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Form Approved OMB NO. 0704-0188

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1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
02-11-2021	Final Report	1-Mar-2019 - 30-Nov-2019

4. TITLE AND SUBTITLE
Final Report: Support of the Sixteenth Conference on the Foundations of Nanoscience (FNANO 2019)

5a. CONTRACT NUMBER
W911NF-19-1-0165

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611104

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
Duke University
C/O Office of Research Support
2200 W. Main St., Ste. 710
Durham, NC 27705 -4677

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
74957-LS-CF.1

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			John Reif
UU	UU	UU	UU		19b. TELEPHONE NUMBER 919-660-6568

RPPR Final Report
as of 04-Nov-2021

Agency Code: 21XD

Proposal Number: 74957LSCF **Agreement Number: W911NF-19-1-0165**
INVESTIGATOR(S):

Name: John Reif
Email: reif@cs.duke.edu
Phone Number: 9196606568
Principal: Y

Organization: **Duke University**
Address: C/O Office of Research Support, Durham, NC 277054677
Country: USA

DUNS Number: 044387793 EIN: 560532129
Report Date: 27-Feb-2020 Date Received: 02-Nov-2021
Final Report for Period Beginning 01-Mar-2019 and Ending 30-Nov-2019
Title: Support of the Sixteenth Conference on the Foundations of Nanoscience (FNANO 2019)
Begin Performance Period: 01-Mar-2019 **End Performance Period:** 30-Nov-2019
Report Term: 0-Other

Submitted By: John Reif Email: reif@cs.duke.edu
Phone: (919) 660-6568

Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 41 **STEM Participants:** 41

Major Goals: This contract was for partial support of honoraria and registration fees for speakers and other expenses at the 16th Conference on Foundations of Nanoscience, Snowbird, Utah from April 15-18, 2019. These funds were used to pay the registration fees and the travel for 5 keynote speakers, the registration fee for 11 invited speakers and 2 conference assistants. The contract funds were not used to support any expenses of any Federal employees.
The Foundations of Nanoscience meeting (FNANO) was established by the International Society for Nanoscale Science, Computation, and Engineering in 2004 as a venue for the wide range of researchers interested in various aspects of self-assembly as it relates to nanoscience and nanotechnology. The meeting features 12 tracks covering recent work in different types of self-assembled architectures and devices, at scales ranging from nano-scale to mesoscale. Methodologies include both experimental as well as theoretical approaches. The conference spanned traditional disciplines including chemistry, biochemistry, physics, computer science, mathematics, and various engineering disciplines. The emphasis is on basic, rather than applied research.?Ensuring Technical Quality of the Conference: The high quality of the conference was ensured by:
1) Track chairs who are responsible for the selection of excellent presenters with new results to share,
2) Vigorous discussions managed by the track chairs, and
3) Annual review of and changes in the track topics to keep them fresh and up to date.
Why the conference was appropriate at the time, date and location chosen: The time and date was chosen to not be in conflict with other related conferences. The location was chosen to a location easily evaluable via air- flight, with a short distance to the nearest airport in Salt Lake City, and with very affordable hotel rooms.
Accomplishments Under Goals:
The FNANO 2019 had a significant impact on the emerging fields of nanoscience and self-assembly by bringing together leading nanoscientists and researchers working in a wide variety of areas of self-assembly. Location and Dates: Snowbird, Utah, April 15-18, 2019.
The 16th Conference on Foundations of Nanoscience had a mixture of invited talks by distinguished nanoscientists as well as contributed posters and open discussion periods to enhance attendee interaction with the goal of creating a vibrant intellectual community in the area of self-assembly. This funding significantly enhanced interdisciplinary basic research in the area of nanoscience and allowed researchers to initiate and maintain cross-discipline collaborations.?Relevance to DOD: Nanoscience has the potential to revolutionize the development of materials, devices, and detectors at the molecular scale.

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as of 04-Nov-2021

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Relevance to DOD: Nanoscience has the potential to revolutionize the development of materials, devices, and detectors at the molecular scale.

Training Opportunities: Nanoscience is at the core of an emerging discipline that brings together researchers from all the branches of engineering as well as from chemistry, physics, biology, computer science, and even mathematics. These cross-disciplinary interactions are crucial for the advancement of nanoscience, but often work that is published in one area is not readily accessible to researchers in another area. Fostering such interactions between individual researchers is the main goal for the FNANO conference series. By bringing top researchers from a variety of fields together in a stimulating environment, with an emphasis on breaking results and discussion, the conference helps researchers communicate new ideas and techniques swiftly and form research collaborations. The common focus for the FNANO meeting was self-assembly, which many nanoscientists think has enormous potential to revolutionize nanofabrication. Top-down methods for construction of nanostructures, such as e-beam lithography, have inherent limitations in scale. Bottom-up methods such as self-assembly appear to have no such scale limitations. While top-down methods are well understood and widely used in engineering and manufacturing processes, self-assembly is a much less well-understood construction process. Although self-assembly is the cornerstone of biological complexity, the “rational” self-assembly methods that can be applied to arbitrary materials/structures (for example, for the self-assembly of lipid or polymer layers) result in structures with limited complexity.

Results Dissemination: Meeting Results were disseminated via conference proceedings (in flash storage), provided to the attendees.

FNANO19 Web page: Caution-http://www.cs.duke.edu/FNANO19/

Honors and Awards: Each year, the FNANO conference usually awards two prizes:
1. (1) The ISNSCE Nanoscience Prize recognizes life-long achievements by a researcher working in any area of nanoscience. It is awarded each year to recognize and encourage outstanding research in all areas of nanoscience. Traditionally the award winner is invited to give the Keynote Lecture in an evening session at FNANO. The ISNSCE Nanoscience Prize was awarded in FNANO19 to David Leigh, University of Manchester, where he is the Royal Society Research Professor Sir: Samuel Hall Professor of Chemistry. Professor Leigh is a leading master of supramolecular knots, catenanes, and rotaxanes. He has inspired us with implementations of a series of synthetic molecular machines, including chemical assemblers and Brownian-ratchet rotary motors powered by various energy sources.
2. (2) The Robert Dirks Molecular Programming Prize recognizes exceptional early-career achievement by a researcher working in any area of molecular programming, whether theory, experiment, computation, or a combination thereof. Traditionally the award winner is invited to give a Prize Lecture at FNANO19. In FNANO19, the award was presented to Dr. Grigory Tikhomirov, of California Institute of Technology for his work engineering hierarchical, disordered, and reconfigurable programmable nanostructures, demonstrating principles for scaling up structural complexity, trading off deterministic control for structural diversity, and implementing autonomous structural reconfiguration.

Protocol Activity Status:

Technology Transfer: None to report, but note that multiple individuals (see FNANO schedule) presenting at FNANO did engage in technical transfer of their group's results.

PARTICIPANTS:

Participant Type: PD/PI
Participant: John Reif
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

RPPR Final Report
as of 04-Nov-2021

Partners

,

I certify that the information in the report is complete and accurate:
Signature: John H Reif
Signature Date: 11/2/21 11:03AM

Foundations of Nanoscience

Self-Assembled Architectures and Devices

16th Annual Conference

Partially Supported By:

The U.S Army Research Laboratory and The U.S. Army Research Office

And Sponsoring Society:

ISNSCE
International Society for Nanoscale Science, Computation and Engineering

Snowbird Cliff Lodge
Snowbird, Utah
April 15-18, 2019

16th Annual Conference on Foundations of Nanoscience (FNANO19):
Self-Assembled Architectures and Devices

Snowbird Cliff Lodge, Snowbird, Utah
Monday, April 15 – Thursday, April 18, 2019

Partial Support from:

The U.S. Army Research Laboratory and the U.S. Army Research Office

Office of Naval Research (ONR)

National Science Foundation(NSF)

Guild BioSciences

Sponsoring Society:
International Society for Nanoscale Science, Computation and Engineering (ISNSCE)

The papers in this volume were presented at the Conference "Foundations of Nanoscience: Self-Assembled Architectures and Devices" held in Snowbird, Utah, Monday, April 15 – Thursday, April 18, 2019.

This meeting is supported in part by the U.S. Army Research Laboratory and the U.S. Army Research Office under grant number W911NF-19-1-01651. The views, options, and or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army or U.S. Government position, policy, or decision, unless so designated by other documentation.

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, engineeering, molecular biology and molecular medicine.

Prior FNANO conferences have had a significant impact on the emerging fields of nanoscience and self-assembly, by bringing together leading researchers in a strongly interdisciplinary forum. This 16th Conference on Foundations of Nanoscience follows in this tradition, featuring invited talks by distinguished nanoscientists in a schedule with many contributed posters and open discussion periods to allow for scientific interaction.

THANKS

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

Special thanks to Camelia Pierson Eaves at Duke University for her excellent work behind the scenes, and for the effort of the Conference Assistants: Abhishek Dubey, Shalin Shah and Ming Yang.

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.

John Reif, FNANO19 General Chair
A. Hollis Edens Distinguished Professor of Computer Science
Department of Computer Science
Duke University, Durham, NC, USA

and

Andrew Turberfield, FNANO19 Program Chair
Professor of Physics
Clarendon Laboratory, Department of Physics,
Oxford University, Oxford, UK

FNANO 2019 CONFERENCE ORGANIZATION

Conference Chair: John Reif <reif@cs.duke.edu>, Department of Computer Science, Duke University, Durham, NC

Program Chair: Andrew Turberfield <a.turberfield@physics.ox.ac.uk>, Department of Physics, Oxford University, Oxford, UK

FNANO 2019 Program Committee

Track on DNA Nanostructures: Semantomorphic Science

- **Chair:** Nadrian Seeman <ned.seeman@nyu.edu>, Department of Chemistry, New York University, New York, NY

Track on DNA Nanosystems: Programmed Function

- **Chair:** Friedrich Simmel <simmel@tum.de>, Department of Physics, Technical University Munich, Germany

Track on Protein and Viral Nanostructures:

- **Chair:** Nicole Steinmetz <nsteinmetz@eng.ucsd.edu>, Department of Nanoengineering, University of California-San Diego, San Diego, CA

Track on Integrated Chemical Systems:

- **Chair:** Jeremiah Gassensmith <gassensmith@utdalls.edu>, Department of Chemistry, University of Texas, Dallas, TX

Track on Principles and Theory of Self-Assembly (Posters Only):

- **Chair:** Rebecca Schulman <rschulm3@jhu.edu>, Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD

Track on Computational Tools for Self-assembly:

- **Chair:** William Shih <William_Shih@dfci.harvard.edu>, Departments of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA

Track on Synthetic Biology:

- **Chair:** Alex Deiters <deiters@pitt.edu>, Department of Chemistry. University of Pittsburgh, Pittshurgh, PA

Track on Nucleic Acid Nanostructures in Vivo:

- **Chair:** Yamuna Krishnan <yamuna@uchicago.edu>, Department of Chemistry, University of Chicago, Chicago, IL

Track on Chemical Tools for DNA Nanotechnology:

- **Chairs:**
  - Andrew Ellington <ellingtonlab@gmail.com>, Chemistry and Biochemistry Department, The University of Texas at Austin, Austin, TX
  - Floyd Romesberg <floyd@scripps.edu>, Scripps Research Institute, LaJolla, CA

Track on Biomedical Nanotechnology:

- **Chair:** Thomas LaBean <thlabean@ncsu.edu>, Materials Science & Engineering, North Carolina State University, Raleigh, NC

Track on Nanophotonics and Superresolution:

- **Chair:** Ralf Jungmann jungmann@biochem.mpg.de, Max Planck Institute for Biochemistry, Martinsried, Germany

Track on Molecular Machinery:

- **Chair:** Andrew Turberfield <a.turberfield@physics.ox.ac.uk>, Department of Physics, Oxford University, Oxford, UK

Special Track on Nanoscience for Computation

- **Chair:** Andrew Turberfield <a.turberfield@physics.ox.ac.uk>, Department of Physics, Oxford University, Oxford, UK

Workshop for Early-Career Nanoscientists:

- **Chair:** Philip Lukeman <lukemanp@stjohns.edu>, Department of Chemistry, St. John's University

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NSF STUDENT TRAVEL GRANT GUIDELINES:

Participants are selected based upon:

1. Status as an high school, undergraduate or graduate student
   a. *No postdocs, faculty or other senior research personnel will be supported* by the grant.
2. U.S. Citizen
   a. Permanent Resident card holders must provide front and back copy of the card
3. Foreign National students must attend U.S. college or university and provide proof of current enrollment; DS-2019; copy of passport picture page with visa that shows the entry stamp; letter indicating that student is not supported by any U.S. funding or grants and is eligible to receive reimbursement for personal expenses outside of the institution in which they are enrolled.
4. Accepted as a contributing speaker, invited speaker or poster presenter
5. Submission of registration request prior to March 30, 2019
6. Compliance with the Fly America Act and transportation allowance cannot exceed the cost of jet economy round-trip airfare between your home or institution and the conference
7. If you request less than jet economy fare or have other support to defray part of the cost of transportation, a corresponding reduction will be made in the allowance from funds provided by this travel grant.
8. Faculty advisors of student applicants' must provide written statements regarding availability (or lack thereof) of travel funds for the students from NSF and other funding sources.
9. Receipts for lodging and airfare must be made by the student requesting the travel grant.
10. Provide demographic information: gender, ethnicity.

Those satisfying the above criteria will each receive an **equal portion** of the travel funds (possibly $100-$300+ per student). If selected to receive funds, you will receive emailed instructions to submit sensitive information and pdfs of receipts. Travel grant stipends will not be processed until 30-60 days after you have registered, signed in at the FNANO registration table, and provided all required documentation. Deadline for submitting all required documentation is May 1, 2019

If you request less than jet economy fare or have other support to defray part of the cost of transportation, a corresponding reduction will occur in the allowance from funds provided by this travel grant.

NOTE:

- We will reserve 20% of the award to women, undergraduates and underrepresented minorities. Announcements will be distributed through our website, track chairs, and colleges/universities with programs that provide student research in conference related fields. The reserve funding will not be redistributed to support students in another category.
- A list of persons with their institutional addresses and demographic information for whom travel funds are provided will be reported per guidelines to NSF.

National Science Foundation Office of the Director
Alexandria, VA 22314

Important Notice No. 144 **February 8, 2018**

Important Notice to Presidents of Universities and Colleges And Heads of Other National Science Foundation Grantee Organizations

The National Science Foundation (NSF) does not tolerate sexual harassment, or any kind of harassment, within the agency, at grantee organizations, field sites, or anywhere NSF-funded science and education are conducted. The 2,000 American colleges, universities and other institutions that receive NSF funds are responsible for fully investigating complaints and for complying with federal non-discrimination law.

As the primary funding agency of fundamental science and engineering research in the United States, NSF is committed to promoting safe, productive research and education environments for current and future scientists and engineers. We consider the Principal investigator (Pl) and any co-Pl(s) identified on an NSF award to be in positions of trust. The Pl and co-Pl and all grant personnel must comport themselves in a responsible and accountable manner, including during the performance of award activities conducted outside the organization, such as at field sites or facilities, or during conferences and workshops.

To bolster our commitment to a safe research environment, NSF is taking the following steps:

1. New Award Requirements: NSF has developed a new award term and condition that will require grantee organizations to report findings of sexual harassment, or any other kind of harassment regarding a Pl or co/Pl or any other grant personnel. The award term and condition also will require the grantee to report the placement of the Pl or co- Pl on administrative leave relating to a harassment finding or investigation. This term and condition will make it clear that NSF may take unilateral action as necessary to protect the safety of all grant personnel, to include suspending or terminating an award or requiring the grantee to replace or remove personnel. NSF will solicit feedback on this new award term and condition through its Proposal and Award Policies and Procedures Guide Federal Register process within the next several weeks.

2. Harassment-Free Research Workplaces: NSF expects all awardee organizations to establish and maintain clear and unambiguous standards of behavior to ensure harassment-free workplaces wherever science is conducted, including notification pathways for all personnel, including students, on the primary and supplemental awards. This expectation includes activities at all research facilities and field sites and during conferences and workshops. All such settings should have accessible and evident means for reporting violations and awardee organizations should exercise due diligence with timely investigations of allegations and corrective actions.

3. Enhanced Web Resources: The NSF Office of Diversity and Inclusion {ODI) is tasked with seeking to ensure that NSF-funded programs and projects are free of discrimination. ODI recently launched a dedicated web portal to consolidate policies and procedures, promising practices, and frequently asked questions relating to sexual and other forms of harassment with the intent of making it easier for the research community and the public to access information. This portal is where NSF will continue to add content related to ending harassment. To access the portal, please visit NSF.gov/harassment.

NSF is working to make certain that awardee organizations respond promptly and appropriately to instances of sexual and all other forms of harassment. A community effort is essential to eliminate sexual and other harassment in science and to build scientific workspaces where people can learn, grow and thrive.

France A. Cordova, Director

Notification requirements:
The term and condition will require awardee organizations to notify NSF of any findings/determinations of sexual harassment, other forms of harassment, or sexual assault regarding an NSF funded PI or co-PI.

The term and condition also will require the awardee to notify NSF if the PI or co-PI is placed on administrative leave or if the awardee has imposed any administrative action on the PI or any co-PI relating to any finding/determination or an investigation of an alleged violation of awardee policies or codes of conduct, statutes, regulations, or executive orders relating to sexual harassment, other forms of harassment, or sexual assault. Finally, the award term and condition specifies the procedures that will be followed by NSF upon receipt of a notification.

Notifications must be submitted by an authorized organizational representative within 10 business days from the date of the finding/determination, or the date of the placement of a PI or co-PI by the awardee on administrative leave or the imposition of an administrative action, whichever is sooner. The term and condition will be effective for any new award, or funding amendment to an existing award, made on or after the effective date.
NSF will consider in its review of each notification submitted:

1. Safety and security of personnel supported by the NSF award;
2. Overall impact to the NSF-funded activity;
3. Continued advancement of taxpayer investments in science and scientists; and
4. Whether the awardee has taken appropriate action to ensure the continuity of science and that continued progress under the funded project can be made.

NSF has developed an electronic capability for submission of the required notifications that will be available on NSF's harassment page. The information will go directly to the Office of Diversity and Inclusion.

Upon receipt and review of the information provided, NSF will consult with the authorized organizational representative, or designee. Based on the results of this review and consultation, the Foundation may, if necessary, assert its programmatic stewardship responsibilities and oversight authority to initiate the substitution or removal of the PI or any co-PI, reduce the award funding amount, or where neither of those previous options is available or adequate, to suspend or terminate the award.

Definitions:
For purposes of the term and condition, the following definitions apply:
Sexual harassment: May include but is not limited to gender or sex-based harassment, unwelcome sexual attention, sexual coercion, or creating a hostile environment, as set forth in organizational policies or codes of conduct, statutes, regulations, or executive orders.
Other Forms of Harassment: Non-gender or non-sex-based harassment of individuals protected under federal civil rights laws, as set forth in organizational policies or codes of conduct, statutes, regulations, or executive orders.
Finding/Determination: The final disposition of a matter involving sexual harassment or other form of harassment under organizational policies and processes, to include the exhaustion of permissible appeals exercised by the PI or co-PI, or a conviction of a sexual offense in a criminal court of law.
Administrative Leave/Administrative Action: Any temporary/interim suspension or permanent removal of the PI or co-PI, or any administrative action imposed on the PI or co-PI by the awardee under organizational policies or codes of conduct, statutes, regulations, or executive orders, relating to activities, including but not limited to the following: teaching, advising, mentoring, research, management/administrative duties, or presence on campus.

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Track on Molecular Machinery. Track Chair: Andrew Turberfield, University of Oxford

Posters Track on DNA Nanostructures: Semantomorphic Science A

Track on DNA Nanostructures I: Semantomorphic Science A

Track Chair

Nadrian Seeman

New York University

Self-Assembly of a Designed Nucleoprotein Architecture Through Multimodal Interactions

Rohit H. Subramanian, Sarah J. Smith, F. Akif Tezcan*

*tezcan@ucsd.edu; corresponding author and presenter

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0356, USA

The co-self-assembly of proteins and nucleic acids (NAs) produces complex biomolecular machines (*e.g.*, ribosomes and telomerases) that represent some of the most daunting targets for biomolecular design. Despite significant advances in protein and DNA or RNA nanotechnology, the construction of artificial nucleoprotein complexes has largely been limited to cases that rely on the NA-mediated spatial organization of protein units, rather than a cooperative interplay between protein- and NA-mediated interactions that typify natural nucleoprotein assemblies. In this presentation, I will describe a structurally well-defined, 2D synthetic nucleoprotein assembly (Figure 1) that forms through the synergy of three types of intermolecular interactions: Watson-Crick base pairing, NA-protein interactions, protein-metal coordination. The fine thermodynamic balance between these interactions enables the formation of a crystalline architecture under highly specific conditions, as thoroughly characterized by X-ray crystallography, SAXS, EM and AFM measurements as well as molecular dynamics simulations.

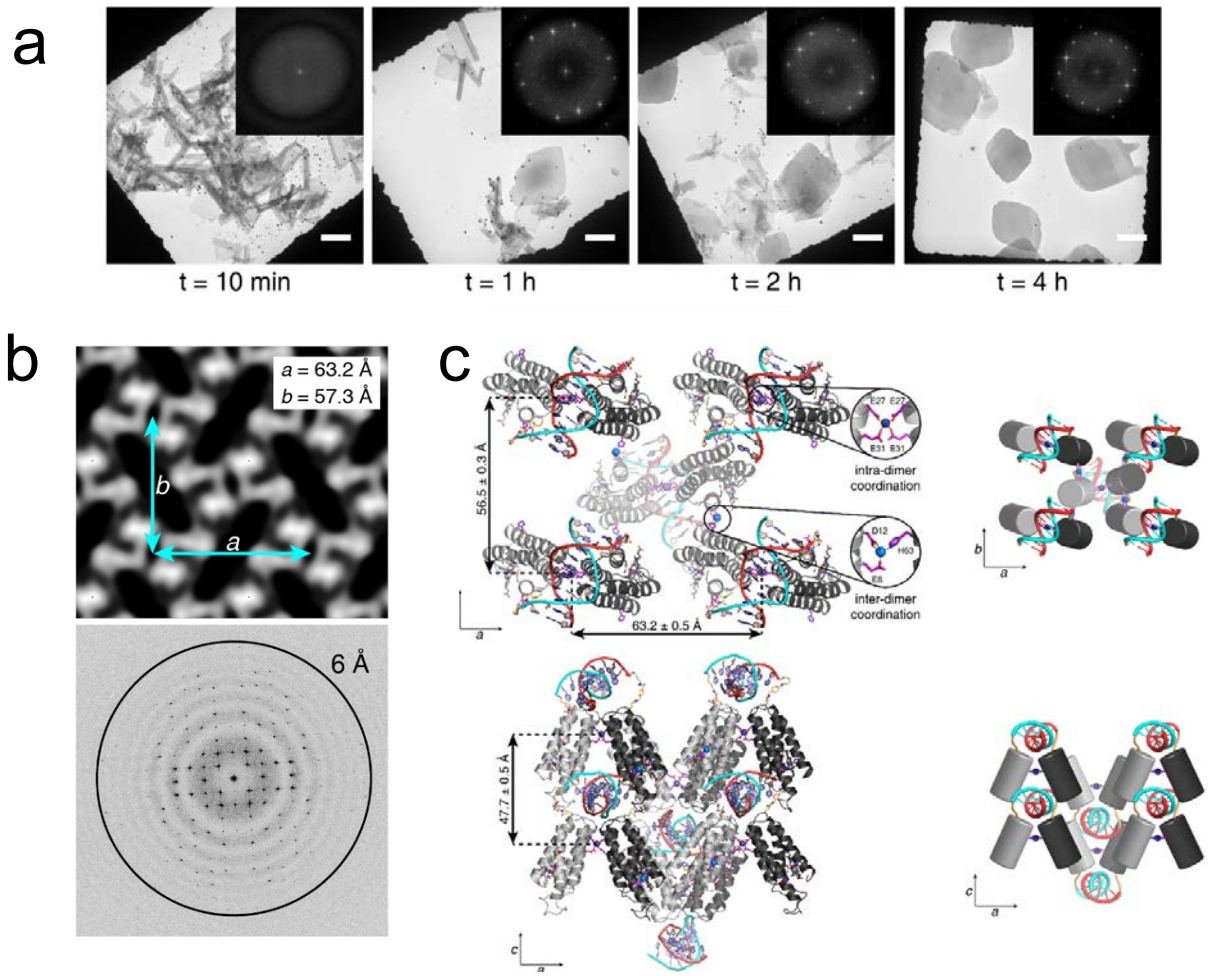

Figure 1. (a) Time course of the self-assembly of 2D nucleoprotein arrays as monitored by transmission electron microscopy. (b) 2D cryoEM projection map and corresponding FFT pattern of 2D nucleoprotein arrays. (c) Structural model of the 2D nucleoprotein arrays: (top) as viewed down the 2D plane; (bottom) along the 2D plane.

Fabrication of a DNA Origami Integrated Hydrogen Peroxide Sensing Platform

*Yun Zeng[1], Wenyan Liu[2], Risheng Wang[1]**

[1] Department of Chemistry, Missouri S&T, Rolla, MO 65409, USA

[2] Center of Research for Energy and Environment, Missouri S&T, Rolla, MO 65409, USA

Contact: wangri@mst.edu

The approach of integrated design is to combine different materials/molecules to work together and produce an enhanced cooperative effect. Recently, DNA nanostructures have shown considerable promise as versatile scaffolds for organizing functional nanoparticles. For instance, DNA origami have been used to spatially deposit multiple enzymes for cascade-catalysis studies,[1] and to construct DNA nanocaged enzymes to enhance catalytic activity and stability.[2] In addition, various shapes of DNA nanostructures have been employed for delivery of cancer drugs, such as doxorubicin (DOX).[3] Encouraged by the diverse capabilities of DNA origami scaffolds, we sought to explore the opportunities for use of DNA origami as a biocompatible nanoplatform to integrate multiple components for potential sensing application.

H_2O_2 is a major causative agent of diseases, including cancer, neurodegenerative disorders, aging, and many others. Hence, accurate quantification of H_2O_2 in complicated biological systems is critical for accurate health risk diagnosis and therapeutic evaluation. We develop a biocompatible and integrated hydrogen peroxide sensing system by immobilizing catalase, an enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen, on a DNA origami platform containing intercalated iridium complex BTP ((btp)2Ir(acac), an oxygen-sensitive phosphorescent probe). Our results demonstrated that the spatially attached enzyme on DNA origami templates facilitated the efficient diffusion of O_2 to BTP, thus enhancing the sensing sensitivity, as compared to that of a mixture of catalase and BTP. This integrated and biocompatible sensing platform might be useful in assisting the diagnosis and treatment of reactive oxygen species (ROS) related diseases.

References:

1. J. Fu, M. Liu, Y. Liu, N. Woodbury, and H. Y,. J. Am. Chem. Soc, 2012, 134, 5516.
2. Z. Zhao, J. Fu, S. Dhakal, A. Johnson-Buck, M. Liu, T. Zhang, N. Woodbury, Y. Liu, N. Walter, and H. Yan, Nature Communication, 2016, 7, 20619.
3. Q. Zhang, Q. Jiang, N. Li, L. Dai, Q. Liu, L. Song, J. Wang, Y. Li, J. Tian, B. Ding and Y. Du. ACS NANO, 2014, 8, 6633.

Investigation of physical properties of i-motif and G-quadruplex in a confined nanospace

Masayuki Endo, Sagun Jonchhe, Shankar Pandey, Tomoko Emura, Kumi Hidaka, Mohammad Akter Hossain, Prakash Shrestha, Hiroshi Sugiyama, and Hanbin Mao
Department of Chemistry, Graduate School of Science, Kyoto University
Department of Chemistry and Biochemistry, Kent State University
endo@kuchem.kyoto-u.ac.jp

It is well known that the physical properties of biomolecules such as thermal stability and folding kinetics change depending on the environment. For investigating the properties of biomolecules in the confined environment, precise design of a nanoscale space to place the molecules is critical. We created a nano-sized space using DNA origami, and found that G-quadruplex placed in the nanospace was thermodynamically stabilized and folding occurred rapidly. [1] In this study, we examined the influence of the nano-sized space on the physical properties of the i-motif (iM) and G-quadruplex (GQ) structure. [2] We examined mechanical unfolding of the iM and GQ in the nanocages using optical tweezers. The nanocages including iM and GQ were prepared by incorporation of iM- and GQ-containing strand into a half-opened nanocage and subsequent closing. In the nanocage, iM structure was formed stably even at a pH close to neutral. By using four different sizes of nanocages, we found that the mechanical and thermodynamic stability of iM and GQ increased with decreasing size of nanocages. It was also found that the water activity reduced by decreasing the size of nanocages. These results revealed that the stability of iM and GQ in nanocages is correlated with the decrease in water activity.

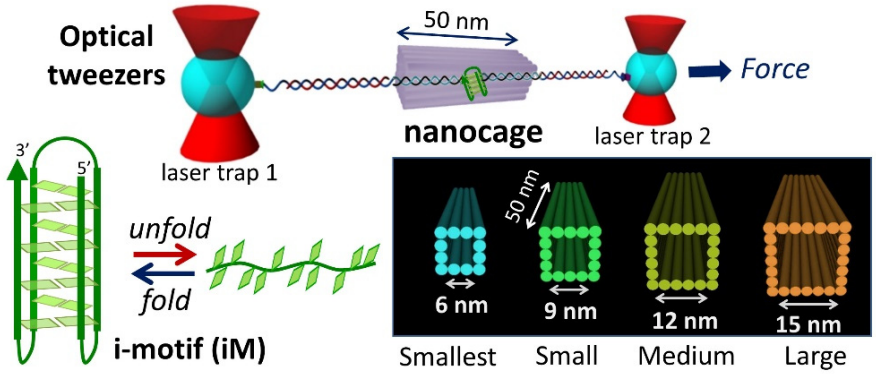

Fig. 1 Schematic illustration of i-motif in the DNA nanocage and the method to unfold/fold i-motif structure inside the four different size of nanocages using optical tweezers.

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Track on Chemical Tools for DNA Nanotechnology

Track Chair

Andrew Ellington

University of Texas at Austin

and

Floyd Romesberg

Scripps Research Institute

Extending Mutualistic Biopolymer Co-Assembly

Chen Liang, Allisandra Rha, Anthony Sementilli, Christella Gordon, Youngsun Kim, David G. Lynn
Departments of Biology and Chemistry, Emory University, Atlanta, GA 30322

Life on Earth is a study in mutualism. From our global ocean ecosystems to eukaryotic microbiomes, interdependencies continue to drive evolution across all length scales. Defining the nanoscale foundations of these associations are clearly manifest in the Central Dogma's management of biological information. We have been particularly interested in ribonucleoprotein granules, the classic example being the nucleolus where the ribosome is constructed, as a framework for how amyloid proteins might function together with mRNA to manage information processing[1,2]. Several paracrystalline amyloid structures, among them Aβ, tau, IAPP, and light-chain amyloids, have now been solved using solid-state nuclear magnetic resonance (NMR) and/or cryo-electron microscopy (cryo-EM). From these and other peptide paracrystalline peptide architectures, assembly can be assigned as occurring along three distinct growth surfaces: (i) the registry and orientation of backbone H-bonding depends on side-chain cross-strand pairing in a fashion energetically related to nucleic acid base-pairing; (ii) sheet-sheet stacking energetics are driven by sheet-facial complementarity[3,4], analogous to base-pair stacking in nucleic acid duplexes; and (iii) the charged external surface of cross-β assemblies that results from precisely stacked sheets, much like the surface phosphates of nucleic acid duplexes, regulates higher order aggregation. As in nucleic acids, all three surfaces contribute distinctly to templating fidelity and provide an opportunity to explore the co-assembly codes for RNA/amyloid co-assemblies. As shown in Fig. 1, short RNA strands (A_{10}) are able to template the assembly of short amyloidogenic peptides (Ac-KLVIIAG-NH_2) into ordered multi-lamellar nanotubes composed of peptides amyloid leaflets pacificated by RNA.

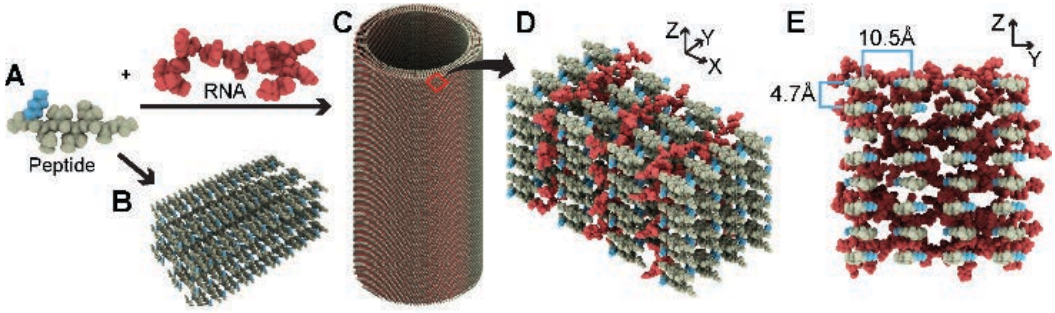

Figure 1. Structural models of peptide and NA/pep co-assemblies. (**A**) Peptide and RNA are depicted as globular structures with spherical atoms. (**B**) Peptides assemble as parallel and anti-parallel β-sheets and as (**C**) multi-lamellar nanotubes when templated by NAs. (**D**) Cross-β monolayers make up the individual lamellae of the RNA/pep nanotubes with RNA passivating the positively charged cross-β positively charge surface. (**E**) The peptides in the co-assembly are in anti-parallel, in-register β-sheets containing 4.7Å and 10.5Å d-spacings assigned to inter-strand and inter-sheet distances, respectively. The blue N-terminal lysine amine and the red RNA highlight their electrochemical distribution in the co-assemblies.

Protein condensation occurs via two-step nucleation[5-8] and our operational model is that this initial liquid-like assembly is captured by the RNA. We will provide evidence that the intermediate metastable phase contains dynamic crystallites, ones that appear to bias the conformational space accessible to the peptides, facilitating the ability of the RNA to template amyloid growth[3,4]. As shown in Fig. 2, structural analyses suggest that the surfaces of amyloid assemblies may be templated in various ways, taking advantage of the energetic constraints controlling assembly. Indeed we further will provide evidence that these assemblies are susceptible to other exogeneous templates, ranging from preformed peptide assemblies to metal ions and RNA, opening the possibility of new dynamic assembly networks controlled by feedback reactions[9-13].

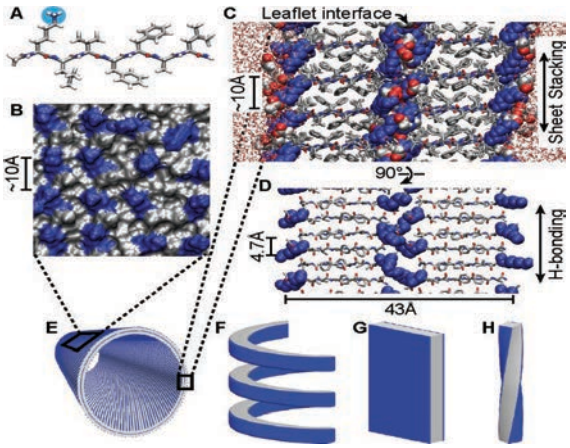

Figure 2. Structures available to peptide assemblies and the surfaces/morphologies susceptible to external templates.

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Movers and shakers: How single molecule fluorescence microscopy helps accelerate nucleic acid based devices

Nils G. Walter
Single Molecule Analysis Group, Department of Chemistry, 930 N. University Ave., University of Michigan, Ann Arbor, MI 48109-1055, USA; nwalter@umich.edu

Dynamic DNA nanotechnology has yielded nontrivial autonomous behaviors such as stimulus-guided locomotion, computation, and programmable molecular assembly. Despite these successes, DNA-based nanomachines suffer from slow kinetics, requiring several minutes or more to carry out a handful of operations. Recently, we have pursued two orthogonal approaches to increase the speed of nucleic acid based devices, using "movers" and "shakers": By implementing a "DNA acrobat" that cartwheels over a field of complementary oligonucleotides using toehold exchange movement at the speed limit[1]; and by coupling a DNA binding motor protein such as RNA polymerase to move one part of a device against another and thus "shake things up"[2]. Single molecule fluorescence microscopy is a powerful tool that allows us to probe both the mechanism and movement of such modified devices for optimization. Our results based on single molecule fluorescence resonance energy transfer (smFRET) and super-resolved single particle tracking suggest that substantial improvements in the operating rates of broad classes of DNA nanomachines are possible.

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Synthetic minimal cell toolbox

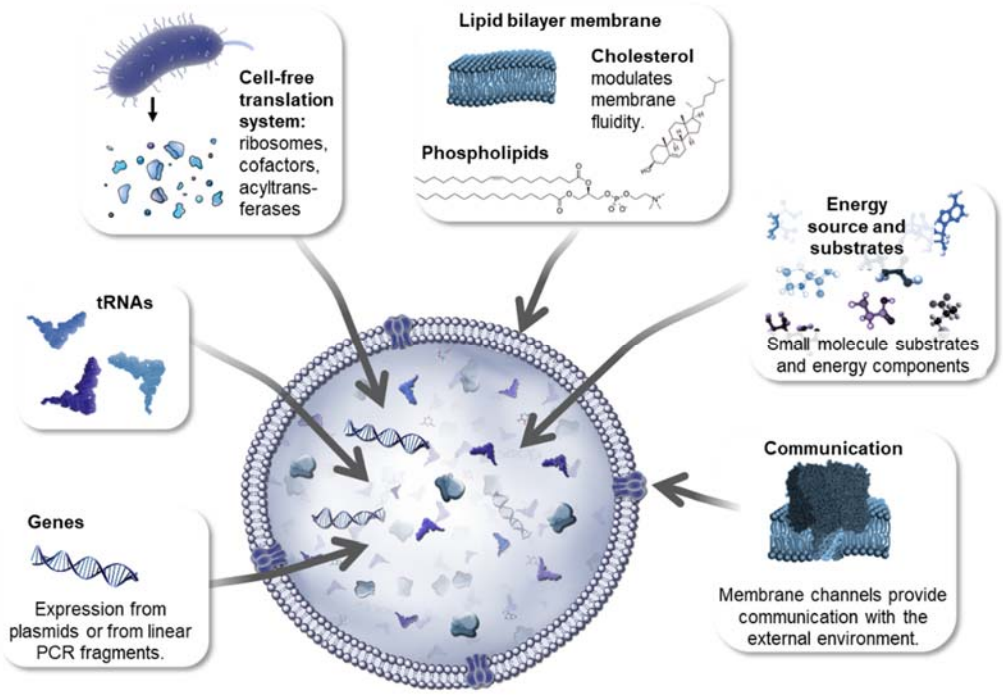
Kate Adamala, University of Minnesota, www.protobiology.org, kadamala@umn.edu

Synthetic minimal cells are liposome bioreactors expressing proteins from synthetic genomes. They are programmable systems that exhibit some, but not all, properties of living organisms. This technology is combining flexibility of cell-free protein expression with the ability to mimic life-like complex biochemical pathways, creating a versatile chassis for developing different types of genetic tools.

All standard DNA editing and processing techniques can be used in synthetic cell systems, including CRISPR, riboswitches, restriction enzymes or recombinases. Complex, multi-gene circuits can be built in programmable synthetic cells, without the noise from endogenous pathways in live cell experiments.

Our lab has developed such programmable platform, for designing and optimizing custom, multi-component genetic circuits, to build DNA-based Biocomputing devices, sensors and actuators. We have engineered a method for specific programming of synthetic cell lineages, which we are combining with new DNA replication methods and high thorough cell-free cloning and expression pipeline. This creates a versatile toolbox for synthetic minimal cell based bioengineering.

With synthetic cells, we can optimize existing processes, or engineer novel pathways and artificial biological systems, for biotechnology, basic science, space exploration, biosafety and biomedical applications. Synthetic cells offer a way to bridge natural synthetic biology with electronic devices, and to engineer biological tools with unprecedented accuracy and precision.



Schematic of a model synthetic minimal cell

Biologically inspired sensors of nucleic acid assembly

Lydia M. Contreras[1], Jorge Vazquez-Anderson[1], Mia Mihailovic[1]
[1]University of Texas at Austin

Given the importance of the formation of structural motifs to nucleic acid functioning, fundamental characterization and applied engineering efforts depend heavily on the understanding of their specific shapes and on their interactions with specific binding partners. However, only a few techniques exist to probe nucleic acid assembly directly **inside** cells and thereby elucidate stretches of nucleotides that can establish intermolecular interactions that could be important to nanostructure formation. In this talk, we will describe our recent advances in developing in vivo sensors, inspired by naturally used biological schemes, that allow for the simultaneous in vivo characterization of thousands of potential interacting interfaces in nucleic acids. As part of our initial efforts in this area, we have exploited mechanisms of post-transcriptional regulation to synthesize a fluorescence-based sensors that can assay nucleic acid structures in vivo via anti-sense RNA hybridization[1]. Our most recent efforts involved the development of a novel high throughput method based on synthetic biology and machine learning approaches to characterize regional accessibility of functional nucleic acid structures in living cells[2]. Specifically, we have engineered a system in which accessibility is correlated to transcriptional elongation, as evaluated by RNA-seq. We will describe how insights obtained from these biological sensors can be used in the characterization of newly discovered nucleic acid motifs and in the discovery of novel assembly mechanisms.

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Track on Nanophotonics and Superresolution

Track Chair

Ralf Jungmann
Max Plack Institute for Biochemistry, Martinsried

MINFLUX Nanoscopy and Tracking

Francisco Balzarotti[1], *Yvan Eilers*[1], *Klaus C. Gwosch*[1], *Jasmin Pape*[1], *Stefan W. Hell*[1]

[1]*Department of NanoBiophotonics, Max Planck Institute for Biophysical Chemistry*
Am Fassberg 11, 37077, Göttingen, Germany
E-mail: fbalzar@mpibpc.mpg.de

Superresolution microscopy methods such as STED and PALM/STORM have revolutionized far-field optical fluorescence microscopy by manipulating state transitions of the emitters, offering potentially unlimited resolution. In practice, however, the resolution of an image is limited by the finite photon budget of fluorescent probes, while their finite emission rate imposes a spatial-temporal trade-off in tracking applications. By synergistically combining the strengths of both superresolution families, the recent MINFLUX concept (1) tackles these limitations by rendering each emitted photon more informative.

MINFLUX localizes an emitter by repeatedly probing its location with an excitation beam that features a zero of intensity (fig. 1A-B). The emitter position is obtained from the knowledge of the beam shape and the number of photons collected at each location of the beam. When compared to conventional centroid-localization techniques, it is possible to reach a given precision by using fewer photons, or conversely, have an improved precision for the same photon budget.

Multiple results of the concept will be presented (fig. 1C-E) for imaging and tracking. Tracking of 30S ribosomal subunits in living E. coli fused with the photoconvertible protein mEOS2 demonstrated a 22-fold reduction of the required photon detections and increased the temporal resolution and the number of localizations per track by 100-fold. Images of DNA origami labeled with Alexa Fluor 647 achieved ~1nm precision, resolving molecules 6 nm apart. Additionally, tracking of the movement of DNA origami constructs (2) resulted in ~2nm precision and sub-millisecond time resolution. Extensions of the concept for ~um-sized multicolor imaging will also be presented.

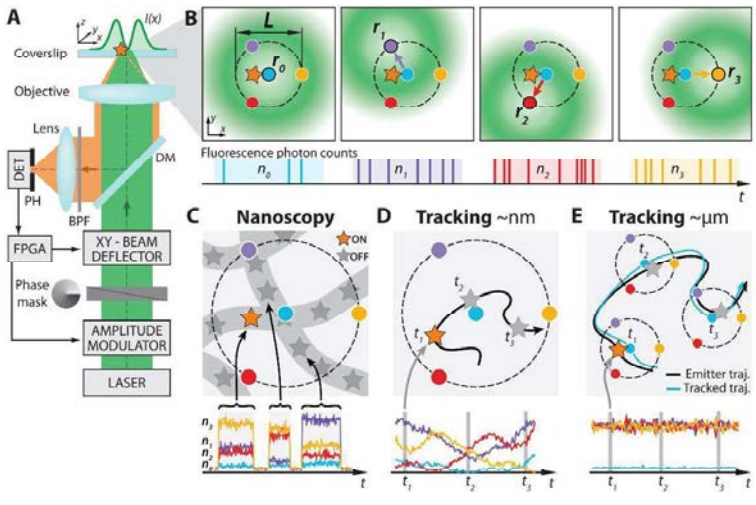

Figure 1. MINFLUX concept. (**A**) Scanning fluorescence microscope with a donut-shaped excitation beam and confocal detection. (**B**) An emiter (star) is exposed secuentially to the excitation beam at four different locations, collecting different fluorescence signals. (**C–E**) Applications of the concept for (C) imaging, by consecutively localizing blinking molecules; (D) nm–range tracking by performing localization very fast; (E) µm–range tracking, by reposition of excitation beam pattern onto the emitter with a feedback loop.

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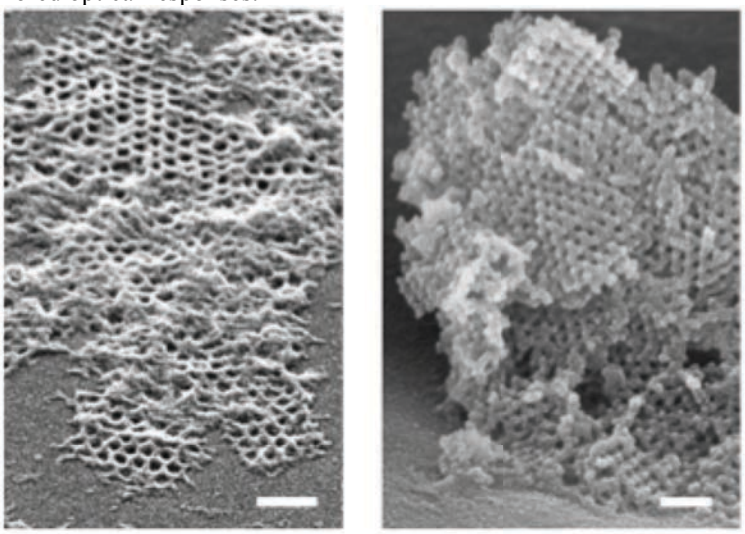
Chiral plasmonic sensing and silicifying DNA materials

L. Nguyen[1], T. Funk[1], A. Heuer-Jungemann[1], K. Martens[1], L. Hu[2], A. O. Govorov[2] & T. Liedl[1]

[1]*Faculty of Physics and Center for Nanoscience (CeNS), Ludwig-Maximilians-Universität, Geschwister-Scholl-Platz 1, 80539 München, Germany*
[2]*Department of Physics and Astronomy, Nanoscale and Quantum Phenomena Institute, Ohio University, Athens, Ohio 45701, United States*

In recent years, DNA self-assembly has reached a level of maturity that DNA today is routinely used by research laboratories to construct functional two- and three-dimensional nanomachines and materials [1,2]. By defining attachment sites for active components on DNA structures, others and we have realized complex and nanometer-precise assemblies of biomolecules, organic fluorophores and inorganic nanoparticles [3]. We employed such nanoassemblies to create new plasmonic effects, which, in turn, enable us to detect proteins and virus-derived RNA molecules with high selectivity and sensitivity [4].
In related work, we have studied plasmon-assisted long-range chiral interactions in nanorod−nanoparticle−nanorod complexes [5]. Due to the plasmonic resonance of a transmitter particle, the chirality of the nanorod pair is coupled over long distances and additionally transferred to the visible spectral interval. Such chiral plasmonic structures could find application as sensors or as materials with novel optical properties.
To, however, fully explore the real-life applicability of DNA nanotechnology, it will be essential to increase the stability of DNA-based structures with respect to thermal, chemical and mechanical demands. We show how silicification of DNA origami crystallites that grow into micrometer-scale 3D assemblies leads to nanomaterials that withstand drying without structural deformation (Figure)[6,7]. The combination of plasmonic architectures with silica-rigidified crystalline assemblies could pave the way to 3D materials that are designed on the molecular level while reaching macroscopic dimensions and exhibiting tailored optical responses.



Scheme 1 *Silica growth on DNA origami crystals. Left: 60° tilted view of bare DNA origami crystals. The crystals flatten upon adsorption to the substrate. Right: 60° tilted view of silica coated DNA origami crystals. The 3D structure is preserved. Scale bars are 200 nm...*

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Imaging single-molecules with temporal DNA barcodes

Shalin Shah[1], Abhishek Dubey[2] and John Reif[1,3]

[1] *Department of Electrical & Computer Engineering, Duke University, US*
[2] *Computational Sciences & Engineering Division, Health Data Sciences Institute, Oak Ridge National Lab, US*
[3] *Department of Computer Science, Duke University, US*
Email: reif@cs.duke.edu

Many biochemical events of importance are complex and dynamic. Fluorescence microscopy offers a versatile solution to study the dynamics of biology at the mesoscale. An important challenge in the field is the simultaneous study of several objects of interest, referred to as *optical multiplexing* [1]. For improved multiplexing, some prior techniques used repeated reporter washing or the geometry of nanostructures; however, these techniques require complex nanostructure assembly, multiple reporters or advanced multistep drift correction. Here we propose a time-based approach, for improved optical multiplexing, that uses readily available inexpensive reporters and requires minimal preparation efforts. We program short DNA strands, referred hereby as *DNA devices*, such that they undergo unique conformation changes in presence of the dye-labeled reporters. The *universal fluorescent reporter* transiently binds with the devices to report their activity. Since each device is programmed to exhibit different hybridization kinetics, their fluorescent time trace, referred to as the *temporal barcode*, will be unique. We model our devices using Continuous-time Markov Chains and use stochastic simulation algorithm to generate their temporal patterns. We first ran several simulation experiments with a small number of our devices, demonstrating several distinct temporal barcodes, all of which use a single dye color [2]. Later, using a nanostructure, we designed a much larger pool of unique temporal barcodes and performed supervised learning using support vector machine [3]. Using the guidelines and principles obtained from the simulation experiments, we experimentally validate eight different devices with three tunable device parameters using TIRF microscopy [4].

Keywords: DNA kinetics, Optical multiplexing, Temporal patterns, Machine learning, Single-molecule imaging, Continuous-time Markov chain

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Track on DNA Nanosystems: Programmed Function

Track Chair

Friedrich Simmel

Technical University Munich

Programming dynamic behaviors in molecular systems and materials

Leopold N. Green[1], Hari K. K. Subramanian[2], Jaimie M. Stewart[1], Siddharth Agarwal[2], Melissa A. Klocke[2], and Elisa Franco[2,3]

[1]*Department of Bioengineering, University of California at Riverside, CA, USA,* — [2]*Department of Mechanical Engineering, University of California at Riverside,CA, USA,* — [3]*Department of Mechanical and Aerospace Engineering, University of California at Los Angeles,CA, USA*

Biological systems reconfigure their shape at the level of single cells, tissues, and organs in response to external stimuli, for a variety of purposes such as growth, development, and self-repair. Cell shape reconfiguration is accomplished by directing molecular materials (such as cytoskeletal proteins) through molecular circuits which sense, process, and transmit information from the environment. Although they are often organized in a modular fashion, these cellular pathways are still too complex to be directly embedded in a biosynthetic material. An alternative route is offered by nucleic acid nanotechnology: sensors, logic, and dynamic circuits [1, 2, 3, 4], and a variety of nanostructures [5, 6, 7] have been successfully demonstrated. Using nucleic acid systems, our group aims at building synthetic biomolecular materials in which, taking inspiration from nature, assembly and disassembly of nucleic acid scaffolds is controlled by dynamic nucleic acid inputs, sensors, and circuits to achieve responsiveness and adaptation properties that rival those of biological scaffolds.

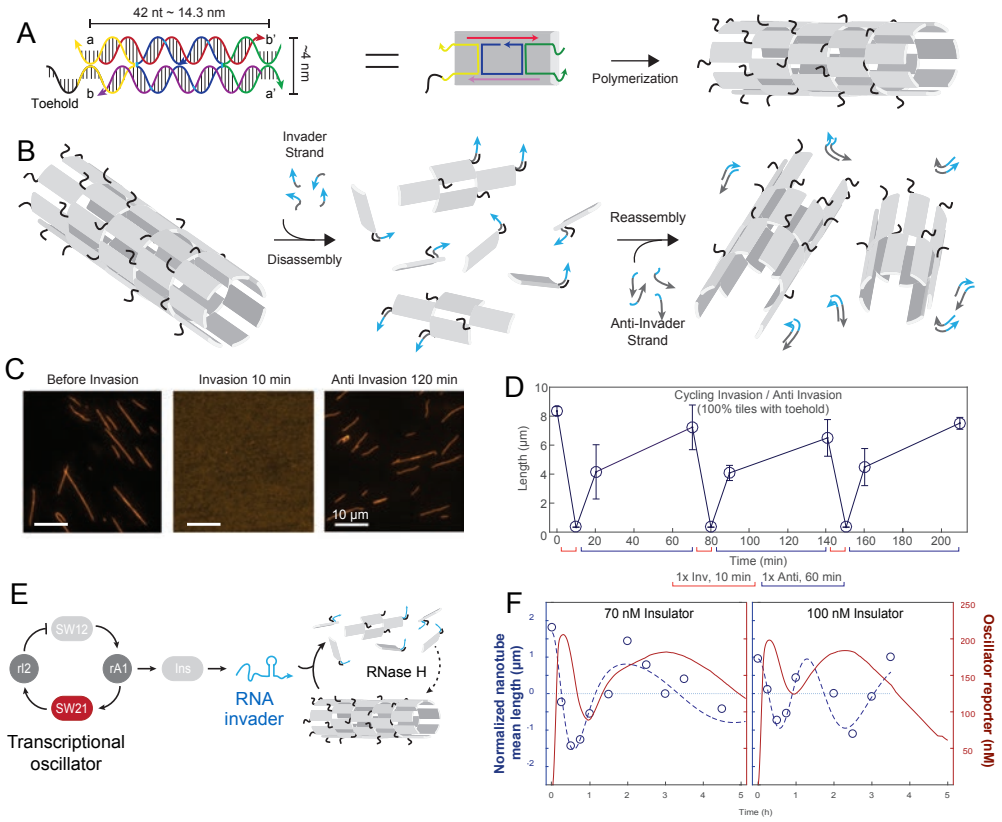

Figure 1: *We controlled DNA nanotube self-assembly using molecular signals including a synthetic oscillator.*

We have built DNA and RNA self-assembling nanotubes that can respond to a variety of inputs that include other nucleic acid molecules, pH, and enzymes. These nanotubes assemble

from nano-scale tiles, and are some of the largest known nucleic acid nanostructured assemblies [5]: they reach tens of microns in length and present mechanical properties similar to actin filaments and microtubules. We have engineered DNA [8, 9] and RNA tiles [10, 11] to include actuation domains to which nucleic acid inputs can bind thereby triggering assembly or disassembly. For example, we added a single-stranded overhang to the tile binding domains, or sticky-ends, to promote disassembly when an "invader" strand is added in solution. Removal of the "invader" species allows the nanotubes to reassemble isothermally, making it possible to control growth reversibly. We have shown that it is possible to control nanotube assembly and disassembly with nucleic acid inputs directly added in solution [8], with a nucleic acid pH sensor [9], and with synthetic transcriptional systems [12]; in particular, we showed that nanotube length can be controlled with an autonomous molecular oscillator [8, 3, 13, 14]. We have also designed hybrid RNA-DNA nanotubes whose assembly and disassembly can be directly controlled by enzymes that produce and degrade RNA. These dynamic assembly processes can be modeled quantitatively with a coarse-grained model of differential equations capturing the nanotube length distributions [15].

These responsive nucleic acid structures could be used as scaffolds for dynamic heterogeneous materials [16], and as scaffolds for artificial protocells. Current studies are aimed at engineering these nanotube systems for resilience in the cellular environment [17] and encapsulation in droplets and vesicles.

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Synthetic cells: Bottom-up assembly with DNA nanotechnology and microfluidics

Kerstin Göpfrich, Kevin Jahnke, Ilia Platzman, and Joachim P. Spatz
Max Planck Institute for Medical Research, Department of Cellular Biophysics, Jahnstraße 29, D 69120, Heidelberg, Germany
Department of Biophysical Chemistry, University of Heidelberg, Im Neuenheimer Feld 253, D 69120 Heidelberg, Germany
Contact: kerstin.goepfrich@mpimf-heidelberg.mpg.de

Bottom-up synthetic biology has been successful at isolating components from cells and reconstituting subcellular functions inside compartments. Progress towards a fully functional synthetic cell, however, requires strategies to recombine and arrange a multitude of components in space and time. We therefore propose to merge two precision technologies, namely microfluidics and DNA nanotechnology, to position and manipulate components in synthetic cells [1,2]. In particular, we demonstrate that DNA can be used as a near-universal linker for responsive and programmable compartment functionalization. Our method relies on the self-assembly of single-stranded cholesterol-tagged DNA handles, which provide an addressable anchoring point for complementary DNA carrying an arbitrary functional group. Using this DNA handle approach, we demonstrate the stimuli-responsive attachment of reactive groups, DNA nanostructures, microspheres, an actin cortex and even living cells to the periphery of surfactant-stabilized microfluidic droplets [3]. We further employ DNA to construct functional components, including a pH-responsive DNA-based cytoskeleton mimic, which serves as a stabilizing cortex inside synthetic cells.

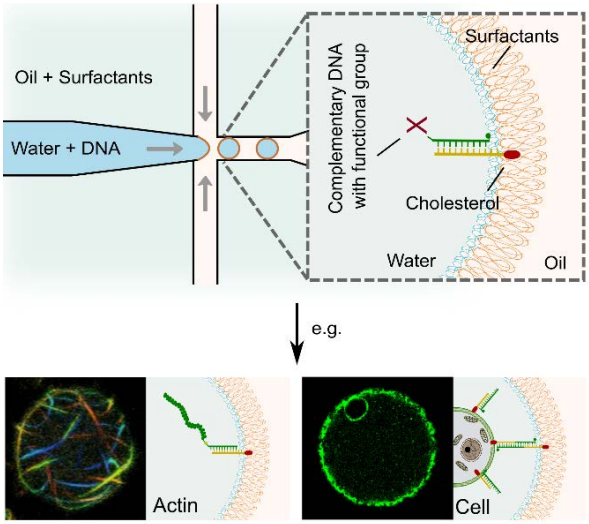

Fig. 1: *Top:* Illustration of the concept for the chemical functionalization of surfactant-stabilized microfluidic droplets via DNA handles. *Bottom:* Confocal fluorescence images demonstrating the attachment of an actin cortex and a living cell to the periphery of microfluidic droplets.

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[2] B. Haller, K. Göpfrich, M. Schröter, J. W. Janiesch, I. Platzman* & J. P. Spatz*, Charge-controlled microfluidic formation of lipid-based single-and multicompartment systems, *Lab on a Chip* 18, 2665–2674 **(2018)**.
[3] K. Jahnke, M. Weiss, C. Frey, S. Antona, J.-W. Janiesch, I. Platzman, K. Göpfrich* & J. P. Spatz*, Programmable Functionalization of Surfactant-Stabilized Microfluidic Droplets via DNA-Tags, *under review*.

Deducing the roles of individual chromophores in energy transport relays using machine-learning tools

Brian Rolczynski, Electronics Science and Technology Division, US Naval Research Laboratory
William Klein, Center of Bio/Molecular Science, US Naval Research Laboratory
Sebastian A. Diaz, Center of Bio/Molecular Science, US Naval Research Laboratory
Reza Zadegan, Department of Materials Science and Engineering, Boise State University
Mario Ancona, Optical Sciences Division, US Naval Research Laboratory
Wan Kuang, Department of Electrical and Computer Engineering, Boise State University, Boise State University
Igor L. Medintz, Center of Bio/Molecular Science, US Naval Research Laboratory
Joseph Melinger, Electronics Science and Technology Division, US Naval Research Laboratory, joseph.melinger@nrl.navy.mil

Biological energy transport pathways are often constructed using multiple copies of a chromophore. The resulting closely spaced energy levels can be beneficial for efficient energy transport, but their heavily overlapping spectral contributions hinder optical spectroscopies attempting to distinguish their individual energies or dynamics. Nonetheless, understanding the contributions of particular chromophore sites is important for deducing the structure-transport relationships. In this study, DNA scaffolds are constructed with 14 sites that can be occupied with chromophores, in order to mimic a biological energy transport relay. A library of over 140 unique combinations (Figure 1) was synthesized by selectively excluding the chromophores at various sites, and the fluorescence spectra were measured to obtain their FRET characteristics. Machine-learning methods were used to infer the dependence of these processes on each chromophore from the large data set. These results indicate, for instance, that the third Cyanine 3.5 chromophore is more important for the wire transfer efficiency than the first. Furthermore, the random forest methods employed here also infer nested higher-order dependencies. These results provide insight into the cooperative effects of particular chromophores in energy transport.

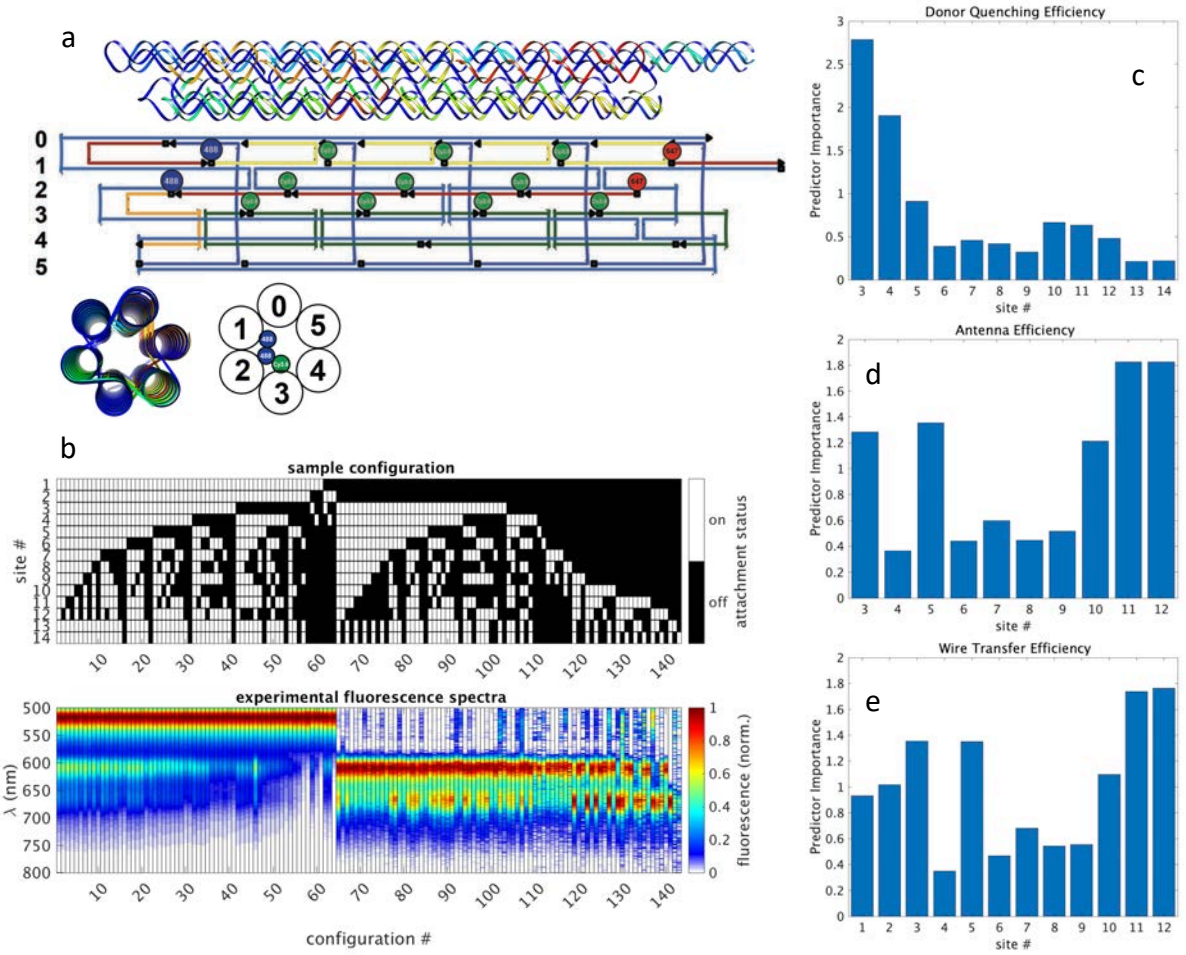

Figure 1. Machine-learning is used to deduce the importance of individual sites on energy transport in an extended chromophore network, including many repeated units. (a) A 14-site scaffold is constructed using a bundle of 6 DNA duplexes, as shown in corresponding ribbon and schematic diagrams. The stitching scheme is indicated in the schematic, as well as the chromophore sites. The first two, next ten, and last two sites are assigned to Alexafluor 488, Cyanine 3.5, and Alexafluor 647, respectively, in order to build a downhill energy scaffold for directional, incoherent energy transfer. (b) More than 140 unique sample configurations are made by deleting chromophores at particular sites on the scaffold. In the top figure, each column represents a unique sample configuration. The white boxes indicate present chromophores, while black gaps indicate deletions. Below, each column plots the corresponding fluorescence spectrum for each configuration. (c-e) Based on the fluorescence spectra, Random Forest machine-learning algorithms are used to deduce the relative importance of individual sites on figures of merit such as the wire transfer efficiency.

A large size-selective DNA nanopore with sensing applications

Rasmus Thomsen[1], Anders Okholm[1], Mette Malle Galsgaard[2], Swati Krishnan[3], Søren Schmidt-Rasmussen[2], Rasmus Sørensen[1], Friedrich Simmel[3], Nikos Hatzakis[2], Jørgen Kjems[1,4,†].*

[1]Aarhus University, iNANO, Aarhus, Denmark, [2]University of Copenhagen, Dept. of Chemistry, Copenhagen, Denmark, [3]Technical University of Munich, Physics, Munich, Germany, [4] Aarhus University, Molecular Biology and Genetics, Aarhus, Denmark
**thomsen3@inano.au.dk, †jk@mbg.au.dk*

DNA nanotechnology has a huge potential to build biological robotic nanostructures. The major advantages of DNA nanotechnology lie within its easy structural predictability combined with numerous possibilities of functionalization[1,2]. Hence, by using DNA nanotechnology, it is possible to position functional moieties and particles at high spatial resolution creating functional molecular assemblies. Interesting structures to mimic includes nanopores which is used by nature to control and guide molecular flow and reactions between enclosed compartments.

This work demonstrates the progress of creating a DNA nanopore device with the ability of programmed insertion into selected barcoded membranes. This is based on a comprehensive DNA origami design introducing a signal-controlled exposure of lipid membrane anchors and a gated channel controlling the translocation of macromolecules which enables the structure to work as a biosensing DNA device.

Several DNA nanopores have previously been reported in the scientific literature[3–6]. Most, however, work as passive channels, remain limited in size and all lack a mechanism of controlled insertion. To address these notions, we have created a DNA nanopore with a multi-helical cylinder and 3 flap-structures as visualized in Figure 1a. The origami structure is formed with a high yield and the predicted structure was visualized by TEM single particle average imaging presented in Figure 1b.

Due to the hydrophilic nature of DNA, functionalization with hydrophobic lipid moieties is important but to avoid aggregation and allow insertional control, the lipids are only exposed when opening of the flap-structures are activated, as visualized in Figure 1c. Using several techniques, the nanopore design has demonstrated its capability of insertion into liposomes (Figure 1d-f). This includes characterization of the insertion of the DNA nanopore both visually by negative stain TEM as shown in Figure 1d and kinetically by single particle measurements using a TIRF setup[7] as demonstrated in Figure 1e-f. From the TIRF setup we also demonstrated how barcoded SUVs is recognized by the DNA nanopore and target for insertion with a higher efficiency compared to non-barcoded controls.

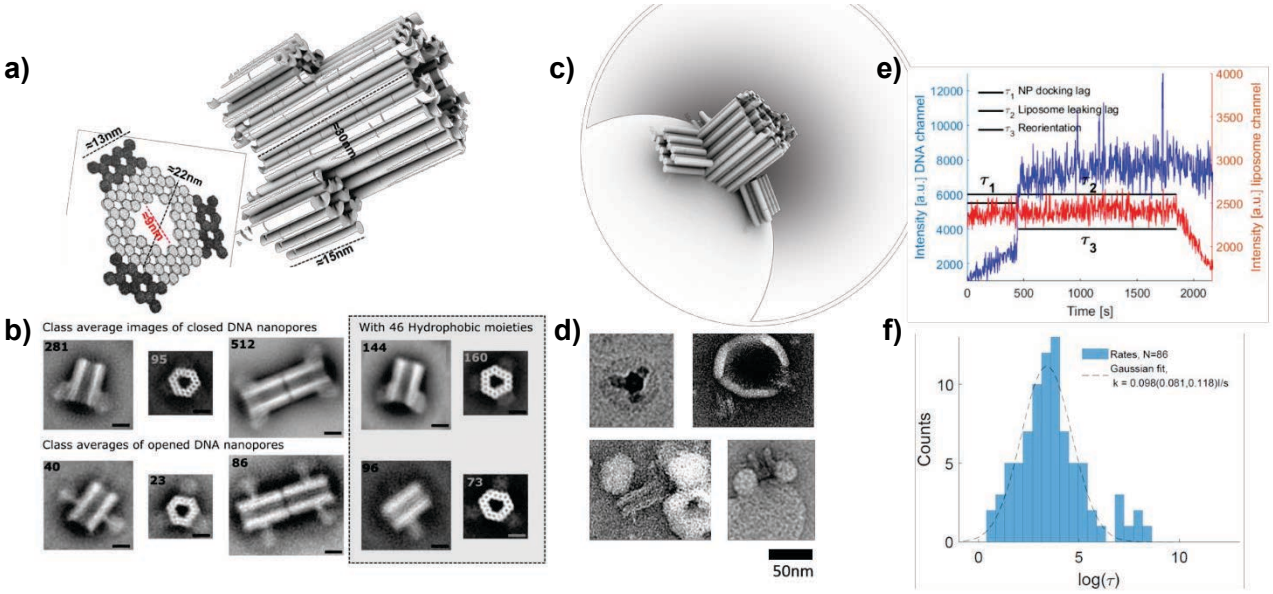

Figure 1: (a) Visual representation of the DNA nanopore shown from a side view. (b) TEM class average images of the DNA nanopore in opened and closed flap conformation. All scale bars are 15 nm. (c) Visual representation of a flap-mediated inserted DNA nanopore. (d) nsTEM cutouts of liposome inserted and interacting DNA nanopores. (e) Fluorescent traces of DNA nanopores (blue) and dye flux (red) of a surface immobilized SUV. (f) Histogram of 86 recorded dye flux traces with mediated by the DNA nanopore.

In addition, by introducing a molecular plug we show how the macromolecular flux across the membranes of individual GUVs can be controlled in a size dependent manner using a confocal setup. With the combination of a rigid mechano-insensitive channel equipped with molecular plug the ability to gate the flow of molecules size-selectively by an external signal, we hypothesize a future development of generic single molecule sensors with a visible readout for the eye.

Furthermore, by design of a preprogrammed end-dimerization pattern allowing dimerization of two orthogonal DNA nanopores, creation of two-headed nanopores for dual targeting is possible, as shown in Figure 1b and 1d. Interestingly, programable multistep chemical and enzymatic reactions between compartments would be possible with such a two-headed DNA nanopore, simply by connecting two barcoded liposomes populations. This would be a step towards the creation of enclosed nanoreactors.

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Track on Computational Tools for Self-Assembly

Track Chair

William Shih

Wyss Institute and Harvard Medical School

A simple mechanical model for synthetic catch bonds

Kerim Can Dansuk, Sinan Keten

Dept. of Civil & Environmental Engineering and Mechanical Engineering

Northwestern University

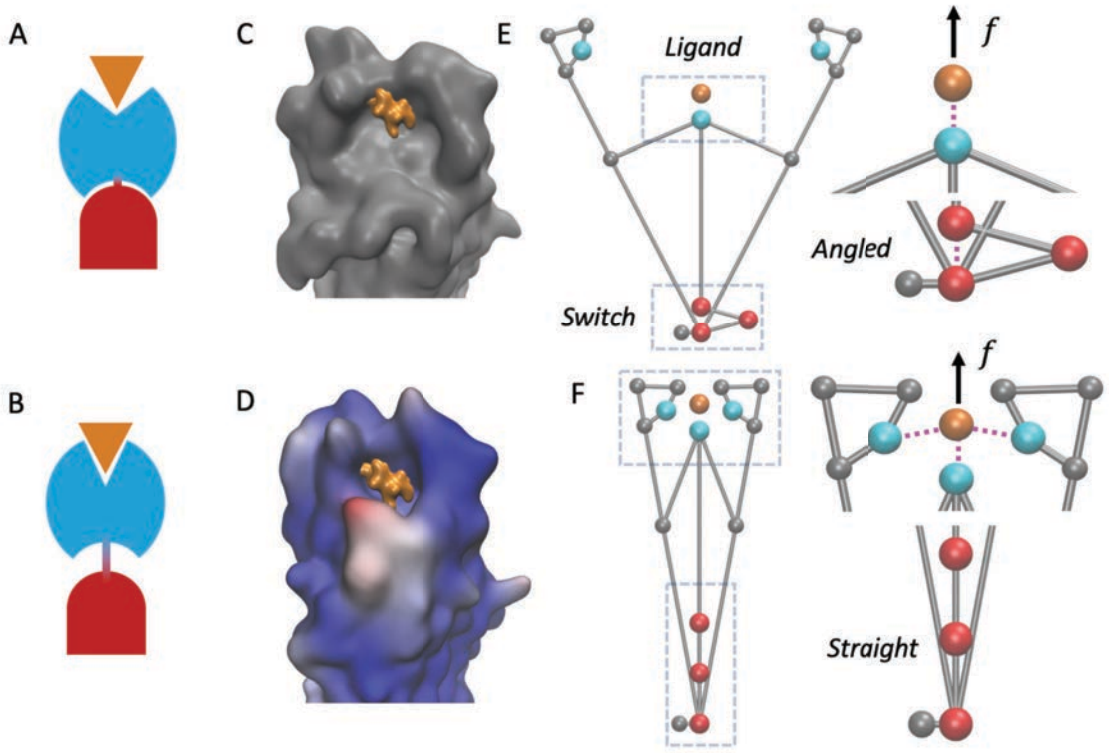
s-keten@northwestern.edu

A challenging problem in designing nanoparticle networks is to engineer nanoparticle interfaces that have tunable cohesive strength and rate-responsive behavior, for which inspiration can be taken from biological systems. An exemplary bio-interface is the Chaperone-Usher (CU) pili, such as type 1 expressed by bacteria Escherichia coli. The pili have unique biomechanical properties that enhance the ability of bacteria to sustain attachment to surfaces under large stresses, such as constant force extensibility, logarithmic velocity-uncoiling force dependence, and adhesive tips with catch bond behavior that exhibit longer bond life-times at greater force levels. Although biophysics of the pili under strain or stress is well-studied for anti-infective applications that aim to compromise pili adhesion, utilizing the biomechanical properties of the pili in material design applications is yet to be explored. In the first part of this talk, I will present a model for the elongation of a single CU pilus with catch bond tip adhesin, where we examined its toughness response using Monte Carlo simulations. We showed that the pilus can act as a ‘‘molecular seat belt’’ that exhibits low toughness when pulled slowly and high toughness when pulled rapidly. Furthermore, we found that systematically varying the catch bond and shaft parameters leads to tunable seat belt behavior at the interface, where the sharpness of the transition from the low toughness to the high toughness regime and the velocity at the start of the transition can be dictated by molecular design parameters. The molecular seat belt mechanism presented here provides insight into how nanocomposite interfaces can be engineered to create molecular networks with linkers that switch on or off depending on strain rate.

In the second part of the talk, I’ll focus on catch bonds, and present a simple mechanical model that captures their behavior. Catch bonds are protein-ligand bonds which become more difficult to break with larger applied force, a counterintuitive phenomenon that is yet to be reproduced in synthetic systems. In our recent work, we have demonstrated that a simple mechanical design based on a tweezer-like mechanism can exhibit catch bond characteristics under thermal excitations. The tweezer has a force-sensitive switch which controls the transition of the system to a high-ligand-affinity state with additional ligand-tweezer interactions. Applying kinetic theory to a two-mass-two-spring idealized model of the tweezer, we show that by tuning the shape of the switch and the ligand-tweezer

interaction energy landscapes, we can achieve greater lifetimes at larger force levels. We validate our theory with molecular dynamics simulations and produce a characteristic lifetime curve reminiscent of catch bonds. Our analysis reveals minimal design guidelines for reproducing the catch bond phenomenon in synthetic systems such as molecular switches/foldamers, DNA linkers and nanoparticle networks.

Figure:



Catch bond models Schematics of A) open and B) closed conformation of FimH protein with lectin (cyan) and pilin (red) domains. Ligand (mannose) is shown in orange. C) Open (PDB 4XOD) and D) closed conformation (PDB 4XOB) of ligand binding pocket, located on the lectin subunit. The closed conformation is colored via a scale (blue=small, white=middle and red=large), which shows the displacement of the atoms relative to the open conformation. As FimH transitions from its open to closed conformation, the loop segment closes on the ligand, forming new interactions. E and F) The tweezer design is inspired from FimH and has main and secondary binding sites (cyan) and pairwise switch (red). In open conformation (E) ligand forms one interaction with the tweezer and the switch members are angled. In closed conformation (F) ligand forms three interactions with the tweezer and the switch members are straight. Interactions and applied external forces are indicated by pink dashed lines and black arrows respectively. Switch and ligand regions are highlighted with boxes, and close-up views of these regions are given on the right side of panels E,F.

Automated Design of Curved DNA Origami Capsules with Multilayer-Reinforced Rigidity with Specified Shape

Daniel Fu[1†], Raghu Narayanan Pradeep[2], Fei Zhang[2], John Schreck[3], Hao Yan[2], John Reif[1]
[1] Department of Computer Science, Duke University, Durham, NC 27516, USA
[2] School of Molecular Sciences and Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA
[3] Department of Chemical Engineering, Columbia University, New York, NY 10027, USA
[†] dfu@cs.duke.edu

Our overall goal is to create artificial molecular-scale enclosures that feature complex shape curvature and a hosting capability for guest molecules within a defined and enclosed cavity. We also desire a scalable path to reinforcing structural rigidity for stalling degradation effects or for shape-sensitive applications. Further, we desire automated design of these from a specified shape as described by a 3D CAD file.

The DNA origami technique [1] has launched numerous design strategies for constructing various solid or hollow 3D DNA nanostructures, such as by the "folded-sheet" [2], DNA bricks [3], polyhedral [4], and DNA "capsule" [5] strategies. In contrast to the other strategies, DNA capsules pioneered by Han *et al.* are the only design strategy that pave a clear approach towards our goal. Yet while software has been developed to expedite the respective design processes of the other strategies [6-9], DNA capsules still lack comparable automated design tools which has hindered the availability of the strategy's feature-rich nanostructures for downstream applications. Such structures may be applicable to the capture, release, and transport of medicinal molecules for drug delivery [10,11] or for the guided growth of metallic nanoparticles via nanocasting [12, 13].

In the present work, we demonstrate an automated method, which given a CAD specification of the 3D desired shape of a DNA capsule design, generates strand sequences for the DNA capsule design. We also report on our investigations of the biophysical properties of DNA capsules, which exhibit shape curvature unique from all other existing design strategies. We demonstrate reinforced rigidity of structures by extending previously known single-layer designs to novel multi-layer analogues. Structures were also simulated and analyzed in oxDNA [14,15] to study the effect of material density on structural rigidity. Fluctuations of molecules are tracked, and overall rigidity of the structure is scored as an average of root-mean-square values taken across all molecules. We find an increase in rigidity as determined by a decrease in overall molecule movement, potentially due to the vastly increased crossover density within the structure corresponding to multi-layer origami [16]. Most significantly, the cavity of these unique structures is stabilized for downstream applications that may require (a) dense walls for protection of cargo from environmental degrading agents or (b) high rigidity requirements to maintain shape specificity. As future work, our workflow also seeks to expand our current integration with molecular dynamics tools such as oxDNA into fluid feedback loops that will enable the research of heuristic optimization algorithms. This will be of great importance as DNA designs increasingly become

laboriously large and complex to design and troubleshoot for even the most expert of designers.

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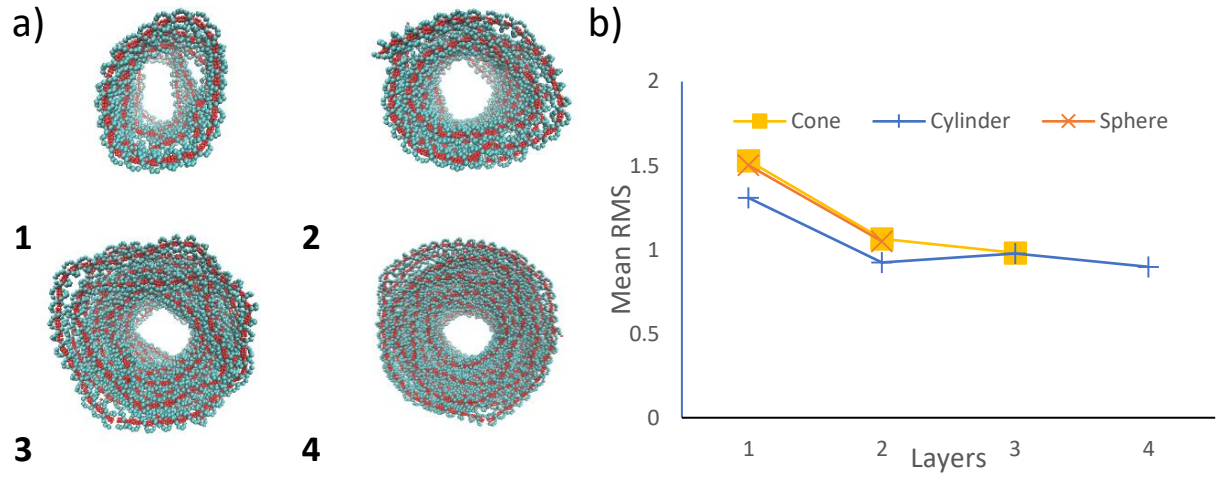

Figure 1 a) Relaxed molecular configurations of DNA origami cylinders with 1, 2, 3, and 4 layers. Relaxed state is obtained from molecular dynamics simulation in oxDNA2. **b)** Overall molecular fluctuation (measured by average RMS of all molecules) trends downward, indicating an increase in rigidity.

Enumeration, condensation and simulation of pseudoknot-free domain-level DNA strand displacement systems

Stefan Badelt[1], *Casey Grun*[2], *Karthik Sarma*[3], *Brain Wolfe*[1],
Seung Woo Shin[1] and *Erik Winfree*[1]
[1]California Institute of Technology, [2]Yale University, [3]UCLA
{badelt,winfree}@caltech.edu

Introduction DNA has been shown to be a robust and versatile substrate for nanoscale construction and computation. It is a common abstraction to describe these nucleic acid systems in terms of **domains**: contiguous sequences of nucleotides that are intended to participate in hybridization as one entity. Complementary domains are intended to interact, and all other pairs of domains are not. This *domain-level abstraction* is often used implicitly in the design process, but it can also serve as an explicit formal abstraction layer with well defined semantics that can be rigorously analyzed. The first step to analyse a **domain-level strand displacement (DSD)** system is to start with a finite set of initially present complexes and generate a **chemical reaction network (CRN)** of all possible reactions and products. We use the term **enumeration** to refer to the process of generating this CRN, given a set of initial complexes and a set of rules for their interactions.

Relevance Several previous efforts have explored enumeration and simulation of DSD systems using a built-in set of reaction rules. Most notably, the DNA circuit analysis tool **VisualDSD**, which in its most recent version [1] supports a more general class of secondary structures including pseudoknots. However, this also requires complex reaction rules, and therefore prior knowledge about the investigated system, otherwise it may fail to point out potential malfunction. A similar argument can be made for a recent approach of enumeration using graph rewrite systems [2].

Enumeration and condensation. Our enumerator, **Peppercorn** [3], separates the enumeration and simulation so that the resulting reaction network can be rigorously analyzed. The enumerator can represent complexes with arbitrary (pseudoknot-free) secondary structures and all possible domain-level reaction types: `open`, `bind`, as well as `3-way`, `4-way`, `proximal`, and `remote branch migration`. It enforces a separation of timescales in order to avoid implausible polymerization that would otherwise result from this degree of generality. We provide a condensation algorithm, that provides a smaller CRN with guaranteed correspondence to the full, detailed reaction network: condensed CRNs contain all overall "slow" reactions, assuming that "fast" reactions happen instantaneously.

Rate model. The enumerated network presents a rate-independent, trajectory-based picture of the system, which can be combined with a suitable rate model. In this work, we present an approximate rate model dependent only on domain length and reaction type. The model uses empirical domain-level reaction rates derived from selected DNA strand displacement and other DNA biophysics experiments. The domain-level reaction rate constants assume perfect Watson-Crick complementary of domains and "typical" designed sequences.

Case studies. Peppercorn presents a generalized model to identify the underlying CRN of a DSD system. We simulate these CRNs using deterministic ordinary differential equations

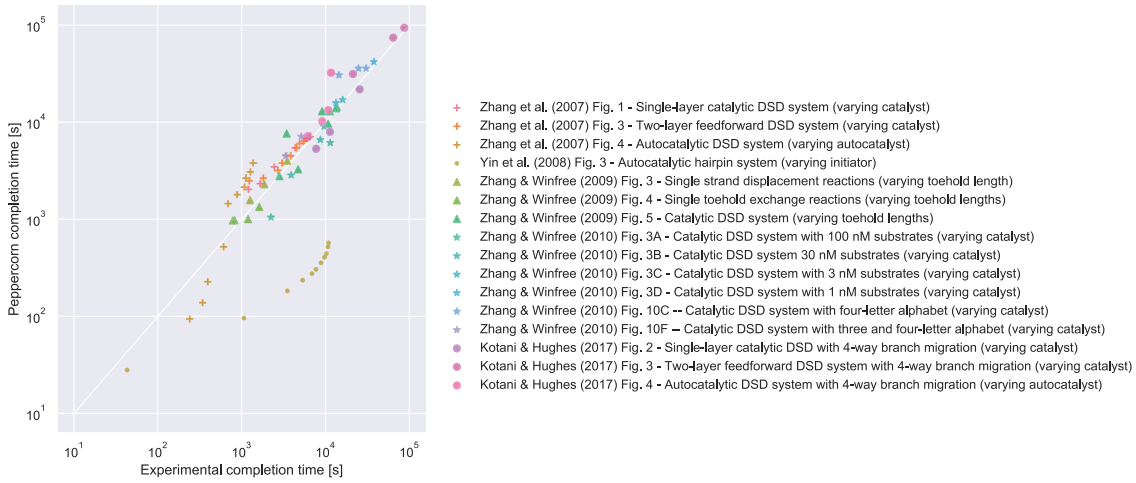

Figure 1: Simulated DNA system completion times in comparison with experimental data from five selected publications. For autocatalytic systems, we compare half-completion time, i.e. the time at which the fluorescent reporter reaches 50%. Since this method cannot capture slow reactions, we use an analogous diagonal-crossing time for all other plots: the diagonal crosses the x and y axes at the maximum clearly visible ticks from experimental data plots.

and compare the simulations to experimental data. By comparing a range of different toehold lengths and branch-migration domain lengths for classic 3-way strand displacement, 3-way toehold exchange, and 4-way strand displacement, we find correspondence of model and experimental data over approximately 9 orders of magnitude. We can use simple adaptations to the energy model to estimate completion times of large **Seesaw** systems (not shown), and we observe a good overall fit to experimental data as shown in Figure 1.

Availability. Peppercorn is available on GitHub [3], either as a standalone program for domain-level enumeration, or ready to embed into other projects using the library interface. As of now, the **peppercornenumerator** library is already a central part of the **DyNAMiC** Workbench Integrated Development Environment [4], the "CRN-to-DSD" compiler **Nuskell** [5], and the automated sequence-level verification software **KinDA** [6].

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Track on Synthetic Biology

Track Chair

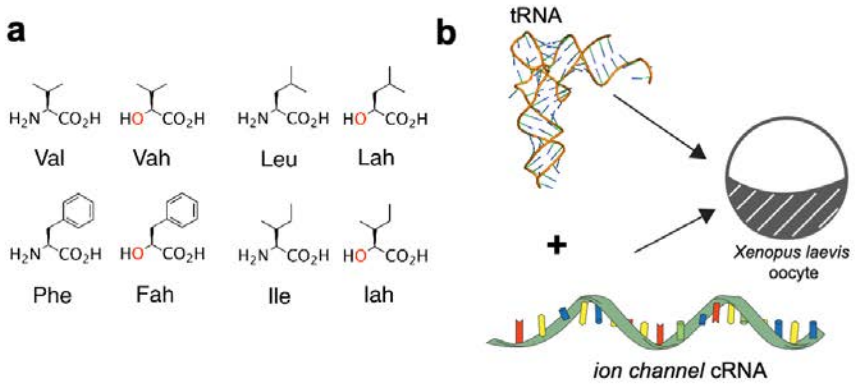
Alex Deiters

University of Pittsburgh

The Power of Small: Atomic Mutagenesis in Ion Channels in a Post-Structural Era

The Ahern laboratory is interested in the pharmacology and function of the voltage-gated ion channels that support electrical signaling in muscle and nerve cells. We use chemical biology, protein engineering and biophysics to quantify ion channel function at high resolution. One of our experimental approaches employs misacylated orthogonal tRNA to encode unnatural amino acids. This approach enables the high-resolution functional examination of ion channel function and the analysis of binding modes of ion channel therapeutics.

In one such example we recently set out to investigate the functional role of main-chain hydrogen bonds in the transmembrane segments of ion channels. To this end amide-to-ester substitutions were synthesized and encoded at sites throughout the S4 voltage-sensing segment of *Shaker* potassium channels, a canonical voltage-gated ion channel. This manipulation removes the i+n hydrogen bond in helical segments, while sparing the side-chain identity. Further, the S4 helical region of the channel undergoes a rapid (millisecond), voltage-driven outward translation of ~15A during channel gating, thus providing an established experimental read-out of the impact of main-chain H-bond removal. Biochemical analysis of base-hydrolyzed channels demonstrates high-fidelity encoding of the alpha-hydroxy variants. Functional analysis of channels expressed in the Xenopus oocyte show that alpha-hydroxy amino acids are highly tolerated at most positions with the S4 segment – that is, at many sites these AH channels display normal voltage-dependence of channel function. However, the data also highlight a transitional region between putative α-helical and 3_{10} segments within the S4 segment where main-chain hydrogen bond removal is particularly disruptive to voltage-gating. Here, H-bond cleavage produces an uncoupled phenotype where voltage-sensing is no longer tightly linked to channel opening. Direct electrophysiological measurements of S4 movement indicate compensatory voltage-sensor perturbation. Computational simulations of an active voltage-sensor reveal that this junctional region between the alpha- and 3_{10}-helicies intrinsically features a dynamic hydrogen bonding pattern and that its helical structure is reliant upon amide support. Overall, the data highlight the specialized role of main-chain chemistry in the mechanism of voltage-sensing; other catalytic transmembrane segments may enlist similar strategies in signal transduction mechanisms.



Encoding chemically modified amino acids into ion channels. a, Structures of amino acids (aa) and α-hydroxy acids (ah): Vah = hydroxyl-isovaleric acid, Lah = 2-hydroxy-4-methyl pentanoic acid, Fah = 3-Phenyl lactic acid, Iah = 2-hydroxy-3-methylpentanoic. Right, these are ligated to the tRNAs (pdb 2ZNI) for nonsense suppression. **b** , *Ion channel* cRNA containing a UAG stop codon at the suppression site is co-injected with an orthogonal amber (TAG) suppressor tRNA chemically ligated to an amino or alpha hydroxy acid (red).

Engineering Synthetic Membraneless Organelles With Tunable Composition

Jorge Dabdoub[1], Michelle Tong[1], Benjamin Schuster[2], Gregory Dignon[3], William Benman[4], Daniel Hammer[5], Jeetain Mittal[3], Matthew C. Good[1,4]
[1]Cell and Developmental Biology, University of Pennsylvania
[2]Chemical and Biochemical Engineering, Rutgers University
[3]Chemical and Biomolecular Engineering, Lehigh University
[4]Bioengineering, University of Pennsylvania
[5]Chemical and Biomolecular Engineering, University of Pennsylvania
Correspondence: mattgood@pennmedicine.upenn.edu

Protein phase separation is a critical regulator of intracellular structure and physiology, driving the assembly of membraneless organelles. However, it has been difficult to discern the function of these structures and the contribution of polypeptide sequence and interaction valency to protein self-assembly. Major challenges include inducible control of membraneless organelle assembly and disassembly and distinguishing the role of scaffold-client binding from scaffold-client condensation. To better understand the mechanisms and consequences of protein phase separation in living cells, we sought to engineer a synthetic membraneless organelle from the ground up using a single polypeptide that can be genetically encoded in cells. Our system is based on the intrinsically disordered N-terminal RGG domain from the P granule protein LAF-1. By altering its primary sequence, its valency and its modular interactions with cargo proteins, we build a protein condensate platform that can function as an orthogonal synthetic compartment within cells. To determine the sequence-determinants of RGG phase separation, we used coarse-grain modeling of protein condensation in silico. We identified critical regions and select types of chemical interactions critical for its phase separation and validated these finding in vitro. Next we re-engineered the sequence to modulate it upper critical solution temperature for phase separation and achieved a broad range sequences capable of phase separation at distinct temperatures and osmolarities. To inducibly alter RGG valency and thus protein phase separation in real-time we leveraged inducible dimerization domains. This approach enabled us to chemogenically trigger protein phase separation in vitro using a small molecule, rapamycin, and to optochemically induce phase separation by uncaging of photocaged rapamycin (dRap). We also demonstrate inducible protein condensation in living cells. To control recruitment of recombinant protein to our synthetic organelle expressed in cells we tagged our RGG scaffold and clients with cognate pairs of coiled coils. We demonstrate both constitutive and inducible cargo recruitment to these organelles in vivo. By using a temperature-sensitive interaction pair we are exploring thermally-mediate cargo release. This platform promises programmed composition and architecture of a synthetic compartment for co-localizing or sequestering proteins within cells. The system can be used to elucidate how protein condensation cells regulate processes and has numerous applications in cellular engineering.

Molecular Control of T Cell-Based Therapeutics

Travis Young

California Institute for Biomedical Research, 11119 North Torrey Pines Road, La Jolla, CA 92037, tyoung@calibr.org

T cell-based therapies, including T cell engaging bispecific antibodies and genetically engineered chimeric antigen receptor engineered T cells (CAR-T cells), have produced remarkable results in clinical trials – achieving complete remissions in patients with hematological malignancies who failed multiple lines of prior therapy. Towards increasing the potency and safety of these therapeutics, as well as expanding them to cancers outside of the hematological space, we have defined how the biophysical characteristics of the antibody-based components of these therapies modulate the physiological response of the T cells that carry out the anti-tumor activity. For example, we have designed a “switchable” CAR-T cell system using antibody-based molecular switches. This platform enables fully tunable control of CAR-T cell activity in a universal format that can be redirected to nearly any therapeutic antigen target. The platform is expected to reduce the risk of severe adverse events that have plagued the development of CAR-T cell therapies clinically. We have demonstrated such a platform can reduce risks related to cytokine release syndrome and double as a safety switch to turn the therapy off in the case of an adverse event. We have further demonstrated the temporal control over CAR-T cell activation enables the development of robust central memory T cells. In preclinical mouse models we’ve demonstrated these central memory cells can be recalled affording on-demand, in vivo T cell expansion. These concepts are expected to be central to clinical efficacy with T cell-based therapeutics and important to ultimately achieving efficacy in solid tumors. A proof of concept clinical trial will be initiated in late 2019 for patients with lymphoma.

Artificial Gel-based Organelles for Spatial Organization of Cell-free Gene Expression Reactions

Lukas Aufinger, Friedrich C. Simmel

Physics-Department and ZNN, Technische Universität München, Am Coulombwall 4a, 85748 Garching, Germany, E-mail: simmel@tum.de

Gene expression reactions in eukaryotic cells occur in a spatially organized manner. On a subcellular level, mRNA is transcribed and processed inside the nucleus, exported to the endoplasmic reticulum, and translated into the encoded protein. In multicellular organisms, a cell's functionality is determined by spatial differentiation during embryonic development. Mimicking such spatial organization with artificial cellular systems, requires compartments that enable programmable localization of reaction components and addressable communication between them [1, 2].

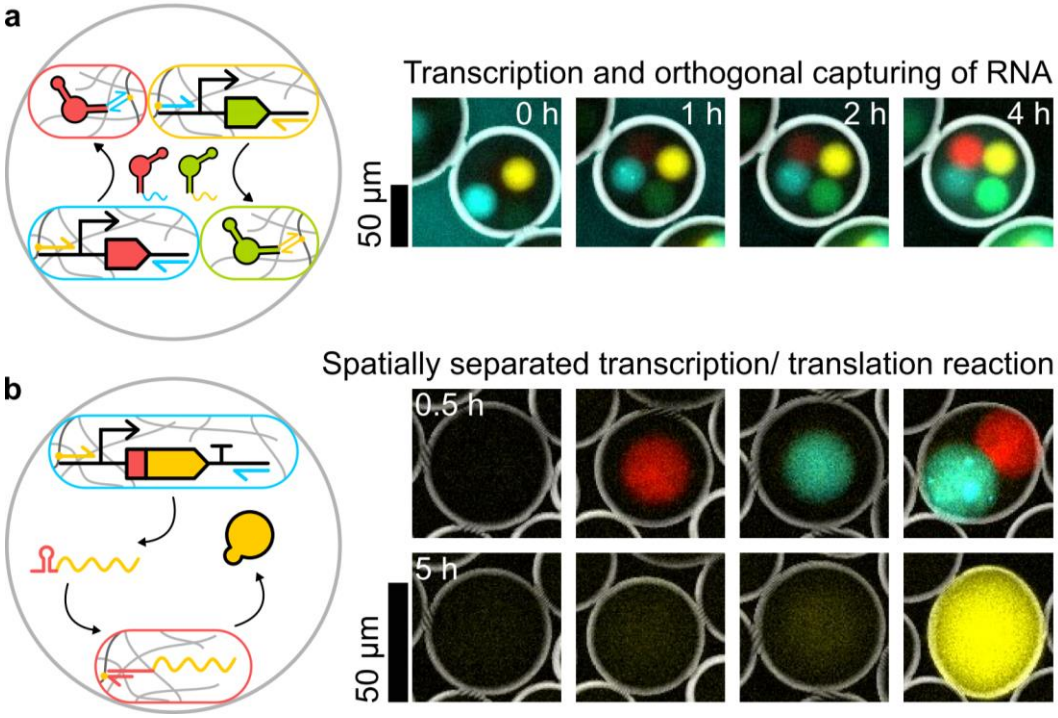


Figure: Schematics (left) and fluorescence overlay inverted bright field images (right) of transcription, capture and translation organelles. **a)** Fluorescent RNA aptamers are specifically localized in capture organelles. **b)** Expression of the reporter protein depends on the presence of transcription and translation organelles.

We therefore utilized droplet microfluidics to generate membrane-free reaction compartments [3] made of porous agarose hydrogel beads that can be functionalized with DNA molecules using copper-catalyzed alkyne-azide cycloaddition “click chemistry". In order to mimic eukaryotic cells, we then encapsulated these artificial organelles within emulsion droplets containing the PURExpress cell-free gene expression system. To assign a dedicated function to an organelle, the immobilized DNA molecules can either act as genetic templates or capture diffusing RNA molecules.

We show that RNA signals can be transcribed from transcription organelles and specifically targeted to capture organelles via hybridization to the corresponding DNA addresses (**Figure a**). We also demonstrate that mRNA molecules, produced from transcription organelles and controlled by toehold switch riboregulators [4], can be captured and activated in translation organelles containing their cognate DNA triggers (**Figure b**). Using artificial gel-based organelles we effectively separated genetic transcription and translation processes, which is similar as in eukaryotic cells, where these processes take place in the nucleus and cytosol, respectively.

More generally, our organelles can be thought of as functional modules, which can be used to compose complex cell-free synthetic biological systems, whose behavior is programmed by the composition of the organelle mixture. Importantly, due to the fact that the organelles are not bounded by membranes, they can potentially be operated for extended periods of time, which is important for the generation of more complex spatiotemporal behaviors.

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Special Track on Nanoscience for Computation

Track Chair

Andrew Turberfield

University of Oxford

Molecular Transistors

Mark A. Reed[1], Hyunwook Song[1,2]; and Takhee Lee[3]
mark.reed@yale.edu
[1]Yale University, New Haven, CT, USA
[2]Kyunghee University, Yongin, Korea
[3]Seoul National University, Seoul, Korea

Electron devices containing molecules as the active region have been an active area of research over the last few years. In molecular-scale devices, a longstanding challenge has been to create a true three-terminal device; e.g., one that operates by modifying the internal energy structure of the molecule, analogous to conventional FETs. Here we report[1] the observation of such a solid-state molecular device, in which transport current is directly modulated by an external gate voltage. We have realized a molecular transistor made from the prototype molecular junction, benzene dithiol, and have used a combination of spectroscopies to determine the internal energetic structure of the molecular junction, and demonstrate coherent transport.[2,3] Resonance-enhanced coupling to the nearest molecular orbital is revealed by electron tunneling spectroscopy, demonstrating for the first time direct molecular orbital gating in a molecular electronic device.

We further demonstrate that energetic orbital positions can be modified by appropriate endgroup and sidegroup substitutions. Modifications of endgroups allows the realization of complimentary single molecule FET devices. Systematic sidegroup substitutions of varying electronegativity allows a systematic engineering of orbital positions, analogous to threshold voltage control. The unique device characteristics that these types of molecular devices enable will be discussed.

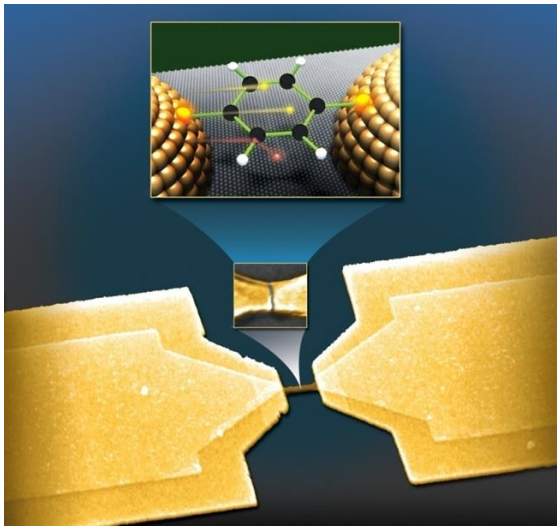

Figure 1. Schematic illustration of a single molecule transistor.

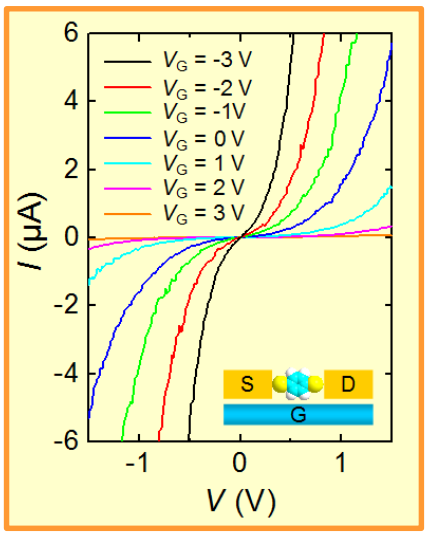

Figure 2. Transfer characteristics of a single benzene dithiol transistor.

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Track on Biomedical Nanotechnology

Track Chair

Thomas LaBean

North Carolina State University

Biomolecules for Biological (and Non-biological!) Things: Materials Construction through Peptide Design and Solution Assembly

Darrin J. Pochan, Materials Science and Engineering, University of Delaware
pochan@udel.edu

Self-assembly of molecules is an attractive materials construction strategy due to its simplicity in application. By considering peptidic molecules in the bottom-up materials self-assembly design process, one can take advantage of inherently biomolecular attributes; intramolecular folding events, secondary structure, and electrostatic interactions; in addition to more traditional self-assembling molecular attributes such as amphiphilicty, to define hierarchical material structure and consequent properties. Two classes of materials will be discussed. First, the local nano- and overall network structure, and resultant viscoelastic and cell-level biological properties, of hydrogels that are formed via beta-hairpin self-assembly will be presented. Importantly, the hydrogels do not form until individual peptide molecules intramolecularly fold into a beta-hairpin conformation. Subsequently, specific, intermolecular assembly occurs into a branched nanofibrillar network. These peptide hydrogels are potentially excellent scaffolds for tissue repair and regeneration due to inherent cytocompatibility, porous morphology, and shear-thinning but instant recovery viscoelastic properties. During assembly and gelation, desired components can be encapsulated within the hydrogel network such as drug compounds and/or living cells. The system can shear thin but immediately reheal to preshear stiffness on the cessation of the shear stress. Recent adaptation of the materials to high throughput screening will be described.

Second, a new solution assembled system comprised of theoretically designed coiled coil bundle motifs will be introduced. The molecules and nanostructures are not natural sequences and provide opportunity for arbitrary nanostructure creation with peptides. With control of the display of all amino acid side chains (both natural and non-natural) throughout the peptide bundles, desired physical and covalent (through appropriate “click” chemistry) interactions have been designed to produce one and two-dimensional nanostructures. One-dimensional nanostructures span exotically rigid rod molecules that produce a wide variety of liquid crystal phases to semi-flexible chains, the flexibility of which are controlled by the interbundle linking chemistry. The two dimensional nanostructure is formed by physical interactions and are nanostructures not observed in nature. All of the assemblies are responsive to temperature since the individual bundle building blocks are physically stabilized coiled coil bundles that can be melted and reformed with temperature. Additional, novel nanostructures to be discussed include uniform nanotubes as well as the templated growth of metallic nanoparticle on and in peptide nanostructures. Included in the discussion will be molecule design, hierarchical assembly pathway design and control, click chemistry reactions, and the characterization of nanostructure as well as inherent material properties (e.g. extreme stiffness, responsiveness to temperature and pH, stability in aqueous and organic solvents).

Genetically Encoding Functional RNA Origami: Anticoagulant

Carson Key[†], Kristen Froehlich[‡], Michael Fergione[†], Sahil Pontula[‖], Matthew Hart[∏], Pedro Carriel[‡], Cynthia Koehler[†], Abhichart Krissanaprasit[†], and Thomas H. LaBean[†]*

[†] Department of Materials Science and Engineering, College of Engineering, North Carolina State University, Raleigh, NC 27695, USA
[‡] Department of Biomedical Engineering, College of Engineering, North Carolina State University, Raleigh, NC 27695, USA
[‖] William G. Enloe High School, North Carolina State University, Raleigh, NC 27610, USA
[∏] Department of Physics, North Carolina State University, Raleigh, NC 27695, USA
**Corresponding Author: thlabean@ncsu.edu*

Abstract
Anticoagulants disrupt the coagulation cascade to prevent the formation of blood clots that can restrict blood flow to vital organs. However, current anticoagulants, such as warfarin and heparin, have a narrow therapeutic window for administration that poses a serious danger of tissue hemorrhaging as well as other adverse side effects.[1] DNA and RNA aptamers which bind to and specifically inhibit thrombin, a central enzyme in the coagulation cascade, have been developed; however, these aptamers display poor pharmacokinetics due to their small sizes and rapid renal clearance.[2-4] Here, we demonstrate a set of single-molecule RNA origami structures bearing RNA thrombin-binding aptamers that provide enhanced anticoagulant activity—seven-fold higher than free RNA aptamers. The RNA origami was designed *in silico* and produced by *in vitro* transcription as shown in Fig 1A.[5] By incorporating 2'-fluoro-modified CTP and UTP, the RNA origami was nuclease-resistant and stable in human blood plasma for at least 24 hours and retained its anticoagulant properties after three months of storage at 4°C. Additionally, we were able to rapidly reverse the anticoagulant activity of the RNA origami through the addition of complementary DNA antidote strands—giving a significant advantage over the clinical anticoagulants used currently (Fig 1B).[3] We will present results demonstrating optimization of the structure by changing the spacing and increasing the number of displayed aptamers. This is the first demonstration of functional RNA origami for a biomedical application. RNA origami tethered RNA aptamers may also find use in a broad range of applications involving other forms of nucleic acid drug delivery.

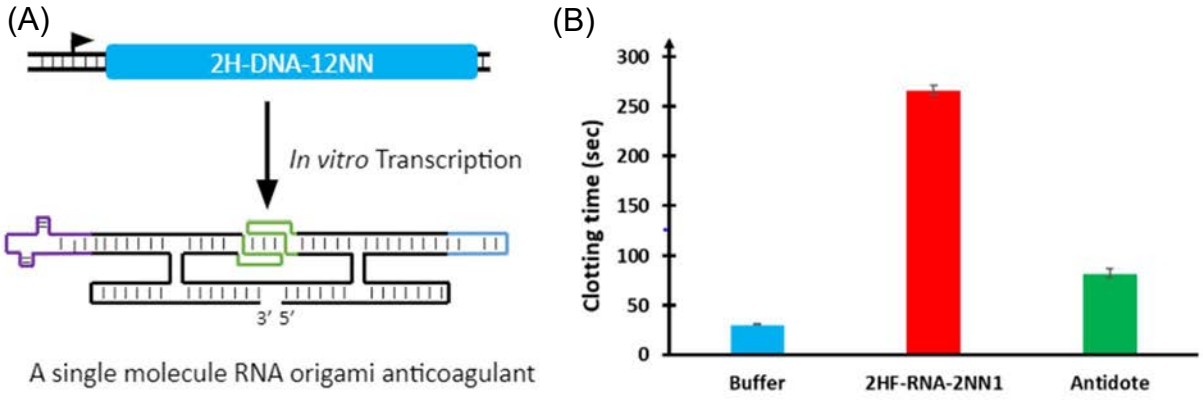

Figure 1: (A) Production of single-molecule RNA origami structures bearing RNA thrombin-binding aptamers. (B) Functional RNA origami provides enhanced anticoagulant activity which can be reversed through the addition of a nucleic acid antidote.

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DNA origami nano-tailored surfaces enhance biomolecular interactions at the biosensor surface

D. Daems[1,*], I. Rutten[1,*], J. Bath[2], A. Turberfield[2], J. Lammertyn[1,+]
[1]KU Leuven, Department of Biosystems, Biosensors group, Belgium
[2]Oxford University, Department of Physics, Clarendon laboratory, United Kingdom
[*] Authors contributed equally; [+] Corresponding author: jeroen.lammertyn@kuleuven.be.

Accurate detection of biomarkers requires robust biosensors with high specificity and sensitivity, which are two of the most important characteristics of a diagnostic tool. The performance of biosensors strongly depends on the nanoarchitecture of the biosensing surface. In many studies the bioreceptor density, orientation and accessibility by the target are often overlooked, resulting in suboptimal analysis systems with uncontrolled surface functionalization[1]. This will lead to decreased affinity of the bioreceptors, as limited bioreceptors are available due to steric hindrance, interfacial heterogeneity and suboptimal orientation[1,2].

Here, we demonstrate that by introducing DNA origami[3] to structure the biosensing interface, biomolecular interactions between bioreceptor and target can be enhanced. The designed and developed DNA origami structures, decorated with DNA aptamers, are studied as a novel tool to nanostructure the biosensing surface with nanoscale precision, favoring the affinity between target and aptamer. Different DNA nanotechnology based bioassays were developed using two microfluidic platforms, namely the innovative Evalution™ (EVA) platform[4] and an in-house developed digital microfluidic platform[5].

First, the EVA platform was used for the fluorescent detection of thrombin. The EVA is an integrated system based on 3 major components: barcoded disc-shaped microparticles to capture target molecules, a disposable microfluidic cartridge and an instrument that integrates all assay steps. Starting from a 24-helix bundle, an antenna-like DNA origami was designed to precisely position thrombin-specific aptamers[6] on the microparticles with a distance of 16.32 nm apart as the aptamer-based sandwich assay covers an area of ± 7 nm. The origami-based technology proved to be not only capable of reproducible thrombin detection (CV: 4.1 %), but revealed also a 7.8-fold increased binding potential compared to directly coupled capturing aptamers (holding 5.3-fold more bioreceptors) due to the application specific design. Furthermore, we demonstrated that DNA origami nanostructured biosensing interfaces outperformed basic aptamer coupling with respect to limit of detection (LOD: 11 × improved) and signal-to-noise ratio (SNR: 2.5 × better).

Second, a digital microfluidic platform was used for the detection of the main peanut allergen Ara h1[7]. Digital measurements were performed by subdividing the sample into a large number of smaller volumes (nL – pL), resulting in the presence or absence of a single molecule within each subvolume according to Poisson statistics. For 10 pM Ara h1, we observed only a slight increase in signal to around 11 % fluorescent microparticles for the structures with 1 - 3 aptamers attached to a 2D planar DNA origami, while a 2.4-fold

increase in signal was measured for the DNA origami with 4 aptamers compared to the setting with 5-fold more directly coupled aptamers (9.2 %). We assume that the enhanced signal could be explained by the maximal distance between the 4 aptamers (10.5 nm), promoting avidity towards the Ara h1 trimeric protein (radius ± 9 nm), next to the favored upwards orientation and enhanced kinetics by crowding effects. Furthermore, we demonstrated that DNA origami nanostructured microparticles outperformed basic aptamer coupling with respect to LOD (15 × improved) and SNR (2 × better).

In conclusion, our results highlight the potential for DNA nanotechnology to improve biomolecular interactions on biosensing platforms. The reported surface functionalization strategy provides a general approach leading to increased affinities that can be directly transferred for the detection of various target molecules. The importance of this work should be recognized as the combination of the intrinsic advantages of DNA origami to structure bioreceptors upwards with nanoscale precision, together with lowering the steric hindrance, unspecific interactions and the interfacial heterogenicity. Further research will focus on improving the sensitivity by DNA-based signal amplification techniques. We believe that these results will be of major interest to scientists and clinicians looking for new molecular insights and ultrasensitive detection of a broad range of targets.

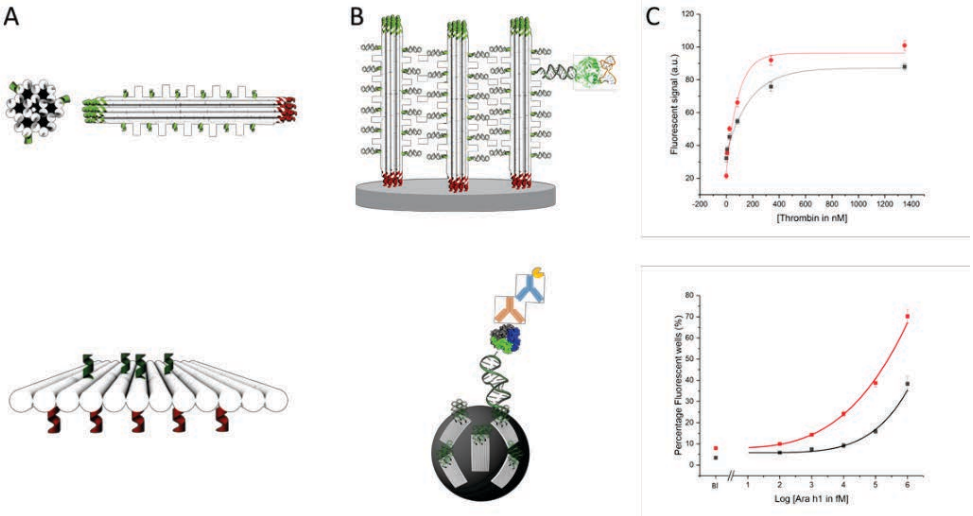

Figure 1: Schematic overview of the DNA-based microfluidic bioassays developed in this study. A) Design and development of modified DNA origami structures with strands of ssDNA for bioreceptor (green) and biosensor surface coupling (red): 24-helix bundle (above) and planar origami (bottom). B) Above: The detection of thrombin is performed in a two-step assay: (1) thrombin is captured by an aptamer (TBA2) immobilized on the barcoded disks, (2) a fluorescent secondary aptamer (TBA1) is used to detect the captured thrombin. Below: The detection of Ara h1 is performed in a three-step assay: (1) Ara h1 is captured by an aptamer immobilized on the magnetic microparticles, (2) a detection antibody is used to detect the captured Ara h1 and (3) the formed complex is labelled with an enzyme-conjugated secondary antibody generating a fluorescent signal. C) Calibration curves for specific target detection using bioreceptors coupled to the biosensing interface with (red) or without (black) DNA origami. Error bars represent one standard deviation (n = 3).

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pH responsive DNA origami nanocapsules for endosome-specific drug delivery

Frances Anastassacos[1,2,3], *Maartje M. Bastings*[1,2,3] *and William M. Shih*[1,2,3]

[1] Department of Cancer Biology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215
[2] Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115
[3] Wyss Institute for Biologically Inspired Engineering at Harvard, Boston, MA 02115

Contact email: William_Shih@dfci.harvard.edu

The therapeutic potential of DNA nanostructures is increasingly being explored, specifically in the domain of smart drug delivery vehicles. Upon endocytosis by mammalian cells, DNA origami specifically travels to the endosome. The release of drugs upon exposure to the endosome would be a very useful mechanism of cargo release that limits off-target delivery exposure to drugs.

With endosome-specific release in mind, we have developed a pH-responsive DNA origami barrel using pH-responsive i-motif DNA sequences, typically found in telomeric DNA. Under acidic conditions, the cytosines in these C-rich sequences become hemi-protonated and preferentially bind other cytosines within the i-motif. This results in the release of the complementary strand. In our structure, the equator of the barrel is lined with up to eighteen i-motifs. Using transmission electron microscopy, we observed a dependence on the kinetics of barrel opening on the number and length of the i-motif sequences present. After optimizing our design, we were able to switch 85% of the barrels to an open-state after 5 minutes of incubation at pH 4.5. Furthermore, we coated our DNA origami with a protective oligolysine-PEG5K layer for downstream survivability *in vivo*. We studied the influence of the coating on the kinetics of acid-induced barrel opening and found only small differences in opening kinetics.

Our plan is to use this barrel as the central component of a large nanocapsule formed through hierarchical assembly of five individual DNA origami components. We plan to evaluate the ability of the nanocapsule to open and release cargos in a pH-responsive manner *in vitro* and subsequently in mammalian cells.

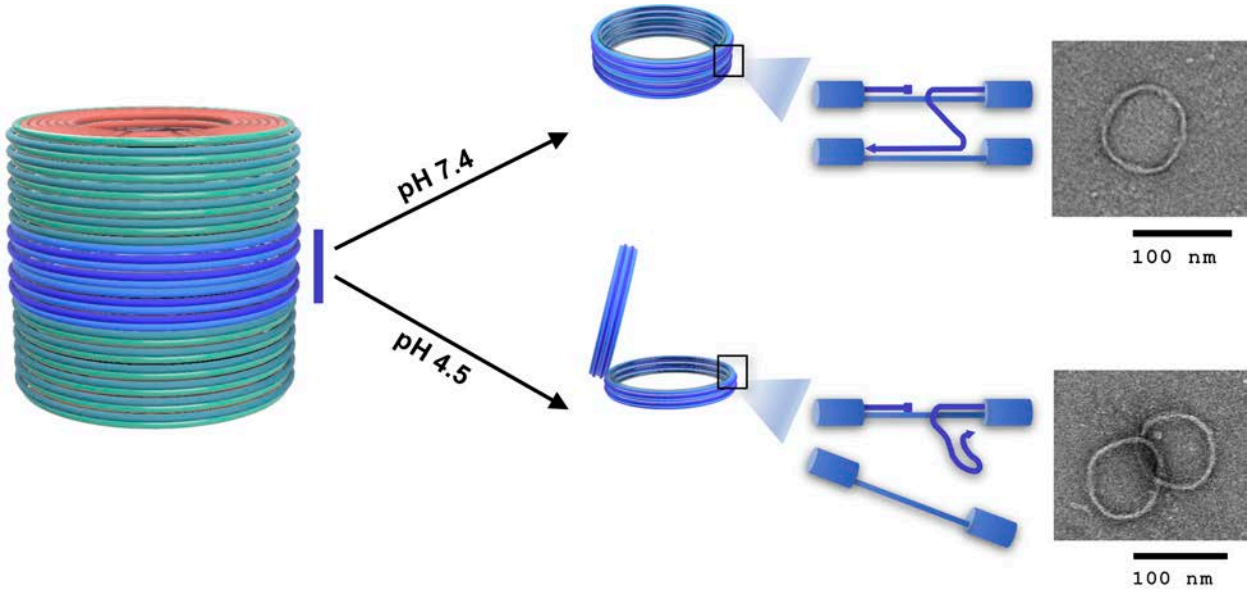


Fig 1. Schematic of the DNA origami nanocapsule (left). The central barrel component of the structure remains closed on incubation at neutral pH, whereas incubation at pH 4.5 results self-binding of the i-motif strands and subsequent release of the lower half of the barrel. These two states are distinguishable under negative stain transmission electron microscopy (right).

Track on Protein and Viral Nanostructures

Track Chair

Nicole Steinmetz

University of California – San Diego

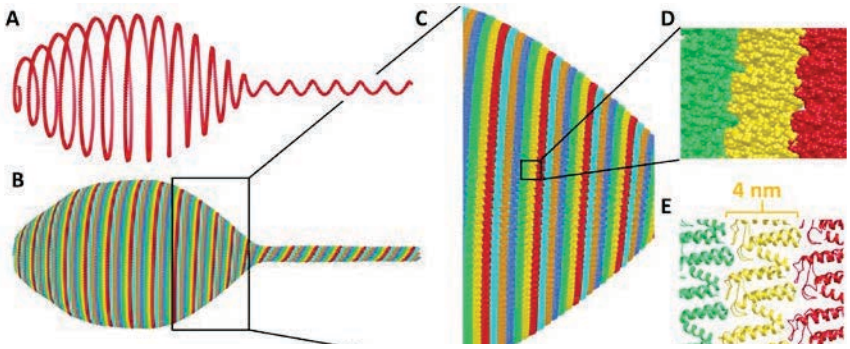
Easy Peasy Lemon Squeezy; *Acidianus* Tailed Spindle Virus Provides a New Paradigm for Viral Capsid Architecture and Genome Delivery

Rebecca Hochstein[a,d], Mark Young[b,d] and Martin Lawrence[c,d]

E-mail: lawrence@montana.edu

Departments of [a]Microbiology and Immunology, [b]Plant Sciences and Plant Pathology, [c]Chemistry and Biochemistry, and the [d]Thermal Biology Institute, Montana State University, Bozeman, MT 59717 USA

The spindle- or lemon-shaped virion morphology is common among archaeal viruses, where it is a defining characteristic of many viral families. However, structural heterogeneity intrinsic to spindle-shaped viruses has seriously hindered efforts to elucidate the molecular architecture of these lemon-shaped capsids (1, 2). We have utilized a combination of cryo-electron microscopy and X-ray crystallography to study *Acidianus* tailed spindle virus (ATSV). These studies reveal the architectural principles that underlie assembly of a spindle-shaped virus (3). Cryo-electron tomography shows a smooth transition from the spindle-shaped capsid into the tubular-shaped tail and allows low resolution structural modeling of individual virions. Remarkably, higher dose 2D micrographs reveal a helical surface lattice in the spindle-shaped capsid. Consistent with this, crystallographic studies of the major capsid protein reveal a decorated four-helix bundle that packs within the crystal to form a four-start helical assembly with structural similarity to the tube-shaped tail structure of ATSV and other tailed, spindle-shaped viruses. Combined, this suggests that the spindle-shaped morphology of the ATSV capsid is formed by a multi-start helical assembly with a smoothly varying radius and allows construction of a pseudo-atomic model for the lemon-shaped capsid that extends into a tubular tail (3).



A pseudo-atomic model for ATSV. A) A single helical strand of the major structural protein covering the lemon-shaped capsid and cylindrical tail. B) A complete 6-start helical model. C) An enlarged view of the section indicated in panel B. D) An enlarged view of the space filling pseudo-atomic model indicated in panel D, showing three adjacent helical strands with an apparent pitch of 4 nm. E) A ribbon representation of the model indicated in panel D, with a 4 nm pitch between adjacent strands. The complete capsid is composed of ~ 10,000 copies of the major capsid protein, with a mas of 160 mega-Daltons.

Studies of spindle shaped viruses from our lab and others also demonstrate remarkable extracellular development of the virus. They are commonly observed to exit the cell as lemon-shaped particles that lack tail structure. In the extracellular milieu, they then grow tails at one or both ends of the lemon-shaped capsid, with a concomitant decrease in the diameter of the lemon-shaped capsid (4-6), suggesting flow of the major capsid protein

into the tails (1, 3). In addition, internal pressures of 10 atm have been measured for halophilic spindle-shaped capsids, indicating the packaged DNA can be under substantial pressure in these lemon shaped viruses (7, 8). Further, for the halophilic spindle-shaped viruses, exposure to detergents or heat results in DNA ejection and complete conversion to empty cylindrical structures (8, 9). Combined, these studies on ATSV and other spindle-shaped viruses suggest their lemon-shaped capsids are metastable structures capable of a spectacular transition from an underwound lemon-shaped capsid to a more tightly wound and stable cylindrical assembly. As the virus matures, tail growth reduces the volume of the capsid, compressing the viral genome, reaching pressures as high as 10 atm. When the pressurized capsid encounters a new host, the container is uncorked, and the pressure provides the initial force for genome ejection. Uncorking, partial genome injection and subsequent depressurization, in turn, further transition to the cylindrical structure, completing of genome injection. Thus, the transition from the meta-stable lemon shape to the lower energy cylinder provides the driving force for genome ejection (3), and in this light, the tailed spindle-shaped morphology of the mature viral capsid is an intermediate in this transition. Finally, the unique properties of this containerized system might be harnessed for bio-nanotechnology purposes.

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Title: Designing Virus Capsids for Controllable Therapeutic Delivery
Presenter: Junghae Suh

Abstract: Viruses are nature's nanomachines that already conduct amazing feats of biomolecular computation – they have evolved to recognize specific biomolecular inputs and produce functional outputs critical to the infection of their host organisms. Viruses have been harnessed over the last several decades as delivery vectors for a variety of biomedical applications. In particular, the adeno-associated virus (AAV) has come to the forefront as a leading delivery vector for human gene therapy. In December 2017, the US FDA approved an AAV-based gene therapy for the treatment of inherited blindness – the first of such therapies in the US. Despite tremendous advances, there are still a number of improvements to make in regards to vector efficiency, specificity, and controllability. To make virus-based delivery a more predictable process, we must obtain control over the naturally encoded biomolecular programs already embedded in the viral capsids. Our lab has investigated ways to rewrite the details of what cues can be accepted as inputs and what functional outputs can be produced by the capsids. I will discuss how virus capsids can be designed to compute different aspects of their environment and to use this information to decide whether or not they perform a user-programmed output.

.

Virus and DNA Origami Directed Nanoparticle Superlattices

Ville Liljeström[1], Veikko Linko[1], Sofia Julin[1], Mauri. A. Kostiainen[1]

[1] Department of Bioproducts and Biosystems, Aalto University, Espoo, Finland, mauri.kostiainen@aalto.fi

Material properties depend critically on the packing and order of constituent units throughout length scales. Structure of colloidal assemblies is strongly affected by the assembly process, and higher structural control can be reliably achieved only if the process is deterministic.[1] Here we show that the well-defined shape of virus particles and DNA origami nanostructures can be used to direct the formation of nanoparticle superlattices.

The self-assembly of cationic spherical gold nanoparticles and anionic rod-like tobacco mosaic viruses yields well-defined binary superlattice wires (Fig. 1a). The superlattice structures are explained by a cooperative assembly pathway that proceeds in a zipper-like manner after nucleation. Curiously, the formed superstructure shows right-handed helical twisting due to the right-handed structure of the virus. This leads to structure-dependent chiral plasmonic function of the material.[2]

Furthermore, we show that the negatively charged DNA origami surface enables the assembly 6-helix bundle DNA origami and cationic gold nanoparticles into well-ordered 3D tetragonal superlattices (Fig. 1b). Further, the results reveal that shape and charge complementarity between the building blocks are crucial parameters for lattice formation.[3]

Our systematic approach identifies the key parameters for the assembly process (ionic strength, electrolyte valence, pH) and correlates the effect of the size and aspect ratio of the building particles with the achieved crystal structure and lattice constant. Results highlight the importance of well-defined colloidal units when pursuing unforeseen and complex assemblies.

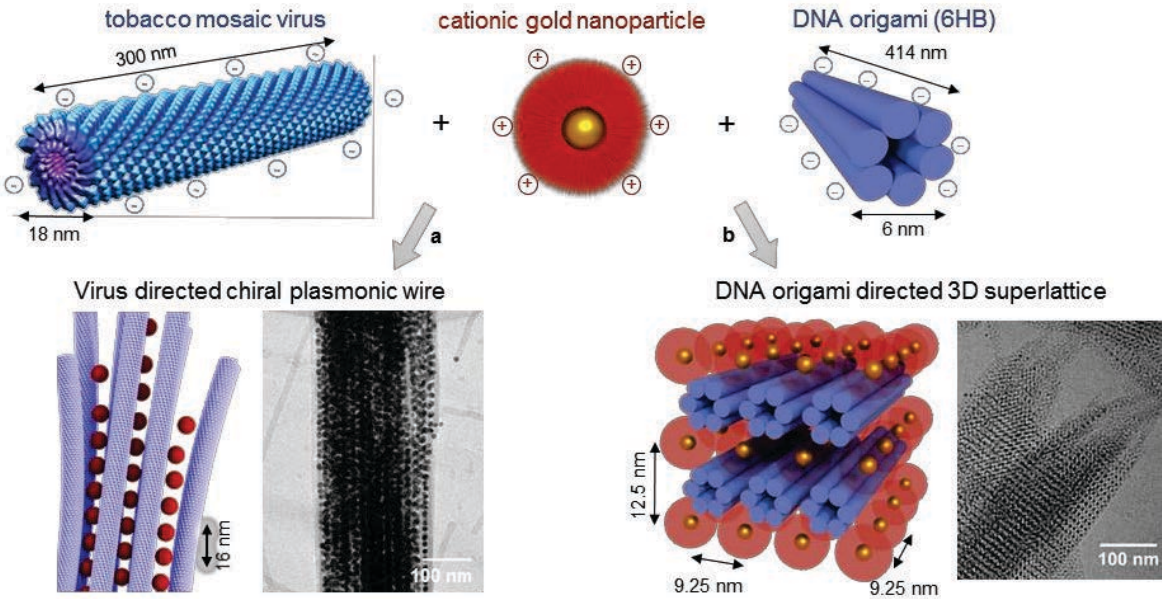


Fig. 1. Electrostatic self-assembly of biomolecule directed superlattices.

[1] *Nature Nanotech.*, **8**, 52-56 (2013) [2] *Nature Comm.*, **8**, 671 (2017) [3] Submitted (2019)

Track on DNA Nanostructures II: Semantomorphic Science B

Track Chair

Nadrian Seeman

New York University

Using the Fluorous Effect to Control the Immobilization and Orientation of DNA and DNA Nanostructures

Gabriella E. Flynn,[a] Jamie M. Withers,[b] Andrea Taladriz Sender,[b] Jiajia Zou,[a] Justin Sperling,[a] Sarah L. Henry,[a] Gerard Macias,[a] Jonathan M. Cooper,[a] Glenn A. Burley,[b] Alasdair W. Clark[a]
Alasdair.clark@glasgow.ac.uk.

[a.] *School of Engineering, Rankine Building, University of Glasgow, Glasgow, UK.*
[b.] *WestCHEM & Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow, UK.*

The immobilization of DNA nanostructures onto solid supports in pre-defined positions is crucial if DNA is to be applied to the self-assembly of electronic and photonic systems. As such, many methods of attachment have been developed, most of which rely on electrostatic or strong covalent interactions. However, this often requires unfavorable conditions, such as high Mg^{2+} concentration, which can lead to the aggregation of carbon nanotubes, metal nanoparticles and other inorganic 'building materials'. Furthermore, it often results in poor control over the 2D orientation of the origami as they are quickly fixed to the surface with no option for *in situ* realignment. Here, we propose a new method for controlling the surface-immobilization and orientation of DNA and DNA nanostructures using the non-covalent fluorous effect.

The fluorous effect refers to the observation that highly fluorinated or perfluorinated compounds have a tendency to exclude themselves from both aqueous and organic phases. Molecular tagging with fluorous "ponytails" has the effect of drawing compounds into fluorous layers. As such, "fluorous affinity" tags have been used as an effective means of immobilizing carbohydrates and peptides on surfaces; techniques we have now applied to DNA and DNA nanostructures.

Here we demonstrate that the DNA can be reversibly immobilized using the fluorous effect, allowing us to immobilize, regenerate and the *re-immobilize* DNA on nano and micro-patterned surfaces, **Figure 1.**
We go on to show that the fluorous effect can be applied to DNA nanostructures in such a way that allows us to control the facial orientation of the nanostructures when immobilized on solid substrates, **Figure 2**. Notably, this technique requires only that we modify the nanostructure with fluorous ponytails, *not the surface.* As a result we can build facial-orientation control into DNA nanostructures and apply them to unmodified surfaces; control that is crucial if DNA nanostructures are to be used as engineering platforms, since essential spatial information presented on one side of a 2D nanostructure will be sequestered if immobilization occurs 'face-down'.

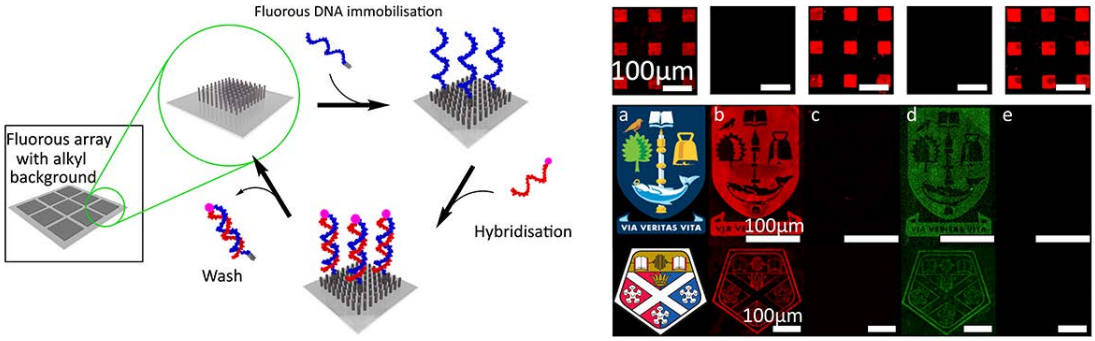


Figure 1. (Left) *Schematic showing the immobilization of a fluorous-tagged ssDNA onto fluorous surface-regions, hybridisation with complimentary ssDNA, and regeneration using a solvent wash.* ***(Right)*** *Reversible surface-patterning. Fluorescence microscopy showing immobilization, hybridisation, removal, and re-immobilization using the same fluorous micro-patterned surfaces.*

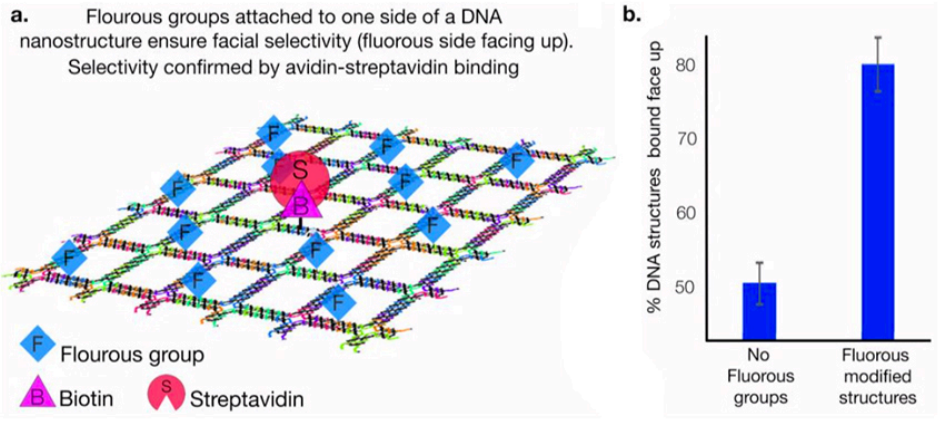


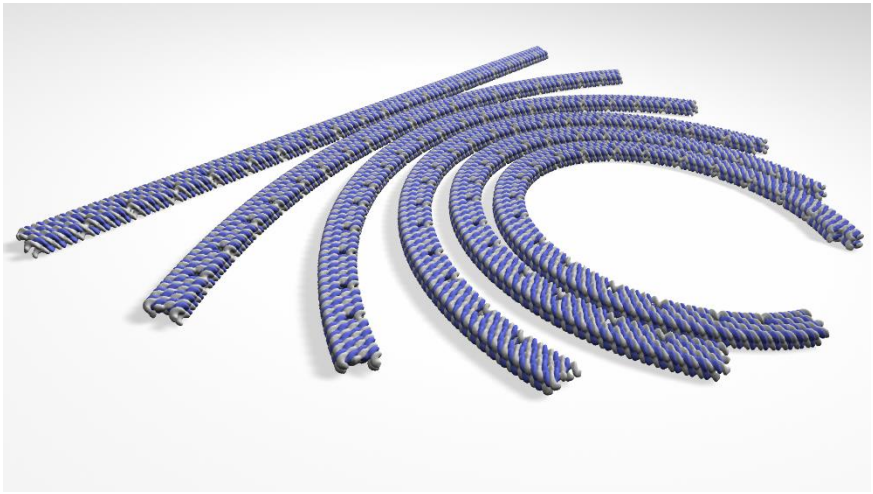
Figure 2. (a) *Schematic showing position of 12 fluorous groups and one central biotin on DNA origami structure. DNA structures were incubated with streptavidin, which is easily visible by AFM.* ***(b)*** *Following immobilisation AFM was used to characterise the orientation of the nanostructures. Fluorous-modified structures were significantly more likely to immobilize 'face-up' compared to un-modified structures.*

Large deformation of a linear DNA origami beam via cumulative actuation of tension-adjustable modules

Yuki Suzuki,[a,b] *Kohei Mizuno*,[b] *Ibuki Kawamata*,[b] *Satoshi Murata*[b]

[a] *Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, Japan*
[b] *Department of Robotics, Graduate School of Engineering, Tohoku University, Japan*
E-mail: ysuzuki79r@molbot.mech.tohoku.ac.jp

The natural muscle undergoes large deformations upon its contraction/relaxation through the cumulative action of the basic mechanical units. Inspired by this cumulative effect seen in biological systems, we herein designed a linear DNA origami beam that consists of repeats of a tension-adjustable module, the cumulative actuation of which results in a large deformation of the entire shape into an arched shape. We demonstrate that the degree of deformation is systematically controlled by replacing only a set of strands that is required for actuation of the module. Moreover, by employing the G-quadruplex-forming sequence for the actuation, we realized K^+-induced reversible contraction and relaxation of the origami structure. The adjustability and expandability of our design provide a versatile approach to design DNA nanostructures that exhibit a large deformation in response to an external stimulus.



Schematic illustration of a series of arched DNA origami structures deformed from a linear shape.

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DNA templates and building blocks for bioinspired molecular assemblies

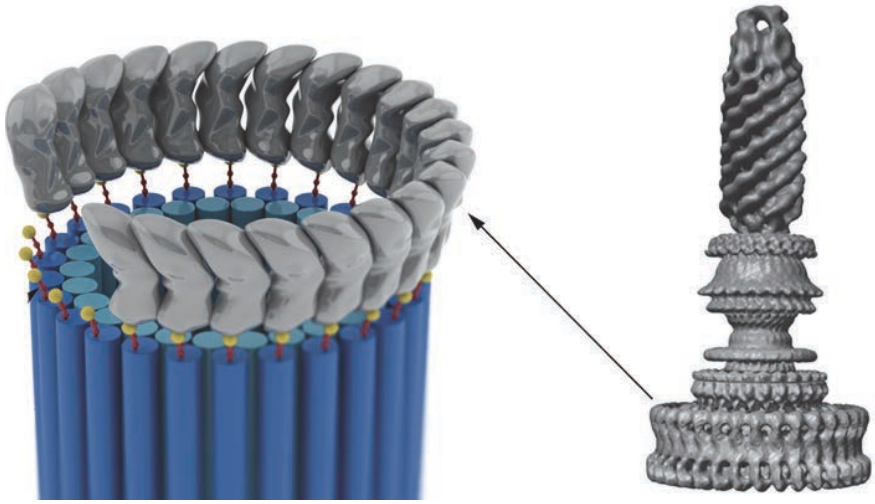
Andrew J Tuckwell, Jonathan F Berengut, Matthew AB Baker, Rokiah G Alford, Sophie Hertel, Chu Wai Liew, Stephanie Xu, Sam E Tusk, Joel Spratt, Keiichi Namba, Richard M Berry, Andrew J Turberfield and Lawrence K Lee

Most proteins tend to function in highly organised and dynamic protein self-assemblies that convert chemical interactions into complex biological phenomena. To understand nature's molecular design rules, we try to control the self-assembly of complex protein machines using DNA templates as structural scaffolds.

Our interest in constructing soluble DNA templates led us to characterise the shape and dimensions of single-layered DNA origami in solution with small-angle X-ray scattering (SAXS). This demonstrated how their shape was extremely sensitivity to crossover periodicity and established methods to measure absolute dimensions of DNA origami structures in solution. We also devised a general approach for designing minimally-strained DNA origami nanotubes with arbitrary internal angles. This allows for novel pleated nanotubes that can be constructed with a continuous range of diameters and where the inner wall can be formed from circular arrays of adjoining parallel helices. The ends of these nanotubes were used as circular scaffolds to mediate the artificial assembly of biomolecular machines to probe their structure and dynamics.

DNA origami structures were also used as components in synthetic systems designed to mimic complex biomolecular phenomena. These include the paradox of stability vs exchange; where some biomolecular assemblies remain stable in isolation yet turnover or exchange components when freely diffusing subunits are available. As well as the formation of linear self-limiting polymers.

This bottom-up approach provides a rigorous test of theoretical models and useful tools to gain new insight into the biomolecular world.



Track on Chemical Systems

Track Chair

Jeremiah Gassensmith

University of Texas - Dallas

Supramolecular Polymers for High Energy Density Rechargeable Batteries

Ali Coskun

Department of Chemistry, University of Fribourg,
Chemin du Musée 9, 1700 Fribourg, Switzerland
Email: ali.coskun@unifr.ch

Three high-energy density electrode materials – namely, silicon (Si) anodes, lithium (Li) metal anodes, and sulfur cathodes operating by alloying, electroplating, and electrochemical reactions, respectively – have gained discernable interest owing to their unparalleled theoretical capacity. Nevertheless, these electrode materials entail new intrinsic drawbacks such as massive volume change for Si, uncontrollable lithium dendritic growth for Li metal, and the formation of soluble lithium polysulfides as well as their shuttling for sulfur cathodes. Accordingly, supramolecular chemistry and/or mechanically interlocked molecules/polymers such as rotaxanes and/or supramolecular polymer networks can play a pivotal role to address these challenges facing rechargeable batteries. In this presentation, the concepts of supramolecular chemistry and their working principles in high energy density electrode materials in Li-ion batteries will be discussed.

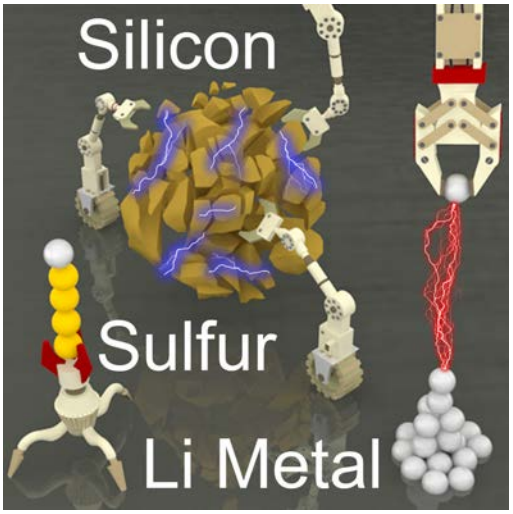


Figure 1. Graphical representation of supramolecular approaches in high energy density Li-ion batteries

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Versatile perfluorocarbon nanoemulsion theranostics stabilized by poly(2-oxazoline) amphiphiles

Ellen M. Sletten, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA, USA 90095
sletten@chem.ucla.edu

Nanomaterials which sequester, protect, and delivery therapeutics are necessary to combat complex diseases. The Sletten Group employs perfluorocarbon (PFC) nanoemulsions as a core delivery scaffold. PFC emulsions are non-toxic, dynamic nanomaterials that have been previously employed as oxygen carriers and contrast agents. We leverage the orthogonal nature of the fluorous phase to, in one step, prepare multifunctional nanomaterials with fluorous-tagged therapeutics loaded on the inside and targeting agents on the outside (Figure 1). A critical component of PFC nanoemulsions is the surfactant, which dictates the size, stability, and surface chemistry of the droplets. This talk will discuss the development of poly(2-oxazoline) amphiphiles that allow for the stabilization, surface functionalization, and triggered disassembly of PFC nanoemulsions. Applications of PFC nanoemulsions toward photodynamic therapy and chemotherapeutic delivery will be highlighted.

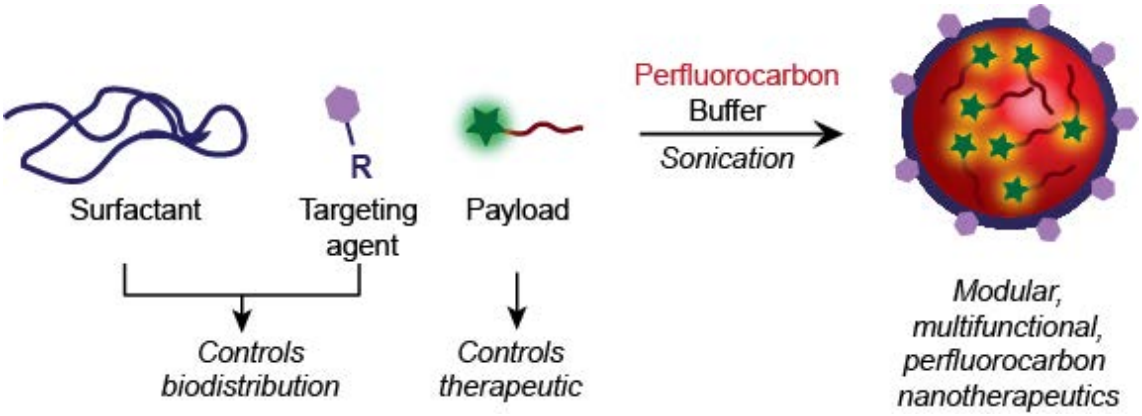

Figure 1. Assembly of functional perfluorocarbon nanoemulsions. The droplets are stabilized by custom surfactants that can have targeting agents attached to control the biodistribution. Payloads can be sequestered to the center of the nanoemulsions through that attachment of fluorous tags. The choice of payload dictates the therapeutic or diagnostic.

SUPRAMOLECULAR POLYMERIZATION DRIVEN BY CHEMICAL FUELS

Thomas HERMANS[1]

[1]Université de Strasbourg, CNRS, 8 allée Gaspard Monge, 67000 Strasbourg, France

hermans@unistra.fr

Looking at nature, we see that living materials with biological functionality, such as the actin or microtubule (MT) cytoskeletal network, achieve dynamics as well as supramolecular structures with the same protein building blocks. In other words, the components can assemble, but also react (i.e., tubulin is also an enzyme that hydrolyses guanosine triphosphate GTP), which in turn affects the assemblies. In this way, living systems use chemical fuels and self-assembly to create a built-in chemomechanical interaction. Moreover, such networks operate at the onset of oscillations,[1–3] which results in rapid response and adaptivity. In the case of MTs, oscillations stem from auto-amplification of MT production by higher concentrations of MTs due to their nucleation–elongation self-assembly mechanism.[4] Negative (delayed) feedback comes from GTP to GDP hydrolysis that creates mechanical stress build-up in the MT leading to sudden bursting called “catastrophes”. Here, we present several systems where chemically fueled artificial supramolecular polymers can be kept in out-of-equilibrium steady states.[5–7] In certain regimes, supramolecular size oscillations are obtained, traveling polymerization fronts can be formed, and large-scale dissipative patterns have been found. Dissipative structures in the Prigogine-sense can be made in this way, where self-assembly is coupled to large scale transport phenomena.

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Track on Nucleic Acid Nanostructures in Vivo

Track Chair

Yamuna Krishnan

University of Chicago

Designer DNA nanodevices to probe endocytic pathways

Ludger Johannes[1], Dhiraj Bhatia[1], Christian Wunder[1], Cesar Augusto Valades-Cruz[1], Yamuna Krishnan[2]*

*[1)] Institut Curie, Cellular and Chemical Biology, U1143 INSERM, UMR3666 CNRS, 26 rue d'Ulm, 75248 Paris Cedex 05, France; [2)] Department of Chemistry, The University of Chicago, 929 E, 57th St., Chicago, Illinois 60637, USA; * ludger.johannes@curie.fr*

Abstract:

Multiple pathways co-exist for endocytic uptake of extracellular cargoes into cells (Doherty & McMahon, 2009). How the dynamic equilibrium between endocytic pathways is regulated and how cargoes are sorted into different endocytic carriers have been long-standing questions in the field of cellular membrane biology. We have characterized molecular mechanisms and functions of uptake processes that operate in a complementary manner to the conventional clathrin pathway (Ewers, Römer et al., 2010, Lakshminarayan, Wunder et al., 2014, Renard, Simunovic et al., 2015, Römer, Berland et al., 2007, Römer, Pontani et al., 2010, Simunovic, Manneville et al., 2017), and have suggested the glycolipid-lectin (GL-Lect) hypothesis on the clathrin-independent construction of endocytic pits by the lectin-mediated co-clustering of glycosylated proteins and lipids. Yet, in-depth understanding of such clathrin-independent uptake processes has been severely hindered due to the lack of precise tools in terms of labeling endocytic ligands and following their intracellular trafficking at high spatio-temporal resolutions.

Structural DNA nanotechnology explores various structural and functional properties of DNA to manipulate matter at nanoscale for diverse applications (Modi, Bhatia et al., 2010). Three-dimensional architectures based on DNA polyhedra have raised particular interest in biomedical applications. DNA polyhedra possess an internal void bounded by a well-defined three-dimensionally structured surface (Bhatia, Surana et al., 2011). I will present the first successful delivery of quantum dots as the internal payload of DNA icosahedra that are monofunctionalized with specific endocytic ligands such as galectin-3, Shiga toxin B-subunit (Bhatia#, Arumugam# et al., 2016), or transferrin. Using advanced lattice light sheet imaging and our ligand-functionalized DNA-caged quantum dots, we dynamically dissect the construction of endocytic sites in live cells and at single molecule resolution. Thus, quantum dot-loaded DNA polyhedra bearing ligands of unique stoichiometry represent a new class of high-precision molecular imaging tools to quantitatively dissect multiple pathways and processes in living cells.

Similarly, magnetic nanoparticles (ferro-fluid) were encapsulated with endocytic ligand-functionalized DNA cages. These magnetic tools were explored to purify galectin-3-containing clathrin-independent carriers and transferrin-containing clathrin-coated vesicles. Quantitative mass-spectrometry revealed pathway-specific and common cargo molecules and trafficking factors. We have validated key cargoes and trafficking factors involved in the biogenesis of clathrin-independent carriers.

Our multidisciplinary approach using DNA nanotechnology, advanced imaging tools, and cellular biochemistry has significantly furthered our understanding of formation of distinct

populations of endocytic pits Further, our results highlight the emerging potential of DNA devices in cell biology and biomedical applications that could enable probing and programming of various biological systems (Bhatia, Wunder et al., in press).

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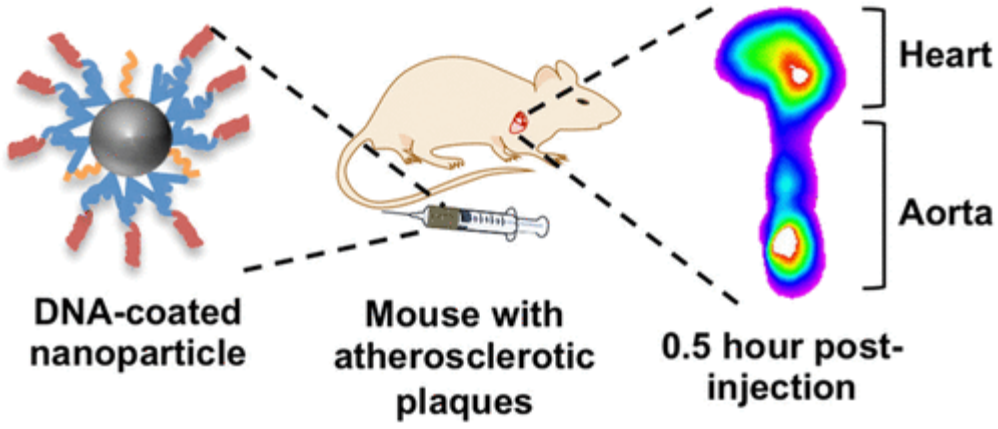
Promoting the delivery of nanoparticles to atherosclerotic plaques by DNA coating

C. H. Jonathan Choi (Email: jchchoi@cuhk.edu.hk)

Department of Biomedical Engineering, The Chinese University of Hong Kong

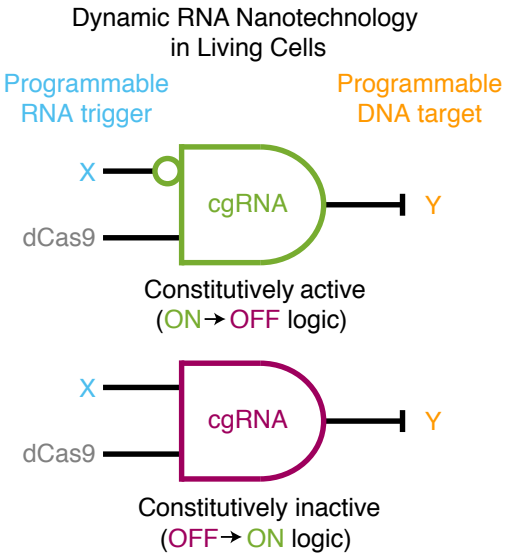
Many nanoparticle-based carriers to atherosclerotic plaques contain peptides, lipoproteins, and sugars, yet the application of DNA-based nanostructures for targeting plaques remains infrequent. In this work, we demonstrate that DNA-coated superparamagnetic iron oxide nanoparticles (DNA-SPIONs), prepared by attaching DNA oligonucleotides to poly(ethylene glycol)-coated SPIONs (PEG-SPIONs), effectively accumulate in the macrophages of atherosclerotic plaques following an intravenous injection into apolipoprotein E knockout (ApoE–/–) mice. DNA-SPIONs enter RAW 264.7 macrophages faster and more abundantly than PEG-SPIONs. DNA-SPIONs mostly enter RAW 264.7 cells by engaging Class A scavenger receptors (SR-A) and lipid rafts and traffic inside the cell along the endolysosomal pathway. ABS-SPIONs, nanoparticles with a similarly polyanionic surface charge as DNA-SPIONs but bearing abasic oligonucleotides also effectively bind to SR-A and enter RAW 264.7 cells. Near-infrared fluorescence imaging reveals evident localization of DNA-SPIONs in the heart and aorta 30 min post-injection. Aortic iron content for DNA-SPIONs climbs to the peak (~60% ID/g) 2 h post-injection (accompanied by profuse accumulation in the aortic root), but it takes 8 h for PEG-SPIONs to reach the peak aortic amount (~44% ID/g). ABS-SPIONs do not appreciably accumulate in the aorta or aortic root, suggesting that the DNA coating (not the surface charge) dictates in vivo plaque accumulation. Flow cytometry analysis reveals more pronounced uptake of DNA-SPIONs by hepatic endothelial cells, splenic macrophages and dendritic cells, and aortic M2 macrophages (the cell type with the highest uptake in the aorta) than PEG-SPIONs. In summary, coating nanoparticles with DNA is an effective strategy of promoting their systemic delivery to atherosclerotic plaques.



Conditional Guide RNAs: Programmable Conditional Regulation of CRISPR/Cas Function in Bacteria via Dynamic RNA Nanotechnology

Mikhail H. Hanewich-Hollatz[†‡], Zhewei Chen[†‡], Jining Huang[‡], Lisa M. Hochrein[‡], and Niles A. Pierce[‡,§,¶,]*

[‡]Division of Biology & Biological Engineering and [§]Division of Engineering & Applied Science, California Institute of Technology, Pasadena, CA 91125, USA
[¶]Weatherall Institute of Molecular Medicine, University of Oxford, Oxford OX3 9DS, UK
[†]Authors contributed equally
**Corresponding author: niles@caltech.edu*



A guide RNA (gRNA) directs the function of a CRISPR protein effector to a target gene of choice, providing a versatile programmable platform for engineering diverse modes of synthetic regulation (edit, silence, induce, bind). However, the fact that gRNAs are constitutively active places limitations on the ability to confine gRNA activity to a desired location and time. To achieve programmable control over the scope of gRNA activity, here we apply principles from dynamic RNA nanotechnology to engineer conditional guide RNAs (cgRNAs) whose activity is dependent on the presence or absence of an RNA trigger. These cgRNAs are programmable at two levels, with the trigger-binding sequence controlling the scope of the effector activity and the target-binding sequence determining the subject of the effector activity. We demonstrate molecular mechanisms for both constitutively active cgRNAs that are conditionally inactivated by an RNA trigger (ON→OFF logic) and constitutively inactive cgRNAs that are conditionally activated by an RNA trigger (OFF→ON logic). For each mechanism, automated sequence design is performed using the reaction pathway designer within NUPACK to design an orthogonal library of three cgRNAs that respond to different RNA triggers. In *E. coli* expressing cgRNAs, triggers, and silencing dCas9 as the protein effector, we observe programmable conditional gene silencing with a median dynamic range of ≈6-fold for an ON→OFF "terminator switch" mechanism, ≈15-fold for an ON→OFF "splinted switch" mechanism, and ≈3.6-fold for an OFF→ON "toehold switch" mechanism; the median crosstalk within each cgRNA library is <2%, <2%, and ≈20% for the three mechanisms. By providing programmable control over both the scope and target of protein effector function, cgRNA regulators offer a promising platform for synthetic biology.

A multiscale approach to investigating cell signaling *in vivo* using DNA origami and colloidal hydrogels

Cynthia Koehler, Dan Chester, Ashley Brown[1], Thomas LaBean[2]
[1]aecarso2@ncsu.edu, [2]thlabean@ncsu.edu

Nanoscale patterning of surfaces is a high potential application of DNA nanotechnology. The use of DNA origami to achieve this goal in an *in vivo* setting has direct applications in the exploration of cell signaling processes. It is known that substrate elasticity directs many cellular responses, such as attachment and migration; however, this direction of investigation is far from straightforward. Most biological tissues possess non-linear elastic properties with time-dependent deformation in response to cellular forces, and it is likely that there is significant interplay between these properties and directed cell clustering. Few model material platforms with non-linear elastic properties have been created and characterized, let alone those which display time-dependent deformation or directed clustering. Given the broad scale of properties required to cellular processes, it is difficult to probe numerous aspects of cell signaling. This project addresses this problem through a multiscale material system with tunable elastic and viscoelastic properties that can be precisely patterned via DNA origami. This enables the examination of the processes involved in myofibroblastic adhesion, spreading, migration, and differentiation. This is done via a composite nanogel thin film/DNA origami structure to organize and present TGFβ to live cells.

TGFβ is a multifunctional cytokine that performs several cellular functions, including the control of cell growth, differentiation, and proliferation. Its performance is impacted by non-linear elastic behavior in substrates; additionally, previous studies have shown that its performance can be impacted through clustering of its ligands, as shown by the use of a DNA origami clustering tool. To create a multiscale system capable of interrogating the interplay between these factors, we combine a DNA origami tool with acrylated colloidal hydrogels that have tunable elastic properties. TGFβ expression will then be monitored to gauge modification of the cell signaling pathway.

The origami consist of staple strand modifications at discrete sites of the tall rectangle to allow covalent conjugation between aminated anchor oligonucleotides and the acrylated colloidal hydrogels. An affinity peptide that conjugates to separate discrete sites on the origami encourages cell clustering. A combination of atomic force microscopy, various fluorescence methods, and 24 hour live cell assays are used to characterize this system. It is anticipated that TGFβ expression will increase in the presence of pre-patterning with DNA origami. This could potentially enable meaningful advances in the development of wound healing treatments, as well as advances in the characterization of composite polymer film/DNA origami structures.

Track on Molecular Machinery

Track Chair

Andrew Turberfield

University of Oxford

Active molecular sensors built with DNA nanotechnology

Ulrich F. Keyser

Cavendish Laboratory, University of Cambridge, JJ Thomson Ave. Cambridge, UK

DNA nanotechnology is transformative for experiments that require molecular control over the shape of nanometer-sized objects. This presentation will provide an overview of novel approaches enabled by DNA based self-assembly.

Nanopore sensing, best known for DNA sequencing, translates the three-dimensional structure of molecules into ionic current signals. Designed DNA molecules enable multiplexed protein sensing with an all electrical approach [1]. The structured DNA carriers also give insights into polymer physics [2] and may pave the way to data storage applications [3].

DNA-origami nanopores can enhance the functionality of solid-state nanopores, are easily combined with fluorescence microscopy [4] and consequently allow for the creation of an all-optical voltage sensor based on Foerster resonant energy transfer [5]. Our DNA origami nanopores are ideally suited for enhanced optical sensing with unprecedented control over the exact arrangement of molecules in plasmonic nanocavities [6].

DNA origami nanopores can be transformed into ion channels via hydrophobic modifications. Our artificial DNA systems span orders of magnitude in molecular weight from single helices to large porins [7,8] and show voltage-activated characteristics similar to ion channel gating. A combination of experiments and molecular simulations show that DNA ion channels act as enzymes that allow mixing of lipids between different leaflets of bilayers [9]. In an outlook, the incorporation of PNIPAM into DNA origami enables the temperature activated motion of DNA based structures [10] offering an attractive alternative for controlled actuation.

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Directional control of a molecular hopper

*Yujia Qing, Sandra A. Ionescu, Gökçe Su Pulcu, Hagan Bayley**

Department of Chemistry, University of Oxford, Oxford OX1 3TA, UK
**hagan.bayley@chem.ox.ac.uk*

Intrigued by technological potential, scientists have long attempted to control molecular motion. We monitor the individual 0.7-nm steps of a single molecular machine, which we have called a 'hopper'. The hopper moves in an electric field along a track within a nanopore controlled by a chemical ratchet. The hopper demonstrates characteristics desired in a moving molecule: defined start- and end-points, processivity, fuel autonomy, directional motion and external control. The hopper is readily functionalized to carry cargos. For example, DNA can be ratcheted along the track in either direction, a prerequisite for nanopore sequencing.

Engineering with Biomolecular Motors

Henry Hess and Gadiel Saper

Department of Biomedical Engineering, Columbia University, New York, NY 10027
hhess@columbia.edu

Biomolecular motors, such as the motor protein kinesin, can be used as off-the-shelf components to power hybrid nanosystems. These hybrid systems combine elements from the biological and synthetic toolbox of the nanoengineer and can be used to explore the applications and design principles of active nanosystems [1].

Efforts to advance nanoscale engineering benefit greatly from biological and biophysical research into the operating principles of motor proteins and their biological roles. In return, the process of creating in vitro systems outside of the context of biology can lead to an improved understanding of the physical constraints creating the fitness landscape explored by evolution. However, our main focus is a holistic understanding of the engineering principles applying to systems integrating molecular motors in general.

To advance this goal, we and other researchers have designed biomolecular motor-powered nanodevices, which sense, compute, and actuate. In addition to demonstrating that biological solutions can be mimicked in vitro, these devices often demonstrate new paradigms without parallels in current technology. Long-term trends in technology toward the deployment of ever smaller and more numerous motors and computers give us confidence that our work will become increasingly relevant.

Here, our discussion aims to step back and look at the big picture. From our perspective, energy efficiency is a key and underappreciated metric in the design of synthetic motors. On the basis of an analogy to ecological principles, we submit that practical molecular motors have to have energy conversion efficiencies of more than 10%, a threshold only exceeded by motor proteins [2]. We also believe that motor and system lifetime is a critical metric and an important topic of investigation. Related questions are if future molecular motors, by necessity, will resemble biomolecular motors in their softness and fragility and have to conform to the “universal performance characteristics of motors”, linking the maximum force and mass of any motor, identified by Marden and Allen. The utilization of molecular motors for computing devices emphasizes the interesting relationship among the conversion of energy, extraction of work, and production of information. Our recent work touches upon these topics and discusses molecular clocks [3] as well as a Landauer limit for molecular robotics [4].

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Self-assembled DNA rotors enable high-resolution tracking of molecular motors

Pallav Kosuri[1], Benjamin Altheimer[1], Mingjie Dai[2], Peng Yin[2], Xiaowei Zhuang[1]

[1]Howard Hughes Medical Institute, Department of Chemistry and Chemical Biology, Department of Physics, Harvard University, Cambridge, MA 02138, USA.
[2]Wyss Institute for Biologically Inspired Engineering, Department of Systems Biology, Harvard Medical School, Boston, Massachusetts 02115, USA.
Correspondence: pallavkosuri@fas.harvard.edu

We report a high-throughput single molecule method that enables precise rotational tracking of DNA with millisecond time resolution. ORBIT (Origami Rotational Beacon Image Tracking) uses fluorescently labeled self-assembled nanoscale rotors to amplify the rotation of a DNA duplex of interest (see Figure). Our method does not rely on an externally applied force but instead achieves high spatiotemporal resolution due to the low drag of the origami rotor and the stiffness of the short DNA duplex. We used ORBIT to track DNA rotation driven by the molecular motors RecBCD and RNA polymerase, revealing new insights into the action mechanisms of these enzymes.

RecBCD is a DNA repair enzyme that recognizes double stranded breaks and initiates homologous recombination to repair the damage. RecBCD starts this process by binding to a double stranded break and unwinding the DNA by peeling apart the two strands of the duplex, thereby inducing rotation. ORBIT allowed us to track DNA rotation generated by individual RecBCD molecules and we directly observed, for the first time, the sequence of events during initiation at a double stranded break. Based on our results we propose a new mechanism for RecBCD activity, wherein one of its subunits acts as a starter motor that initiates processive motion.

RNA polymerase (RNAP) serves as a gatekeeper for the flow of genetic information in cells, and its mechanism and regulation are consequently of central importance to many aspects of biology. We used ORBIT to track RNAP activity by measuring the unwinding of a DNA template during transcription. Our data show in unprecedented detail the rotational motion produced by RNAP and reveal previously unseen step-wise rotational movements corresponding to RNAP translocation across single bases in the DNA template strand.

Compared to previous single-molecule studies of these two motor proteins, ORBIT achieves higher resolution without the need to apply an external force. Our method can easily be parallelized and requires no specialized equipment beyond a standard fluorescence microscope. The simplicity and customizable nature of this method should enable high resolution studies of a wide range of protein-DNA interactions.

In summary, our work demonstrates the power of DNA nanotechnology to amplify and visualize molecular movements, enabling new insights into biomechanical systems.

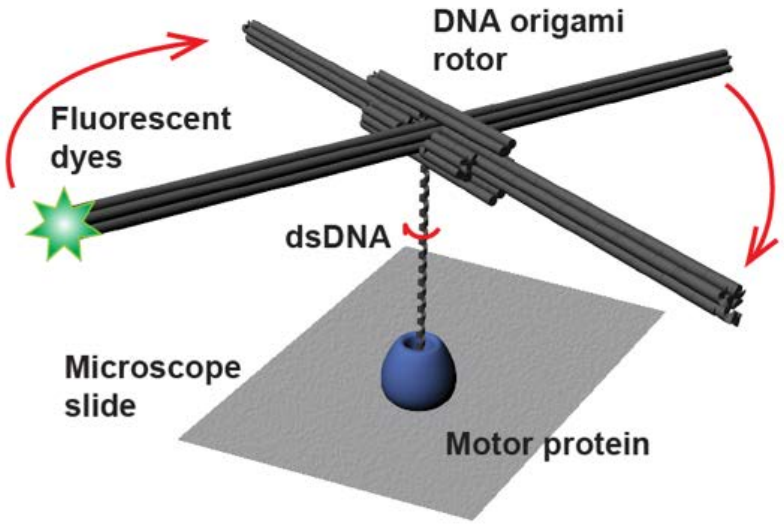

Figure. Schematic depiction of the ORBIT method to measure DNA rotation. Rotation of a double stranded (ds) DNA segment is amplified by a DNA origami rotor and detected by tracking the position of fluorescent dyes attached to the tip of a rotor blade. To measure motor protein-induced DNA rotation, the protein molecules are attached to the surface of a microscope slide. DNA substrates with attached origami rotors are then added. Substrate binding and subsequent DNA rotation is captured using a fluorescence microscope.

Nano-electronic components built from DNA templates

Jingjing Ye[1], Richard Weichelt[2], Alexander Eychmüller[2], Ralf Seidel[1]

[1]Peter Debye Institute for Soft Matter Physics, Universität Leipzig, Linnéstr. 5, 04103 Leipzig, Germany

[2]Physical Chemistry, TU Dresden, 01069 Dresden, Germany

Corresponding author: ralf.seidel@physik.uni-leipzig.de

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

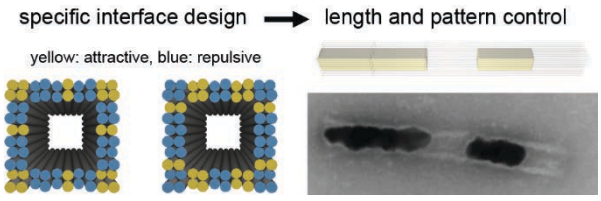

Figure 1. Sketch and tSEM images showing the modular DNA platform. With specific interface design by choosing different helix positions for attractive and replusive reaction, the length and pattern of the metal structures can be controlled.

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POSTERS

Track on DNA Nanostructures I: Semantomorphic Science A

Track Chair

Nadrian Seeman

New York University

Exploring the addressability of DNA-decorated multifunctional gold nanoparticles with DNA origami template

*Rachel Nixon[1], Wenyan Liu[2], Shuo Yang[1], Risheng Wang[1]**

[1] Department of Chemistry, Missouri S&T, Rolla, MO 65409, USA

[2] Center of Research for Energy and Environment, Missouri S&T, Rolla, MO 65409, USA

Contact: wangri@mst.edu

Gold nanoparticles have attracted extensive research interest due to their unique optical, electrical, and physical properties. For gold nanoparticles (AuNPs), specifically, additional capabilities can be achieved upon surface-functionalization of the nanoparticles with oligonucleotides, a process typically achieved by alkyl thiol adsorption. DNA oligos, with their unique programmable sequences and predictable base-pairing, can enable the AuNPs to anchor wherever complementary DNA sites are positioned. Numerous reports describe explorations of structures that involve AuNPs coated with identical strands of a single DNA sequence. However, these mono-functional AuNPs were limited in their recognition capabilities as they could only hybridize to a single complementary sequence.

Herein, we present the facile assembly of multi-functional DNA-decorated stable AuNPs which demonstrates the capability of simultaneously recognizing multiple DNA sequences, thereby enabling the bottom-up assembly of complex nano-particle systems. By loading multiple variations of DNA strands onto AuNPs, we have further elucidated the effects of several parameters on their hybridization capabilities with a DNA origami template, including varied DNA ratios and DNA lengths. Our investigation demonstrated that it is possible to synthesize multi-functional DNA decorated AuNPs with unaltered binding efficiency, as compared to mono-functional particles by selectively controlling the properties of the DNA strands (such as the DNA sequence, ratio, and differences in lengths of the DNA). We expect that the concept of multi-functional AuNPs, described here, will prompt development of further applications in the assembly of heterogeneous structures (containing various types of nano-objects) and promote more precise geometric control over the organization of these materials in space. This will further enhance the possibilities of customized nano-systems in materials science and bioscience.

pH-Driven hierarchical assembly of DNA origami nanostructures

*Shuo Yang[1], Wenyan Liu[2], Risheng Wang[1]**

[1] Department of Chemistry, Missouri S&T, Rolla, MO 65409, USA

[2] Center of Research for Energy and Environment, Missouri S&T, Rolla, MO 65409, USA

Contact: wangri@mst.edu

The development of DNA nanotechnology has resulted in tremendous advanced applications in materials science, and bionanotechnology. For example, DNA origami nanostructures have shown considerable ability in organizing nanomaterials through their high level of spatial addressability. However, to expand the capabilities of DNA origami, a major challenge that needs to be overcome is how to control a scale-up in the size and complexity of DNA origami structures. Recently, different types of environmental-responsive triggers, such as pH, metal-ion, and light, have been exploited to construct DNA origami nanoclusters that have demonstrated potential applications in dynamic, and multi-scale sensing systems. Nevertheless, the majority of dynamic association/dissociation processes has been controlled by a two-state conversion. The environmental stimuli controlled hierarchical assembly of larger DNA origami nanostructures (> 5 units), with multiple consecutive processes, has not yet been reported.

In this study, we present the pH-regulated, stepwise cyclic self-assembly of DNA origami multiple-tile nanoclusters by employing DNA triplexes as dynamic linkers. The DNA triplex is formed by the interaction between a pH-insensitive Watson-Crick duplex and a single-stranded DNA (ssDNA) through pH-sensitive parallel Hoogsteen base-pairing. Once the DNA triplex is in an unfolding status, its single-stranded DNA domain is available for complementary binding through Watson-Crick pairing that links components together. While refolding of the DNA triplex will disrupt above complementary pairing, leading to the dissociation of the components. Therefore, we tested the selective self-assembly of DNA origami trimers and more complex nine-unit DNA origami clusters in a step-wise fashion, based on the pH-dependent duplex-to-triplex transition in response to three different pH environments. This process is reversible upon a decrease in the pH value. The ordered assembly and disassembly processes are demonstrated by AFM images and gel analysis. The pH-driven assembly of DNA nanostructures could be potentially utilized in biomedical fields such as drug delivery systems.

A DNA Origami based multi-input logic gate for modifying DNA by enzyme nano-factory

Banani Chakraborty

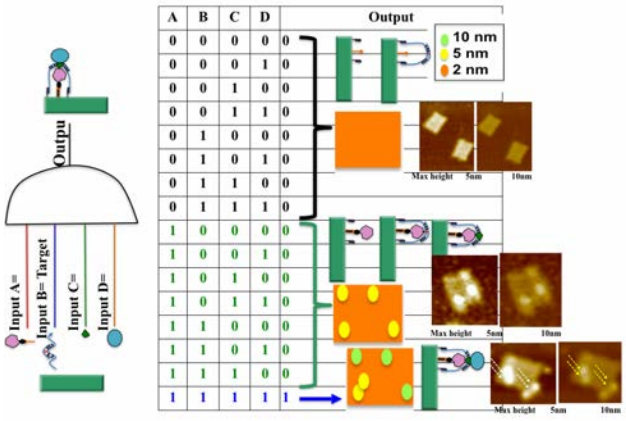
Department of Chemical Engineering

Indian Institute of Science

Bangalore, India

Abstract:

Past decade has seen enormous progress in DNA nanotechnology through the advent of DNA origami. Functionalizing the DNA origami for multiple applications is the recent focus of this field. Here we have constructed one of the first DNA enzyme nano- factory, which modifies target DNA embedded on a DNA origami platform. We have immobilised the DNA methyltransferase M.TaqI next to the target DNA on the DNA origami and used this enzyme to sequence-specifically modify the target DNA with biotin using a cofactor analogue. This enzyme nano-factory exemplifies a multi-input AND gate logic. The four components of this particular AND logic gate are: [A] M.TaqI, [B] target DNA, [C] biotinylated cofactor and [D] streptavidin, a topographic marker for AFM imaging which binds to biotin with very high affinity. We have successfully regenerated the origami surface by removing the output products of the enzyme nano-factory to complete the nano-factory cycle. This logically controlled nano-factory is reversible and holds the potential to be a prototype for variable and multiple input logic gates for multi- enzyme, multi-substrate nano-factories controlled by simple to complex logical operations on DNA origami surface.



Scheme 1: Truth table and schematic representation of 4-input AND logic gate and corresponding AFM images for the nano-enzyme factory

Kinetics of Strand Displacement and Hybridization on Wireframe DNA Nanostructures: Dissecting the Roles of Size, Morphology, and Rigidity

Casey M. Platnich, Amani A. Hariri, Janane F. Rahbani, Jesse B. Gordon, Hanadi F. Sleiman, Gonzalo Cosa
Correspondence to hanadi.sleiman@mcgill.ca or gonzalo.cosa@mcgill.ca

In recent years, wireframe DNA structures have gained significant attention due to their minimalistic construction and their potential for encapsulation and sensing. Dynamic reconfiguration of these assemblies is often achieved using strand displacement, wherein a constituent DNA strand bearing a single-stranded portion (called an overhang or toe-hold) is removed from the structure upon hybridization to a fully complementary DNA stand. For dynamic DNA devices to reach their full potential, however, the kinetics of strand displacement and hybridization on these constructs must be determined.

We have recently reported the use of single-molecule fluorescence methodologies to observe the reversible switching between double- and single-stranded forms of triangular wireframe DNA nanotubes. Specifically, by using fluorescently labeled DNA strands, we were able to monitor changes in intensity over time as we introduced different sequences. This allowed us to extract detailed kinetic information on the strand displacement and hybridization processes. Due to the polymeric nanotube structure, the ability to individually address each of the three sides, and the inherent polydispersity of our samples as a result of the step polymerization by which they are formed, a library of compounds could be studied independently yet simultaneously. Kinetic models relying on mono-exponential decays, multi-exponential decays, or sigmoidal behavior were adjusted to the different constructs to retrieve erasing and refilling kinetics. Correlations were made between the kinetic behavior observed, the site accessibility, the nanotube length, and the structural robustness of wireframe DNA nanostructures, including fully single-stranded analogs. Overall, our results reveal how the length, morphology, and rigidity of the DNA framework modulate the kinetics of strand displacement and hybridization as well as the overall addressability and structural stability of the structures under study.

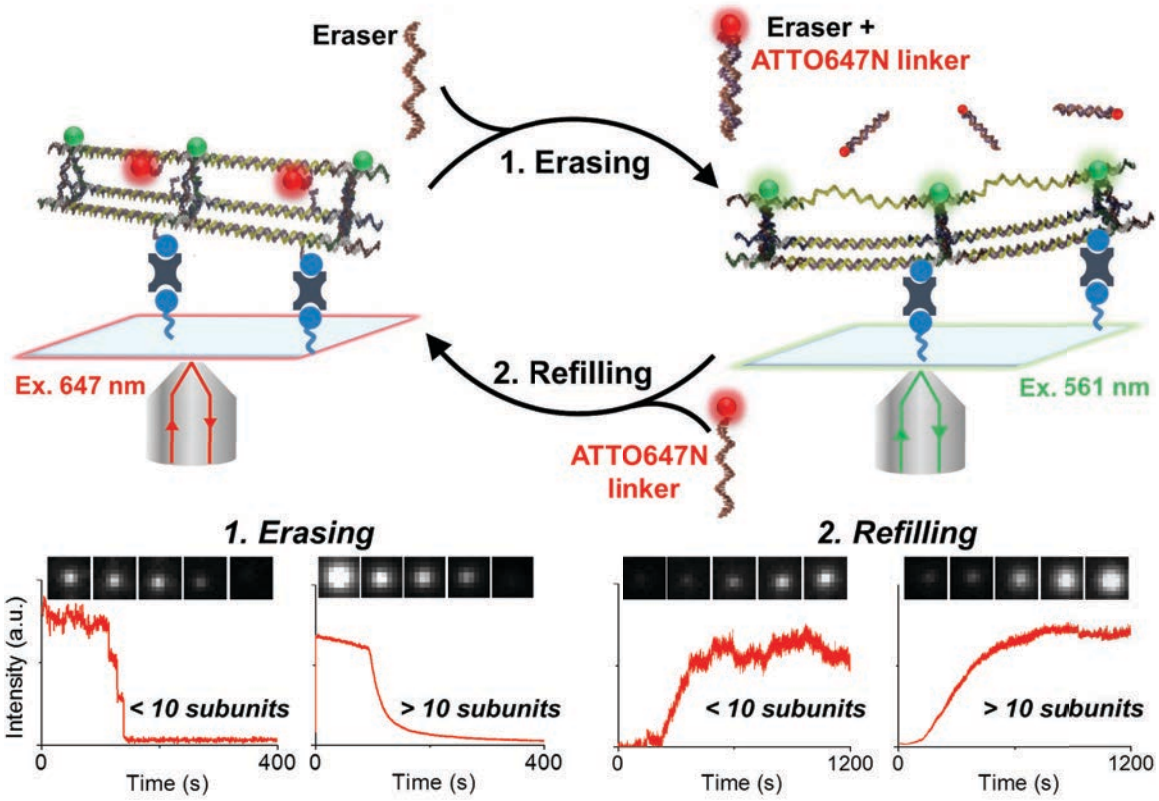


Figure 1: Erasing and refilling of ATTO647N labeled strands from wireframe DNA nanotubes. The intensity of each nanotube was monitored over time using single molecule total internal reflection fluorescence microscopy, allowing for the kinetics of erasing or refilling to be determined for each construct.

1. Platnich *et al. ACS Nano*, **2018**, *12* (12), pp 12836–12846.

Construct Complex Gold Nanoparticle Structures using DNA as a Chaperon

Xin Luo, Pongphak Chidchob, Hanadi F. Sleiman
Department of Chemistry, McGill University
E-mail: xin.luo3@mail.mcgill.ca

DNA nanotechnology has revealed the exceptional properties of DNA as a molecular building block for nanostructure construction due to its programmable interactions and conformations.[1] Moreover, it also has the potential to precisely organize other nanomaterials at the nanometer scale.[2] Gold nanoparticles (AuNPs) endowed with anisotropic DNA valency are an important class of functional materials, as they can assemble into complex structures with a minimal number of DNA strands.[3] However, methods to encode three-dimensional DNA strand patterns on AuNPs with a controlled number of unique DNA strands in a pre-designed spatial arrangement remain elusive. In this presentation, a simple one-step method to yield such DNA-decorated AuNPs is demonstrated, through encapsulating AuNPs into DNA minimal nanocages.[4] The AuNP@DNAcage encapsulation complex inherits the 3D anisotropic molecular information from the DNA nanocage with enhanced structural stability, which can be further applied as a building block for the self-assembly of complex architectures. In addition, we demonstrate an Assemble-Grow-Lift Off (AGLO) strategy to construct robust gold nanostructures in aqueous solution using DNA origami as a template. Firstly, DNA-functionalized AuNPs are assembled onto a DNA origami template to form a pre-designed AuNP pattern; we then slowly deposit supplementary gold onto the surface of the assembled AuNPs with the optimized surfactant; eventually the growing AuNP surface will merge with the adjacent AuNPs to generate one integrated gold nanostructure consisting of connected AuNPs from the origami-templated AuNP assembly. Strand displacement can be carried out subsequently to release the gold nanostructure and regenerate the DNA origami template.

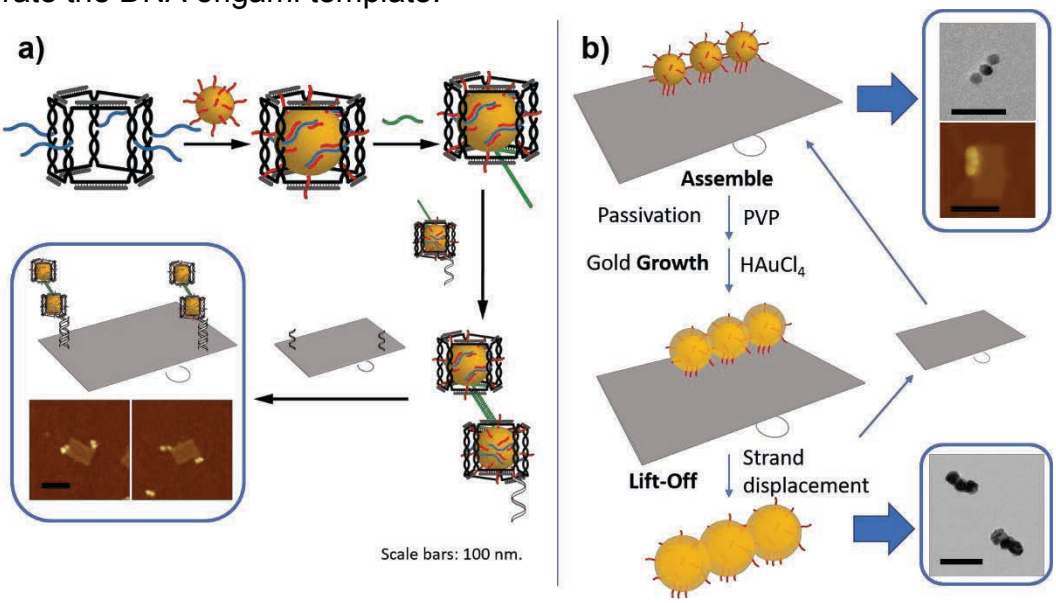


Figure 1. Schematic illustration of **a)** the encapsulation of AuNPs into DNA minimal pentagonal prism cages and the assembly of AuNP@DNAcage complex with DNA origami; and **b)** the Assemble-Grow-Lift Off (AGLO) strategy to construct robust gold nanostructures using DNA origami as a template.

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3D self-replication of DNA nanostructures

Feng Zhou[1,2], Ruojie Sha[2], Nadrian C. Seeman[2] & Paul M. Chaikin[1]
[1]Center for Soft Matter Research, New York University, New York, NY 10003, USA
[2]Department of Chemistry, New York University, New York, NY 10003, USA

Corresponding Author: Feng Zhou: zfedward1988@gmail.com; Nadrian C. Seeman: ned.seeman@nyu.edu; Paul M. Chaikin: chaikin@nyu.edu.

Self-replication is a natural process that can generate materials and pass along information. We have seen several examples of artificial self-replication in which the template assembles, organizes and directs formation of the target nanostructure. However, the self-assembly procedure increases the template's dimensionality, which makes it challenging to template and replicate a 3D object. Here, we report the direct self-replication of a 3D object. First we fabricate our 3D object: a three face cube corner by folding a planer self-assembled set of three DNA origami tiles. This three face cube corner is our template. The replication proceeds by self-assembling three daughter origami tiles to three edges of the cube corner. DNA single strands on each cube face and daughter tile hybridize to fold the tiles inward and complete the cubic box. The daughter tiles are then crosslinked into a new cube corner. Heating releases the two complementary cube corners. The 1st cycle of self-replication produced 45% of 3D trimers out of the templates, and the yield of the 2nd cycle is 42%, indicating the successful replication of the 3D nanostructure. We have found that accurate positioning is essential to the success of this type of self-replication. This method provides a general breakthrough for conducting high-order self-replication by organizing the materials *via* folding. Considering that the 3D DNA nanostructure is a functional platform, this type of 3D self-replication is expected to open a new way to produce new materials, such as the plasmonic nanomaterials, by passing the steric information through successive generations.

Acknowledgements

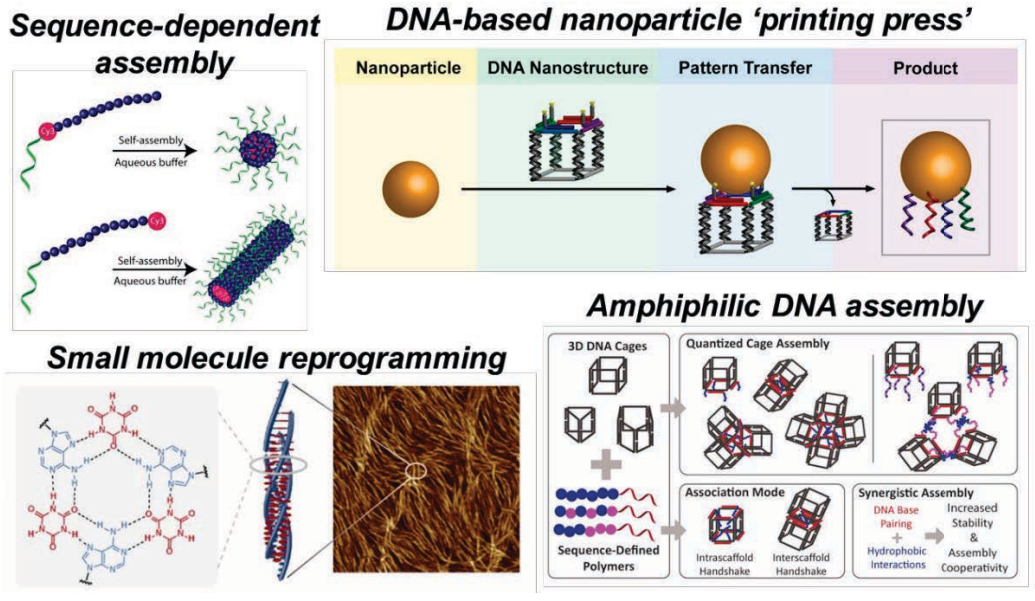
This research has been primarily supported by DOE DE-SC0007991 and DE-SC0000989.

Reprogramming self-assembly pathways with amphiphilic DNA and small molecules

Felix J. Rizzuto and Hanadi F. Sleiman

felix.rizzuto@mcgill.ca, hanadi.sleiman@mcgill.ca

The introduction of abiological motifs into DNA-based constructs enables new functionality. Over the last decade, the Sleiman group have used small molecules and amphiphilic DNA strands to produce new molecular materials: muscle-like fibres, anisotropic nanoparticles, spherical nucleic acids, and monodisperse, asymmetric polymer clusters. Key to the formation of these materials is the programmability of DNA interactions, which dictate structural morphology and enable secondary recognition sites to express new self-assembly pathways. The Sleiman lab interfaces polymer chemistry with DNA nanotechnology to selectively bind proteins, 'print' perfect particles, reprogram DNA assembly and open new routes for biological operation.



POSTERS

Track on Chemical Tools for DNA Nanotechnology

Track Chair

Andrew Ellington

University of Texas at Austin

and

Floyd Romesberg

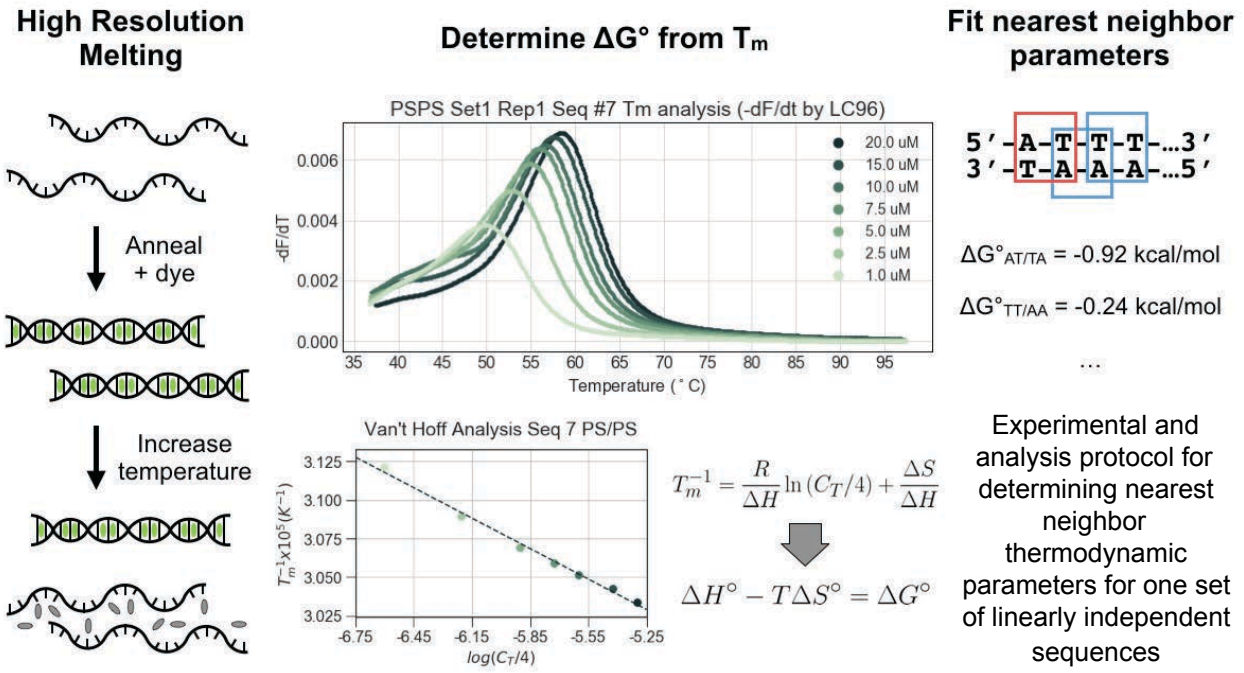
Scripps Research Institute

Modeling thermodynamic stability of phosphorothioate-modified DNA

Siyuan S. Wang[1]*, *Erhu Xiong*[2], *Andrew D. Ellington*[1]

[1] Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712, United States
[2] College of Life Science, South China Normal University, Guangzhou 510631, P. R. China
*Corresponding author email address: siyuanswang@gmail.com

Phosphorothioate (PS)-modified oligonucleotides form less stable duplexes than their unmodified phosphodiester (PO) counterparts. Combined with the possibility of PS-modified oligo synthesis by chemical and enzymatic means, this property has led to the use of PS modifications in nucleic acid diagnostics circuits to improve signal amplification. We present here a nearest-neighbor model of hybridization for PS-PS and PS-PO DNA interactions derived from high resolution melting data collected on 40 sequences with PS modifications of mixed chirality. For any given sequence, the order of stability is PO-PO>PS-PO>PS-PS. Nearest-neighbor pair stability rankings are similar to PO-PO pair rankings reported in previous literature, with CG/GC and GC/CG among the most stable and TA/AT among the least stable. Finally, we fitted our data to variations of the nearest-neighbor model and assessed the quality of fit for each variation. We found that inclusion of terminal basepairs as an additional 8 parameters to the 16 nearest-neighbor pair parameters in the fit leads to overfitting and poorer predictions than using the 16 pair parameters alone. We envision that our predictive model could enable precise tuning of components used in DNA nanotechnology via PS modifications.



High-throughput thermodynamic profiling of mismatched nucleic acid hybridization on Next-Generation sequencing chips

Siyuan S. Wang[1]*, *Andrew D. Ellington*[1], *Ilya J. Finkelstein*[1]

[1] Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712, United States
*Corresponding author email address: siyuanswang@gmail.com

Next-generation sequencing has revolutionized biology by enabling novel approaches to collecting and analyzing data while dramatically reducing costs through massively parallel platforms. While the most straightforward advantage brought about by next-generation sequencing is the ability to rapidly sequence large volumes of DNA, a more subtle albeit valuable use is the application of the parallel format to profile biophysical properties for a large sequence space. Previous works have leveraged next-generation sequencing platforms to evaluate binding interactions between various biomolecules (including CRISPR-Cas complexes and protein targets) and libraries of millions of unique sequences presented in different forms (such as dsDNA or RNA aptamers) at a level of throughput not attainable by other methods. We demonstrate here that next-generation sequencing chips can be used to profile the effects of mismatched hybridization on DNA-DNA or DNA-nucleic acid variant duplex stability. We envision that this approach may be used to construct a more detailed understanding of mismatched hybridization that could inform biophysical models of nucleoprotein-nucleic acid interactions. Additionally, comprehensive models of hybridization for nucleic acid variants could lead to new mechanisms for engineering DNA-based circuitry.

Thermodynamic and Kinetic Optimization of Aptamer Switches for Real-time Molecular Recognition

Brandon D. Wilson[1], Amani Hariri[2], Ian Thompson[2], and H. Tom Soh[2,3,4*]

[1]Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA.
[2]Department of Electrical Engineering, Stanford University, Stanford, CA 94305, USA.
[3]Department of Radiology, Stanford University, Stanford, CA 94305, USA.
[4]Chan Zuckerberg Biohub, San Francisco, CA 94158, USA.
* Correspondence to tsoh@stanford.edu

Biosensors use molecular recognition to transduce analyte concentration into an observable signal. Thus, the operational properties of a biosensor—LOD, detection range, etc.—are ultimately dictated by the physics of molecular recognition. In nature, these interactions have been finely tuned over millions of years to function over well-defined concentration ranges. However, artificially generated affinity reagents such as aptamers often do not have the thermodynamic parameters needed to generate an optimally performing biosensor. A generalized approach to controlling the apparent affinity of aptamers would greatly increase their utility as affinity reagents in biosensors.

In this work, we develop a method of precisely controlling the thermodynamics and kinetics of molecular recognition in an aptamer-based system. Our linked displacement strategy (LDS) uses the same quencher functionalized displacement strand of the widely used duplexed aptamer but anchored to the aptamer via a poly-T linker [Fig. 1a]. The LDS construct offers two control parameters—displacement strand length and linker length—that allow us to independently tune the effective affinity and temporal response of the probe [Fig. 1b]. As a proof-of-concept, we converted a previously described ATP aptamer (K_D ~10 μM) into a number of LDS constructs that sense ATP with apparent affinities ranging from 1 μM to >1 mM [Fig 2a/b], and kinetic response rates ranging from

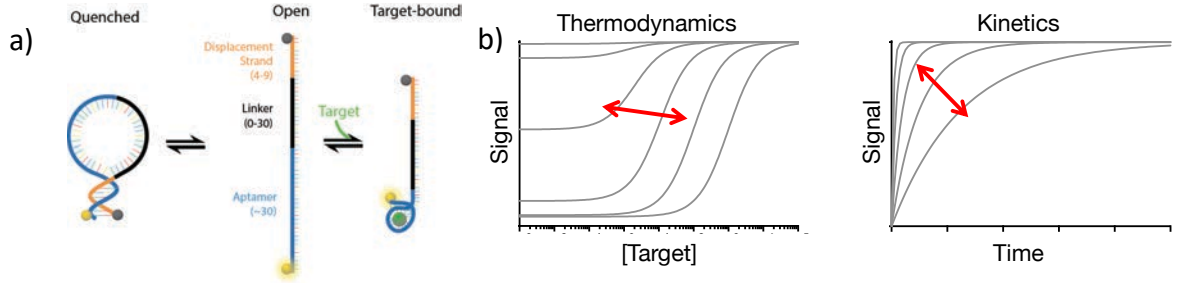


Figure 1: Linked displacement strand ("LDS") scheme. A single molecule switch is comprised of an aptamer functionalized with a fluorophore linked via a polyT linker to a complementary strand functionalized with a quencher. (a) This system is well characterized with a three-state population shift mechanism. The lengths of the linker and the displacement strand can be varied to control the resultant thermodynamic and kinetic properties of the construct (b).

hundreds of milliseconds to minutes. Our experimental results show excellent concordance with mathematical predictions, suggesting that this method enables the

design of novel LDS constructs without the need for lengthy trial and error. Finally, we demonstrate the first example of an aptamer's kinetic response being tuned nearly independently of the thermodynamic performance [Fig. 2c/d].

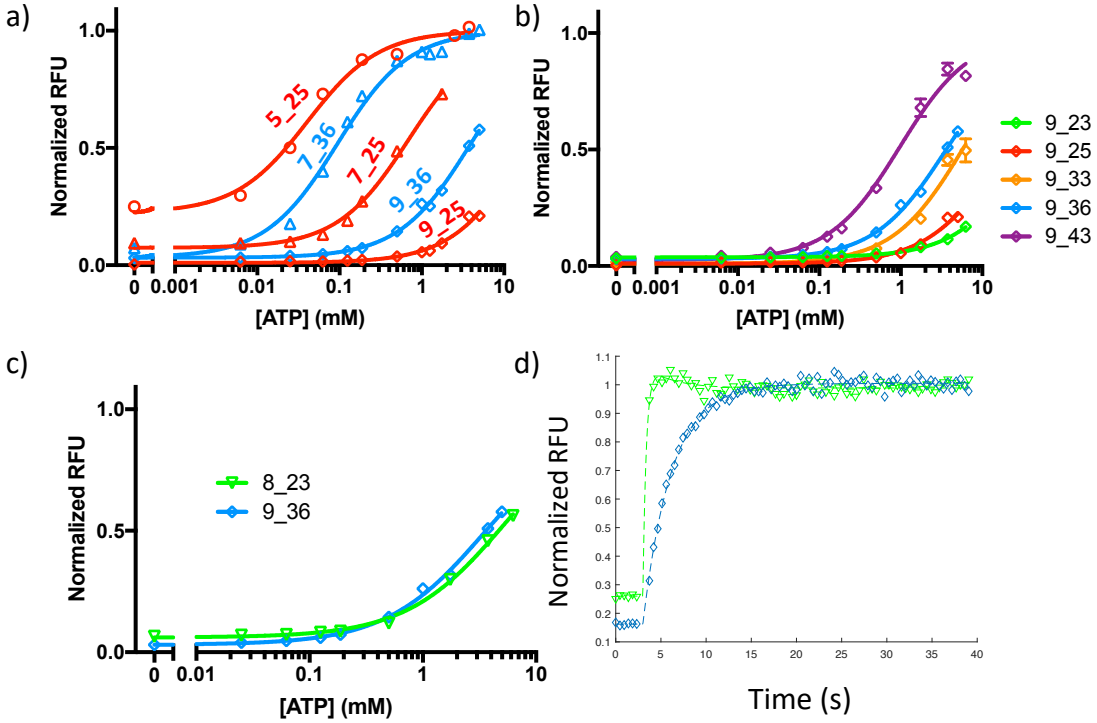


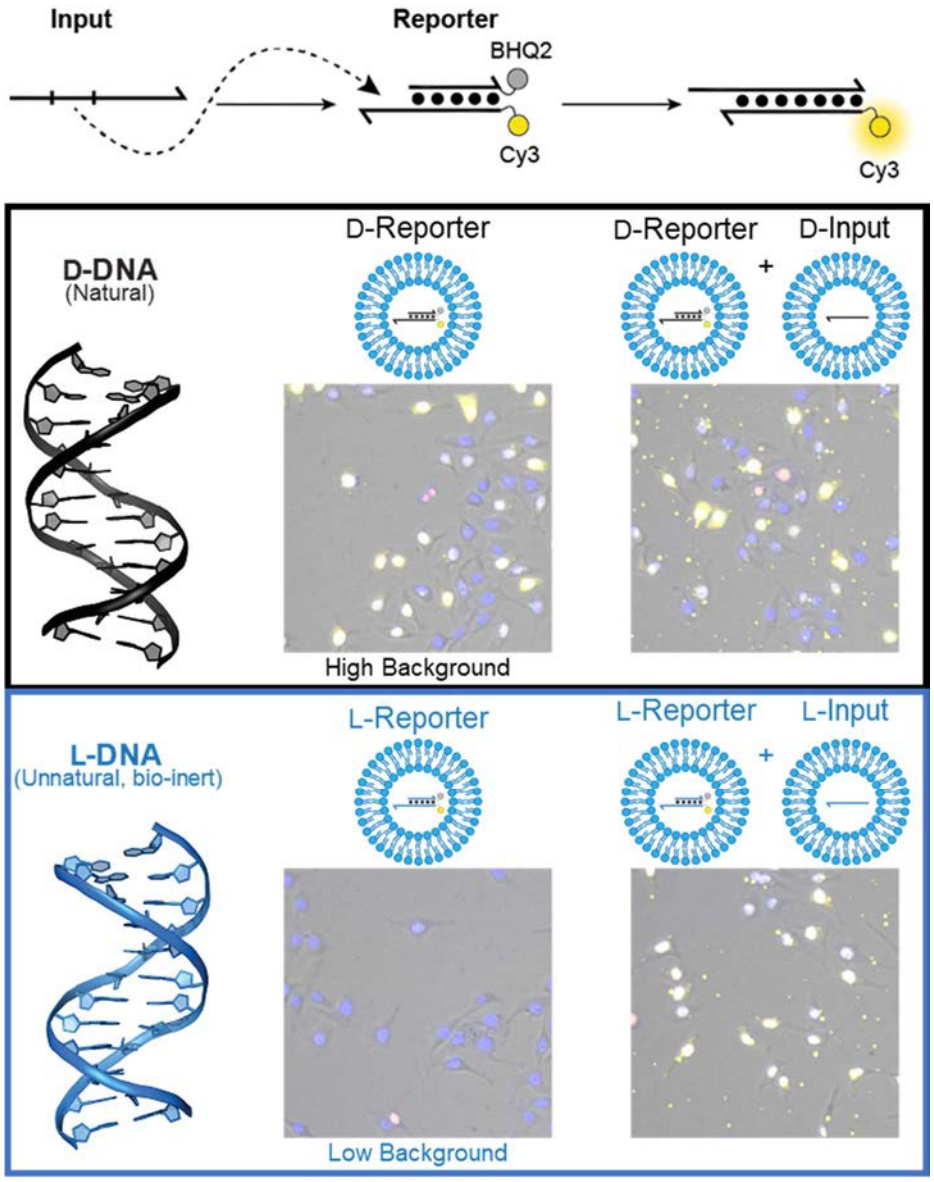
Figure 2: Binding curves for the ATP aptamer in various LDS constructs. Our nomenclature lists LDS constructs as "displacement strand length"_"linker length". (a) Linker length is held constant at 25 (red) and 36 (blue) while the displacement strand is changed from 5 to 7 to 9 nucleotides. A single additional base to the displacement strand can shift the effective affinity by an order of magnitude. (b) By holding the displacement strand length constant at 9 nt and varying the linker length from 23 to 43 nts, we can achieve fine-grained control over the aptamer's effective affinity. Notably, we can design pairs of constructs that have the same thermodynamics (c) but very different temporal resolutions (d).

The battle of enantiomers: comparing the performance of D-DNA and L-DNA strand-displacement circuits in living cells

Brian Young[1], *Adam Kabza*[1] *and Jonathan Sczepanski*[1,*]

1 Texas A&M University, *jon.sczepanski@chem.tamu.edu

While DNA is most widely known as a genetic polymer, it has recently emerged as a powerful material for constructing nanotechnologies. These technologies exploit the well understood properties of Watson–Crick (WC) base pairing in order to program the assembly of static DNA structures and carryout dynamic operations based on DNA strand displacement.[1-2] Given the ease by which DNA can be programed to interact with living systems, it is not surprising that many of the most promising applications of DNA nanotechnology are in the areas of biomedical research and disease diagnosis/treatment.[3] Unfortunately, the environment inside live cells is incredibly harsh towards exogenously introduced nucleic acids. Exogenous DNA is rapidly degraded by cellular nucleases and high concentrations of endogenous proteins and nucleic acids often lead to non-specific interactions and spurious operation. In order to overcome these issues, researchers most often rely on chemical modification of the DNA backbone (i.e. 2' OMe/Fluoro groups, phosphorothioate linkages, etc.). While these modifications have been shown to increase the stability of exogenously delivered DNA, they also alter the kinetic and thermodynamic properties of hybridization compared to natural DNA, thereby undermining the well-established design parameters of these systems.[4] Additionally, chemical modifications have been shown to increase the toxicity and immunogenicity of DNA *in vivo*, further decreasing the appeal of this approach. We propose that many of the challenges associated with bringing DNA nanotechnologies into the cell can be overcome by simply looking into the mirror. L-deoxyribose nucleic acid (L-DNA), which is the synthetic enantiomer (or mirror image) of native D-DNA, is resistant to both nuclease degradation and non-specific interactions with cellular macromolecules, yet maintains identical hybridization kinetics when compared to the D-DNA.[5] On this basis, we constructed a series of L-DNA-based strand-displacement circuits and examined their behavior in living cells.[6] By directly comparing the performance of D-DNA and L-DNA versions of each circuit, we show that L-DNA circuits are dramatically more stable and reliable in living cells than their D-DNA counterparts. Furthermore, using heterochiral strand displacement, we show that L-DNA-based circuits can be interfaced with native D-nucleic acids in living cells. Together, this work lays the foundation for interfacing more complex L-oligonucleotide-based circuits within living cells and organisms for exciting applications in bioengineering, synthetic biology, and clinical diagnostics.



L-DNA-based strand-displacement circuits have improved performance and reliability in living cells. This result is exemplified by the dramatic difference in the stability of D- and L- reporter modules in HeLa cells.

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POSTERS

Track on Nanophotonics and Superresolution

Track Chair

Ralf Jungmann

Max Plack Institute for Biochemistry, Martinsried

Toward 1-D Arrays of Two Color Molecular Beacons

David Neff, Sanaz Jafarvand, Nathan Shin and Michael Norton

Department of Chemistry, Marshall University, Huntington,WV 25755
(Norton@Marshall.edu)

Discrimination among splice variants, varied linear assemblies of segments of specific nucleic acid sequences, remains a challenge at the single molecule level. Combining molecular beacons and DNA origami is a powerful approach with many potential applications[1], and may represent one approach to enabling transduction of this sequencing information. Additionally, the size scale of origami arrays potentially provides a mechanism for detection with high spatial resolution[2]. In this presentation we will discuss our early steps in the development of the systems required in order to pursue this approach, including the imaging system, a two color model molecular beacon and 1-D arrays[3] of cross shaped origami[4].

The use of single color molecular beacons for single molecule detection suffers from the potential of a photobleaching event aliasing for a negative detection event. The use of two color molecular beacons, in principle, enables a mechanism for the assurance of beacon integrity, assuring that each state (open or closed) of the beacon is capable of reporting.

Design and characterization experiments have been performed with a two color molecular beacon system, in solution and bound to origami. Preliminary implementations employing approximations of the Alternating Laser Excitation (ALEX)[5] technique will be presented.

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Programmable DNA-nanostructures for rapid and facile molecular counting

Nathan D. Williams[1], Wenjiao Zhou[1], John Powell[1], Ravi Kasula[1], Derek Toomre[1], Julien Berro[1,2], Chenxiang Lin[1]
[1]Department of Cell Biology, Yale School of Medicine, New Haven, CT 06520
[2]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520
Corresponding author: chenxiang.lin@yale.edu

DNA-origami-based fluorescence standards have been demonstrated as valuable tools for molecular counting, but their utility in live cell quantitation is only beginning to be realized[1-5]. Established methods for counting molecules in live cells are robust, but also laborious and poorly suited for counting 10-100 molecules[6-8]. We present a method for fast and easy molecular quantification of dye- or fluorescent-protein-tagged targets, in living or fixed cells, using only a fluorescence microscope and barcoded[9], DNA-origami-based, fluorescence standards. Our standards do not require antibodies, long acquisitions, or complex processing. They are easily redesigned, and multiple new configurations may be prepared in under a week. We leveraged the power of a genomically-recoded organism (GRO) and "Click Chemistry"[10-11] to generate DNA-conjugated fluorophores, which may be precisely and strongly attached to our DNA-nanostructures via DNA hybridization. Structures self-assemble with high fidelity, and fluorophore occupancy was estimated by quantitative gel electrophoresis and stepwise photobleaching to be ≥80%. We successfully assembled brightness standards for

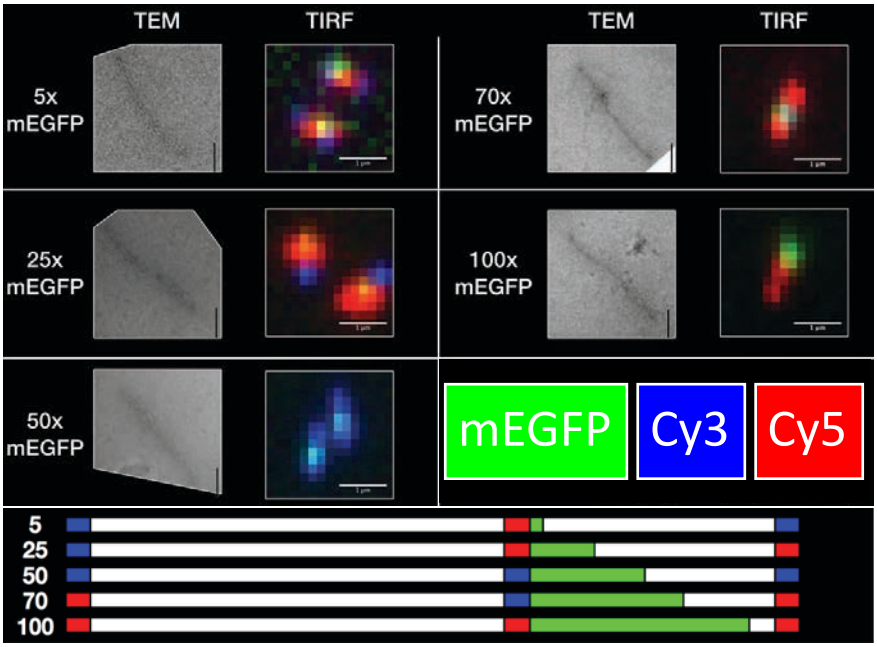

Figure 1: Barcoded mEGFP Standards.
Top: TEM (left) & TIRF micrographs (right). Number of mEGFP molecules to the left of each pair. Structures are ~800 nm long. Blue: Cy3; red: Cy5; green: mEGFP. LUTs have identical settings, apart from the 5x mEGFP TIRF micrograph, which has boosted mEGFP levels for clarity. TEM Scale bars: 200 nm. TIRF scale bars: 1 μm. Bottom: diagram of barcoding patterns (same color scheme).

quantifying molecules tagged with mEGFP (monomeric enhanced green fluorescent protein) or the far-red dye, SiR (siliconized rhodamine).

To ascertain the accuracy of our method, we used barcoded standards with 5, 25, 50, 70, or 100 mEGFP molecules (Figs. 1 & 2). to quantify cytokinesis node proteins genomically-tagged with mEGFP in *S. pombe*. Standards and cells were imaged together using a TIRF microscope, and spots were picked using a custom TrackMate script. Spots from standards were sorted by type (i.e. #mEGFP) by an expectation-maximization Gaussian-unmixing algorithm, and sorting was validated by reviewing barcoding. The number of mEGFP per yeast-spot was estimated by plotting along the calibration curve generated by the standard spots. To account for the quenching effect of the imaging media, standards with 5 mEGFP were photobleached alongside yeast with ~3 mEGFP/node, and the bleaching step sizes were compared. After correcting for the media's quenching effect, our quantification fell within the expected range previously published[12].

We have achieved promising results from preliminary in-situ testing with genomically mEGFP-tagged cytokinesis proteins in *S. pombe* and in SiR-labled, Halo-tagged, vesicle fusion proteins in mammalian cells. The generality and flexibility of our approach, and the unprecedented nanoconstruction potential of DNA-nanotechnology, will fill a gap between the dynamic ranges of stepwise photobleaching and ratiometric comparison to internal fluorescent standards, making our standards an integral part of the biologist's toolkit.

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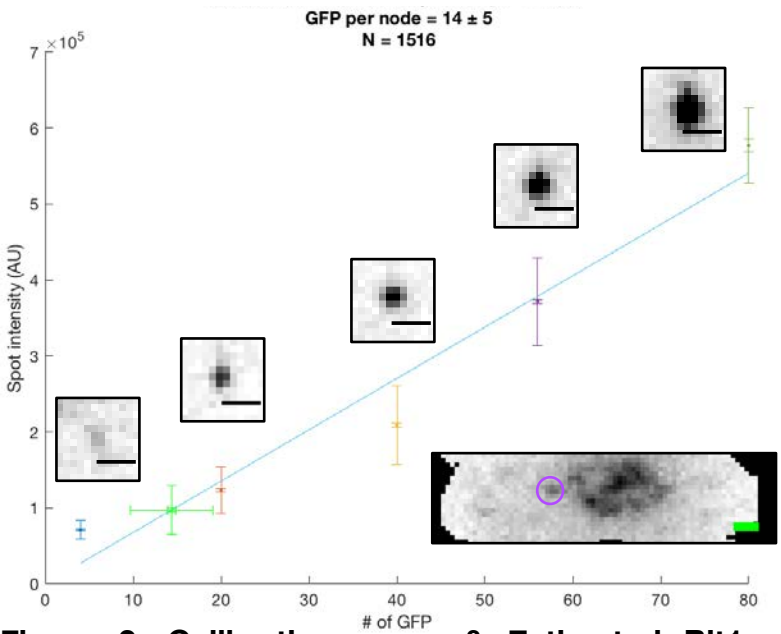

Figure 2: Calibration curve & Estimated Blt1p-mEGFP Copy Number Per Node. (Green error bars; expected: 10-15). Y-axis error bars from barcoded mEGFP standards. Inner and outer error bars represent SEM and SD, respectively. Insets above curve: typical spots used in calibration. Inset below curve: typical cell with circled single node picked by TrackMate script. All scale bars: 1 μm.

Amplified Uncaging of Functional Molecules through the Hybridization Chain Reaction

Alexander L. Prinzen,[a] *and Hanadi F. Sleiman*[*b]
[a] *Department of Chemistry, McGill University, Montreal, Canada.* [b] *Department of Chemsitry, McGill University, Montreal, Canada.*
E-mail: alexander.prinzen@mail.mcgill .ca

Triggering the uncaging of functional molecules in response to a particular stimulus has practical applications in a variety of different areas including smart therapeutics and bio-imaging.[1] Nucleic acids are a favorable stimulus for uncaging due to their uniqueness and diversity across individuals. Additionally, aptamer sequences allow nucleic acids to respond to more specialized cues such as small molecules or proteins. Many molecular beacon and catalytic systems exist that release functional molecules.[1-2] However, these systems either do not amplify release or are limited to only being responsive to nucleic acid signals. The hybridization chain reaction (HCR) is an isothermal amplification method that can be adapted to be responsive to a variety of biological signals.[3] While HCR has been used extensively for the detection of different substrates it has yet to be employed to amplify the release of caged molecules. Here we report the progress made towards combining a nucleic acid templated disulfide exchange, with HCR for the selective and amplified release of a caged fluorescein in response to signals such as nucleic acids, small molecules and protein (Fig 1.).

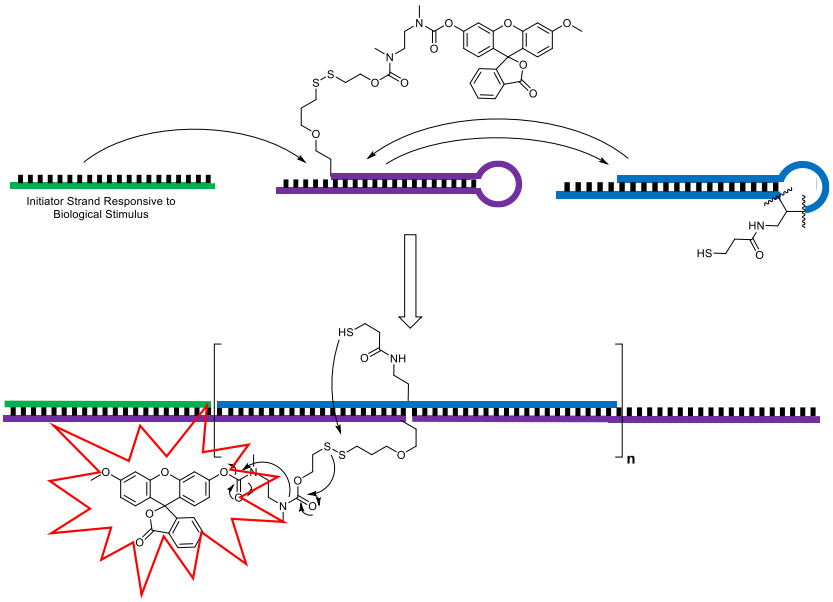

Figure 1. HCR resulting in a DNA templated disulfide exchange, releasing caged fluorescein

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POSTERS

Track on DNA Nanosystems: Programmed Function

Track Chair

Friedrich Simmel

Technical University Munich

Self-Assembly of Three-Dimensional Electronic Systems with Neuromimetic Network Architectures

Ming Gao[1], Nikolay Frick[1], Mahshid Hosseini[1], Abhichart Krissanaprasit[1], Thom LaBean[1]*
[1]Department of Materials Science & Engineering, North Carolina State University, Raleigh, NC 27606, USA.
E-mail: mgao4@ncsu.edu, thlabean@ncsu.edu

Self-assembling materials have the unique strength of using molecular recognition to program the final architecture and the assembly process. Inspired by biological neural networks, we are motivated to use molecular recognition to integrate three-dimensional neuromimetic networks. Electronic systems with these types of network architectures are trainable, learnable, and error-tolerant. They show interesting non-linear behaviors, and can potentially be applied in classification, memory, and information processing.

We are developing three-dimensional neuromimetic networks using conducting nanoparticles and nanowires assembled in insulating polymeric matrices. The architecture uses nanoparticles and nanowires as functioning components to form networks of extremely high connectivity. As matrix for the networks, we explored different types of polymeric materials, including thermoplastics, thermosets, and biopolymers. Among all thermoplastic matrices we explored, polycaprolactone shows the best overall performance in maintaining percolation with minimal amounts of embedded conducting nanomaterials. We used polycaprolactone extensively for embedding of silver nanowires and silver nanowires with silver sulfide nanocrystals, and optimized the fabrication process. The constructed electronic system with polycaprolactone can also be reshaped with heat to modify the morphology of the embedded network. We particularly analyzed dispersion conditions of silver nanowires and other nanomaterials in different solvents. Adequate dispersion of nanomaterials before embedding is essential to fabricating a homogeneous nanocomposite of randomized network architecture. As for biopolymer matrix, we are especially interested in using Y-tile and ds-linker to construct a DNA hydrogel with embedded gold nanoparticles and silver nanowires. The mechanical properties of the hybrid hydrogels were also studied by bulk rheological testing. We also investigated fabrication of hydrogels and aerogels with agarose matrix and embedded nanostructures. The structures we embedded in hydrogels include networks of carbon nanotubes, silver nanowires, and copper nanoparticles with silver dendrites. We also explored changes in properties after converting these hydrogels to aerogels through supercritical drying and freeze-drying.

Real-Time Observation of The Movements of DNA Origami Pinching Devices on Mica Using High-Speed AFM

Akinori Kuzuya

Department of Chemistry and Materials Engineering, Kansai University, 3-3-35 Yamate, Suita, Osaka 564-8680, Japan, kuzuya@kansai-u.ac.jp.

We have recently developed DNA origami pinching devices; DNA Pliers with two lever portions in 2D conventional DNA origami design, and DNA Chopsticks with 3D tubular levers.[1] These devices can take three forms; open cross, parallel closed, and antiparallel closed forms. When the lever portions are modified with a specific ligand, these devices can pinch exactly one target molecule between the levers and turn into parallel closed form. This structure change can be visualized by AFM. Real-time monitoring of the structure change was also possible with fluorescently modified DNA origami pinching devices, but this observation was not in single-molecular resolution.

In this study, we examined suitable conditions for DNA Origami devices to have sufficient mobility on mica for target binding and structure change, without coming off from the substrate, and successfully observed their movement with high-speed AFM in real-time.

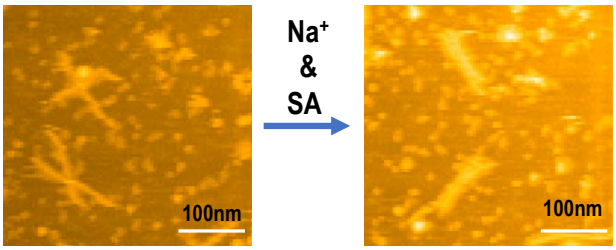

Figure 1. High-speed AFM images of DNA Chopsticks before and after adding Na^+ and SA.

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Self-assembled DNA Light-Pipes

Rajan Sharma,[1] Iwona Mames,[2] Eugen Stulz[2] & Christoph Wälti[1]
[1] Bioelectronics, The Pollard Institute, School of Electronic and Electrical Engineering, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT
[2] School of Chemistry, University of Southampton, Southampton U.K.

**corresponding author: C.Walti@leeds.ac.uk*

Many photonics applications, including artificial light-harvesting systems, fundamentally rely on the ability to transport photonic excitations over large distances. Photonic wires employing fluorescence resonance energy transfer (FRET) cascades are promising candidates to address this need, but despite having received significant attention in recent years, the integration of such photonic wires into complex devices remains a significant challenge. Nature employs a large number of chromophores to collect cooperatively photons and channels the resulting excitons to specific reaction centers with extraordinary efficiency. To mimic such highly optimised energy-transfer processes, synthetic electronically active light-harvesting molecules, notably porphyrins, have been employed. However, a highly ordered network of porphyrins is required to achieve efficient transport of excitons over large distances.

DNA owing to its highly efficient molecular recognition and thus programmable assembly properties has been exploited to create a wide range of nanoscale structures with well-defined geometries and topologies. However, advanced applications often require additional complementary molecular techniques for positioning a wide range of heterogeneous functional elements on complex DNA nanostructures and devices with high precision and control over the orientation, location and assembly.

Here, we demonstrate the creation of efficient, self-assembled light-pipes using porphyrin-modified DNA in conjunction with the homologous recombinase protein RecA to drive the programmable assembly and molecular-scale positioning. These light-pipes can transport excitons between two chromophores of a FRET pair separated by distances much larger than the relevant Förster distance. The light-pipe devices are assembled from a single-stranded (ss)DNA molecule containing covalently attached and electronically active porphyrins distributed along the length of the DNA. RecA nucleoprotein filaments (NPFs) are formed by polymerising the homologous recombinase RecA onto the porphyrin-containing ssDNA. RecA then catalyzes the alignment of the NPF at regions of sequence homology onto double-stranded (ds)DNA scaffolds.

We demonstrate the light-piping by self-assembling the porphyrin-NPF between the two constituent chromophores of a FRET pair which are attached to a dsDNA scaffold. In the absence of the light pipe, no FRET is observed, however, once a porphyrin-NPF is self-assembled between the FRET chromophores, efficient FRET transfer from the donor chromophore to the acceptor chromophore via the porphyrin-NPF is observed. Both, steady-state fluorescence and fluorescence life-time measurements are presented. This

approach of self-assembling DNA-based photonic wires will find application in, for example, novel photonic DNA–based materials and molecular photovoltaic devices.

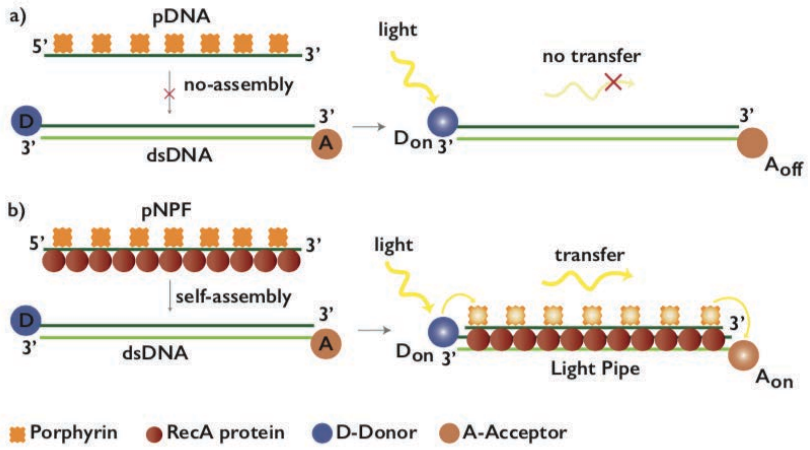

Figure 1: Light-pipes are assembled from a ssDNA containing electronically active porphyrins distributed along the length of the DNA. RecA nucleoprotein filaments (NPF) are formed by polymerising RecA onto the porphyrin-containing ssDNA (pDNA), which site-specifically self-assembles onto a homologous dsDNA with chromophores attached at either ends. (a) In the absence of the light pipe no FRET between the donor and acceptor is expected. (b) Once a pNPF is correctly assembled between the chromophores, efficient FRET from the donor to the acceptor chromophore via the spatially arranged porphyrins on the pNPF is enabled.

POSTERS

Track on Computational Tools for Self-Assembly

Track Chair

William Shih

Wyss Institute and Harvard Medical School

Engineering Kinetically Reproducible DNA Devices

Michael Tobiason[1], Bernard Yurke[1,2], and William L. Hughes[1,]*
[1] Micron School of Materials Science & Engineering, Boise State University
[2] Department of Electrical & Computer Engineering, Boise State University
[*] To whom correspondence should be addressed. Tel: 208.426.4859; Email: WillHughes@BoiseState.edu

DNA duplex-formation and strand-displacement reactions are fundamental to the field of DNA nanotechnology, however the relationship between DNA sequences and the rate of these reactions is still not fully understood. Based on this, it is hypothesized that observed kinetic variations arise due to unintentional base pairing and that by eliminating such interferences, kinetically uniform devices can be generated. It is found that ranking sequences based on both the size and number of their largest unintentional intramolecular complements results in statistically significant (p-values < 0.05) sub-populations with improved kinetic reproducibility consistently across three sets of duplex-formation rates reported in the literature (1,2). This is taken to be evidence that the majority of observed kinetic variation in the devices arises from intramolecular interferences. To engineer devices based on this principle the “Sequence Evolver” software — which combines an evolution-inspired heuristic algorithm with an intuitive fitness function — is presented. By experimentally characterizing DNA devices generated using the software, it is demonstrated that both duplex-formation and strand-displacement kinetics can be expected to vary by a factor of two or less when sequences satisfy four conditions: [1] No intramolecular interferences longer than 2 bp, [2] no intermolecular interferences longer than 4 bp, [3] no stretches of consecutive Cs or Gs longer than 3 bp, and [4] no stretches of consecutive As or Ts longer than 6 bp. These results are presented as experimental validation of the software’s effectiveness. In addition, these findings support the initial hypothesis, suggest that small intramolecular interferences play a more substantial role in device kinetics than previously thought, and indicate that engineering sequences based on these principles will enable the creation of devices with kinetic reproducibility not achievable using alternative state-of-the-art methods.

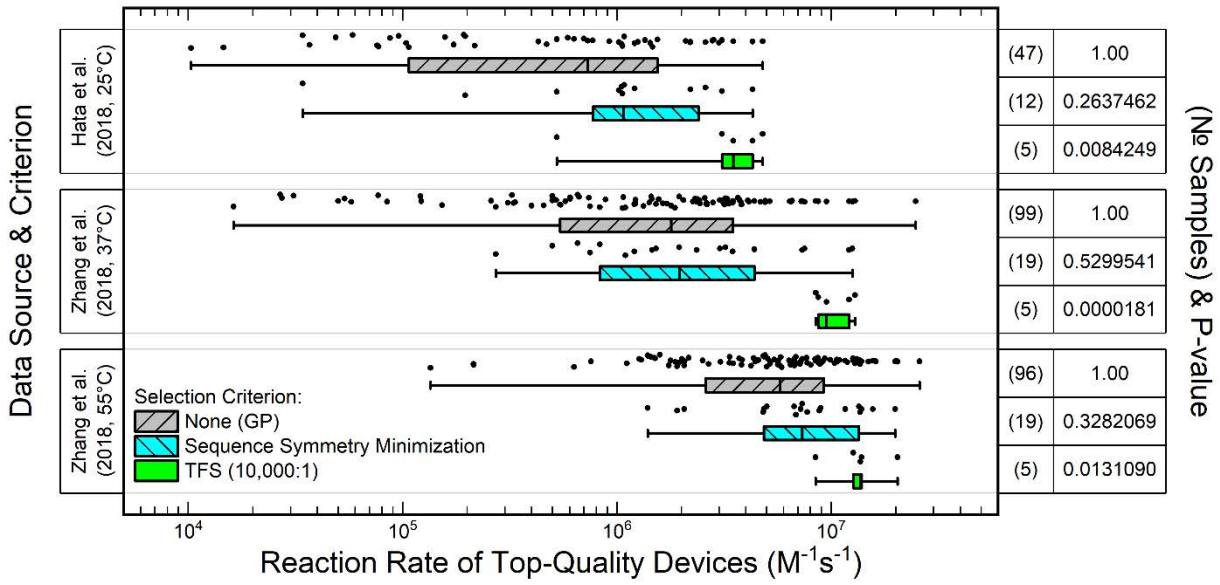

***Figure 1**. Reaction rates present in three large and self-consistent data sets (left axis) were ranked using one of three potential sequence-selection criteria (plot legend). Based on each ranking, the reaction rates of "top-quality" devices were identified and are graphically depicted (black dots, horizontal axis). Each population of devices is statistically summarized using a median line, a box connecting the 25th and 75th percentiles, and whiskers connecting the min and max values. The null hypothesis that the top-quality population was drawn from the same kinetic distribution as the unfiltered general population (GP) was evaluated using a Kolmogorov-Smirnov test. The resulting p-value and the number of samples in each top-quality population are reported to the right of the data.*

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Design automation for DNA origami mechanisms

Chao-Min Huang[1], Anjelica Kucinic[2], Carlos E. Castro[1, 3] and Hai-Jun Su[1*]*

[1]Department of Mechanical and Aerospace Engineering, [2]Department of Chemical and Biomolecular Engineering and [3]Biophysics Graduate Program, The Ohio State University, Columbus, Ohio 43210, USA

*Corresponding authors: castro.39@osu.edu su.298@osu.edu

DNA nanotechnology and scaffolded DNA origami take advantage of programmed Watson-Crick base pairing to construct self-assembled structures on nanometer scale. DNA origami mechanisms coordinate the rigidity of dsDNA and the flexibility of ssDNA to create dynamic structures with desired motions [1]. While designing DNA origami structures can be challenging, recent efforts have led to automated approaches for design [2]; however, these design tools focus on static objects and the rigidity of the components limits their usage in dynamic mechanism designs. Current design processes for DNA origami mechanisms and machines still rely on heavily manual DNA routing approach within caDNAno software [3] that can be highly challenging for multi-component structures. Hence, designing complex mechanisms requires prior expertise or significant training, and although dynamic mechanisms have a range of applications, their broader development is significantly limited by the challenges of design.

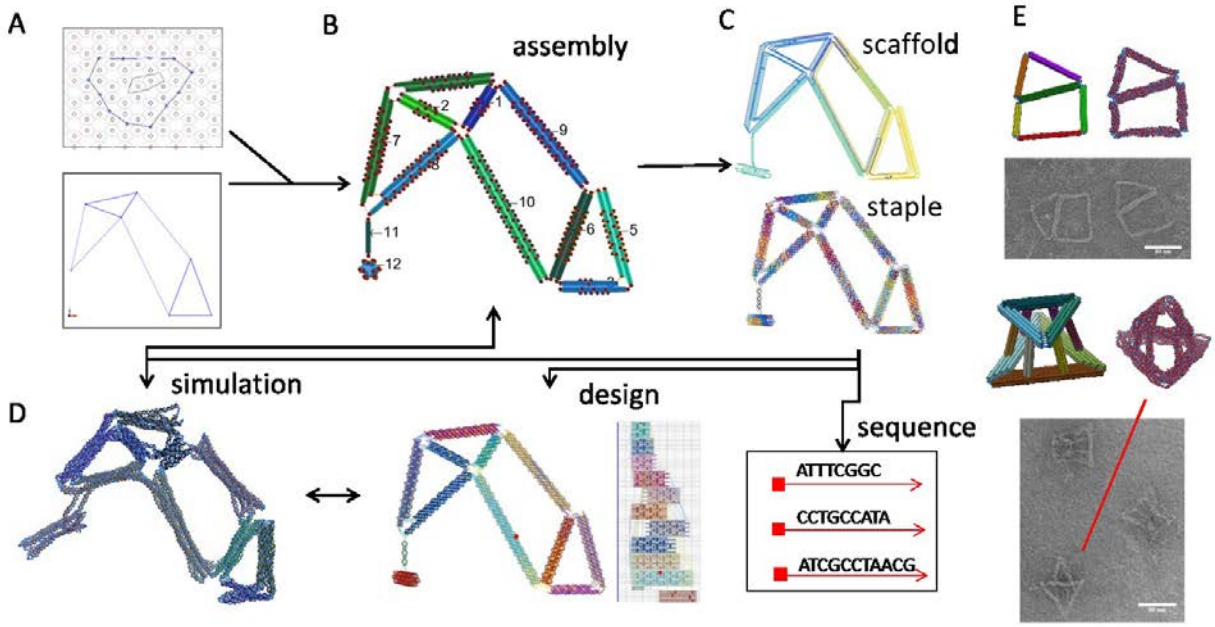

Figure 1 Design process of this software. (A)Start with embedded basic extrusion tool or STEP files from other CAD software. (B) Assemble components in a graphical user interface. (C) Automate the scaffold and staple routing. (D) Result outputs: (Left) oxDNA topology and configuration files. (Middle) caDNAno JSON file and its analogue in the software with 3D mapping for modifying. (Right) staple sequences for experiment (E) Design, simulation and experiment for a four-bar linkage and Stewart platform. Scale bar =50 nm.

As shown in Fig 1, we present a GUI-based design software in which users are able to insert bundles as components with various lengths, cross-sections, and underlying cross-section lattices. The software allows for easy design of multiple components and translation/rotation of individual components to approximate positions. Then, the software automatically (or manually for expert users) specifies connection points between components for flexible or rigid joints. Once the joint connection points have been specified, a graph-theory algorithm is used to generate the overall scaffold routing, followed by automated determination of staple routings as well. Finally, this software is able to export the staple sequences for fabrication and experiments, compatible JSON files for design post-processing or future modification in caDNAno, and oxDNA files for simulation [4] to ultimately facilitate optimizing the design through iterations of simulation and experiment. The software also includes a module to facilitate the design of DNA-based actuation mechanisms.

In addition, the scaffold algorithm has two primary options: the first option is to use the conventional routing where staple crossovers are the major source of constraining structures with occasional scaffold crossovers; the other is to maximize the scaffold crossovers(Fig 2A) so that staples are routed with minimum crossovers, similar to a recent study [5]. Since the staples are almost all straight in the design panel, this design strategy can facilitate the modularity and versatility to create structures that can be folded with many configurations or properties (Fig 2B).

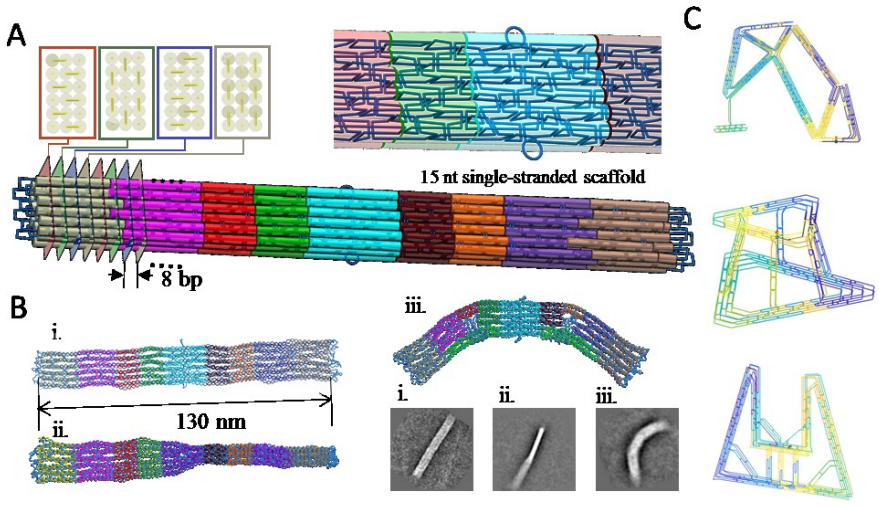

Figure 2 Additional scaffold routing option: maximize scaffold crossovers. (A) Design of a 3×6 square-lattice bundle. (B) Simulations and TEM image average of rigid, twist and bend modes. (C) Apply this strategy to other mechanisms.

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POSTERS

Track on Synthetic Biology

Track Chair

Alex Deiters

University of Pittsburgh

Enhanced Catalysis of a Three-Enzyme Pathway on a DNA Triangle

William P. Klein[1,2] Rasmus P. Thomsen[3], James Vranish[4], Kendrick Turner[1], Mario Ancona[5], Jørgen Kjems[3] and Igor L. Medintz[1,]*

[1]Center for Bio/Molecular Science and Engineering, [5]Electronic Science and Technology Division, U.S. Naval Research Laboratory, Washington D.C. 20375, USA
[2]National Research Council, Washington, D.C. 20001, USA
[3]Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, Denmark
[4]Ave Maria University, Ave Maria, FL 34142, USA
**igor.medintz@nrl.navy.mil*

The field of synthetic biology looks to exploit powerful enzymatic systems found in nature for the efficient production of complex molecules of commercial interest such as pharmaceuticals, specialty chemicals, and solar fuels. Traditionally this has been done *in vivo* which offers many advantages, but drawbacks include the need to maintain the health of the cell, the need to transport reactants and products across the cell wall, etc. An alternative is cell-free synthetic biology where the natural enzymatic system is instead implemented outside the cell. A common challenge to this type of approach is enzyme instability, an effect often mitigated by affixing the enzymes to a solid support. One such support has been DNA, and it has been found that arranging the enzymes on a DNA scaffold can also enhance the turnover.[1-3] To explore this further and hopefully to better understand the underlying mechanisms,[4,5] we here investigate a system consisting of glycolysis enzymes situated on a DNA nanostructure.

Our multienzyme cascade was composed of three enzymes, namely, amylase, maltase, and glucokinase, arranged on a DNA origami triangle.[6] Excitingly, we observed a peak, ~17 fold increase in the pathway's turnover rate. The enzymes were conjugated to the DNA using a previously developed procedure that can be summarized as follows.[7] The enzymes were expressed with six histidines and two lysine residues added using site-directed mutagenesis. An NTA-modified DNA guide strand was added to act as a temporary binding site for a complementary DNA reacting strand that was terminated with an aldehyde group. A covalent bond was then formed between the aldehyde-modified DNA strand and a proximal lysine residue on the enzyme. By using unique ssDNA sequences for each enzyme, the DNA-conjugated enzymes could then be attached to specific staples of the DNA triangle. Different configurations of the three enzymes on the triangle were investigated, with four such arrangements shown in Figure 1a. One set of structures shown in the AFM images in Figure 1b-d varied the number of maltases as this was likely to be the rate-limiting enzyme. The configurations are referred to as 222, 242, and 262, respectively, with the middle number representing the number of maltases present. In addition, we investigated how the separation of the enzyme into a non-proximal conformation affected the turnover rate of the cascade by using the 2S2S2 configuration shown in Figure 1e, where "S" denotes increased enzyme separation.

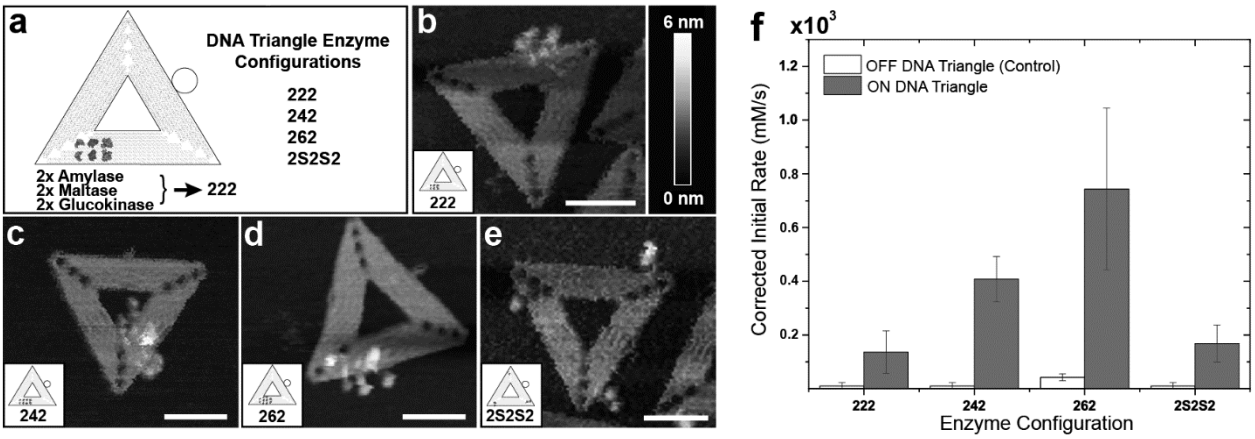

Figure 1 a) A schematic of the DNA triangle enzyme cascade showing the enzyme attachment locations for the DNA triangle consisting of two amylase, two maltase, and two glucokinase. The configuration was abbreviated as 222. A configuration consisting of a total of four and six maltase were also investigated and were referred to as 242 and 262, respectively. A fourth enzyme configuration, where the enzyme was attached at the three triangle corners was also investigated and was referred to as 2S2S2. b-e) AFM images of the 222, 242, 262, and 2S2S2 DNA triangle enzyme cascades. The white dots in the AFM images are the individual enzymes attached to the DNA triangle. f) A plot of the average corrected initial rates for each of the enzyme configuration investigated.

The kinetic assays were performed by recording the formation of NADH (via its absorbance at 340 nm) as a function of time using maltoheptaose as the initial substrate. A bar graph of the initial rates is shown in Figure 1f, scaled by the specific enzyme attachment probability as determined through analysis of FPLC spectra of the dye-labeled enzymes attached to the DNA triangle. The gray and white bars represent the average initial rates for the enzyme configurations with the enzymes either ON or OFF the DNA triangle, respectively. A peak turnover rate was observed, for the 262 enzyme configuration with a ~17-fold increase, despite an average enzyme attachment yield of only ~35%. The overall turnover enhancement is believed to result from a complex interplay of substrate channeling and the formation of a favorable local environment like a hydration layer around the DNA origami nanostructure, resulting in increased enzyme stability and/or retention of the intermediates. Indeed, kinetic simulations that include such retention can account for at least part of the observed enhancement. This work has demonstrated a functional cell-free three-enzyme cascade with potential future applications ranging from complex drug synthesis to the biodegradation of chemical warfare agents.

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Towards synthetic cells using peptide-based reaction compartments

Kilian Vogele[1], Thomas Frank[1], Lukas Gasser[1], Marisa A Goetzfried[1], Mathias W Hackl[2], Stephan A Sieber[2], Friedrich C Simmel[1,3], and Tobias Pirzer[1]

[1]Physics of Synthetic Systems - E14, Physics-Department and ZNN, Technische Universität München, 85748 Garching, Germany.
[2]Departmant of Chemistry, Center for Integrated Protein Science (CIPSM), Technische Universität München, Lichtenbergstrasse 4, 85748 Garching, Germany.
[3]Nanosystems Initiative Munich, 80539 Munich, Germany.

Corresponding author: pirzer@tum.de

Membrane compartmentalization and growth are central aspects of living cells, and are thus encoded in every cell's genome. For the creation of artificial cellular systems, genetic information and production of membrane building blocks will need to be coupled in a similar manner. However, natural biochemical reaction networks and membrane building blocks are notoriously difficult to implement *in vitro*. Here, we utilized amphiphilic elastin-like peptides (ELP) to create self-assembled vesicular structures of about 200 nm diameter. In order to genetically encode the growth of these vesicles, we encapsulate a cell-free transcription-translation system together with the DNA template inside the peptide vesicles. We show *in vesiculo* production of a functioning fluorescent RNA aptamer and a fluorescent protein. Furthermore, we implement *in situ* expression of the membrane peptide itself and finally demonstrate autonomous vesicle growth due to the incorporation of this ELP into the membrane.

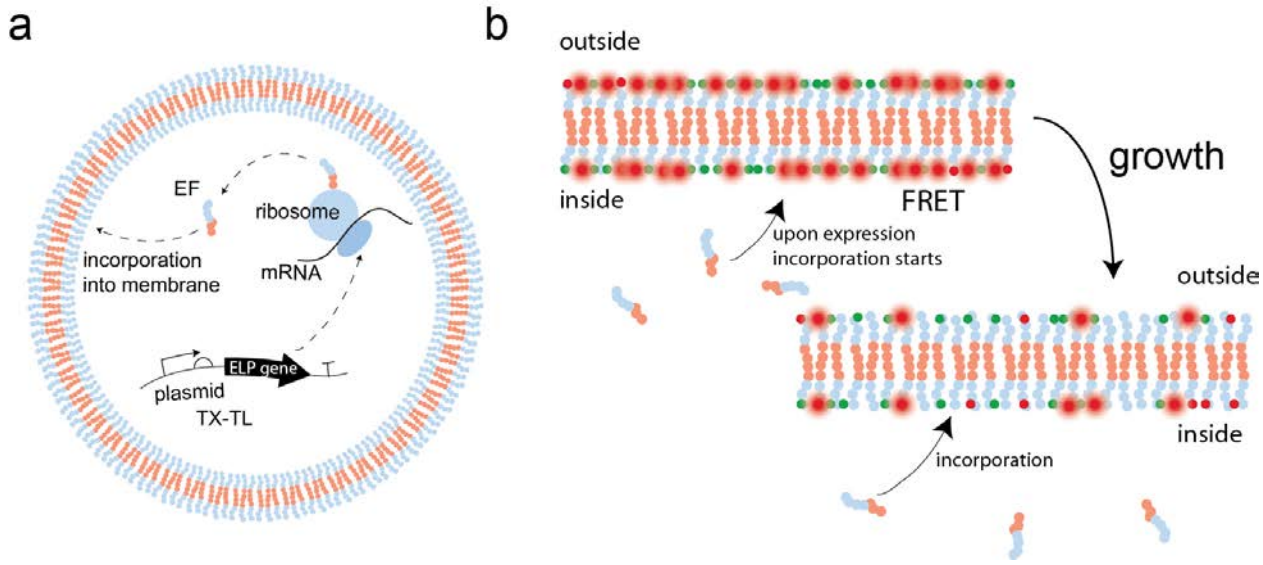

Figure. a, Sketch of the expression of the amphiphilic elastin-like peptide EF inside a vesicle using TX-TL. **b,** Illustration of the FRET assay used. The vesicles formed using Cy5-EF and Cy3-EF. Upon expression of unlabeled EF and its incorporation into the membrane the mean distance between FRET pairs rises and the donor signal increases.

Engineering dynamic nucleic acid nanotubes in cell-sized compartments

Siddharth Agarwal, University of California, Riverside
Melissa A. Klocke, University of California, Riverside
Elisa Franco, University of California, Los Angeles, efranco@seas.ucla.edu

Programmable, synthetic cells have broad applications in sensing and drug-delivery. Current work in the development of synthetic cell components focuses on on membranes and compartmentalization, and as well on developing the minimum required cellular machinery for synthetic cells to carry out different processes [1, 2]. In native cells, cytoskeletal filaments are a key structure for cell division, motility, and intra-cellular transport. Harnessing these filaments for use in synthetic systems is limited by the complexity of the proteins and processes responsible for the dynamic behavior of the filaments. Synthetic tile-based DNA nanotubes, however, are comparable in length and stiffness to cytoskeletal filaments, but can be engineered to demonstrate dynamic behavior while requiring few reacting components in comparison to the native system [3, 4]. To move towards using DNA nanotubes as cytoskeletons in synthetic systems, their dynamic behavior must be integrated with co-transcriptional circuitry and characterized in compartments.

We demonstrate the characterization of nanotubes that polymerize in water-in-oil droplets [5, 6]. These droplets can be produced very quickly, and serve as a simplified compartmentalization system. While the nanotubes exhibit complex networks during polymerization in confinement, nanotubes mature into a more rigid and aligned morphology in under 24 hours. Ongoing efforts aim at controlling nanotube assembly using in vitro transcriptional networks encapsulated and operating in the droplets.

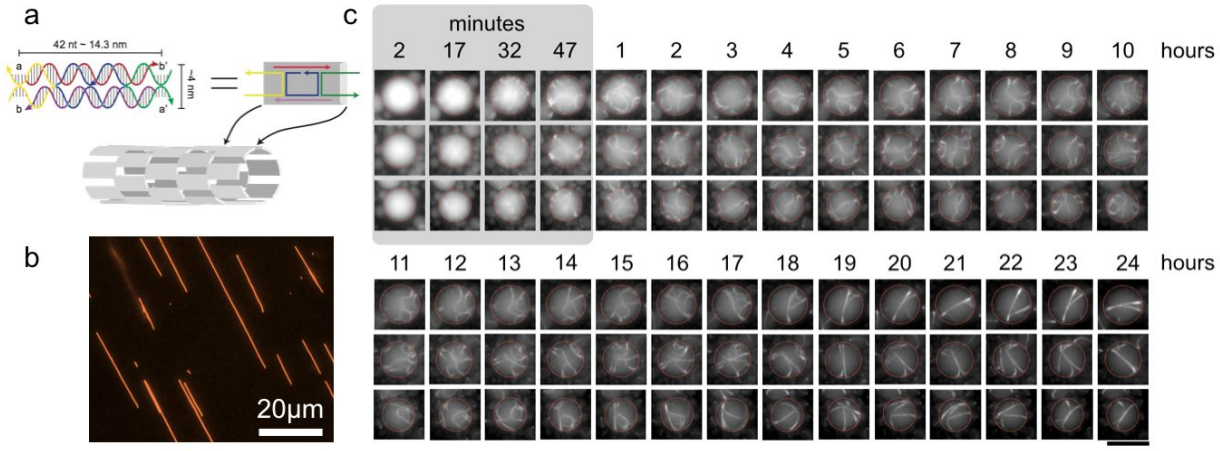

Figure 1. a) Cartoon schematic of tile-based DNA nanotubes. b) Representative fluorescence microscopy image of DNA nanotubes. c) Representative fluorescence microscopy images of DNA nanotubes in water-in-oil droplets. Scale bar: 20 μm.

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Isothermal formation of DNA origamis at room temperature in an unchanging buffer: robust self-assembly through multiple folding pathways

Caroline Rossi-Gendron,[1] Koyomi Nakazawa,[1] Masayuki Endo,[2] Léa Chocron,[1] Mathieu Morel,[1] Sergii Rudiuk,[1] Hiroshi Sugiyama,[2,3] Damien Baigl[1] *

[1] PASTEUR, Department of chemistry, Ecole Normale Supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France
[2] Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa Oiwakecho, Sakyo-Ku Kyoto 606-8502, Japan
[3] Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Yoshida Ushinomaecho, Sakyo-Ku Kyoto 606-8501, Japan

damien.baigl@ens.fr ; caroline.rossi-gendron@ens.fr

When it comes to DNA origami formation, the large majority of research papers use the same method: the M13 template and the staples of a given shape are mixed together in a magnesium-containing buffer (usually TAE 1x + ~10 mM $MgCl_2$) and submitted to a temperature ramp starting with a denaturing step at high temperature and going down to 20 °C. This temperature ramp allows the system to go through several distinct equilibrium states, each of which stable at a given temperature. But what happens when the primitive system (meaning the M13 template and the staples in the buffer, before annealing) is incubated at a constant temperature? Can the system self-organize to create a flawless origami structure after a certain incubation time? In other words, is it possible to form DNA nanostructures by replacing the thermal annealing with an isothermal annealing? A few isothermal protocols have been developed, but they are based either on the addition of organic denaturing agents[1–4] or on the use conventional saline buffers but at a high temperature[5,6].
In this presentation, I will describe experimental results on a new original method for the isothermal formation of DNA nanostructures at room temperature, which does not involve any structural modification of DNA, nor any environmental modification before, during or after the isothermal folding process. I will demonstrate the versatility of this method by describing the formation of different origami shapes at room temperature (25 °C) and at 30 °C in an unchanging buffer. I will also describe the kinetics of the folding process and, as the most significant result, show the first *in situ* observation by AFM of the isothermal folding process at room temperature. This constitutes the first direct characterization and visualization of the folding mechanism(s) of DNA origamis[7].

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POSTERS

Track on Principles and Theory of Self-Assembly

Track Chair

Rebecca Shulman

Johns Hopkins University

Concentration dependent exchange of subunits on DNA origami: a 'fuel-free' design from nature's molecular machines.

*Rokiah G. Alford *[1], James C. Walsh[1], Lisanne M. Spenkelink[2], Jonathan F. Berengut[1], Sophie Hertel[1], Till Böcking[1], Antoine M. van Oijen[2], Lawrence K. Lee[1]*

[1] EMBL node for Single Molecule Sciences, UNSW, Sydney, Australia, [2] School of Chemistry, University of Wollongong, Wollongong, Australia, * Corresponding Author(s): rokiah.alford@unsw.edu.au

Many biomolecular complexes appear stable and well-defined when observed in isolation, but the same complexes have also been observed dynamically exchanging their parts in the presence of free subunits. This phenomenon, known as concentration dependent exchange (CDE), provides a means for biomolecular assemblies to dynamically alter their shape or function without the need for an independent fuel source.

The replisome, responsible for DNA replication, is one such biomolecular machine that uses CDE to swap polymerases when functionally required. A mechanism known as 'multi-site competitive exchange' (MSCE)[1] has been proposed to explain CDE in the replisome: DNA polymerase binds to the replisome via an interaction with the leading DNA strand and to one of multiple binding sites on a helicase subcomplex (fig 1a). In isolation, a single DNA polymerase remains stably bound because the probability of releasing both binding sites simultaneously is low. However, if competing polymerases occupy the additional helicase binding sites (fig 1a(i)), once the initial polymerase releases the DNA strand (fig 1a(ii)), there is equal probability for a competing polymerase to bind to the DNA (fig 1a(iii)) thereby exponentially decreasing the affinity of the initial polymerase (fig 1a(iv)). CDE occurs because occupancy of the helicase binding sites is dependent on the concentration of freely diffusing polymerases.

Here, we construct a synthetic system that uses the MSCE principle with DNA origami (fig 1b&c), and use single-molecule fluorescence microscopy to demonstrate the concentration-dependent exchange of subunits. We further developed mathematical models (fig 1d) to explore how MSCE can allow varied complex phenomena to arise from simple interactions. The versatility of DNA as a building material allows us in turn to rationally control these parameters and directly test predictions to recreate these phenomena artificially.

This research used DNA nanostructures and mathematical models to probe an important biological phenomenon, while simultaneously utilising lessons from natural molecular systems to broaden the design space in DNA nanotechnology.

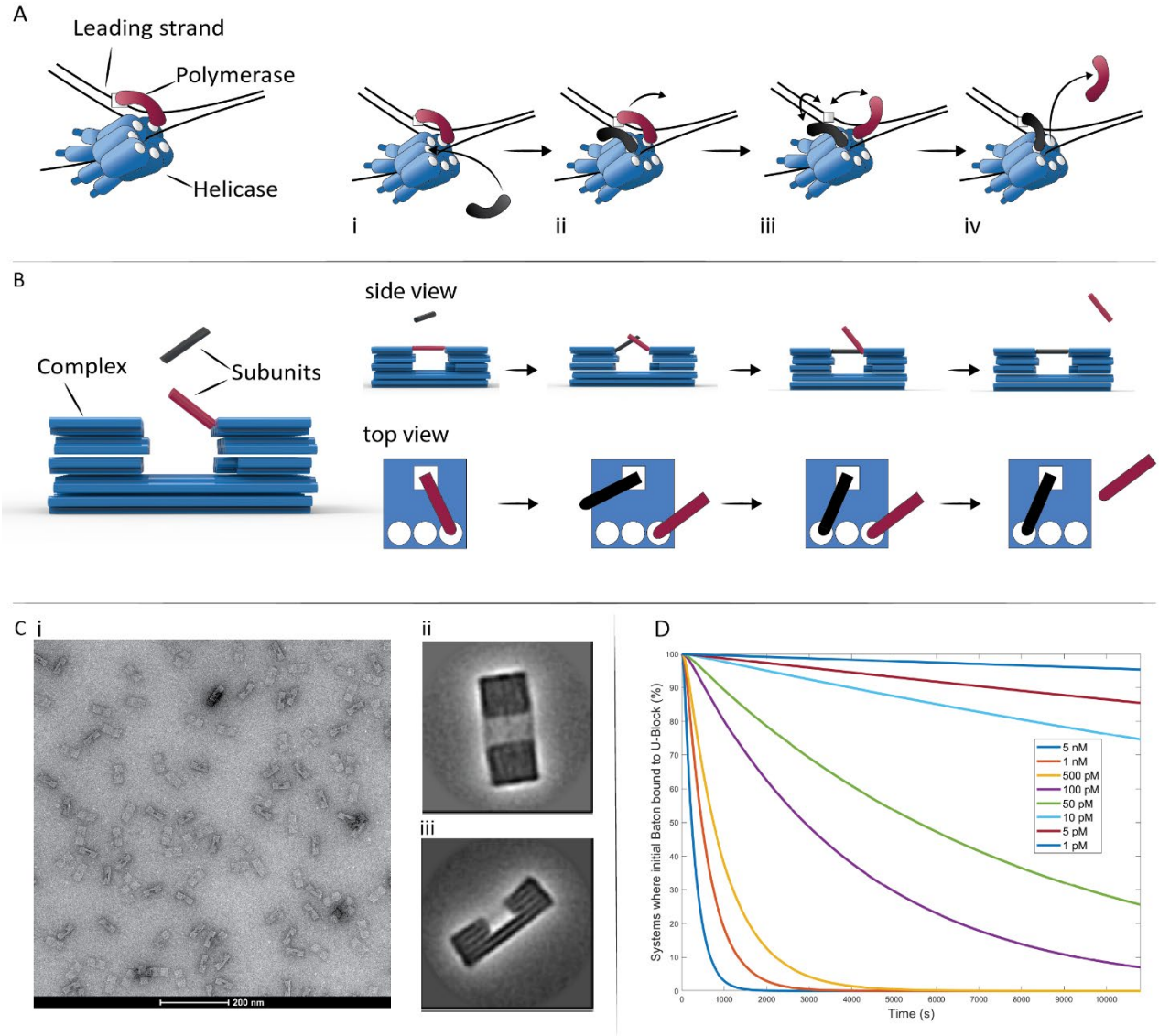

Figure 1. (A) Simplified representation of the T7 bacteriophage replisome performing concentration dependent exchange (i-iv), with the initial and competing polymerases in red and black respectively. (B) Schematic depictions of synthetic multi-site competitive exchange system performing exchange with side and top views, where the initial and competing subunits are also in red and black respectively. (C)(i) Exemplary TEM micrograph of structures (scale bar 200 nm) with (ii) top and (iii) side views from single particle averages. (D) Simulation data predicting that the loss of initial subunits through exchange with competitors over 3 hours would occur in a concentration dependent manner as the dissociation rate of the initial subunit increased as the concentration of competitor subunits increased (1 pM to 5 nM).

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Measuring Energetics and Cooperativity of Origami Folds

Jacob M. Majikes, Daniel Schiffels, Michael Zwolak, Paul Patrone, Anthony Kearsley, Samuel P. Forry, J. Alexander Liddle
National Institute of Standards and Technology
jacob.majikes@nist.gov

Realizing the extraordinary variety and versatility of structural DNA nanotechnology requires reliable rules that connect yield with nanostructure design and processing. Determining these rules is complicated by the cooperativity mediating the thermodynamics and kinetics of folding. Characterizing this cooperativity represents a new challenge as the theoretical and experimental tools developed for biological systems do not readily translate to the folding process in artificial ones.

We begin to address this problem using a simple model system comprising a single fold of the M13 scaffold and explore entropic and enthalpic contributions by varying fold distance, scaffold persistence length, and staple concentration. Real-time Polymerase Chain Reaction (rtPCR) equipment enables the high-throughput measurements needed to test the large experimental parameter space, while a carefully-designed modular fluorescent melt curve reporter system minimizes extraneous signals. In combination with a novel affine transformation technique, melt curve baseline and background correction are improved to a level where robust extraction of thermodynamic parameters becomes possible.

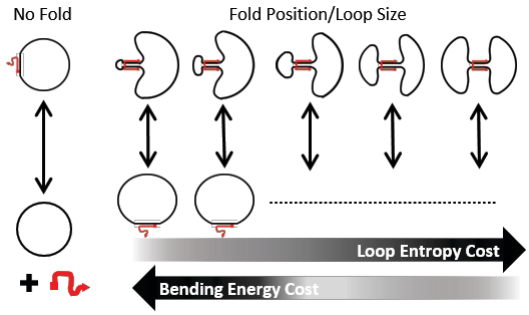

Figure 1. Schematic of folding states used to probe entropic penalties and cooperativity

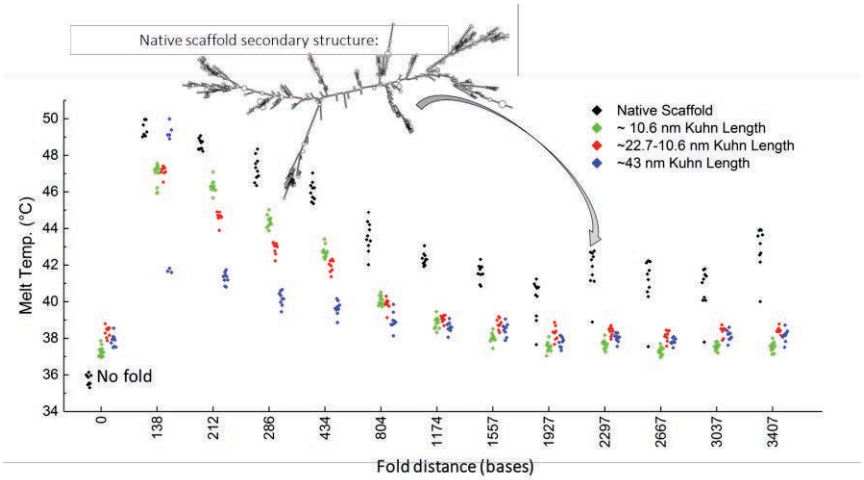

Figure 2. Melting temperature as a function of fold distance and scaffold persistence length.

We find that entropic contributions for folding are in reasonable agreement with extrapolations of existing biological models. More interestingly, we observe that single fold yield decreases with increasing staple concentration, once the concentration reaches a critical value, as shown in Figure 3. This reduction in yield for a single fold in the presence of staple excess is not observed in typical origami containing hundreds of folds. This difference, while not intuitive, is consistent with our understanding of entropic contributions to cooperativity.

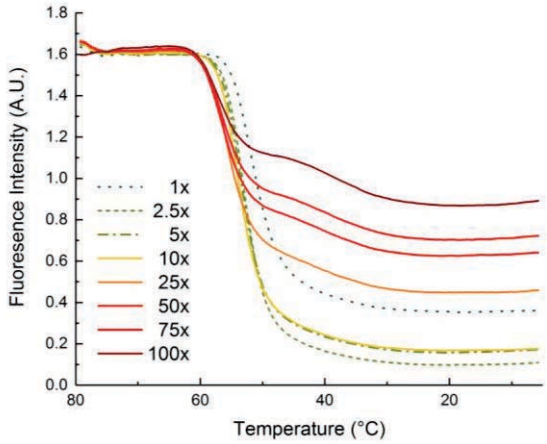

Figure 3. Reporter intensity as a function of temperature and staple excess for a 138 base fold. Scaffold folding reduces reporter intensity while side product formation does not.

POSTERS

Track on Biomedical Nanotechnology

Track Chair

Thomas LaBean

North Carolina State University

Reversible Self-Assembly Guides Phenotype of Cells

Ronit Freeman, Applied Physical Sciences, Adjunct associate professor of Biomedical Engineering, University of North Carolina Chapel Hill, Chapel Hill, NC

Abstract Text:

Soft structures in nature such as protein assemblies can organize reversibly into functional and often hierarchical architectures through noncovalent interactions. Molecularly encoding this dynamic capability in synthetic materials has remained an elusive goal. My talk will describe hydrogels of peptide-DNA conjugates and peptides that organize into superstructures of intertwined filaments that disassemble upon the addition of molecules or changes in charge density. The storage moduli of the hydrogels change reversibly as superstructures form and disappear. Experiments and simulations demonstrate that this response requires large scale spatial redistribution of molecules directed by strong noncovalent interactions among them. Interestingly, when astrocytes were placed on the material made from only simple filaments, the astrocytes had a naïve phenotype, but when the superstructures formed they became reactive. The astrocytes then reverted back to the naïve phenotype when the hierarchical structure disassembled. This discovery links the architecture of the cell's microenvironment to the critical changes of phenotype that occur when the central nervous system is diseased or injured and it gives new ideas on how to undo the scars in injured or diseased brain and spinal cord.

(Freeman *et al*, **Science**, 2018, Vol. 362, Issue 6416, pp. 808-813).

DNA-based nanofabrication for antifouling application

Liwei Hui, Anqin Xu, Haitao Liu*

Nano- and microstructured surfaces recently attracted extensive attention due to their potentials in self-cleaning, antifouling, superhydrophobic / superolephobic, and antireflection applications. The fabrication techniques to produce such surfaces include laser interference patterning, thermo oxidation, plasma etching, reactive-ion etching and patterned deposition. DNA-based nanofabrication is particularly attractive for such applications due to its low costs, access to arbitrary/sophisticated shape, and scalability to large substrate size. Our preliminary data showed that DNA triangle-templated surface could significantly inhibit bacterial adhesion and biofilm growth.

Enzyme-to-DNA Conversion Using Ultra-Small Circular DNAs

H. Bui[1], M. G. Ancona[2], E. R. Goldman[3], Y. Kim[4], D. Mathur[5], I. L. Medintz[3]

[1]National Research Council, Washington DC, USA, [2]Electronic Science and Technology Division, Code 6800, [3]Center for Bio/Molecular Science and Engineering, Code 6900, [4]Material Science & Technology Division, Code 6300, Naval Research Laboratory, Washington DC, USA, [5]College of Science, George Mason University, Fairfax VA, USA.
Email: hieu.bui.ctr@nrl.navy.mil

Abstract: Regular plasmid/circular DNAs with more than several hundred to thousand bases (bp) are abundant in cells where they encode genetic instructions and are important for survivability. Smaller circular DNAs (i.e., less than 100bp) are, however, unknown in nature though they have been studied in the laboratory. In particular, researchers began synthesizing[1] and exploring[2] the utility of ultra-small circular single-stranded DNAs for genetic detection[3] and as catalytic templates for DNA synthesis[4] in the 1990s. An advantage of circular DNAs is that they are completely resistant to degradation by exonucleases[5], and when ultra-small one would expect also resistance to endonucleases. On this basis, we study ultra-small circular ssDNAs as elements for inclusion in nuclease-resistant DNA circuits with potential *in vivo* applications. Specifically, we create a novel enzyme-to-DNA signal converter and then use this in proof-of-concept Boolean gates.

A general scheme to synthesize ultra-small circular ssDNAs is outlined in Fig. 1. A linear ssDNA strand of 80 nucleotides (abbreviated GL) was modified by phosphorylation at the 5' end. A short oligomer (GS) that was complementary to both ends of the GL strand was then hybridized to the GL strand and the nick in the latter was healed using a T4 ligase. Once the ligation was complete, the GS strand was removed using toehold-mediated strand displacement (TMSD), thereby leaving a circular oligomer. Gel electrophoresis and fluorescence-based methods were used to confirm the successful formation of the product as well as to demonstrate nuclease-resistance against *ExoI* and *ExoIII* as shown in Fig.1. Using this general approach we then designed and assembled an enzyme-to-DNA signal converter or transducer as illustrated in Fig. 2. For this purpose, the circular DNA included three distinct functional domains (see Fig. 2): (i) A backbone domain that can form a stable duplex with a substrate strand that serves to stretch the loop, (ii) a cleavage domain that contains a short cleavage site for a specific target enzyme, and (iii) a sequestered domain that remains un-paired and hybridizes to a downstream hairpin if and only if the target enzyme has cleaved the circular DNA. In the absence of the target enzyme, the converter and the hairpin do not hybridize as a result of their closed structures and steric hindrance. But when the target enzyme is present, it cleaves the converter, opens up its closed structure, and allows hybridization with the hairpin to occur by strand displacement. This results in the observed structural changes that are detected by the gel, and by spectral changes that result from dye-labeling the hairpin (molecular beacon) as seen in Fig. 2. To achieve the desired functionality, multiple aspects of the system had to be carefully optimized in both the converter itself and in the hairpin.

Having demonstrated the DNA signal converter, we next looked to exploit it in DNA detection circuits that build on prior work with deoxyribozymes[6, 7], restriction enzymes[8], or enzyme-free via DNA hybridization[9]. One simple way of doing this is to modify the converter itself, e.g., a multiple-input Boolean OR-gate can be realized by choosing the recognition site to be cleavable by isoschizomers. As illustration, we created 2-input OR-gate that detects either or

both *HaeIII* and *BsNI*. A more general approach would be modular in form with multiple enzyme-to-DNA converters in parallel that would transduce the catalytic action into DNA which would in turn serve as inputs to a combinatorial DNA logic. This is illustrated in symbolic form for the simple cases of OR and AND in Fig. 3, and analogous experimental results will be reported at the conference. All of our work has been *in vitro* to date, but the ability to withstand nucleolytic attack could potentially make this a valuable approach also *in vivo*.

Acknowledgment: This work was supported by ONR's Biomaterials and Bionanotechnology Program (Dr. Laura Kienker, Code 34) and NRL's Nanoscience Institute.

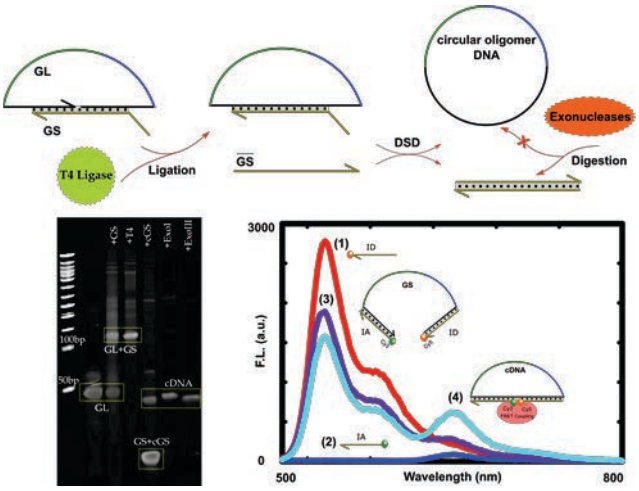

Figure 1. Synthesis and purification of circular oligomers using T4 ligase and TMSD, and verification using gel electrophoresis and FRET.

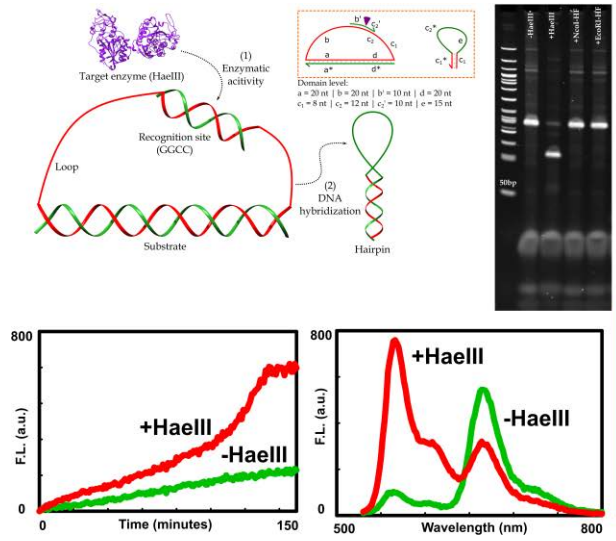

Figure 2. Enzyme-to-DNA converter design capable of converting a target nuclease into a DNA signal. Gel electrophoresis and FRET provide verification.

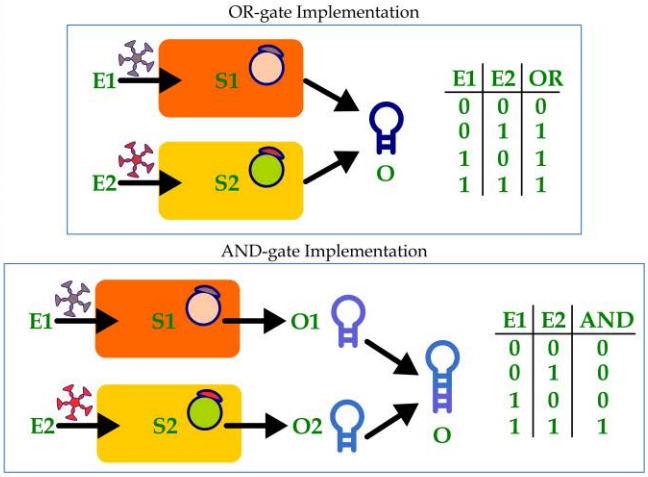

Figure 3. Schematic illustrating the modular combination of converters and DNA logic to form OR and AND gates capable of processing enzymatic information.

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Reconfigurable and Programmable pH-Responsive DNA Origami Nanocapsule for Loading, Encapsulation and Displaying of Cargo

Heini Ijäs[*†‡], Iiris Hakaste[†], Boxuan Shen[†], Mauri A. Kostiainen[†§], and Veikko Linko*[*†§]*
[†] Biohybrid Materials, Department of Bioproducts and Biosystems, Aalto University, 00076 Aalto, Finland
[‡] Nanoscience Center, Department of Biological and Environmental Science, University of Jyväskylä, 40014 Jyväskylä, Finland
[§] HYBER Center of Excellence, Department of Applied Physics, Aalto University, 00076 Aalto, Finland
** Email: heini.e.ijas@jyu.fi or veikko.linko@aalto.fi*

DNA nanotechnology provides a toolbox for creating custom and precise nanostructures with nanometer-level accuracy. In the recent years, a growing interest to add dynamic properties to the otherwise static nano-objects has led to the introduction of various examples of dynamic nanoscale devices based on the DNA origami technique [1]. An intriguing aspect of these devices is the possibility to make them perform predefined tasks when triggered with external stimuli. In biological applications, devices aimed to function as delivery vehicles need to be able to shield and transport their payload until they receive a specific external trigger to release or display the cargo at the targeted cells or tissues. One example of such a biologically relevant trigger for cargo display is a pH change in the environment – such as an elevated cytoplasmic pH, a distinct characteristic of cancer cells [2].

Here we present a dynamic, pH-responsive DNA origami nanocapsule for the encapsulation and display of various types of molecular cargo [3] (see Figure 1). The design has been functionalized with multiple “pH latches” – triplex-forming pairs of a DNA double helix and a single-stranded DNA. In low pH, Hoogsten interactions between the latch counterparts lock the capsule in a closed state. When the pH is raised above the pK_a of the system (here pH 7.24, and adjustable by the T-A·T base triad content of the latch strands [4–5]), the nanocapsules open. By using Förster resonance energy transfer (FRET) based measurements, we have shown that the opening takes place extremely rapidly after being triggered by a modest pH increase. We have also demonstrated that switching between open and closed states can be performed repeatedly in physiologically relevant conditions [6]. By applying both gold nanoparticles and enzymes as cargo mimics, we have shown that this can be appended into a full cycle of cargo loading, encapsulation, and display while preserving the functionality of the enclosed molecules.

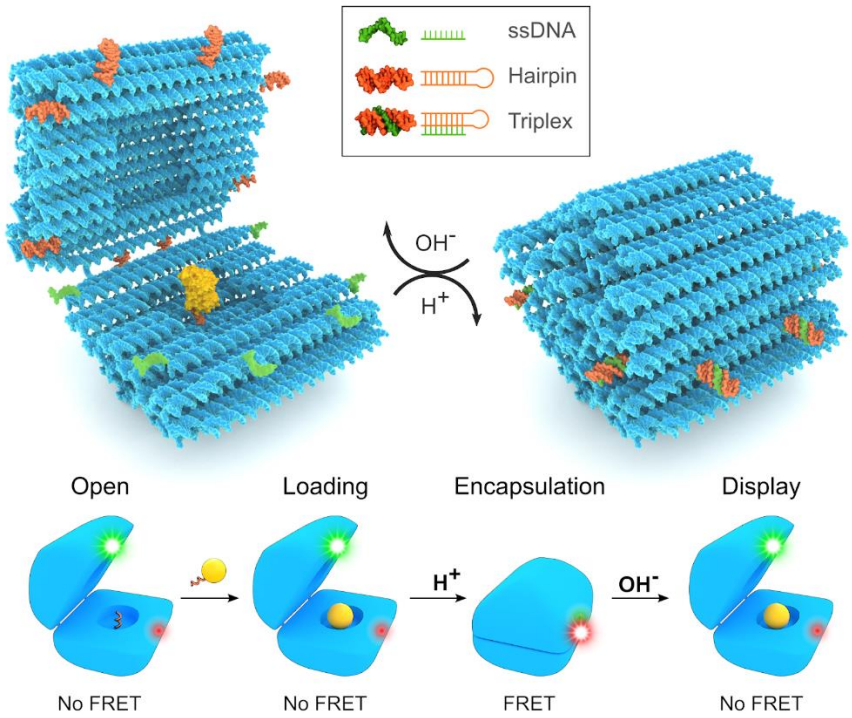

Figure 1. **DNA origami nanocapsule design and function.** *Top panel:* The hinged nanocapsule with a horseradish peroxidase (HRP) payload is equipped with eight programmable pH-responsive latches, which hold the nanocapsule closed when pH < pKa. *Bottom panel:* Cargo loading cycle of the nanocapsule, Open/closed state of the structures during each step of the cycle is determined by FRET analysis of fluorescent dyes (green: energy donor, red: energy acceptor) positioned into the opposing halves.

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DNA dendrimer-coated DNA nanostructures

Youngeun Kim[1,2,*], *Peng Yin*[1,2]
[1]*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston MA 02115*
[2]*Department of Systems Biology, Harvard University, Boston MA 02115*
* youngeun.kim@wyss.harvard.edu

Though DNA structures have been demonstrated in a few bio-related applications, maintaining the structural stability of such DNA architectures in biological conditions still remains a significant challenge.[1,2] The shape and size of DNA origami were considered as important factors that influence the sturdiness of such structures when exposed to the presence of nucleases and low concentration of divalent cations.[3,4] Consequently, multiple strategies were studied to stabilize DNA structures via incorporation of chemical moieties, exposure to UV light, or surface coating with lipids or polymers.[5-9] However, most methods still cannot guarantee stability in biological serum (e.g. 10% FBS) for longer than 24 hours and/or the entire outer surface of the DNA structure becomes entirely coated such that accessing individual DNA strands becomes difficult. In this work, we present a strategy to controllably modulate the surface density of DNA brick nanostructures by programmably locating overhang sequences and attaching "brush-like" DNA dendrimers to coat the outer surface of DNA brick nanostructures. As a result, higher surface density leads to better structural stability and the "brush-like" DNA strands can become locked onto DNA brick structures for further stabilization.

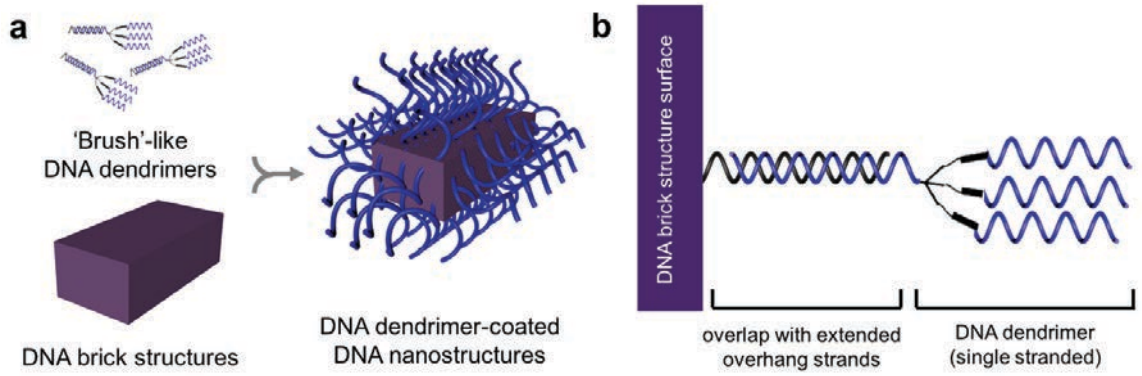

Figure: (a) 'Brush'-like DNA dendrimers can be added to DNA brick structures to densely coat the outer surface of DNA nanostructures. (b) DNA dendrimers attach to DNA brick structures by forming a complementary duplex with the overhang sequence.

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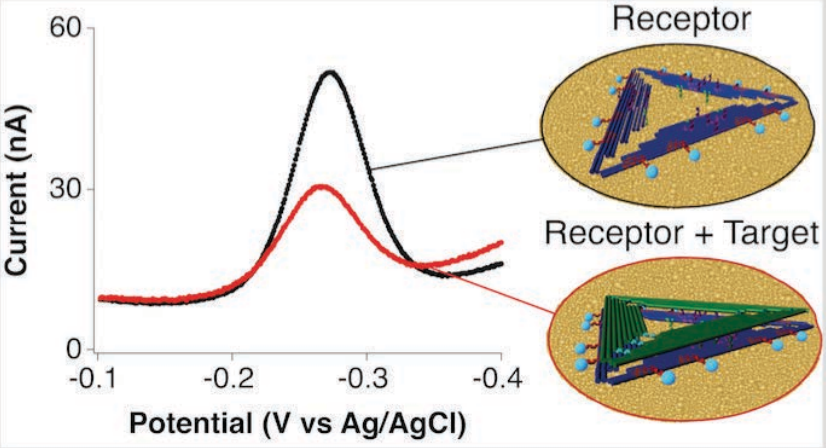
An Electrochemical Biosensor Exploiting Conformational Changes in Electrode-Attached DNA Origami to Detect Hundred Nanometer-Scale Targets

Netzahualcóyotl Arroyo-Currás,[†] Muaz Sadeia,[‡] Alexander K. Ng,[‡] Yekaterina Fyordova,[‡] Natalie Williams,[‡] Tammy Afif,[‡] Nathan Ogden,[§] Roberto C. Andresen Eguiluz,[§] Kevin W. Plaxco[§] and Philip S. Lukeman[‡]*

‡ M Sadeia, AK Ng, Y Fyordova, N Williams, T Afif, PS Lukeman*
Department of Chemistry, St. John's University, Queens, NY 11439, USA . E-mail: lukemanp@stjohns.edu
†N Arroyo-Curras. Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
§ N Ogden, RC Andresen Eguiluz, KW Plaxco. Department of Chemistry and Biochemistry, Center for Bioengineering and Department of Chemical Engineering, University of California Santa Barbara, Santa Barbara, CA 93106, USA

Abstract: The specific detection in clinical samples of "mesoscale" analytes with dimensions in the tens to hundreds of nanometers, such as viruses and large proteins, would improve prospects for disease diagnosis. Detection of these analytes (as opposed to their sub-components), is challenging, as it requires the simultaneous binding of multiple recognition sites spaced over tens of nanometers. We have thus adapted DNA origami, with its unparalleled customizability to precisely display multiple target-binding sites at this scale, to an electrochemical biosensor platform. Our proof-of-concept employs triangular origami covalently attached to a smooth gold electrode and functionalized with redox reporters; electrochemical interrogation successfully monitors mesoscale target-binding-induced changes in electron transfer from the reporters. This approach enables the specific detection of analytes displaying recognition sites that are separated by ~ 40 nm, a spacing significantly greater than that achieved using antibodies or aptamers.



POSTERS

Track on DNA Nanostructures II: Semantomorphic Science B

Track Chair

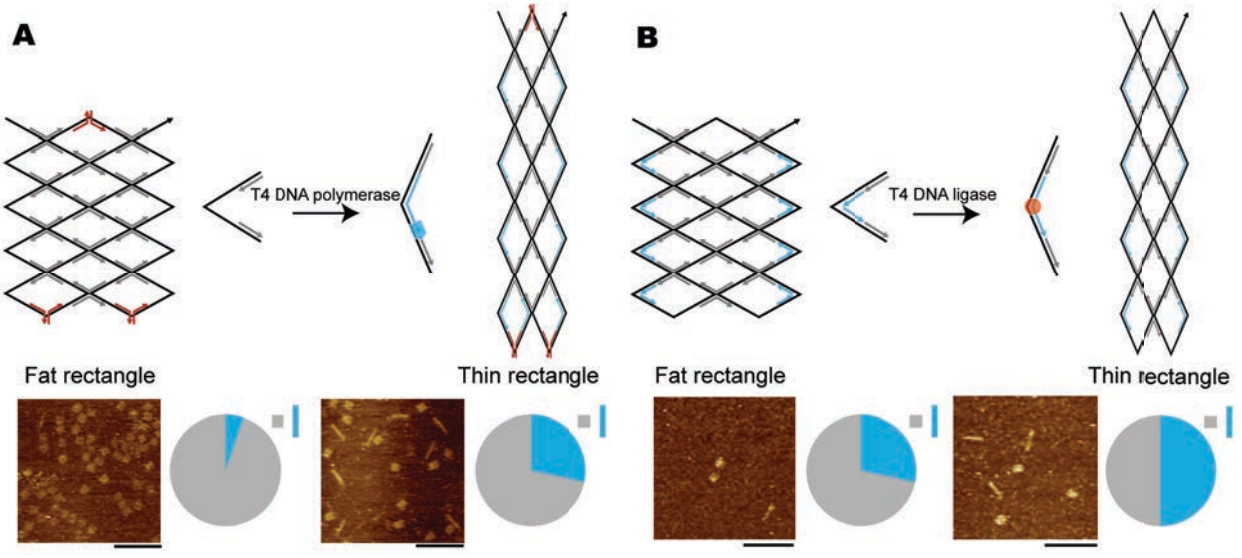
Nadrian Seeman

New York University

Conformational isomerization of DNA nanostructures based on enzyme treatment

Qi Yan, Yaqi Wang, Bryan Wei*
School of Life Sciences, Tsinghua University-Peking University Center for Life Sciences, Center for Synthetic and Systems Biology, Tsinghua University, Beijing 100084, China
*Correspondence and requests for materials should be addressed to B.W. (email: bw@tsinghua.edu.cn)

In a carefully designed DNA origami nanostructure, the local conformational change of a few individual junctions can lead to a global conformational isomerization[1,2]. In our dynamic DNA nanostructure system, two conformational isomers with different aspect ratios, the fat rectangle and the thin rectangle, are available. According to our earlier investigation, the inclusion of a chosen set of conformational tie staples results in one of the conformational isomers, and the isomerization between two conformations can be achieved by strand displacement. Here we show that the conformational isomerization can also be triggered by enzyme treatment[3]. When enzymes widely used in molecular cloning, such as DNA polymerase and DNA ligase, are applied to trim the tie staples, conformational isomerization is presented and the corresponding morphological changes are characterized by atomic force microscopy imaging.



Summary Figure. Schematics of conformation isomerization based on enzyme treatment. The enzymatic treatment on tie staples (shown in blue) results in fat rectangle (left) changing to thin rectangle (right). (A) Conformational isomerization based on T4

DNA polymerase treatment. (B) Conformation isomerization based on T4 DNA ligase treatment. Scale bars: 500 nm. The pie charts illustrate the population percentage of the two conformational isomers.

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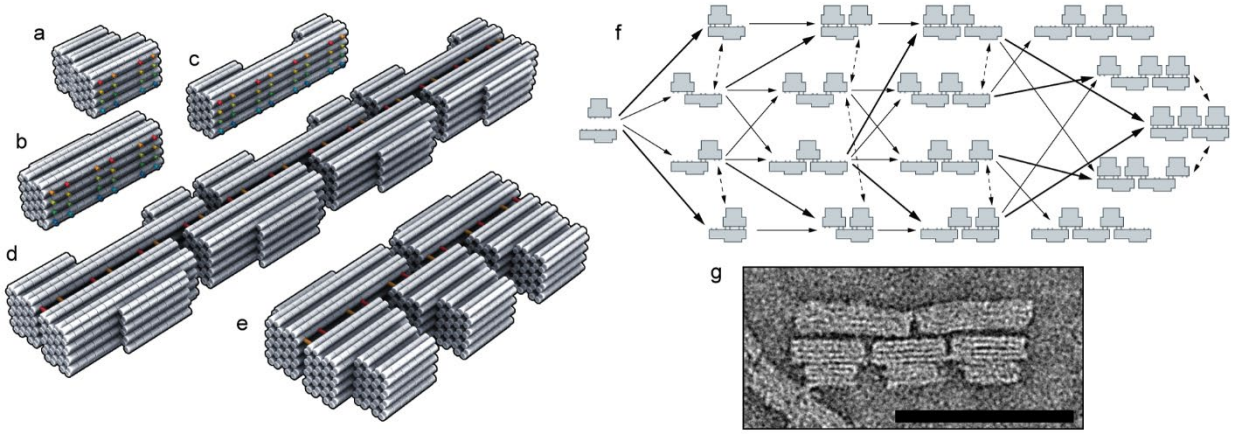
Vernier Assembly of Multi-Subunit DNA Origami Nanostructures

Jonathan F. Berengut (jberengut@gmail.com) & Lawrence K. Lee
EMBL Australia node for Single Molecule Science, UNSW Sydney, Australia

Methods for the synthesis of self-limiting, multi-subunit DNA origami structures allow for nanoscale assemblies with greater dimensions and versatility. One such method that is yet to be explored with DNA origami is the use of Vernier assembly to construct polymers of fixed length from different subunits. The versatility and tuneability of DNA origami allows for the exploration of parameters that are important for increasing the yield of fully-formed Vernier assemblies. These include the affinity of binding sites and the inclusion of cooperative elements as well as assembly conditions such as subunit stoichiometry, salt concentration and temperature. In this study, we construct several types of DNA origami subunits with different numbers of binding sites and use electrophoretic assays and electron micrographs to explore the yields of correctly-assembled finite polymers. This work will shed light on the design and assembly pathways of complex multi-subunit nanostructures.



a Schematic of the 2-site Vernier DNA origami subunit, with double-stranded DNA domains depicted as cylinders. This nanostructure has two binding sites, each comprised of an array of single-stranded staple extensions. The binding sites have tuneable affinity by varying the number and length of staple extensions. **b** The 3-site Vernier subunit. **c** The 4-site Vernier subunit. **d** Schematic of a 4:3 Vernier assembly comprised of four 3-site subunits and three 4-site subunits. **e** Schematic of a 3:2 Vernier assembly comprised of three 2-site subunits and two 3-site subunits. **f** Exploration of assembly pathway of 3:2 Vernier assembly. **g** TEM micrograph of a correctly-assembled 3:2 Vernier assembly (Scale bar: 100 nm).

DNA origami tubes with reconfigurable cross-section

Anjelica Kucinic[2], Chao-Min Huang[1], Hai-Jun Su[1] and Carlos E. Castro[1, 3*]*

[1]Department of Mechanical and Aerospace Engineering, [2]Department of Chemical and Biomolecular Engineering and [3]Biophysics Graduate Program, The Ohio State University, Columbus, Ohio 43210, USA

[*]Corresponding authors: Castro.39@osu.edu, Su.298@osu.edu

Structural DNA nanotechnology, more specifically DNA origami has enabled the precise design and construction of complex nanoscale structures. The DNA origami self-assembly process has also been leveraged to create devices with a variety of functional dynamic and mechanical properties. Advanced 3D dynamic devices include objects with 1D motion such as hinges or pistons, and mechanisms with more complex motion such as crank-sliders or Bennet linkages with programmed kinematic motion or actuation capabilities[1]. A number of recent efforts have expanded DNA origami to larger scales through hierarchical assemblies that integrate many individual structures in a controlled way[2,3,4,5], and prior efforts have demonstrated integration of reconfigurable constructs into arrays[2,3,4,5]. However, the reconfigurability and complex motion of higher order assemblies remains significantly limited compared to individual devices. To address this challenge, we developed a reconfigurable 6-component DNA origami mechanism (DOM) that can be reconfigured into several different shapes and can be assembled into a linear array such that the shape of the device forms the cross-section of the array. Our goal is to demonstrate the reconfiguration of DNA tubes into a variety of stiff cross-sections, which can also exhibit a range of mechanical properties such as anisotropy.

The reconfigurable mechanism consists of a 6-bundle structure where each bundle comprises 12 dsDNA helices arranged in a 3x4 square lattice cross section. These 6 bundles are connected via ssDNA hinges to form a closed loop mechanism (Fig 1A). The initial configuration is flexible and contains 172nt-long scaffold loops on each bundle. These scaffold loops are present to enable formation of struts, which determine the shape of the mechanism. Additional staples are added that connect two scaffold loops to form a strut that connects two bundles (Fig. 1B). The struts can create bundle angles of 90º, 120º, 160º, and 180º. Each folded configuration contains three full struts. Varying bundle angles and the connectivity between bundles (via struts) can create different geometries from the initial configuration (Fig. 1C). In this work, we folded a rectangle, triangle, hexagon, and compacted configuration. The

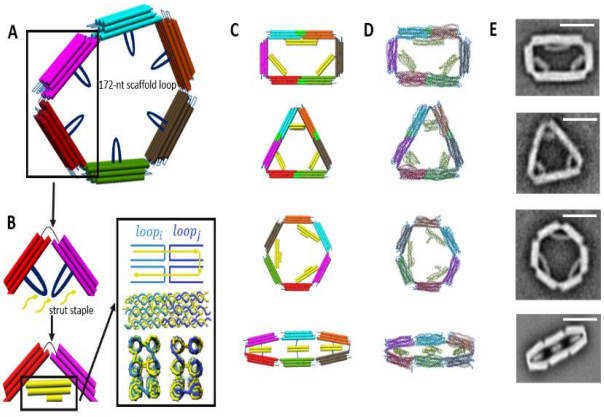


*Figure 1: Design of 6-Bar. **A** depicts the 6-bar structure with 6 bundles and 172nt scaffold loops. **B** depicts a detailed view of the formation of the truss staples with the scaffold loops. **C** depicts cylinder models with scaffold routing of four geometries. **D** OxDNA simulations of the 6-bar geometries. **E** TEM image averages of the 6-bar geometries. Scale bars = 50nm.*

rectangle and triangle configuration contain a 180º truss, therefore we have added setting staples to fortify the two co-linear bundles.

Each geometry is capable of forming a higher order assembly via the addition of polymerization strands that connect individual structures into an array (Fig. 2A). Each bundle contains 12 overhangs, 6 overhangs on the front face and 6 overhangs on the back face of the bundle. The overhangs are either 8 nt or 12 nt long and allow for the configurations to polymerize into geometrical tubes through the addition of polymerization strands (Fig. 2A, inset). We have folded polymers with the rectangle, triangle, hexagon and compacted configuration on the order of ~10s of individual structures (Fig 2B). Each geometrical configuration can reconfigure to a different geometry with the use of strand displacement, changing the cross section (Fig 2C). Our current work is focusing on optimizing the polymerization and reconfiguration of the tube assemblies.

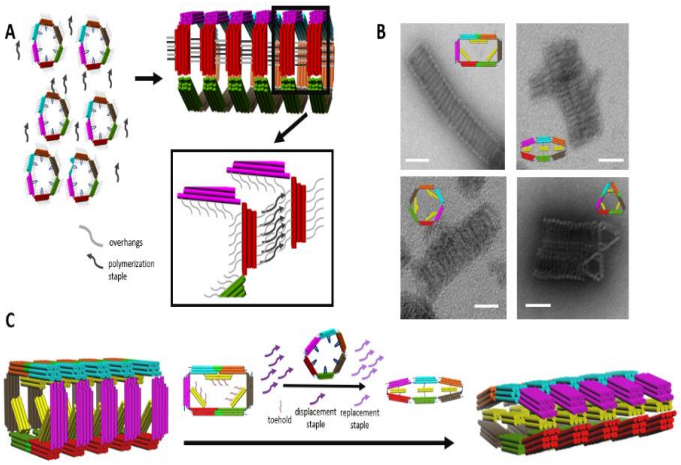


*Figure 2: Polymerization and Reconfiguration of tubes. **A** depicts the polymerization schematic of the 6-bar using 8bp and 12bp overhangs on each bundle. Polymerization strands are added to bind the monomer structures together. **B** TEM images of polymerized 6-bar structures. Top left rectangle, top right compact configuration, bottom left hexagon, bottom right triangle. Scale bars = 50nm **C** Schematic depicting a rectangle reconfiguring to compact configuration. Toeholds are placed on the truss staples, then strand displacement occurs to form an intermediate state. Replacement staples are then used to reconfigure the polymer to a compacted configuration.*

Broadly speaking, this work integrates the design and construction of dynamic DNA origami structures capable of polymerization into higher order structures to make tubes with reconfigurable cross sections. This work expands the current capabilities of complex design and motion at the device scale to enable actuating dynamic DNA origami higher order assemblies and reconfiguration. Future work consists of creating block copolymers consisting of heterogeneous geometries that can reconfigure multiple cross sections.

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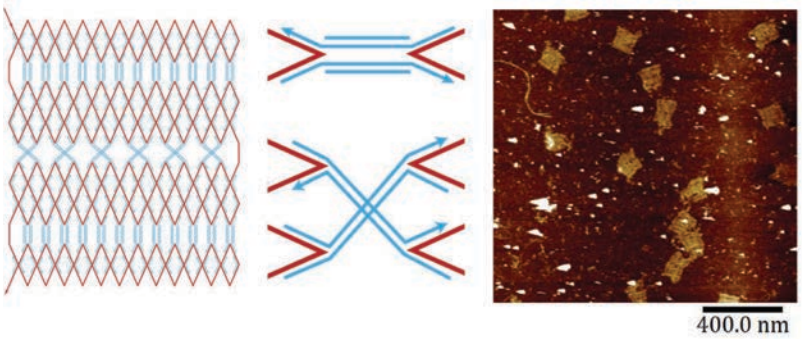
Hybrid Wireframe DNA Nanostructures

*Yan Cui, Jun Yan, Mingxuan Kai, Bryan Wei**

School of Life Sciences, Tsinghua University-Peking University Center for Life Sciences, Center for Synthetic and Systems Biology, Tsinghua University, Beijing 100084, China

**Correspondence and requests for materials should be addressed to B.W. (email: bw@tsinghua.edu.cn).*

Wireframe framework has been adopted in DNA nanostructures recently and a variety of design and construction schemes of scaffolded wireframe origami structures have been demonstrated. However, the scaffold that serves as the key enabler of the resulted structures, also demands design restrictions. For example, there is size and sequence dependence and the routing challenges associated with the scaffold.
In our hybrid approach, scaffolded parts with single duplex helix edges are laid out first, and then scaffold-free components are added to connect the scaffolded parts.
We have designed and constructed two basic scaffolded wireframe junction modules with different numbers of arms around, and applied several types of scaffold-free components for each specific module. As a consequence, there are fewer design restrictions for the hybrid structures thus a much broader design space. Especially, we present multi-layer 3D wireframe structures with simple routing solution in this study, and routing of an origami counterpart would be extremely challenging.



Hybrid wireframe structure with two types of scaffold-free components.

DNA Origami Supports for Protein and RNA Structural Studies

John Powell[1,2], Joshua Temple[3], Yong Xiong[3], Meaghan Sullivan[3,4], Matthew Simon[3,4], and Chenxiang Lin[1,2]

[1]Department of Cell Biology, Yale University, New Haven, CT
[2]Nanobiology Institute, Yale University, West Haven, CT
[3]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT
[4]Chemical Biology Institute, Yale University, West Haven, CT

Corresponding author can be contacted at chenxiang.lin@yale.edu.

Cryo-electron microscopy (cryo-EM) 3D reconstruction is a structural biology approach with mounting promise for characterization of RNAs and proteins – especially those RNAs and proteins with chemical properties that render them uncongenial toward conventional structural biology techniques. However, the quality of any given cryo-EM reconstruction may be limited by the relatively low image contrast of small biomolecules and the presence of disruptive interactions between the molecule of interest and the air-water interface. Here, we outline our progress toward engineering several DNA origami-based supports for structural studies of RNAs and proteins using cryo-EM imaging.

We have produced a DNA origami "Frame" that can capture and hold otherwise low-contrast non-coding RNAs such as GEMM I riboswitch, with the nanodevice serving as a fiducial marker for particle averaging. Class averages of negatively stained TEM images indicate appropriate placement of GEMM I in the DNA origami frame, and we are now transitioning to cryo-EM imaging experiments to gather higher-resolution and native structure class averages and 3D reconstructions from the fiducial-bound RNA.

Additionally, we have also developed multiple DNA origami nanodevices that can polymerize to form "Grid" architectures composed of either square or triangular monomeric subunits and capable of loading assorted proteins of interest. Using this approach, the proteins of interest can be enclosed within the DNA origami array – guarded from structural disruptions stemming from the air-water interface of the cryo-EM grid. Our efforts on this front are now focused on scaling up production of our DNA origami nanodevices for cryo-EM imaging. We are working to characterize to what extent our polymeric structures define the thickness of the ice layer, to what extent the loaded proteins of interest are protected from denaturation by the air-water interface, and whether these changes correlate with alterations to the preferred orientations of our proteins within the ice layer. Furthermore, we are now testing versions of each grid type with varying stoichiometries of protein-loading in order to increase protein density per EM image and to facilitate even more efficient structural analysis.

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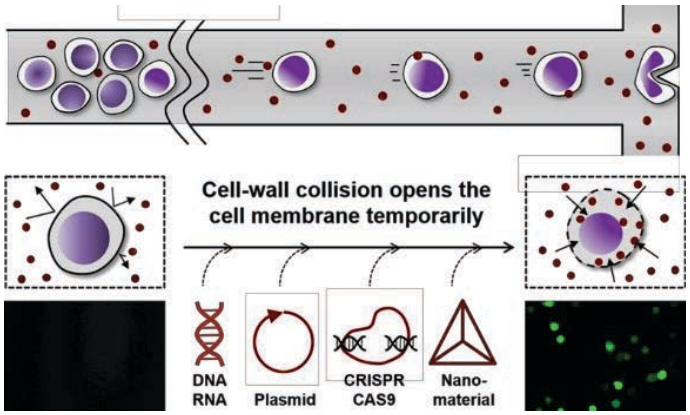
Microfluidic Delivery of DNA Nanostructures and Investigation of *In Vitro* and *In Vivo* Stability

Megan Kizer[1,2], Yanxiang Deng[3], Aram Chung[3*], Robert Linhardt[1,2], and Xing Wang[1,2*]*

[1] Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180
[2] Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180
[3] Mechanical, Aerospace and Nuclear Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180
** Corresponding authors: kizerm@rpi.edu, ac467@korea.ac.kr, wangx28@rpi.edu*



DNA nanostructures have proven to be useful tools for various biotechnological applications including drug delivery, cellular computation, new therapeutics and sensing. For these nanostructures to perform their intended cell-based applications, they must physically enter living cells. This is non-trivial and nanostructure lifetime is reduced by cation availability and nuclease action inside the cell and cell culturing media or blood plasma. Traditional means of delivering these nanostructures to the cell site of action include passive (incubation) and active (electroporation, and viral, lipid, and cationic carriers) methods which suffer from toxicity, inconsistency, and low-throughput. Here we present a microfluidic approach to deliver these nanomaterials to cells without utilizing carrier molecules, electrical fields, or long incubation times. Two microfluidic hydroporators which rapidly and efficiently deliver DNA nanostructures were developed. The devices focus cells at a T-junction or a cross-junction, where compression and shear or fluid inertia and diffusion respectively generate membrane nanopores and allow for cellular delivery of nanomaterials. After successful delivery, we investigate the stability of the nanostructures in surrounding media to validate their lifetime and applicability in the cellular environment.

Figure: Inertial microfluidic cell hydroporator (iMCH) for intracellular delivery of nanomaterials. Schematic illustrating the design and operating principles of iMCH. Cell-wall collision and fluid-shear create nanopores allowing nanocargos to diffuse through.

POSTERS

Track on Molecular Machinery

Track Chair

Andrew Turberfield

University of Oxford

Kinesin-propelled microtubules exhibit collective motion and extended life-time under confinement

Gadiel Saper[*], *Stanislav Tsitkov, Neda Bassir Kazeruni, Henry Hess*

Department of Biomedical Engineering, Columbia University, New York, NY, USA

[*]*Corresponding Author: gs3025@columbia.edu*

Engineering with linear biomolecular motors and filaments is a promising direction for nano-motor technology. One of the applications is an engine constructed from an array of motor protein and filaments, which converts chemical energy directly to a macroscopic amount of mechanical work. There are two main engineering obstacles: (1) aligning and synchronizing the movement of the filaments and motor proteins, and (2) wear of the filaments affecting the engine life time. Here we address these two issues and investigate the collective motion and alignment, as well as the depolymerization of microtubule filaments propelled by the motor protein kinesin under confinement. We perform the gliding assay within a flow cell and control the height of the cell with Convex Lens-induced Confinement (CliC). Our initial measurements indicate that under vertically confined conditions the kinesin-propelled microtubules adapt a collective motion. This swarming behavior likely originates because in the vertically confined conditions, there is not enough vertical space for one microtubule to pass over the other microtubule. Therefore, the collision forces the microtubules to align and move in the same direction. The microtubules also appear to show high stability over time, that is instead of getting shorter they become longer. We suggest that within confinement the tubulins released by shortening microtubules cannot diffuse away into the solution and stay at a high concentration near the surface leading to polymerization of long microtubules. This research suggests that vertical confinement can be used to align and induce microtubules collective motion as well as extend their life-time, which can benefit motor proteins and filaments applications.

Control of DNA Origami Mechanisms and Assemblies via Gold Nanoparticles

Joshua A. Johnson[1], Abhilasha Dehankar[2], Jessica O. Winter[1,2], Carlos E. Castro[1,3,*]

[1]Interdisciplinary Biophysics Graduate Program, [2]Department of Chemical and Biomolecular Engineering, [3]Department Mechanical and Aerospace Engineering, The Ohio State University, Columbus OH, 43210, USA
**Corresponding Author: castro.39@osu.edu*

A major direction of research in DNA origami nanotechnology is the folding of dynamic nanostructures with motion that mimics macroscale machines [1]. One application of these nanomachines is the organization and control of other components such as proteins or nanoparticles (NPs). In particular, the ability to control arrays of NPs provides a promising approach to modulate their interactions and the material properties that emerge from those interactions. This has led to several efforts to organize and control multiple NPs on DNA origami platforms[2]. In these approaches, generally the DNA origami structures are reconfigured while the NPs remain statically attached and are carried along with the structure reconfiguration. Here we demonstrate a distinct approach where the NPs are an integral component that both controls the DNA origami conformation and facilitates rapid thermal actuation. We further integrated the NP-controlled DNA constructs into higher order assemblies so distributed actuation of the DNA devices results in dynamic control of spacing in NP arrays.

Dynamic DNA nanostructures are typically actuated via the addition of DNA "fuel" strands that form or displace connections to reconfigure a structure [3]. DNA origami mechanisms actuated in this manner usually require several minutes to several hours to transition between states. Although recent work has demonstrated that DNA origami can change configuration close to its diffusive limit, these methods still use additives such as salt, which may undesirable and require flow through the system for each actuation step [4]. Other methods for rapid control of DNA origami devices utilize electrical or magnetic fields [5], but these methods are difficult to expand for distributed control of composite assemblies. The goal of this work is to demonstrate a novel actuation method employing nanoparticles as control elements in a thermal actuation scheme for both rapid and reversible control of composite DNA origami-NP devices and assemblies.

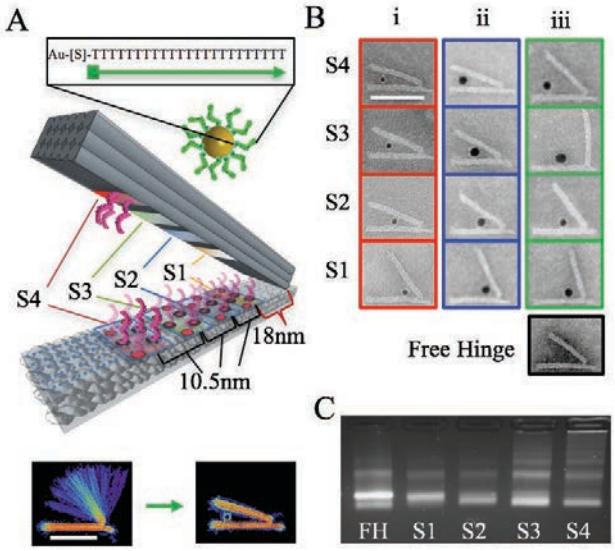


Figure 1 - Controlling DNA origami hinges with nanoparticles. (A) Clusters of overhangs make up various NP binding sites. (B) TEM images showing closed hinges with (i) 5 nm or (ii) 15 nm AuNPs bound to sites 1-4 or (iii) open hinges with 15 nm AuNP barriers. (C)Gel shift of 5 nm AuNP bound hinges compared to free hinges (FH).

Using a DNA origami hinge mechanism, we have included overhangs along the interior surfaces of each hinge arm (Fig. 1A), which bind to a DNA-conjugated gold NP to latch the hinge into a closed configuration. By varying NP binding position and size, we have shown the angular distributions of the hinge are highly controllable as verified by transmission electron microscopy (Fig. 1B, i and ii). Additionally, we have shown that NPs can serve as rigid barriers when bound to only one arm to sterically occlude a range of small angles from otherwise freely fluctuating hinges (Fig. 1B, iii).

We further demonstrated that tuning the NP binding affinity of the bottom arm relative to the top arm allows for actuation of the hinge via DNA melting without releasing the NP entirely. Steady state thermal actuation as well as rapid temperature-jump assay show rapid, reversible, and tunable actuation based on the affinity of latching strands. In T-jump assays we found that NP-hinge composites actuate on the timescale of seconds, which is limited by the rate of heating of the bulk solution.

We have also polymerized hinges into dynamic assemblies, which retain their ability to thermally actuate using nanoparticle as control elements. The nanoscale conformational changes characterized for each individual hinge unit translates into micrometer scale changes for the multiunit assembly. The polymer hinges in combination with effective actuation schemes will establish a basis for rapidly reconfigurable higher-order assemblies.

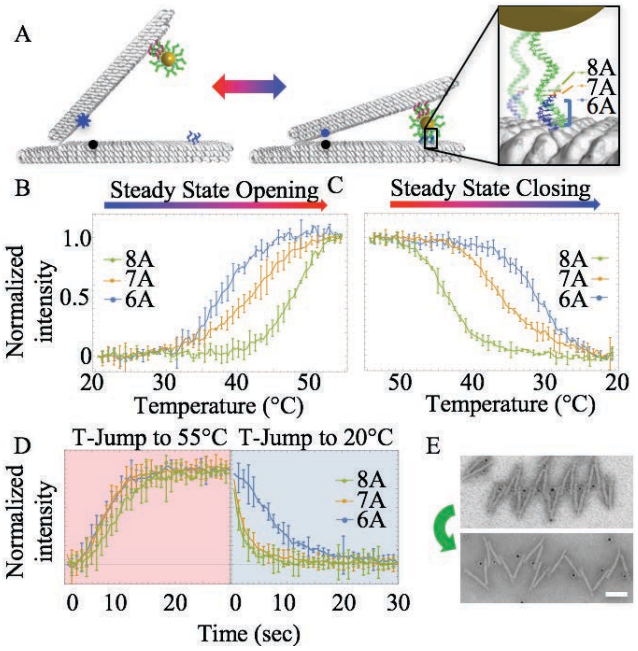


Figure 2 - Thermal actuation of NP-hinge composites. (A) Scheme for detection and tuning of hinge actuation. (B) Steady state opening of hinges with varying bottom overhang lengths. (C) Corresponding steady state closing demonstrates reversible actuation. (D) Rapid temperature-jump assays demonstrating rapid and reversible actuation over many cycles. (E) Actuation of nanoparticle arrays in dynamic hinge assemblies.

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DNA-Templated Protein Assembly: An Engineering Approach to Understanding Nature's Molecular Machines

Andrew Tuckwell[1], *Sophie Hertel*[1], *Chu Wai Liew*[1], *Stephanie Xu*[1], *and Lawrence Lee**[1]
[1]*EMBL Node for Single Molecule Sciences, UNSW, Sydney, Australia*
*Lawrence.Lee@unsw.edu.au

The Bacterial Flagellar Motor (BFM) and Type III Secretion System (T3SS) are mega Dalton scale protein machines that function as a rotary motor and virulent syringe apparatus respectively. Their complexity and efficiency in structure and function epitomise nature's mastery of protein nanotechnology.

This same complexity and scale present challenges in understanding the mechanistic basis of their assembly and function. *In vivo* assembly of these biomolecular machines occurs in an all-or-nothing manner making it difficult to understand the assembly mechanisms of intermediate scale sub-structures at a local level and how they integrate with the larger whole.

Here we present a 'bottom-up' engineering approach to studying the self-assembly of sub-structures of the BFM and the T3SS. DNA nanostructures were designed to mediate the self-assembly of protein subunits by templating them in a configuration that mimics their spatial arrangements *in situ*. Furthermore, the synthetic nature of DNA templates allows manipulation of assembly conditions previously beyond our control.

We demonstrate using electron microscopy that such DNA templates can mediate artificial assembly of T3SS and BFM sub-complexes. Kinetic measurements of templated assembly with surface plasmon resonance allowed the observation of cooperative mechanisms, providing a novel means to probe weak inter-subunit interactions and their structural origin. Finally, protein sub-complexes can be cross-linked non-specifically and analysed with mass-spectroscopy to approximate inter-subunit interfaces and construct pseudo-atomic models from high-resolution crystal structures.

This study demonstrates the use of synthetic DNA templates to probe the molecular mechanisms underlying how complex protein superstructures self-assemble into functional machines from simple chemical interactions.

Catalytic molecular walker teams

David Arredondo[2,3] and Darko Stefanovic[1,2,3]
darko@cs.unm.edu
[1]Department of Computer Science
[2]Nanoscience and Microsystems Engineering Program
[3]Center for Biomedical Engineering
University of New Mexico

We study the motion of random walkers with residence time bias between first and subsequent visits to a site, as a model for synthetic molecular walkers based on catalytic DNA known as molecular spiders [3, 2, 1, 5]. Previous studies have discovered exceptionally long superdiffusive transients, which would be relevant in experiments and applications. The mechanism of the transient superdiffusion was explained via the emergence of a boundary between the new and the previously visited sites, and the tendency of the multi-legged spider to cling to this boundary, provided there is a residence time bias between the first and the subsequent visits to a site. Detailed results were obtained for a two-legged spider with hand-over-hand gait in one dimension. The key insight is that the spider alternates between being on the visited/new boundary (and moving forward ballistically) and drifting in the sea of previously visited sites; with each period this sea becomes bigger, hence the eventual breakdown of superdiffusion.

Approaches for improving the transient and perhaps asymptotic behavior of molecular spiders include modified chemistry, different walker body and leg geometry, and the use of walker teams. Walker teams are inspired by nature's molecular motor teams [6]. Rank et al. [4] provided a detailed analysis of teams of two-legged molecular spiders, on parallel one-dimensional tracks, connected by a "leash", i.e., a kinematic constraint that no two spiders can be more than a certain distance apart. They showed that teams of two, three, and four spiders successively outperform a single spider, for a range of leash lengths and chemical kinetics.

Here we ask: can we separate the effects of having a team of walkers from the effects of each walker having multiple legs? Our model system uses single-legged walkers, each on its own one-dimensional track, connected by a leash. Each track is prepared with fresh substrates for $x \geq 0$, and consumed products for $x < 0$; walkers start at $x = 0$. Using both kinetic Monte Carlo simulation and an analytical approach, we recapitulate the method of [4]. Even though a single one-legged walker does not exhibit directional, superdiffusive motion, we find that a team of one-legged walkers on parallel tracks, connected by a flexible tether, does enjoy a superdiffusive transient. Thus, the essential effects of a walker team are present even when each single team member is a single-legged walker rather than a two-legged spider. Intuitively, the geometric constraint of the leash unites the multiple walkers into a soft-bodied walker with multiple legs.

This material is based upon work supported by the National Science Foundation under grant CCF-1422840.

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APPENDIX

Exciton quantum walks over DNA-assembled dye aggregates – a path to quantum computing?

Bernard Yurke

Department of Materials Science and Engineering and the Department of Electrical and Computer Engineering, Boise State University, Boise, Idaho 83725, BernardYurke@BoiseState.edu

The quantum of energy that a dye absorbs, when it transitions from the electronic ground state to the lowest optically accessible excited state, is referred to as an exciton. This packet of energy exhibits particle-like behavior. An exciton can hop from one dye to another, mediated by transition dipole-dipole interactions between neighboring dyes. This interaction enables an exciton to spread out in a quantum-mechanical wave-like manner over an aggregate of dyes. In addition, excitons undergo two-body interactions mediated by an electrostatic interaction, which in the dipole approximation results from the difference between electronic ground-state and electronic excited-state static electric dipoles. This enables excitons to scatter off of each other. These two interactions are modeled by the Frenkel-exciton Hamiltonian [1], which well characterizes the behavior of excitons on dye aggregates. A system governed by a Frenkel-exciton Hamiltonian is an example of a Bose-Hubbard system. It was shown by Childs et al. [2] that universal quantum computing can be performed as a many-particle quantum-walk on Bose-Hubbard systems. This makes many-exciton quantum walks over DNA-assembled dye aggregates a potential candidate for quantum computing. An advantage this system has over some other proposals for quantum computing is the manufacturability afforded by DNA-assembly. A drawback this system has is the strong coupling of the electronic degrees of freedom to the vibronic degrees of freedom, which causes decoherence. The degree to which this drawback can be mitigated remains to be seen. Here I will describe how dye aggregates can be configured to function as exciton transmission lines and quantum gates and I indicate what the architecture of a many-exciton quantum-walk computer might be. I will also describe work preformed at Boise State University directed toward the assembly of dye aggregates that can function as quantum gates [3-5].

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APPENDIX

Do Active Enzymes Exhibit Enhanced Diffusion?

Jan-Philipp Günther,[†,‡,] Michael Börsch,[§] Günter Majer,[†] and Peer Fischer[†,‡]*

[†]Max Planck Institute for Intelligent Systems, 70569 Stuttgart, Germany

[‡]Institute of Physical Chemistry, University of Stuttgart, 70569 Stuttgart, Germany

[§]Jena University Hospital, Friedrich-Schiller University Jena, 07743 Jena, Germany

**email address: guenther@is.mpg.de*

Enzymes, nature's smallest machines, are the driving force of life itself. Fundamental understanding of the function and motion of enzymes is therefore essential for biology, pharmacy and medicine. Additionally, enzymes can act as inspiration for de novo nanomachines or power nano- and micromotors. It is well known that motor proteins are the molecular engines for muscle action. Similarly, enzymes are motors for the active transport mechanisms found in a living cell. Recently, it has been proposed that an enzyme that freely diffuses through solution can show ballistic motion and an increase in its diffusion constant, when it is catalytically active.[1-4] The main experimental method to study the diffusion of enzymes is fluorescence correlation spectroscopy (FCS). Here, the diffusion of single fluorescently labeled enzymes is observed through a confocal volume of an FCS microscope (Fig. 1a). Typically, the diffusion of enzymes in a solution without substrate (D_0) has been compared to one with substrate (D), which enables the calculation of a diffusion enhancement factor (D/D_0). The observed enhancements were reported to be as high as D/D_0=1.8, and this in turn raises the question how to explain the enhancement and the amount of energy needed to reach such high values.[5-7]

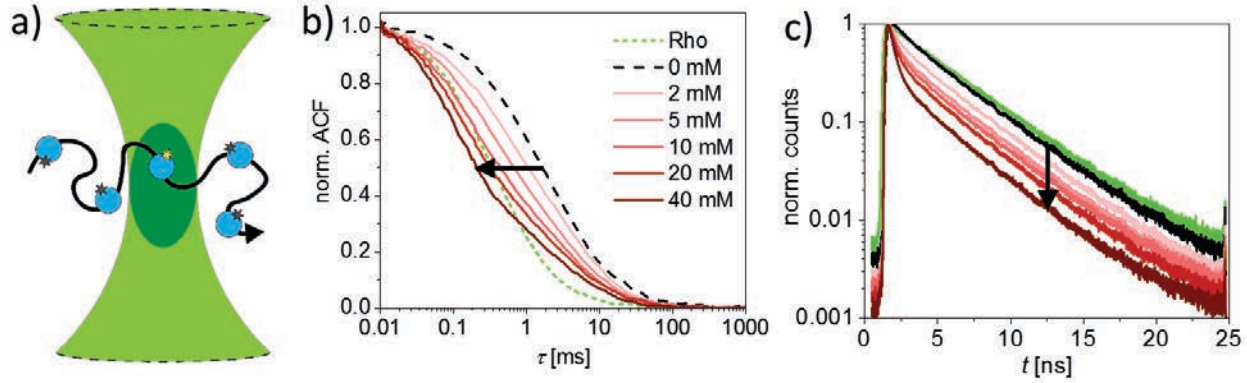

Fig. 1. FCS of phosphatase labeled with Alexa488. a) Principle of FCS. The enzyme (blue) is diffusing freely through the confocal volume (dark green), which excites the fluorophore (asterisk). b) Autocorrelation function (ACF) of phosphatase in presence of various concentrations of NPP shows apparent diffusion enhancement. c) In situ fluorescence lifetime measurements extracted from the measurements in b).[9]

APPENDIX

We were able to show that in the cases of ATPase[8] and phosphatase,[2] the diffusion enhancement upon substrate interaction has been due to artefacts in sample preparation or analysis of the FCS data.[9] For phosphatase we could show that the apparent enhancement reported in ref. 2 is due to quenching of the fluorophore by the enzyme's substrate p-nitrophenyl phosphate (NPP) and not because the enzyme actively propels itself. NPP changes the lifetime of the fluorophore's dark-state and therefore shortens the correlation time in the ACF similar to a diffusion enhancement (Fig. 1b). We were able to support this with in situ fluorescence lifetime measurements (Fig. 1c) and controls including a non-quenching substrate.[9]

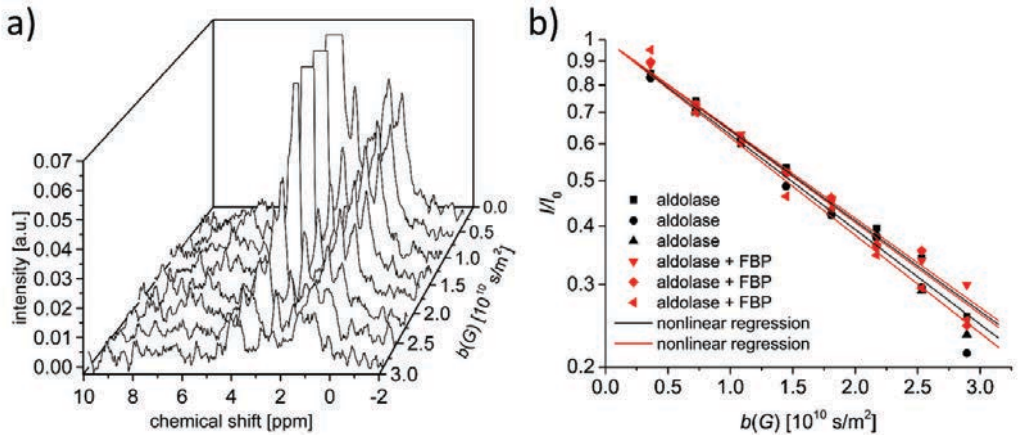

Fig. 2. PFG-NMR Diffusion experiment of active aldolase. a) Exemplary PFG-NMR spectra showing the signal attenuation of aldolase during conversion of its substrate FBP. b) Regression of the attenuation of the aliphatic aldolase signal between chemical shift δ=0.5 ppm and 1.6 ppm. The experiments were performed three times with, and three times without the substrate FBP.[10]

We recently turned to another technique where labeling of the enzyme is not necessary: Pulsed field gradient nuclear magnetic resonance (PFG-NMR). Therefore, with PFG-NMR, photo-physical effects cannot distort the diffusion measurement. We tested for the diffusive behavior of active aldolase,[10] which has been reported to exhibit a diffusion enhancement of up to D/D_0=1.35 even though the enzyme is endothermic.[3] To the best of our knowledge, we were able to report the first diffusion measurement of an active enzyme with NMR. As with phosphatase, no evidence for any diffusion enhancement of aldolase upon activity is seen in PFG-NMR.[10] Possible reasons for the diffusion enhancements reported by others could be artefacts such as dissociation of aldolase oligomers, which has been reported to be substrate concentration dependent.[11]

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