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Self-Assembled Architectures and Devices

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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, molecular biology and molecular medicine and various engineering disciplines including MEMS.

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. FNANO 2020 follows in this tradition, featuring invited talks by distinguished nanoscientists in a wide-ranging schedule of talks and posters.

This year, the physical meeting had to be cancelled. We have instead created a virtual FNANO to allow us to continue to share our research despite the restrictions that are affecting us all. The format is simple – we are making available (universally) a programme of the conference and a ‘workbook’ containing all abstracts with embedded links to videos, slide shows and posters provided by the presenters (all abstracts not specifically withdrawn are included – not all have links). We hope that you will take advantage of this informal ‘virtual conference’ to maintain communication and encourage progress in our research community.

Please send notes of any problems in accessing the online presentations to fnano20@easychair.org – we will forward them to the presenting authors.

THANKS

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Special thanks to Camelia Pierson Eaves at Duke University for her excellent work behind the scenes.

*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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Track on
DNA Nanostructures:
Semantomorphic Science

Track Chair
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Self-replication, Exponential growth, Mutations, Directed evolution, Colloidal Architecture, and DNA Micro-Machines.

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Self-replication and evolution under selective pressure are inherent phenomena in life, but few artificial systems exhibit these phenomena. We have designed a process and a system of DNA origami tiles that exponentially replicate a seed pattern, doubling (or more) the copies in each diurnal-like cycle of temperature and UV illumination, producing more than 7 million copies in 24 cycles. We demonstrate environmental selection in growing populations by incorporating pH sensitive binding in two sub-populations. We also use DNA origami to self-assemble complex arrangements of colloids and emulsion droplets with highly specific geometry showing control over valence, position, dihedral angles, chirality and to make DNA micro-machines.

Objectives - Control of:
Valence
Particle Positions
Dihedral Angles
Bond Angles (cis-trans - arbitrary)
Chirality

Build Sequentially
Make and Fold Linear Structures

Activate

Self-Replication and exponential growth

Strategy
Use the Molecular scale control of
DNA Nanotechnology
Meta-DNA Structures

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https://www.dropbox.com/s/g8nk7zv13i2375o/Meta-DNA-Guangbao%20Yao.mp4?dl=0

DNA origami has emerged as a highly programmable method to construct customized objects and functional devices in the 10-100 nanometer scale. Scaling up the size of the DNA origami would create many potential applications, including but not limited to, metamaterial construction and surface-based biophysical assays. Recent efforts to enlarge DNA origami architectures have focused on the hierarchical self-assembly of the DNA origami through using of short sticky-end cohesion and/or blunt end adhesion. These techniques also involve geometric matching to form 2D patterns and 3D objects. Here, by using meta-scale building blocks to form more diverse and complex structures in the micrometer scale, we are able to mimic the molecular behaviors of DNA strands and their assembly strategies. We demonstrate that a 6-helix bundle DNA origami nanostructure in the sub-micrometer scale (meta-DNA) could be used as a meta-scale analogue of a single-stranded DNA (ssDNA). By programming the molecular self-assembly of meta-DNA, a wide variety of micrometer-sized DNA architectures have been obtained. First, we demonstrated that two meta-DNAs containing complementary “meta-base pairs” can form double helices with programmed handedness and helical pitches. Then, a series of sub-micrometer to micrometer scale DNA architectures, including meta-multi-arm junctions, 3D polyhedrons, and various 2D/3D lattices were constructed to show the versatility of the meta-DNA. Furthermore, we demonstrated a hierarchical strand-displacement reaction on meta-DNA to transfer the dynamic features of DNA to the meta-DNA. The meta-DNA is an ideal universal building block for scaling up DNA origami nanostructures due to several of its distinctive properties, such as programmable interactions, tunable rigidity/flexibility, controllable helical chirality, and versatile self-assembly directionality. This meta-DNA self-assembly concept may transform the microscopic world of structural DNA nanotechnology.
Fig. Schematics of DNA and Meta-DNA self-assembly structures.
Left: schematics of three DNA self-assembly strategies for creating DNA nanostructures. 1) Self-assembly of short strands; 2) Scaffold origami that contains many short strands and a single long DNA strand; and 3) single-stranded DNA origami that contains one long DNA strand with a designated sequence.
Right: Models of dsM-DNA that can be created as right-handed or left-handed helix. A 6-helix bundle DNA origami (blue) mimics the “backbone” of M-DNA. The different colored rods represent an array of 10-nt double-stranded DNA that mimic the “base pairs” between the M-DNA. Examples of sub-micrometer-scale structures based on M-DNA, including self-folded, self-linked, and self-assembled 2D and 3D structures.
Diverse recipes of DNA self-assembly

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Hybridization of complementary DNA segments have been utilized in DNA self-assembly to create all kinds of DNA nanostructures. A few self-assembly methods and a large collection of assembly building blocks have been introduced in the field. Phenomenal progresses have been achieved in upgrading size and complexity of programmable DNA nanostructures. In our development of applying molecular building blocks of different geometries and sizes, addressable structures in compact helices and wireframe architectures have been demonstrated. Notably, the 6-arm junction motif presented in one of the earliest blueprint of DNA nanotechnology, was eventually realized in our DNA self-assembly system. Watson-Crick base pairing is just one of the many types of binding force that can be utilized in DNA self-assembly. Besides, other guest molecules such as proteins can co-assemble in molecular self-assembly systems. In general, we present our recent efforts to try more diverse recipes of DNA self-assembly.
Triangulated wireframe structures: Polymerase-assisted gap filling, and assembly from single-stranded tiles

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Video of the talk: https://youtu.be/iQw2beg9Dgo

We have explored the possibility to synthesize the complementary sequences to single-stranded gap regions in DNA origamis cost effectively by a DNA polymerase rather than by a DNA synthesizer. For this purpose, wireframe DNA origami structures consisting of equilateral triangles were folded with single-stranded gap regions. This reduced the number of chemically synthesized nucleotides needed to determine the shape and size of the final structure by up to 66%. Optimized gap-filling reactions were completed in less than 3 min. The introduction of flexible gap regions in folded origamis resulted in fully collapsed or partially bent structures due to entropic spring effects. We demonstrated structural transformations with DNA polymerases including the expansion of collapsed structures and the straightening of curved tubes. We anticipate that this approach will become a powerful tool to build DNA wireframe structures more material-efficiently, and to quickly prototype and test new wireframe designs that can be expanded, rigidified, or mechanically switched.

Moreover, we extended the single-stranded DNA tile method to cover a range of anisotropic, finite, triangulated wireframe structures as well as one-dimensional crystalline assemblies. These structures are composed of six-arm junctions with a single double helix as connecting edges that assemble in physiologically relevant salinities. For a reliable folding of the structures, 2–4 nucleotides of single-stranded spacers have to be introduced in the junction connecting neighboring arms. Coarse-grained molecular dynamics simulations using the oxDNA model suggest that the spacers prevent the stacking of DNA helices, thereby facilitating the assembly of planar geometries.

Architectured metamaterials are engineered artificial systems with mechanical properties defined by their structure rather than chemical composition. They display unique auxetic behaviors that are distinct and different from those of regular materials. The difference is manifested by the Poisson’s ratio which measures the proportional decrease or increase in a lateral measurement to the increase in length in a material under elastic stretch:

\[ v = -\frac{\Delta y / y}{\Delta x / x} = -\frac{\varepsilon_y}{\varepsilon_x} \]

Auxetic structures exhibit negative values of Poisson’s ratio (thus, often termed negative Poisson’s ratio or NPR materials), whereas regular materials show positive values. This means that, for example, they can be compressed horizontally, when compressed vertically. This unique property may be exploited for energy absorption mechanisms and adaptive, reconfigurable materials with enhanced toughness and shear resistance.

Here we demonstrate two dimensional (2D) architectured metamaterials from DNA that exhibit negative Poisson’s ratio. We characterize their auxetic properties and investigate underlying mechanics. We constructed several auxetic nanostructures from DNA origami, including re-entrant honeycomb and re-entrant triangle. Given nanoscale dimensions, we implemented chemical loading (strand displacement) to demonstrate auxetic deformations. Simultaneously, a coarse-grained molecular dynamics (MD) simulations on the oxDNA platform was performed to study structural behaviors under external loads, determining mechanical properties such as Young’s modulus. We found that auxetic behaviors under chemical and mechanical loads are similar and qualitatively consistent, and that not only structure but chemistry also plays a role in behaviors of architecture metamaterials. Finally, we explored the classical elasticity theory to develop design guidelines for auxetic structures.

This work provides detailed insights into the mechanics of deformable DNA structures. Such understanding could be critical for constructing adaptable, self-healing materials via biomolecular self-assembly. Compared to other biomolecules, DNA-based approach should be superior in achieving structural complexity and controllability. Finally, with advent of strategies that can address the scalability, the findings from this study may open up new engineering opportunities.

Figure 1. (a) Theoretical Poisson’s ratio as a function of internal angle. (b) Atomic force microscope (AFM) images of a re-entrant triangle at two different angles. The structural
transformation is completed by strand displacement (chemical loading). (c) Coarse-grained MD calculations of a re-entrant triangle under mechanical loads, simulating auxetic behaviors in (b).
Self-assembly of nanotubes from distinct gamma-modified peptide nucleic acid oligomers in organic solvent mixtures

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Link to invited talk:

https://youtu.be/pZEbD4wZHXs

Protein and nucleic acid biomolecules are known to form an enormously diverse range of supramolecular assemblies in nature. Inspired by these natural ordered assemblies, self-assembly of peptides and DNA have emerged as two important branches of structural nanotechnology. While peptide structures offer chemical versatility and structural complexity, they lack the precise base-pairing and robust molecular recognition available with DNA self-assemblies. The sub-nanometer precision in self-assembly enabled by structural DNA nanotechnology, however, has been limited to aqueous or substantially hydrated media. Bridging the two worlds of peptide and DNA self-assembly using a synthetic building-block such as peptide nucleic acid (PNA) could combine the rational design of DNA with the chemical versatility of peptides. This work demonstrates the semantomorphic considerations in the design of 3-helix nanotubes using gamma-modified peptide nucleic acids (γPNAs) to form micron-scale filaments. In select polar aprotic organics solvent mixtures, γPNA self-assemble into ‘bundled’ nanotubes, and the conventional surfactant SDS can be used to reduce bundling and achieve a tight width distribution. Further, the morphologies of these γPNA structures can be tuned by means of solvent compositions and by strand substitution with DNA and unmodified PNA. This work introduces a science of γPNA nanotechnology and raises numerous questions around the potential for this material. As a manufacturing tool, PNA nanotechnology may be able to bring nanometer-scale programmability to polymer synthesis systems and may enable novel bottom-up manufacturing approaches to microsystems in organic solvents. We believe future work in the systematic investigation of structural motifs, backbone chemical modifications, and solvent conditions could extend the capabilities of nucleic acid nanotechnologies towards diverse nanosensing applications.
Figure: Semantomorphic considerations to the design of 3-helix γPNA nanotubes

A) Schematic comparison between a full helical turn of DNA and PNA double helix. B-form DNA is typically stable with 10.5 bases per helical turn while PNA has a helical pitch of 18 bases per turn. B) A schematic representation of the structural motif adapted from DNA SST design that shows 6-base domains and an overall repeat unit of 18 bases. γPNA oligomers are classified into contiguous (teal and red arrows) or crossover (yellow arrows) PNAs based on their position in the motif. Specific γPNA oligomers (P8 and P6) are labelled with fluorescent Cy3 (orange star) and biotin (purple oval) respectively, to enable detection of structure formation using fluorescence microscopy. 6-base domains correspond to 120° rise in helical rotation enabling the structural motif to program the assembly of 3-helix nanotubes that can polymerize lengthwise. C) TIRF (5μm scale bar) and TEM (100nm scale bar) images of (top panels) showing nanotube formation in optimized organic solvent conditions with ‘bundling’ of nanotubes in the absence of anionic surfactant SDS. TIRF and TEM images (bottom panels) show decreased bundling and tight width distribution of nanotubes in the presence of appropriate concentrations of SDS observed via fluorescence intensity and width profile measurements.
Track on
Biomedical Nanotechnology

Track Chair
Thomas LaBean

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Mimicking the form and function of natural systems remains a holy grail of materials science. To address the challenges of biomimetic materials synthesis, our group pioneered a spectrum of self- and directed-assembly methods that serve as a tool kit to fabricate synthetic structures with architectures rivaling the complexity of natural materials. In a review of published work, this lecture first discusses the assembly and function of nanosized cellular constructs referred to as protocells wherein mesoporous silica nanoparticles are loaded with diverse cargos and encapsulated within conformal supported synthetic or natural lipid bilayers. Using intravital imaging within the chick chorioallantoic membrane model, we demonstrate targeted drug delivery to individual disseminated cells of interest for treatment of leukemia and metastatic cancer. We then discuss the process of silica cell replication wherein all cellular features are transformed into exact silica replicas in a self-limiting process. The bio-composite replicas preserve indefinitely selective surface antigen binding and multiple protein functions in their native 3D contexts. Silicified red blood cell (RBC) replicas serve as a starting point for rebuilding completely synthetic RBCs via layer-by-layer polymer deposition, silica etching and fusion of native RBC membranes. Rebuilt RBCs can be loaded with multiple cargos and circulate much like native RBCs, providing an unusual example of a long circulating synthetic macroparticle. Silicified cancer patient cell replicas preserve cancer neo-antigens and serve as the basis for a personalized cancer vaccine.

Molecular probe recognition of intracellular and membrane bound components of A549 cell after silica cell replication, drying, room temperature storage, and buffered HF silica etching to re-reveal the biomolecular interfaces.
Viral sensing and inhibition with DNA star strategy
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Recorded talk link: https://youtu.be/ijKcbpSMDa0

Abstract: Many infectious diseases including viruses, bacteria, and toxins, present unique spatial patterns of antigens on their surfaces.2-4 These specific patterns facilitate multivalent binding to host cells, resulting in enhanced pathogenic infectivity. Based on this naturally occurring multivalent binding mechanism, synthetic multivalent entry blockers were previously introduced by linking epitopes-binding ligands to a scaffold to improve multivalent binding avidity.5-8 Recently, Kwon et al.9 carried out anti-influenza assays in mice to demonstrate that matching ligand spacing with the distance of viral epitopes is critical in inhibiting viral infection, while higher ligand densities have resulted in null or much weaker inhibition of influenza infection. However, existing scaffolds, which include polymers, dendrimers, nanofibers, inorganic nanoparticles and lipid nanoemulsions, have shown toxicity.10,11 Furthermore, some viruses, such as dengue, Zika and hepatitis viruses, present very complex geometric patterns of epitopes that are needed to match but cannot be addressed by existing scaffolds because they are not as precise in ligand spacing or provide limited control over the scaffold shape and ligand valency. A customizable molecular scaffold strategy capable of incorporating pathogen-specific ligands and patterns may address these issues on both therapeutic and diagnostic fronts.

DNA, when folded into nanostructures with a specific shape, is capable of spacing and arranging binding sites into a complex geometric pattern with nanometer-precision. Here we demonstrate a designer DNA nanostructure that can act as a template to display multiple binding motifs with precise spatial pattern-recognition properties, and that this approach can confer exceptional sensing and potent viral inhibitory capabilities. A star-shaped DNA architecture, carrying 5 molecular beacon-like motifs, was constructed to display 10 dengue envelope protein domain III (ED3)-targeting aptamers into a two-dimensional pattern precisely matching the spatial arrangement of ED3 clusters on the dengue (DENV) viral surface. The resulting multivalent interactions provide high DENV-binding avidity. We show this structure is a potent viral inhibitor and that it can act as a sensor by including a fluorescent output to report binding. Our molecular-platform design strategy could be adapted to detect and combat other disease-causing pathogens by generating the requisite ligand patterns on customized DNA nanoarchitectures.
DNA star sensor. 3’ overhangs on the DNA star allow aptamer incorporation. The 10 incorporated aptamers match the pattern and spacing of ED3 clusters. 5 fluorophore-quencher pairs along the inner pentagon of the star remain in a quenching FRET until binding interactions between aptamers and ED3 domains unzip the hairpins into ssDNA, enabling a fluorescent readout.

References:
Membraneless Organelles by Design

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Liquid-liquid phase separation (LLPS) of proteins is a fundamental process in living cells. It underpins the formation of functional, non-membrane bound, liquid-like compartments involved in cell function and development, such as the nucleolus and Cajal bodies, and many more. These membraneless organelles can separate and concentrate specific sets of molecules in a highly dynamic manner. They also exchange components with their microenvironments rapidly. Within the liquid droplet, proteins maintain or acquire a folded structure, and conserve functions such as specific protein recognition.

We are exploiting LLPS to generate designer membraneless organelles that recapitulate complex functions, and incorporating them within a synthetic protein-based cell, the "ProteoCell". This is a functional, lipid-free, 'living' cell in which proteins pick up the function of lipids to create multi-compartment 'ProteoCells' that can self-generate, house reactions to produce products, interact with other ProteoCells, and in total, define a new type of cell-like structure with fundamentally novel properties.

We aim to design organelles that incorporate desired enzymes by tagging them with intrinsically disordered sequences that can optimally solvate them, and colocalizing elements of reaction cascades. To guide the design of the LLPS tags, we are using a multiscale simulation approach that predicts the ability to undergo phase separation in response to variations in concentration, solution temperature and electrolyte concentration. The model simulates the behavior of micrometer-sized systems and captures experimentally determined temperature-dependent LLPS. We have demonstrated the incorporation of HPR in LLPS droplets formed by Ddx4 tags, and explored the reactivity of the enzyme within the droplet.
Single-Molecule Sequencing with Polymerase Nanocircuits

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Next-generation DNA sequencing has made rapid progress over the past decade, with single-molecule techniques enabling rapid advancements in the field. Still faster and cheaper DNA sequencing demands that technologists successfully merge nanoscale biofunction with the type of device density and scaling that is more typical of solid-state electronics. Recently, excitement has been growing about the potential for achieving this convergence with single-molecule electronics. Single-molecule devices with functioning proteins as the active element represent a new concept for recording biochemical activity. Multiple research teams have collected signals from individual proteins by linking biomolecules to nanoscale electrodes.

Figure 1 illustrates the concept of direct electronic readout from a molecule of DNA polymerase [1,2]. The active enzyme is tethered to a sufficiently sensitive electrode, depicted here as a single-walled carbon nanotube. In a physiological buffer solution of single-stranded DNA template and complementary nucleotides, DNA polymerase spontaneously processes the template into a double-stranded helix. Upon the successful formation of each new base pair, the enzyme’s activity induces dynamic fluctuations $\Delta I(t)$ in the underlying nanotube that are a detailed, real-time record of activity.

Once operating, a single polymerase molecule may be monitored continuously as it sequentially processes different DNA templates. We have followed single enzymes through $>10,000$ bond-forming events, providing new insights into their processivity, kinetic variability, sensitivity to genetic sequence, and tolerance toward synthetic dNTP analogs. Polymerases from four different organisms have been studied to test the generality of the technique, to compare and contrast signals generated by modest structural differences, and to determine the necessary characteristics for accurate sequencing. For example, $\varphi 29$ DNA polymerase is an enzyme with extremely high fidelity and processivity but a tendency for long, unpredictable pauses between bursts of activity. Taq DNA polymerase, on the other hand, is the premier commercial enzyme for polymerase chain reaction (PCR) because of its stable activity at 72 °C.

These hybrid bioelectronic devices are proving to be a versatile and reproducible new tool for single-molecule science and potentially a high-density, high-throughput DNA sequencing platform [3]. Furthermore, these hybrid solid-state devices have many advantages over more traditional single-molecule techniques. Single-molecule activity can be continuously monitored without fluorescent labeling or the oxidation and bleaching that accompany laser illumination. Electronic transduction can resolve sub-microsecond
dynamics [4], and temperature insensitivity allows high-temperature operation with thermophiles like Taq polymerase.

This research is supported by NIH/NHGRI R01-HG009188.

References

Figure 1. (A) Cartoon of a DNA polymerase duplicating single-stranded DNA template while attached to a carbon nanotube transistor. (B) Discrete current pulses occur with each nucleotide incorporation. (C) Detailed illustration of KF’s crystal structure and the movement of charged amino acids. Adapted from [2].
Track on
Chemical Tools for DNA Nanotechnology

Track Chair
Andrew Ellington
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Complex order in self-assembled nanocrystal superlattices

Michael Gruenwald, University of Utah

Watch the presentation at: https://vimeo.com/418322061

Self-assembly of nanocrystals into functional materials requires precise control over nanoparticle interactions in solution, which are dominated by organic ligands that densely cover the surface of nanocrystals. Recent experiments have demonstrated that small nanocrystals can self-assemble into a range of superstructures with different translational and orientational order of nanocrystals. The origin of this structural diversity remains unclear. In this presentation, I will discuss our recent efforts to understand the self-assembly of these nanocrystals over a broad range of ligand lengths and solvent conditions using molecular dynamics computer simulations. We show that small differences in nanoparticle shape, ligand length and coverage, and solvent conditions can lead to markedly different self-assembled superstructures due to subtle changes in the free energetics of ligand interactions. Our results rationalize the large variety of different reported superlattices self-assembled from seemingly similar particles and can serve as a guide for the targeted self-assembly of nanocrystal superstructures.
Dynamic Self-Assembled Hydrogels Crosslinked with Tunable, Orthogonal Reversible Covalent (TORC) Bonds

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https://www.arosalesgroup.com/fnanoposter

Tunable, orthogonal, reversible, and covalent (TORC) chemistries provide a hierarchical approach to many biotechnological applications, such as directed assembly, templated reactions, and biomaterial platforms. When incorporated into hydrogels, dynamic TORC bonds confer adaptability to the network, enabling rearrangement on the molecular scale that is dependent on bond lifetime and reaction kinetics. This rearrangement can be translated to several useful bulk properties, such as the ability to relax an applied stress or re-form crosslinks across an induced network defect. To assess their suitability as drivers for the self-assembly of soft materials with diverse properties, we have first investigated the use of a reversible conjugate addition to crosslink poly(ethylene glycol) (PEG)-based hydrogels (Figure 1A).

Two different conjugate acceptors were investigated for the ability to selectively control reaction kinetics and therefore hydrogel mechanics. Small changes to substituent groups at the reactive site can lead to large changes in the corresponding crosslinking kinetics. Specifically, a strong electron withdrawing nitrile group on the conjugate acceptor increased the forward reaction rate constant by nearly 20-fold, while the reverse rate constant increased by only 6-fold. This preferential tuning of the reaction kinetics allowed for an increase in the equilibrium constant due to the higher $k_1$. When attached to PEG macromers, these reactions readily enabled gelation within seconds. Excitingly,

![Figure 1. A) Reversible conjugate addition between the conjugate acceptor functionalized PEG and a 4-arm PEG-thiol. B) Plateau storage modulus of hydrogels crosslinked with either an unsubstituted (R=H, orange) or nitrile-substituted (R=CN, blue) benzalcyanoacetamide acceptor. C) Self-healing behavior of a hydrogel crosslinked via reversible conjugate addition.](image-url)
mechanical tests showed the difference in equilibrium translated to a nearly identical increase in the shear plateau modulus (Figure 1B). Because the reverse reaction rate constant did not change as much, bulk hydrogel properties that depend on \( k_r \) were also held relatively constant (e.g., stress relaxation behavior). Furthermore, the dynamic crosslinks enabled the hydrogel to rapidly and spontaneously self-heal (Figure 1C).

Overall, these results demonstrate that decoupling the kinetic rate constants of TORC bond exchange allows systematic control over dynamic hydrogel bulk properties, such as plateau modulus and stress relaxation. In addition, dynamic crosslinking with TORC bonds results in spontaneous self-healing behavior, which may be leveraged for the programmed assembly of other soft materials with diverse biotechnological applications.
Building star-like nanostructures with protein-dsDNA assemblies

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In DNA nanotechnology, to build DNA-based star-like nanostructures with large size (>100 nm) is challenging because requires complex design and self-assembly processes that demand a large amount of different short ssDNA oligonucleotides. Recently, we have developed a simple and economic approach based on DNA-protein assemblies, to prepare stiff and large branched nanostars. First of all, nanostar-like DNA templates with up to six arms were prepared by self-assembly of PCR-produced dsDNA fragments (211 to 722 bp) into a small DNA junction. Then, the assembled star-like duplex DNA fragments are subsequently coated with a virus-inspired protein based in the archaeal Sulfolobus sulfataricus Sso7d DNA-binding domain, which has a large stiffening effect on the dsDNA nanostar. The protein binds dsDNA with high affinity and not sequence specificity, largely reducing the high structural flexibility of naked dsDNA, thus creating a more structurally regular assembly. Furthermore, the protein coating significantly enhances the stability of the nanostars after incubation at high temperatures and protects them against nuclease degradation for more than 10 hours. This strategy opens a new way to prepare structurally defined hybrid protein–dsDNA nanostructures that could be exploited as building blocks for novel multivalent nanomaterials.

Link to see the presentation: https://youtu.be/UsznU0Tu_m8
Track on
DNA Nansystems: Programmed Function

Track Chair
Fritz Simmel

Technische Universität München
Munich, Germany
Microscopic Perspective on Programming Function in Biological and Man-Made DNA Systems

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Department of Physics, University of Illinois at Urbana-Champaign, USA

Spontaneous assembly of DNA molecules into compact structures is ubiquitous in biological systems and has emerged as a new paradigm for practical nanotechnology. The properties of such assemblies are programmed at the molecular level by the handful of atoms that differentiate the letters of the genetic alphabet. In this lecture, I will describe our efforts to elucidate the microscopic rules that govern DNA self-assembly in biological systems using a computational microscope. Further, I will describe how computer simulations are used to design synthetic molecular systems that reproduce and sometimes outperformed the functionality of biological machines.

A recorded version of the talk is available at: http://bionano.physics.illinois.edu/talks/fnano20/
Soft matter biophysics study of DNA capsules and DNA droplets toward micrometer-sized molecular robots

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To see the video of this talk, please visit http://takinoue-lab.jp/virtualfnano2020/

Molecular robotics is an interdisciplinary research field that aims to construct nanometer-/micrometer-sized dynamical robots made of organic polymer materials and biomaterials such as DNAs, proteins, and lipids. Molecular robots are expected to have molecular sensors, molecular computers, and molecular actuators in their body and to work in a tiny space in an autonomous manner, such as healing in human bodies, although the integration of those functions in one molecular robot body is still difficult. In our research group, to integrate multiple functions for molecular robots and to control those functions in a programmable manner, we have currently been challenging to construct micrometer-sized DNA capsules and DNA droplets, which will be shown in this presentation.

First, we will report a DNA origami nanoplate-based emulsion with designed nanopore function (Fig. 1) [1]. To date, many functional microencapsulation technologies including lipid bilayer vesicles and water-in-oil emulsions (droplets). Recently, nano/microparticle-stabilized emulsions called Pickering emulsion have been reported in the view point of functional microreactors. To extend the possibility, we constructed DNA-nanoplate-stabilized emulsion because DNA nanoplate produced by DNA origami method will have the design programmability, which will be expected as the basis of integrated multifunctional capsular microstructures for high-performance molecular robots in the future. Here, we generated hexagonal DNA nanoplate with about 100 nm in diameter and 2 nm in thickness using DNA origami technology (Fig. 1a,b). The DNA nanoplates were amphiphilized by the modification of the only one face of the hydrophilic DNA nanoplate with hydrophobic cholesterol groups. An aqueous solution of the amphiphilic DNA nanoplate was put into an oil phase of mineral oil, and then the two-phase solution was emulsified (Fig. 1c). By the microscopic observation, microemulsion stabilized with the amphiphilic DNA nanoplates was confirmed (Fig. 1d). Finally, we confirmed the ion transportation between contacted two droplets stabilized with the amphiphilic DNA nanoplates with a nanopore using a microdevice for ion current measurement. This result suggests that the integration of programmed function on DNA origami nanoplate-based emulsion (Fig. 1e).

Second, we will report a DNA droplet produced through liquid-liquid phase separation of a DNA nanostructure (named DNA Y-motif) solution (Fig. 2a,b) [2]. In this study, we found that the phase behavior of DNA Y-motif could be controlled based on the sequence design of the terminal sticky end of the branches of DNA Y-motif. Also, we found that the fusion of the liquid-like DNA droplets could be controlled based on the sticky-end sequence (Fig. 2c) and that the autonomous fission of DNA droplet could be achieved with enzymatic reaction (Fig. 2d). Finally, we demonstrated protein positioning in the DNA droplets based on the sequence (Fig. 2d). These results show that
the condensed soft matter phase of DNA nanostructure can be used as a molecular robot body that has the integration ability of functional molecules such as proteins.

We believe that these technologies will promote the construction of molecular robots with integrated functions. Also, in the future, by the combination with biomembrane technology such as a DNA cytoskeleton [3] and the integration with electrical control device such as a computer-controlled artificial cell reactor [4], the functional molecular robots will be helpful in many fields mentioned above.

![Diagram](image)

Fig. 1. DNA origami nanoplate-based emulsion with designed nanopore function [1]. (a) Conceptual illustration. (b) DNA origami nanoplate with a nanopore. (c) Photo of emulsion in a test tube. (d) Microscopic image of DNA origami nanoplate-based emulsion. (e) Investigation of ion transportation function of the nanopore using a microdevice.

![Diagram](image)

Fig. 2. Sequence design-based control of DNA droplets [2]. (a) Design of DNA Y-motif. Each branch has 16 base pairs (~5 nm). The terminal of each branch has an 8-base single-stranded sticky end. (b) Phase behavior of the Y-motif DNA: dispersed phase, liquid-like droplet phase, and elastic gel phase. (c) Fusion of DNA droplets. (d) Fission of DNA droplet with enzymatic reaction. (e) Selective positioning of proteins.

References


Cell motility is a crucial function that gives rise to various complex biological processes such as embryogenesis and wound repair. Chemotactic cell migration on surfaces, in particular, plays a critical role in immune response. For example, neutrophils chase bacteria by sensing biochemical signals and crawling with a sustained increase in speed (i.e. chemokinetic response), and ultimately engulf them via phagocytosis. Recently, synthetic minimal cells were developed as a versatile platform for advancing fundamental understanding in biology as well as for creating novel biomedical applications including drug delivery and chemical microreactors. Engineering cell-mimicking functions such as directed motility in synthetic protocells will thus provide new opportunities. While various molecular machinery was developed to create biomimetic functions in synthetic protocells, few mechanisms have been proposed for control of cell migration and chemotactic motility.

Here we demonstrate dynamic synthetic vesicles (SVs) capable of chasing one another on two dimensional (2D) surfaces by programming DNA components. As a programmable material, DNA has been engineered for generating synthetic molecular systems such as nanostructures, affinity reagents, motors, and logic gates. We show that directed motility in DNA functionalized SVs can be achieved by combining toehold switchable oligonucleotides with signaling strands and that the ‘follow’ vesicle recognizes the path that the ‘lead’ vesicle has travelled and tracks the trajectory with enhanced speed. To demonstrate such synthetic chemotaxis, we first self-assembled vesicles using phospholipid-oligonucleotide conjugates whose sequence contains our motility designs. The vesicle has an average diameter of ~200 nm and decorated with multiple DNA walkers such that it can migrate on an RNA fuel decorated glass coverslip.

Figure 1 illustrates our mechanism for directed and chemokinetic motility using signaling oligonucleotides. The surface RNA consists of two fuel domains for lead (grey) and follow (blue) vesicles. The two fuels contain 10-nt complementary segments and initially form a hairpin structure (state (i)). An 8-nt toehold is available for binding the lead SV (state (ii)). The hybridization of the lead SV activates the RNA switch through toehold mediated strand displacement and exposes the follow fuel domain. After fuel activation, a nuclease will find and cleave the RNA fuel such that the vesicle will thus explore the fuel surface and migrate toward new unexplored regions with intact RNA fuels (state (iv)). After lead SV movement, the follow vesicle (green) will hybridize to the now-activated fuel and start migration (state (v)). The follow vesicle is designed with shorter DNA walkers than the lead vesicle so that the follow SV will chase the lead SV with increased velocity on track. The most critical step in this process is that the follow vesicle will have a strong bias to stay on the activated fuels such that it will move only along the path that the lead SV has explored, thus chasing the lead vesicle (state (vi)).

Figure 2 shows our experiments where the lead vesicle first demonstrates autonomous migration dynamics powered by nuclease, exhibiting average velocity of ~1 nm/s. After lead vesicle migration, follow vesicles were introduced and randomly searched for activated fuels. If a follow vesicle intersected the lead SV’s path as its initial position, it started to follow the track. Note that
for follow vesicles that did not intersect with lead vesicle paths, no significant movements or speed were observed as all RNA fuels were inactive for follow SV migration. The follow SV in tracking experiments starts randomly on the lead SV trajectory and can move towards either direction. Once it migrates in a particular direction, it will continue to trail the path, because its motion outside the path will be suppressed. The trajectories show that the follow vesicle moved in the opposite direction on the lead vesicle path.

Further analysis confirms the follow vesicle’s designed chemokinetic behaviors. In natural chemotaxis, neutrophils for example, chemokinetic motility plays a critical role in chasing and eliminating bacteria. In the path tracking experiment, the follow vesicle migrated with significantly greater velocity (increased by ~50%) than the lead vesicle. This results from our DNA motif design as the follow vesicle has a shorter walker length (15-nt) than the lead vesicle (18-nt). To the best of our knowledge, this is the first demonstration of DNA programmed coordination between two migrating vesicles. This work opens new possibilities for DNA programmable biochemical communication and coordination among protocells. The extensive library of dynamic DNA systems may allow researchers to create complex behaviors in synthetic cells that could ultimately match the complexity of their natural counterparts.

**Figure 1.** (a) Schematic of an engineered vesicle with DNA programmable motility on 2D surface. (b) A follow vesicle (green) chases a lead vesicle (red), enabled by switchable RNA fuel, which contains two fuel domains (gray and blue) for lead and follow vesicles. The follow vesicle fuel is initially shielded in a hairpin structure (i). A lead vesicle first associates with the lead fuel (gray) (ii) and activates the fuel through strand displacement (iii). After the lead vesicle migrates toward adjacent unexplored fuel area (iv), the follow vesicle will then find the activated fuel (v) and experience a strong bias that forces it to move only along the trajectory of the lead vesicle, thus demonstrating the path tracking behavior (vi).

**Figure 2.** Programmed chemotactic vesicle migration. The trajectories of lead (red) and follow (green) vesicles as a function of time and dimensionless time, Fourier number, $Fo = Dt/r^2$ where $D$ is a diffusion coefficient, $t$ is time, and $r$ is the vesicle radius.

**References:**

Transmembrane signal transducing DNA nanosensors for intact membrane enclosed biomarker detections

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Click here or visit https://youtu.be/J77JyjR6olw to view the recorded talk.

Signal transduction across phospholipid bilayer allows cells to respond to their external environment and communicate with neighboring cells. Trans-membrane signal transduction by highly-evolved membrane proteins such as G-protein coupled receptors (GPCR’s) play crucial role in regulating physiological processes such as proliferation, differentiation, metabolism and apoptosis.\textsuperscript{1} De novo engineering of the aspects of the cellular signal transduction machinery with artificially designed molecular devices has enormous opportunity in synthetic biology for potential application in artificial tissue signaling, biosensing, and controlled drug delivery.\textsuperscript{2}

In principle, GPCR’s consist of a dynamically-reconfigurable hydrophilic–hydrophobic–hydrophilic (Hi–Ho–Hi) molecular structure where the hydrophobic part is buried in the lipid bilayer while the two hydrophilic ends remain on the two sides of the bilayer. Membrane spanning DNA nanostructures have been demonstrated previously\textsuperscript{3} that mimic several type of membrane proteins such as ion channels, membrane sculpting proteins or lipid flippase. Here, we demonstrate Transmembrane signal transduction by a Transmembrane Nano Sensor (TraNS) DNA nanodevice. Our TraNS device inserts through lipid membrane and dynamically reconfigures upon sensing a membrane-enclosed DNA or RNA target, thereby transducing biomolecular information across lipid membrane. Four
interwoven DNA strands are self-assembled to form the scissor-shaped nanostructure of the TraNS device. Hydrophobic cholesterol anchors are covalently conjugated to each nanostructure to create a hydrophobic belt around it, similar to the $Hi-Ho-Hi$ molecular signature of GPCR’s, that helps it to span the lipid bilayer. Using the precise design principles of DNA nanotechnology, we design the TraNS device such that after insertion to lipid bilayer, it selectively switches from a closed to an open state upon sensing a nucleic acid target encapsulated inside the membrane. Gel electrophoresis and fluorescence spectra confirms the formation and configurational switch of the TraNS device. Current efforts are focused on employing the TraNS device for biosensing application by detecting the presence of non-small cell lung cancer specific micro-RNA – miR-21-5p in exosomes.

References

Track on Synthetic Biology

Track Chair

Alex Deiters

University of Pittsburgh

Pittsburgh, Pennsylvania
Synthetic Genetic Systems for Rapid Mutation and Continuous Evolution in vivo

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Abstract: We are interested in building genetic systems that have extremely high mutation rates in order to speed up the evolution of target proteins and enzymes in vivo as well as to record transient information, such as lineage relationships or exposure to biological stimuli, as durable genetic information in situ. I will primarily discuss our work on building OrthoRep, a highly error-prone orthogonal DNA replication system that mutates user-selected genes at a rate of 1e-5 substitutions per base (s.p.b.) without any increase in the genomic mutation rate (1e-10 s.p.b.). This ~100,000-fold mutational acceleration allows for the rapid continuous evolution of target biomolecules entirely in vivo using a simple serial passaging process amenable to extensive repetition. I will discuss the application of OrthoRep in exploring drug resistance, studying protein evolution, and evolving useful enzymes and proteins. I will also comment on the value of deep and scalable continuous evolution in searching for and understanding old and new biomolecular function going forward.

To access this talk, please follow this link:

https://youtu.be/s7JuStV7u0U
Stimulus-responsive self-assembly of protein-based fractals by computational design

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

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

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Membrane transport proteins mediate the exchange of ions, small molecules, and macromolecules across the cell membrane. These important cellular gateways are involved in signaling, neurotransmission, metabolism and nutrient uptake across the tree of life. Despite their fundamental biological importance, the study of membrane proteins using directed evolution and deep mutational scanning remains challenging due to their requirement for the lipid membrane environment. We have developed a cell-free platform for high-throughput screening of membrane transport proteins. Our approach uses microfluidics to generate monodisperse giant unilamellar vesicles containing transporter genes and cell-free protein expression reagents. We apply our screening platform to analyze all mutants in the de novo designed transporter Rocker and identify a unique variant with enhanced kinetic properties. Our approach provides a general framework for high throughput experimentation of membrane proteins, including deep mutational scanning and directed evolution.
Track on
Principles and Theory of Self-Assembly

Track Chair
Rebecca Schulman
John Hopkins University
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Colloidal crystal metallicity

Monica Olvera de la Cruz, Northwestern University, m-olvera@northwestern.edu

Talk at https://northwestern.app.box.com/file/661470460083

Abstract: Colloidal crystals have been extensively designed using classical atomic models. In particular, numerous compounds have been devised with DNA-functionalized nanoparticles following design rules akin to ionic crystals, where individual colloids behave as programmable atom equivalents (PAEs) in superlattices. Here we show PAEs that behave as electron-equivalents (EEs) when their sizes and DNA grafting density are reduced [1]. In mixtures with large PAEs, the EEs functionalized with complementary DNA strands to the large PAEs, roam the crystal holding the large PAEs in specific lattice sites, akin to electron clouds in atomic metals (Fig. 1). As the number of strands increases or the temperature decreases, the EEs localize. Continuous and discontinuous localization-delocalization transitions are observed. Asymmetric in charge and size mixtures of oppositely charged particles also undergo a localization-delocalization transition as the temperature increases that resemble sublattice melting in atomic superionics [2]. This electron-atom-equivalent duality produces metallic, intermetallic and compound phases, and lays the foundation to explore colloidal metallic alloy analogues.

Figure 1. (A) Snapshots from the molecular dynamics (MD) simulation depicting “ionic” bonding behavior (top) and “metallic” bonding behavior (bottom) [1]. (B) MD simulation of PAEs with 150 DNA linkers hold in crystal positions by EEs with eight complementary DNA linkers at a 6:1 ratio of EEs:PAEs (Credit: Martin Girard).

References
Feedback regulation of crystal growth with monomer buffering

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Molecular self-assembly \textit{via} crystallization is a powerful technology that can produce millimeter- or larger-scale order from atomic or molecular components. However, crystal growth is highly sensitive to conditions such as temperature and monomer concentration. To control the types of crystals that grow, seed crystals are often used but there is typically a narrow range of monomer concentrations that favor isothermal growth from nucleating seeds and monomer depletion during growth eventually causes growth to halt if monomers are not replenished. Living systems can sustain crystal growth by using chemical reactions to regulate active monomer concentrations, enabling robust growth even as physical conditions vary. For example, the concentrations of active tubulin monomers are tightly controlled by the cell during microtubule self-assembly \textit{via} reactions that regulate tubulin turnover and availability. In addition to enabling robust microtubule growth, the regulation of active tubulin concentrations exhibits feedback to ensure that growth continues even as the number of microtubule growth fronts (load) changes over time. The ability to implement feedback regulation \textit{via} chemical reactions in synthetic crystallization processes could make it possible to achieve robust growth and sustain crystallization during complex hierarchical assembly processes with time-varying loads.

Here we develop a chemical reaction network that regulates crystallization by buffering monomer concentrations during crystal growth. Like a pH buffer that regulates hydrogen ion concentration, monomer buffering is achieved with a set of reversible reactions whose equilibrium dictates the setpoint monomer concentration. As the monomer buffering reactions are in dynamic equilibrium, Le Chatleier’s principle provides feedback that resists changes to the setpoint concentration during growth. To study regulation of crystal growth \textit{via} monomer buffering we use seeded DNA nanotubes as a model system. To buffer DNA nanotube monomer concentrations during growth, we adopt a DNA strand displacement reaction network that facilitates the reversible exchange of active and inactive monomers. Through simulations and experiments we demonstrate that regulating nanotube growth with monomer buffering reduces sensitivity to monomer depletion effects by maintaining the monomer concentration within a seeded growth regime during crystallization. Monomer buffering is able to enhance growth capacity by an order of magnitude compared to unregulated growth and, through feedback, adapt to changes in load over time. This work introduces a new paradigm for regulating crystal growth through chemical feedback that controls the flow of chemical potential during growth. Further, the simplicity of the buffering mechanism suggests it should be generalizable to a range of chemical processes.
Bronze, Iron, Plastic age, how will the next material revolution look like?
Nikolaus Correll, University of Colorado at Boulder

Talk online: https://youtu.be/nrvkrOGcz5A

We are observing increasing miniaturization of computation, tighter integration of functionality into volumes of decreasing size, and a trend across all material science disciplines – from basic physics/chemistry to composite materials – to integrate computational processes inside materials. While such materials are a consequence of the “silicon age”, I argue that there is opportunity for a completely novel class of materials that are, akin to natural systems and in contrast with engineered materials that are homogeneous or composites, engineered from a small number of identical cells. Such cells could take the role of sensors, actuators, computation, communication or structure and interact with each other and the environment to create a new level of multi-functionality. This idea is not new and has been explored in architecture (“programmable matter”, Goldstein, 2005), in art (“mutant materials”, Antonelli, 1995), in computer science (“amorphous computing”, Abelson, 1995), in design (“radical atoms”, Ishii, 2012), and robotics (“morphological computing”, Pfeifer, 2006, “robotic materials”, Correll, 2017). Available as a powder, they could be dissolved into liquids or rubbers, and repairing a structure would simply require to replace those parts of a structure that are actually broken.

Computation will become an important part of future material systems. Computation will allow materials to analyze, change, store and communicate state in ways that are not possible using mechanical or chemical processes alone. What “computation” is, when a mechanical or electrical process is “computation”, and what its possibilities are, is unclear to most material scientists, while computer scientists are largely unaware of recent advances in so-called active and smart materials. For example, a nervous system is a critical part of natural material systems such as an octopus arm, a cuttlefish skin or even a bone, but it is hard to see – in the absence of an interdisciplinary definition of the following terms – what is computed and communicated. Yet, everything that can be computed can also be achieved by smart arrangement of mechanical processes, an insight rooted in the concept of Turing universality of mechanical computers. Here, a dialog and formal understanding of what is physically possible, e.g. stimulus-responsive “smart materials” and DNA computers, and how an abstract treatment of these concepts allows for mathematical reduction might enable the creation of new materials with unprecedented functionality without requiring von Neumann architectures.

This problem is currently being attacked from multiple frontiers. On one end, computer scientists have embraced neural networks and material scientists actively researching novel substrates such as memristors and other neuromorphic computing devices. On the other end, computer scientists have understood to leverage the computational and kinematic abilities of DNA, with biologists and chemists contributing novel tools to realize such computing and robotic systems. Looking back at biological multi-cellular systems these two processes are at different orders of magnitude: DNA doubles as memory, code and actuator for the cellular “operating system”, with “neurons” a particular class of cell that is able to perform high-level computation in concert with others.

There are two fundamental questions that might be of interest to the DNA self-assembly community: First, how can we close the gap between the multiple orders of magnitude of operating system-level DNA computing and meso-scale cells that can provide macroscale
functionality like sensing, actuation, computation, communication and energy metabolism, and second, “how can basic building blocks achieve complex functionality such as a cell, an ant, or a person, out of exclusively physical interactions?” In addition, through the lens of DNA self-assembly, this problem can be tackled at different levels of abstraction and has received considerable interest from the computing community in the subfields of “swarm intelligence” (Bonabeau, 2001) and “swarm robotics” (Brambilla, 2013). Which problems are most relevant today is closely tied to what kind of sensors, actuators, and finally building blocks can reasonably be manufactured now and in the future. Here, the playing field is still wide open as we still have even not fully understood the relationship between local rules and the resulting global behavior of most social insects, despite their numbers being comparably low (when compared to the number of cells in a small mammal, for example), their communication graph being sparse (when compared to the number of synapses connecting to a single neuron in the brain) and them being observable with the eye. Making inroads toward the so-called “global-to-local problem”, however, might not only allow us to design intelligent objects from a large number of identical building blocks, but also help to lift our understanding of the world around us to a new plateau.

References


Pattern recognition in the nucleation kinetics of molecular self-assembly

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Phenomena in nature often exhibit computational behavior, and many processes have been used in constructed systems to explore the computational ability of basic molecular processes. One example is algorithmic self-assembly, which explores how information can be stored and processed during the self-assembly of tile-like molecular components. Initial theoretical investigations showed how cellular automata, formal languages, and Turing machine computations could be implemented\textsuperscript{1–4} through the specific programming of tile attachments during the growth of assemblies, and compelling experimental demonstrations of computation within crystalline growth have been successful.\textsuperscript{5–7} Recent theoretical investigations have identified a very different mode of information processing: how concentration-biased nucleation processes can select among a multifarious variety of shapes consistent with a set of tiles.\textsuperscript{8,9} Here we establish the reality underlying these theories by demonstrating a DNA tile assembly system that uses nucleation to reliably recognize and classify a series of 18 grayscale images, as well as degraded counterparts. The process, which operates through the stochastic exploration of possible nuclei, takes as input a pattern of monomer concentrations, and, rather than being dependent upon the concentrations of single monomers, depends upon the patterns, and co-localization, of tiles in many possible nuclei. As such, the form of computation is particularly suited to pattern recognition, and bears similarities to (having been inspired by) neural networks such as Hopfield Associative Memories.\textsuperscript{10}

We constructed a system of 917 DNA single-stranded tiles (SSTs) that assemble into three different shapes (Fig. 1(a)), each positioning a set of 317 shared tiles in different arrangements through the use of programmed glues and 600 tiles that each appear in only one shape. When slightly below the melting temperature of the shapes, the initial nucleation process requires a number of energetically-unfavorable steps, growing small fragments of each shape until a sufficiently large nucleus has grown to allow favorable growth. The system’s exploration of small, unfavorable fragments is the limiting step to growth of complete shapes. Thus, while the complete shapes have different thermodynamic properties, if all tiles are at equal concentrations, the pathways to nucleation of each shape will be similar, and comparable numbers of each structure will grow.

Figure 1: The multifarious self-assembly system of 917 tiles, at equal tile concentrations, will nucleate and grow three distinct shapes in comparable quantities (a), with 317 shared tiles (blue) appearing in different locations in different shapes, and 600 shape-specific tiles (beige) that appear only in one shape. However, by changing the 917-dimensional vector of initial tile concentrations (b), the kinetic barrier to the nucleation of each shape can be tuned such that the system will have a strong preference for nucleating a particular target shape (c).
However, the favorability of the myriad pathways to nucleation of each shape will depend on the concentrations of tiles that can bind to form small fragments of the structures: fragments that contain more high concentration tiles will have tiles attach more frequently, and may serve as nuclei that reduce the kinetic barrier to nucleation for the corresponding shape. As such, the nucleation process of the multifarious system naturally performs a form of pattern recognition on the 917-dimensional vector of initial concentrations, translating the input pattern into different nucleation rates for the three possible structures.

To explore the pattern recognition capabilities of DNA tile nucleation, we collected 18 30×30 grayscale images. Pattern recognition was trained in silico, without changing tile sequences, by choosing the map from locations on the 30×30 input canvas to the system’s 917 tiles. Using a simple optimization algorithm, we tuned the mapping so that each image corresponded with a concentration vector where high concentration tiles tended to be clustered in the assembly corresponding to the first letter (H, A, or M) of what the image represented. Using quenching of fluorescent tile probes during narrow temperature ramps in a quantitative PCR (qPCR) machine, and counts of final assemblies via atomic force microscopy (AFM), we determined that the system correctly recognized all 18 training images, and all but one of the 12 degraded training images.

Our results demonstrate that, in addition to the widely-studied computational abilities of crystal growth, the nucleation of crystal structures can also perform computation. The computation, and determination of what structures will nucleate, is carried out by simple interactions of many monomers, and is highly dependent not on the concentrations or properties of individual monomers, but on the patterns of concentration and interactions, thus allowing the system to naturally perform a form of pattern recognition. The computational capacity of nucleation, a strong model of the process, and further application of the ability nucleation processes to stochastically explore large state spaces, remain to be explored.

(1) Winfree, E. In DNA Based Computers, 1996, pp 199–221.

Figure 2: 18 training and 18 test images (a) were translated using the learned mapping to initial tile concentrations (examples in b). In a 150 hr temperature ramp from 48 to 45°C in a qPCR machine, we observed the pattern recognition process via fluorescent labels on each shape (c), then counted assemblies in AFM images (d). Analysis of quenching times and counts of final assemblies showed that our system recognized all training images, and most test images (e).
Special Track on Encapsulated Systems

Track Chair

Jack Szostak

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Nonenzymatic copying of RNA into phosphoramidate DNA inside model protocells

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https://www.cs.duke.edu/FNANO/SzostakKeynote.mp4

Copying mixed-sequence oligonucleotide templates nonenzymatically is a long-standing problem both with respect to the origin of life, and with regard to the synthesis of artificial living systems. We have recently found that RNA templates can efficiently direct the synthesis of a complementary strand composed of N3′→P5′ phosphoramidate DNA (3′-NP-DNA) using 3′-amino-2′,3′-dideoxyribonucleotides activated with 2-aminimidazole. Using only the four canonical nucleobases (A, G, C, and T) of modern DNA, we demonstrate the chemical copying of mixed-sequence RNA templates up to 25 nucleotides long, both in solution and within fatty acid vesicles. In an exciting recent development, we have been able to demonstrate primer extension with displacement of a pre-existing strand that is complementary to the template. This strand displacement synthesis is catalyzed by short oligos that open up the sequential regions of the template by cycles of toehold binding and branch migration. Our current efforts are aimed at demonstrating repeated cycles of replication of oligonucleotides long enough to encode active ribozymes inside model protocells. If that can be accomplished, it may become possible to synthesize artificial, evolving cellular systems.
DNA nanotechnology enabled membrane engineering

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Lipid-bilayer membranes form barriers to define the boundaries of a cell and its subcellular compartments. They undergo modulated structural changes and mediate biochemical reactions to sustain the cell’s life cycle. Inspired by such elegance in nature, engineers and biologists have aspired to build artificial membranes to recapitulate the cellular membrane structure and dynamics. Such in vitro preparations provide a complexity-reduced system for the study of functional interactions between membranes and their associating molecules. Here I present our technical innovations in programmable, high-precision membrane engineering. Our general approach is to use self-assembled DNA nanostructures as templates to guide the assembly of lipid bilayers and transduce the programmable feature of the DNA nanostructures to the templated vesicles. We show the assembly, arrangement, and remodeling of liposomes with designer geometry, all of which are exquisitely controlled by a set of modular, reconfigurable DNA nanocages, giving rise to membrane curvatures present in cells yet previously difficult to construct in test tubes. Incorporating membrane-interacting proteins into these DNA-templated liposomes allows us to systematically study protein-mediated membrane dynamics, such as SNARE-mediated membrane fusion and extended synaptotagmin-mediated lipid transfer.

In addition to “growing” liposomes inside the DNA templates, we have developed a DNA-brick assisted sorting method to generate liposomes with a wide range of uniform sizes and chemical contents. The sorting technique presents a scalable way to prepare milligrams of monodispersed liposomes while preserving the membranes’ preexisting features. In proof-of-concept experiments, we show the curvature-sensitive activities of two membrane proteins (SNARE and ATG3) using sorted liposomes with a fine gradient of sizes.

5. Yang et al. Sorting liposomes of distinct sizes by DNA-brick assisted centrifugation. BioRxiv 2020.02.01.930321
Initiating biological reactions via DNA-mediated vesicle fusion

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Bilayer vesicles have great potential to encapsulate and control biological reactions in complex environments. As synthetic vesicles continue to be designed with increasingly complex activities, such as housing protein expression systems, an important question has arisen about how to control the progress of such reactions. Vesicle fusion driven through membrane bound DNA tethers is a promising route to control fusion and content mixing between specific vesicles. In this talk, I will demonstrate how DNA-mediated vesicle fusion efficiency may be increased by introducing liquid-ordered and disordered lipid phases into vesicle membranes. We further explored the extent to which hydrophobic mismatch between lipid phases enhances vesicle fusion kinetics. Lastly, we showed that vesicle fusion between DNA-tethered vesicles can be used to deliver and mix cargo required for in vitro protein expression by synthesizing a model soluble and membrane protein. This study illustrates how membrane biophysics can be used to enhance DNA-mediated fusion events and advances our capabilities in controlling localized reactions, an important step in developing more complex vesicle-based systems.

Complementary DNA tethers promote fusion of specific vesicles, allowing for the initiation of cell-free expression reactions.
Track on
Nanophotonics and Superresolution

Track Chair
Ralf Jungmann

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Super-resolution Microscopy to Investigate Cellular Organization at the Nanoscale

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Cell biological research relies heavily on the capabilities of light microscopes to resolve structures or processes of interest at the nanoscale [1]. My research group is developing super-resolution technology which achieve down to about 20 nm resolution in 3D and applies them to cell biological questions. We are, in particular, focused on improving the 3D resolution, speed, live-cell compatibility, throughput and depth penetration of STED and PALM/STORM microscopes [2, 3].

In my presentation, I will provide an overview of recent developments in my lab. I will highlight our development of multicolor 4Pi-SMS imaging at 20 nm 3D resolution and live-cell applications of STED microscopy to investigate the nanoscale organization of the endoplasmic reticulum [4] and other cellular organelles.

I declare financial interests in Bruker Corp. and Hamamatsu Photonics.

Chiral assembly of DNA-coated Au/Ag core-shell nanorods on DNA origami with strong optical properties


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Video link: https://www.youtube.com/watch?v=mcysrkBD8Mo

DNA has proven to be an excellent choice of molecule for programmable self-assembly and single-molecule sensing. DNA self-assembly has surpassed its early stages and today is routinely used for constructing functional nanodevices and materials. By defining active sites on the DNA origami structures, we have realised a variety of sensors, including autonomous force spectrometers and highly sensitive plasmon-based detectors of virus-derived RNA and proteins.

We here present a reliable method to synthesize highly stable silver nanoparticles (AgNPs) and Au/Ag core/shell nanorods (NRs) that are conjugated in a one-pot reaction with functional ligands such as thiolated DNA. Our particles are stable for months in buffers containing sodium or magnesium ions, allowing for direct incorporation of the particles in DNA self-assembly applications. Figure 1a shows a negative stain transmission electron microscope (TEM) image of two Au/AgNRs that are mounted on two sides of a 44 helix-bundle DNA origami structure. The dense layer of single-stranded DNA (ssDNA) that is coating the NRs can be seen as a light “halo” around the NRs.

Figure 1a TEM image and schematic illustration of DNA-functionalized Au/AgNRs conjugated with a 44-helix-bundle DNA origami. Scale bar = 50 nm. b Absorption spectra of Au/AgNRs@DNA synthesized with different AgNO3 concentrations (1, 3, 5, 7.5, and 10 mM) resulting in distinct Ag-shell thicknesses.
Owing to their superior plasmonic properties, silver particles and rods perform a variety of tasks substantially better than their golden counterparts. By arranging pairs of Au/AgNRs with exceptionally high yields on DNA origami structures we implemented devices for plasmonic chirality sensing and surface-enhanced Raman spectroscopy (SERS).

In our experiments, we also show that the localized surface plasmon resonance (LSPR) of the Au/AgNRs can be tuned through the optical spectrum with LSPR peaks reaching much lower wavelengths compared to that of AuNRs (Fig. 1b). Additionally the bimetallic chiral assemblies shown in Fig. 1a display strongly increased CD responses in comparison to their Au counterparts (g-factor as high as $7 \times 10^{-2}$), which could hold great promise for plasmonic sensing with increased sensitivity as urgently required for antibody or pathogen RNA testing. In prospective work our presented method may be also employed to create other particle geometries to further expand the variety of such ordered plasmonic systems.
For photonics applications that require ultrafast transport of photoexcitations it is important to organize dye molecules with close intermolecular spacing and with control of orientation. DNA has been investigated as a scaffold in part because established chemical attachment methods allows for dyes to be positioned on DNA with sub-nanometer positioning and, in some cases, with orientation [1-3]. This capability has been exploited for designing dye networks that interact through dipolar coupling, and which show potential utility for applications such as light harvesting [1,4] and information processing [5,6]. While most DNA-organized dye networks have operated in the weak coupling limit, where the distance between dyes is large compared to their size, recent work has begun to assess the ability of DNA to arrange dyes with strong dipolar coupling [2,3,7]. Here, we explore the case where cyanine dyes are positioned on DNA duplexes with relatively small (<20Å) intermolecular separations. Using a variety of steady state and time-resolved spectroscopy, as well as theoretical methods, we demonstrate that the DNA duplex is a robust enough scaffold to allow the formation of delocalized molecular excitons leading to a rich set of spectroscopic signatures. We demonstrate a consequence of the strong dipole coupling through the observation of new excited state absorption bands, which we tentatively assign to optically accessible two-exciton states. Such states formed from strongly coupled dyes on DNA could ultimately play a role in excitonic circuits for information processing [5].

In this summary we focus on Cy3 and Cy5 homodimers. A structural model for Cy3 dimers attached to dsDNA is shown in Fig. 1a. The Cy3s are attached to opposite strands at the same position along the duplex (referred to as a 0bp separation). To help localize dye position the dyes are doubly attached to the DNA backbone by short 3-carbon linkers. A molecular dynamics simulation was performed to obtain insight into how the dyes arrange on DNA when in solution. Fig. 1b shows a representative snapshot from the simulation with the dyes having inserted into the base stack region with an oblique orientation. Figs. 2a,b show absorption spectra of the Cy3 and Cy5 homodimers. Both cases show evidence of strong dipolar coupling as revealed by a splitting of the low energy absorption band into J-like and H-like components, consistent with an oblique orientation. When cooled to near 100K these absorption bands narrow by about a factor of two, suggesting the bands are not dominated by inhomogeneous distributions of dye position and orientation. Figs. 3a,b show ultrafast transient absorption (TA) spectra used to characterize the excited state dynamics of the cyanine dimers. The TA spectra show ground state bleach (GSB) and stimulated emission (SE) bands that resemble the absorption and emission spectra. Most notable is the appearance of new excited state absorption (ESA) bands, which are located near 550nm and 640nm for Cy3 and Cy5 homodimers, respectively. These bands result from the strong dipolar coupling and do not appear in our TA spectra for weakly coupled dimers or monomers on DNA scaffolds. We discuss a theoretical model that suggests the new ESA bands are transitions from one-exciton to two-exciton states.


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**Fig. 1.** (a) Structural model of a 18 bp DNA duplex with two Cy3 dyes (green) that are doubly attached to opposite strands. (b) MD snapshot of the DNA duplex in (a). The MD simulation is performed using 350 mM NaCl and shows the dyes inserting into the DNA base stack.

**Fig. 2.** (a) Normalized absorbance of the (a) 0bp Cy3 dimer and (b) 0bp Cy5 dimer at 295K (black) and cooled to ~100K (blue). The 0-0, 0-1, and 0-2 vibronic bands are indicated, as are the H-like and J-like contributions to the 0-0 splitting.

**Fig. 3.** Transient absorption spectra measured at 295K. The 0bp Cy3 dimer photoexcited at 550 nm (a) and the 0bp Cy5 dimer photoexcited at 665 nm (b) show new excited state absorption bands (ESA) near 545 nm and 640 nm respectively. Ground state bleach (GSB) and stimulated emission (SE) bands are indicated.
Single molecule FRET Assay: A sensitive Protein-based Assay for accurate molecular detection at the single molecule resolution

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Imunoassays play a critical role in clinical diagnostics with their ability to detect specific target molecules in complex biological samples. These tools hold great promise in the early diagnosis of many slow-developing diseases, including infectious, oncological, autoimmune and neurological diseases. The current gold-standard in the field of biomolecular detection is the enzyme-linked immunosorbent assay or ELISA$^{(1)}$. However, the sensitivity of conventional ELISA-based diagnostic immunoassays is fundamentally limited by noise. Noise may result from sampling or partition error, biological variability, detectors, cross-reactivity, and non-targeted bindings (non-specific). There is hence a need for an ultrasensitive and specific assay that can reject interference from complex biological samples and discriminate true binding of the target molecules.

Recently, novel assay formats have been developed to improve sensitivity and overcome noise and interference. For instance, proximity ligation assays (PLA$^{(2)}$, antibody colocalized microarray assay (ACM)$^{(3)}$ as well as force-based approaches including fluidic shear force$^{(4)}$, have been engineered to reduce reagent-driven cross reactivity or interference. More recently, our group developed a sensitive and quantitative protein detection technique that combines dual affinity reagents with surface-enhanced Raman spectroscopy (SERS) and chemometric analysis to efficiently distinguish between specific and nonspecific target binding$^{(5)}$. However, most of these methods would require tedious quantification steps, long incubation time and specialized devices to perform the assay.

An alternate set of techniques are based on single-molecule fluorescence microscopy (i.e. TIRFM), which have advanced to observe a single fluorescently labeled biomolecule$^{(6)}$. More recently, far-field fluorescence microscopy has undergone major advances since the advent of methods circumventing the classical diffraction limit, i.e., super-resolution microscopy. Notably, DNA-PAINT has been used to obtain multicolor imaging of DNA nanostructures with ~5-nm spatial resolution$^{(7)}$. In this work, we developed a Single-Molecule FRET protein-based Assay that consists of arrays with individually addressable antibody sandwiches, providing the capability to discriminate non-specific binding at high resolution. In fact, we used FRET as a readout modality to improve our lateral resolution and achieve a more accurate counting at the nm resolution. We evaluated the assay sensitivity by testing various concentration of a target protein from 1pM to 10nM (Figure 1). We also confirmed that our assay format and readout methodology enabled us to detect sub-picomolar concentrations of proteins in a serum sample containing large amounts of other, non-target blood proteins. Thus, our technique provides a generalizable way to achieve much greater immunoassay performance...
characteristics than currently achievable through standard methods. This method will be an integral step that can lead the way to devices capable of accurate analyte quantification in low-resource settings. Advances in point-of-care techniques for sample treatment, target pre-concentration as well as novel fluorescence-enhancing materials will potentially enable single-molecule detection to be achievable by instruments such as smartphone cameras. Future perspectives aim to make a super-resolved digital assay using methodologies such as DNA-PAINT, and involve extending the single-plex digital assay into a multiplexed format.

![FRET schematic and graphs](image)

**Figure 1:** Schematic of FRET-based readout mechanism upon target binding in a sandwich protein assay format. Single molecule fluorescent images showing fluorescence signal in the donor/acceptor channels upon donor excitation before and after target protein binding. Graphs showing FRET efficiency computed at increasing concentration of target protein (0-10nM).

**References**

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Track on
Computational Tools for Self-Assembly

Track Chair
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Hybrid top-down and bottom-up approach for engineering DNA assemblies

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Over the last decade, major advances in DNA nanostructure fabrication methods have enabled the self-assembly of DNA devices and materials with increased size and complexity[1-3]. However, realizing the potential of these advances also requires new design methodology. Currently, design of these DNA nanostructures relies largely on a bottom-up approach where strands are arranged manually, which becomes tedious for complex structures. This has driven recent efforts to automate the design process, but currently automated design methods are limited to specific types of static, primarily wireframe geometries. We have developed a new approach that merges top-down and bottom up methods together with feedback from coarse-grained Molecular Dynamics simulation using oxDNA [4] or mrDNA [5] to enable design of DNA origami [6] assemblies with features including i) complex geometries with many components (lattice-based, surface-based, wireframe, or hybrids thereof, Fig. 1A), ii) programmed dynamic properties (e.g. mobility, Fig. 1B), and iii) ability to actuate and assemble into higher order systems (Fig. 1B, middle and right). We expanded this framework to enable direct design of assemblies with multiple scaffolds as illustrated for a DNA origami airplane comprised of four orthogonal sequence [7] scaffolds (Fig. 1C). We are optimistic this design approach can significantly broaden the scope of DNA-based design, especially for complex reconfigurable assemblies with many components or sub-systems.

Fig. 1: A) Our design approach covers a wide design spectrum including lattice, surface, and wireframe structures and enables design hybrid structures that integrate components of each type. Images show average structures from oxDNA simulations. B) Design of a flexible joint allows for relative motion of a “butterfly” mechanism, that can be actuated to close along the upper
edge and then formed into a well-defined higher order assembly containing six units. Actuation and higher order assembly were carried out by addition of DNA strands that bind to overhang strands. Scale bars are 50nm. (C) Utilizing the multi-scaffold algorithm, we designed and fabricated a DNA origami airplane comprising 4 scaffolds, which was fabricated following protocols similar to those reported in [7].

Link to talk: https://mediasite.osu.edu/Mediasite/Play/99a9cbf977bf4553a639391597ee66021d

REFERENCES:

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

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In this presentation, we introduce a multiscale modeling approach to predicting the shape and mechanical properties of DNA origami nanostructures¹-⁷ from the constituent base sequences and their connectivity information. Unknown geometric parameters and stiffness values of various structural motifs including normal/nicked dinucleotide steps and crosslinking Holliday junctions are characterized from the trajectories of molecular dynamics simulation at the molecular resolution. They are then fed into corotational beam elements to construct the finite element model of a DNA nanostructure and predict quickly its overall shape and structural properties. In addition, the entropic effect of single-stranded DNAs and the electrostatic repulsive forces between helices are incorporated efficiently into the model. The capability and efficiency of the proposed method are demonstrated by solving various DNA nanostructure designs reported previously. Due to the multiscale nature of the proposed method, the predicted shape and properties can be obtained at the molecular resolution in minutes. As it estimates the model parameters from finer-scale simulations, we ultimately aim to circumvent any arbitrary parameterization in the model specific to certain designs.

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scadnano: A browser-based, easily scriptable tool for designing DNA nanostructures

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Abstract

We introduce scadnano (short for “scriptable cadnano”), a computational tool for designing synthetic DNA structures. Its design is based heavily on cadnano [1], the most widely-used software for designing DNA origami [2], with two main differences:

1. scadnano runs entirely in the browser, with no software installation required.
2. scadnano designs, while they can be edited manually, can also be created and edited by a well-documented Python scripting library[2,3] to help automate tedious tasks.

A secondary goal, closely aligned with the goal of the scripting library, is that the file format should be easily human-readable, to help when debugging scripts or interfacing with other software.

Support. The author thanks Benjamin Lee for contributing unit tests and setting up the hosting for GitHub and readthedocs, and Tristan Sterin for contributing cadnano import/export features. The author was supported by NSF awards 1619343, 1900931, and CAREER award 1844976.

1 Summary

The scadnano graphical interface largely mimics that of cadnano,[4] with the main distinguishing features at present being those cited in the abstract. Fig. 1 shows a screenshot. The graphical interface is described in more detail at the GitHub repository.[5]

The architecture of scadnano uses software engineering principles that have gained prominence only in the past decade, notably the unidirectional data flow architecture for graphical user interfaces employed by React and Redux[6] (also the web-specific programming language Elm[7]). These frameworks greatly reduce the burden of maintaining large, complex, interactive software, which is notoriously difficult to reason about. It is hoped that going forward, this will help make maintenance, bug-fixing, and feature enhancement a straightforward task.

[4]Though a few terms are different, e.g., “skip” in cadnano is “insertion” in scadnano.
Copy/paste. For example, one feature of scadnano lacking in cadnano, which was straightforward to implement within this architecture, is the ability to move and copy/paste strands. We have found this feature to ease the burden of creating large designs quickly, even without using the scripting library. This is because many DNA origami designs are very repetitive, having more than 200 staples but fewer than 10 different “shapes” of staples. Large designs can then be created rapidly by recursive copy/pasting of staples. A tutorial at the GitHub repository shows how to rapidly create a DNA origami rectangle.⁸

Checking strain due to backbone rotation. Another feature available in scadnano is the ability to change the assumed rotation angle of the backbone, useful for avoiding undertwist or overtwist of the DNA helix. cadnano assumes an immutable angle at offset 0 on a helix, and the angle elsewhere on the helix is calculated as $360d/10.5$, where $d \in \mathbb{N}$ is the distance of the offset from the leftmost offset of the helix. scadnano allows this angle to be updated. One can either set it directly, or set it by the following operation: by clicking on a crossover, the angles of the two helices are updated to point at each other. We have found this useful for checking that the helices are not too “locally strained”, while keeping the tool from being opinionated about what sort of rotations are allowed.

References


**In-silico** design, visualization, and analysis of large DNA and RNA nanostructures

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Talk: [https://youtu.be/0lCRULkqirg](https://youtu.be/0lCRULkqirg)

This work seeks to remedy three deficiencies in the current nucleic acid nanotechnology software environment: the lack of a fast and user-friendly visualization tool, no standard for common structural analyses of simulated systems, and a lack of user-friendly tools for initializing and running molecular simulations of DNA/RNA nanotechnology designs. This work builds on the coarse-grained modelling tool, oxDNA/RNA, which has grown significantly in popularity in recent years and is frequently used to study nucleic acid nanotechnology design, model biophysics of DNA/RNA processes, and rationalize experimental results.

We introduce here oxView (Fig 1a), a web browser-based visualizer and editor that can load structures with over 1 million nucleotides, create videos from oxDNA simulation trajectories and allows users to perform basic edits to DNA and RNA nanotechnology designs. OxView also implements simple rigid body dynamics for relaxing and interactively rearranging structures (Fig 1b). Since all major design formats can be exported to the oxDNA format, oxView is intended as a bridge between other design tools, allowing re-use of older designs in complex, multicomponent designs. We additionally introduce open source software tools for extracting common structural parameters to characterize large DNA/RNA nanostructures simulated in oxDNA. Available characterizations include mean structures and structure flexibility (shown in Fig. 1a), hydrogen bond fraying, and interduplex angles. The output of these tools can be loaded into oxView, allowing user to interact in a graphical interface with the simulated structure in the 3D graphical environment and interactively modify structures to achieve desired properties.

OxDNA simulations can be a powerful tool for prototyping designs prior to synthesis or used to further explore features observed in experiments in greater detail. However, the barrier to entry to performing meaningful simulations is currently quite high, requiring fluency in Unix operating systems, statistical physics and computer programming. To lower the barrier somewhat, we have set up a new public webservice at oxdna.org to provide high-performance computing resources with a user-friendly interface to the DNA/RNA nanotechnology community. This resource facilitates the process through exporting the user’s design from a design tool, performing an initial relaxation, producing an equilibrium sampling trajectory in oxDNA/oxRNA, and using the previously mentioned general analysis tools to characterize the structure. As this tool will be integrated with oxView, this will provide an opportunity for users to make iterative modifications to designs. We hope that the combination of analysis, visualization and hardware resources will inspire tighter integration between experimental and simulation characterization of nanostructures.

All tools described here are available under the Gnu Public License at:
[https://github.com/sulcgroup](https://github.com/sulcgroup)
Examples of oxView usage: a) The oxView interface with a DNA origami wireframe loaded. A color overlay has also been added showing the per-nucleotide RMSF as calculated by the analysis tools. b) Relaxing an icosahedron designed in CaDNAno using oxView's rigid body dynamics simulator. Such structures are impossible to relax using oxDNA's relaxation protocols.
Track on
Integrated Chemical Systems

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An Approach to Identifying Molecular Aliens & Making Life in the Laboratory

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The search for evidence of life elsewhere in the universe has relied upon data collected from probes in our solar system, or astronomical observations. Equally, the quest to make life in the lab is confused by the eternal argument centering around a working definition for life. Knowing what signatures can be assigned to living systems is difficult as alien life has never been seen before. A solution would be to identify a feature exclusively associated with all life, and develop a detection system for that feature that could be used for the search for alien life, and also making life in the lab. We postulate living systems can be distinguished from non-living systems as they produce complex molecules in abundance which cannot form randomly in the absence of biology or technology[1]. In my talk I will present an approach to universal life detection based upon a new theory of molecular complexity called molecular assembly. I will show results attempting to validate this theory on a set of diverse samples from around the world and outer space. I will also show how we are trying to synthesise life forms in the lab using a new programmable chemical robotic system called The Chemputer[2].

Tailoring Supramolecular Polymers for High Capacity Electrodes in Lithium-ion Batteries

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High capacity anode (Silicon, Si) and cathode (Sulfur) electrode materials\(^1\) are receiving significant attention from the battery community due to their exceptionally large capacities that could accelerate the advent of future lithium ion battery applications represented by advanced portable electronics and hybrid electric vehicles. A consequence of the large Li capacity, however, is that the Si anode undergoes significant volume expansion, indeed up to 300%, resulting in severe mechanical and interfacial failures of the electrodes. Hence, for Si anodes, polymeric binders may not play simple passive roles as in conventional battery electrodes, but could rather contribute significantly to decent cell performance. Our systematic investigation of polymeric binders incorporating Meldrum’s acid revealed\(^2\) that most critical binder property for silicon anodes in lithium ion batteries is the self-healing effect facilitated by series of noncovalent ion-dipole interactions. Accordingly, we transferred\(^3\) millipedes’ adhesion mechanism, that is enabled by a synergistic effect between many contacting points based on continuous pairs of legs and several micron-sized adhesive pads decorated with ionic moieties located on each leg, at macroscale to polysaccharide binders at nanoscale in order to clearly demonstrate the effect of ion-dipole interactions in polysaccharide binders for high capacity Si anodes in lithium ion batteries. Recently, we have also reported that supramolecular crosslinking of polymer binders via dynamic host-guest interactions between hyperbranched \(\beta\)-cyclodextrin polymer\(^4\) and a dendritic gallic acid crosslinker incorporating six adamantane units enable\(^5\) intimate silicon-binder interaction, structural stability of electrode film, and controlled electrode-electrolyte interface, yielding enhanced cycling performance for silicon anodes in lithium ion batteries. More recently, we have also demonstrated that by incorporating small amount of...
polyrotaxanes into the conventional binders, it is possible to form binder networks with unparalleled elasticity, which enabled stable cycle life for silicon microparticle anodes at commercial-level areal capacities.[6]

References

Exploring New Building Blocks for Self-Assembled Architectures

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We describe our exploration into using new molecular building blocks for various self-assembled architectures. Cyclic cis-diamides can form four hydrogen bonds and their 90° bent shape allows easy access to macrocyclic structures. Nitrones are synthesized by dynamic covalent chemistry between aldehydes and hydroxylamines. Here, we discuss their C–H hydrogen bond donor moieties for self-assembled structures and anion binding properties. Finally, we serendipitously discovered that metallatetrahedranes can be dynamically formed between a Mo(VI)-alkylidyne and alkyne substrates during alkyne metathesis. Strategies to utilize metallatetrahedranes to construct cage architectures will be discussed.

Figure. Hydrogen bonded assemblies of cyclic cis-diamides and nitrones. Formation of a metallatetrahedrane via alkyne metathesis catalysts.
Track on
Nucleic Acid Nanostructures In Vivo

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Elevated lysosomal cysteine protease activity in tumor-associated macrophages restrains anti-tumor immunity.
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Abstract

A powerful way to eliminate tumors is to activate CD8\(^+\) cytotoxic T lymphocytes (CTLs) through antigen cross-presentation. Although dendritic cells are traditionally considered to perform this function, tumor-associated macrophages (TAMs) can also cross-present antigens. Given that TAMs are the most abundant tumor-infiltrating leukocyte, harnessing their ability to activate CD8\(^+\) CTLs would be advantageous. However, the mechanisms limiting antigen cross-presentation by TAMs are incompletely understood. Here we show that M2-like TAMs harbor hyperactive lysosomal cysteine protease (LCP) activity that blocks them from activating CD8\(^+\) CTLs through antigen cross-presentation. Using a DNA nanodevice, we developed an LCP inhibitor (E64-nano) that preferentially targets TAMs in vivo. E64-nano improves cross-presentation by TAMs and attenuates tumor growth via CD8\(^+\) CTLs. When combined with cyclophosphamide, E64-nano showed sustained tumor regression in a triple-negative-breast-cancer model. Our studies identify LCPs in TAMs as an important immune blockade in cancer and provide a potential therapeutic that alleviates this blockade.

Summary model

![Summary model diagram](attachment:image.png)
A DNA-based voltmeter for organelles

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Abstract:
Membrane potential is a key property of all biological membranes and underlies how membranes respond to electrical impulses and transduce chemical signals. It is therefore a fundamental signaling cue in all cells1. The role of membrane potential in most intracellular organelles remains unexplored because of the lack of suitable tools. We describe a fluorescent DNA-nanodevice that reports absolute membrane potential and can be targeted to specific organelles in live cells2. It is equipped with a voltage sensitive fluorophore, a reference fluorophore for ratiometric quantification, and acts as an endocytic tracer (Fig 1A). Further, one can display molecular trafficking motifs on DNA nanodevices and localize the latter within subcellular organelles3–5 (Fig 1B). We could thereby measure the membrane potential of different intracellular organelles in situ in live cells, which has not been possible previously (Fig 1C). By quantitatively reporting electrical properties at the biotic-abiotic interface, Voltair can potentially guide the rational design of biocompatible electronics6. Additionally, our understanding of how membrane potential regulates organelle biology is poised to expand through this new technology.

References:
Figure 1: (A) Schematic of the working principle of DNA voltmeter Voltair. Measuring probe (M, Green) is a voltage sensitive dye (RVF) conjugated to a DNA duplex that is membrane-tethered by attachment to a lipid anchor (POPE). Reference probe (R, red) is DNA duplex with a reference dye (Atto647N, red sphere) that together with RVF reports membrane potential ratiometrically. (B) Schematic of targeting strategy: VoltairIM undergoes scavenger receptor mediated endocytosis by binding scavenger receptors. Endocytosed VoltairIM traffics in a time-dependent manner from the plasma membrane to the early endosome, the late endosome and then the lysosome. Modified Voltair probes, VoltairTGN and VoltairRE access the retrograde and recycling pathways and measure the membrane potential of these respective organelles. (C) Tabular column summarizing approximate ionic concentration and voltage difference in organelles along the endosomal pathway. Cytosolic (Cyt), extracellular (Ec) and organellar concentrations of main ions that maintain membrane potential.
A DNA-based fluorescent probe maps NOS3 activity with sub-cellular spatial resolution.

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Abstract: Nitric oxide synthase 3 (NOS3) produces the gasotransmitter, nitric oxide (NO) that drives critical cellular signaling pathways by S-nitrosylating target proteins. Endogenous NOS3 resides at two distinct sub-cellular locations - the plasma membrane and the trans Golgi network. However, NO generation arising from the activities of both these pools of NOS3 and its relative contribution to physiology or disease is not yet resolvable. We describe a fluorescent DNA based probe technology, NOckout, to quantitatively map the activities of endogenous NOS3 at both sub-cellular locations in live cells. We have found that though NOS3 at the Golgi is ten-fold less active than at the plasma membrane, its activity is essential for the structural integrity of the Golgi. The newfound ability to spatially map NOS3 activity provides a platform to discover selective regulators of the distinct pools of NOS3.

Fig. 1. NOckout devices simultaneously map activities of NOS3 sub-populations in live cells.
DNA mechanotechnology: super resolution traction force orientation mapping with DNA hairpin tension probes
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Presentation video link:
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DNA mechanotechnology1, which consists of DNA-based devices that are engineered to sense, transmit, and generate mechanical forces, has recently emerged at the intersection of DNA nanotechnology and single molecule biophysics. These DNA-based mechanical devices have fundamentally changed the manner in which biologists study mechanical forces at the nanoscale and has opened new avenues for the design of force-generating nanomaterials1.

The most common application of DNA mechanotechnology thus far has been the use of DNA-based molecular tension probes for the measurement of piconewton (pN)-scale forces at the cell-surface interface2 (Fig. 1a). Mechanical forces between ~1 and ~100 pN in magnitude regulate many cellular processes at the plasma membrane via mechanosensitive receptor proteins such as integrins and the T-cell receptor3. Molecular tension probes are central tools for the visualization and control of these molecular forces. The most precise molecular tension probes are DNA hairpin-based tension probes. A DNA hairpin tension probe is composed of a DNA hairpin that is flanked by a fluorophore-

Figure 1: a) Molecular tension probes are surface-anchored DNA hairpins flanked by a fluorophore-quencher pair that unfold and dequench when pulled on with force exceeding a sequence-specific threshold. b) The de-quenched fluorophore rigidly stacks perpendicular to the opened tension probe. It’s polar ($\theta_{force}$) and azimuthal ($\phi_{force}$) angles can be inferred by imaging with polarized excitation light, rotating the polarization angle, and c) fitting the intensity to a $\cos^2$ function. d) Applying this treatment to each pixel in a widefield image allows diffraction-limited force orientation mapping. As an example, the orientation map of platelet integrin forces is shown where the force orientation measured in each pixel is denoted by the dipole orientation and color.
quencher pair. These probes are anchored to an underlying surface (e.g. a glass microscopy slide) and present a receptor-binding ligand on the other end. Cells are then cultured on top of the tension probe-functionalized surface and the cells’ receptors bind to the probes’ ligands. Each tension probe has a force threshold that can be tuned from ~2 pN to ~19 pN by changing the GC content and/or length of the hairpin. While these probes are immensely useful for visualizing receptor forces, they typically only report on force magnitude. However, the orientation of force is a key factor in regulating receptor mechanics⁴,⁵.

We recently presented molecular force microscopy⁶, which uses polarized fluorescence to report on the 3D orientation of receptor forces; by using linearly polarized excitation light and imaging the sample multiple times while rotating the light’s polarization state, we can measure the orientation of the opened tension probes’ fluorophores and, therefore, the orientation of the receptors’ forces (Fig. 1b, c).

MFM enables mapping of pN-scale cellular traction force orientation with the highest spatial and temporal resolution presented to date (Fig. 1d). However, fluorescence microscopes are generally not equipped to perform the polarization modulation necessary for MFM. Here, we present one solution to this challenge by demonstrating that MFM can be implemented using conventional structured illumination microscopy (SIM). SIM microscopes are relatively common and are maintained by core facilities at many research institutions. We show that SIM-MFM leverages the inherent polarization modulation associated with SIM imaging⁷ to correctly measure force orientation. Our results of cellular experiments show that SIM-MFM can reproduce our previous observations while increasing the temporal resolution of image acquisition ~3.6-fold (Fig. 2a). Because SIM was originally designed as a super resolution technique, we also show that SIM-MFM can produce super resolution force orientation maps with ~2-fold higher spatial resolution than conventional MFM (Fig. 2b).

Track on
Molecular Machinery

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At nanoscale, the peculiar functioning principles and the synthesis of individual molecular actuators and machines has been the subject of intense investigations and debates over the past 60 years. In this lecture, we will focus on the design of collective motions that are achieved by integrating, in space and time, several or many of these individual mechanical units together. In particular, we will provide an in-depth look at the intermolecular couplings used to physically connect a number of artificial mechanically active molecular units such as molecular switches and motors. We will highlight the various functioning principles that can lead to their collective motion at various length scales. We will also emphasize how their synchronized, or desynchronized, mechanical behavior can lead to emerging functional properties and to their implementation into new active devices and materials.
Handhold-mediated strand displacement: a DNA-based mechanism to generate out-of-equilibrium complexes through templated reactions

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Toehold-mediated strand displacement (TMSD) is a spontaneous enzyme-free reaction in which an input nucleic acid strand replaces an incumbent strand in a duplex with a target strand1 (Fig.1A). This process is driven by an overhanging single-stranded domain, known as toehold, located in the target. TMSD, despite its simplicity, can act as the basic motif of arbitrarily complex biologically-inspired networks2,3. However, the underlying biochemistry of the TMSD motif is always that an invading strand recognises part of a target, and subsequently forms a stronger connection with the target. Natural systems, however, also employ templated reaction mechanisms that can produce specific products4,5. In a templated process, like RNA transcription or protein translation, two molecules recognise a third (the template) and bind to it transiently. Whilst attached to the template, the two molecules can bind to each other. In this way, recognition of the template can drive the specific binding of two other molecules. If the product, as in transcription and translation, eventually releases the template, the resultant products are inherently far-from-equilibrium – unlike the outputs of conventional TMSD.

Here we introduce handhold-mediated strand displacement (HMSD), an independent method to modulate strand displacement reaction rates that naturally allows for non-equilibrium templating in DNA nanotechnology (Fig.1B). Handholds are toehold analogues located in the incumbent strand and recognised by the input strand. We measure and model the kinetics of 98 systems, demonstrating that handholds can accelerate the formation of input/target duplexes by up to five orders of magnitude (Fig. 1C). Crucially, handholds of moderate length accelerate reactions whilst allowing for eventual detachment of the produced input/target duplex. We are thus able to perform further assays to demonstrate the formation of far-from-equilibrium complexes via HMSD-based templating.

Figure 1 | A) Toehold-mediated strand displacement. The presence of a toehold (t) in the target strand (T) mediates the detachment of the incumbent (N) by the input strand (I). B) Handhold-mediated strand displacement (HMSD). The incorporation of a handhold (h) (an independent overhang in the incumbent strand) spatially constrains the input strand to the vicinity of the target. The handhold binding is intended to enhance the speed of binding to short proximal toeholds in the target, resulting in faster reaction rates. For sufficiently short handholds, the product can then detach. C) Handholds can substantially enhance invader/target binding rates for short toeholds. A fluorescent reporter detects the progress of the HMSD reaction by binding after branch migration is completed. Trajectories are fitted with a single set of reaction parameters. Conditions: System involving a 7nt handhold and a 2nt toehold 15 nM reporter complex, 10 nM target duplex, [7-4] nM input strand, 1M NaCl in 1x TAE at 25°C. Inset: The same reaction shown on a scale illustrates the kinetics for 6nM of input that is complementary to the toehold but not the handhold.
A biochemical DNA nanoscope that identifies and localizes over a hundred unique features with nanometer accuracy

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Video link to presentation: https://bit.ly/dnananoscope

Techniques that can both spatially map out molecular features and discriminate many targets would be highly valued for their utility in studying fundamental nanoscale processes. In spite of decades of development, no current technique can achieve both nanoscale resolution and discriminate hundreds of targets. Here, we developed a DNA nanoscope that acquired a detailed class average image from a homogenous collection of DNA origami by purely biochemical means. We successfully identified and spatially localized over a hundred unique elements, some spaced just 6 nm apart, with an average spatial localization accuracy (RMS deviation) of \(~2\) nm. The bottom-up, sequencing enabled mechanism of the DNA nanoscope is fundamentally different from top-down imaging, and hence offers unique advantages in precision, throughput and accessibility.

DNA nanoscope applied to various patterns

A. DNA nanoscope workflow. B. Many different patterns reconstructed with high accuracy. Each pattern is drawn to the same scale (scale bar = 5 nm). The numbers below the pattern are the RMS deviation between the designed and reconstructed pattern. Points missing from the reconstruction are indicated with red solid circles as opposed to gray solid circles. C. We encoded ‘color’ in auxiliary sequence tags that were then read out with the DNA nanoscope. D. Color wheel pattern with 77 distinct colors. Each auxiliary tag is unique. D. Holiday tree with 21 distinct colors. Each separate column of the pattern is a distinct auxiliary sequence, while points within the same column share the same sequence. All 13 points that make up the trunk share the same auxiliary sequence. E. An aggregate view of the accuracy of all the reconstructions from B and D. Each dot corresponds to the offset error vector between the reconstructed and the designed point. Each offset vector is translated to the center of the bulls-eye, whose each ring is 1 nm wide.
Enzymes are versatile biomolecular machines. Their tasks in living organisms range from peptide synthesis and energy conversion to intracellular transport. However, enzymes can also change fluid properties in their vicinity, which affects diffusion and transport on the micro- and nanoscale. Here we discuss how fluid interactions enable different kinds of low Reynolds number enzyme-powered nanosystems.

Products from enzymatic reactions may for instance lower the viscosity of fluids, which can facilitate transport. This mechanism is utilized by the bacterium \textit{H. pylori}, which excretes urease to induce a gel-sol transition in mucin gels and thereby enables its motion through the gel. Such mechanism can be mimicked with enzyme-decorated micropropellers and used for targeted transport in minimally invasive medical applications.\textsuperscript{1}

Direct fluid transport can also be realized with enzymatic micropumps. Here, the density gradient created by the enzymatic reaction causes a convective flow. We improved this method by introducing bacteriophages as beneficial nanotemplates for enzymes, which yielded higher activities and flow speeds in the enzymatic micropumps.\textsuperscript{2} This principle can readily be implemented in lab-on-a-chip and other devices.\textsuperscript{3}

Finally, it is interesting to ask if free diffusion of enzymes themselves can be changed by their catalytic activity. Due to its implications for biological processes it is of fundamental interest to ask if a self-diffusiophoretic mechanism also plays a role in enzyme diffusion. In our search for the smallest enzyme-propelled freely diffusing structure we focus on active enzyme diffusion with different high precision diffusion measurement techniques and find that individual enzymes may not show any measurable enhanced diffusion.\textsuperscript{4,5} We further discuss the implications of these findings for the realization of self-propelled nanosystems consisting of enzyme-decorated biotemplates.
Fig. 1. Various enzyme-powered nano- and microsystems. a) Enzymatically active biomimetic micropropeller penetrates mucin gel.\textsuperscript{1} b) Enzymatic micropump based on enzyme–phage–colloids.\textsuperscript{2} c) Diffusion of active enzymes and interaction with its substrate.\textsuperscript{4}

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POSTERS

Track on
DNA Nanostructures:
Semantomorphic Science

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Standard solid phase synthesis of DNA limits the length of DNA strands to ~ 150 bases. The Sleiman group has recently developed the process of sequential growth, which allows the facile preparation of extended DNA strands with full control over length (e.g., 2000 bases) and sequence\textsuperscript{1,2}. Using this strategy, we generated different monodisperse backbones with a range of sizes and with repetitive patterns of sequence. The repetitive domains offer simplicity, as only e.g., 10 strands are needed to assemble a structure, versus the hundreds of strands necessary for the assembly of DNA origami. These backbones can be mass produced, in a cost-efficient manner, using bacteriophage to generate single-stranded precursor DNA, containing the strand of interest incorporated within self-excising DNA domains. Circular ligation of these backbones gives circular templating strands, which allows the assembly of DNA tiles into size-defined nano-ring structures. The resultant circumference of these rings is much larger than those reported in literature, allowing necessary space for organization of materials in applications such as size-controlled liposome assemblies and gold nanoparticle scaffolding with interesting plasmonic properties. Moreover, these backbones with repetitive domains are used in the asymmetric elongation of the unique arms of a branched DNA-small molecule motif\textsuperscript{2}. The extended arms provide necessary space for organization of different materials with full control over the number, separation, location and type of nanoparticles. Also, the core of these branched units presents a dynamic behavior that can induce the specific folding of these arms, via biotin-streptavidin interaction, into a potential 3D nanotube-like DNA nanostructure.

Building high-performance electronics from structural DNA nanotechnology

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Abstract Text

Structural DNA nanotechnology encodes the spatial positioning information into the linear sequences of composing single-stranded DNA. Arbitrary geometries, such as tube, cuboid, and polyhedron, could thus be in silico designed and scalable fabricated. The dimensions of self-assembled DNA structures have been scaled up from nanometer to micrometer. Diverse functional nano-materials, including gold nanoparticles, quantum dots, CNTs and oxides, have been assembled onto self-assembled DNA template. However, building high-performance electronics from self-assembled DNA templates is still difficult, owing to the presence of contaminations from bio-lattices and limited dimension scalability.

In recent years, carbon nanotube (CNT) electronics have been widely explored for their outstanding transport properties. Theoretical simulations suggest that patterning CNTs into uniformly parallel arrays at 5 nm to 10 nm inter-CNT pitch will exceed the performance of Si electronics. However, thin-film approaches fail to
meet the requirements on both high density and uniform inter-CNT pitch (figure 1). As a result, current CNT FETs exhibit the performance tradeoff between the high on-state performance of fast on/off switching.

To address these obstacles from both structural DNA nanotechnology field and the CNT electronics, we reported precise scaling of inter-CNT pitch using a supramolecular assembly method called Spatially Hindered Integration of Nanowire Electronics (SHINE) (figure 1). First, we used the self-assembled DNA nano-trenches to confine CNT assembly. The assembled CNTs exhibited consistent inter-CNT pitches down to 10.4 nm and uniform orientation, meeting the patterning requirements of the projected sub-5 nm technology node for the first time. Furthermore, using the spatial confinement from PMMA cavities, we demonstrated centimeter-scale alignment for the CNT-decorated DNA templates. Finally, to build up the high-performance transistors, clean CNTs surface was the prerequisite. We explored a rinsing-after-fixing approach to gently remove contaminations without degrading CNT alignment. Building on these foundations, we demonstrated, for the first time, DNA-template transistors with performance exceeding thin-film FETs.

At the interface of high-performance electronics and bio-molecular self-assembly, precise bio-fabrication could provide ultra-scaled devices or circuits compatible with or sensitive to local biological environments.

Figure 1. Spatial Hindered Integration of Nanowire Electronics. Left panel: CNT arrays made from conventional thin-film approaches. Right panel: assembly CNTs onto the DNA brick crystal.
particles oriented in the wrong direction. We believe DNA origami goniometer will facilitate cryo-EM studies of small DNA binding proteins by providing positional information that would be essential in determining the correct structure. Currently we are adding new functionality to our DNA goniometer to display other targets such as RNA and proteins that do not bind to DNA.
DNA Origami Goniometer for single particle cryo-EM

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In recent years, single-particle cryo-electron microscopy (cryo-EM) has emerged as a powerful technique for high-resolution structure determination of biological molecules that are challenging to study using other high-resolution techniques such as X-ray crystallography or NMR. Recent technological advances in sample preparation, data acquisition, and data processing have streamlined structure determination using cryo-EM, and thus cryo-EM became an attractive method also for small proteins and complexes that were previously considered to be only suited for X-ray crystallography or NMR.

Although there have been many technological advances in single-particle cryo-EM, there are some challenges that remain to be addressed pertaining to the positional control of particles on the grid surface. Cryo-EM sample preparation involves the deposition of particles randomly onto a cryo-EM grid and high-resolution structure determination of the target molecule is achieved from the randomly oriented particles. For some targets, the conventional approach yields a high-resolution structure. However, for some targets, the high-resolution structure cannot be determined due to 1) misalignment of particle images during 3D reconstruction or 2) particles adopting only limited preferred orientations on the grid surface.

Here, we developed a DNA origami goniometer for single-particle cryo-EM studies of DNA binding proteins. The goniometer serves as a fiducial marker to locate the target protein and it has structural elements that are used to construct visual barcodes to encode and distinguish goniometer variants for different orientations of the target protein. We tested the utility of DNA goniometer with an 82 kDa, TALEN family protein. We designed 14 different DNA goniometer structures corresponding to 14 different orientations of BurrH by altering the barcode pieces. We developed a 2D classification scheme to decode the DNA goniometer barcode and separate structures corresponding to the different orientation of BurrH into different classes. When we use the orientational information provided by the DNA goniometer, we reach a final resolution of 6.5Å with all particles oriented in the correct direction. However, when we do not use the orientation information, the 3D reconstruction of BurrH yields a wrong model with 20-50% of
particles oriented in the wrong direction. We believe DNA origami goniometer will facilitate cryo-EM studies of small DNA binding proteins by providing positional information that would be essential in determining the correct structure. Currently we are adding new functionality to our DNA goniometer to display other targets such as RNA and proteins that do not bind to DNA.
POSTERS

Track on
Biomedical Nanotechnology

Track Chair

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Detection of G-quadruplex structures using hybrid DNA-origami silicon-nitride nanopore

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https://sohinipl.wixsite.com/fnano2020-sub18

INTRODUCTION

The nanopore measurement technique has gained prominence as a single-molecule sensor, where the passage of a single molecule through a nanopore (5-10 nanometers diameter) fabricated on a thin (20-50 nm) dielectric membrane separating two fluidic chambers results in a blockade of ion transport through the nanopore. Parameters associated with such ionic current blockades such as its amplitude and duration contain information about the molecule passing through the nanopore.

METHODS

In our experiments, we functionalized a silicon-nitride nanopore of diameter ~20nm with thrombin binding aptamer (TBA15) with sequence 5’-GGTTGGTGTGGTTGG-3’ using DNA origami. An increase in the normalized current power spectral density confirmed the docking of the DNA origami on the nanopore. The TBA15 aptamer binds specifically with potassium ions and human alpha-thrombin by forming G-quadruplex structures. We used thrombin molecules and different buffer solutions to detect these dynamic G-quad formations.

Fig1. (a) SEM image of a 20nm silicon nitride nanopore. (b) AFM images of rectangular origami structures. (c) Noise profile of before and after Origami docking on nanopore. (d) Schematic of thrombin binding with TBA15.
RESULTS & DISCUSSION

Interestingly, we observed that interaction of single thrombin molecules with TBA15 resulted in ionic current blockades quite similar to the case when the buffer solution contained potassium ions (Fig.2). In case of the thrombin experiments, the buffer used contained 0.1M of NaCl so the current changes were caused solely by the binding of thrombin with TBA15. In the absence of thrombin, no such current blockades were observed. However, when the buffer was changed to 0.1M KCl, blockages started appearing.

This work demonstrates the fact, that using the hybrid DNA-origami silicon nitride nanopore structures we can detect the dynamic behavior of aptamer-protein interactions and G-quad formations.

![Fig2](image_url)

*Fig2.* Current traces for (a) Thrombin in NaCl buffer (b) KCl buffer with no thrombin (c) NaCl buffer with no thrombin.

REFERENCES

Evaluating Toxicology, Immunogenicity, Pharmacokinetics, and Bio-Distribution, of DNA Origami Nanostructures In Vivo

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Scaffolded DNA origami nanotechnology allows for the generation of nanoscale objects with predefined shapes via molecular self-assembly as well as a robust platform for drug delivery applications. Previous studies reported that DNA origami nanostructures could be functionalized with targeting moieties (1) and effectively loaded with anthracyclines (e.g. doxorubicin and daunorubicin) (2-6) and thrombin (1). Furthermore, drug-loaded DNA origami nanostructures were reported to induce an enhanced anti-cancer effect relative to free drug in solid tumors in vitro (2, 3) and in vivo (4, 5) and hematologic model systems in vitro (6). In addition, recent findings showed that thrombin-loaded nanostructures induce tumor necrosis and inhibit tumor growth in vivo in a targeted manner (1). Despite this exciting promise, the toxicology, immunogenicity, bio-distribution, and pharmacokinetics of a high dose of DNA origami nanostructures in vivo remain ill defined. Here we evaluate two DNA origami nanostructures that vary in shape, a flat single-layer triangle and rod-shaped multi-layer nanostructure. A repeat dosing regimen of unloaded triangle and rod-shaped structures at clinically relevant levels revealed that DNA origami nanostructures were non-toxic in vivo as shown by weight, histopathology analysis of sections from major organ systems, and a complete biochemical panel assessing liver function. A modest pro-inflammatory molecular and cellular immune response was evident among mice treated with both triangle and rod-shaped DNA origami nanostructures that dampened by the conclusion of the dosing regimen. Cellular internalization ex vivo experiments revealed myeloid cell populations preferentially internalized DNA origami nanostructures. Distribution findings assessed by fluorescence-based live animal in vivo imaging system (IVIS) showed DNA nanostructures distribute throughout the periphery immediately after i.v. injection and within ~30 minutes after i.p. injection. Taken together, our in vivo findings suggest that DNA origami nanostructures are non-toxic, generate a modest immune response, distribute effectively into the periphery, and, therefore represent a promising novel platform for future cancer drug delivery studies in vivo.

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

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Nucleic acid therapeutics, such as small interfering RNA (siRNA) and antisense oligonucleotides (AON), offer unique potential for gene therapy because of their effectiveness and directed silencing of the targeted gene of interest. However, significant challenges have impeded their translation into clinical applications, such as instability in biologically relevant media, off-target effects, and poor cellular uptake.

An attractive solution is the use of drug delivery nanomaterials that protect and deliver the oligonucleotide drugs to their desired target site, such as liposomes and polymeric nanoparticles. However, the approval of these drug delivery materials has been slow due to many hurdles blocking their translation from lab prototypes to actual clinical applications. Most synthetic carriers are a mixture of polydisperse molecules that are not precisely controlled in size, shape, and composition. This leads to heterogeneity in properties, toxicity, and off target effects which are highly undesirable.

DNA nanotechnology offers a very promising alternative, which utilizes nucleic acids as a material to build nanostructures that act as targeted drug carriers. This is due to the ease of manipulating DNA’s structural parameters (size, shape, rigidity, functionalization) as well as chemical composition. These structures are monodisperse, bio-degradable, non-toxic, and can themselves be therapeutic.

An especially powerful DNA functionalization is the attachment of hydrophobic polymers, which has led to the emergence of a new class of amphiphilic DNA block copolymers. We have developed a highly efficient solid-phase method to generate monodisperse and sequence-defined DNA-polymer conjugates. Based on this method, we have designed a range of molecules and vehicles with various functionalities and applications in drug delivery, with total control over their properties.

In this work, we will be highlighting the importance of this sequence-controlled DNA-polymer conjugate method, which has allowed us to fabricate multiple systems for therapeutic applications. Specifically, we will be focusing on two systems developed from this method and their drug delivery applications: spherical nucleic acids (SNA) and albumin-binding Dendritic-DNA (DDNA) molecules. Studies such as characterization, stability, cell work, including gene silencing and conditional drug release, as well as in vivo investigations will be discussed.
Figure 1 Representative scheme of the 3 particles developed and used for drug delivery (left to right): Responsive Spherical Nucleic Acid, FANA-modified Spherical Nucleic Acid, and Dendritic DNA-albumin complex.

References:

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Self-Assembled Plasmonic DNA Origami Nanoantennas for Diagnostics Applications with Low-Tech Devices

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The DNA origami technique provides an unprecedented method to create multiple copies of well-defined self-assembled nanostructures. Methods of modern chemistry allow to functionalize DNA with molecules and functional groups of interest. Exploiting these features we designed a pillar-shaped 3D DNA origami nanostructure functionalized with biotins for the surface immobilization and docking strands allowing to precisely position plasmonic nanoparticles. Upon illumination with freely propagating light, the local electric field between nanoparticles increases and a dye placed in the plasmonic hotspot exhibits a fluorescence gain of several orders of magnitude. In our present work, we modified a hotspot region with molecular recognition units (molecular beacon or sandwich assay) to detect an enhanced signal only in the presence of a specific nucleic acid target (Figure 1).

Figure 1. Schematic representation of the DNA origami plasmonic nanoantenna for single DNA detection. a) Pillar-shaped DNA origami structure immobilized on a glass surface via biotin-
streptavidin interaction. Protruding docking strands allow precise positioning of plasmonic nanoparticles. The inset demonstrates the comparison of fluorescent traces of the dye located in a plasmonic hotspot and the same dye in usual conditions. b) Fluorescence-quenched hairpin (molecular beacon) incorporated into a plasmonic hotspot as a molecular recognition unit. Upon the detection of the target DNA in the hotspot, fluorophore-quencher pair spatially separates that leads to appearing of the fluorescent signal enhanced several orders of magnitude. c) Sandwich assay incorporated into the hotspot region allows sensing of the enhanced fluorescent signal after the subsequent binding of the target DNA and imager DNA to a capturing strand.

We discuss the requirements and limitations of the recognition units’ design, and present results demonstrating efficiency, specificity, selectivity, and stability of the self-assembled plasmonic DNA origami nanoantennas in diagnostics applications with outstanding signal-to-noise ratio signal. Several orders of magnitude enhancement of the fluorescent signal after detecting the target DNA allowed integration of the designed sensing assays into low-tech devices. We demonstrate a strategy to use spotted DNA nanoantennas to detect single DNA molecule with non-immersion optics using LED excitation as well as the sensing of a single DNA using a smartphone camera as a detector. Obtained results give a high promise of using self-assembled plasmonic DNA origami nanoantennas for the development of inexpensive point-of-care diagnostic platforms.

Figure 2. Schematic representation of the low-tech devices for single DNA detection using DNA origami plasmonic nanoantennas. a) Microfluidic chip with spotted nanoantennas illuminated with LED and imaged with non-immersion optics; b) Battery driven smartphone-based hand-hold device allowing imaging nanoantenna at a single-molecule level.

References
Rational Design of Aptamer Switches with Tunable pH Response

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Physiological pH conditions are maintained through tight homeostatic control within cells and tissues, and local variations in pH play a critical role in many important biological processes. Accordingly, there is considerable interest in engineering molecular systems to achieve programmable pH-selective behavior. When integrated with highly specific molecular recognition elements such as DNA aptamers, these mechanisms could enable precise control of nanodevices for applications including drug delivery, imaging, and clinical diagnostics. However, existing approaches for achieving pH-dependent molecular recognition rely on tight coupling between pH- and ligand-responsive DNA motifs, imposing stringent design constraints. Thus, aptamer switches cannot readily be modified to work outside their intrinsic pH-response range or tuned in terms of the sensitivity of their response, greatly limiting their utility in real-world applications.

In this work, we demonstrate a general approach for rationally designing aptamer switches that can be tuned to respond to a wide variety of pH conditions—including highly selective target binding within a narrow pH window. We build upon the intramolecular strand displacement (ISD) aptamer design, in which an aptamer is linked to a complementary displacement strand (DS) through an inert DNA linker. By inserting pH-responsive motifs into the linker or DS, we induce pH dependence through changes in intramolecular binding inhibition without directly affecting aptamer function (Fig 1).

Figure 1 | Mechanism of tunable pH-responsive aptamer switches. (a) Fluorescent signaling occurs through competition between target binding and DS hybridization within the ISD construct. ISD constructs can be modified to include pH-responsive motifs in the linker or DS. (b) Selective binding at low pH is achieved through pH-dependent folding of an intramolecular triplex linker, which confines the DS close to the aptamer at neutral pH and far from the aptamer at low pH. (c) Selective binding at neutral pH is achieved through the inclusion of a pH-dependent mismatch within the DS that strengthens binding competition at low pH.

As a proof of concept, we designed a range of strongly pH-responsive aptamer switches based on an established ATP aptamer. We engineered selective binding at acidic pH through insertion of an intramolecular triplex DNA motif in the linker. The
resulting construct showed over 1,000-fold higher binding affinity at pH 5 ($K_D = 2.6$ μM) as compared to pH 8.5 ($K_D = 3.3$ mM). Moreover, our design accommodates a range of triplex sequences, enabling tuning of the pH dependence range by ~1 pH unit and tuning of the pH-response strength by ~25-fold (Fig 2a/b). To instead achieve selective binding at neutral pH, we introduced a pH-responsive mismatch within the DS, achieving ~20-fold stronger binding at pH 8.5 ($K_D = 310$ μM) compared to pH 5 ($K_D = 5.6$ mM). This strategy can leverage both A-C and A-G mismatches, thus it can be extended to almost any aptamer sequence (Fig 2c/d).

Finally, because these two tuning strategies are non-interfering, we show that they can be applied in tandem to achieve even greater control over the range of pH response. We design a construct combining both linker and DS modifications that achieves near-native aptamer binding affinity over only a narrow “pH window” near the physiological pH range of 6–7 ($K_d = 32–42$ μM) with reduced affinity both at higher pH ($K_d = 410$ μM) or lower pH ($K_d = 110$ μM) (Figure 2e).

**Figure 2** | (a) A triplex-based linker design leads to large shifts of the binding curve towards low $K_D$ as solution pH is decreased, indicating high binding affinity only at low pH. (b) Varying the triplex sequence content (% of TAT triplets vs. CGC triplets) enables tuning of this pH dependence. (c) Inserting a single pH-responsive mismatch into the DS achieves high binding affinity specific to neutral pH. (d) These effects can be achieved using A-C or A-G mismatches. (e) Both mechanisms can be combined in one construct to produce complex effects, such as a preferential binding within only a narrow “pH window.”
Label-free and ultrasensitive electrochemical biosensor based on artificial nanoparticles

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Nanomaterials have been extensively utilized in biosensing systems for highly sensitive and selective detection of a variety of biotargets. Detection of ultra-low content of DNA in samples has also attracted tremendous research attention. Thus, in order to further improve the sensitivity and detection level of electrochemical DNA biosensing, a variety of signal amplification strategies have been reported, including rolling circle amplification (RCA), nanocomposites, catalyzed hairpin assembly (CHA), etc. Among them, nanocomposites that are typically constructed by combining two or more nanomaterials, have gained increasing interest owing to their unique physical and chemical properties.

In this work, a facile, label-free, and ultrasensitive electrochemical DNA biosensor has been developed, based on “urchin-like” carbon nanotube-gold nanoparticle (CNT-AuNP) nanoclusters, for signal amplification. Specifically, electrochemical polymerization of dopamine (DA) was employed to modify a gold electrode for immobilization of DNA probes through the Schiff base reaction. Upon sensing the target nucleic acid, the dual-DNA (reporter and linker) functionalized AuNPs were introduced into the sensing system via DNA hybridization. Afterwards, the end modified single-wall carbon nanotubes with DNA (SWCNT-DNA) were attached to the surface of the AuNPs through linker-DNA hybridization that formed 3D radial nanoclusters, which generated a remarkable electrochemical response. Because of the larger contact surface area and super electronic conductivity of CNT-AuNP clusters, this novel designed 3D radial nanostructure exhibits an ultrasensitive detection of DNA, with a detection limit of 5.2 fM (a linear range of from 0.1 pM to 10 nM), as well as a high selectivity that discriminates single-mismatched DNA from fully matched target DNA under optimal conditions. This biosensor, which combines the synergistic properties of both CNTs and AuNPs, represents a promising signal amplification strategy for achieving a sensitive biosensor for DNA detection and diagnostic applications.
Iterative Kinetic Proofreading for High-Specificity DNA Sequence Discrimination

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Genomic single-nucleotide variants (SNVs) can result in significant functional consequences to an organism and can give rise to diseases such as cancer. As a result, the ability to detect rare DNA mutations in biofluids is a crucial diagnostic tool for the early detection of disease. We outline a strategy for detecting SNVs in DNA sequences against a large background of similar sequences using the principles of kinetic proofreading. The T790M mutation of the epidermal growth factor (EGFR) is used as a model SNV target sequence.

Kinetic proofreading is a natural mechanism by which an incorrect substrate is selectively removed from a chemical reaction pathway through one or more irreversible “exit” reactions, thereby achieving a multiplicative increase in molecular specificity with each additional proofreading (selective exit) step [1]. We propose an autonomous kinetic proofreading system that uses repeated DNA strand displacement (DSD) to enable the detection of SNVs in DNA sequence with extremely high specificity.

DNA nanotechnology provides a programmable method for extending kinetic proofreading into artificial systems. Specifically, we will be utilizing toehold-mediated DNA strand displacement (DSD), a flexible approach for designing cell-free molecular circuits for computational problems [3]. In toehold mediated DSD, an invading strand of DNA displaces the incumbent strand of a partially double-stranded complex via two steps: (1) binding to an adjacent short, single-stranded “toehold” region, which significantly accelerates displacement, and (2) branch migration, or the replacement of complementary base pairs within the existing duplex [3].

Kinetic proofreading with DSD works via competitive hybridization of DNA probes to SNV-containing target sequences followed by selective liberation of the target sequence toehold for additional rounds of competitive DSD [2]. This process can be performed iteratively, where the target is captured by mutant-selective probes during each round.
and wildtype sequences are ejected from the reaction pathway via irreversible hybridization with wildtype-selective probes [1]. The target is freed after each round through re-exposure of the toehold.

While the discrimination of each round of proofreading is finite, it can be repeated multiple times on the same pool of molecules, reducing the rate of false positives each time, and permitting theoretically unlimited selectivity as the number of rounds increases [1]. This contrasts to the strictly finite selectivity of conventional hybridization assays, such as quantitative polymerase chain reaction (qPCR). However, it should be noted that as the number of proofreading rounds and ratio of true positives to false positives increases, leak reactions become more probable and may ultimately impose an upper limit on the maximum selectivity achievable, as wildtype sequences begin to enter and exit the biochemical pathway at the same rate.

Early investigations suggest that using mutant-selective DNA:RNA chimeric duplex probes and RNase H enzyme is a reliable method for freeing the target toehold between rounds of proofreading. Additionally, using ultraviolet (UV) light and mutant-selective DNA probes with photocleavable spacers separating the toehold domain from the rest of the probe is another promising approach to enable multiple proofreading iterations. Future work will explore DSD with four-way branch migration for more efficient hybridization reactions. While four-way branch migration is slower than three-way branch migration, it offers the advantage of avoiding secondary structures that may obscure toeholds or result in premature degradation of RNA nucleotides.

This work will represent a fundamentally new capability for molecular recognition, which we predict will bypass the thermodynamic limits of molecular discrimination of DNA hybridization at equilibrium and may achieve virtually unlimited selectivity [1]. In the wider scope, this project is an application of molecular programming to the problem of molecular-scale recognition, a crucial step for integrating computation in molecular devices and systems.

Continuous detection of glucose and insulin using a device-driven approach

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Abstract
Current continuous monitoring systems are limited to a few small molecules, including glucose. Continuous glucose monitors have improved diabetes management and reduced patient burden. A system that can continuously monitor insulin could enable the identification inter-patient and intra-patient variability of insulin kinetics. Current methods to determine insulin pharmacokinetics are laborious, and modeled population insulin kinetics are used to make treatment decisions in combination with trial and error. In response, we have developed a real-time assay capable of continuously and simultaneously measuring glucose and insulin. Our system, called real-time enzyme-linked immunosorbent assay or RT-ELISA, combines molecular probes into a bead-based fluorescence assay wherein a microfluidic platform enables target recognition within <1 minute and a highly sensitive camera measures target abundance.

RT-ELISA detection strategy
RT-ELISA employs two different bead-based target capture strategies to detect both insulin and glucose in parallel. For glucose, we used aptamer probes and a strand displacement strategy²²–²⁴. Briefly, we hybridized Cy5 fluorophore-conjugated glucose aptamers²⁵ with a DNA competitor strand that has been conjugated to a quencher (BHQ2) molecule and coupled these complexes to polystyrene microbeads. The aptamer-DNA competitor complex keeps the fluorophore and quencher in close proximity, producing no signal in the absence of target. When the aptamer binds to glucose, the competitor strand dissociates and alleviates quenching of the fluorophore, producing a signal (Figure 1A, left). For insulin, we developed a fluorescence-based sandwich immunoassay in which microbeads were functionalized with anti-human insulin antibodies as capture reagents, with detection achieved with a second anti-insulin antibody labeled with R-phycoerythrin (R-PE) (Figure 1B, left).

Figure 1. Overview and validation of RT-ELISA assay strategy.
We initially validated the performance of our glucose and insulin assays in buffer. After functionalizing microbeads with the aptamer-DNA competitor complex for glucose detection or insulin capture antibodies, we incubated the beads with different concentrations of glucose (Figure 1A) or insulin plus detection antibodies (Figure 1B). After an hour, we were able to detect bead-target complexes under a fluorescence microscope.

The RT-ELISA device integrates three modules to achieve continuous, real-time monitoring: (1) a mixing module, which combines molecular probes for analyte detection with the whole blood sample; (2) a depletion module, which minimizes background by reducing the number of blood cells in the sample; and (3) a detection module, which brings the target to the detection window to quantitatively measure its abundance (Figure 2).

**Continuous measurement of glucose and insulin in whole blood**

To characterize the sensitivity and temporal resolution of the RT-ELISA device, we tested it with human whole blood spiked with known concentrations of glucose and insulin (Figure 3). We used these data to construct standard curves that correlate fluorescence signal intensity to glucose or insulin concentration in whole blood (Figure 3A, B, insets). Data reflect individual bead readouts, where at least 200 beads were measured for each concentration.

**Conclusion**

We have demonstrated that the RT-ELISA device can achieve sensitive and accurate continuous monitoring of insulin and glucose in the whole blood of live animals, enabling real-time analysis of insulin pharmacokinetics and pharmacodynamics.
Preparation of DNA Quadruplex Meso/Nanogels for DDS Application

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We have recently and successfully developed DNA quadruplex hydrogels, which instantly gelates in response to K⁺ or Na⁺ addition, by directly coupling several deoxyguanosine residues to the both ends of polyethylene glycols utilizing High-Efficiency Liquid-Phase (HELP) large-scale DNA synthesis technique [1, 2]. DNA quadruplex hydrogel has various advantages such as biodegradability, intelligence and self-healing capacity. Studies on application as a cell culture substrate and a drug release device have already been started [3]. In this study, we examined a utility of this hydrogel as a Drug Delivery System (DDS) carrier.

We found that nanoparticles of 100–200 nm diameter can be efficiently prepared by forming gels in sufficiently diluted solution of dG₄-PEG-dG₄ macromonomer. The mesogels were fully characterized by dynamic light scattering (DLS), atomic force microscopy (AFM) and transmission electron microscope (TEM). High-speed video-rate AFM measurements enabled direct and real-time observation of selective dissolution of DNA Quadruplex mesogels triggered by the addition of a crown ether into the system. Drug loading of G-quadruplex-binding anticancer drug and its delivery into cells were also accomplished. Finally, the macromonomer solution was injected via tail vein into mice and in vivo experiments were performed. Three hours later, DNA mesogels were found to accumulate in liver.

Figure 1. Schematic illustration of the system..

REFERENCES
Counting individual molecules: DNA nanostructures for diagnostic applications

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Rapid and low-cost detection of disease biomarkers is becoming increasingly important in modern healthcare where a growing focus is placed on early diagnosis. The ability to detect small concentrations of biomarkers in patient samples is one of the cornerstones of modern healthcare, and Ideally, a diagnostic assay should be able to detect the presence of very few biomarkers in a small volume of a complex clinical sample.

In current state-of-the-art biosensors for such clinical applications, an antibody or antibody mimic is used to capture relevant biomarkers in the sample, and in general, each antibody–biomarker interaction contributes a small amount to the accumulated assay signal. However, the individual immuno-interactions cannot be identified anymore and only manifest themselves as part of this ensemble-averaged signal. Arguably, the ability to detect biomarkers with single entity resolution rather than via ensemble-averaging techniques would provide significant advantages for the detection of ultra-small biomarker concentrations.

Of the many single molecule methods which have been developed over recent years, the use of nanopores, where a voltage is applied across the nanopore and pulses in the time-varying electrochemical current are used for detecting individual proteins, is a promising approach. Here, single molecules translocate through the nanoscale pore causing a momentary modulation in the otherwise steady ion current. However, it is very difficult to detect a specific molecule from a complex mixture as the translocation speed of single entities is generally high when the diameter of the nanopore is much larger than the size of the molecule, and, importantly, the signals resulting from different proteins are generally very similar and thus difficult to differentiate.

Here, we report a biosensor platform using DNA origami featuring a central cavity with a target-specific DNA aptamer as carriers for translocation through nanopores, which enables individual biomarkers to be identified and counted to compile a sensing signal.

We show that modulation of the ion current through the nanopore upon the DNA origami translocation strongly depends on the presence and in fact the size of a central cavity. While DNA origami without a central cavity cause a single peak in the ion current, DNA origami of the same dimensions but featuring a central cavity lead to double peaks in the ion current. We also show that the peak characteristics, in particular the peak amplitude and the dwell time, are different depending on the presence or absence of a central cavity.

In this work, we exploit this to generate a biosensing platform capable of detecting human CRP in clinically relevant fluids.

We designed DNA origami frames with cavities large enough to lead to clear ion current double-peaks and introduced a CRP-specific DNA aptamer into the cavity. We show that
upon binding of CRP, the ion current peak changes to a single peak and the peak characteristics (dwell time and amplitude) change. Using this three-parameter classification approach, we can distinguish between CRP-occupied and unoccupied carriers when they translocate through the nanopore. We demonstrate CRP biosensing by computing the ratio of occupied vs total number of frames with a limit of detection of 3 ng/ml.

Figure: (a) DNA-origami nanopore biosensing concept. (b) Scatter plot of translocation peaks for (i) unoccupied carriers and (ii) carriers incubated with CRP. Ion current events which are double peaks are plotted orange, and the ones that fall inside the 95% confidence ellipse are plotted as circles, the others as triangles. Only events that fall within the 95% confidence ellipse are considered double peaks. The same analyses were carried out for single peaks (blue). Ion current events which resembled neither a double nor a single peak are shown as black triangles and are excluded from the analysis. (c) Normalised single peak count, i.e. ratio of single peaks vs total classified peaks against CRP concentration. The data were fitted with Langmuir isotherm.
Protein-Facilitated Small Molecule Detection Using CRISPR/Cas

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https://www.dropbox.com/sh/yujf7kjbndjnxb6/AABeQxLbxs0UWIPefjYDobFCa?dl=0

The ability to detect small molecules, metal ions, and proteins is essential for identifying biomarkers of disease and assessing the safety of food and drinking water. However, existing detection methods for many analytes lack sensitivity, are not specific, or require expensive instruments and time-consuming methods to implement. Molecular detection systems based on CRISPR/Cas enzymes provide built-in amplification capabilities and high specificity, but have thus far been mainly limited to nucleic acid detection. In this study, we aim to expand the capabilities of CRISPR/Cas based diagnostics to the detection of other molecules such as small molecules and proteins. To do this, we have designed a nanoplatorm that employs protein-facilitated toehold-mediated strand displacement to activate CRISPR/Cas12a. This is done by incorporating transcription factor binding sites into a strand exchange complex, allowing the transcription factor to bind and preventing strand exchange. In the presence of a target small molecule, the transcription factor is unbound, enabling strand displacement to occur and providing a new substrate for Cas12a. Activation of Cas12a is monitored using ssDNA reporters that are cleaved through the collateral cleavage activity of Cas12a. This cleavage can be observed using a fluorescence plate reader or on a paper-based platform such as a lateral flow assay. With the capacity to incorporate readout on a paper-based system, this method can be used to create low-cost, easy-to-use diagnostics for the detection of diverse molecules, such as metals, drugs, and proteins. At this stage, we have successfully designed and implemented this method for the detection of small molecules such as IPTG and Anhydrotetracycline (Atc). We have also been able to generate orthogonal strand displacement sets, meaning multiple reactions can occur without crosstalk or nonspecific activation. With these orthogonal sets, small molecule detection has the potential to be multiplexed, resulting in screening for multiple molecules at a time. For the future, we aim to move towards water sample testing, looking for toxic metals and potential drugs in an easy to use paper-based platform that can be run at home.
Figure 1 Small Molecule Detection using CRISPR/Cas: Fig. 1a shows the overall mechanism for small molecule detection using toehold mediated strand displacement. Here, the transcription factor binds to the DNA complex, blocking displacement. In the presence of a small molecule, the transcription factor is removed, and displacement occurs. 1b shows the single-stranded target going on to activate the gRNA-Cas12a complex. Activation initiates cleavage of a ssreporter which can be read out on a lateral flow assay as shown in 1c.
Toward ultra-sensitive digital diagnostics for protein biomarkers: Sensing and anti-fouling surface treatments

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The detection and quantification of low concentrations of disease biomarkers, such as protein biomarkers from biological fluids is crucial for medical diagnostics as well as basic research. Array-based single-molecule high-throughput biomolecule analysis methods, in particular, combine the merits of digitized quantification with ultra-high sensitivity as compared to previous bulk analysis methods. However, integration of a protein sensing module to single-molecule, high-throughput biomolecule analysis array is non-trivial. Moreover, analysis of proteins through this approach presents significant challenges attributable to non-specific surface adsorption and their structural heterogeneity. Thus, the development of a low-cost, single-molecule protein sensing array with ultra-high sensitivity is crucial for early-stage disease diagnosis and prognosis. In this work, we introduce a modular platform that addresses the critical bottleneck in cheap, high throughput, highly sensitive, single-molecule detection of protein biomarkers. The platform harnesses a nanoarray of the bistable DNA origami nanodevices whose state change is dependent on individual capture of analyte molecules (nucleic acid, peptides or proteins) rigidly bound to the structure. Next, we integrate the DNA origami nanodevices with our patent-pending low-cost DNA origami nanoarray platform for high-throughput analysis of protein binding using optical microscopy. Total internal reflection fluorescence (TIRF) microscopy images and agarose gel-electrophoresis demonstrates the synthesis of fluorophore-labeled DNA origami nanodevices. We also demonstrate our ability to differentiate its bound and unbound states through the use of photo-cleavable linkers on the nanodevices as a proof of concept. We have also successfully shown the presence of the bistable DNA nanodevices on a periodic grid, fabricated using microsphere lithography. Lastly, we present an improved anti-fouling method for preventing non-specific binding of both analyte molecules and bistable DNA sensors to the glass microscope coverslip. One of the challenging aspects has been the integration of the sensor configuration onto the nanoarray platform while retaining minimal non-specific interaction of biomolecules with the surface of the platform. Current efforts are focused on screening the optimal approach for effective anti-fouling of the platform to perform sensitive quantification of the target bio-molecules.
POSTERS

Track on
Chemical Tools for DNA Nanotechnology

Track Chair
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Evolution and structure of cubane-modified aptamers as new tools for DNA nanotechnology

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Nucleic acid aptamers are ideal tools for molecular recognition for various DNA nanotechnology applications. However, nucleotide chemical diversity can be limiting when one compares to amino acid counterparts. Nucleotide chemical diversity has been extended in a number of ways including the introduction of chemistries similar to amino acids into nucleotide bases. Amino acid-like chemistries at the 5-position of pyrimidines are known to be tolerated by polymerases, thus allowing Darwinian molecular evolution through SELEX (selective evolution of ligands by exponential enrichment). Here, we investigate how far this idea can be stretched into evolutionary selections of synthetic chemistries well beyond the typical confines of biology.

Our aptamer target was Plasmodium vivax lactate dehydrogenase (PvLDH), an important malaria diagnostic biomarker for which specific, conventional DNA aptamers had eluded us. We performed an aptamer selection using dU carrying the extraordinary platonic solid cubane as an unusual non-biological benzene isostere which is hydrophobic, surprisingly water soluble, yet biocompatible. Tight-binding, specific cubane-modified aptamers (cubamers) were selected and were specific for PvLDH over the closely related Plasmodium falciparum lactate dehydrogenase (PfLDH). An X-ray crystal structure of the cubamer in complex with PvLDH showed cubanes clustering with hydrophobic amino acids in a variable alpha helix as key to recognition and specificity. Non-classical hydrogen bonds from the cubyl hydrogens were also observed, demonstrating how such aptamer selections with non-natural chemistries can lead to non-biological macromolecular binding interfaces that otherwise could not be imagined. The cubamer was incorporated into assays which could distinguish PvLDH and PfLDH at clinically relevant concentrations in serum, with potential application for malaria diagnosis.

Such approaches to apply evolutionary macromolecular selections integrating divergent synthetic chemistries may significantly extend the horizons of applications of aptamers in DNA nanotechnology.

Customizable nanostructures built through the DNA-origami technique hold tremendous promise in nanomaterial fabrication and biotechnology. Despite the cutting-edge tools for DNA-origami design and preparation, it remains challenging to separate structural components of an architecture built from — thus held together by — a continuous scaffold strand, which in turn limits the modularity and function of the DNA-origami devices. To address this challenge, here we present an enzymatic method to clean up and reconfigure DNA-origami structures. We target single-stranded (ss) regions of DNA-origami structures and remove them with CRISPR-Cas12a, a hyper-active ssDNA endonuclease without sequence specificity. We demonstrate the utility of this facile, selective post-processing method on DNA structures with various geometrical and mechanical properties, realizing intricate structures and structural transformations that were previously difficult to engineer. Given the biocompatibility of Cas12a-like enzymes, this versatile tool may be programmed in the future to operate functional nanodevices in cells.
Data analysis and measurement sensitivity for DNA thermodynamics

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Traditional calorimetry can be difficult to apply to nucleic acid nanofabrication systems. Often, these systems contain more components than can be resolved independently and have enough mass to make preparation of high molarity samples difficult. Given this, it is common to use ensemble techniques, or to image numerous samples, and use the relative population of states to extract thermodynamics via van’t Hoff analysis.

While van’t Hoff analysis is sufficiently common to appear in most undergraduate curricula, it is notoriously fickle. This is due to the complex propagation of uncertainty from melt curves to the equilibrium constant and from the equilibrium constant to the extracted parameters, $\Delta H$, $\Delta S$, and $\Delta C_P$.

We present both an introduction to advanced data analysis techniques as well as an evaluation of the sensitivity of the van’t Hoff analysis to measurement uncertainties.

Figure 1. Affine transformation of experimental optical melt data.

Figure 2. Monte Carlo sensitivity analysis of thermodynamics extraction for increasing noise, showing statistical $\Delta H/\Delta S$ compensation artifact.
POSTERS

Track on

DNA Nanosystems: Programmed Function

Track Chair

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Dynamic Modification of Lipid-DNA Probes on Live Cell Membranes: A Quantitative Evaluation

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Poster Link:
https://drive.google.com/file/d/1GEd-M9S9aEt68jZYWVqFWzglCNj8yXC/view?usp=sharing

Cell membrane provides a physical barrier between the intracellular compartments and the extracellular environment. Moreover, cell membrane plays important roles in the signal transmission between cells and extracellular matrix or among neighboring cells. Better understanding and modulation of the compositions and functions of cell membranes is critical for regulating cell signaling and interactions. Among different methods developed for cell membrane study and regulation, lipid-DNA conjugates are powerful tools with great potentials. These conjugates have recently attracted much attention for cell membrane analysis, transmembrane signal transduction, and regulating intercellular networks. These lipid-DNA probes can be spontaneously inserted into the membrane simply by incubation. The highly precise and controllable DNA interactions have further allowed the programmable manipulation of these membrane-anchored functional probes. However, our understanding is still quite limited on how these lipid-DNA probes can interact with the cell membranes and what parameters determine this process.

Here, we aim to provide an in-depth understanding of how lipid-DNA conjugates interact with cell membranes. With quantitative assessment using several methods, we have determined the cell membrane insertion kinetics, magnitudes, and durations of different lipid-DNA probes. We have systematically studied the effect of lipid/DNA structure, hydrophobicity, concentration, temperature, and cell type on these membrane interactions. We studied the probe modification density on different cell membrane and realized that, modification is highly subjected the lipid linker (Figure 1a). By measuring the relative hydrophobicity of each probe, we found that there is an optimum probe hydrophobicity for maximum probe density on the cell membrane. (Figure 1b). We further discovered that probe detachment from the cell membrane usually happens through two main mechanisms which result in probe density decay (Figure 1c). Probes either internalize into the cells through endocytosis or diffuse out into the solution. Understanding these pathways quantitively, helped us to develop a general mechanism of probe interaction with membrane and their removal from cell surface (Figure 1d).

First, there is equilibrium between the monomeric and aggregated forms of lipid–DNA probes in the solution. Monomeric form lipid-DNA probes insert into the cell membranes. Then, some of the cell membrane-anchored probes are internalized into the cells through
clathrin- or caveolae-mediated endocytosis. Most of the internalized probes are then located inside the late endosomes or lysosomes where they are degraded or rejected out of the cells. Some probes are likely transferred to the Golgi apparatus or endoplasmic reticulum as well. On the other hand, the membrane-anchored probes can also directly flow out into the extracellular solution, which is the reverse process of the initial probe membrane insertion. It is worth mentioning that direct probe exchange between neighboring cells can also occur at the cell–cell junctions.

**Figure 1:** (a) Effect of different lipid linker on probe modification efficiency on MDCK cell membrane. (b) Membrane probe density on four different types of cell lines plotted against probe corresponding hydrophobicity. (c) Probe density decay from the MDCK plasma membrane. (d) Schematic of the dynamic process of lipid-DNA probe modification on cell membranes.

Detailed understanding of Lipid-DNA conjugates interaction with cell membrane enabled us to minimize probe removal from the cell membrane and extend their effective time window to over 24 hours. Therefore, these lipid-DNA conjugates now can be applied for various membrane studies that require long-term immobilization on the cell membrane. In conclusion, our quantitative data have dramatically improved our understanding on how lipid-DNA probes can dynamically interact with the cell membranes. These results can be further used to allow broad applications of lipid-DNA probes for cell membrane analysis and regulations.

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Metal nanomaterial synthesis and placement on DNA nanotubes to make electrically conductive wires

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Self-assembly aided nanostructure fabrication is increasingly appealing, as it requires fewer materials and has potential to reduce feature sizes in making complex nanostructures. DNA, with its nanoscale dimensions, functional groups and complementary base pairs, is a powerful template for making nanostructures via self-assembly.1 We use DNA nanotubes that have a hollow interior and variable length as templates. Figure 1A shows a schematic diagram of the growth of DNA nanotubes. Atomic force microscopy (AFM) of deposited DNA nanotubes on an oxidized Si wafer shows separate 30 nm wide DNA structures that can be as long as 10 μm (Figure 1B). These DNA nanotubes offer a route to electrically conductive nanowires that are several micrometers long and have controlled orientation that may be useful in constructing integrated nanoelectronic devices. We metallize DNA nanotubes by using two different methods with the ultimate goal of creating long connections. One approach involves seeding DNA nanotubes with gold nanorods (Figure 2A) and connecting them by electroless plating (Figure 2B).1 Another method utilizes Pd ionic seeding3 in a three-step process: Pd activation, Pd reduction to form seeds and electroless plating (Figure 2C).

We utilize electron beam induced deposition (EBID) of conductive Pt contacts to study electrical properties (Figure 3A) with resolution <100 nm. Initial conductivity measurements revealed that the formed Au seeded and plated structures have ohmic character (Figure 3B); the average resistance values for three structures were 59 kΩ, 84 kΩ and 98 kΩ, and more measurements are in progress. Our work yields new insights into high-density nanomaterial attachment on DNA structures, making them potentially useful templates for wires in complex electronic circuitry.

References


![Figure 1](image1.png)

Figure 1 (A) Schematic diagram of the growth of DNA nanotubes; reprinted from ref. 2. (B) AFM image of DNA nanotubes.

![Figure 2](image2.png)

Figure 2 SEM images of (A) DNA nanotubes seeded with Au nanorods. (B) Plated DNA nanotube structure after Au nanorod seeding. (C) Structure formed after ionically seeding and plating with Au.

![Figure 3](image3.png)

Figure 3 (A) SEM image of EBID-connected DNA nanotube structure. (B) Current-voltage curve for a plated DNA nanotube.
DNA Origami Cryptography for Secure Communication

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Biomolecular cryptography exploiting specific biomolecular interactions for data encryption represents a unique approach for information security. However, constructing protocols based on biomolecular reactions to guarantee confidentiality, integrity and availability (CIA) of information remains a challenge. Here we develop DNA origami cryptography (DOC) that exploits folding of a M13 viral scaffold into nanometer-scale self-assembled braille-like patterns for secure communication, which can create a key with a size of over 700 bits. The intrinsic nanoscale addressability of DNA origami additionally allows for protein binding-based steganography, which further protects message confidentiality in DOC. The integrity of a transmitted message can be ensured by establishing specific linkages between several DNA origamis carrying parts of the message. The versatility of DOC is further demonstrated by transmitting various data formats including text, musical notes and images, supporting its great potential for meeting the rapidly increasing CIA demands of next-generation cryptography.
**Fig. 1** Protocol of DOC for message confidentiality. **a** The process is composed of three layers—pattern encryption as the outer layer (gray), followed by the steganographic intermediate layer (green), and DNA origami encryption (DOE) as the innermost layer (pale green). **b** Alice holds the DNA scaffold and can generate the M-strands. Alice firstly encoded the plaintext message “HEY” letter by letter into binary numbers, and then encrypted the numbers for each letter (navy) and their respective positions in the message (teal) into a braille-like spot pattern. Then Alice encrypted the patterns into a combination of scaffolds carrying several M-strands, according to a defined DNA origami folding scheme. **c** Bob holds streptavidin and generate the staples. With the staples Bob was able to fold the DNA origami, revealing biotinylated patterns on the M-strands. Subsequently, Bob added streptavidin to make the patterns recognizable under the AFM. Finally, the plaintext message was decrypted letter by letter into binary numbers and decoded. **d** The fluorescent pattern under the STORM. **e** Braille-like streptavidin patterns under the AFM. Scale bar: 50 nm.

**Fig. 2** Transmitting music and image with DOC. **a** The custom keypad indexing the music to binary numbers. **b** From spot pattern to music. **c** AFM images of streptavidin patterns conveying the music. **d** A 256-pixel panda image. The pixel boxed in orange is presented by a spot pattern (an analogic map of China). **e** 256 streptavidin patterns conveying the panda. Scale bar: 50 nm.

Reference
DNA dynamics and computation based on toehold-free strand displacement

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Web link of poster: https://cloud.tsinghua.edu.cn/d/ca95b4cd0ef84b1c9763/

Instead of toehold mediated strand displacement, we have shown in this study that controlled dynamics can be achieved by the simpler implementation of toehold-free strand displacement. Under such a scheme, when a single-stranded DNA (ssDNA) blocker (e.g., \( n' \)) fully complementary to a certain segment of paired duplex (e.g., \( nn^* \)) is presented at an excess amount, it can displace the specific segment by pairing competition (e.g., \( nn' \)) (Fig. a). The blocking can be applied to an arbitrary set of segments of paired duplexes (e.g., \( NN^* \), \( N = \{n_1, n_2, \ldots, n_i\} \); \( N^* = \{n_1^*, n_2^*, \ldots, n_i^*\} \)). When a counterpart set of ssDNA blockers (e.g., \( N' = \{n_1', n_2', \ldots, n_i'\} \)) is presented at an excess amount, the original pairing scheme will be outcompeted and displaced (Fig. b). In other words, when the original pairing scheme of \( NN^* \) without strand displacement is defined as an ON state, the pairing after strand displacement (\( NN' \)) can be defined as an OFF state. Basic dual-unit systems and a more complex quadruple-unit system are designed based on the concept. With blockers serving as a controller which switch ON/OFF a certain binding by toehold-free strand displacement, a 4-bit input/2-bit output and a 16-bit input/8-bit output Boolean functions are then implemented based on the reliable dynamic switch. In general, toehold-free strand displacement has been applied in a number of DNA nanostructure systems.
Summary Fig. Schematic diagrams of controllable dynamics of DNA nanostructures based on toehold-free strand displacement. Tree maps of binding reactions of (a) one species of blocker to displace one species of prespecified segment (nn*) or (b) two species of blockers to displace two species of prespecified segments (n1n1*, n2n2*).
DNA-scaffolded envelope assembly for HIV Electron Microscopy Polyclonal Epitope Mapping (EMPEM)

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With the recent success of single particle electron microscopy (EM), a revolutionary strategy to characterize heterogeneous immune responses to HIV envelope (Env) has been developed by the Ward lab[1]. **Electron Microscopy Polyclonal Epitope Mapping (EMPEM)** enables structural characterization of heterogenous complexes of polyclonal antibody (pAb) responses to antigens. The original study demonstrated mapping of the polyclonal immune response of rabbits to vaccination with an experimental HIV trimer immunogen, but we have now adapted the assay to a wide variety of antigens and sera derived from different animals including non-human primates[2] and humans. However, with limited amount of serum available, especially from human trials, testing cross-reactivity of pAbs against diverse antigens remains extremely challenging.

Self-assembled DNA nanostructures have emerged as promising biomolecular scaffolds to precisely organize functional elements at nanoscale, due to their sequence-coded, addressable nature.[3] Super-molecular constructs of proteins that are scaffolded by DNA nanostructures exhibit good control over nanometer scale inter-component distances, orientations, and relative molecular ratios.

Combining the strength of both DNA nanotechnology and EM

We propose to assemble HIV Env antigens onto a 24 helix bundle (24HB) 3D DNA nano-scaffold to exert precise control over HIV Env copy number and geometry. We will then use this tool to investigate the cross-reactivity of both monoclonal antibody (mAb) and polyclonal antibody (pAb) responses to diverse HIV strains. This method would not only save a large amount of serum and experimental time, but also achieve parallel epitope mapping across multiple HIV strains in a single experiment (FIG. 2A).

![FIG. 1. Assembly scheme of Env-DNA complex. (A) Conjugation of Env with ssDNA. (B) Assembly of Env on to 24HB DNA origami. (C) Control of number/ position of envs.](image-url)
The engineering of the DNA-Env chimera must take several factors into consideration. Primary among these, is that the antigenic surface of Env must be left undisturbed and intact for antibodies to access. To achieve this, we genetically modified the C-term of HIV Env and used a cysteine-maleimide based conjugation protocol (FIG. 1A). HIV Envs were then assembled onto DNA nanoscaffolds through hybridization (FIG. 1B). Inter-molecular distances can be successfully controlled by placing probe strands at different positions along the 24HB rod to achieve optimum density for EM imaging (FIG. 1C). MAbs hitting a variety of epitopes on HIV Env have been imaged and characterized on the DNA scaffolded Env complex (FIG. 2B). We are currently testing the conjugation protocol on multiple strains of HIV Envs and assembling them onto the same 24HB DNA scaffold in defined positions. This approach using antigens bound to DNA scaffolds can be easily combined with EMPEM studies of other viral systems, such as influenza and coronavirus.

References


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Nanoscale application and measurement of high force using DNA Origami nanocalipers

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The ability to apply and measure large forces (>10pN) on the nanometer scale is critical to the ongoing development of nanomedicine, molecular robotics, and the understanding of several high-force biological processes such as chromatin condensation, membrane deformation, and viral packaging. Current techniques in force spectroscopy to apply nanoscale forces are lacking in throughput and applicability in vivo. DNA Origami nanocalipers are uniquely positioned both as a high-throughput measurement technique and as a bio-compatible device which can be applied to both in vivo and in vitro measurements to meet these challenges.

We show that DNA origami devices are well suited to meet these needs through transmission electron microscopy (TEM) and Forster resonance energy transfer (FRET). We demonstrate that FRET is an accurate readout of open/closed state as confirmed by direct measurement of the angle of the hinge through TEM. The nanocalipers are trapped temporarily by a base-paired DNA internal strut which is fluorescently labeled to exhibit high FRET in the closed state and low FRET in the open state. This work is supplemented by a partition function model predicting the closed fraction as a function of sequence. In addition to direct contributions from base-pairing, the model incorporates the effect of shortening the internal strut by increasing the number of paired bases. This effect is due to the difference in contour length between single-stranded and duplex DNA. We additionally predict, through the computational modeling platform oxDNA, as well as directly observe, bending of our structure and its ensuing effect on the apparent angle of the nanocaliper under high force. We continue by measuring FRET in single molecule total internal reflectance microscopy, allowing us to directly measure the kinetics of opening and closing. Kinetics within the hinge are compared to a direct measurement of the kinetics of internal strut binding in the absence of the hinge using a fluorescence quencher system. By comparing the dissociation rate between these samples, we can measure the average force applied by that hinge in the closed state. To summarize, we characterize the nanocalipers through their closed fraction, output force, kinetics, and bending to understand the effects of sequence, vertex design, strut length, and placement on the mechanics of the nanocalipers through modeling, ensemble, and single molecule techniques. By directly measuring and engineering forces up to 25pN, we elucidate new DNA Origami design principles specifically for nanoscale high-force applications which will likely be necessary to study and engineer high-force phenomena.
Fig 1. a) The high-force DNA Origami nanocaliper with FRET readout. Measured force is increased by increasing the length of an oligonucleotide attached to one arm of the nanocaliper, resulting in a larger number of complementary bases. b) The dissociation of conjugated quencher-oligonucleotide is observed through monitoring the fluorophore signal on a single molecule TIRF microscope. Biotin-PEG passivated surfaces are used to anchor the fluorophore construct to the imaging surface.

Fig 2. a) Example single molecule trace for dsDNA quenching experiment, 8bp in length, at 20nM concentration. The trace was fit to a hidden Markov model using ebFRET and a histogram of the raw data collated over time is shown at right. b) Cumulative sum of melting events at 20nM oligonucleotide concentration. A single exponential fit yields a characteristic rate of .03 events per second. c) A summary of kinetics for the 4 concentrations sampled for the 8bp oligonucleotide. d) Example single molecule trace for a nanocaliper closed by an 8bp internal strut with FRET reporter. e) Cumulative sum of opening events for 8bp internal strut nanocaliper, which single exponential fit yielding a characteristic rate of .15 events per second. f) Estimation of the force from kinetic measurements. The distance over which the force is applied is estimated with an upper bound of .3nm, the distance on-axis between paired bases in B-form duplex DNA.
1 Introduction
This abstract demonstrates a novel scheme for storing information and performing computation on randomly nicked DNA. Previous research has shown that hydroxyl radicals can be used to cleave DNA — we will use the term “nick” DNA — randomly along its backbone [1]. One can exert fine-grained control on the rate of nicking. We exploit this process to store fractional values: the value stored in DNA is a fraction between 0 and 1, relative to a maximum rate of nicking. We use toehold-mediated DNA strand displacement, a powerful tool for performing computation on DNA [2], [3]. We also use DNA enzymes such as ligase, which repairs nicks on the DNA backbone, and flap endonuclease, which snips off overhanging single-strand flaps [4],[5]. With random nicking, we can exploit the theory of stochastic computing to transform stored fractional values [6]. We demonstrate the basic operations of data storage and computation in this paradigm.

2 Encoding Data on DNA
We propose nicking DNA strands using hydroxyl radicals. Nicking produces double-stranded DNA complexes with random cuts in the phosphate backbone. After denaturing, bases opposite adjacent nicks are exposed. Call the fractional value that is stored the probability that a given base is exposed. Equivalently, by the law of large numbers, it is the ratio of the total number of bases exposed to the total number of bases. We will call contiguous exposed regions in this strand gaps and their counterparts covers. For example, in Figure 1 (4), the complex has 11 bases exposed out of a total of 38 bases, so the value stored by the strand is $11/38 \approx 0.2894$. There are 4 gaps and 4 covers.

3 Nicking Rate Transformation
This process of data storage assumes that random nicking can be performed selectively on the phosphate backbone of only one of the two strands in a double-stranded complex. When the complex is lightly denatured, bases between adjacent nicks fall off. We call the minimum distance between adjacent nicks for this to occur the threshold for denaturing. The strand can be treated with ligase to repair any extraneous nicks after this stage. Call the nicking rate, the rate at which nicks are produced, relatively to a maximum rate. We have determined that the following function predicts the fractional value of a strand $f$ based on the nicking rate $x$ and threshold for denaturing $k$:

$$f = 1 - (1 + kx)(1 - x)^k$$

This result is based on simulations as well as analytical reasoning.

4 Logical AND operation
Based on the theory of stochastic computing, a logical AND operation produces a result that is the product of two fractional values [6]. Figure 2 depicts how the operation can be performed: the probability of a certain base being exposed in the final strand $C$ is the probability of it being simultaneously exposed in strand $A$ and in strand $B$; this is the product of the fractional values of both strands, assuming these were created randomly and independently. Note that with $f_A = 11/38$ and $f_B = 13/38$,
we have $f_C = 4/38 \approx 0.1052$. This approximates $f_A \times f_B \approx 0.0990$.

5 Logical NOT operation

Based on the theory of stochastic computing, given a fractional value $x$, a logical NOT operation produces a value $1 - x$. Figure 3 shows a NOT operation: the denaturing step separates out the covers for the output strand that exactly fill the gaps in the input strand.

6 Conclusion

In this abstract, we presented a novel scheme for storing data in DNA using random nicking with hydroxyl groups. We also proposed methods for implementing basic computation, namely products with AND operations and $1 - x$ computations with NOT operations. The methods are fundamental yet powerful. Research in stochastic computing has shown that complex functions can be performed by composing these operations [6]. For instance, a Taylor series expansion of $e^{-x}$ can be computed with only 7 such operations:

$$e^{-x} \approx 1 - x + \frac{x^2}{2!} - \frac{x^3}{3!} = 1 - x\left(1 - \frac{x}{2}\left(1 - \frac{x}{3}\right)\right)$$

We have designed and verified computation of such functions through simulation. We are collaborating with the Soloveichik and Milenkovic groups at the Univ. of Texas and the Univ. of Illinois, respectively, to demonstrate the computation experimentally on DNA.

7 References


Characterizing the force dependent kinetics of a DNA origami force probe


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DNA origami nanotechnology is a rapidly developing field that shows promise in scientific applications such as mechanically aided drug delivery, molecular sensing, force sensing, and probing of single molecule dynamics. Complex and dynamic 3-dimensional structures can perform a prescribed function through controlled actuation making their use precise and reproducible. The endogenous nature of the DNA building blocks makes it well suited for biological applications due to its ability to hybridize with both biological and inorganic systems. This versatility makes DNA origami a valuable tool for mechanistic studies of biological systems in which physical perturbation is necessary.

Scaffolded DNA origami consists of a several kilobase (kb) single stranded (ss) plasmid that is annealed into a three-dimensional nanoscale structure by short (~50 base) ss staples. By systematically choosing the positions of cross-linking staples, countless unique structures can be created. This is achieved by mixing the DNA scaffold strand with a specific set of staple strands, heating the sample to denature any base pairing interactions, then slowly cooling the reaction (hours to days). This slow cooling ensures that the base pairing interactions reach their lowest energy configuration.

The device in our study, called a nanodyne, acts as a binary force sensor (See Figure 1). Consisting of two origami bundles linked by six crossover strands, the device can exist in either an open or closed configuration. With careful design of the crossover strands, the nanodyne can be programed to open at a prescribed force. By characterizing nanodynes with different crossover configurations we accrue a library of force sensing nanoprobes. These nanoprobes can then be used in biological systems where traditional force spectroscopy techniques are more challenging to implement. For instance, shear forces due to fluid flow can be challenging to determine in non-idealized environments, such as in a blood vessel or extracellular matrix.

In order to achieve high resolution data to build our library of devices, we have built a magnetic tweezer from an inverted microscope body. With this new instrument we have a

Figure 1: DNA Origami Nanodyne Structure. The nanodyne consists of 2 DNA origami barrels connected by 6 ssDNA linkers. With the addition of specific oligos, these linkers can be constrained (red), unconstrained (green), or able to fluctuate (blue). The diagram only shows 2 of the 6 linkers for clarity. The force dependent opening and closing kinetics will be modulated by the number and type of integrated linkers.
maximum resolution of 3-4 nm in the axial direction (Figure 2a) and a camera frame rate between 150-250 Hz (depending on the size of the field of view). This is more than sufficient for measuring the nanodyne opening which has a gap size of around 34 nm and opening/closing rates on the order of seconds to minutes (Hudoba et. al.). We have shown with this new instrument that we can collect force extension curves of dsDNA tethers that fit to the Worm-Like Chain model (Figure 2b). This is essential for identifying single tethers which act as the ‘handle’ for pulling on DNA origami devices.

Previous experiments with the nanodyne by Hudoba et. al. focused on low force detection on the order of 100 fN. This was achieved by monitoring Forster Resonance Energy Transfer (FRET) fluctuations due to thermal forces exerted on a nanodyne with a 10 bp zipper. Our ongoing work will expand on these results to include force sensors calibrated to detect forces in the 0.1-20 pN range. Instead of FRET, we will utilize the axial resolution of our magnetic tweezer to detect the change in length of the nanodyne-tether complex due to the rupture of the embedded zipper. To accommodate zipper rupture over a range of forces, we can alter the length of the DNA zippers as well as the number and orientation in the structure. In combination with constrained and unconstrained loops, we can modify the 6 loops in the nanodyne to fluctuate under a prescribed force. After we have characterized the opening and closing dynamics in a range of devices exhibiting distinct behaviors, we can incorporate them into non-ideal systems for the purpose of force sensing.

References

Using strand displacing polymerase to program chemical reaction networks

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Abstract

Chemical reaction networks (CRNs) provide a powerful abstraction to formally represent complex biochemical processes. DNA provides a promising substrate to implement the abstract representation (or programming language) of CRNs due to its programmable nature. Prior works that used DNA to implement CRNs either used DNA-only systems or multi-enzyme DNA circuits (1, 2). Architectures with DNA-only components had the rationale of being biologically simple systems while multi-enzyme system aimed at following nature and its complexity. In this work, we explore an alternative architecture which lies at the center of the spectrum between DNA-only systems and multi-enzyme DNA systems. Our architecture relies on a strand displacing polymerase enzyme (3) for implementing CRNs. We, first, introduce the theory and design of our framework and then use in silico and in vitro demonstrations as a proof-of-principle. We design a simple protocol that approximates arbitrary unimolecular and bimolecular reactions using polymerase strand displacement reactions. Then
we use these fundamental reaction systems as modules to show *in silico* demonstrations three large-scale applications of our architecture, including an autocatalytic amplifier, a molecular-scale consensus protocol, and a dynamic oscillatory system (4). Finally, we experimentally engineer a catalytic amplifier as a use-case of our framework since such amplifiers require intricate design of DNA sequences and reaction conditions (5).

**Keywords:** DNA kinetics, Polymerase strand displacement, CRNs, Oscillatory protocols, Consensus networks

**References**


POSTER

Track on Synthetic Biology

Track Chair

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A DNA-origami scaffolded artificial nuclear pore complex

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In eukaryotic cells, the Nuclear Pore Complex (NPC) is the sole gateway that controls molecular traffic between the nucleus and cytoplasm1-3. At the core of this massive protein complex is a central channel lined with intrinsically disordered proteins called Phe-Gly-rich nucleoporins (FG-nups). Despite its ubiquity, the detailed mechanisms underlying the NPC’s barrier formation and selective permeability remains elusive. This is in part because of the NPC’s structural complexity and heterogeneity in living organisms and a lack of means to controllably arrange FG-nups in a confined space to recapitulate their natural physiochemical environment2,4.

Our approach to unlocking the nuclear transport mechanisms is to use the DNA-origami technique to create a nanoscale channel with FG-nups attached at programmed locations with defined stoichiometry, which we term NuPODs (NucleoPorins Organized by DNA). Such artificial constructs mimic key structural features of the NPC central channel, allowing for the systematic study of the influence of FG-nup configuration on their perm-selective properties. We previously constructed NuPODs with yeast central channel nups (Nsp1 and Nup100), where we discovered that the cohesiveness, density, and spatial organization of nups within a cylindrical confinement profoundly impact the structure and dynamics of the FG-nup collectives5.

To assess how unique combinations of FG-nups impacts the permeability of NuPODs, we have assembled a basket-shaped DNA structure with a rectangular prism that houses up to 48 copies of FG-nups and a chemically modified baseplate that traps incoming macromolecules. Using such a FG-nup gated nanochamber, we show that Nsp1 and Nup100 form very different diffusion barriers. While a NuPOD lined with 48× Nsp1 is permeable to proteins over 100 kD, 48 copies of Nup100 form a barrier that blocks the entry of ~50 kD proteins. The strength of the barrier depends on Nup100 copy number and likely on cohesive interactions as reducing Nup100 density or mutating its GLFG motifs to GLSG reduces the strength of the diffusion barrier. Lastly, to evaluate the kinetics of nuclear transport receptor-mediated transport through NuPODs, we have engineered an artificial nuclear envelope that features a GUV with membrane-embedded NuPODs. Therefore, these programmable NuPODs present an enabling platform for investigating the nuclear transport mechanism.

REFERENCES


POSTERS

Track on
Principles and Theory of Self-Assembly

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Complexity and modularity in a simple model of self-assembling polycubes

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It seems intuitively clear that some shapes will be easier to self-assemble than others. Recent theoretical results based on algorithmic information theory (AIT) [1, 2] have quantified this intuition by predicting that the number of assembly rules that lead to a particular shape scales exponentially with the Kolmogorov complexity of the simplest rule needed to make such a shape. Here we study this very general prediction using a simple model of 3D polycube structures whose assembly is controlled through face-face interaction rules.

The model, which is a generalisation of the 2D polyomino model [3, 4], stochastically assembles a set of cubic building blocks on a lattice, each of which can have an oriented and coloured patch on its six faces, into an output polycube. See Figure 1 for an example.

Starting with the first cube in the input, additional cubes are attached, similar to patchy particles, wherever colours and orientations match, and will bind to $-n$, while the orientation is in any of four possible rotations: $\text{I} (0)$, $\text{I} \left( \frac{\pi}{2} \right)$, $\text{II} (\pi)$ or $\text{II} \left( \frac{3\pi}{2} \right)$.

Figure 1: Illustration of how a genotype input (top), is mapped onto the phenotype output polycube by stochastic assembly from an initial seed. The growth stops when no more patches are exposed, making this particular output bounded.

Colours can be any integer number $n$.
As can be seen in Figure 2, there is a clear log-linear relationship between the probability of finding a rule that assembles into a particular structure, and the information needed to specify the structure, as predicted in [1, 2].

![Graph showing the power-law distribution of bounded and deterministic polycubes found by randomly sampling input rules. Each point represents a particular phenotype (five of them illustrated), with the x-axis being a measure of the smallest rule required to assemble the structure (a proxy for Kolmogorov complexity), while the y-axis is the approximated probability of finding the phenotype by random sampling. The outliers at the bottom right are caused by unique structures only found once in the finite sampling.](image)

Finally, we comment on how these rules apply to the design of large-scale self-assembled nanostructures. For example, it is much easier to design modular or symmetric structures than asymmetric structures, and the former may be much more robust to errors than the latter.

References


Ab-Initio Studies of Exciton Interactions of Cyanine Dyes

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Recently, cyanine dyes covalently bound in DNA complexes have been studied for their exciton delocalization properties that include J- and H-aggregate behavior and Davydov splitting1,2. The self-assembly properties of DNA can bring dyes within distances (≤2nm) that induce shifts in absorption maxima. Thus, DNA is used as a scaffold, allowing the manipulation of the orientations of the dyes. To improve the viability of dye-DNA systems for excitonic applications, the control of the positions of dyes in DNA systems is crucial. Density functional theory (DFT) calculations can be used to optimize the structures of molecular systems. However, proper choice of the exchange-correlation (XC) functional is necessary to model systems accurately. Therefore, various XC-functionals were employed and their effects on the ground state orientations and excited state properties of Cy5 dye molecules were explored in this study.

The orientations and excited state properties of cyanine dye Cy5 aggregate systems covalently bound to DNA were studied using DFT3 as well as an in-house program based on the theoretical model of Kühn, Renger, and May (KRM)4. The KRM model can predict the orientations of dyes within an aggregate from its absorbance and circular dichroism (CD) spectra and vice versa. DFT calculations were performed on the solvated Cy5 dimer structures to explore the effects of various DFT XC-functionals on the geometry optimization process. Time-dependent DFT calculations (TD-DFT) were also performed on the Cy5 monomer to explore the effects of XC-functionals and Franck-Condon (FC) approximations on the predicted absorbance spectra. Fig. 1a shows the Cy5 monomer optimized using DFT. Fig. 1b shows the Cy5 dimer in the orientation determined using the KRM program by fitting experimental absorbance and CD spectra. The center of mass separation is 1.47 nm and the angle of the dimer is 60°.

Fig. 1: The Cy5 (a) monomer optimized using DFT and (b) dimer determined by fitting experimental absorbance and CD spectra using the KRM program.
Comparing the experimental absorbance spectra to the spectra obtained with the KRM program, the dimer system optimized using the ωB97-XD XC-functional provides the best agreement with experiment, as shown in Fig. 2a. For Cy5 monomer systems, it was found that FC approximations provide good predictions of absorbance spectra when using an XC-functional with no long-range or dispersion correction (B3LYP), as shown in Fig. 2b. For the dimer system, it was determined that the ωB97-XD XC-functional provides the best agreement with experiment since it accounts for long-range and damped empirical dispersion corrections, whereas the other functionals tested do not. The long-range dispersion corrections are necessary to accurately estimate the dye-dye interactions in the dimer system. Furthermore, the B3LYP XC-functional is optimal for a single molecule because it does not have long-range dispersion correction which could overestimate the vertical absorbance transition energy.

![Fig. 2: (a) Predicted absorbance of the DFT-optimized Cy5 dimer system generated using the KRM program compared with experiment. (b) Comparison of the predicted absorbance of the Cy5 monomer using TD-DFT with FC approximations for various XC-functionals.](image)

Our computational results advance an understanding of the XC-functional effect on the structural stability and excitonic phenomenon in Cy5 dyes. DFT-based electronic structure calculations can help determine orientations of Cy5 dyes in the ground state. Furthermore, TD-DFT results reveal the effect XC-functionals have on the excited state properties and improve our understanding of how to best simulate dye systems.

References


Stretching DNA origami: effect of nicks and Holliday Junctions on the axial stiffness

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Abstract

The programmed self-assembly of synthetic DNA strands offers the unique ability to build complex architectures with precision at the nanoscale. These DNA nanostructures can act as templates for both organic and inorganic molecules, thus having tremendous potential in many areas, including drug delivery, super-resolution imaging and nano-manufacturing. Although there have been numerous works that focus on the self-assembly conditions, aiming at an increased yield and stability of the synthesized nanostructures, the mechanical properties of the resulting assemblies have been relatively unexplored, with the exception of their bending resistance and the corresponding persistence length. Despite a few previous studies [1-6], the mechanics of DNA nanostructures under external forces is yet to be systematically examined, in order to estimate their effective stiffness and improve the robustness of the designed assembly.

Here we systematically design nanobeams with controlled characteristics, and subsequently synthesize and test them to extract their mechanical properties under tensile forces. To evaluate the effect of nicks and immobile Holliday junctions (HJs) on the axial stiffness of DNA nanostructures, we use two base designs of DNA origami nanobeams. All nanobeams consist of two DNA duplexes connected through HJs, creating a series of periodic DX-tiles. The first design, C172N, has a double crossover every 21 nucleotides (172 HJs total) while in the second design, C86N, the distance between successive HJs corresponds to 42 nucleotides (86 HJs total). In both designs, shown in Fig. 1, the two helices are nicked in every center between consecutive crossovers, resulting in their total number of nicks being 171 and 85 respectively. The two designs were synthesized using a one-pot thermal process and successful assembly was confirmed by gel-electrophoresis and TEM. Treating T4 DNA ligase in both designs leads to two more nanobeams, C172L and C86L, which exhibit the same crossover density as the C172N and C86N, respectively, but with zero nicks. This allows us uncouple the effect of HJs and nicks on stretching stiffness of DNA origami.

To stretch DNA origami beams using hydrodynamic forces, the nanobeams are immobilized on a glass surface of the microfluidic device. The stretching force is applied through a micron-sized particle bound to the free ends of the nanobeam.
end of the DNA origami while the buffer is flown through the microfluidic device (Fig. 2). By adjusting the flow rate, the stretching force magnitude exerted on the DNA origami can be modulated ranging from 1 pN to 30 pN. The stretching force was applied step-wise, with 4.9 pN as the starting magnitude and a 2.5 pN unit increment every 10 seconds, until reaching a magnitude of 25 pN. The displacement of the micron-sized particle is tracked and the elongation of the DNA origami as a function of the applied force is recorded.

Force displacement responses show that the stiffest response corresponds to the ligated beam with the smaller number of HJs (C86L) while the more compliant beam is the nicked beam with the double density of HJs (C172N). Our measurements indicate a ligated nanobeams are 60% axial stiffer compared to their counterparts with the nicked helices, suggesting nicks significantly reduces the stiffness of the origami. Furthermore, we also observed that reducing the number of HJs by 50% results in more than twofold increase in stiffness. This result demonstrates that adding HJs in DNA nanostructures may increase bending rigidity and the corresponding persistence length, but counterintuitively decrease stretching stiffness substantially. To gain insights to our measurement results, we construct a computational model, where each helix is regarded as a continuum elastic rod with the effective geometric and material properties of B-form DNA. Our computational model leads us to conclude the local stiffness for both HJs and nicks is two orders of magnitude smaller than the corresponding one of the double helix.

References
Thermodynamics of DNA looping for origami folding

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

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

Figure 1. Single fold yield: Reporter intensity as a function of temperature and staple excess for a 3407 base fold. Scaffold folding reduces reporter intensity while side product formation does not.

Figure 2. Single fold thermodynamics: Fitted $\Delta H$, $\Delta S$ and $\Delta C_p$. H.O. is a hybridization-only control sample.
A Computationally Derived Technique for the Precision Placement of Heterogeneous Nanoscale Species on a Single Substrate

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Despite fantastic achievements in the field of DNA nanotechnology, it remains very difficult to place DNA devices at precise locations on substrates. Most devices float freely in solution, limiting their applications. For instance, in plasmonics, device function would be strongly enhanced if scientists had more control over the relative orientation of each device; or in biomolecule detection, spatially registered arrays of devices capable of detecting specific DNA sequences and proteins could enable screening for thousands of biomolecules at the same time. While recent progress has enabled the placement of a single species of DNA nanodevice on a substrate with arbitrary orientation,¹,² precisely placing multiple species on a single substrate is not currently possible. This is a major challenge because the interactions between DNA and the surface to which it adsorbs are nonspecific, which points toward a much broader question in nanoscience: how does one arrange a set of small, nonspecific interactions into a larger interaction that is specific? Our ability to realize multifunctional self-assembled systems will be restricted without this technology.

Using energy landscape analysis and a Brownian dynamics model, we propose a technique that should allow for the specific placement of two species of DNA devices on a spin-coated silica substrate with lithographically defined binding sites as outlined in Ref. 1. Based on our method, one DNA device is smaller than the other, with steric brushes attached in the manner of Gopinath and Rothemund² to provide an effective radius that is larger than the region which interacts with the silica binding site. Carefully tuning the relative sizes of the species and the size of the repulsive brushes creates a condition which prevents multiple smaller devices from occupying a larger binding site in a metastable state, which we call “single occupancy”, while still allowing the large device to displace smaller devices from their binding sites without any metastable states. This solution is generally applicable to any heterogeneous nanoscale species onto which polymers can be grafted.
Figure 1: Assembly state diagram for the entire design space of this concept. The x axis represents the size of the repulsive brushes relative to the large tile. The y axis represents the size of the small tile relative to the large tile. Purple, orange, red, and blue regions contain metastable states which are expected to adversely affect yield or prevent organization altogether. The green region satisfies both the single occupancy requirement and the metastability-free rejection requirement, thereby enabling two-species placement on a surface.

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POSTER
Special Track on Encapsulated Systems

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Oily ocean surface films as a cradle for the emergence of life

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Progress in the bottom-up construction of synthetic cells\textsuperscript{1} has given insights into how biological organisms function and on the origins of life. Despite advances in the last decades, a demonstration of an open-ended \textit{in-vitro} evolution of synthetic cells that is simple enough to serve as a plausible model of the prebiotic/biotic transition has yet to be demonstrated. Here, we develop a synthetic cell platform based on geophysical considerations.

We model the protocell as oil-in-water droplets. While there have been several works demonstrating oil-in-water droplets exhibiting the behaviors that protocell may encounter,\textsuperscript{2,3} there have yet a complete demonstration of oil-in-water droplet that can undergo a complete life cycle involving birth, growth, replication, and death, within a plausibly prebiotic environment.

The proposed model centers on the ubiquitous mechanical processes in the dispersal of natural and man-made oil slicks in modern oceans. In the prebiotic world, the organic matter, primarily delivered to the earth’s surface by micrometeorites, would have naturally accumulated to ocean surface as oil slicks.\textsuperscript{4} Additionally, these organic compounds were, perhaps, prevented from reaching shore by entrapment in ocean gyres. Hydrodynamics forces such as raindrops or breaking waves on these thick organic films, resembling a primordial soup, would have produced droplets (Fig. 1A). Similar notion was proposed by Oparin where hydrodynamic forces play an important role on the origins of life by enabling Darwinian evolution in the fragmentation of coacervates through hydrodynamic shear.\textsuperscript{5} Comparably, the oil droplets could have undergone
growth via accumulation of organic compounds (Fig. 1B–i), fission via locally intense hydrodynamic shear produced by rainfall or breaking waves (Fig. 1B–ii), and eventually death via the loss of buoyancy through mineral accumulation (Fig. 1B–iii). In short, these droplets could have functioned as simple self-replicators that natural selection could act to select droplets possessing more fit chemical and structural composition, and ultimately giving rise to the protobionts.

Finally, we will present results from preliminary laboratory experiments that explore the rich fluid-mechanics phenomena involved in the production of oil droplets from hydrodynamics forces acting on oil films residing on water surfaces and in the fissioning of oil droplets (Fig. 1C). In particular, raindrop impacting oil film residing on water also produces water-in-oil-in-water (w/o/w) droplets that resemble lipid vesicles in that a volume of water is surrounded by film of oil (Fig. 1D). Such droplets may have facilitated the prebiotic/biotic transition. Stability test reveals that the droplets can maintain its identity up to three days if PEG-octyl-ether is used as the surfactant. In contrast, the droplets are only stable on a time scale of minutes for PEG600-cholesterol surfactant. Further experimental development may lead to a bench-top system in which oil droplets undergo growth and fission, and are maintained at a steady state concentration through continual removal of oil droplets which is analogous to a death process in natural environment. The population of oil droplets maintained by such a system could serve as a platform for in-vitro evolution.

References

POSTERS

Track on
Nanophotonics and Superresolution

Track Chair

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Strongly coupled dye aggregates are of interest for a variety of applications, including light harvesting and energy conversion, optoelectronics, and quantum computing. Although dye aggregates can form via spontaneous aggregation in solution, or even intercalation into the major or minor groove of DNA, greater control over aggregate structures and properties (e.g., spacing, orientation, and subsequent coupling strength) is desirable. Templating dye aggregates via covalent attachment to the DNA backbone provides a novel means by which to more precisely control aggregate structure. Despite the fact that the static electronic structure of DNA-templated dye aggregates has been studied in some detail, there is a paucity of studies that directly probe their excited-state dynamics. While accelerated lifetimes have been reported in some recent studies of DNA-templated dye aggregates, the predominant mechanism of decay remains unclear. In this talk, we describe how nonradiative decay governs exciton lifetimes in DNA-templated constructs of strongly-coupled cyanine 5 (Cy5) dye aggregates. In particular, we have employed simple DNA duplex strands and Holliday junctions to create a variety of oligomeric dye aggregate structures with interesting optical properties indicative of strong excitonic coupling [1,2]. Steady-state and time-resolved (time correlated single photon counting, TCSPC) fluorescence measurements suggest and confirm, respectively, that nonradiative decay is the primary decay pathway in these DNA-templated cyanine aggregates [3]. Femtosecond transient absorption measurements quantify the singlet
exciton lifetimes, which are on the order of tens to hundreds of picoseconds for the aggregates (Figure 1). In addition, the results indicate that nonradiative decay in the form of internal conversion contributes 99.5 and 99.9% to the overall decay in J- and H-type aggregates, respectively [3]. These results imply that suppressing or eliminating nonradiative decay channels in DNA-templated dye aggregates is essential for their use in optoelectronic applications.

Figure 1. Excited state lifetime decreases by several orders of magnitude for strongly-coupled DNA-templated cyanine 5 (Cy5) dye aggregates relative to that of the monomeric dye covalently bound to DNA due to enhanced nonradiative decay via internal conversion in the aggregates. Figure adapted from [3].

Acknowledgments

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References


Optical Properties and Nonradiative Decay in Solutions of DNA-Templated Dye Aggregates


The use of DNA as a scaffold for controlled assembly of dye aggregates is a promising technique for future artificial light harvesting, optoelectronic, and quantum computing device fabrication. Using a combination of steady-state fluorescence, time correlated single photon counting, and ultrafast transient absorption spectroscopy, we have found that exciton lifetimes in J- and H-aggregates of cyanine 5 (Cy5) dyes formed via covalent attachment of the dyes to DNA templates (in particular, double stranded DNA and Holliday junctions) are governed by nonradiative decay [1]. Here we describe additional detailed optical characterization of the structures present in these DNA-templated dye aggregate solutions, as well as quantitative analysis of the excited state dynamics of these structures. While fluorescence is suppressed in the J- and H-aggregates due to enhanced nonradiative relaxation, fluorescence excitation spectroscopy shows that even after purification via gel electrophoresis there exists a low concentration, but highly emissive, subpopulation of species in both the J- and H-aggregate solutions. We have identified this bright subpopulation as “optical monomers” (i.e., weakly coupled dyes), and in the case of the J-aggregates, have developed an approach to extract the emission spectrum of the weakly fluorescent J-aggregate species from the overall solution emission spectrum (Figure 1). This analysis also provides quantitative and mechanistic insight into the accelerated lifetimes observed for these DNA-templated dye aggregates. In particular, the expected lifetime of the J-aggregates in the absence of increased nonradiative decay upon aggregation was derived for the limiting cases of either lack of or complete superradiance, then compared to the experimentally measured lifetime.
These results confirmed that nonradiative decay is the predominant decay pathway in these structures, accounting for 99.5% and 99.9% of the total decay rate for the J- and H-aggregates, respectively.

Figure 1. UV-vis absorbance spectra of cyanine 5 (Cy5) dyes covalently attached to DNA templates with associated fluorescence spectra for the monomer and J-dimer species. Figure adapted from [1].

Figure 2. Predicted (complete superradiance case, i.e., N = 2) versus measured fluorescence decays for the ground state recovery of the excited Cy5 J-dimer. Figure adapted from [1].

Acknowledgments

Work at Boise State University was supported by the NSF through INSPIRE No. 1648655 and MRI Award 0923541. Portions of the work were also supported by DOE LDRD No. 154754. Z.S.D.T. and G.D.S. acknowledge support from DOE Grant No. DE-SC0019370.

References

Single Molecule Detection on a Portable Smartphone Device with DNA Origami Nanoantennas

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Detection of single molecules represents the ultimate sensitivity one can achieve in a diagnostic assay. While fluorescence is the method of choice for numerous diagnostic applications, the utilization of single-molecule fluorescence detection to the fields of medical diagnostics and point-of-care sensing is still limited by the number of photons that can be detected from a single fluorescent label. Often the single-molecule approach is limited to expensive and elaborate optical setups that are required to observe a single molecule. One strategy to bridge this gap relies on physical fluorescence signal amplification by plasmonic nanostructures that can act as optical nanoantennas concentrating the incident excitation light into zeptoliter volumes and enhancing the photon stream of single fluorescent molecules.1-2 In this contribution we describe our recent progress in development of such self-assembled DNA origami nanoantennas that could be used for point-of-care diagnostics.3-5 Exploiting the unique positioning precision and addressability of DNA origami,6 we designed plasmonic nanoantennas with diagnostic assays incorporated directly in the plasmonic hotspots of gold and silver nanoantennas (Figure 1a, right). To this end, we designed a two-pillar shaped DNA origami structure (Figure 1a, left), which not only provides high fluorescence enhancement but also an accessibility to the hotspot region required for single-molecule diagnostic applications. Single-molecule fluorescence enhancements reaching few hundred folds could be achieved using DNA nanoantennas composed of this DNA origami structure and 100 nm silver or 100 nm gold nanoparticles (Figure 1b). This signal amplification provided by plasmonic DNA nanoantennas allowed us to achieve single molecule detection with a smartphone camera. We designed a home-build, portable and battery-driven smartphone device capable of detecting single fluorescent dyes when placed in the hotspot of plasmonic silver nanoantennas (Figure 1 c and d). We used this
portable device to carry out a diagnostic sandwich assay for the detection of DNA specific to antibiotic-resistant *Klebsiella pneumoniae* (fragment of the Oxa-48 gene).\(^7\)

**Figure 1.** a) Sketch of DNA nanoantenna utilized for fluorescence amplification (left) and DNA origami nanostructure used for DNA nanoantenna assembly (right); b) Fluorescence enhancement histograms obtained for ATTO647N dye when placed in monomer and dimer nanoantennas composed of 100 nm gold and 100 nm silver nanoparticles; c) Portable smartphone device for single-molecule detection; d) Examples of single-molecule fluorescence trajectories of Alexa Fluor647 used for detection of DNA specific to antibiotic-resistant *Klebsiella pneumoniae* and recorded on a portable smartphone device.

**References:**

Origami Arrays Supporting Analog and Digital Sensing

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Origami platforms provide one of the very few methods for high resolution placement of single molecules. Such placement precision has been shown to be useful for the representation of multiple single molecule recognition events in the form of nanoscopic patterns. Regardless of the readout method (AFM vs Optical), the appearance of data in a prescribed pattern can be used as a differentiator against nonspecific, adventitious signals (noise or clutter) which are unlikely to mimic the intended pattern. For optical reporting Abbe’s law may set constraints on the density of sensor sites useful in pattern production. Several examples of Origami based platforms developed to span the size scale representing a crossover from analog to digital sensing domains are provided. The application of structures topographically chiral in two dimensions to gain insight into photonic and mechanical properties of reporters is also presented.

AFM image of multiple copies of an origami platform which is chiral in two dimensions.
POSTERS

Track on
Computational Tools for Self-Assembly

Track Chair
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A multiscale approach to the analysis of structured DNA assemblies at the molecular resolution

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Structural DNA nanotechnology has enabled the construction of scalable and complex objects through self-assembly of complementary sequences. Demand for a more efficient computational approach to analyzing and designing DNA nanostructures keeps increasing as a result. Here, we present a multiscale strategy for modeling and analyzing structured DNA assemblies that allow the rapid prediction of their shape and properties with base-level precision. First, the equilibrium geometry and mechanical properties of local structural motifs classified according to connectivity between bases or base-pairs were characterized through molecular dynamics simulations. Second, coarse-grained structural models were developed to describe these mechanical characteristics accurately. Those for electrostatic repulsive forces between helices were also developed. Finally, the equilibrium shape and derived properties were obtained by constructing the global stiffness matrix of the entire structure in the finite element analysis framework. The validity of the proposed method has been tested via comprehensive example designs.

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Overcoming convergence issues in predicting the three-dimensional shape of DNA origami structures through partition and relocation strategy

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As structural DNA nanotechnology for constructing sophisticated three-dimensional structures has advanced considerably over recent years, computational methods to predict the shape of DNA origami structures more accurately and efficiently become important [1-3]. The expected final shape of a DNA origami design can be very different from its initial configuration particularly when highly curved and/or twisted parts exist as it is often designed on a lattice for convenience [4]. As a result, the solution procedure for shape prediction may suffer from convergence issues such as non- or slowly-converging calculation steps and converging to a wrong configuration. To overcome these convergence problems, we developed an algorithm to automatically partition the original DNA origami design into multiple sub-structures connected to one another and relocate them in 3D space. Relocated positions are determined such that sub-structures would not (or hardly) be tangled or overlapped during analysis steps. We could obtained the three-dimensional shape of several DNA origami structures correctly without any convergence problem.

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References

A versatile robust framework for automating design of multi-component DNA nanostructures

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URL: https://go.osu.edu/fnano20_MagicDNA_poster

Structural DNA nanotechnology¹ utilizes programmable properties of DNA to construct nanodevices for a range of applications in science and technology. Currently, design tools for these DNA nanostructures rely largely on a bottom-up manual approach² where challenges with the routing process limits the number of components, or top-down automated approaches³,⁴ that are limited to selected types of static geometries. Mimicking modern computer-aided-design (CAD) of mechanical assemblies with multi-components, we developed a new design software that combines the benefits of both bottom-up and top-down approaches into a versatile framework that automates design of multi-component DNA nanostructures with complex geometries, selected mobility (static or dynamic) and a large size based on incorporation of multiple scaffolds. We call this software Multi-component Assembly in a Graphical user Interface guided by Computation for DNA self-assembly (MagicDNA). Enabled by several computational algorithms and graphical user interfaces, the proposed framework also enables feedback from coarse-grained molecular dynamics simulations to facilitate rapid design iteration for complex structures. Fig. 1 details the workflow for our design process in the MagicDNA software. We validated the capabilities and robustness of the framework through design and fabrication of several DNA assemblies. One example is shown at the bottom right of Fig. 1D.

Fig. 1. Schematic of the proposed design framework for multi-component DNA origami structures. (A) Top-down line modeling for specifying the geometry. By defining the cross-section of each line, a cylinder model composed of multiple bundles is created. A cylinder model can be saved into part library individually or in a group for use in future designs. (B) In the assembly process, each bundle can be subjected to translate or rotate in 3D space. After specifying the connectivity between bundles, the mechanism is assembled with single-stranded scaffold connections. (C) Automatic routing algorithms search the strand routings for both multi-scaffold and staple. (D) Visualization of the design as both 2D and 3D diagrams and two-way interface with the caDNAno software for fine tuning. (E) Simulation of the design profile before fabrication to examine the routings, including generating the topology and configuration files of the coarse-grained oxDNA model and analysis of the trajectory file. (F) After iterative optimization, export a list of staple sequence and further experimental validation.


DNA nanotechnology as a field has demonstrated the capability of making almost any desired shape of interest. For the field to reach its full potential there is a requirement to make it accessible to the larger scientific community and thus a need for automation. Although there have been a few developments in this regard, there has not yet been an automated tool to make smooth curvature based closed DNA origami nanostructures (herein termed as DNA capsules). In this work, we present the software-automated design of various axially symmetric geometrical shapes which we thereafter tested extensively via experiments. Folding staple sequences were automatically generated for these hollow shell DNA origami capsules. The software developed also takes into account various design parameters such as crossover positions, nucleation sites and strand routing paths. The formation of DNA nanostructures based on these generated sequences were then validated experimentally. These experiments helped us in readjusting certain parameters in the process of automation. During this process, we realized that certain structures did not form very well when they were single layered, however, they jumped in yield and formation intactness when they were multi-layered. In this work, a few experimental demonstrations of this new design strategy has been validated through some example shapes like cone, mushroom and dumbbell, wherein
the single layered structure had either poor yield or bad structural integrity, but when they were multi-layered the formation capability increased.

Figure. 1. The design process of software. (A) The 3D model is sampled to (B) generate a custom circular mesh. Each mesh line is regarded as a DNA helix and helical geometric properties are applied. A network of crossovers and nicks are systematically applied to (C) produce staples within a certain length range (30-60), which can be (E) used to simulate and thereafter synthesize the DNA nanostructure.
In vitro disassemble and reassemble of the encapsulin nanocompartment of Myxococcus xanthus

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Encapsulins are shell proteins that assemble in nanocompartmentalization systems found in prokaryotes (ENCs, encapsulin nanocompartments). These systems are responsible for encapsulating cargo proteins which allow control over cell metabolism by creating unique microenvironments. Their biological importance is reflected in the widespread distribution and variation of these systems and their cargo proteins.

Shell proteins form encapsulin nanocompartments are structural homologs of the HK97 viral capsid protein, varying in diameter, number of protomers that assemble them, and the presence of pores. The ENCs are assembled by 180 protomers that in the join have a diameter of 31 nm. ENC from Pyrococcus furiosus and Myxococcus xanthus present three conserved domains: peripheral domain (P), domain axial (A) and an elongated loop (E) which align well with the gp5 protomer of the phage capsid HK97. The multiple pores that have encapsulin nanocompartments have diameters of 5 to 6 Å, which vary according to their chemical nature. The encapsulation of cargo proteins as ferritins (FLP), DyP, hemertrine and rubretryhin peroxidases is directed by a short C-terminal targeting sequence called cargo loading peptide (CLP), a conserved short sequence of 10 amino acids that directs the highly specific encapsulation process. The importance of cargo proteins lies in the physiological function of nanocompartments and their assembly. The way in which these cargo proteins interact with shell proteins occurs in different ways: 1) through a CLP in the N-terminal position of the cargo protein, or 2) the cargo protein is merged with the shell protein as a single polypeptide, lacking CLP.

The ENC of M. xanthus is formed by a cover protein (EncA, 32.5 kDa) and three internal proteins (EncB, 17 kDa; EncC, 13 kDa; EncD, 11 kDa). The shell forms a T=3 icosahedral structure with a diameter of 30–32 nm composed by 180 protomers. The internal proteins EncB and EncC have ferritin-like domains and are adhered to the internal surface of EncA. EncB and EncC allow M. xanthus to store iron in the form of ferrihydrite, storing an order of magnitude more iron than ferritins, based on their larger amount. In this work, we studied the physicochemical conditions for in vitro disassemble and reassemble of M. xanthus ENC, and the encapsulation of heterologous proteins throughout the reassemble process. Our results demonstrate that in vitro reassemble and encapsulation of heterologous proteins is possible.
POSTERS

Track on
Integrated Chemical Systems

Track Chair

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Intracellular Protein Delivery by Means of Glutathione Sensitive Cleavable Linker

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Figure 1. Bioconjugation scheme of DB-TPP linker onto a protein model followed by glutathione disulfide exchange

Improving intracellular protein delivery can be the next best thing in curing protein-related diseases, because proteins serve a wide range of functions, such as: catalyzing reactions, transport and storage of molecules and gene expression regulation. These processes are affected by misfolding or mutations of proteins, often leading to diseases such as: diabetes, cancer, or neurodegenerative diseases. Therefore, there is an essential need to deliver therapeutic proteins into the cell. The size, charge, and polarity of proteins along with their hydrophilic nature makes their delivery challenging. A number of approaches have been used to overcome these limitations, and from cell-penetrating peptides (CPPs) and liposomes to nanoparticles, they all have a common goal of delivering outside cargo into the cell. However, they suffer from lysosomal degradation, premature leakage, and low dosage concentration of delivered protein to the target site respectively. In this work, we address this therapeutic challenge by modifying the surface of a protein of interest and render it able to cross the cell membrane.

When designing an effective protein delivery system, we took advantage of the cysteine residues on the surface of a protein and their ability to perform bioconjugation reactions through disulfide bonds. Disulfide bonds can be reduced to free thiols followed by conjugation onto a lipophilic cation that will help the protein perform endosomal escape. Once in the cytosol, glutathione will cleave the linker and restore the protein its original bioavailability and functionality. This system provides intracellular protein delivery by the means of a glutathione-sensitive cleavable linker. Bacteriophage Qβ is the model used, a 28 nm icosahedral nanoparticle, with 180 solvent exposed disulfide groups that can form hexametric and pentameric subunits by linking five or six monomers. Qβ is the protein of choice, because it can account for both large and small sized proteins. The idea behind this is to modify a Dibromomaleimide (DB) molecule by attaching a nontoxic lipophilic positively charged compound, Triphenylphosphine (TPP), to it then conjugate
the linker to Qβ. This modified linker can utilize the reversible nature of the disulfide bonds on the surface of Qβ. Due to TPP’s positive lipophilic character, the linker aided the carrier in overcoming endosomal escape and delivered Qβ safely inside the cell. Conceivably, once in the cytosol, the protein encountered a sulfur-rich environment due to relatively high concentration of glutathione. This caused a disulfide exchange to take place and cleaved the glutathione-sensitive linker, allowing Qβ to regain its individual characteristics of biological activity, biodistribution, and metabolism.

Consequently, efficient bioconjugation of DB-TPP to the protein model Qβ is the means by which any protein rich in disulfide bonds can help the protein perform endosomal escape and get delivered safely into the cytosol.


Combining Photothermal Therapy with Immunotherapy for Breast Cancer Treatment and Metastasis Inhibition.

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Cancer is one of the leading causes of death in the world despite recent advances in therapeutic strategies still there is a substantial number of patients are confronted with disease recurrence and low survival rates. The main reason for the insufficient therapeutic efficacy is an inability to fully eliminate cancer cells in a tumor environment and eradicate metastases at distant sites. Therefore, there is a need for an approach to not only destroy solid tumors selectively but also eliminate disseminated and metastatic lesions simultaneously. One way to reach this approach is combining available therapeutic strategies such as immunotherapy and photothermal therapy to capitalize on the advantages of both methods and amplify the treatment efficacy compared to either immunotherapy or photothermal therapy alone. In this work we took advantage of virus like particles (VLPs) unique properties such as ease of synthesis, functionalization ability, and most importantly their immunogenicity to introduce a novel in situ photothermal agent to successfully suppress primary breast cancer tumor in BALB/c mice bearing a highly metastasis 4T1 tumor, prolong survival time and reduce metastases.
in distant sites. For this purpose, we used the VLP, Qβ, as an immunogenic agent as well as a multivalent scaffold for the attachment of a NIR dye (croconium). After the conjugation of croconium (croc) dye, Qβ has been able to act as a NIR-triggered mediator and convert light energy into heat, causing cell ablation by hyperthermia and heat stress activated inflammatory signals. Also, Qβ by itself can work as an immunoadjuvant, owing to its encapsulated bacterial RNA, and pathogen associated molecular pattern to further enhance antigen presenting cells activation to amplify the subsequent immune response. In the experiments, intratumoral administration of Qβ-croc followed by laser radiation could apply the synergistic effect of PTT and immunotherapy that resulted a higher tumor suppression on the treated tumor-bearing mice and effective lung metastasis prevention by inducing long-term anti-tumor immunity.

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Hierarchical Assembly of Nucleic Acid/Coiled-Coil Peptide Nanostructures

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DNA and peptides are two of the most commonly used biomolecules for building self-assembling materials, but few examples exist of hybrid nanostructures that contain both components. In the past three decades, DNA has been the forefront the most useful

Figure 1. Overview of strategy for assembling DNA origami with coiled-coils. A) Site-specific functionalization of two peptides comprising a coiled-coil with DNA. Mixing the two peptide-DNA conjugates yields a self-assembling coiled-coil with two orthogonal oligonucleotide handles. B) Assembling a DNA origami structure (e.g. a cuboid) with multiple ssDNA handles into 1D nanofibers driven by the coiled-coil bearing complementary handles. C) Agarose gel electrophoresis analysis of assembly. Lane M: dsDNA ladder; 1: M13 scaffold; 2: cuboid with A* and B* following purification. D-E) AFM and TEM images of cuboid nanofibers assembled by coiled-coil interactions.
building blocks for self-assembled materials due to the programmable nature of Watson-Crick pairing. This has led to a wealth of complex, anisotropic, and highly functional with programmable and dynamic properties. Polypeptides, by contrast, have the advantage of greater chemical diversity made available through the twenty canonical amino acids, and a huge range of synthetic non-canonical amino acids if solid-phase synthesis is used. Peptides also possess multiple structural motifs (e.g. α-helices, β-sheets) and self-assembly modes (e.g. coiled-coils, collagen triple helices) that impart unique functional and mechanical properties. The unification of these two separate materials into one self-assembling system offers an untapped well of opportunities utilizing the structural characteristics of DNA well imparting new functionality and chemical diversity with the polypeptides.

Herein, we report the synthesis by and use of coiled-coil peptide-DNA conjugates to link together DNA origami structures bearing complementary strands, and to generate one-dimensional (1D) supramolecular polymers. Our design relies on chemically modifying each peptide of a heterodimer coiled-coil pair with a short DNA strand, resulting in a self-assembled core with two addressable handles (Figure 1A). These handles were then used to link together a DNA origami cuboid bearing a tunable number of complementary handles at its two ends. We demonstrate the formation of nanofibers consisting of rigid origami units linked by multiple coiled-coil pairs (Figure 1B). These fibers were analyzed by agarose gel electrophoresis, atomic force microscopy, and transmission electron microscopy (Figure 1C-E) with the longest fibers consisting of almost 80 origami units and exceeding three micrometers in length. By spatially confining multiple coiled-coils on the DNA cuboids, we effectively create a polypeptide-based interface between origami structures, akin to protein-protein interactions. We probed the effect of peptide multivalency on the efficiency of nanofiber assembly, and explored multiple self-assembly pathways: one-pot vs. sequential annealing, hierarchical formation of dimer and trimer structures as well as alternating copolymers, and linking of purified origami with coiled-coils. Taken together, our results show that peptide-DNA conjugates can be used to create hierarchical nanomaterials that integrate more than one self-assembly “mode” to create biomolecular scaffold in a controlled and programmable fashion.

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Hydrogen Bonded Self Assembly of Cyclic Cisoid Diamides

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POSTER LINK:

https://drive.google.com/open?id=1t45RR4VBa4RGsotmRpJRQB6x_fE5Xoqk

Cyclic Cisoid Diamides (CCD) are simple building block with two cis-diamides that can form up to four hydrogen bonds. CCD contains a non-planar 8-membered ring which allows two phenylene groups to be close to 90° to each other. Such structural feature provides opportunities to design tetrameric macrocycles with CCD groups on the corners. In addition, CCD has planar chirality, that commonly racemizes in room temperature. Here, we will utilize CCD as a building block for macrocyclic compounds that self-assemble into framework architectures.

![Figure](a) Two Configurations of CCD; (b) Zigzag Chains in the CCD Single Crystals.

*Figure.* (a) Two Configurations of CCD; (b) Zigzag Chains in the CCD Single Crystals.
Anion Receptors with Dinitrone C-H Hydrogen Bond Donors

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Nitrones are commonly used as a source of 1,3-dipole for [3+2] cycloadditions. Nitrones are easily prepared by condensation between aldehydes and hydroxyl amines in a reversible (dynamic) process. Furthermore, we recognized that the nitrone C–H groups are significantly polarized due to the neighboring electronegative atoms and the positive formal charge on the nitrogen. In fact, there were a handful of crystallographic precedence of nitrone C–H groups forming hydrogen bonds in solid state. Therefore, we hypothesized that nitrone C–H groups can serve as hydrogen bond donors for binding anions. Here, we discuss nitrone-based anion receptors that are synthesized via dynamic covalent chemistry.

Figure. Preparation of a nitrone-based anion receptor.

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https://drive.google.com/open?id=1IAKFQf6T2OOxkCy8M_fiuHRKUf1iC3J
Self-healing Hydrogels with Reversible Covalent Crosslinking

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Tunable, orthogonal, reversible, and covalent (TORC) chemistries provide a hierarchical approach to many biological applications, such as directed assembly, templated reactions, and biomaterial platforms. In terms of biomaterial platforms, viscoelastic hydrogels have shown promise as powerful tools for such biological applications as injectable drug delivery or cell culture. Reversible covalent crosslinks provide a useful approach to achieve these viscoelastic hydrogels due to their high mechanical stability and tunability. Little work, however, has been done to investigate how the kinetics of reversible covalent crosslinks influences the resulting hydrogel mechanical properties. Gelation rate, plateau modulus, and stress relaxation are three key mechanical characteristics that are substantially controlled through crosslinking kinetics. This work answers those questions by investigating a pair of thia-conjugate addition reactions with different electron withdrawing strengths (nitrile and hydrogen) at the reaction site, and connects these to their hydrogel mechanical properties when used as a crosslinker.

Reversible thia-conjugate addition (also known as a Michael type reaction) allows for facile study of the connection between kinetics and mechanics. Normally requiring high temperature for reversibility, the addition of an electron withdrawing group at the α site of an α,β-unsaturated carbonyl allows the reverse reaction to proceed at physiological conditions. An additional substitution of an aromatic ring at the β site provides an easily tunable chemical substituent in order to vary the kinetics of the reaction. The mechanism for this reaction is shown in Figure 1. The forward reaction relies on a deprotonated thiol adding to the β carbon, while the reverse reaction relies on deprotonation of the α carbon.

Figure 1. The reaction mechanism for thia-conjugate addition reaction. The squiggly lines represent polymer backbones that will create the foundation of the hydrogel. Both the forward and reverse rate rely on deprotonation of either the attacking thiol or α carbon. This demonstrates the strong dependence this reaction has on pH.

Results of this study show that certain kinetic effects play larger roles in the mechanical properties than others. The nitrile group at the R position leads to a 19 fold increase the forward rate constant and only about a 6 fold increase in the reverse rate
constant. The reverse reaction rate is the primary influencer of stress relaxation in the hydrogels, while the equilibrium of the reaction is the primary influencer of the plateau modulus. This is confirmed through a frequency sweep on a rheometer, where the plateau modulus shows strong correlation to the equilibrium of the reaction, while the crossover point demonstrates a stronger dependence on the reverse reaction rate. Additionally, since the crosslinks are reversible, this material displays self-healing properties. Two hydrogels were cut in half and the halves were rejoined. The rejoined hydrogel demonstrated recovery of mechanical properties within a minute. This research helps further elucidate the effects that crosslinking kinetics have on hydrogel mechanical properties, and the reversible thia-conjugate addition is a tunable platform for viscoelastic hydrogels. This knowledge of TORC based chemistries can also be more broadly applied to a host of biological applications, where dynamic systems are garnering increased interest.
POSTERS

Track on

Nucleic Acid Nanostructures In Vivo

Track Chair

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Genetically Encoded Ratiometric RNA-based Sensors for Quantitative Imaging Live-Cell Dynamics of Small Molecules

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https://drive.google.com/file/d/1je-Z-8V-0Wf9vEQJ2N1JusIku10uIEvY/view?usp=sharing

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Key words: Nucleic acids, Sensors, Quantitative, Fluorescence

Monitoring the changes of distribution and dynamics in cellular metabolites and signaling molecules is crucial for understanding cellular physiologies, metabolite and signal transduction pathways. For example, one of the signaling molecule called bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is participated in the biofilm formation. Antibiotics such as tetracycline is used in clinical treatment, but is facing the challenge of antibiotic resistant. There is an urgent need to develop real-time quantitative sensor to better understand these signaling and resistance pathways. Genetically encoded RNA-based sensors are powerful tools for detecting various target molecules in living cells. However, it is still challenging to precisely determine the intracellular concentration of target molecules using traditional single-fluorescent-RNA sensors due to the variations in the cell distribution or expression of the RNA sensor. To achieve this goal, we have recently developed a novel type of ratiometric RNA-based sensors using an orthogonal pair of RNA/fluorophore conjugates, DNB/SR-DN ($\lambda_{ex}/\lambda_{em}$, ~571/591 nm) and Broccoli/DFHBI-1T ($\lambda_{ex}/\lambda_{em}$, ~480/503 nm). Fluorescence of sulforhodamine B (SR) was quenched by a quencher called dinitroaniline (DN) by contact quenching mechanism. Upon binding with a dinitroaniline-specific DNB RNA aptamer, the spatial separation of the SR and DN will recover the fluorescence signal of SR dye. By fusing with a small molecule binding RNA aptamer to the DNB RNA through a transducer sequence, an RNA sensor can be developed specific for the target molecule. Upon the presence of target
molecule, the RNA sensor can be turned on and provide high fluorescence signal. By co-expressing this DNB sensor with the internal reference, Broccoli, on a same F30 scaffold, we have achieved a ratiometric sensor system that can normalize the RNA expression level from cell-to-cell (Fig. 1a). Herein, cellular DNB-to-Broccoli fluorescence intensity ratio can be directly applied to quantify the target concentrations at the single-cell level. Unfortunately, due to the instability of the SR-DN dye, this ratiometric sensor was difficult to monitor the target dynamics. It is known that dinitroaniline is a universal quencher for varieties of dyes including SR and tetramethylrhodamine (TMR). We realized that TMR-DN (λex/λem, ~555/582 nm) is much more stable compare to SR-DN. By simply replacing the SR-DN with TMR-DN dye, we have further demonstrated second generation of a stable Broccoli/DFHBI-1T and DNB/TMR-DN detection system. This stable monitoring system had been used for dynamic monitoring of tetracycline in the cells (Fig. 1b). We believe these advanced genetically encoded ratiometric sensors can be widely utilized for intracellular studies of various small molecules.

Figure 1. Schematic of ratiometric sensor and dynamic imaging of tetracycline in the cells. (a) Schematic of the ratiometric sensor that comprises an F30 scaffold (black), a Broccoli (green) and a DNB-based sensor. The DNB-based sensor is composed of a DNB (red), a target-binding aptamer (blue) and a transducer (gray). Target binding to the aptamer (blue) stabilizes the transducer, enabling DNB to fold and activate the fluorescence of SR-DN or TMR-DN. (b) Monitoring tetracycline accumulation dynamics in live BL21 (DE3)* cells after adding 500 μM tetracycline at 0 min. DNB sensor channel (red), Broccoli channel (green) and DNB-to-Broccoli (R/G) ratiometric images were shown. Scale bar, 5 μm.
Genetically Encoded Hybridization Chain Reactions for RNA Imaging in Living Cells

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https://drive.google.com/file/d/1p_5zIDfNAjjNVMm_R2BBjBK62hH2bKH/view?usp=sharing

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Key words: Nucleic acids, In situ amplification, Subcellular locations imaging, Fluorescence

With high sensitivity and low detection limit, in situ amplification methods such as hybridization chain reaction (HCR) and HCR based fluorescence in situ hybridization (HCR-FISH), have emerged as a powerful technique for measuring the cellular concentration and subcellular location of target biomolecules. However, these techniques are still suffering from challenges including difficulties in the probe delivery, enzymatic degradations, and potential biosafety risks. Genetically encoded RNA sensors emerge as valuable tools for detecting target molecules in living cells, but it remains a challenge for imaging and spatial tracking low-abundance targets, especially in mammalian cells. We report here an IN SItu Genetic Hybridization Amplification Technique, termed INSIGHT, for the sensitive imaging of the subcellular distributions and locations of RNA targets in living cells. The INSIGHT design is based on a combination of fluorogenic RNA reporters with RNA-based HCR. The RNA Broccoli was split into two non-fluorescent fragments and separately conjugated to the end of H1 and H2 hairpins. In the absence of initiator RNAs, H1 and H2 coexisted under a metastable state and two fragments of Broccoli cannot be spontaneously hybridized to generate fluorescence. Upon the addition of an initiator RNA, the H1 hairpin will be opened and expose a sequence that can invade and hybridize with the H2 hairpin. A cascaded hybridization between these two hairpins was then initiated and induced the reassembly of a chain of Broccoli to activate an amplified fluorescence signal (Figure 1a). Importantly, the generated chain of Broccoli will be associated with the initiator strand, which can be used for the cellular tracking. By further introducing an RNA molecular beacon, we developed a modular INSIGHT system to image different RNA targets (Figure 1b). The loop sequence of the molecular beacon can be changed to hybridize with different RNA targets. These molecular beacons also contained a conserved stem region. Part of the initiator strand was blocked in this stem. Target binding induced the dehybridization of the stem, activated the initiator RNA, which further induced a cascaded hybridization between H1 and H2. As a result, the same sequence of H1 and H2 can be used to detect various RNA targets. By simply changing the loop region of a molecular beacon, the INSIGHT system has been conveniently designed for the detection and subcellular location imaging of SgrS and bglF RNA targets in bacterial cells (Figure 1c) and survivin mRNA in mammalian cells (Figure 1d). We believe this modularly in situ genetical hybridization amplification technique will provide an effective and versatile platform for sensing and tracking various analytes in living cells.
Figure 1. (a) Schematic of the genetically encoded in situ amplification system. After the cellular transcription of the H1 and H2 strands (blue) from a delivered vector, the binding of initiator RNA (red) triggered the cascaded H1/H2 hybridization to reassemble the Broccoli (green) for imaging the subcellular location of the initiator strand. (b) Schematic of a general INSIGHT platform for target RNA detection. The target RNA hybridizes with the molecular beacon and releases an initiator strand (red) to trigger the hybridization chain reaction between H1 and H2. (c) Imaging the subcellular location of SgrS and bgIF in bacterial cells. Confocal fluorescence imaging of cells expressing pETDuet-H1/H2 + pCDFDuet-SgrSMB-7B or pCDFDuet-bglFMB-7B (molecular beacon that targets SgrS or bgIF), or pETDuet-Broccoli. DAPI was used to stain the nucleoid of E. coli. Scale bar, 5 μm. (d) Imaging the survivin mRNA in HeLa cells at 24 h after Lipofectamine® 3000-based transfection. Images were taken in the presence of 40 μM DFHBI-1T. 5 nM YM155 (inhibitor) was added to inhibit the generation of survivin mRNA. Scale bar, 10 μm.
POSTERS

Track on
Molecular Machinery

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Nanopore decoding for solution of Hamiltonian path problem in DNA computing

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DNA computing has been attracted as one of the molecular computing which can perform massively parallel computation. However, it usually is time-consuming to detect and decode the output information in the conventional system. We have proposed the nanopore decoding, which can perform rapid and electrical recognition of oligonucleotides with label-free. We here report the nanopore decoding for the output in a directed Hamiltonian path problem (HPP). In this method, the output molecules were purified by gel electrophoresis and passed through the nanopore to determine the correct answer. As proof of concept, we demonstrated nanopore decoding with small graph encoding HPP. The results showed the feasibility of nanopore decoding that can decode the output rapidly and label-free.

In 1994, Adleman solved HPP, which is classified into NP-complete, using DNA molecules [1]. Since then, DNA computing has been attracted as a tool for solving mathematical problems because of its huge parallelism and low energy consumption. However, the approach to detecting and decoding the output DNA molecules was time-consuming because it required multiple-steps of biological operation, including repetitive magnetic beads experiments. We have proposed a rapid and electrical decoding method using nanopore technology for DNA computing with label-free [2-3]. In this work, based on this technology, we attempted to demonstrate the nanopore decoding of Adleman’s parallel DNA computations. In this approach, the output molecules were decoded by the following steps. Step 1: Amplify the random paths generated by DNA computation as single-stranded DNA by PCR and one-side PCR. Step 2: Separate the PCR products depending on its length by gel electrophoresis, and extract correct-length DNA encoding a route visiting all nodes only once. Step 3: Each path strand hybridizes to the extracted DNA, and the duplex passes through an α-hemolysin (αHL), a pore-forming protein, nanopore with the path strands unzipping. We measure the time of ion current blockage, unzipping...
time, and sequence the Hamiltonian path by analyzing the time.

**Fig. 1.** (a) The scheme of nanopore decoding. (b) The result of nanopore measurement.

In this experiment, we prepared a small graph to demonstrate nanopore decoding (Fig. 1 (a)). There are 6 kinds of routes satisfying the requirement of the answer of HPP, so we prepared the 6 types of chemically synthesized barcode-like DNA (bcDNA) which coded each of the route. One of the routes coded the Hamiltonian path in the graph. It was confirmed by thermodynamic simulation that each bcDNA formed duplex with each path strand with different Gibbs free energy ($\Delta G$). We reconstituted $\alpha$HL into lipid bilayers formed by “droplet contact method” [4] with a microfabricated device and measured the unzipping time of the 6 kinds of routes. As a result of nanopore measurement, the unzipping time increased with increasing of $\Delta G$. And also, it was confirmed that the small differences of $\Delta G$ in thermodynamic simulation can be distinguished by nanopore measurement (Fig. 1 (b)). Then, we measured the unzipping time using the output of DNA computation. As we expected, the result of nanopore measurement after entire computing mostly corresponded with that of chemically synthesized bcDNA which coded the Hamiltonian path in the graph. Our data suggested that nanopore technology can be applied to DNA-based parallel computation as a decoding method.

**References**

DNA mechanotechnology: highly polyvalent DNA motors generate 100+ piconewtons of force via autochemophoresis

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Poster Link: https://drive.google.com/file/d/1wQQYPYAelkM1-2zxT3TRNUPyXM4_OCe/view?usp=sharing

DNA mechanotechnology1, which consists of DNA-based devices that are engineered to sense, transmit, and generate mechanical forces, has recently emerged at the intersection of DNA nanotechnology and single molecule biophysics. These DNA-based mechanical devices have fundamentally changed the manner in which biologists study mechanical forces at the nanoscale and have opened new avenues for the design of force-generating nanomaterials1.

A forefront of DNA mechanotechnology research is the development of synthetic machines that convert chemical energy associated with duplex hybridization to mechanical work. Such synthetic motors could perform engineered functions similar to those performed by motor proteins such as kinesin, myosin, and dynein. DNA walkers2, which “walk” along linear or planar tracks via burnt-bridge hybridization interactions, are perhaps the most promising synthetic analogs of such molecular motors. DNA walkers can precisely transport nanoscale cargo but cannot generate piconewton-scale force. This primary function of motor proteins is necessary for countless processes including muscle contraction, clotting, immune sensation, embryogenesis, and mechanosensation. We present progress towards the design of force-generating nanomachines by showing that highly polyvalent DNA motors (HPDMs) generate 100+ piconewtons of force via a mechanism that we term autochemophoresis3.

HPDMs are DNA-coated microparticles that connect to planar RNA-functionalized surfaces via DNA-RNA hybridization4 (Fig. 1a). Ribonuclease H enables translocation by cleaving the RNA-DNA duplexes, resulting in an RNA depletion track in

Figure 1: a) HPDMs translocate via burnt bridge interactions between DNA “feet” and RNA “fuel”. b) As the HPDM translocates it consumes fuel, leaving a “depletion track”.

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the HPDM's wake (Fig. 1b). Translocating for hours at micron/minute speeds, HPDMs are the fastest, most processive DNA-based motors reported to date\(^4\). To test HPDMs' force generation capability we designed a single molecule fluorescence microscopy experiment which enables direct visualization of mechanically-ruptured molecular bonds. Surprisingly, we found that HPDMs generate forces that mechanically rupture 25 basepair DNA duplexes and biotin-streptavidin bonds (Fig. 2), which is often considered one of the strongest noncovalent bonds found in nature with a 100+ pN force threshold.

HPDMs lack directed tracks and conformational switching such as ATP-fueled powerstrokes, thus underscoring the novelty of this result. Our study shows that the DNA strands in massively parallel DNA walker-type motors will spontaneously coordinate to generate summative forces. To study this fundamental mechanism of force-generation, we developed a simulation method that accurately reproduces most properties of HPDM motion via direct modeling of the distance-dependent biophysics of DNA-RNA interactions. These simulations highlight the mechanism of HPDM force generation and demonstrate that motion is driven by autochemophoresis, which has been observed in biological systems\(^5-7\). Our work supports the hypothesis that autochemophoresis may be a third fundamental method of force generation in molecular motors and living systems.


**Figure 2:** a) Cartoon depiction of process used to measure force generation. Biotin-capped strands on the HPDM and underlying surface both bind to a single streptavidin molecule. In order for HPDM translocation to occur, the HPDM must generate mechanical force that is sufficient to rupture the biotin streptavidin bond – on the order of 50-150 pN. b) Single molecule imaging of fluorescent streptavidin reveals the occasional deposition of streptavidin within depletion tracks, thus suggesting that HPDMs generate enough force to rupture the biotin-streptavidin bond.
Enhanced Diffusion of Catalytically Active Enzymes

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The past decade has seen an increasing number of investigations into enhanced diffusion of catalytically active enzymes [1]. These studies suggested that enzymes are actively propelled as they catalyze reactions or bind with ligands (e.g., substrates or inhibitors). In this Outlook, we chronologically summarize and discuss the experimental observations and theoretical interpretations and emphasize the potential contradictions in these efforts. We point out that the existing multimeric forms of enzymes or isozymes may cause artifacts in measurements and that the conformational changes upon substrate binding are usually not sufficient to give rise to a diffusion enhancement greater than 30%. Therefore, more rigorous experiments and a more comprehensive theory are urgently needed to quantitatively validate and describe the enhanced enzyme diffusion.

We utilized dynamic light scattering (DLS) to measure the diffusion coefficient of aldolase in the absence and presence of its substrate [2]. The DLS measurements have an experimental error of 3% and do not find a statistically significant change of the aldolase diffusion coefficient even at a saturating substrate concentration. This finding lends support to the contention that photophysical artifacts may have affected the prior FCS measurements and challenges the idea that enzymes can be self-propelled by their catalytic activity.


Rotary DNA nanotube microswimmers driven by electrophoresis

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Link for viewing the poster: Dropbox-link

Motility of micro-organisms in low Reynolds number relies on different physical principles than large-scale objects in high Reynolds number. An example of this in biological system is found in bacteria flagella that allows non-reciprocal motion by a rotating motion along its center-line axis. These physical principles have been tested by \textit{de novo} engineering of artificial flagella by subjecting helical microstructures magnetic bead chimeras in a magnetic field\textsuperscript{1,2}. Despite the observed flagella-like, the presence of the relatively-large magnetic bead is expected to perturb the fluid flow near the helical microstructures. Here, we report a fluid mechanics study of helical SST DNA nanotubes under influence of uniform electric field. The electrophoresis-based setup eliminates the need for the magnetic bead and yield a simpler hydrodynamic flow near the helical microstructures.

The helical microswimmers are based on the SST DNA nanotubes design from Maier et al.\textsuperscript{3}. Briefly, the nanotubes have the dimensions of \~{}10 \textmu m long with a diameter \~{}1 \textmu m with a typical length and diameter of 8 \textmu m and 1 \textmu m, respectively. The helical nanotube structures 13HTs-4s are composed of 13 unique single-stranded DNA, with 3 of which tagged with Cy3B dye for light microscopy imaging. The helical SST nanotubes are prepared by annealing in 12.5 mM MgCl\textsubscript{2}. The helical nanotubes were then placed inside a 1.5 cm \times 0.5 cm flow chamber subjected to a 30 V potential in order to drive the nanotubes by electrophoresis. The motility was then recorded with an epi-fluorescence microscope. We measured translation and rotation speed of the helical motif by performing image analysis from the two-dimensional microscopy images. The average translation and rotation speeds are at 21.2 \textmu m/s and 7.5 rad/s, respectively. We found a non-linear relationship between the translation and rotation speeds suggesting non-trivial mechanical interactions. Demonstration of flagella motility without the necessity of attaching a relatively-large magnetic bead could lead to a more accurately study of the interactions between helical motif and fluid flows in low Reynolds number regime.

References