatures using scaffolded DNA origami and single-stranded DNA bricks. These achievements, combined with the ability to control enzyme functions, enhance our understanding of the fundamental principles governing cellular reactions and enable the creation of non-living artificial systems that replicate cellular mechanisms. Here, we reported a groundbreaking method to engineer the sigmoidal kinetics of enzymes on rectangular DNA origami. This involved utilizing an aptamer switch to modulate substrate concentration near the enzyme. In more details, by increasing the ATP concentration, the complementary strand will leave the aptamer and its affinity will rise to the ATP. Therefore, by enriching the surface with ATP, the apparent km will be decreased so the velocity of the enzyme will increase sharply at certain concentration of the substrate. A sepharose size exclusion column was employed to precisely control enzyme activity by removing free enzymes from the cascade reaction. This innovation opens new ways for engineering artificial enzymes with applications in molecular sensing, biocatalysis, and therapeutics.



Enzyme cascade reaction on origami

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Triggered contraction of self-assembled micron-scale DNA nanotube rings

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Contractile rings formed from cytoskeletal filaments mediate the division of cells. Ring formation is induced by specific crosslinkers, while contraction is typically associated with motor protein activity. In the past, we have reconstituted DNA nanotechnology-based mimics of cytoskeletons inside cell-sized compartments, including giant unilamellar lipid vesicles [1,2]. Here, we aim for a new function, namely the formation of contractile DNA rings. We engineer DNA nanotubes as mimics of cytoskeletal

filaments and a synthetic crosslinker based on a peptide-functionalized starPEG construct [3]. The crosslinker induces bundling of ten to hundred individual DNA nanotubes. Importantly, the DNA nanotube bundles curve into closed micron-scale rings in a one-pot self-assembly process yielding several thousand rings per microliter. Coarse-grained molecular dynamics simulations reproduce detailed architectural properties of the DNA rings as observed by electron microscopy. Furthermore, theory and simulations predict DNA ring contraction – without motor proteins – upon increasing attraction or decreasing bending rigidity of the DNA nanotubes, yielding mechanistic insights into the parameter space relevant for efficient nanotube sliding. We experimentally realize a variation of these parameters by addition of molecular crowders or temperature increase, respectively. In agreement between simulation and experiment, we obtain ring contraction to less than half of the initial ring diameter [3].

DNA-based contractile rings could be a future element of an artificial division machinery in synthetic cells or of contractile muscle-like materials.

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Figure 1: **a** Schematic illustration of synthetic peptide starPEG-(KA7)₄ bundling DNA nanotubes.

b-d As observed in experiments, starPEG-(KA7)4 induces the self-assembly of multiple DNA nanotubes into closed micron-scale DNA rings that contract upon addition of a molecular crowder or heating. b Transmission electron micrographs of bundled DNA nanotubes. Scale bar: 200 nm (top) and a self-assembled and kinetically trapped DNA nanotube ring (bottom, compare to e). Scale bar: 500 nm. c Confocal overview image of self-assembled DNA nanotube rings. Scale bar: 20µm. d (from left to right) Confocal images of uncontracted (top) and contracted (bottom) DNA nanotube rings without and with 25 wt% 500 kDa dextran. Scale bar: 2 µm. DNA nanotube ring diameter for different molecular weights of 25 wt% dextran and for heating from room temperature up to 40 °C. e-f Coarse-grained MD simulations of DNA nanotube rings and their contraction. e Kinetically trapped structure in incomplete ring formation after simulated annealing (bottom). starting at temperature T_1 (top) and annealing to a high temperature $T_2 = 8T_1$ (middle). f MD simulation snapshots of an isotropic initialization (top) and a DNA nanotube ring (bottom). g Bundle contraction during equilibration (ring diameter as a function of MD simulation time) for different potential strengths ε/kBT . Smaller equilibrium diameters result from increasing ε . Snapshots for $\varepsilon/kBT = 0.8$. Scale bar: 60 σ . σ being the diameter of the bead in the coarse-grained MD simulation and thereby the diameter of the simulated DNA nanotube with a mean (Poisson-distributed) length of 580 $\sigma \sim 6.96 \mu m$.

Free Energy Profiles of Geometrically Frustrated DNA Origami

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DNA origami has proven to be a powerful method for constructing complex nanostructures from bottom up. Paired with methods for dynamic structural transformation, they are an excellent platform to study mechanical principles in action at the nanoscale. As a proof of concept, the current work delves into understanding the mechanical principle of 'geometric frustration', that is, the inability of a system to minimize its energy due to its lattice arrangement.¹ Using a single DNA origami wireframe, we observe the ability to elicit distinct responses based on the edges chosen for deformation. The two selected pathways reconfigure the initial 'extended structure' to either 'compatible' or 'incompatible' conformations as illustrated in Fig. 1(a). To elucidate the divergence, we utilize coarse-grained molecular dynamics (MD) simulations using the oxDNA platform². Using umbrella sampling³, we bias the simulations to explore the complete configuration space, varying the end-to-end (E2E) distance of a selected edge to extract the free energy profiles of both structures^{4,5}. The free energy profiles offer critical insights into the behavior with the incompatible structure showing distinct double minima while the compatible design has only a single minimum (Fig. 1(b)). This work presents a potential platform to study geometric frustration at the nanoscale, and in turn gain insights into DNA mechanics as well. This work may be further extended to deformable structures with tunable free energies by strategically designing the DNA lattice.



Fig. 1. (a) Schematic and origami designs of compatible and incompatible structures. (b) Free energy profiles of the compatible and incompatible states as a function of the end-to-end (E2E) distance.

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Generation of DNA Oligomers with Similar Chemical Kinetics via In-Silico Optimization

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Networks of interacting DNA oligomers are useful for applications such as biomarker detection, targeted drug delivery, information storage, and photonic information processing. However, differences in the chemical kinetics of hybridization reactions, referred to as kinetic dispersion, can be problematic for some applications. Here, it is found that limiting unnecessary stretches of Watson-Crick base pairing, referred to as unnecessary duplexes, can yield exceptionally low kinetic dispersions. Hybridization kinetics can be affected by unnecessary intra-oligomer duplexes containing only 2 basepairs, and such duplexes explain up to 94% of previously reported kinetic dispersion. As a general design rule, it is recommended that unnecessary intra-oligomer duplexes larger than 2 base-pairs and unnecessary inter-oligomer duplexes larger than 7 base-pairs be avoided. Unnecessary duplexes typically scale exponentially with network size, and nearly all networks contain unnecessary duplexes substantial enough to affect hybridization kinetics. A new method for generating networks which utilizes in-silico optimization to mitigate unnecessary duplexes is proposed and demonstrated to reduce in-vitro kinetic dispersions as much as 96%. The limitations of the new design rule and generation method are evaluated in-silico by creating new oligomers for several designs, including three previously programmed reactions and one previously engineered structure.

Employing DNA origami to elucidate protein structure and function using cryo-EM

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Over the past 10 years, TEM has become a technique capable of resolving atomic features of non-symmetric biomolecules. Modern TEM practices are able to resolve atomic features, routinely obtaining resolutions of 2-3 Å for non-symmetric biomolecules. However, there are limitations within the type of biological macromolecules which the cryo-EM methodology can be applied to. Namely, the targets need to have sufficient size, mass and rigidity in order to produce a strong enough signal for alignment during the reconstruction of their 3D electrostatic potential from 2D TEM micrographs (Wu and Lander, 2012, Li *et al*, 2021). In this study, we aim to design and apply DNA origami structures to reconstruct non-optimal targets.

In the case of insufficient size and mass, a stable DNA origami rod structure was designed, which can symmetrically bind small protein targets for reconstruction. In order to successfully apply this technique, two criteria need to be met: (1) the structure itself needs to be stable enough to resolve to high resolution, and (2) the protein decorating the structure needs to be bound tightly enough to not have any motion between the structure and the protein. We have established that for the DNA structure we can achieve resolutions of \leq 3 Å, providing a solid foundation for resolving the structural intricacies of the bound biological molecules. In order to achieve the crucial spatially tight binding to the rod structure we are inserting a compact 61aa DNA-binding domain into the protein targets, which is designed based on the Engrailed protein from *Drosophila melanogaster* (Tucker-Kellogg *et al*, 1997).

Meanwhile, in the case of preferred orientation, a DNA origami sphere is decorated with the target protein in order to push it towards adopting views it otherwise would not. In this case, standard, already established conjugation methods can be used, such as bis-alkylation of Histidines from His-tags (Cong *et al*, 2012) and Sortase coupling (Fabricius *et al*, 2018). Currently we are testing the system with various targets, including both protein complexes and other DNA origamis which are known to suffer from preferred orientation, and establishing a cryo-EM processing pipeline for such samples.

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Microscale DNA Tethers Can Connect Cells Separated by Large Distances

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Abstract

Cell networks that rely on functional cell-cell connections play important roles in developing cell-based therapeutics. However, physically connecting cells separated by micron-scale distances remains a challenge. Here, we report the development of microscale tethers built from DNA nanostructures for physically connecting cells to form a cell network. DNA origami-seeded DNA tile nanotubes are first conjugated to target cells. These nanotubes can then grow and join to connect cells. Microscale tethers can be attached to different types of cells and cells can form multiple connections to create networks. The tether can grow long enough to span at least one non-connected cell. These microscale tethers might enable the transfer of chemical or physical messages between cells in multicellular assemblies or artificial cell communities.

Coarse-grained modelling of DNA-RNA hybrids

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DNA-RNA hybridisation is involved in biological processes such as transcription or DNA replication and is also highly relevant to biotechnological applications including antisense therapy and CRISPR-Cas9 gene editing. Here we introduce oxNA, a coarse-grained model combining oxDNA and oxRNA, enabling the computational study of DNA-RNA hybrid systems on timescales inaccessible to all-atom simulations. As an example application, we use the model to study an R-loop, which reveals a free energy landscape governed by entropic effects. Simulations of hybrid toehold-mediated strand displacement show good agreement with existing experimental data on reaction kinetics. Confirmed experimentally, our modelling has also uncovered strong sequence-dependent effects in hybrid strand displacement systems, which allows for independent control over thermodynamics and kinetics—reaction rate can be tuned across several orders of magnitude, while the thermodynamic drive of the reaction remains essentially unchanged.

Enhancing Interstrand Thymine Photo-crosslinking Efficiency in DNA-Templated Dye Aggregates through the Use of Shorter Linkers

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The programmable assembly of dye molecules on DNA templates is a promising strategy for promoting exciton delocalization in molecular aggregates. Enhanced structural rigidity of DNA scaffolds can improve the stability of these aggregates, which is crucial for exciton transport. Our approach centers on the strategic implementation of photo-crosslinking reactions between thymine bases within a DNA HJ's core, thereby forging additional covalent bonds to reinforce the aggregate structure. The study investigated how the molecular distance between two thymines affects the effectiveness of photocrosslinking ([2+2] cycloaddition) reactions, utilizing linkers of various lengths to connect the dyes to the DNA templates.

By anchoring squaraine dyes to the HJ core via extended linkers attached to thymine modifiers, we explored the spatial arrangement of these dyes and its influence on the photo-crosslinking reaction. We scrutinized the influence of thymine proximity on photo-crosslinking efficiency by employing linkers of varying lengths to attach dyes to the DNA templates. The investigation revealed that shorter linkers promote a higher yield of interstrand thymine photo-crosslinking, attributed to reduced intermolecular distances and intensified dye-dye interactions. The insights gained from this study not only validate the use of shorter linkers for improved cross-linking efficiency but also lay the groundwork for enhancing the stability of dye aggregates on DNA scaffolds. This advancement opens new avenues for the application of these constructs in exciton-based technologies, including light harvesting, nanoscale and quantum computing, and organic optoelectronics.

This research was fully supported by the U.S. Department of Energy (DOE), Office of Basic Energy Sciences, MaterialsSciences and Engineering Division, and DOE's Established Program to Stimulate Competitive Research (EPSCoR) program under Award No. DE-SC0020089.

FNANO 2024

Tuesday April 23rd, Morning Track on DNA Nanosystems: Programmed Function

Track Chair **Friedrich Simmel** Technical University, Munich
A Tale of 2 Strands: From Genomes to Origami, Vaccines, Data Storage, and Back

Mark Bathe, PhD

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ABSTRACT

DNA and RNA are most familiar as the sequence-controlled polymers used to store and propagate genetic information. Concurrent advances in nucleic acid nanotechnology and nucleic acid synthesis, however, have now enabled the fabrication of custom DNA- and RNA-based virus-like particles for subunit vaccines and gene therapeutic delivery; nucleic acid based archival digital data storage; and nucleic acid barcoding of encapsulated genomes for room temperature biobanking of human clinical samples, pathogens for pandemic surveillance, and organisms for ecological preservation. In this presentation I will share our work in these areas, focusing on virus-like DNA-based particulate vaccines and DNA "hard-drives" with random access capabilities for large-scale archival data storage, as well as genome biobanking. Future research efforts will be discussed together with commercial activities emerging from past research in these areas.

Single-stranded RNA nanopores without chemical functionalization

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Building mimetics of biological components has long been a goal of the molecular programming field to better understand the fundamental building blocks of life and to engineer life-like function with specific parameters. Membrane-spanning nanopores, which bestow size- and chemistry-specific permeability to lipid bilayers, have been successfully developed by the DNA nanotechnology community in many sizes and with a variety of chemical functionalization and control schemes [1].

Generally, interaction between DNA pores and membranes have been mediated by hydrophobic moieties, such as cholesterol, covalently attached to strands during chemical synthesis. These hydrophobic moieties embed themselves in the bilayer core, providing an energetic landscape favorable to pore insertion.

However, in-cell production and application of nanopores is difficult to achieve with chemically-modified and multistranded architectures. Here, we present rational design, molecular simulations, cryo-electron microscopy and single-channel ionic current recordings of the first transcribable RNA origami transmembrane pores.

We also extend the library of membrane-active moieties for nucleic acid pores, starting with the traditional cholesterol-modified DNA strands attached via complimentary loops, then extending a previously-reported [2] strategy which used streptavidin-modified strands and biotinylated lipids to instead use integrated biotin aptamers, and finally show promising characterization and miniaturization of RNA aptamers which bind and disrupt phospholipid bilayers on their own [3].



(A) oxRNA simulation of an RNA transmembrane pore showing its dimensions and flexibility. (B) Singleparticle reconstruction from cryo-electron microscopy of the pore from A. (C) Example single-molecule ionic current recordings and conductance histograms for cholesterol- and biotin aptamer-modified pores.

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Higher-order assembly of DNA origami: new strategies, new functions

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DNA nanotechnology is a rapidly growing field that holds great promise for creating nanodevices capable of complex functions, including drug delivery, molecular sensing, nanomanufacturing, and molecular computing. However, for many of these applications to become a reality, the devices need to be assembled into macroscopic arrays to gain high throughput and/or exhibit complex functions. As the PI of a molecular modeling lab, I will discuss how our lab is addressing this challenge using statistical mechanics, molecular simulations, and machine learning, often in close collaboration with experimentalists. Specifically, I will describe our ongoing efforts in creating brush-functionalized DNA origamis [1,2] and assembling them into target architectures using a neural-adjoint inverse-design approach [3], achieving assemblies of dynamic origami devices capable of exhibiting emergent functions like signal communication [4,5] and macroscopic order-disorder transitions [6], and controlling the energy landscape of a species-level binding displacement "reaction" to achieve orthogonal placement of multiple DNA species on surfaces [7].

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FNANO 2024

Tuesday April 23rd, Afternoon Track on Integrated Chemical Systems Track Chair Jeremiah Gassensmith University of Texas at Dallas

Integrated Chemical Systems for Cancer Immunotherapy Applications

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Biological systems have exquisite complexity, responsiveness, and interactions at multiple scales. We have developed synthetic materials inspired by nature to address unmet medical needs. In the first example, unique aptamers with high affinity for T cell markers were discovered and applied as alternatives to antibodies for T cell isolation in the manufacturing process for CAR T cells. In the second example, a polymer was developed that mimics the endosomal release mechanism of adenovirus, selectively displaying a membrane-disrupting peptide in acidic pH. This polymer promotes efficient endosomal release and has been used to deliver biologic drugs such as nucleic acids and peptides into the cell for applications such as cancer immunotherapy.



Schematic of delivery mechanism for VIPER (Virus-Inspired Polymer for Endosomal Release).

Multifunctional mesoporous silica nanoparticles for cancer treatment

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Nanoparticles are an innovative platform for cancer treatment that reduces systemic toxicity and allows for active targeting of tumor sites to enhance therapeutic efficacy. Mesoporous silica nanoparticles (MSNs) have emerged as an attractive drug delivery system due to their vast functionalization potential, biocompatibility, and high surface area. The surface of these nanoparticles can be modified with targeting agents that allow not only the specific interaction with cancer cells, but also targeting organelles inside the cells. In addition, active molecules such as photosensitizers and anticancer drugs can be chemically attached or physically loaded into the interior and/or exterior surface of MSNs. Herein, I will present the use of MSNs for the treatment of breast and skin cancer by combining chemo and gene therapy; the target-specific combination therapy mediated by MSNs to modulate the tumor microenvironment to improve the therapeutic outcome against pancreatic cancer; and our more recent results on the use of MSNs for immunotherapy.



Figure caption: Advantages and applications of mesoporous silica nanoparticles as drug delivery systems for cancer treatment.

Sequence-Based Recognition of Peptides and Proteins for Programmable Assembly

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The development of rules for the sequence-selective molecular recognition of peptides and proteins by synthetic compounds should greatly facilitate their programmable selfassembly. This presentation will discuss several rules that we and others have developed for the sequence-based recognition of peptides and proteins by cucurbit[n]uril synthetic receptors (Figure). Recognition occurs in neutral aqueous solution with binding affinities ranging from high micromolar to sub-nanomolar. Binding sites are remarkably small, 1-3 residues, which has enabled the installation of minimally sized affinity tags onto proteins to control their assembly. The vast majority of the work in this area has targeted the Nterminus, but due to practical limitations with engineering N-terminal affinity tags we recently investigated the scope and limitations of binding at non-terminal sites on polypeptides.[1]



Figure. Three binding modes of cucurbit[8]uril with peptides, each with unique sequence-selectivity and applications. (a) binding a single peptide at an N-terminal Trp (W), which can also be mediated by an auxiliary guest to facilitate sensing. (b) binding two peptides, each at an N-terminal Phe (F), which has been used to dimerize, oligomerize, polymerize, and trap/release peptides and proteins. (c) binding a single peptide at a non-terminal Leu-Tyr (LY) or Lys-Phe (KF), which has been used to fold peptides and is the main topic of this presentation.

A small library screen of the binding of cucurbit[8]uril (Q8) to peptides, each with a dipeptide target site, resulted in several leads, which were then characterized by a combination isothermal titration calorimetry (ITC), ¹H NMR spectroscopy, mass spectrometry, and X-ray crystallography. The equilibrium dissociation constant values for two of the dipeptide binding sites, Lys-Phe (60 nM) and Phe-Lys (86 nM), were of unprecedented affinity for a synthetic receptor at a non-terminal site. A high-resolution crystal structure of a related complex revealed that Q8 induces the peptide to fold into a type-II beta turn at the dipeptide binding site, which is the first reported evidence for sequence-selective beta-turn formation by a synthetic ligand. A high-resolution crystal structure of Q8 bound to the heptapeptide Gly-Gly-Leu-Tyr-Gly-Gly-Gly revealed the molecular basis of recognition to be inclusion of the Leu and Tyr side chains within the Q8 cavity and a network of hydrogen bonds between the peptide, Q8, and nearby water molecules. Unexpectedly, the crystal structure revealed that the peptide was folded into a type II β -turn upon binding to Q8.

The Lys-Phe dipeptide target site was moved to the N-terminus, and binding with Q8 was measured with an affinity of 0.3 nM, which is the strongest interaction reported between a peptide and synthetic ligand.[2] Crystallographic analysis revealed several features that may drive the 100- to 1000-fold sequence selectivity in this system. The development of applications of cucurbit[n]uril•peptide interactions to the directed assembly of peptides and proteins in one- and two-dimensions will be discussed.

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FNANO 2024

Tuesday April 23rd, Poster session Track on Integrated Chemical Systems Track Chair Jeremiah Gassensmith University of Texas at Dallas

Title

Carrier gas triggered controlled biolistic delivery of DNA and protein therapeutics from metal-organic frameworks.

Authors

Yalini H. Wijesundara, a Fabian C. Herbert, Orikeda **Trashi**, Ikeda Trashi, Olivia R. Brohlin, Sneha Kumari, Thomas Howlett, Candace E. Benjamin, Arezoo Shahrivarkevishahi, Shashini D. Diwakara, a Sachini D. Perera, Samuel A. Cornelius, Juan P. Vizuet, Kenneth J. Balkus, Jr, Ronald A. Smaldone, Nicole J. De Nisco and Jeremiah J. Gassensmith *

Abstract

Proteins, DNA, and RNA-based drugs are widely used in clinical settings due to their effectiveness and specificity. However, their administration via injection necessitates trained medical personnel and results in the generation of hazardous waste. In this study, we propose a method for the controlled delivery of these drugs, enabling either rapid or gradual release without altering their composition. By encapsulating them within zeolitic-imidazolate framework eight (ZIF-8), we demonstrate that these biomolecules remain stable in powdered form and can be introduced into living tissues of animals and plants using a cost-effective, gas-powered "MOF-Jet" device. Furthermore, we show that the release kinetics of these drugs can be adjusted by selecting appropriate carrier gases for the MOF-Jet. Through in vitro and in vivo experiments, we observe that the use of CO2 creates a temporary acidic environment, leading to immediate release of the drugs upon dissolution of ZIF-8, whereas the use of air results in gradual degradation of ZIF-8 and sustained release of the drugs over a week. This study represents the first instance of controlled delivery of biomolecules via biolistic methods using ZIF-8, offering a valuable tool for both basic and applied scientific research.

The Influence of Steric Hindrance in Nanotechnological Devices and Processes

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Molecular interactions are often influenced by the structure of participating molecules. Some molecules possess obstructive features that reduce their reactivity, a phenomenon known as steric hindrance. Although cited as having significant impact on nanoscale systems, steric hindrance is not well-studied. Here, we detail the effects of steric hindrance on DNA strand displacement, investigate its impact on CRISPR/Cas activity, as well as study its importance in coacervate solutions. Within strand displacement networks, steric hindrance studies helped elucidate the viscosity and geometrical factors on CRISPR/Cas activity and also informed how coacervate microenvironments may help control steric effects. The results indicate that steric hindrance has profound effects and should be more heavily considered in the design of nanoscale devices and processes.

Signal amplification of biosensors through DNA-scaffolded assembly

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Biosensors are devices that detect the presence of an analyte with a bioreceptor and transduce a measurable signal. They are used broadly in medical devices such as glucose monitors, rapid antigen tests and pregnancy tests, and for the detection of pollutants and pathogens in food, water, and the environment. Protein biosensors have been developed to target a large variety of molecules by using antibodies and ligand-binding proteins as bioreceptors. Colourimetric or electro-chemical enzymatic reactions may be used for signal transduction. Most protein biosensors consist of two protein components that form a complex upon ligand detection. While two-component protein biosensors are simpler to develop, there are several disadvantages to their design, including their limited utility at low concentrations and when immobilised on a surface.

Here we show the conversion of a two-component protein biosensor to a fully integrated single-component biosensor by assembling the protein components on a tunable DNA scaffold. We also show through numerical simulations that single-component biosensors are more sensitive than two-component biosensors due to signal amplification, without increasing the binding affinity of the proteins to their ligand. The biosensor consists of a beta-lactamase enzyme that is unfolded by an insertion of calmodulin, but refolds upon the binding of a ligand to receptors fused to a peptide and the unfolded enzyme. The proteins are attached to DNA through a SpyTag peptide-DNA conjugate. We demonstrate the synthesis, assembly, and function of the single-component biosensor, and compare its activity and sensitivity to the two-component system.

We model the expected enhancement gained from converting a two-component biosensor to single component, observing a linear amplification in signal at concentrations below the dissociation constant of protein binding in the absence of ligand, or the false positive signal. Single-component sensors display increased dynamic range and sensitivity to the ligand at lower concentrations, and the magnitude of change in these properties is dependent on linker properties. We anticipate that this work will inform the design of ultra-sensitive single-component biosensors, enabling accurate detection and quantification of molecules that are critical for environmental and human health.



Figure 1. (A) In a two-component biosensor, a signal is produced when the two components bind, mediated by an analyte ligand. (B) In a single-component biosensor, the two components are attached to each other. (C) A single-component sensor will have faster binding of the two protein components compared to a two-component sensor, increasing the true positive and false positive signal. (D) Our design of a generalisable, calmodulin-split beta-lactamase biosensor, assembled on a DNA scaffold. Upon ligand binding, the beta-lactamase is refolded and the nitrocefin substrate is hydrolysed to a coloured product. (E) Experimental data of the two-component sensor. An increased ligand concentration increases the rate of change in absorbance. (F) Numerical simulations demonstrate the increased signal of single-component sensors, which extends their dynamic range and increases their sensitivity to the ligand.

Leveraging DNA Intercalation to Tune Bulk Properties of Supramolecular Hydrogels

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Polymeric hydrogels cross-linked with non-covalent interactions offer advantageous properties (viscoelasticity, self-healing, and stimuli responsiveness) for various applications like drug delivery, tissue mimicking materials, and 3D printing.¹⁻⁴ The macroscopic properties (plateau modulus, G_{P} , intrinsic relaxation time, \mathbf{r}) of these hydrogels are determined by the thermodynamic and kinetic parameters (Kea, ka respectively) of the non-covalent interactions.^{3,5} However, the challenge lies in the susceptibility of these interactions to water, electrolytes, and biomacromolecules, limiting their use in complex aqueous and biological environments.³ To address this, we explored the use of dsDNA as a generic polymer cross-linked with bifunctional intercalators, avoiding the need for designing a complex supramolecular system de novo (Fig. 1A). We selected three intercalators and attached them to **PEG** ($M_W = 2k$) to form cross-linkers (Fig. 1A). In order to confirm intercalation, UV-Vis measurements were conducted using increasing concentrations of each intercalating cross-linker (0 - 40 mM). We observed a significant increase in fluorescence intensity until each cross-linker was saturated (Fig. **1B**). This corelates to roughly half the number of base pairs in a 5% wt/wt solution of salmon sperm DNA (This was before we had enough bioreactor DNA to make gels), which is known to be the point where further binding is unfavored.⁶ Interestingly, UV-Vis could be a means to directly observe the number of cross-links at any given time, allowing



Figure 1. A) cartoon depicting how intercalation can be used to cross-link two DNA strands and the identity of each intercalating cross-linker. B) Each **DISH** under ambient light and UV-Light. Increased fluorescence intensity arises from intercalation. C) Frequency sweeps showing a enhancement in shear modulus of each **DISH** vs a DNA control. D) Normalized stress-relaxation curves of each **DISH** compared to two controls E) Fit of each curve to the Kohlrausch equation. F) stress-relaxation plots of acridine at different temperatures. G) Stress-relaxation plots of psoralen at different temperatures.

unique study of the effects of different conditions on supramolecular cross-linking. While many supramolecular interactions exhibit a wide range of K_{eq} , the range of available k_d often remains quite limited (usually 1 - 2 orders of magnitude) and can vary wildly in different solvent conditions. Intercalators not only span an impressive range of K_{eq} (10⁻⁸) -10^{-2} M⁻¹) but also span a large range of k_d (10⁻³ – 10³ s⁻¹).⁷ First we must similarly prove that the disassociation of intercalating molecules will contribute to an observed r. Stressrelaxation experiments conducted at 37 °C show distinct relaxation behavior between the intercalating cross-linkers (**Fig. 1C - E**). These plots were normalized to G_{ρ} and then fit to the Kohlrausch-Williams-Watts function to determine \mathbf{r} and a fitting parameter ($\boldsymbol{\alpha}$) (Fig. **1E**). Each intercalating cross-linker gave unique values for τ , with **Acr-PEG** relaxing the slowest (126 s), and Pso-PEG relaxing the fastest (49 s). Each DNA intercalating supramolecular hydrogels (DISH) exhibited distinct mechanical properties and the disassociation rates of the intercalators were leveraged to tune the gel properties. Moreover, each **DISH** was stable and unchanged in 10% serum. These materials open the door to a new supramolecular system where material properties can be programed via strategic choice of intercalator.

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Tuesday April 23rd, Poster session Track on DNA Nanostructures: Semantomorphic Science C

Track Chair

Hao Yan

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Electrochemical Impedance Spectroscopy of a DNA Origami Hinge

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Obtaining kinetic information on dynamic DNA nanostructures, for which a limited number of orthogonal measurements exist, is non-trivial. The interpretation of these measurements can be improved with appropriate physical modeling. However, validating the physical models to provide robust information, whether to quantify ensembles of structures or remove outliers in super resolution measurements, require several independent and orthogonal measurements to help constrain the data.

Electrochemical measurements of DNA nanostructure dynamics are appealing as they can be rapidly acquired and have many tunable parameters. Electrochemical Impedance Spectroscopy (EIS) is one such technique for measuring binding and conformational changes in macromolecules. In EIS, the macromolecule or nanostructure is bound to a passivated electrode surface and is subjected to an AC field, while the resulting conductance across the electrode surface is measured. The transfer signal, which relates input and output signal is then fitted to an equivalent circuit model with circuit elements approximating the physical system.

EIS is a powerful approach in part because the choice of AC excitation frequency allow one to freeze out different sources of signal, while a DC offset allows the system to be perturbed as it is measured. Given this complexity, and the wide variety of possible equivalent circuit models, care is required to extract meaningful physical insights. Fortunately, the modularity of DNA origami allows for rational validation of the equivalent circuit model. We present our preliminary attempts to do so for an origami hinge.







DNA Origami Cage Assembled Inorganic Nanoparticles

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Plasmonic molecules are small assemblies of metal nanoparticles with definitive bond angles and gap sizes. Simulations predict plasmonic molecules support strong plasmonic coupling and have other interesting plasmonic effects, which make them attractive for numerous optical, sensing applications and investigative tools for fundamental plasmonic theories. Despite the promising simulation results, the syntheses of those plasmonic molecules are challenging because the current assembly approaches cannot precisely control the geometry of these assemblies, especially when the constituent nanoparticles have disparate sizes. Consequently, the plasmonic coupling within these assembled structures is often much lower than that predicted by simulations of ideal plasmonic molecules. To unleash the enormous potentials in plasmonic molecules, it is critical to organize different-sized nanoparticles with well-defined bond angles and gap sizes. Two new stepwise assembly approaches to form linear trimeric plasmonic molecules that consist of two large nanoparticles flanking a small nanoparticle, which can serve as plasmonic lenses concentrating intense electric fields in the interparticle gap. Both approaches use a DNA origami cage to encapsulate the DNA functionalized central particle. In the first approach, termed docking to DNA origami cage (D-DOC), the two DNA functionalized terminal nanoparticles bind to the openings of the cage via hybridization with capture strands. In the second approach, termed cageconstrained inter-particle hybridization (CCIPH), the terminal nanoparticles are connected to the central nanoparticle as their ligands hybridize with the ligands of the central nanoparticle exposed at the two cage openings. These two approaches have been used to align the centers of 10 nm, 30 nm, and 50 nm gold nanoparticles into plasmonic heterotrimeric molecules. Two symmetric timers and an asymmetric trimer are synthesized, and each assembly step is investigated. For all three trimers, structural analyzes are conducted by scanning electron microscopy (SEM) to assess the bond angles and gap distances. The plasmonic effects of two symmetric trimers are evaluated by UV-Vis absorption spectroscopy and Raman spectroscopy. In accordance with experimental data, extensive finite-difference time-domain (FDTD) simulations are performed. The bond angles and gap sizes of our assembled plasmonic molecules are precisely defined, and one of our model trimers shows strong plasmonic coupling and expected surface enhanced Raman scattering enhancement factor. The optimizations and future directions of these two assembly approaches are discussed.



Figure 1. Two-step DNA origami cage gold nanoparticle assembled trimer schematic.



Figure2. SEM images of DNA origami cage encapsulated 10nm gold nanoparticle flanked by 30nm gold nanoparticles. All scale bars are 100 nm.



Figure3. SEM images of DNA origami cage encapsulated 10nm gold nanoparticle flanked by 50nm gold nanoparticles. All scale bars are 100nm.

Fully addressable, designer superstructures assembled from a single modular DNA origami

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Intricate self-organization is essential in many biological processes, underpinning vital functions and interactions. In an effort to mimic such processes, synthetic biology aims to engineer dynamic structures with controllable functions using nanotechnological tools. A key requirement of engineered building blocks is the ability to assemble and disassemble superstructures with precision. Making use of the highly controllable nature of DNA selfassembly, we here present the moDON¹, a modular DNA origami nanostructure, which is capable of assembling into 18,225 distinct monomers, forming complex and controlled superstructures with connections in three dimensions. While shape and addressability of DNA origami are nearly arbitrary, its overall size is limited by the length of the scaffold. Previous methods of extending the size of DNA origami (e.g. hierarchical assembly², modified scaffolds³, etc.), either led to loss over control of shape and addressability beyond a single monomer or led to proportionally increased cost and design effort if a plethora of distinct monomers had to be formed. With the moDON we are able to overcome both issues. The modular design combines xy- and z-plane assembly methods, enabling the one-step assembly of finite, fully addressable structures of > 40 MDa and periodic structures reaching several µm in size and > 1 GDa mol. Weight (see Fig 1). We demonstrate complete xy-z orthogonality, by enabling controlled selective or parallel assembly and disassembly using distinct orthogonal triggers. While xy assembly and disassembly are triggered by an increase or decrease in MgCl₂ concentration, assembly and disassembly in the z direction are triggered by the presence of linker or invader strands. Interestingly, the kinetic profile of assembly and disassembly aligns with biological time scales, paving the way for applications in dynamic nanomachinery and advanced biomaterials. Finally, we showcase the conjugation of gold nanoparticles to specific positions within superstructures, underscoring the efficacy of this approach for creating intricate and orthogonal nanoscale architectures with preserved site-specific addressability. The moDON thus offers an efficient, cost-effective solution for constructing large, precisely organized, and fully addressable structures with vast potential in synthetic cellular systems design and beyond.



manifold of fully addressable superstructures

Figure 1: Modular approach to DNA origami superstructure design (a) The moDON as a fundamental building block is just one single DNA origami, with modularity incorporated in two different ways in the design: **(b)** in xy-direction modularity is introduced by alternative scaffold routing of the outer, connective sides of the moDON. **(c)** Connectivity in z-direction is introduced by a set of orthogonal three-strand-systems. Combined, both modes of modularity allow to form 18,225 moDON monomers with unique connectivity combinations. **(d)** By defining the exact connectivity of each monomeric building block, superstructures of precise form and size are created. Since each of the moDON monomers in each superstructure has a unique connectivity, it has a unique position in the superstructure. Thus, each part of the superstructure fully addressable, just as in a single DNA origami. Scale bars are 50 nm.

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oxDNA Simulations of Nicked Minicircles

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DNA minicircles are useful systems for the study of DNA's physical properties under high degrees of curvature and twist. These kinds of mechanical stress are common in cells and viruses; for instance, most eukaryotic DNA is wrapped around histone octamers at a radius of ~4.5 nm, just 10% of its persistence length. It is common textbook knowledge that the helical repeat of DNA is ~10.45 base pairs/turn and independent of curvature, but experimentally we found strong twist-bend coupling, or that DNA unwinds when tightly bent (1).

As part of this project, we simulated nicked DNA minicircles with varying twist using oxDNA. The preferred helical repeat was determined primarily from the base pair counts of energetically stable, topologically closed minicircles. We developed several new pieces of analysis software to measure kinking, i.e. sharp bending, as well as broken stacking, fraying, and twisting. We used some previously published methods and designed new criteria for kink detection which are more precise and generalizable. This oxDNA study revealed no significant change in the helical repeat as a function of curvature for circles larger than 80 base pairs.



(A, B) Representative oxDNA simulation snapshots of an 89 bp and 95 bp minicircle. **(C)** Plot of the fraction of unkinked, or "stacked", configurations found in each simulation trajectory as a function of number of base pairs. Minicircles with higher stacked fractions are under less torsional stress and considered more stable.

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Metal-Mediated Molecular Electronics in DNA: A Use Case for Semantomorphic Crystals

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DNA as a vehicle for molecular programming relies on the predictability and information storage capability of the canonical Watson-Crick base pairs, A:T and G:C. With only four Watson-Crick nucleobases and the insulating behavior of natural DNA, it has become clear that there are strong limitations on DNA-based electronic systems. To address this limitation, we present an expanded DNA alphabet based on metal-mediated DNA (mmDNA) base pairing in which we substitute hydrogen bonds between pyrimidine nucleobases with templated metal ion coordination.

Here we use a semantomorphic 3D DNA crystal system as a diffraction platform to determine the biomolecular structures of metal ion (Ag^+ , Hg^{2+} , Au^+ , and Cd^{2+})-mediated DNA (mmDNA) base pairs; and we further use this comprehensive structural library to elucidate fundamental design rules for an expanded DNA metal coding system [1]. We demonstrate the effectiveness of using self-assembled crystals for the rapid screening of metal base pairs, with over 100 published mmDNA structures and many hundreds of failed combinations to inform rational design.

Transport calculations further elucidate an electronic fingerprint of the base pair classes in library. Energy gap calculations showed added LUMO states in mmDNA, rendering them attractive molecular electronic candidates. We further manipulate our self-assembled DNA crystals using pH to capture a series of reversible, pH-driven chemical reactions [2]. Importantly, we identify titration points at which mmDNA pairs exist in a heterobimetallic state, where a single DNA base pair binds to both Ag^+ and Hg^{2+} in tandem. Our self-assembly system was further able to capture the intermediates in pH-driven transmetallation, and modeling identifies the conductance modulation that accompanies these reactions. The precision self-assembly of bioinorganic DNA chemistry at the sub-nanometer scale will drive atomistic design frameworks for nanodevices and nanotechnologies based on semantomorphic architectures.



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Self-assembled cell-scale containers made from DNA origami membranes

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The construction of large, closed superstructures from DNA origami subunits represents a major milestone in DNA nanotechnology, requiring innovative self-assembly strategies beyond those traditionally employed for DNA origami. Current methods [1, 2, 3] face challenges including escalating complexity, cost, and development time with increasing superstructure dimensions, often accompanied by low yields of the desired structures.

Here, we introduce radially symmetric DNA origami subunits inspired by the structure and interactions of lipids, termed 'Dipids'. A single Dipid variant can self-assemble into giant DNA origami monolayer membranes, which can be programmed to form closed containers or hollow tubes, with diameters ranging from 100 nm to over 1 μ m.



Figure 1: TEM micrographs of containers formed by XS, L, XL, and XXL Dipid variants. Container diameters were measured along the dotted lines shown in each image and back-calculated to their 3D diameters. Mean experimental diameters of XS, S, and M container designs are shown on the axis below, next to selected biological (CBS: Carboxysome) and state-of-the-art synthetic systems. Scale bars: 1 μ m.

The base monomer for all assemblies is derived from a DNA origami barrel design by Wickham et al. [4]. We engineered 30 ssDNA binding strands distributed symmetrically on the barrel's outer surface to mimic the isotropic and flexible monomer interactions, and the preferred in-plane assembly, characteristic for lipid molecules. Local curvature is introduced through the computationally guided, asymmetric extension of the ssDNA binding strands. We experimentally tested a subset of 6 container and 6 tube-forming Dipid designs

with diverse assembly curvature. Using TEM and confocal microscopy, we found that all designs work as intended, assembling into the designed structures.

The Dipid framework enables rapid and economic development of DNA origami superstructures, where the geometry and diameter are programmable by adjusting only up to 24 staples for containers and 8 for tubes. Such DNA origami membranes present an unprecedented approach for compartmentalization that opens up new possibilities for bottom-up biology and cell-scale soft robotics.

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Rational Design and Engineering of Complex 3D DNA Tensegrity Triangle Variants

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The predictable conformation and reliable programmability of DNA has made it an attractive material for bottom-up nanoscale construction. This fact led to Ned Seeman's proposal of the field of Structural DNA Nanotechnology as a means of organizing matter in two- and three-dimensions using Watson-Crick-Franklin base parity and immobile Holliday junctions. The full realization of this proposal was the development of the DNA tensegrity triangle – a robust motif that self-assembles into macroscopic three-dimensional crystals of diffraction guality.¹ The original design of this nanostructure reliably self-assembled into rhombohedral (R3) unit cells of various sizes. Herein, we demonstrate significant advances in DNA tensegrity triangle modification and supramolecular self-assembly, crystal engineering, and free energy tuning for polymorphic and polycrystalline crystallization. Firstly, we introduce sequence and geometric asymmetries that allow for fine, incremental control over unit cell edges and angles, and the programmable augmentation of crystalline parameters such as cavity and channel size.² The design principles of algorithmic self-assembly in these 3D DNA tiles has also been systematically described, categorized, and exploited to form lattices of hexagonal (P6₃), cubic (P4₁32), and trigonal (P3₂21) space groups.^{3,4} Finally, we introduce incremental rotational steps between pairs of immobile junctions within the tensegrity triangle to study the control and programmability of DNA tertiary chirality.^{5,6} These recent advances allow for increased customization capability when designing periodic DNA lattices and allow for a greater variety in the organization and orientation of guest matter in three-dimensions – the foundational goal of Structural DNA Nanotechnology.

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Switchback DNA: A tale of two duplexes

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Switchback DNA is a synthetic motif in DNA nanotechnology in which two DNA strands fold back after every half-turn to form a left-handed parallel double helix. Although the Xray structure of switchback DNA was reported recently, the effect of the unique structural features on its properties is still unexplored. Here, we compare the biochemical and biophysical properties of switchback DNA and conventional double-stranded DNA duplex. Our results show that while switchback DNA is stable at room temperature at high divalent ion concentrations, its thermodynamic stability is lesser than that of conventional duplex. Strand competition and strand displacement experiments reveal that DNA strands show an absolute preference for their duplex complements rather than switchback complements. While switchback DNA is more stable against DNase I, it is more vulnerable to some other nucleases than conventional duplex. Compared to conventional duplex, fluorescent intercalators and minor groove binders showed lower fluorescence emission when bound to switchback DNA. A comparative evaluation of the immunogenic properties of the two structures in HeLa cells revealed that switchback DNA induced less CXCL-8 expression than conventional duplex while showing similar cell viability. Further, we hypothesize that switchback DNA could be a potential alternate structure adopted by short-tandem repeats involved in repeat-expansion diseases. These results shed light on the unexplored biophysical and biochemical properties of this unique DNA structure. which may help integrate it in the design of novel nucleic acid nanostructures for various applications.

FNANO 2024

Tuesday April 23rd, Afternoon Track on DNA Nanostructures: Semantomorphic Science II

Track Chair

Hao Yan

Arizona State University

Bending Unwinds DNA

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Exactly 30 years ago, Marko and Siggia postulated twist-bend coupling (TBC) of DNA in a theoretical paper (1). They predicted that due to its groove asymmetry, DNA should unwind when it is bent. This challenges the standard mechanical model of DNA and decades-old textbook knowledge that the helical repeat of relaxed DNA h_0 is 10.4-10.5 bp/turn and independent of curvature. Surprisingly, experimentalists have failed to prove

or disprove this extremely consequential prediction so far. We developed an assay with nicked DNA minicircles of variable length **(A-C)** and demonstrate that the helical repeat h_0 of tightly bent DNA indeed unwinds to over 11 bp/turn, exactly as predicted (**D**, blue curve). We furthermore characterized thermodynamic stability and kinetics of nicked circles by coarse-grained and all-atom molecular dynamics simulations, as well as theoretical models (2).

Our discovery requires reassessing the molecular mechanisms and energetics of all biological processes where DNA is tightly bent or relaxed again, including DNA packaging, gene regulation and



A nicked minicircle in a stacked conformation (A) is in equilibrium with a kinked (B) and a kinked and twisted conformation (C). (D) Relaxed helical repeat h_0 of circles of N_{bp} base pairs (bottom axis). Blue: experimental datapoints from this study, black: classical circularization experiments. Blue fit according to twistbend-coupling theory by Marko and Siggia (1).

gene expression. The widening of the helical repat also needs to be considered in the design of DNA nanostructures that include tightly bent DNA including many DNA origami structures, catenanes and rotaxanes. Finally, physical models such as the twisted worm-like chain model and computational models including oxDNA need to be corrected.

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Thermodynamic optimization of staple routing improves DNA origami folding accuracy

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Fundamental advances in our understanding of molecular design principles have the potential to transform all subsequent work in a field, especially when coupled with computational tools that facilitate access to those principles. A prime example is Alphafold2, a protein structure prediction tool, which illustrates the impact of combining molecular-modeling innovations with user-friendly software. We are excited to report a comparable leap in DNA origami design: a breakthrough in design principles, translated into a practical and freely accessible software tool.

DNA origami is a powerful method for nanoscale fabrication. Researchers design a structure by routing sequences of DNA scaffold and staple strands to fold into a desired shape. This process, akin to protein design, presents a vast solution space. Given the inherent time and cost constraints of experimental validation, it is critical to select the most promising design variants for synthesis, as only a limited number can be feasibly evaluated. This bottleneck has left us with a limited understanding of how design choices impact folding. While some heuristics have emerged, they must be applied by hand and have lacked rigorous exploration in the absence of a quantitative framework. Consequently, a persistent question for all complex structures designed over the past 15 years has been: What design variant will fold most accurately?

Here, we introduce a comprehensive solution to this question for any structure. We established a quantitative framework to encode and evaluate design rules, allowing us to systematically determine the key drivers of folding accuracy. We translated these factors into a thermodynamic model that can be used to score any staple route and an algorithm that leverages the model to optimize a full design. To validate, we recreated multiple challenging structures and observed unprecedented improvements in folding accuracy. Our approach has surpassed 100% of manual designs tested, even those extensively revised by trial and error.

In an effort to elevate the new design principles from theory to practice, we encapsulated our methodology in a computational notebook that can be used to optimize any design within minutes.

DNA origami self-assembly with complex curved surfaces defined in 3D space

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Curved structures found in both artificial constructions and natural biomolecular assemblies enable the gain of sophisticated properties and adaptability. They provide a source of inspiration for the DNA de novo design of innovative biomimetic architectures, materials, and devices with enhanced functionalities. However, the DNA nanotechnology field encounters challenges in achieving complex curved structures in 3D space. While already existing methodologies enable the design and production of DNA origami with curvatures [1][2][3][4], the inherent constraints imposed by the DNA geometry pose significant limitations in achieving high yields of well-defined structures with precise curvatures and complexities.

In response to these challenges, we present a general method to fold arbitrary 3D curved structures using a routing algorithm that traces scaffold strands along curved surfaces. Crossover positioning is made by the user using the 3D view panel, directly from the intended geometry of the desired shape. Our new method has been implemented in our design software ENSNano which offers a highly automated workflow for designing complex curved DNA nanostructures. We applied our procedure to design ten origami structures with unprecedentedly complex curvatures defined in 3D space. The accuracy of the programmed and folded objects has been validated through cryo-electron microscopy.

We believe that the sleek and user-friendly geometric interface of ENSnano will catalyze the design of biomimetic nanostructures with the potential to expand the range of achievable shapes in DNA nanotechnology.



Fig. 1: Our new method enabling the design of three-dimensional curved-DNA origami. A: The four steps process; B: Structures designed and produced with this method

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Wednesday April 24th, Morning Track on Nanophotonics and Superresolution

Track Chair

Ralf Jungmann

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DNA-assembly for photonics, plasmonics and biosensing

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Over the last decades, DNA self-assembly in general and DNA origami in particular have matured at a breathtaking pace and DNA architectures are today routinely used for the constructing of functional two- and three-dimensional nanomachines and materials [1,2]. Our group has contributed complex and nanometer-precise assemblies of biomolecules, organic fluorophores and inorganic nanoparticles [3].

We now demonstrated diamond-type DNA origami lattices exhibiting structural color. For this we first grew single crystals with overall dimensions of 10 - 20 micrometers. This step was followed by sol-gel type silicification [4] and subsequent atomic layer deposition (ALD) of several nanometers of TiO_2 to ensure a high enough refractive index contrast to open a photonic band gap, which could then be observed in the reflection spectrum of the crystals [5]. We are currently exploring further properties of this unique type of material.

Another interest of our group is to employ gold and silver nanoparticles for plasmonic sensing by making use of circular dichroism, absorbance and scattering properties of such versatile and nanoscopic reporters [5].

In this talk I will report on our ongoing efforts to build functional plasmonic devices on the one hand and materials that are designed on the molecular level while reaching macroscopic dimensions on the other.

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Building a DNA-based platform for studying the interactions between plasmonic nanoparticles and fluorescent nanodiamonds

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The presence of plasmonic nanoparticles can influence optical response of near-by molecules¹ and spatial signal detection in super-resolution microscopy². Unravelling this phenomenon could help in the conceptualization and design of nanoscale magnifiers or improved single molecule sensors. To probe this so-called single molecule mirage, we aim to construct a platform to precisely address different parameters influencing the observed phenomena and will help understand them.

We employ DNA origami structures to assemble different metallic nanoparticles with fluorescent nanodiamonds. The nitrogen-vacancy center-containing nanodiamonds exhibit photoluminescence properties that distinguishes them vastly from classic organic fluorophores³ make them excellent candidates for studying the described phenomenon. For example, photobleaching can be almost completely neglected and more importantly, the Stoke's Shift of roughly 100 nm covering the spectral range of the plasmonic resonance of gold nanoparticles from 10 to 100 nm makes it possible to separately address absorption and emission of these solid-state fluorophores.

Here, we present a novel DNA-surface modified fluorescent nanodiamonds enabling binding to DNA nanostructures, together with gold nanoparticles of different sizes. We use the 12-helix bundle, a rod-like origami structure, to create heterogeneous particle assemblies with modulated distances to each other to probe the influence of the gold nanoparticles on the nanodiamond emission. The fluorescent nanodiamonds are functionalized via copper-free click reaction, forming a dense coating of oligonucleotides on their surface with around 200 strands per particle. Gold nanoparticles are functionalized via monothiolated oligonucleotides as shown before. To achieve binding of

the two different particles, the influence of different parameters on the assembly has been investigated, e.g. mixing ratios, annealing protocols, and purification techniques. The final assemblies have been investigated via AFM and TEM to evaluate assembly yield, structural integrity, and distance between particles. The optical properties of the fluorescent nanodiamonds have been probed by Fluorescence Lifetime Imaging and spectroscopy, showing a photoluminescence lifetime modulation in the presence of gold nanoparticles but no alteration of spectral properties.

Future endeavors will focus on larger gold nanoparticles and super-resolution imaging of the fabricated structures. Further, we believe that our approach has great potential in diamond color center research and applications such as spintronics and magnetometry.

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Figure 1: (a) shows the proposed principle of light modulation. A projection of the emitted light is expected when the PL emission band falls within the plasmon resonance of the AuNP. In case of overlap with the PL absorbtion band, no such projection is expected. (b) highlights the assembly pipeline from origami synthesis to full structure fabrication with (c) showing the different means of particle binding to the origami substrate. In (d) the different aspects of assembly analysis are shown with the respective information extracted from each technique.
Energy transfer process controlled by DNA

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Light harvesting complexes (LHC) are highly structured to convert efficiently absorbed light into chemical energy. So far, only DNA nanotechnology provides the necessary stoichiometric and positional control for placing multiple fluorescent dyes on an artificial structure. We harness this power of DNA nanotechnology in two ways:

First, we develop multichromophoric DNA origami structures to mimic the excited state processes in LHCs and OLEDs. To this end, the photon stream contains valuable information of the dyes nearfield environment that we extract in new ways.

Second, we use the finding, that dsDNA with an ssDNA tail stands vertically on graphene. In combination with a fluorescent dye, graphene acts as a two dimensional FRET acceptor up to a dye-graphene distance of 40 nm. With the axial precision from graphene energy transfer, we can either monitor precisely protein induced DNA bending or perform 3D super-resolution imaging with DNA-PAINT with an axial localization precision down to 0.3 nm.

Programmable DNA-based Super-Resolution Microscopy for Spatial and Single-Molecule Proteomics

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Recent advances in single-cell sequencing and spatial transcriptomics have broadly transformed biological research and biomedicine. While transcriptomic profiling is powerful in identifying cell types and mapping complex tissue microenvironment, proteins reflect functional and dynamic state of the cell, and are the targets of most therapeutics. Comprehensive proteomic characterization in the cell requires not only accurate abundance measurement, but also their sub-cellular localization, local "molecular microenvironment", as well as their isoform and post-translational modification state. Current spatial proteomics profiling methods fell short in both imaging resolution and multiplexing capacity to faithfully report complex molecular architectures. In terms of single-molecule protein analysis, an effective method for accurate identification, sequencing and proteoform analysis on the single-molecule level has yet to be established.

DNA-PAINT super-resolution microscopy uses short DNA oligo binding to achieve Single-Molecule Localization Microscopy (SMLM), and provides unique advantages of repeated probe binding, as well as programmable sequence and blinking kinetics. In particular, DNA-PAINT achieves unprecedented spatial imaging resolution (5 nm or lower), high molecular sensitivity and coverage (>90%) in macromolecular complexes, and the programmable probe design makes it ideally suitable for highly multiplexed imaging. In addition, using a UV-activatable probe combined with real-time image analysis allows active molecular "writing" in complex cellular environment, with equal super-resolution precision (Action-PAINT). Our lab takes these unique advantages of DNA-PAINT and develops new imaging-based methods to tackle current limitations in spatial and single-molecule proteomics.

Single molecule localization microscopy imaging with fluorescent gold nanoclusters.

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Gold nanoclusters smaller than 2 nm containing few to hundreds of gold atoms exhibit fluorescence due to the presence of discrete energy levels at this small size¹. Taking advantage of the ease in surface modification of gold nanoclusters, the presence of large stokes shift and tuneable fluorescent emission across the visible region to the near infrared region, we aim to apply this material as fluorescent probes for single molecule localization microscopy, as well as, to provide better understanding of spectral enhancement and shift in projected fluorescent position that occurs during plasmonic coupling with the excitation and/or emission spectrum of fluorescent materials.

Using a bottom-up synthesis approach in the presence of auric chloride, lipoic acid, sodium borohydride, and polyethylene glycol, a highly stable gold nanoclusters was synthesized. Activation of the carboxyl group on lipoic acid was done using carbodiimide reagent before conjugation with 4-lodobenzyl amine.

Transmission electron microscope showed that the average size of the synthesized gold nanoclusters was 1.67 nm. The emission intensity of the gold nanocluster conjugated with 4-iodobenzyl amine was five orders of magnitude brighter than gold nanoclusters without 4-iodobenzyl amine as confirmed by fluorescence spectroscopy. Conjugation of resulting gold nanoclusters to thiolated-oligonucleotide is achieved through thiol – gold affinity. We accomplished the attachment of gold nanoclusters onto a 12-helix bundle DNA Origami structure, a novel achievement in this study, this was also utilized to confirm the presence of gold nanocluster-oligonucleotide conjugates.

Fluorescent gold nanoclusters having emission in the far-red region was synthesized from auric chloride as precursors, with the emission intensity boosted by conjugation with an electron-rich ligand. Successful conjugation of synthesized gold nanoclusters with oligonucleotide was achieved, although optimization of the conjugation process is still in progress. Future experiment will involve application of gold nanoclusters for bioimaging using the DNA-PAINT technique, also, the presence of well separated excitation and emission spectrum will allow us to provide better understanding of the plasmonic coupling phenomenon observed between gold nanoparticles and fluorescent materials.



Figure 1. Fluorescent maps of gold nanocluster (a) and gold nanoclusters conjugated with electron-rich ligand (b). The presence of electron-rich ligand is associated with the boosting of fluorescence emission intensity as observed in (b). Figure 2. Oligonucleotide conjugated gold nanoclusters are immobilized onto the 3 attachment sites of the 12-helix bundle DNA Origami having complimentary oligonucleotide elongations. The presence of gold nanoclusters on the 12-helix bundles DNA Origami (a and b) indicates success in functionalization of gold nanoclusters with oligonucleotide. Scale bar = 50 nm.

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Wednesday April 24th, Poster session Track on Nanophotonics and Superresolution

Track Chair

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Variable-Length Chemical Linkers as a Tool for Controlling Relative Dye Orientation on DNA Scaffolds

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The purposeful control of electronic coupling between dye molecules is vital for controlling the transport of excitonic energy within a multi-dye aggregate for applications in synthetic light harvesting, sensing, and quantum information science. Achieving such control requires the ability to position and orient dye molecules with high precision. DNA nanotechnology provides versatile scaffolds that can organize dye molecules into programmable 3D arrangements with spatial precision of up to about four Angstroms. However, control of dye orientation on DNA has proven to be more challenging. Several groups have exploited the hydrophobic interactions between dye molecules and the DNA scaffold,¹⁻³ and between the dyes themselves,⁴ to characterize the degree of dye orientation on DNA, though there have been relatively few demonstrations of tuning dye orientation between specific configurations³. In this presentation we show that the length of the chemical linker that attaches a Cy5 dye to DNA can tune the relative orientation of Cy5 aggregates on dsDNA and DNA Holliday junctions (HJ). In the case of Cy5 dimers we hypothesize that short aliphatic dye linkers will restrict the ability of pairs of hydrophobic Cy5 dyes to maximize the overlap of their π -electron systems, thus favoring the formation of J-like dimers (near end-to end dye orientation). In contrast, relatively long linkers have greater flexibility to allow hydrophobic Cy5 dyes to maximize overlap of their π -electron systems, favoring formation of H-like dimers (near co-facial dye orientation). To test this hypothesis, we assemble Cy5 dimers and tetramers (Fig. 1a) on DNA while systematically varying the number of carbon atoms (nC; n = 2,3,4) of the two-point phosphodiester linker. In one key result, our steady state spectroscopy (Fig. 1b) shows that short 2C linkers produce red-shifted optical spectra from the monomer, suggestive of J-like dimer orientations, while 3C and 4C linkers produce optical spectra with an increasing blue shift from the monomer, suggesting dimers with increased H-like character. In a second key result, our photo-selective transient absorption (TA) spectroscopy (Fig. 1c) demonstrates that the short 2C linkers produce structurally homogeneous J-like dimers on HJs, indicating that using short linkers can be an effective strategy for suppressing heterogeneous mixtures (i.e., sub-populations of J-like and Hlike configurations) that are sometimes found in DNA-templated cyanine dye aggregates.⁵ In the case of Cy5 tetramers on HJs we will present optical spectra that suggest the tetramer acquires increasing H-like character as the linker length increases. For deeper physical insight, we will present molecular dynamics simulations of Cy5-nC dimers on dsDNA for the series n = 1-6 and use them to help understand the experimental results. We will also discuss how our findings might impact the future construction of strongly coupled dye aggregates on DNA scaffolds for the transport of excitonic energy.

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Fig. 1. a) Chemical structure of Cy5 internally attached to DNA using variable-length linkers (left) and schematics of four possible adjacent Cy5 dimers on a DNA HJ, a Cy5 tetramer on a DNA HJ, and a Cy5 dimer on dsDNA (right). b) Absorbance and CD spectra of Cy5-nC adjacent BC dimers on a DNA HJ (left) and a Cy5 dimer on dsDNA (right). The dashed line is the reference absorbance spectrum for a Cy5-2C monomer on each scaffold. C) Photo-selective TA spectra for Cy5-nC adjacent BC dimers on a DNA HJ at 1 picosecond after excitation with a 100 femtosecond laser pulse. Top: Cy5-2C. Middle: Cy5-3C. Bottom: Cy5-4C. Insets: The pump wavelength is color matched with TA spectrum. The blue curves are difference TA spectra generated by subtracting the TA spectrum from the 662 nm pump from the TA spectrum from the 606 nm pump.

Signatures of Exciton Relaxation Dynamics in DNA-Assembled Molecular Dimers Measured with Two-Dimensional Electronic Spectroscopy

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DNA templating is a versatile method of assembling molecular dye aggregates such that their excited states form delocalized excitons. The relaxation dynamics of these excitonic states are of intrinsic relevance to studies of natural light-harvesting as well as applications including devices for quantum-information processing. A selective technique to measure these relaxation dynamics is two-dimensional electronic spectroscopy (2D ES), in which we have determined that the contrasting selection rules of H- and J-type molecular dimers yield diagnostic peak locations and dynamics that have not previously been reported. In this study, we develop the theory for third-order nonlinear spectroscopy signals using a purely electronic model of a molecular dimer, which predicts a distinct spectral shift arising from exciton relaxation. Using DNA tethering and self-assembly, we construct aggregates of cyanine molecules and perform 2D ES measurements to support the signatures predicted by the theoretical model. The results lay the foundation for future studies that will include the complicating effects of vibronic states and additional electronic levels, which will isolate and quantify the dynamics of exciton relaxation in DNA-assembled aggregate nanostructures.

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Probing homogeneous and inhomogeneous broadening in DNA-templated cyanine heteroaggregates using two-dimensional electronic spectroscopy

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DNA has broad applications in the field of nanotechnology due to its self-assembly, simple design rules, re-configurable nature, and ease of functionalization with light absorbing molecules (dyes). For example, DNA Holliday junctions are an excellent scaffold for dye aggregates that host delocalized excitons. However, the interactions between such excitons and DNA are not broadly understood. Here, we use femtosecond two-dimensional electronic spectroscopy (2D ES) to study line broadening in the 2D spectra, which informs on the delocalization of the exciton state, of a series of cyanine-based heterotetramers composed of varying quantities of Cy5 and Cy5.5, as well as the constituent monomers tethered to various DNA configurations. A lineshape analysis of the heterotetramer 2D spectra reveals that the contribution to exciton decoherence induced by inhomogeneity increases with increasing Cy5.5 content, which we attribute to multiple conformations in dye packing caused by additional steric hinderance between dyes. In the monomer 2D spectra, we observe signatures of DNA motion on the fs-ps time scale impacting the homogeneous broadening of the monomer excitons. Our results provide insight into the line broadening in DNA-assembled aggregates, and provide a framework for future 2D ES studies on the impacts of aggregate composition and the DNA environment on exciton delocalization.

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Towards Sequence Parameterized Simulations of DNA-PAINT

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DNA-PAINT is a powerful and flexible implementation of Stochastic Reconstruction Microscopy (STORM), a super resolution technique that enables researchers to produce images with subresolution accuracy^{1,2}. In its most rudimentary implementation, this imaging system requires two DNA strands: a fluorophore containing imager strand and a docking strand which is anchored to a substrate of interest and is complimentary to the imager strand. The strands are designed in such a manner that they spontaneously hybridize and dehybridize. In the seminal DNA-PAINT publication, it was demonstrated that the rate of detected localizations is directly related to the concentration of the imager strand and independent of the length of the hybridization³. These rates of localizations in turn determine the 'on-time' of a localization which is an important parameter to control in order to avoid overlaps.

Currently, Picasso is the primary DNA-PAINT simulator that allows one to input custom kinetic parameters such as k_{on} and dark time². While important parameters to be sure, we hypothesize that these parameters can be computed from the sequences that are to be used as the imager and the docking strands when the problem is articulated in a statistical mechanical framework: What is the probability of observing the micro-state in which the imager and docking strands are hybridized?

The Boltzmann distribution is a powerful tool when computing macroscale thermodynamic parameters of chemical systems from its molecular components. Certain formulations of the distribution use three parameters: the number of lattice sites and ligands denoted as Ω and L respectively, and the free energy of a microstate ΔG^4 . The ΔG of hybridization can be computed using the NUPACK software, while Ω and L can be set by the user^{5–7}. In systems such as DNA-PAINT, $\Omega >> L$ as the concentration of the imager strand is dilute. The Boltzmann distribution parameterized by these three parameters can output a probability that in turn parameterizes a Monte Carlo model that simulates an observed localization of the imager strand.

Our initial simulations using this sequence informed framework demonstrate that the frequency of localizations and consecutive localizations, indicated by a broad peak in the time-intensity trace diagram, is directly proportional to L when the sequences are complimentary to one another. This is consistent with expected experimental results as STORM necessitates a trace amount of the fluorescent molecule to promote sparse localizations to prevent overlap of adjacent signals.

This work is supported through NSF award # MCB 2027738 and NIH award # 1R16GM145671.

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Decoupling sphere diameter and binding site size in microsphere/nanosphere lithography

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DNA Origami constructs, or tiles, provide excellent breadboards to support single molecule and molecular scale experimentation inaccessible via any other approach due to the difficulties associated with manipulating soft matter. Due to the small dimensions of 2D tiles, on the order of 100nm in diameter, the dominant method for placing individual tiles into arrays has been electron beam lithography, a method which is inaccessible to many researchers. Recently a much more accessible method, based on nanosphere lithography utilizing polystyrene spheres, has been employed with great success (1) to produce arrays of origami platforms suitable for research.

A potential impediment to the generalization of this method of single molecule positioning, which has broad utility for many applications, including for the implementation of digital diagnostic arrays is that the same "soft" mechanical properties of polymer spheres which are central to enabling this lithographic approach also determine that the binding site diameter increases linearly with the particle diameter. It would be useful to be able to independently adjust the spacing and the binding site diameter, rather than adapting to the reported fixed binding site size/particle diameter ratio of 0.27, which severely limits the separations practically achievable between single molecule origami platforms and presents a barrier to the application of non-super-resolution widefield optical methods.

In order to address this barrier this lab has performed a series of Oxygen plasma etching experiments to reduce the diameter of the polystyrene spheres, and therefore the diameter of the binding sites. The observed etching is very sensitive to local environment and order within the hexagonal arrays. Decreasing the diameter by a factor of two does not result in a factor of two reduction in the binding site diameter. This is presumably due to a combination of anisotropic etching by the plasma and other aspects related to the polymeric composition of the spheres.

Experiments are in progress to further extend the separation of binding sites in order to relax imaging system requirements and further enable the implementation of digital analytical chemistry through use of nanosphere/microsphere lithography approaches.

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Wednesday April 24th, Poster session Track on DNA Nanosystems: Programmed Function B

Track Chair **Friedrich Simmel** Technical University, Munich Programmable capture of diverse biomolecules on a DNA-functionalized polymer

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The ability to isolate specific molecular targets from complex biological samples is crucial for many applications such as diagnostics, genetic testing, and personalized medicine. A variety of commercial methods exist for this purpose; however, they are often tailored to one specific biomolecule with restrictive sample requirements, a short shelf life, and high cost. In contrast, synthetic polymers offer a stable, inexpensive base material for the capture of diverse biomolecules in homogeneous solution. By blending the principles of DNA nanotechnology with traditional polymer chemistry, we can further engineer a programmable nanomaterial whose structural and functional properties can be dictated through DNA sequences.¹

Herein, we report a simple, cost-effective, and generalized method for the capture of target biomolecules based on a DNA-functionalized smart polymer.² Our system programmably binds either DNA, RNA, or proteins through the addition of target-specific catcher strands (Figure 1A). Gentle purification is achieved through phase separation triggered by a unique polymer crosslinking mechanism³ (Figure 1B). To highlight its broad utility towards biomedical applications, we demonstrate selective capture of several highly relevant biomolecular targets, including DNA oligonucleotides, SARS-CoV-2 RNA, human ribosomal RNA, and human thrombin. Target molecules are sequestered with high specificity and >80% efficiency (Figure 1C).

Due to the programmable nature of our system, it can be easily modified to capture a wide variety of biomolecules. Notably, capture occurs under physiological conditions, hence chemically sensitive molecules can be targeted. Furthermore, it does not rely on expensive enzymes or magnetic beads, yielding a cost of <\$0.05 per sample. All together, we anticipate that this method will simplify preparative workflows in diverse applications including biosensing, next-generation sequencing, and transcriptomics.

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Figure 1. (A) Schematic of the polymer system for the programmable capture of biomolecules. Catcher strands encode specificity to the target while crosslinkers trigger phase separation for sequestration of the polymer via centrifugation. **(B)** Representative capture of a fluorescent DNA target on the polymer system. **(C)** Pulldown efficiencies for different biomolecules. Nonspecific pulldown is measured in the absence of catcher strands (n = 3 independent experiments).

Switchable DNA Catalysts for the Detection of Intercellular Protein–Protein Interactions

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Intercellular protein-protein interactions (PPIs) play essential roles in governing various biological processes, including immune recognition and response, synapse formation and synaptic transmission. A powerful approach called proximity labeling (PL) has been employed to unravel molecular interactions occurring at cell-cell contacts. This method involves the introduction of either enzymes or synthetic catalysts to specific proteins or subcellular compartments, mediating the covalent tagging of endogenous proteins in close proximity. However, current PL techniques either require the use of genetically engineered proteins, potentially perturbing native PPIs, or lack precise spatiotemporal control in response to PPIs.

We have previously reported DNA-based switchable photocatalysts capable of specifically activating PL at the sites of protein homo- and hetero-dimers on the surface of living mammalian cells.^[1] Here, we applied switchable DNA catalysts to detect intercellular PPIs at cell-cell contact sites with high spatial and temporal resolution. The DNA-directed PPI detection system is composed of two split invaders anchored onto PPI partners and a partially complimentary double-stranded DNA substrate conjugated with photocatalyst and quencher which is catalytically inactive by default. The occurrence of PPIs leads to the proximity of two split invaders, triggering the reconstitution of the full invader and driving toehold-mediated strand displacement to activate the photocatalyst, enabling selective PL at cell-cell contacts. We implemented the switchable DNA catalysts to accomplish in situ detection and discrimination of varied intercellular PPIs triggered by specific environmental cues with high spatiotemporal precision. This approach offers a new tool for deciphering the cell-cell interactions at the molecular level and holds promise for broader biomedical applications beyond PL by activating photochemical catalysis triggered by molecular interactions.

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Strand displacement reactions beyond room temperature

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Toehold-mediated DNA strand displacement reactions have been widely applied to realize dynamic nano-devices and chemical reaction networks [1]. In these reactions, an invader strand binds to the toehold overhang of a target strand, replacing a target-bound incumbent strand (Figure 1A). Typically, a strand displacement reaction system transits into a thermodynamic final state due to the irreversible replacement of the incumbent strand by the invader strand.

Recently, there has been a growing interest in driving these reactions out-of-equilibrium to mimic reaction networks of living systems [2]. This is achieved by incorporating dissipative elements, such as the enzymatic degradation of nucleic acids strands [3-5]. Notably, these elements often require physiological temperatures for optimal enzyme efficiency. In contrast, most currently established principal reactions as well as complex reaction networks operate at room temperature. To design and realize complex reaction networks that include dissipative as well as temperature-responsive elements, an extensive understanding of the temperature-dependence of strand displacement reactions is essential, which at present is neither systematically characterized nor completely comprehended.

Here, we systematically investigated the temperature dependence of the strand displacement process by using bulk fluorescence experiments. Interestingly, the temperature dependence of the strand displacement rate constant initially increases but subsequently decreases with rising temperature (see Figure 1B). By employing simple thermodynamic modeling based on a previously established Markov chain model [6], we demonstrate that the temperature dependence is dictated by a dynamic interplay between reversible toehold hybridisation and irreversible strand replacement. We anticipate that our results will be particularly valuable when designing DNA-based chemical reaction networks at elevated and/or changing temperatures.



Figure 1: Temperature dependence of the toehold-mediated DNA strand displacement reaction. (A) Schematic representation of the strand displacement process. The invader strand reversibly binds to the toehold overhang of the preformed incumbent-target duplex. Displacement of the incumbent leads to the irreversible formation of the invader-target duplex. The displacement reaction can be described by a single second-order rate constant, or by a three state system using three rate constants. (B) Second-order rate constant of the strand displacement reaction involving a 6-nucleotide long toehold measured at different temperatures (solid symbols). The dashed line represents a fit of a simple thermodynamic model to the experimental data.

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DNA Origami-gold Nanostructures for Enhancing Sensitivity in Fiberoptic Surface Plasmon Resonance Biosensors

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Recently, a novel approach for enhancing the sensitivity of fiber-optic SPR (FO-SPR)based biosensors was demonstrated¹. Instead of only relying on changes in refractive index, this method also exploits the coupling phenomenon between the continuous surface plasmons on a gold surface and the localized surface plasmons of gold nanoparticles (AuNPs). Dillen *et al.* effectively harnessed this phenomenon by tethering AuNPs onto a gold-plated fiber-optic probe through a single-stranded DNA tether. This technique resulted in a sixfold increase in signal intensity compared to a label-free system and generated a calibration curve with a limit of detection (LoD) of 230 nM for a ssDNA target. However, clinically relevant biomarkers often fall within the low nanomolar, picomolar and even femtomolar range.

Enhancing the biosensor's sensitivity can be achieved by maximizing the signal change per construct. To explore this approach, we utilized the programmability of DNA origami, constructing a 14-helix bundle capable of housing multiple AuNPs (Figure 1). Augmenting the number of AuNPs was possible due to the presence of specific DNA extensions protruding from the origami. These strands could hybridize to complementary DNA on the AuNP surface. In all four designs, the purified yield of desired structures was above 70%.



Figure 1: (a) Schematic of a DNA origami nanostructure with AuNPs. (b) TEM images and yields of structures with one to four AuNPs.

Upon analyte binding, the coordinated movement of multiple AuNPs on a single origami structure induces a substantial signal change, surpassing the capabilities of the current setup. Therefore, the primary objective of this project was to explore the impact of

arranging varying numbers of AuNPs onto an origami structure and assessing whether increasing the number of AuNPs per structure could amplify the FO-SPR shift, thereby creating a more analytically sensitive biosensor.

When increasing the number of AuNPs on the origami and monitoring the binding of these structures to the FO-SPR surface, we observed an exponential increase in signal (Figure 2). This outcome confirms that incorporating more AuNPs per construct indeed amplifies the signal shift.



Figure 2: (a) Schematic of AuNP-origami constructs binding onto FO-SPR surface. (b) Mean end shift following a 4-hour incubation of origami complexes with an increasing number of AuNPs on a FO-SPR surface.

Moreover, through the toehold-mediated strand displacement of constructs with four AuNPs, we established a calibration curve with a LoD of 70 nM (Figure 3). This heightened sensitivity represents a threefold improvement over the sensitivity reported in the existing system, underscoring the potential of using DNA origami and multiple AuNPs for constructing bioassays with enhanced sensitivity.



Figure 3: (a) Schematic of displacement of AuNP-origami constructs from FO-SPR surface. (b) Sensorgrams following incubation of varying concentrations of ssDNA target and (c) corresponding calibration curve.

The next phase of this project entails tethering the origami onto the fiber surface, thereby establishing an integrated system for detection and signal amplification without the need of additional steps and regents. This endeavour aims to create a DNA origami-based continuous biosensor with heightened sensitivity.

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Probing and Controlling the Sensitivity of a Dynamic DNA Origami Device to Multiple Stimuli

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The non-cellular component of living tissues is the extracellular matrix (ECM), which is a

3D scaffold that supports various cell functions such as differentiation, proliferation, apoptosis, and migration. Maintenance of tissue is orchestrated by the reciprocal crosstalk between cells and the host ECM. Dysregulation of the biochemical and biophysical properties of the ECM contributes to several diseases such as cancer, arthritis, and back pain injuries. While the effects of certain biochemical biophysical and properties have been extensively studied, such as ECM composition and stiffness respectively, other properties lack extensive measurements in the context of a 3D ECM. Two such properties are the local osmolarity and interstitial flow that percolates through the 3D ECM.

Here we leverage recent advances on molecular sensors as means to address these challenges. Specifically, we present a DNA origami sensor that can be tuned to measure different biochemical and biophysical cues in the microenvironment. The sensor we present is around 100 nm in length and is composed of two barrels of DNA connected by six flexible scaffold linkers (Figure 1A). The sensor can reversibly assume two stable



Figure 1. Schematics of the DNA origami sensor. A) The sensor is designed to have two stable states: an open state, and a closed state. The interactions on the six flexible scaffold linkers can be optimized to control the energy landscape of the sensor. B) The sensor can be optimized to sense different biochemical and biophysical stimuli, including changes in osmolarity and fluid flow forces. Scale bars = 50nm.

states: an open state and a closed state. The energy landscape of the sensor can be tuned by controlling the interactions on the six scaffold linkers. On one of the linkers, we add a (Förster resonance energy transfer) FRET pair that enables us to monitor C) opening and closing of the sensor. In an earlier work, we have shown that the sensor can be used to measure changes in the compressive depletion forces due to molecular crowding [1] or tensile forces with tunable sensitivity [2]. In this work, we show that the careful tuning of the scaffold linker interactions can enable the channel. measurement of changes in



Figure 2. Sensor response to external stimuli. A) Probing the sensitivity of different sensor designs to changes in salt concentrations. B) Sensor response to an osmotic gradient created in a microchannel, showing the spatial sensitivity of the sensor. C) Characterizing the single molecule kinetics of the sensor. D) Sensor response to changes in flow forces imposed by changes in flow rates in straight flow channel.

the osmolarity in the microenvironment, as well as changes in the flow forces induced by interstitial flow (Figure 1B). We use various techniques, ranging from ensemble FRET measurement and imaging in microfluidic devices to single molecule FRET (smFRET) measurements for tuning and calibrating our sensors (Figure 2A,C). We show that we can design sensors that are sensitive to changes in the osmolarity in the 400 – 1200 mOsm range (Figure 2A,B). We also show that, by tuning the design of the sensors, we can measure changes in shear stress induced by fluid flow forces at and below 1.7 Dyn/cm² (Figure 2D).

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Gated transport of DNA nanostructures across lipid membranes by amphiphilic DNA hairpins

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ransmembrane proteins, including ion channels and transporters, serve as essential molecular mechanisms that enable communication between living cells and their surroundings. These proteins enable cells to detect, compute, and respond to various molecular cues in their environment.¹ Recent studies have successfully conjugated hydrophilic DNA with hydrophobic components as a building block for engineering bioinspired membrane-spanning devices that recapitulate the functions of biological channels and nanopores.²⁻⁴ In this research, we designed, engineered, and characterized amphiphilic DNA harpins designed to facilitate the transport of DNA cargos of increasing sizes across lipid bilayers, controlled by the presence of a specific target strand. Our transmembrane DNA hairpins, after inserting into lipid bilayers, recruited specific Cy3-labeled DNA targets, previously encapsulated inside Giant Unilamellar Vesicles (GUVs), forming a distinctive halo ring of Cy3-target strands. Upon detection of the target, the DNA hairpin structures dynamically opened, partially or entirely, enabling an influx of DNA cargos into the GUVs. Negative controls with empty GUVs decorated with transmembrane DNA hairpins do not exhibit leakage. Systematic experiments with different DNA cargos of varying sizes, ranging from 13-nt ssDNA $(\sim 2 \text{ nm})$ to streptavidin-labeled DNA triangles $(\sim 20 \text{ nm})$, show a decrease in the transport rate with increasing DNA cargo size. These small amphiphilic hairpins pave the way for the design strategy of gated DNA channels with ultimate simplicity.

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Illuminating the mechanism of DNA origami folding with a new mesoscopic model

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Scaffolded DNA origami exploits the highly specific canonical base pairing rules of DNA to fabricate intricate and dynamic nanostructures. In this method, single-stranded oligonucleotides (staples) are designed to collectively hybridize with a single long strand (scaffold), causing it to fold into the target shape. Despite the centrality of folding to DNA origami, the details of this process are unclear because current models cannot access the long timescales necessary to simulate the folding of full-sized origamis in standard conditions. Here, we present a new mesoscopic model that captures the entire folding process, from freely floating strands to the eventual product. To accomplish this, the model coarse grains DNA at levels up to 8 base pairs per bead, integrates motion with Brownian dynamics, and uses switchable force fields to depict the change in mechanical properties of DNA after hybridization and dehybridization. Simulations of a 4-helix-bundle (4HB) with a straight scaffold routing show hierarchical folding with first-order kinetics in two regimes. The folding starts with fast initial constraint of the scaffold associated with a transitional zipping action, leading to gradual incorporation of the remaining staples. Conversely, simulations of a 32-helix bundle (32HB) reveal heterogeneous staple incorporation, frequent trapping in metastable states, and lower yield compared to the more accessible 4HB structure. In addition to these analyses, we investigate the role of hybridization strength, scaffold routing, and staple design on the folding process. This model opens an avenue for predicting and understanding how origami design impacts the speed and quality of the folding.

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Figure 1: Mesoscopic representation of DNA origami in our model. (A) Fully disassembled and assembled structures before and after folding. Not all staples are shown for clarity. The underlying DNA strands are shown as helical tubes for reference. The beads used to represent DNA are shown in dark blue for scaffold and in different colors for each staple oligonucleotide. **(B-E)** Mathematical and visual description of bead interaction potentials used to replicate the mechanical properties of DNA.



Figure 2: Folding behavior of a 4HB structure with straight scaffold routing. (A-C) Dependence of the folding mechanism on the strength of staple binding as characterized by the variation of three quantities over the course of the simulation: staple concentration normalized by their initial concentration (A) which is observed to experience a change in slope between 0.7 s and 1 s, Landau-De Gennes crystallinity parameter describing global order (**B**), number of unrealized contacts during folding describing local order (**C**), and the mean fraction of incorporated staple strands which are bound to the scaffold (**C**, **inset**). The plotted lines represent averages of data collected from 10 independent simulations and the shaded envelopes surrounding those lines represent SEMs. (**D**) Representative images of scaffold conformations during assembly. These correspond to the open circles shown in panels **A**, **B**, and **C**. Arrows indicate zipping direction.

Wednesday April 24th, Morning Track on DNA Nanosystems: Programmed Function II

Track Chair **Friedrich Simmel** Technical University, Munich

Site-directed placement of three-dimensional DNA origami

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Molecular self-assembly approaches that offer atomic precision, ultimate design freedom, and scalability in nanofabrication are projected to have a major impact on the engineering of future artificial light-managing surfaces. Structural DNA nanotechnology, particularly DNA origami self-assembly, allows the high-yield synthesis of a wide variety of hybrid organic-inorganic optical nanostructures at a resolution unachievable by top-down fabrication. The integration of these nanostructures into real devices, however, remains a challenge. More than a decade ago, DNA origami placement was established, a technique based on the site- and shape-selective deposition of DNA origami objects onto lithographically patterned substrates, creating large-scale arrays of precisely placed DNA structures [1, 2]. However, the DNA origami placement methods developed so far were limited to planar DNA origami and could only fabricate two-dimensional arrays and patterns. We extend DNA origami placement to the third dimension by mounting threedimensional DNA origami onto nanopatterned substrates, followed by silicification to provide hybrid DNA-silica structures with a height of ~ 50 nm exhibiting feature sizes in the sub-10-nm regime [3]. Our versatile and scalable method relying on self-assembly at ambient temperatures offers the potential to three-dimensionally position any inorganic and organic components compatible with DNA origami nanoarchitecture. Such nearnanometre-precise spatial positioning of nanoscale building blocks could be crucial for future applications of this method, such as scalable self-assembled optical metamaterials and quantum light sources.

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Cryptic binding sites for polymorphic materials self-assembly

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DNA nanotechnology enables the design of dynamic nanodevices that reconfigure in response to molecular, biophysical, or environmental cues such as nucleic acids, proteins, pH, temperature, and forces. However, these have largely been limited to individual devices that convert inputs into fluorescence readouts. Here we demonstrate proof-of-concept for nanodevices that transduce one or more cues into materials selfassembly. We achieve this through the design of DNA nanodevices where binding sites for self-assembly are initially occluded on the core of the structure, and input cues drive reconfiguration of the nanodevice to expose these binding sites. Our approach is inspired by the concept of cryptic binding sites in proteins that are exposed by cues like forces or binding events to trigger downstream functions. (1) Similarly, our DNA origami nanodevices require triggered structural reconfiguration before they can partake in any hierarchical assembly reactions. We have designed and fabricated multiple unique and modular structures to realize different outcomes, (2, 3) depending on the initial environmental cues. We use electron microscopy, rheometry, and computational approaches to characterize our systems, and show that depending on the superstructure different viscoelastic properties are realized. Figure 1A displays the fundamental unit, a three-arm junction in different conformations: (A-C) closed, and (D-E) open, which can only undergo multimerization when the trigger is present (F). Finally, we demonstrate exemplary oligomers assembled from different units (G). Combining these devices together with a modular spacer unit leads to polymorphic assemblies, which allows to tailor materials as needed from the same reaction and sets a foundation for materials on demand fabrication using DNA origami.

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Figure 1: Modular DNA origami for cryptic self-assembly. oxDNA models of the closed structure (side view (A), front view (B), and inside (C); cryptic binding sites are highlighted in green), and TEM micrograph of the closed structure. oxDNA model (D) and TEM micrograph of the open structure (E). Agarose gel showing dimerization only in the presence of a trigger (F). AFM images of Dimers (G top) and Tetramers (G bottom). Scale bar: 100 nm.

Data storage and encryption using a 5-bit DNA nanoswitch library

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DNA-based construction allows the creation of molecular devices that are useful in information storage and processing. Here, we combine the programmability of DNA nanoswitches and stimuli-responsive conformational changes to demonstrate information encoding and graphical readout using gel electrophoresis. We encoded information as 5-bit binary codes for alphanumeric characters using a combination of DNA and RNA inputs that can be decoded using molecular stimuli such as a ribonuclease. We also show that a similar strategy can be used for graphical visual readout of alphabets on an agarose gel, information that is encoded by nucleic acids and decoded by a ribonuclease. Our method of information encoding and processing could be combined with DNA actuation for molecular computation and diagnostics that require a nonarbitrary visual readout.



Figure 1 – *Encoding information using DNA nanoswitch libraries.* Information can be encoded into DNA by utilizing the DNA nanoswitch, where different loop sizes correspond to 5 different "bits" which can be read using gel electrophoresis. This information is initially encrypted, and can be decoded using ribonuclease to reveal the encoded information in a graphical readout.

FNANO 2024

Wednesday April 24th, Afternoon Track on Molecular Machinery

Track Chair **Andrew Turberfield** University of Oxford

Al based design of genetically encodable nanomachines

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Generative AI approaches offer an unprecedented way to systematically explore protein structural and functional landscape, far beyond what evolution has sampled so far. Some of the most fascinating protein structures, the molecular nanomachines processing energy and information within cells, have until now remained inaccessible to synthetic approaches to biology.

In this presentation, I will illustrate how cutting-edge AI tools now enable the *de novo* design of increasingly complex mechanically constrained protein assemblies. Our research demonstrates that computational protein design can be used to accurately fabricate mechanical systems operating at the nanoscale, opening avenues towards a post-evolutionary era of custom genetically encodable nanomachines.

DNA Nanoscaffolds to Enhance Synthetic Catalysis

<u>Jeffrey D. Martell</u>,* Edward B. Pimentel, Ashley. N. Ogorek, Caleb A. Cox, Xu Zhou, Justice L. Merrifield, Jean Paul Habumugisha, Trenton Peters-Clarke, Joshua J. Coon *Email: jdmartell@wisc.edu

The Martell group develops catalysts that merge the benefits of enzymes and synthetic chemistry, with applications spanning sustainable synthesis and chemical biology. Synthetic catalysts drive a broader scope of chemical reactions compared to enzymes, but they lack the selectivity, activity under mild conditions, and stimuli-responsiveness of enzymes. We use nongenomic DNA to construct hybrid catalysts that accelerate reactions through pre-organization of multiple functional groups, akin to enzyme active sites but not limited to the natural amino acids and cofactors. Through DNA barcoding and combinatorial synthesis, we have developed a platform to rapidly evaluate millions of DNA nanocatalysts, and we developed switchable DNA catalysts that activate in response to specific chemical stimuli.

Synergistic catalysis has expanded the toolkit of eco-friendly synthetic reactions. However, synergistic catalyst systems require high cocatalyst loadings and are discovered through low-throughput methods. We combine synergistic with catalvsis DNA nanostructures enhance to sustainable synthesis. We achieved >100-fold rate acceleration by holding synergistic



Figure 1. DNA-scaffolded synergistic catalysis

co-catalysts in proximity on DNA scaffolds (Figure 1). We are applying this platform for selective transformations by attaching synergistic catalysts to DNA aptamers that bind small-molecule and biomolecular substrates. To accelerate catalyst discovery while minimizing waste, we developed a combinatorial discovery platform, in which millions of supramolecular DNA scaffolds can be screened for catalytic activity in a single test tube. By merging biotechnology and synthetic chemistry, we rapidly screens millions of candidate catalysts, thus accelerating the process of catalyst discovery.

The expanding availability of bio-compatible synthetic catalysts has enabled their application in unmasking therapeutic agents, coating cells with polymers, and tagging endogenous proteins. However, synthetic catalysts are constitutively active, limiting

spatial control over activity. We are usina conformation-switching DNA-based catalysts to regulate the activity of abiotic reactions in response to chemical triggers. We created switchable DNA photocatalysts to control abiotic radical polymerization (Figure 2), and we showed that switchable DNA photocatalysts can be activated at sites of protein-protein interactions on the surface of living cells, triggering the tagging of proximal



Figure 2. Switchable DNA nanocatalysts activate polymer synthesis in response to chemical stimuli.

endogenous proteins for microenvironment mapping.

Representative Publications

Ogorek, A.N.; Zhou, X.; Martell, J.D. Switchable DNA Catalysts for Proximity Labeling at Sites of Protein–Protein Interactions. *J. Am. Chem. Soc.*, **2023**, *145*, 16913–16923. <u>https://pubs.acs.org/doi/full/10.1021/jacs.3c05578</u>

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Pimentel, E.B.; Peters-Clarke, T.M.; Coon, J.J.; Martell, J.D. DNA-Scaffolded Synergistic Catalysis. *J. Am. Chem. Soc.*, **2021**, *143*, 21402–21409. <u>https://doi.org/10.1021/jacs.1c10757.</u>

Nanopore-powered DNA turbines: towards bio-inspired nanorobotics

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Abstract

Our societies have flourished because of macroscale machinery powered by engines and motors. And we are not alone in our reliance on active machines: life itself depends on energy-consuming nanoscale machines, as work at the nanoscale is being done by millions of sophisticated molecular motors. However, until today, designing and building active energy-consuming machines at the nanoscale has remained challenging.

In this talk, I will be presenting our latest results on designing and building nanoscale DNA turbines: DNA nanostructures on nanopores that can autonomously convert transmembrane electrochemical potentials into rotary motion, similar to natural rotary motor proteins such as F_0F_1 -ATP synthase and bacterial flagella motors. We have successfully designed and built two generations of such nanoturbines: a self-organised DNA active rotor (1), and a bottom-up designed chiral-shaped DNA turbine (2). We observed sustained unidirectional rotary motion of these nanoturbines at the single-molecule level as we applied a voltage or salt gradient across the nanopore. These exciting results lay the groundwork, both theoretically and experimentally, for further studies and the development of autonomous nanomachines that leverage autonomous, unidirectional rotational motion.

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Wednesday April 24th, Poster session Track on Molecular Machinery

Track Chair **Andrew Turberfield** University of Oxford
A Modular DNA Origami Model of an Unfolding-Assisted Protease Machine

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Compartmentalization is a cellular strategy for controlling matter distribution in space and time.^[1] Various compartmentalized systems have been selected and perfected by evolution to perform nanoscale tasks under specific control.^[2] In the proteasome, for example, the confinement of the proteolytic action to specialized sites ensures selectivity and efficiency of the protein degradation process.^[3] Inspired by this elegant molecular machine, we constructed a selfcompartmentalized and modular chimera that is capable to perform unfolding and proteolytic degradation of a target substrate. The cell-free nanoscale model was composed of two connected DNA origami nanocages. One nanocage hosted the p97, a segregase/unfoldase molecular machine that is crucial in chromatin remodelling, transcription, and repair processes.^[4] The other nanocage hosted alpha-chymotrypsin, a serine protease which can hydrolyse peptide bonds after hydrophobic amino acids.^[5] We found that the physical confinement of the protease reduced autoproteolysis. One intriguing feature of the model was the unidirectional immobilization of p97 inside the nanocage, as revealed by cryoEM. This provided a 'gateway' mechanism to control access to the compartment and the direction of substrate processing. We observed that both unfolding and proteolytic degradation were faster upon spatial confinement of the enzymes within the DNA nanocage. Moreover, physical connection of the two enzyme-loaded nanocages resulted in a chimera with improved catalytic performance and minimized off-target proteolysis. We envision that the programmability and modularity of our approach can be used to engineer complex reaction cascades within specialized compartments, with promising applications in biomedical and biotechnological fields.

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Polyethylene glycol-modified DNA-based nanodiscs for incorporation and characterization of membrane proteins

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Membrane proteins (MPs) are central to life processes. They constitute about a third of all human proteins and perform vital cellular functions such as transport of ions and molecules across an otherwise impermeable membrane. Characterization of the structure and function of MPs has been challenging due to altered or loss of activity and function outside a native phospholipid environment. Single-particle cryo-EM has become the method of choice for membrane protein structure determination as it provides unprecedented resolution with atomic precision and provides structures in a hydrated, native environment while crystallographic studies often involve detergents that perturb native protein structure. Several bilayer mimetic systems (MSP, peptidiscs, SMALPs, Saposin nanodiscs) have been developed in order to simulate a native surrounding to understand the structure-activity relationship of membrane-associated proteins. However, they suffer from inherent drawbacks associated with lack of control over size and disruption of native protein structures. Hence, alternate tools for the creation of custom-sized nanodiscs without laborious protein engineering procedures would be necessary to achieve the required resolution to study structure and function of MPs using single-particle cryo-EM.

We envision DNA-based tools would be an excellent alternative to the current bilayer mimetic systems as the size of the nanostructure is programmable and consequently, we would be able to efficiently create lipid nanodiscs of variable sizes for study of small MPs to large complexes. Our lab created DNA-based nanodiscs where we modified DNA with hydrophobic alkyl groups in order to enhance interactions with phospholipids. Soon, we found that DNA extensively modified with alkyl groups no longer hybridized with complementary nucleobases. Melting temperature analyses revealed dramatic changes in hybridization capabilities of DNA depending on the extent of backbone neutralization.

Hence an alternate chemical modification was necessary to create stable DNA-lipid nanodiscs. In the current study, we constructed DNA minicircles functionalized with amphiphilic PEG molecules to interact efficiently with a phospholipid bilayer to create a lipid-DNA nanodisc. This method does not influence DNA hybridization as it does not

involve chemical modifications in the backbone and was shown to efficiently recruit lipids depending on the length and number of PEG chains used. All-atom molecular dynamics simulations show that DNA predominantly interacts with lipid headgroups and the PEG chains hold the bilayer stably within the DNA-ring. Next, we will perform protein reconstitution and characterization studies which will aid in discovery of underlying molecular mechanisms of MP function.



Fig 1: Top and side view of PEGylated DNA nanodiscs. Brown ring shows double stranded DNA minicircle with oligonucleotides modified with PEG chains (green). Interior of the ring shows a lipid bilayer made of DMPC and DMTAP (dark blue) lipids. Light blue chains indicate the detergent, DTAB.

FNANO 2024

Wednesday April 24th, Poster session Track on Biomedical Nanotechnology

Track Chair **Thomas LaBean**

North Carolina State University

DNA based programmable nanodevices to instruct biological systems

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Abstract: My laboratory ask how nanometer-sized biomolecules transmit and integrate information across much larger length scales of the orders of cells and tissues. We seek to explore how collections of macromolecules work together to establish a common functional system like cellular pathways, organelles, living cells and further into tissues, organs and entire organisms. Different biomolecules establish long-range orders in living systems by self-assembling into much larger structures, such as molecular complexes, membranes, and cytoskeletal organelles, intra- and intercellular contacts, and long range contacts. The main theme of our lab will be understand the assembly principles of biological systems and the roles they play in living cells, tissues and full organisms...and further developing technologies to modulate the same.

To address these problems, we adapt multidisciplinary, bottom-up approach using DNA nanotechnology. DNA has immense potential to arrange the matter at nanoscale with extreme robustness and spatial specificity. The compatibility of DNA to interface with other biomolecules like proteins, carbohydrates, lipids, small molecules make DNA a natural choice of material for bottom-up self-assembly. Thus, we will merge the complex programmability of DNA nanotechnology with the structural and functional diversity of other biomolecules. Our interdisciplinary research, along with national and international collaborations with experts, will leverage expertise from chemistry, nanotechnology, biophysics, biology, engineering, and medicine.

The overarching goal of the my team would be to translate laboratory findings into the development of new therapeutic strategies.

Keywords: Structural DNA nanotechnology, 3D cages, Monofunctionalized quantum dots, Single particle tracking, Lattice light sheet microscopy

DNA Origami Affinity Zones: A Customisable Testbed for Multivalent Interactions

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Accurate detection and quantification of molecular biomarkers (proteins, DNA/RNA, small molecules, etc.) is essential for rapid disease diagnosis, monitoring and medication[1]. Most diagnostic systems rely on 'affinity reagents' (e.g. antibodies or aptamers) to selectively bind to a target. Unfortunately, the laborious experimental protocols (e.g. SELEX for aptamers) required for affinity reagent development severely limit the potential throughput for developing detection assays to novel biomarker targets. Apart from affinity reagents, many small molecule ligands are known to exhibit weak binding affinity to certain protein motifs. Multiple ligands concurrently binding to a target protein or other complex molecule can result in multivalent interactions, which elevate the binding affinity of the complete assembly to a level close to that of an affinity reagent. Understanding how to predict and design multivalent systems could be the key to improving affinity reagent development and synthesis.

Various DNA- or DNA origami-based ligand positioners for inducing multivalent binding have been proposed, based on the ability to use DNA to suspend ligands in pre-designed positions/orientations[2]. These typically employ either simple wireframe structures (down to a single double helix) or entire origami assemblies which fully encapsulate a target protein. However, the relative bulkiness of DNA (helix diameter of 2nm, base-base distance ~0.34nm) restricts its ability to accurately position ligands in the clefts/active sites of typical globular proteins which are often just ~4-5nm in diameter (Fig.1A). We hypothesise that a system which can achieve higher ligand placement resolution could allow for the creation of specific and overall stronger multivalent interactions, even with small globular proteins.

In this work, we employed the unique geometry of hinge-like DNA origami structures[3] to construct an 'affinity zone', a 3D space where ligands can be accurately positioned in close proximity with a theoretical resolution close to 1 Ångstrom. We anchored multiple

hinge structures on a fixed origami baseplate (Fig.1B) and attached ligands to individual hinges, controlling ligand position via the angle of the hinge. The ligands can be placed in close enough proximity to individually target different binding sites on small proteins, together forming the affinity zone. We employed a combination of purification and microscopy techniques to assemble, validate and characterise our design as well as visualise the introduction of a globular protein into an affinity zone (Fig.1C). If combined with molecular dynamics simulations of the affinity zone, the concept could become a configurable testbed for multivalency in small globular proteins, at a previously unattainable resolution.



Fig. 1: A) The scale of DNA compared to a typical 4nm globular protein is such that precise placement of ligands in active sites is very difficult. B) Mockup of the affinity zone design, where hinge-like origami are used to place ligands in a central 3D area, with the position of each ligand controlled by its corresponding hinge angle. C) 2D Cryo-EM class average of a single hinge origami, TEM snapshot showing an affinity zone with 4 hinges attached (along with a 5nm gold nanoparticle tracer at its centre) and a TEM snapshot of streptavidin bound inside a bi-ligand affinity zone.

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DNA nanopores as artificial membrane channels for bioprotonics

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Biological membrane channels mediate information exchange between cells and facilitate molecular recognition. While tuning the shape and function of membrane channels for precision molecular sensing via de-novo routes is complex, an even more significant challenge is interfacing membrane channels with electronic devices for signal readout, which results in low efficiency of information transfer - one of the major barriers to the continued development of high-performance bioelectronic devices. To this end, we integrate membrane spanning DNA nanopores with bioprotonic contacts to create programmable, modular, and efficient artificial ion-channel interfaces. Here we show that cholesterol modified DNA nanopores spontaneously and with remarkable affinity span the lipid bilayer formed over the planar bio-protonic electrode surface and mediate proton transport across the bilayer. For the first time, we explored the kinetics of DNA nanopore bilayer interactions experimentally through ensemble experiments. Using the ability to easily modify DNA nanostructures, we illustrate that this bioprotonic device can be programmed for electronic recognition of biomolecular signals such as presence of Streptavidin and the cardiac biomarker B-type natriuretic peptide, without modifying the biomolecules. We anticipate this robust interface will allow facile electronic measurement and quantification of biomolecules in a multiplexed manner.



Figure 1. Schematic depiction of the DNA nanopore based bioprotonic device

Polymer-regulated pDNA nanoarchitectures expressing virus functions and challenges to cancer therapy <u>Kensuke OSADA</u>

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Viewing the scheme of life, it may be possible to create a life by designing molecules and controlling their assembly process over several hierarchies in right way. This is theoretically yes but technically too challenging. Nevertheless, it may still be possible to construct a high order architecture prior to the emergence of life. With this hope, we have attempted to prepare a polymeric nanoarchitecture from synthetic polymers and DNA hoping that virus functions emerge in the structure. The structure of virus is an assembly of a polyelectrolyte complex formed between negatively charged DNA and polyamines as a core, protein capsid as a shell, and spike proteins attached on the surface for targeting. Respecting this structure, we prepared a core-shell structure from a plasmid DNA (pDNA) and block copolymers comprising poly(ethylene glycol) (PEG), polyamine segment, and a ligand molecule attached to the PEG distal end. The structure packages a pDNA condensed by polyamine segment in a core and is covered by a PEG shell with ligand moieties on the surface, and showed a size comparable to a virus, ~100 nm.[1]

The structure exhibits a polymorphism including a globular shape, which is not appeared as spherical, rod-shape, and toroid-shape (Fig. 1). These are controllable by modulating interaction potency between pDNA and polyamine, and steric hinderance effect of PEG against condensation process.[2] The rod-shape follows a specific scheme of pDNA folding, "quantized folding". It is a bundle of a

folded pDNA with its length regulated to multiples of 1/2(n + 1) of the contour length of the pDNA folded n times.[3] The toroid is a spooled pDNA preferably by 6 which thereby times. include 7 strands of DNA packed in the orthogonal cross section of the structure.[4]

These structures are curious given the inherent rigidity of DNA because its persistence



Fig. 1. Control of polymorhic folding structures of single pDNA by block copolymers into globular-collapsing (a), rod-folding with folded more (b1) or less (b2), and ring-spooling (c). Single-strand DNA is folded into spherical (d).

length ($l_p = 50$ nm for double-stranded DNA) does not allow the folding of pDNA to rod-shape nor globular shape. We found that DNA undergoes double-strand dissociation to single-strand, which is characterized as a flexible chain with l_p of a few nm, to achieve DNA back folding to adopt the rod-shape while dissociation occurs partly and randomly in the globular shape. Toroid formed by spooling of pDNA maintain the double-strand entirely without dissociation. In contrast, a singlestrand DNA obtained from unpairing of pDNA by heating resulted in a spherical. Thus, it is accounted that the specific nature of DNA in rigidity; *i.e.*, rigid but foldable, permits pDNA to form polymorphic structures in the condensation.[2]

If these nanoarchitectures could access a nucleus of cells and the packaged pDNA permit transcription for gene expression in cell, they could be assumed to have served the comparable function as a virus. Moreover, if they achieved gene expression at targeted cells after systemic injection, they might further be assumed as even beyond virus because virus vectors are still difficult in systemic application. To this end, nanoarchitectures have been refined by controlling the structure and installing various functionalities to serve as gene vector alternative to viral vectors and challenged to cancer therapy.[1] Rod-shaped vector installed with cRGD peptide to target tumor vasculature demonstrated tumor growth suppression effect in pancreatic tumor model mice when systemically dispatched to pancreatic tumor xenograft.[5] The smallest sized spherical vector constructed from unpaired single strand from pDNA penetrated thick stroma of pancreatic tumor, accessed tumor nest cells, and demonstrated improved tumor growth suppression effect in systemic application.[6]

In this way, the nanoarchitectures constructed from synthetic polymers and pDNA performed likewise viral vector, and this fact alternatively indicated that virus function emerge in the nanoarchitectures.

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Investigating the Effects Ionic Strength on the Electric Response of DNA Nanostructures

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We examine the impact of ionic strength on the response characteristics of DNA nanostructures designed to interact strongly with an electrode surface. In particular, we have evaluated the reversible structural transformations of the DNA nanostructures upon hybridization with nucleic acid analytes using electrochemical impedance spectroscopy (EIS), a technique extremely sensitive to changes in the density of charged species near a surface. The ionic strength of the EIS testing solution influences the thickness of the electrical double layer near the surface and, consequently, the double layer capacitance at the electrode interface in EIS measurements. Furthermore, variations in ionic strength can alter the conformation and stability of DNA nanostructures, affecting their hybridization efficiency and, consequently, the sensitivity and specificity of detection. Understanding the impact of ionic strength on these processes is essential to optimize conditions for enhanced detection capabilities.

A notable aspect of our measurements is the impact of the double-layer capacitance, which, if left uncontrolled, can overwhelm the measured signal. Considering that DNA nanostructures are estimated to cover less than 10 % of the electrode surface, passivation of the unreacted gold surface with small-chain carbons, such as hexanethiol, becomes critical for reproducible results. Here, we discuss surface passivation strategies to maximize the measured specific response of the nanostructures. By employing effective passivation strategies, we facilitate the precise detection of nucleic acid analytes through the amplification of signal changes associated with variations in ionic strength and DNA origami structural transformations upon hybridization with specific DNA strands.

FNANO 2024

Wednesday April 24th, Poster session Track on DNA Nanostructures: Semantomorphic Science D

Track Chair

Hao Yan

Arizona State University

inSēquio: A Programmable 3D CAD Application for Designing DNA Nanostructures

DNA nanotechnology is advancing rapidly, and software for designing DNA nanostructures is evolving in a manner reminiscent of the historic trajectory seen in the electronics design automation industry in the 1970s. However, current DNA and RNA nanostructure (DN) design software limits users to either manual design with minimal automation or a constrained range of automated designs. Here we present inSēquio Design Studio, which bridges this gap as a programmable 3D computer-aided design (CAD) application, integrating a domain-specific graphical editor with a Python application programming interface (API) for versatile DN design.

Developed in C++ for Windows⁻ and Macintosh⁻ systems, inSēquio features a user-friendly graphical user interface with advanced 3D CAD features, enabling the design, visualization, and analysis of complex 3D DNs that can incorporate DNA, RNA, peptides, linkers, residue modifications, and arbitrary PDB molecules. To integrate with other design tools, it supports import of cadnano designs and export of all-atom and oxDNA DN design representations. An extensible services architecture accelerates the execution of compute-intensive analysis and simulation tasks by running them as cloud services hosted on GPU servers. As a commercial product, it includes sample designs, detailed documentation, and responsive technical support. With the inclusion of its powerful Python API, inSēquio enables a code-centric design (CCD) approach, enhancing DN construction with improved precision, scalability, and efficiency.

In this presentation, attendees will learn the advantages of the CCD workflow, illustrated by the creation of complex DNs using the inSēquio API with Python code executed from a Jupyter notebook. The session will provide a comprehensive understanding of inSēquio's features, showcasing how Python scripting combined with 3D graphical editing can facilitate the design of highly complex DNs, thereby opening new paths for research and innovation in nucleic acid nanotechnology.

Modeling the collective behavior of FG-Nups in the nuclear pore

complex by dynamic DNA nano-rings

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The nuclear pore complex (NPC) is a large, dynamic channel composed of up to 1000 nucleoporins (Nups) that spans the nuclear envelope. The intrinsically disordered phenylalanine-glycine (FG)-rich Nups line the central channel and form a permeability barrier that accounts for the highly selective yet rapid nucleocytoplasmic transport. The collective FG-Nup behavior in vivo has been conceptualized as a polymer brush [1], dominated by excluded-volume interactions, and a hydrogel [2], dominated by attractive interactions between FG domains, among other models. Most existing techniques provide decent description on the morphology of densely grafted FG-Nups [3] but the quantitative characterization of their collective interactions within a nanopore remains limited. Herein, we present novel DNA nano-rings that can dilate and contract, the extent of which depends on the collective force generated by the Nup interactions grafted inside the ring. We measure that the diameters of such dynamic DNA nano-rings as the function of FG-Nup configurations with or without nuclear transport receptors (NTRs), providing a quantitative measurement on the collective effect of Nup-Nup and Nup-NTR interactions [4]. We anticipate that these dynamic NPC mimics will be a transformative tool in studying collective properties of FG-nups in an NPC-like environment and elucidating the mechanism of nucleocytoplasmic transport.



Fig. 1 | A tetrameric dynamic DNA nano-ring for evaluating the collective interaction of **FG-Nups.** Schematics and negative-stain electron micrographs of the dynamic DNA nano-ring show that the inner ring diameter changes in response to DNA oligonucleotides (orange and green) that rigidify specific regions of the scaffold strand (left) or FG-Nups (yellow) that cluster in the middle of the ring. Scale bars: 50 nm.

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Molecular self-assembly is a promising route to construct biomimetic or bioinspired materials that leverage the diverse properties and interactions of biomolecules. DNA origami nanodevices have contributed to creating series of new structures, which achieve programmable structure with tunable and dynamic properties by leveraging the sequence specific interactions of nucleic acids¹. Previous advances have also established DNA origami as a useful building block to make well-defined micron-scale structures through hierarchical self-assembly², but these efforts have largely leveraged the structural features of DNA origami. The tunable dynamic and mechanical properties also provide an opportunity to make assemblies with adaptive structure and properties. Here we present two approaches to controlling higher order assembly. First, we show the integration of DNA origami hinge nanodevices and coiled-coil peptides into hybrid reconfigurable assemblies (Figure 1A). With the same dynamic device and peptide interaction, we make multiple higher order assemblies (i.e. polymorphic assembly) by organizing clusters of peptides into patches or arranging single peptides into patterns on the surfaces of DNA origami to control the relative orientation of devices. We use coiledcoil interactions to construct circular and linear assemblies whose structure, and mechanical properties can be modulated with DNA-based reconfiguration. Reconfiguration of linear assemblies leads to micron scale motions and ~2.5-10-fold increase in assembly stiffness, quantified in terms of an effective persistence length. In circular assemblies, modulating the hinge angle distribution allows control over the size of the assembly. We also present a different approach to control higher order assembly of polyhedral structures through tuning the structure of a 3-arm DNA origami structure. For the case where the 3-arms exhibit flexible rotating the high order self-assembly yields many different shapes, while modulating the inter-arm angles constrains the mostly likely assembly pathways. The main focus of this study is to leverage coarsegrained molecular dynamic simulation to understand higher order assembly pathways. We used oxDNA³ to simulate the design (Figure 1B), and construct the model of higher order assembly (e.g. 4-structure assembly, Figure 1B bottom) and then break a single interface to consider the energy costs of various higher order assembly

configurations. We evaluate the distances between the two ends at the newly cut interface as a proxy for the likelihood of that particular assembly forming. Our results suggest this approach can predict which higher order assemblies are most likely to form. Next steps will include leveraging this simulation approach to tune designs for targeted higher order geometries.

Overall, our results do not only provide a foundation for stimulus responsive higher order assemblies that can adapt their structure and properties in response to nucleic acid, peptide, protein, or other triggers. In addition, we demonstrate a method of analysis and predicting configurations of high order self-assemble structures, which can support the analysis and improvement of existing design and integrate into future design pipelines for complex higher order self-assemblies.



Figure 1: A) Schematic of DNA origami hinge and coiled-coil peptide. Overhangs are functionalized the outer side of both arms of the hinge which respectively complementary to the DNA handles of each peptide conjugate. By manipulating the pattern of overhang, the binding orientation is controlled to either opposite or same, which can form respectively linear polymers and circular patterns. B) The schematic of 3-arms structure and simulation model for analysis of self-assembly structure configuration. AFM figure of experimental result shows one monomer can form the high order structures with variable numbers of monomers. All scale bar is 100nm.

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Modeling driven synthesis of DNA origami superlattice and complex finitesize structures

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Sophisticated statistical mechanics approaches and human intuition havedemonstrated the possibility to self-assemble complex lattices or finite size constructs, but have mostly only been successful in silico. The proposed strategies guite often fail in experiment due to unpredicted traps associated to kinetic slowing down (gelation, glass transition), as well as to competing ordered structures. An additional challenge that theoretical predictions face is the difficulty to encode the desired inter-particle interaction potential with the currently available library of nanoand micron-sized particles. To overcome these issues, we conjugate here SAT-assembly --- a patchy-particle interaction design algorithm based on constrained optimization solvers --- with coarse-grained simulations of DNA nanotechnology to experimentally realize trap-free selfassembly pathways. As a proof of concept, we investigate the assembly of the pyrochlore lattice, a highly coveted 3D crystal lattice due to its promise in construction of optical metamaterials. We confirm the successful assembly with two different patchy DNA origami designs via SAXS as well as SEM visualization of the silica-coated lattice. Besides the lattice target, we show that complex finite size assemblies, including 3X3X3 "hollow" cube, 4x4x4 "filled" cube and a capsid structure, are achievable with high yield while only a minimal set of DNA origami species is required. Our approach offers a versatile modeling pipeline that starts from patchy particles designed in silico and ends with wireframe DNA origami that self-assemble into the desired structure.



Figure 1: Design pipeline for the self-assembly of a pyrochlore lattice. **a** The topology of the targeted structure is abstracted into SAT clauses and solved by **b** SAT solver for **c** an interaction matrix corresponding to the patch species to assign. **d** The mapped patchy particles are simulated in which **e** undesired states could be identified and avoided for a patchy assignment that only results in **f** successful nucleation and assembly of the desired structure to emerge after iterated optimizations.



Figure 2: Experimental characterization of the assembled pyrochlore lattice and finite size structures. **a** We used icosahedral DNA origami as the building block to pattern the patch assignment and verified the design with oxDNA simulation. **b** SEM characterization of a single crystal, with zoom-in view shown as the inset, scale bar 5 μ m. Model and TEM characterization of the **c** 3x3x3 "hollow" cube **d** 4x4x4 "filled" cube **e** capsid proves the capability for the pipeline to design complex finite size structures.

Dimeric DNA origami nanocapsules for controllable cargo accessibility

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The construction of nanoscale containers using scaffolded DNA origami has been repeatedly reported since the invention of the scaffolded DNA origami method. There is consensus on the potential for applications in drug delivery systems, aiming to transport and release drugs to targeted locations, and in nanoreactors for the precise control of reactions. Conversely, numerous reports detail the formation of multimeric DNA structures to realise various and complex molecular machines.

Although many capsules have been constructed using DNA origami since its inception, with a few exceptions [2], most have been limited to single DNA origami structures, resulting in limitations in their internal volume. Additionally, cargo protection has generally been challenging, with it either not being evaluated [3] or being limited [4]. We attempted to construct a 3D DNA origami capsule capable of protecting cargoes from the external environment by combining shape complementarity [5] and strand displacement for post-assembly zipping. The capsule dimer structure, made from two 8064 bp scaffold DNAs, has an internal volume of 7.5 zL (7500 nm^3) with double-layered walls.

While the DNA capsule was successfully constructed, a significant fraction remained monomeric or in aggregated states, necessitating the development of purification methods. Finally, purification of the dimeric capsule was achieved using streptavidin beads to concentrate only dimers.

As cargo, 2 kDa peptide moieties of split GFP were placed inside, and the capsule's permeability to external protein moieties (25 kDa) was evaluated based on the rate of split GFP reconstitution. Approximately 38% of cargo protection was observed. Similarly, when 25 kDa protein moieties were fixed inside and the permeability for a 2 kDa peptide moiety was evaluated, comparable results were obtained. This implied that leakage occurred with molecules larger than 25 kDa, which was consistent with preliminary SPA analysis by Cryo-EM.



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Wednesday April 24th, Afternoon Track on Biomedical Nanotechnology *Track Chair*

Thomas LaBean

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Reversible Protection and Targeted Delivery of DNA Origami with a Disulfide-Containing Cationic Polymer

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DNA origami technology has shown great potential in the field of drug delivery. This technology offers an attractive alternative to conventional drug carriers that lack control over shape or size and can in turn trigger a lot of uncontrolled off-target effects. Owing to the programmable nature of DNA, control over size and shape, and even mechanical motion can be achieved. Moreover, multiple other functionalities can be added to DNA nanostructures at precise positions and numbers. However, protecting the DNA-based nanostructures from degradation in physiological medium remains challenging. A possible solution can be achieved by using polymers or viral proteins to protect DNA origami and sustain its integrity in physiological conditions for relatively long durations. However, when it comes to intracellular delivery, the delivered cargo should be released inside the cytoplasm or the nucleus especially when properties of bare DNA is required. While most protective coats succeeded in maintaining the structures and aiding in the cell uptake, the protective layer would likely hinder the release of DNA nanostructures along with their delivery cargo or otherwise obstruct their function.

In this project, we, are using bioreducible a poly(disulfide-amine) polymer as a protecting agent for DNA origami structures. This type of bioreducible polymer protects DNA nanostructures, but is readily reduced once they enter the cell cytoplasm. Moreover, the disulfide bonds in the polymer backbone can aid in the direct cytoplasmic cellular entry through disulfide exchange mechanisms that takes place at the cell membrane. The polymer showed strong affinity towards DNA due to its cationic nature and significantly increased the DNA nanostructure stability against degradation. Gel electrophoresis and FRET studies proved the removal of the polymer protection in reducing environments. Cell studies revealed a synergistic increase in cell uptake when combining polymer protection with targeting antibodies. Finally, the study of the uptake mechanism suggested that a fraction of the internalized structures can be directly delivered to the cytoplasm through membrane di-sulfide exchange. The prepared constructs were further tested for their ability to deliver cytotoxic agents conjugated to short DNA strands and showed a promising knockdown to cancer cells relative to the single strands alone.^[1]



Figure 1 Schematic diagram representing flat DNA origami coated with bioreducible poly(disulfide amine) polymer, where the polymer coating remains intact outside cells to protect DNA from degradation. After cellular uptake is and reduction by GSH the polymer is reduced and the DNA origami is released inside the cell cytoplasm

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Biomimetic Nanosystems by Spatial Organization of Protein Components on DNA nanostructures

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Background: Cellular functions rely on a series of multienzyme cascade reactions to catalyze chemical transformation, signal transduction and energy production. Spatial organization of cascade reactions not only facilitates the chemical diffusion and transfer in a catalysis, but also plays critical roles in triggering protein cascades for triggering immune response. If these cellular structures and mechanisms are mimicked and translated to a non-living, artificial system, it could be useful in a broad range of applications with significant scientific and economic impact.

Objective and Methodology: Here, we described the use of DNA-scaffolded assembly to position the spatial pattern between catalytic components to mimic substrate channeling, regulate enzyme-substrate cooperative binding and probe distance-dependent activation of complement cascade for triggering immune response.

Results: We demonstrated a modulation of a multienzyme system with the cascade activity impacted by the local enrichment and exclusion of substrate molecules (Figure A). A DNA origami modified with aptamers was used to bind and enrich ATP molecules in the local area of immobilized enzymes, thereby enhancing the activity of an enzyme cascade by more than 2-fold. Alternatively, DNA nanostructure modified with blocked aptamers does not bind with ATP, thereby reducing the activity of the enzyme cascade. The Michaelis-Menten kinetics showed decreased apparent Km values (~3-fold lower) for enzyme nanostructures modified with aptamers, suggesting the higher effective substrate concentration near enzymes due to the local enrichment of substrates. Conversely, increased apparent Km values (~2-fold higher) were observed for enzyme nanostructures modified with blocked aptamers, possibly due to the exclusion of substrates approaching the surface. As a continuous effort, we aim to engineer an allosteric enzyme complex with mimicking cooperative substrate binding and sigmoidal kinetics of reaction. Toward this, the DNA origami is modified to respond to concentrations of substrate molecules with a low surface-substrate binding affinity initially, as adding of more substrates, the surface affinity to substrate molecules also increases. This results in a positively cooperative binding between DNA origami and ATP, like hemoglobin, and shows a non-traditional Michaelis-Menten kinetics with a high hill coefficient. The similar concept of this modified surface-substrate interaction should be applicable to other multienzyme systems immobilized on nanostructures, which could be useful in the development of biomimetic nanoreactors.

Another on-going project uses DNA nanostructures to control the spatial arrangement of surface nucleophiles for studying complement protein cascade (**Figure B**). The complement cascade is a part of the immune system that enhances the ability of antibodies and phagocytic cells to attack the pathogen's cell membrane. For a long time, it is hypothesized that C3-nucleophile adducts will catalyze addition of new C3s to neighboring nucleophiles only if the nucleophiles have certain spatial pattern accommodating catalysis. To study this, we evaluated how the activation of complement protein cascade depends on the geometric arrangement of surface nucleophiles on DNA nanostructures.

Significance and Impact: DNA nanostructure-templated assembly promises to deliver breakthroughs toward the engineering of novel biomimetic nanosystems, which have great potential for broad applications from chemical synthesis, functional biomaterials and biofuel production to molecular sensing and nanomedicine.



Figure. Biomimetic systems engineered by spatial organization of protein components on DNA nanostructures. (A) Modulation of Enzyme Cascade Activity by Local Substrate Enrichment and Exclusion. (B) Distancedependent activation of complement protein re actions on DNA nanostructures.

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Base modifications eliminate the innate immunogenicity of singlestranded RNA origami

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RNA nanotechnology, an emerging field that integrates the distinct structural and functional attributes of RNA with the design principles of nanotechnology, is beginning to unfold its potential in therapeutic applications. This growing significance is further highlighted by the recent successful development of mRNA vaccines against COVID-19, which has invigorated research into the utilization of RNA nanostructures for improved RNA-based therapeutics. However, it is imperative to acknowledge that the double-stranded RNA regions in these RNA nanostructures are intrinsically immunogenic, acting as innate immune stimulators that are recognized by the pattern recognition receptors (PRRs) including both endosomal (such as Toll-like receptor 3, TLR3) and cytosolic (such as retinoic acid-inducible gene I, RIG-I, and melanoma differentiation-associated protein 5, MDA5) [1]. The activation of innate immune response to RNA nanostructures could potentially compromise the therapeutic efficacy of certain RNA-based medicines, and, more severely, might even lead to cell apoptosis or necroptosis through the downstream pathways of PRRs [2].

Inspired by the finding that certain base modifications on in vitro transcribed mRNA enhance the protein expression by reducing its immunogenicity [3,4], we explored the effects of base modifications, including 5-methycytosine (m⁵C), pseudouridine (Ψ) and N1-methylpseudouridine (m¹ Ψ), on the innate immunogenicity of RNA nanostructures. We employed the square-shaped single-stranded RNA origami (SQ) containing parallel crossovers [5], which shows potent innate immune stimulatory effects both *in vitro* and *in vivo* [6], as a case study. We successfully synthesized SQs with m⁵C and/or $\Psi/m^{1}\Psi$ modifications by in vitro transcription and found that the shapes are not obviously altered by the modifications. While the cellular uptakes of base modified SQs remain largely unaffected, the innate immune responses in mouse macrophage cell line Raw264.7 are reduced to different extents for both TLR3-mediated and RIG-I/MDA5-mediated immunities. Of note, incorporating both m⁵C and m¹ Ψ into SQ almost completely eliminate

the potent immune response of unmodified SQ. Though we are still in the process of investigating the underlying mechanisms, our findings open up promising opportunities for improving the safety profile of RNA nanostructure-enabled or -enhanced therapeutics.



Figure 1. The single-stranded RNA origami without base modifications triggers strong innate immune response through cytosolic dsRNA sensors RIG-I/MDA5 or endosomal TLR3. The cytosolic signal is transduced to mitochondrial antiviral signaling (MAVS) while the endosomal signal from TLR3 is transduced to TIR domain containing adaptor molecule 1 (TRIF). Then both signaling pathways can mediate NF- κ B and IRFs activation for innate immunity. When the m⁵C, Ψ and m¹ Ψ were incorporated to the single-stranded RNA origami, the innate immune responses are be suppressed to different extent. The innate immune response is found to be eliminated by a combination of m⁵C and m¹ Ψ modifications.

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FNANO 2024

Thursday April 25th, Morning Track on Nucleic Acid Nanostructures In Vivo

Track Chair **Björn Högberg** Karolinska Institutet

DNA-origami nanostructures in zebrafish embryos

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DNA nanotechnology is increasingly finding applications in drug delivery. Therefore, relevant animal models are of critical importance to development of DNA nanotechnologybased drugs. We have previously investigated the functional effects of multivalent versus monovalent delivery of insulin using insulin-DNA origami nanostructures. To analyze their effects in vivo, we used a zebrafish (Danio rerio) model engineered for targeted β -cell ablation, thereby modeling the onset of type I diabetes. In this transgenic zebrafish model, the enzyme nitroreductase (NTR) is expressed under the control of the insulin promoter and converts the compound metronidazole (MTZ) to a toxic by-product that ablates β -cells. We induced β -cell ablation by MTZ treatment at two days post-fertilization resulting in increased glucose levels at three days post fertilization. We found that the treatment with multivalent insulin nanostructures significantly decreased glucose levels to below those of the control while monovalent nanostructures did not have an effect.

Further, the zebrafish embryo is a relevant model system for analyzing the biodistribution and clearance of fluorescently labeled DNA nanotechnology-based drugs because it allows for truly whole organism analyses in live animals. Imaging of live zebrafish embryos injected with fluorescently labeled DNA origami nanostructures can be analyzed using different microscopy modalities, including confocal microscopy, light-sheet microscopy, and super-resolution Airyscan microscopy, which enables subcellular detection. In addition, the transparency of the embryos allows for analyzing the nanostructures while in the circulation in live embryos using fluorescence correlation microscopy (FCS) to determine their aggregation over time.

Single cell RNA sequencing enables detecting of the biodistribution of fluorescently labeled DNA origami nanostructures in whole embryos in an unbiased manner. We have previously analyzed whole zebrafish embryos injected with fluorescently labelled DNA nanostructures and obtained an accurate picture of all cell types that interacted with the DNA nanostructures and how their distributions changed when the structures were coated with oligolysine-PEG. In conclusion, we propose that the zebrafish embryo is a relevant model for analyzing the fates of DNA origami nanostructures in vivo with subcellular resolution.

A DNA-Based Robotic Device Exhibiting Controlled and Autonomous Presentation of Nano-patterned Cytotoxic Ligands *in vivo*

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The phenomenon where death receptors (DRs) aggregate on cellular membranes is a critical process leading to programmed cell death or apoptosis. This mechanism is particularly targeted in the treatment of cancerous tumors through the creation of multivalent molecular tools that can activate these receptors. However, the challenge arises due to the ubiquitous presence of DRs across both malignant and healthy cells. In this extended discourse, we delve into the intricacies of a novel device designed to independently and selectively activate the display of cytotoxic ligand patterns specifically in cancerous environments, thus mitigating damage to healthy tissue. We present our groundbreaking findings on a DNA origami construct that is engineered to conceal six ligands within its framework. This construct undergoes a transformation to reveal these ligands, arrayed in a hexagonal pattern with a precise diameter of 10 nanometers, in response to increased acidity levels typical of tumor sites. The strategic display of ligands is calibrated to effectively induce clustering of DRs, thereby initiating apoptosis in human breast cancer cells at a pH of 6.5, while maintaining a passive state at the normal physiological pH of 7.4. The efficacy of this device is further corroborated by its therapeutic potential demonstrated in mice models with human breast cancer xenografts, where it substantially reduced tumor growth by up to 70%. These findings not only underscore the device's efficacy but also highlight the promising therapeutic avenues such devices open up for the development of highly precise cancer treatment modalities.

In vivo Testing and Commercial Development of an RNA-Origami Direct Thrombin Inhibitor Anticoagulant with Reversal Agent

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The engineering of nucleic acid nanostructures has advanced to the point where highly active drug molecules can now be designed, created, tested, and developed for use in human health. Injectable anticoagulants are widely used in medical procedures to prevent unwanted and potentially deadly blood clotting, however, many modern anticoagulants on the market today lack safe, effective reversal agents, exposing patients to the possibility of dangerous uncontrolled bleeding events. Here, we describe our efforts to develop an RNA origami-based, direct thrombin inhibitor anticoagulant (HEX01) in conjunction with its rapid, specific, DNA-based reversal agent (HEX02). We present in vivo data demonstrating efficacy (in trauma and anti-thrombotic models), rapid reversibility, preliminary safety (high biocompatibility, low immunogenicity, etc.), initial biodistribution data, and evidence of desired, long half-life during blood circulation. HEX01 contains multiple, thrombin-binding aptamers appended on a two-helix RNA origami. It exhibits excellent anticoagulation activity in vitro and in vivo. The DNA antidote (HEX02) reverses anticoagulation activity of HEX01 in human plasma within 30 seconds in vitro and functions effectively in a murine liver laceration model as quickly as we are able to measure. Biodistribution studies of HEX01 in whole mice using ex vivo imaging show accumulated mainly in the liver over 24 hours and with 10-fold lower concentrations in the kidneys. Additionally, we show that the HEX01/HEX02 system is non-cytotoxic to epithelial cell lines and non-hemolytic. Furthermore, we found no serum cytokine response to HEX01/HEX02 in a murine model. We conclude that HEX01 and HEX02 represent a safe and effective coagulation control system with fast-acting, specific reversal showing great promise for medical purposes. We will argue that these characteristics make our engineered nucleic acid nanostructures promising candidates for use in coagulation control during transcatheter structural heart procedures in humans.

DNA microscopy in two and three dimensions

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Lymphatic, nervous, and tumoral tissues, among others, exhibit physiology that emerges from three-dimensional interactions between genetically unique cells. Technologies capable of volumetrically imaging transcriptomes, genotypes, and morphologies in a single de novo measurement can provide a critical lens into biological complexity of living systems. We present experimental and computational work to develop DNA microscopy: a modality of imaging that captures physical images of specimen genetic content using a massive distributed network of DNA molecules inside it. We demonstrate DNA microscopy in two-dimensional cell cultures and genome-wide in intact zebrafish embryos.



Figure: DNA microscopy as an imaging modality works by assembling a massive intercommunicating network of uniquely DNA-barcoded molecules in fixed tissue (A-B). This inter-communicating network encodes the geometry (via proximity) and genetic composition of specimens (C). In 2D (Weinstein et al, Cell 2019) using unconstrained diffusion experiments (D-E) image inference (of 6e4 sequenced transcript molecules) recapitulates fluorescence microscopy (fluorescence microscopy scale bar 100µm, DNA microscopy unit corresponds to 1/e fall-off in UEI-rate). Solid-state rolling circle amplification (F-H) provides 3D reconstructions (Qian and Weinstein, bioRxiv 2024) of intact zebrafish embryos (I-J) with genome-wide transcript readouts (bright field scale bar 200µm) from 4e6 molecules. FNANO 2024

Thursday April 25th, Poster session Track on Nucleic Acid Nanostructures In Vivo

Track Chair **Björn Högberg** Karolinska Institutet
Stabilization of Functional DNA Nanomaterials through Minimal Photochemical Methods

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DNA nanomaterials offer an unparalleled degree of control over matter on the nanoscale. In addition to their structural role, DNA nanomaterials also have functional applications in sensing, stimuli response, and as nucleic acid therapeutics. Despite their addressability, ease of synthesis, and biocompatibility, these materials have been challenged by issues of low stability. By harnessing the natural photochemistry of the canonical nucleobases, we create ultra-stable DNA-DNA crosslinks at sequence-defined regions of our structures, vastly increasing stability without compromising function. We demonstrate the application of this method to several classes of functional DNA nanomaterials including nucleic acid therapeutics.

We then extend this method of DNA irradiation to create a one-dimensional platform for presentation of ligand molecules. We demonstrate increased stability of this construct and show application through the presentation of folate ligands designed to enhance cellular uptake. Finally, we use our construct to investigate the effect of ligand density, positioning, and copy number of folate to disentangle the variable mechanisms of internalization.



Figure 1. DNA irradiation yields stable nanomaterials, while retaining the integrity of functional motifs. Three classes of functional DNA nanomaterials are presented with enhanced stability and retained function.

Regulation of Ordinal DNA Translocation in Bacteriophage Φ29 through Trans-subunit Interactions

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Certain viruses such as the tailed bacteriophages, herpes simplex, and pox viruses package double-stranded DNA (dsDNA) into procapsids via powerful, synchronized, ringshaped molecular motors that attach to portals of empty procapsids. These motors consist of individual subunits that coordinate ATP hydrolysis with adjacent subunits to maintain the proper cyclic sequence of dsDNA translocation steps about the subunit ring. Here we explore how the pentameric bacteriophage $\Phi 29$ (Fig. 1A) regulates its packaging mechanism by timing key events, such as ATP binding/hydrolysis and dsDNA gripping, through trans-subunit interaction. Specifically, we considered a dimer of two adjacent subunits of two nucleotide occupancy states: ATP-ATP representing two adjacent subunits (Fig. 1A) before hydrolysis in any of the two subunits (referred to as "previous and "current" in a translocation cycle) and ADP-ATP representing the same subunits but where the previous subunit has hydrolyzed ATP. Umbrella sampling using molecular dynamics simulations were carried out on subunit dimers to compute the free energy landscape associated with the catalytic glutamate residue (E119) approaching the gamma-phosphate of ATP for catalyzing hydrolysis in the current subunit (Fig. 1B). We found that the nucleotide state of the previous subunit introduced a large energy barrier that makes it more difficult to hydrolyze ATP when the previous subunit is bound to ATP rather than ADP, that is, it has not yet hydrolyzed ATP (Fig. 2A). Even though the glutamate switch is a cis-interaction (mechanism contained within one subunit), the previous subunit can be positioned to obstruct the catalytic glutamate from approaching the gamma-phosphate of ATP, through trans-subunit interactions. When the previous subunit hydrolyzes ATP, the subunit releases its grip on dsDNA and moves out of the path of the catalytic glutamate (Fig. 2B). Mutual information analysis of the simulation trajectories revealed direct communication pathways linking the binding pockets (trans) and dsDNA gripping residues of subunits (cis) (Fig. 3). This work shows that the sequential order of DNA translocation events among subunits can be preserved through trans-interactions across subunits and that key events in one ATP binding pocket can be communicated through allosteric pathways to neighboring subunits.



Figure 1. (A) Top view of the Φ 29 motor showing its five subunits (tan, orange, purple, salmon, and green) and the pore containing the dsDNA (red and blue). The subunits are alphabetically labeled. (B) The glutamate switch mechanism of Φ 29, wherein catalytic Glu119 residue is either bound to Arg53 residue or the gamma phosphate of ATP to catalyze hydrolysis.



Figure 2. (A) Free energy profiles as a function of the distance between the catalytic Glu119 and gamma phosphate were determined through umbrella sampling simulations. The dimers consist of the B and C subunits from the pentamer. The C subunit hydrolyzes first, so it is termed "previous", and subunit B hydrolyzes next, so it is termed "current". ADP-ATP dimers, regardless of being dsDNA bound or not, have a smaller energy barrier to overcome for the catalytic glutamate to come closer to the gamma phosphate. (B) Energy barrier comes from the adjacent subunit obstructing the path for the catalytic glutamate to approach the gamma phosphate.



Figure 3. Mutual information of dsDNA-bound ATP-ATP dimer. The target residue is E119*. All residues marked with * belong to subunit C. Residues without an * belong to subunit B. Mutual information shows strong allosteric pathways between E119* to K105* and R146* and across the interface to the neighbor's binding pocket and R146. The mutual information is normalized after outliers are removed.

FNANO 2024

Thursday April 25th, Poster session Track on Biomedical Nanotechnology

Track Chair **Thomas LaBean**

North Carolina State University

Targeted Drug Delivery of Chemotherapy Drugs using Antibody-Labelled DNA Origami Nanostructures

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Targeted cancer therapy aims to selectively kill cancer cells avoiding healthy cells and tissue, thus preventing harmful side effects. Employing therapeutics including small molecules, antibody drug conjugates, and liposomal drugs, while clinically effective, exhibit complications including suboptimal targeting of cells, multidrug resistance, and limited stability [1]. Therefore, maximizing payload delivery to intended cancer target cells while minimizing toxic side effects is essential for effective precision medicine. To achieve these goals, innovative alternative therapies must be utilized. DNA origami (DO) is a molecular self-assembly process that allows for construction of custom designed complex objects at the nanoscale. The process involves folding a long strand of scaffold DNA with several short DNA strands into a pre-desired shape. Among its many applications, DO engineered nanostructures can be utilized as a nanocarrier for chemotherapy drugs [2]. Here, we employ a previously designed rod-shaped nanostructure, referred to as the Horse [3], to selectively deliver chemotherapy drugs to only cancer target cells. CD37 is a transmembrane protein that is expressed on both myeloid and B cells and present an ideal target for Acute Myeloid Leukemia (AML) due to selective uptake due to robust internalization properties [4]. We hypothesized that incorporating 1 or 2 anti-CD37 antibodies (Abs) onto the edge of Horse-DO would target CD37⁺ AML leukemic cells only. Well-formed Horse-DO nanostructures and effective anti-CD37 functionalization with both 1 and 2 anti-CD37 Abs was confirmed with agarose gel electrophoresis (AGE) and transmission electron microscopy (TEM). AlexaFlour-647 labeled anti-CD37-Horse-DO nanostructures having both 1 or 2 anti-CD37 Abs showed time dependent fluorescence increases in CD37⁺ target THP1 AML cells, with an observed anti-CD37-(2)-Horse-DO mediated enhancement. Horse-DO and Horse-DO conjugated to 1 or 2 isotype control Abs showed no increase by both flow cytometry and fluorescent microscopy. This CD37 targeted delivery effect was not observed in CD37-deficient THP1 cells, both in single culture and under co-culture conditions with CD37⁺ THP1 AML target cells as shown by fluorescent and confocal microscopy. In addition, all versions of anti-CD37 targeted and untargeted Horse-DO showed effective daunorubicin(d) loading. Furthermore, (d)-loaded anti-CD37(2)-DO induced a significant reduction in relative cell viability in CD37⁺THP1 AML cells relative to d-Horse-DO and d-isotype(2)-Horse-DO, while the effect was not observed in CD37-/- THP1 AML cells at low daunorubicin concentration. Taken together, these findings

suggest that the anti-CD37-Horse-DO nanostructure elicits both targeted vehicle delivery in an antigen-specific manner, and targeted efficacy *in vitro* when loaded with daunorubicin. These proof-of-concept findings support the pre-clinical development of a CD37 targeted DO drug delivery system for the treatment of AML.



Figure 1. Schematics and Fabrication of Antibody-labelled DNA origami

A) Schematic of CD37 antibody-labelled origami targeting CD37 to facilitate cell uptake. B) Transmission Electron Microscopy (TEM) image of anti-CD37-Horse-DO design. (Scale bar 100 nm). C) THP1 (CD37+) and THP1(CD37-, GFP) cells targeted by anti-CD37-Horse-DO with AF-647 labelled staples. Image was taken through epifluorescence microscopy after 2 hours of incubation (Scale bar 10 μm). D) Flow cytometry analysis of THP1 (CD37+) cells with AF-647 labelled Horse-DO over time.

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Rapid Glioblastoma Diagnosis Using Glial Fibrillary Acidic Protein (GFAP) targeted multivalent aptamer nanoprobe

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glioblastoma (GBM), a prevalent primary malignant brain tumor, affects around 16,000 Americans annually.^[1] In a recent US-based prospective registry of central nervous system (CNS) tumors, glioblastoma accounted for 54% of brain gliomas, with an annual incidence rate of 3.19 per 100,000.^[2] Surgical removal is a crucial component of solid tumor treatment. However, the effectiveness of surgery hinges on the complete removal of cancerous cells. Incomplete excision can lead to a high risk of tumor relapse, representing a significant unmet need in cancer surgery outcomes. In this case, accurate imaging diagnosis is one crucial strategy during the surgical resection, given GBM's infiltrative nature and the necessity to conserve vital brain areas. Traditional intraoperative histopathological analysis, employing antibody staining methods in frozen sections, is effective but time-consuming. We propose aptamers as a more efficient alternative for glial fibrillary acidic protein (GFAP) staining due to their rapid binding and stronger specificity, as evidenced in multiple studies.^[3,4,5] Additionally, the incorporation of DNA nanotechnology^[6] to construct multivalent aptamers could further enhance binding affinity and signal intensity.^[7,8] This approach, we hypothesize, could markedly accelerate staining processes, providing a potential pathway for rapid and precise GBM diagnosis. In summary, this research investigates the application of trivalent aptamer-based staining for rapid GBM diagnosis, emphasizing the imaging biomarker GFAP (Figure 1).



Figure 1. Three aptamers targeted to the GFAP are loaded on a three-helix bundle (3HB) conjugated with Alexa Fluorophore 647 to construct a trivalent aptamer. GFAP is specifically overexpressed in GBM as a cytoskeletal component. By targeting GFAP, GBM cells could be stained in a fiber-like pattern, assisting the resection decision during the surgery.

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Integration of DNA Origami-Based Molecular Sensors for Real-Time Monitoring of Cell-Extracellular Matrix Interactions

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In this study, we present novel methodologies that leverages DNA origami technology for real-time comprehensive monitoring of molecular interactions in cell microenvironments within a physiologically realistic 3D extracellular matrix (ECM). Our approach encompasses two strategies of integrating DNA origami devices at specific sites within the microenvironment including the membranes of *cells* embedded within the ECM model to enable measurements directly at the cell surface and anchored to *collagen or fibrin throughout the hydrogel* to enable measurements over the entire microenvironment.

This work has taken significant strides to enable real-time detection and monitoring of cell-ECM interactions through DNA origami technology. We have previously established a robust method for anchoring DNA origami structures on the membranes of live cells across various cell types ⁽¹⁾. Building on this, we have recently introduced a novel approach for observing live cell interactions with environmental biomolecules within a 3D tissue model, facilitated by a microfluidic device (Figure 1 - left) ⁽²⁾. We developed a DNA origami device, capable of detecting two specific nucleic acid sequences, attached those onto the membrane of B cells (suspension) and dendritic cells (adherent) and demonstrated the multiplexed detection of biomolecules in real-time. These findings underscore the potential of combining live cells with DNA nanodevice-engineered membranes on microfluidic chips, offering a powerful biosensing method to explore subcellular interactions within physiologically relevant 3D environments and under controlled biomolecular transport conditions.

In the cellular microenvironment, the ECM network not only provides structural support but also influences various cellular processes, including migration and differentiation, via signaling molecules. This study also introduces an innovative approach that functionalizes collagen fibers with DNA origami constructs for the simultaneous measurement of multiple molecular targets within hybrid ECM hydrogels, effectively preserving the inherent properties of the collagen fibers. We confirmed the stability of DNA origami constructs attachments to the collagen-based hydrogels, ensuring their suitability for extended applications in antibodies. This advancement facilitated the creation of collagen fibers equipped with DNA-based nucleic acid sensors, enabling the specific detection of nucleic acid targets within collagen hydrogels (Figure 1 - Right). To expand the capability of these sensing modules to detect non-nucleic acid targets, we are currently developing hinge-shaped DNA-origami molecular sensor that incorporate aptamers for the precise detection of PDGF molecules in the ECM. In the future, these DNA origami sensors can serve as a general platform where the aptamers or other sensor constructs are interchangeable.

These methodologies not only offer a robust method to investigate live cell interactions in physiologically relevant 3D environments but also bridge the gap in detecting multiple molecular targets in hybrid ECM hydrogels without altering their inherent characteristics. This work sets a foundational framework for engineering cell, collagen, fibrin, and hybrid ECM model systems with DNA probes, facilitating the detection of various extracellular molecules and offering significant progress in diagnostics and drug discovery.



Figure 1: Left – Detection of biomolecular interactions with live dendritic cells in a 3D collagen matrix using a DNA origami sensing platform. Functionalized cells in collagen gel were seeded into the middle channel of the microfluidic device, and the side channels were used to introduce the flow of target molecules. The apertures between middle and side channels allow the connection of the target channels with the cell channel. Right - Engineered collagen fibers with DNA nucleic acid sensors. Schematic showing a microfluidic device setup for incubating fibers and introducing DNA targets. B) Confocal images of fibers with and without DNA targets. C) Cy5 and Cy3 intensity comparison, indicating target presence. Scale bars: 100 µm. ** and *** denote p-values of 0.01 and 0.001, respectively.

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Using silica beads to improve the quality of low-cost DNA origami nanoarrays

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 ${\sf N}$ anosphere lithography (NSL) leverages hexagonal packing of beads, such as polystyrene and silica beads, to produce a mask for the construction of nanoarrays for biophysical assays and diagnostics. 1 To achieve optimal throughput while precisely controlling the positioning of target molecules, it requires close packing, close to the diffraction limit of light. Recent studies have used polystyrene nanospheres to fabricate cleanroom-free cm-scale hexagonal DNA origami nanoarrays on glass surfaces at intervals of 0.4-1 μ m, without the need for traditional top-down fabrication methods.² Despite previous success in reducing the cost/chip by >2 orders of magnitude, NSL-based DNA origami nanoarrays suffer from multiple 100 nm DNA origami structures per spot due to the >100 nm patch size. Here, we report our attempts to decrease patch size to less than 100 nm using silica beads, with a Young's modulus of $\gtrsim 10$ GPa greater compared to ≤ 0.5 GPa for polystyrene beads. In turn, the smaller size of the sticky patch is expected to reduce the number of spots with multiple DNA origami structures. Using a self-assembled monolayer of 1 μ m silica nanoparticles as masks for NSL, we obtained periodic sticky patches at 1 μ m spacing. Each sticky patch is surrounded by a ring of hexamethyldisilazane (HMDS). The presence of surface-bound water within the interstices of the silica beads facilitated the formation of silane rings.³ The resulting array had inner ring diameters averaging 100 ± 20 nm, compatible with circular DNA origami structures. We anticipate an improvement in the maximum loading efficiency of single DNA origami onto the nanoarray compared to previous findings², thus facilitating the development of a next-generation DNA nanoarray platform for deterministic, high-throughput biophysical applications.

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Thursday April 25th, Morning Track on Biomedical Nanotechnology II Track Chair Thomas LaBean North Carolina State University

No pulling required? - Using DNA origami to decipher the molecular mechanism of Notch activation

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Our lab has explored the use of DNA origami to investigate the effects of ligand spacing and multivalency in biological signalling. By conjugating proteins or small molecules to staple oligonucleotides in DNA origami we are able to produce patterns of those ligands on the surface of our structures and then use these to look at receptor signalling (Shaw et al. Nat. Methods 2014, Wang et al. ACS Nano 2021) and antibody binding and clustering (Shaw et al. Nat. Nanotechnology 2019). Over the last years using a similar strategy, we have investigated the effect of multivalency on the Notch receptor.

We have produced nanoscale patterns of the Notch ligand Jag1 and then used these to stimulate neuroepithelial-like stem cells derived from human dermal fibroblasts (NES cells) from solution, not surface patterns. These induced pluripotent stem cells resemble the cells in the early neural tube – where Notch signalling is thought to be highly important during development.

Through the use of these stimulation experiments, where different types of Jag1-DNA origami nanopatterns are added to NES cells and via the use of molecular assays for investigating endogenous Notch activation (Proximity Ligation Assays, qPCR and mRNA sequencing), we uncover that Notch activation is dependent on multivalency in a manner that is inconsistent with the prevailing pulling-force hypothesis for Notch activation. Our data make us conclude that the Notch receptor possess another mode of activation that is dependent on the time of binding at the receptor.

We thus present how DNA nanotechnology can help us understand biological processes at the nanoscale, with experiments that would have been difficult to perform with classical methods and, at the same time, present how this method gives us new knowledge of previously unknown biophysical mechanisms of Notch activation.

Most of this work is published in: Smyrlaki, I., Fördős, F., Rocamonde-Lago, I. et al. Soluble and multivalent Jag1 DNA origami nanopatterns activate Notch without pulling force. *Nat Commun* **15**, 465 (2024). https://doi.org/10.1038/s41467-023-44059-4

Enhanced PSMA-targeting by Precision Control of DNA Scaffolded Nanoparticle Ligand Presentation

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Targeted nanoparticles have been extensively explored for their unique ability to deliver their payload to a selective cell population while reducing off-target side effects. The design of actively targeted nanoparticles requires the grafting of a ligand that specifically binds to a highly expressed receptor on the surface of the targeted cell population. Optimizing the interactions between the targeting ligand and the receptor can maximize cellular uptake of the nanoparticles and subsequently improve their activity. Here, we evaluated how the density and presentation of targeting ligands dictate the cellular uptake

of nanoparticles. To do so, we used a DNA scaffolded PLGA nanoparticle system to achieve efficient and tunable ligand conjugation. A prostate-specific membrane antigen (PSMA) expressing prostate cancer cell line was used as a model. The density and presentation of PSMA targeting ligand ACUPA was precisely tuned on the DNA scaffolded nanoparticle surface

Receptor-mediated Cell Internalization Efficiency



and their impact on cellular uptake was evaluated. It was found that matching the ligand density with the cell receptor density achieved the maximum cellular uptake and specificity. Furthermore, DNA hybridization mediated targeting chain rigidity of the DNA scaffolded nanoparticle offered ~3 times higher cellular uptake compared to ACUPA-terminated PLGA nanoparticle. Our findings also indicated a ~3.7-fold reduction in the cellular uptake for the DNA hybridization of the non-targeting chain. Finally, we showed that nanoparticle uptake is energy-dependent, and follows a clathrin-mediated pathway. Our results provide a rational guideline for designing actively targeted nanoparticles and highlight the application of DNA scaffolded nanoparticles as an efficient active targeting platform.

DNA Origami Nanoarrays for Dissection of Multivalent Cancer Cell Signalling

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Integrins play a critical role in cancer; they are transmembrane proteins that promote the contact of cells to the extracellular matrix (ECM) regulating cell migration and invasion¹. Around one-third of all solid tumours upregulate epithelial-specific integrin $\alpha\nu\beta6$, which has been associated with a worse overall survival rate in several malignancies. As $\alpha\nu\beta6$ functions partly to enhance receptor tyrosine kinases (RTKs) dependent breast cancer migration, invasion, and carcinogenesis, including HER2-dependent and EGFR-promoted breast cancers, it is important to find the spatial control of these integrin/RTK dependent biological activities at the molecular level^{2,3}.

We present a biomimetic platform with versatile and designable surfaces using DNA origami to achieve nanoscale resolution and multivalency of immobilised ligand spacing at the single molecule level to investigate spatial requirements for maximal cooperative integrin/RTK-dependent biological activities. We tested DNA origami covalently decorated with different numbers of an EGFR-aptamer and/or the avß6-specific peptide, A20FMDV2. When cancer cells were plated onto ligand-decorated DNA origami, we saw EGFR aptamer pairs induce the membrane proximity and phosphorylation of EGFR. Simultaneous engagement of $\alpha V\beta 6$ with A20FMDV2 promoted downstream signalling associated with EGFR, including increased phosphorylation of Erk and Akt, part of the Ras-MAPK and PI3K-Akt pathways. In cells, crosstalk between integrins and EGFR affects the activity, expression level, signalling and trafficking of both, that relays diverse intracellular signals mediating core processes such as cell survival, proliferation, spreading and migration.⁴ Our study highlights the significance of the precise nanoscale arrangement of integrin and RTK ligands required for the spatial control of integrin/RTKdependent biological activities at the molecular level. This strategy has great potential in cancer research and development of novel therapeutics.

Keywords: DNA origami, cancer cell signalling, single molecule, biomimetic nanoarray, spatial control, multivalency



Figure 1. Schematic of multivalent functionalised DNA origami in cancer cell-adhesion investigations.

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Multivalent, IgG-mimetic protein-DNA nanostructures for high-affinity binding to biomolecular targets

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Multivalency enables nanostructures to bind molecular targets with high affinity¹. Although antibodies can be generated against a wide range of antigens, their shape and size cannot be tuned to match a given target. DNA nanotechnology provides an attractive approach for designing customized multivalent scaffolds due to the addressability and programmability of the nanostructure shape and size^{2,3}. Here, we design a nanoscale synthetic antibody ("nanosynbody") based on a three-helix bundle DNA nanostructure with one, two, or three identical arms terminating in a mini-binder protein that targets the SARS-CoV-2 spike protein (Figure 1). The nano-synbody was designed to match the valence and distance between the three receptor binding domains (RBDs) in the spike trimer, in order to enhance affinity (Figure 1). The protein-DNA nano-synbody shows tight binding to the wild-type, Delta, and several Omicron variants of the SARS-CoV-2 spike trimer, with affinity increasing as the number of arms increases from one to three. The effectiveness of the nano-synbody was also verified using a pseudovirus neutralization assay, with the three-arm nanostructure inhibiting two Omicron variants against which the structures with only one or two arms are ineffective. The structure of the three-arm nano-synbody bound to the Omicron variant spike trimer was solved by negative-stain transmission electron microscopy reconstruction, and shows the protein-DNA nanostructure with all three arms attached to the RBD domains, confirming the intended trivalent attachment. The ability to tune the size and shape of the nano-synbody, as well as its potential ability to attach two or more different binding ligands, will enable the highaffinity targeting of a range of proteins not possible with traditional antibodies.



Figure 1. The design of protein-DNA nano-synbody. Structure of the three-arm nanosynbody, with three LCB1 proteins at the tips of its identical "arms." This strategic design enables the nano-synbody's arms to precisely align with the spatial arrangement of target binding domains (e.g. the RBD on the SARS-CoV-2 spike trimer).

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