

Abstracts for

# The 21st International Conference on DNA Computing and Molecular Programming (DNA21)

August 17-21, 2015

Wyss Institute for Biologically Inspired Engineering  
at Harvard University,  
Cambridge, Massachusetts, USA

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DNA21

August 17-21, 2015

Wyss Institute for Biologically Inspired Engineering  
at Harvard University,  
Cambridge, Massachusetts, USA

Author names with an underline indicate the presenters of the contributed talks. Those also prefaced with † are student/postdoc presenters who are eligible for the ISNSCE Best Presentation Award. If you believe there is an error, please notify the organizing committee.

Day 1: Monday, August 17<sup>th</sup>

Start Time	Activity	Chair
8:00 am	Registration/Check in	
9:00 am	Introduction to Tutorials - Peng Yin	
9:05 am	<b>Tutorial 1 - Design Three-Dimensional DNA Brick Structures</b> Yonggang Ke (Emory University and Georgia Institute of Technology)	
10:05 am	Break (15 min)	
10:20 am	<b>Tutorial 2 - DNA-PAINT: Super-Resolution Microscopy with DNA Molecules</b> Ralf Jungmann (Max Planck Institute of Biochemistry and Ludwig Maximilian University of Munich)	
11:20 am	Break (15 min)	
11:35 am	<b>Tutorial 3 - NUPACK: Analysis and Design of Nucleic Acid Systems</b> Nick Porubsky (California Institute of Technology)	
12:35 pm	Lunch on your own	
2:00 pm	Opening Remarks - William Shih	
2:15 pm	<b>Plenary Talk 1 - Limits to Polymer I/O</b> George Church (Harvard Medical School)	Ralf Jungmann
3:15 pm	<b>A Rewritable, Random-Access DNA-Based Storage System</b> †Seyed Mohammadhossein Tabatabaei Yazdi, Yongbo Yuan, Jian Ma, Huimin Zhao and Olgica Milenkovic	
3:40 pm	Break (15 min)	
3:55 pm	<b>DNA barrels: Cylindrical NanoPegboards Assembled from DNA</b> †Shelley Wickham, Jianghong Min, Nandhini Ponnuswamy and William Shih	Yonggang Ke
4:20 pm	<b>High-Confidence Amplification-Free Counting of Single Nucleic Acid Biomarkers by Repetitive Probing</b> †Alexander Johnson-Buck, Xin Su, Maria Giraldez, Meiping Zhao, Muneesh Tewari and Nils Walter	
4:45 pm	<b>Simulation-Guided DNA Probe Design for Consistently Ultraspecific Hybridization</b> †J. Sherry Wang and David Zhang	
5:05 pm	Poster Session 1	
6:30 pm	Day 1 close	

Day 2: Tuesday, August 18<sup>th</sup>

Start Time	Activity	Chair
9:00 am	<b>Plenary Talk 2 - Evolution as Learning</b> Leslie Valiant (Harvard University)	Scott Summers
10:00 am	<b>Dominance and T-invariants for Petri Nets and Chemical Reaction Networks</b> †Robert Brijder	
10:25 am	<b>Synthesizing and Tuning Chemical Reaction Networks with Specified Behaviours</b> Neil Dalchau, Niall Murphy, Rasmus Petersen and Boyan Yordanov	
10:50 am	Break (25 min)	
11:15 am	<b>Degradation Controlled Non-linearities in DNA Networks</b> Kevin Montagne, Guillaume Gines and Yannick Rondelez	Jinglin Fu
11:40 am	<b>Microscopic Agents Programmed by DNA Circuits</b> †Guillaume Gines, Anton Zadorin, Teruo Fujii and Yannick Rondelez	
12:05 pm	<b>Universal Computation and Optimal Construction in the Chemical Reaction Network-Controlled Tile Assembly Model</b> †Nicholas Schiefer and Erik Winfree	
12:30 pm	Lunch on your own	
2:00 pm	<b>Plenary Talk 3 - Integrating DNA Origami with Microfabricated Photonic Crystal Cavities</b> Paul Rothemund (California Institute of Technology)	Elton Graugnard
3:00 pm	<b>Toward Fast and Efficient Computer Controlled DNA-Based Molecular Machines: using Single-Molecule Fluorescence and Microfluidics</b> †Toma Tomov, Roman Tsukanov, Yair Glick, Miran Liber, Yaron Berger, Doron Gerber and Eyal Nir	
3:25 pm	Break (20 min)	
3:45 pm	<b>Designing DNAzyme-Based Walkers</b> Jing Pan, Tae-Gon Cha and Jong Hyun Choi	Hareem Maune
4:10 pm	<b>Controllable Ribonucleoprotein Motors Engineered from Myosin and RNA</b> †Tosan Omabegho and Zev Bryant	
4:35 pm	<b>Tuning the Collective Behavior of Myosin Ensembles Using DNA Origami Scaffolds and DNA Nanotubes</b> †Rizal Hariadi, Ruth Sommese and Sivaraj Sivaramakrishnan	
5:00 pm	Poster Session 2	
6:30 pm	Day 2 close	

Day 3: Wednesday, August 19<sup>th</sup>

Start Time	Activity	Chair
9:00 am	<b>Plenary Talk 4 - Exploring the Space of DNA Signatures</b> Lila Kari (University of Waterloo and University of Western Ontario)	Shinnosuke Seki
10:00 am	<b>Reflections on Tiles (in Self-Assembly)</b> Matthew Patitz, Jacob Hendricks and †Trent Rogers	
10:25 am	<b>Optimal Program-Size Complexity for Self-Assembly at Temperature 1 in 3D</b> Scott Summers, David Furcy and Samuel Micka	
10:50 am	Break (25 min)	
11:15 am	<b>Molecular Ping-Pong Game of Life on a 2D Origami Array</b> Natasha Jonoska and Nadrian C. Seeman	Matthew Patitz
11:40 am	<b>Flipping Tiles: Concentration Independent Coin Flips in Tile Self-Assembly</b> †Cameron Chalk, Bin Fu, Alejandro Huerta, Mario Maldonado, Eric Martinez, Robert Schweller and Tim Wylie	
12:05 pm	<b>Efficient Universal Computation by Molecular Co-transcriptional Folding</b> Cody Geary, Pierre-Etienne Meunier, Nicolas Schabanel and Shinnosuke Seki	
12:30 pm	Lunch on your own for attendees (Steering Committee Meeting/Luncheon in conference space)	
2:00 pm	<b>Plenary Talk 5 - Digital Alchemy for Optimized Self Assembly</b> Sharon Glotzer (University of Michigan)	Yossi Weizmann
3:00 pm	<b>Designs and Algorithms for DNA Folding of Custom 3D Polyhedra</b> †Abdulmelik Mohammed, Eugen Czeizler, Erik Benson, Johan Gardell, Sergej Masich, Björn Höglberg and Pekka Orponen	
3:25 pm	Break (20 min)	
3:45 pm	<b>New Geometric Algorithms for Fully Connected Staged Self-Assembly</b> Erik D. Demaine, Sándor Fekete, Christian Scheffer and †Arne Schmidt	Björn Höglberg
4:10 pm	<b>Leader Election and Shape Formation with Self-Organizing Programmable Matter</b> Zahra Derakhshandeh, Robert Gmyr, †Thim Strothmann, Rida Bazzi, Andrea W. Richa and Christian Scheideler	
4:35 pm	<b>Coarse-Grained Modelling of RNA</b> †Petr Sulc, Flavio Romano, Thomas Ouldridge, Christian Matek, Jonathan Doye and Ard Louis	
5:00 pm	Poster Session 3	
6:30 pm	Day 3 close	

Day 4: Thursday, August 20<sup>th</sup>

Start Time	Activity	Chair
9:00 am	<b>Plenary Talk 6 - Production of DNA Nanostructures in Bacteria</b> Chris Voigt (Massachusetts Institute of Technology)	Yannick Rondelez
10:00 am	<b>Guiding Systematic Improvements of a DNA-Actuated Enzyme Nanoreactor through Single Molecule Analysis</b> † <u>Soma Dhakal</u> , Matthew Adendorff, Minghui Liu, Hao Yan, Mark Bathe and Nils Walter	
10:25 am	<b>Leakless DNA Strand Displacement Systems</b> † <u>Chris Thachuk</u> , Erik Winfree and David Soloveichik	
10:50 am	Break (20 min)	
11:10 am	<b>Supervised Learning in an Adaptive DNA Strand Displacement Circuit</b> <u>Matthew R. Lakin</u> and Darko Stefanovic	Andrew Phillips
11:35 am	<b>Automated Design and Verification of Localized DNA Computation Circuits</b> † <u>Michael Boemo</u> , Andrew Turberfield and Luca Cardelli	
12:00 pm	Group photo & Lunch (provided by Guild Biosciences)	
1:10 pm	<b>On Low Energy Barrier Folding Pathways for Nucleic Acid Sequences</b> † <u>Leigh-Anne Mathieson</u> and Anne Condon	Darko Stefanovic
1:35 pm	<b>Stochastic Simulation of the Kinetics of Multiple Interacting Nucleic Acid Strands</b> <u>Joseph Schaeffer</u> , Chris Thachuk and Erik Winfree	
2:00 pm	Board Bus <b>Important information:</b> * Your name badge has designations for the outing and banquet if you signed up to attend one or both. Please understand that due to limited space, staff will be checking badges before boarding buses and before entering the banquet and only registered attendees will be allowed.	
Buses depart 2:20pm from Northwest Building	Social Activity – Boston Harbor Sightseeing Cruise (3-4 pm) Rowes Wharf Harbor Charles Riverboat Company  ~20 minute scenic walk to Banquet (Staff will lead banquet attendees to the banquet hall for dinner. If anyone chooses to take a cab, one can use Metro Cab Boston 617.254.6060 or any taxi on the road. Or Uber/Lyft is a good alternative option.)	
4:30 to 4:45 guests arrive at venue Banquet	Banquet Dinner (three course dinner/bar) The Exchange Conference Center Boston Fish Pier 212 Northern Avenue Boston, MA 02210  Dinner ends at 8:00pm Buses return to Sheraton Commander Hotel 8:00pm (Buses will be outside of the banquet from ~7:45pm. Cruise guests not attending the banquet are free to enjoy the city at their leisure and ride the bus at this location.)	

Day 5: Friday, August 21<sup>st</sup>: Nanoday

Start Time	Activity	Chair
8:25 am	Introduction - Sungwook Woo	
8:30 am	<b>DNA and Nanotechnology</b> Nadrian C. Seeman (New York University)	Kurt Gothelf
9:00 am	<b>Designer DNA Architectures for Programmable Self-Assembly</b> Hao Yan (Arizona State University)	
9:30 am	<b>Programming Functional Structured DNA Assemblies</b> Mark Bathe (Massachusetts Institute of Technology)	
10:00 am	Break	
10:30 am	<b>Self-Assembly of Polymers and Other Molecules Conjugated to DNA</b> Kurt Gothelf (Aarhus University)	Hao Yan
11:00 am	<b>The Frame Guided Assembly</b> Dongsheng Liu (Tsinghua University)	
11:30 am	<b>Creating Combinatorial Patterns with DNA Origami Arrays</b> Lulu Qian (California Institute of Technology)	
12:00 pm	Lunch (provided) & Awards ceremony	
1:30 pm	<b>Knowledge-Driven Design of Probes and Primers for Nucleic Acid Analysis</b> David Yu Zhang (Rice University)	Friedrich Simmel
2:00 pm	<b>DNA Circuitry and NextGen Sequence Informatics</b> Andrew Ellington (University of Texas at Austin)	
2:30 pm	<b>Synthetic Biology: Reprogramming Life</b> James J. Collins (Massachusetts Institute of Technology)	
3:00 pm	Break	
3:30 pm	<b>Nucleic Acid-Based Components for Artificial Cells</b> Friedrich Simmel (Technical University Munich)	Lulu Qian
4:00 pm	<b>Expansion Microscopy</b> Edward Boyden (Massachusetts Institute of Technology)	
4:30 pm	<b>DNA Nanoswitches: From Force Spectroscopy to Instrument-Free Interaction Analysis</b> Wesley P. Wong (Harvard Medical School)	
5:00 pm	Closing Remarks - William Shih and Peng Yin	
5:15 pm	Day 5 close	

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# Tutorial 1: Design Three-Dimensional DNA Brick Structures

*Yonggang Ke*

*Department of Biomedical Engineering at Georgia Tech and Emory University  
Emory School of Medicine*

Recently, we have demonstrated design and assembly of massive, complex, three-dimensional DNA structures using modular building blocks call DNA bricks. Prescribed nanometer-size 3D shapes and micrometer-size lattice with complex features were constructed using this new method. We will talk about design process, software development in this presentation.

## **Tutorial 2: DNA-PAINT: Super-Resolution Microscopy with DNA Molecules**

*Ralf Jungmann*

*Max Planck Institute of Biochemistry and Ludwig Maximilian University of Munich*

Fluorescence microscopy has been transformed by the invention of methods circumventing the classical diffraction limit, namely super-resolution techniques. In this talk, I will first concentrate on applications of these emerging single-molecule techniques to problems in DNA Nanotechnology. In the second part, I will discuss how we use the programmability of DNA molecules to enable DNA-based super-resolution microscopy, termed DNA-PAINT. Applications of DNA-PAINT do include multiplexed target detection, 3D as well as quantitative imaging of synthetic DNA structures as well as cellular targets such as proteins or nucleic acids.

## **Tutorial 3: NUPACK: Analysis and Design of Nucleic Acid Systems**

*Nick Porubsky*

*Department of Chemical Engineering, California Institute of Technology*

NUPACK, the Nucleic Acid Package, is a software suite used for analyzing and designing systems of interacting nucleic acids. This tutorial will demonstrate the capabilities of NUPACK through several relevant case studies. The examples will run using the NUPACK web interface at [www.nupack.org](http://www.nupack.org) to explain the user interface and workflow. This will allow for real time progress monitoring and examination of results for each example.

## **Plenary Talk 1: Limits to Polymer I/O**

*George M. Church*

*Founding Core Faculty Member & Platform Lead, Synthetic Biology, Wyss Institute*

*Department of Genetics, Harvard Medical School*

*Department of Health Sciences and Technology, Harvard and MIT*

Various polymers inspired by biology can be used for construction of atomically precise devices in one to four dimensions. What limits the precision, speed and costs of input and output to and from such polymeric systems? We can capture analog and digital information in the form of nucleic acids and proteins -- this involves much lower energy per bit copied than comparable electronic systems. Interfacing such systems with neural systems is a particularly interesting challenge.

# A Rewritable, Random-Access DNA-Based Storage System

S. M. Hossein Tabatabaei Yazdi<sup>1</sup>, Yongbo Yuan<sup>2</sup>, Jian Ma<sup>3,4</sup>, Huimin Zhao<sup>2,4</sup>,  
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## One Page Abstract:

With the rapid growth of data arising from social networks, communication systems, particle physics, biology and astronomy, one is faced with the need to develop ultra-high density storage systems that will preserve information under volatile conditions. One potential storage media - DNA - stands out in terms of its durability and unprecedented coding rates. Only two working DNA storage systems have been implemented so far, neither of which allows for random access and data rewriting. The first limitation represents a significant drawback for all storage applications, as one usually needs to accommodate access to specific data sections; the second limitation prevents the use of current DNA storage methods in architectures that call for moderate data editing, for storing frequently updated information and memorizing the history of edits.

Moving from a read-only to a rewritable DNA storage paradigm requires major advances in coding methodology, as: a) Editing in the compressive domain may imply rewriting almost the whole information content; b) In the uncompressed domain, rewriting is complicated by the current DNA storage format that involves shifted reads: In order to rewrite one base, one needs to selectively access and modify four consecutive reads; c) The addressing (indexing) methods used in the current DNA storage format only allow for determining the position of a read in a file, but cannot be used to precisely select reads of interest for sequencing/read-out, as undesired cross- hybridization between the target primer and parts of the data sequences may occur.

To overcome the aforementioned issues, we developed a new, random-access and rewritable DNA storage architecture based on DNA sequences endowed with addresses that may be used for selective information access and encoding with inherent error-correction capabilities. Our system uses DNA editing and new constrained coding techniques that ensure data reliability, specificity and sensitivity of access, and at the same time, provide exceptionally high data storage capacity. The coding methods involve error-correcting balancing codes and prefix-synchronized codes. As a proof of concept, we encoded in DNA parts of the Wikipedia pages of six universities in the USA, selected specific content blocks and edited portions of the text within various positions in the blocks. For editing, we used Overlap-Extension PCR and gBlock methods. We Sanger-sequenced the resulting DNA sequences to verify the accuracy of the process, and detected no errors. The results suggest that DNA media may be used in the near future for both archival and rewritable storage applications.

# DNA barrels: Cylindrical NanoPegboards Assembled from DNA

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## ABSTRACT

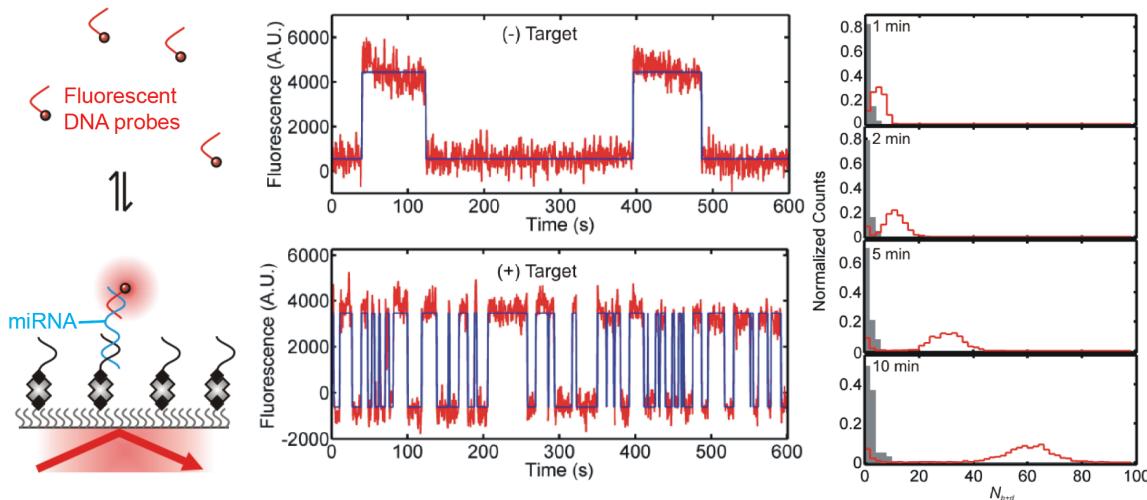
DNA origami, in which a long scaffold strand is assembled with a large number of short staple strands into parallel arrays of double helices, has proven a powerful method for custom nanofabrication. Although diverse shapes in 2D and 3D are possible, one simple shape has remained the most popular for subsequent applications: the single-layer rectangle. One reason is that custom patterns can be created on the surface of the rectangle by functionalizing existing staple strands, without altering the underlying design. The rectangle has many attractive features, such as fast and robust folding and high yield. Modular design of staple strands enables simple abstraction to a regular pixel surface. Here we introduce a family of architectures, DNA barrels built as stacked rings of double helices, that retain the appealing features of the 2D rectangle, but extend construction into 3D, plus reduce unwanted behaviors of the rectangle, such as non-target stacking along the helical ends. We have optimized design parameters for robust folding, and demonstrate versions with outer diameters of 30, 60, 90, and 120 nm. We demonstrated controlled co-axial stacking of these structures, along with addressable decoration with surface functionalities, observed by TEM and DNA-PAINT super-resolution fluorescence microscopy. These structures will provide 3D nanoscale pegboards for future nanoconstruction.

# High-Confidence Amplification-Free Counting of Single Nucleic Acid Biomarkers by Repetitive Probing

Alexander Johnson-Buck<sup>†</sup>, Xin Su<sup>†</sup>, Maria D. Giraldez, Meiping Zhao, Muneesh Tewari, Nils G. Walter

<sup>†</sup>These authors contributed equally to this work.

Due to their dysregulation in many diseases and surprising stability in body fluids such as saliva and blood, microRNAs (miRNAs) have emerged as promising diagnostic biomarkers. However, the most sensitive assays for these biomarkers involve time-consuming sample extraction and amplification by PCR. While single-molecule hybridization-based assays permit sensitive detection without amplification, they typically suffer from significant false positives and finite target specificity. Inspired by the super-resolution imaging technique DNA-PAINT, we introduce kinetic fingerprinting based on Single-Molecule Recognition by Equilibrium Poisson Sampling (SiMREPS) as a novel approach to the amplification-free counting of single unlabeled RNA biomarkers, bypassing thermodynamic limits of specificity and virtually eliminating false positive signals. Monitoring transient binding of fluorescent DNA probes to immobilized analytes using total internal reflection fluorescence (TIRF) microscopy, we demonstrate zero-background single-molecule detection of five exemplary miRNAs as well as more than 500-fold discrimination between two members of the *let-7* miRNA family that differ by a single nucleotide. Through theory and experiment, we show that extending the observation time can yield arbitrary improvements in the specificity of target recognition. We further illustrate the rapid and specific detection of endogenous *let-7* in crude human cancer cell lysate, and quantification of the clinically relevant *miR-141* at the single-copy level in a matrix of minimally treated human serum. High-confidence detection through SiMREPS exploits the high sensitivity of nucleic acid hybridization kinetics to target identity, and may find use in both clinical diagnostics and research.



# Simulation-guided DNA probe design for consistently ultraspecific hybridization

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Hybridization of complementary sequences is one of the central tenets of nucleic acid chemistry; however, the unintended binding of closely related sequences limits the accuracy of hybridization-based approaches to analyzing nucleic acids. Thermodynamics-guided probe design and empirical optimization of the reaction conditions have been used to enable the discrimination of single-nucleotide variants, but typically these approaches provide only an approximately 25-fold difference in binding affinity. Here we show that simulations of the binding kinetics are both necessary and sufficient to design nucleic acid probe systems with consistently high specificity as they enable the discovery of an optimal combination of thermodynamic parameters. Simulation-guided probe systems designed against 44 sequences of different target single-nucleotide variants showed between a 200- and 3,000-fold (median 890) higher binding affinity than their corresponding wild-type sequences. To combat the high oligonucleotide-synthesis costs for the above testing volume, we developed the X-Probe, a conditionally fluorescent nucleic acid probe in which the two functionalized oligonucleotides (fluorescent (F) and quencher (Q)) have sequences decoupled from the SNV/WT sequence. Thus, the same F and Q species could be used for any number of X-Probe designs that target different sequences. From our experiments, the average price of each X-Probe was more than 80% lower than that of a molecular beacon. Furthermore, as a demonstration of the usefulness of this simulation-guided design approach, we developed probes that, in combination with asymmetric PCR amplification, detect low concentrations of variant alleles (1%) in human genomic DNA.

## **Plenary Talk 2: Evolution as Learning**

*Leslie Valiant*

*School of Engineering and Applied Sciences, Harvard University*

Living organisms function according to protein circuits. Darwin's theory of evolution suggests that these circuits have evolved through variation guided by natural selection. However, the question of which circuits can so evolve in realistic population sizes and within realistic numbers of generations has remained essentially unaddressed.

We suggest that computational learning theory offers the framework for investigating this question, of how circuits can come into being adaptively from experience, without a designer. We formulate evolution as a form of learning from examples. The targets of the learning process are the functions of highest fitness. The examples are the experiences. The learning process is constrained so that the feedback from the experiences is Darwinian. We formulate a notion of evolvability that distinguishes function classes that are evolvable with polynomially bounded resources from those that are not. The dilemma is that if the function class, say for the expression levels of proteins in terms of each other, is too restrictive, then it will not support biology, while if it is too expressive then no evolution algorithm will exist to navigate it. We shall review current work in this area.

# Dominance and T-invariants for Petri Nets and Chemical Reaction Networks

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**Abstract.** Inspired by Anderson et al. [J. R. Soc. Interface, 2014] we study the long-term behavior of discrete chemical reaction networks (CRNs). In particular, using techniques from both Petri net theory and CRN theory, we provide a powerful sufficient condition for a structurally-bounded CRN to have the property that none of the non-terminal reactions can fire for all its recurrent configurations. We compare this result and its proof with a related result of Anderson et al. and show its consequences for the case of CRNs with deficiency one.

# Synthesizing and tuning chemical reaction networks with specified behaviours

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We consider how to generate chemical reaction networks (CRNs) from functional specifications. We propose a two-stage approach that combines synthesis by satisfiability modulo theories and Markov chain Monte Carlo based optimisation. First, we identify candidate CRNs that have the *possibility* to produce correct computations for a given finite set of inputs. We then optimise the reaction rates of each CRN using a combination of stochastic search techniques applied to the chemical master equation, simultaneously improving the *probability* of correct behaviour and ruling out spurious solutions. In addition, we use techniques from continuous time Markov chain theory to study the expected termination time for each CRN. We illustrate our approach by identifying CRNs for majority decision-making and division computation, which includes the identification of both known and unknown networks.

## DEGRADATION CONTROLLED NON-LINEARITIES IN DNA NETWORKS

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Cellular information processing is implemented by networks of biochemical reactions. Molecular programming creates conceptually similar architectures *in vitro* with the double goal of i) delivering insights on the inner workings of cellular regulation<sup>1</sup> and, ii) providing amorphous information-processing abilities to man-made chemical systems<sup>2</sup>.

The success of DNA as a molecular programming medium is based on predictable Watson-Crick base-pairing, DNA-to-DNA catalysis, and straightforward interfacing with various physical, chemical or biological signals to be used as inputs, outputs or readouts. These elements can be combined to form chemical reaction networks, where the topology of the network guides the behavior of the whole system. However, the precise kinetic laws of the individual reactions that connect different nodes of the network also play a fundamental role. In particular, the linear or non-linear nature of these interactions strongly affects the network's dynamics.

In order to build versatile and easy-to-model DNA-based reaction networks, we previously introduced a 'DNA toolbox'<sup>3</sup>, a set of reaction that can be arbitrarily cascaded. This toolbox uses only two generic modules encoded by single-strand DNA templates: *activation*, which mimics the basic stimulation of gene expression by a single transcription factor, and the converse process, *inhibition*.

Here we introduce the missing element: an additional mechanism to tune the linearity associated with each individual network vertices: by using species-specific saturable pathways, we can individually adjust the shape of the degradation curve of each DNA species or, equivalently, associate an activation threshold to each node within the molecular circuit. This approach is based on strand design and adds little complexity to the building process. We argue that this provides an efficient, versatile and general way to tune the nonlinearities of network interactions, and hence to access the full functional potential of each topology. We demonstrate this approach through the reprogramming of a single-node homeostatic network into a bistable switch. Further, we prove its versatility by adding new functions to the library of reported molecular systems: an octo-stable system with 3 addressable bits of memory, and the first DNA-encoded excitable system. We also discuss this strategy from a theoretical perspective and provide models in good agreement with the experimental data.

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## MICROSCOPIC AGENTS PROGRAMMED BY DNA CIRCUITS

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Molecular programming (MP) rests on the promise that information can be processed using molecules as carriers and chemical reactions as computational primitives. Among these molecules, DNA offers the robustness and versatility that has already allowed the building of complex molecular systems, many of them being inspired by existing biochemical networks from the living world. However the similarity between *in vitro* and *in vivo* networks does not extend well to the outcome of these computations. Artificial MP output mostly changes in fluorescence, whereas in living systems, the molecular computation actually drive the life and death of macroscopic objects such as cells and organisms. Moreover, a second up-scaling were networks of molecular circuits exchanging chemical signals between groups of cells generate collective behaviors<sup>1</sup> within brains, organisms or ecosystems, brings the result of molecular logic to our everyday, macroscopic level of consciousness.

In this study, we introduce a project that attempts to recapitulate those scale-integration steps: we adapt the solution-phase Polymerase/Exonuclease/Nickase Dynamic Networks Assembly toolbox (PEN DNA) toolbox<sup>2,3</sup> to program the behavior of microscopic particles, themselves embedded in a population of cross-interacting agents. To that purpose, DNA-encoded programs are tethered to microbeads conferring them the ability to receive and send information (as short DNA single strands) but also locally compute according to their own network dynamic. In a preliminary adjustment phase, we highlight a few differences in the chemistry of tethered templates compared to their unbound equivalent and propose offsetting strategies to make supported programs work properly. We then demonstrate that particles functionalized with a positive feedback loop program (PFL encoded by an autocatalytic template) exhibit a standalone reactivity visualized by a sharp and sustained amplification from a single bead in a pseudo-infinite reactor. This autonomous reactivity of particles allows a spatially discrete and time-resolved processing of DNA-encoded programs -a prerequisite to bridge molecular programming with the engineering of mesoscopic collective behaviors. As a first demonstration, we record the cooperative behavior arising from two beads batches, each bearing one part of a split PFL. In this case, the amplification signal production gets correlated to the local beads density, reminding quorum sensing displayed by bacteria or insects. In another development, we used a dynamic bistable mechanism to stabilize the dormant state of agents bearing a PFL. Accordingly, the amplification occurs only if an external stimulus forces a bead above its activation threshold. These bifunctionalized particles now behave as agents with two well-defined states and switch from one to the other only upon detection of a thresholded stimulus. This allowed us to observe a bistable traveling front where the signal is initiated by self-ignition of monostable agents and spread step-by-step across a population of bistable particles. Bistable beads provide a straightforward way to experimentally create heterogeneous signal production and transmission that could be applied to mimic epidemic spread and control, *in vitro*.

The present work demonstrates the successful programming of microparticles by DNA circuits. These achievements open the door to spatially implemented systems and possibly complex networks based on the chemical communication between discrete agents in a heterogeneous environment.

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# Universal Computation and Optimal Construction in the Chemical Reaction Network-Controlled Tile Assembly Model

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**Abstract.** Tile-based self-assembly and chemical reaction networks provide two well-studied models of scalable DNA-based computation. Although tile self-assembly provides a powerful framework for describing Turing-universal self-assembling systems, assembly logic in tile self-assembly is localized, so that only the nearby environment can affect the process of self-assembly. We introduce a new model of tile-based self-assembly in which a well-mixed chemical reaction network interacts with self-assembling tiles to exert non-local control on the self-assembly process. Through simulation of multi-stack machines, we demonstrate that this new model is efficiently Turing-universal, even when restricted to unbounded space in only one spatial dimension. Using a natural notion of program complexity, we also show that this new model can produce many complex shapes with programs of lower complexity. Most notably, we show that arbitrary connected shapes can be produced by a program with complexity bounded by the Kolmogorov complexity of the shape, without the large scale factor that is required for the analogous result in the abstract tile assembly model. These results suggest that controlled self-assembly provides additional algorithmic power over tile-only self-assembly, and that non-local control enhances our ability to perform computation and algorithmically self-assemble structures from small input programs.

## **Plenary Talk 3: Integrating DNA Origami with Microfabricated Photonic Crystal Cavities**

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A wide class of classical and quantum optical devices are based on the coupling of the electromagnetic field to individual atoms, molecules, quantum dots, or other emitters within nanofabricated optical cavities. The coupling efficiency, which can be precisely simulated using numerical codes, is primarily governed by the position of the emitter within the optical modes of the device. For example, the enhancement of an emitter's fluorescence is proportional to the mode-dependent local density of optical states (LDOS) via the Purcell effect. A number of existing experimental techniques variously combine AFM, SEM, and sophisticated lithography to position single emitters within single devices but currently there is no scalable technique to deterministically position emitters within nanooptical environments. This limits our ability to make and study devices based on cavity-emitter interactions---entire papers are often based around the performance of a single, heroically-fabricated device.

Here we experimentally demonstrate the deterministic coupling of fluorescent molecules to photonic crystal nanocavities (PCC) at a large scale. Individual DNA origami molecules carrying discrete numbers of fluorescent molecules are positioned with the resolution of e-beam lithography, in thousands of microfabricated devices. We first validated our approach by taking spectra of 21 different cavities in which each cavity featured an origami placed at a different position along the midline of the cavity. Periodic variation in the peak intensity of the emission demonstrated our ability to control emitter-cavity coupling by "walking" the origami in 50~nanometer steps through the modal pattern of the cavity. Next we used the same technique to create a complete two-dimensional map of one mode of our PCCs with 25 nanometer resolution. For each of 600 precise x-y locations within the mode, a separate device was constructed having a DNA origami at location  $xy$ . The devices are arranged to recapitulate the  $xy$  position of the devices at a large scale, so that simple epifluorescence microscopy creates an "image" of the LDOS. Lastly, we demonstrate the programmability and scalability of our approach by building a 3-bit 65,536 nanocavity array in which the intensity of each pixel is independently programmed by controlling the location and number of molecules within a specific nanocavity.

# **Toward Fast and Efficient Computer Controlled DNA-Based Molecular Machines: using Single-Molecule Fluorescence and Microfluidics**

*Toma E. Tomov\*, Roman Tsukanov, Yair Glick, Miran Liber, Yaron Berger, Doron Gerber and Eyal Nir*

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## **Abstract**

The manufacturing of well-functioning machines is a major challenge of modern technology and science. DNA nanotechnology is probably the most promising path towards realization of this goal on the molecular level. It was demonstrated that DNA based devices and motors could potentially be used in a variety of applications. However, despite variety of architectures and operation principles, devices and particularly motors, still suffer from slow and incomplete stepping reactions along with harmful side products. For the technology to be applicable, it is essential that the devices will operate as commanded, with high operating efficiency and speed, while maintaining structural integrity.

Wishing to understand the reasons for these unwanted reactions and offer solutions, we used a non-autonomous bipedal DNA motor. Upon sequential introduction of DNA fuels and anti-fuels, the motor strides on a DNA track which is embedded on a DNA-origami. We utilize single-molecule FRET/ALEX and TIRF spectroscopy along with computer controlled microfluidics device to study the structure, interaction, kinetic and yield.

We demonstrate that careful analysis of motor kinetics promotes rational design that significantly can improve motor operation over unprecedented distance, processivity and speed. The motor preforms 36 consecutive steps, crossing a distance of 400 nm, with 50% overall yield, which is equivalent to more than 99% yield per chemical reaction, and the stepping rate can be as fast as several seconds per step.

This novel combination of DNA nanotechnology, microfluidics technology and single-molecule fluorescence spectroscopy holds a great promise for future computer control molecular machines.

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## Designing DNAzyme-based Walkers

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Intracellular protein motors have evolved to perform specific tasks critical to the function of cells such as intracellular trafficking and cell division. For example, kinesin and dynein motors transport intracellular cargos to required destinations within a cell, because large intracellular components such as vesicles and mitochondria are too big to diffuse to their destinations. The motor proteins achieve their progressive walking along microtubules by converting the energy from adenosine triphosphate (ATP) hydrolysis into mechanical motion. During the course of ATP binding, hydrolysis and product release, the proteins undergo a series of conformational change, thereby making discrete 8-nm center-of-mass steps and travelling over 1  $\mu\text{m}$ . Inspired by remarkable molecular machineries of protein motors, synthetic analogues have been developed including self-assembled DNA walkers that can make stepwise movements on RNA/DNA substrates or can function as programmable assembly lines. The DNA walker dynamics is usually achieved through a series of reactions, including hybridization, enzymatic cleavage, and strand displacement. However, the kinetics of their stepping reactions is not well understood, and thus, it is unclear how to design them.

In our research, we focus on (i) demonstrating all-synthetic DNA walkers that can transport inorganic cargos along non-DNA tracks autonomously and (ii) studying how design parameters affect the kinetics and processivity of the DNA walkers. Our approach is to use RNA-cleaving DNA enzymes as a model walker, carrying CdS nanocrystal cargos along single-walled carbon nanotube tracks. Similar to the protein motors, DNA motors extract chemical energy from RNA molecules decorated on the nanotubes and use that energy to fuel autonomous, processive walking through a series of conformational changes along the one-dimensional track. Initially, a nanoparticle-capped DNA enzyme strand is bound to an RNA fuel strand through base-pairing of the upper and lower recognition arms on the nanotube track. The enzyme core cleaves the prearranged part of the fuel strand into two fragments in the presence of divalent metal cations. After cleavage reaction, the upper RNA fragment is displaced by the next unbound RNA strand. The lower recognition arm subsequently displaces from the initial RNA and migrates to the next RNA strand, completing a single turnover event. Repeated completion of the single turnover reaction propels autonomous, processive, unidirectional movement of the DNA walker.

To probe the motor operation at the single-molecule level, we have developed a new two-color optical microscopy, termed single-particle/single-tube spectroscopy. Here we use optical properties of the nanoparticle cargo and carbon nanotube track, which fluoresce in the visible and near-infrared spectra, respectively. We monitor the nanoparticle emission against the fluorescent, immobilized nanotube track over time. The spectroscopic measurements provide rich information for motor kinetics, which we theoretically model within the framework of single-molecule kinetics. From the combined experimental and theoretical studies, we elucidate several key parameters that govern the kinetics and processivity of DNA enzyme-based walkers. These parameters include the catalytic core type and structure of DNA enzymes, lengths of the upper and lower recognition arms, and environmental factors such as the type and concentration of divalent metal cations. A better understanding of kinetics and design parameters enables us to demonstrate a walker movement near 5  $\mu\text{m}$  at a speed of ~1 nm/s. It is noted that the displacement and speed are 10-fold greater than previously reported DNA walkers. We also provide a set of design guidelines to construct highly processive, autonomous DNA walker systems and to regulate their translocation kinetics, which should facilitate the development of functional DNA walkers. This presentation will include our benchmark study of DNA walkers against protein motors.

# Controllable ribonucleoprotein motors engineered from myosin and RNA

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To engineer transport systems at the nanoscale that can be used inside<sup>1</sup> or outside<sup>2</sup> cells, we and others have sought to develop molecular motors that respond to a range of targeted signals. Protein motors such as myosins<sup>3,4</sup> and kinesins<sup>4</sup>, which perform transport and force generation functions in biology, have been modified to change speed or direction in response to metal ions<sup>3</sup> or light<sup>4</sup>. However, it is a challenge to extend these control strategies to allow high levels of multiplexing. In contrast, synthetic DNA motors<sup>5,6</sup> are inherently amenable to multiplexed control via sequence addressability, but do not yet compete with biological motors in terms of critical motor properties such as transport velocity. DNA nanotechnology and protein motors have previously been combined in hybrid assemblies where DNA structures act as scaffolds to dictate the spacing, number, and composition of attached motors, in both fixed and dynamically controllable configurations<sup>7-10</sup>. Here, we have designed and tested myosin motors that incorporate rigidly attached lever arms constructed from RNA, introducing a new category of hybrid assembly in which conformational changes in protein motor domains are amplified and redirected by nucleic acid structures that function as integral mechanical elements of engineered nucleoprotein machines. Following principles established in protein engineering studies<sup>3,4</sup>, the RNA lever arm geometry determines the speed and direction of motor transport, and can be dynamically controlled using programmed transitions in lever arm structure. We have used *in vitro* assays of propelled actin filaments to characterize the function of a series of RNA-myosin motors. Our results confirm the successful operation of motors designed to reversibly change direction under the control of strand-displacement reactions<sup>11</sup>. We are also using single-molecule tracking to measure the processive motion of individual multimeric RNA-myosin motors on actin filaments. The myosin-RNA motors described here self-assemble *in vitro* and may also be expressible in living cells. The sequence addressability of RNA lever arm structures will allow multiplexed control of speed and direction in collections of engineered motors, enabling sophisticated programming of complex transport systems. In principle, the signaling inputs to the motors can originate from cellular RNA or transcriptional circuits<sup>12</sup>, or upstream outputs from DNA computations<sup>13,14</sup>, allowing integration with a wide variety of existing technologies in synthetic biology and nanoscience.

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# Tuning the collective behavior of molecular motor ensembles using DNA origami scaffolds and DNA nanotubes

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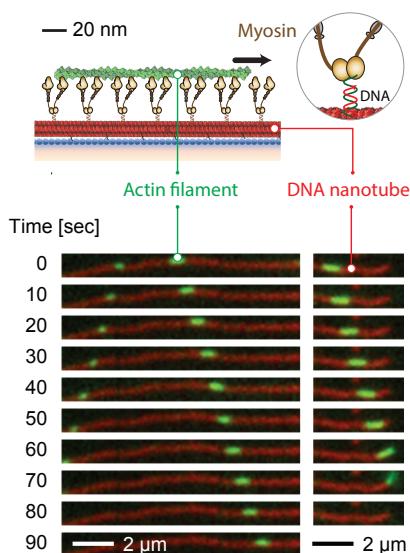
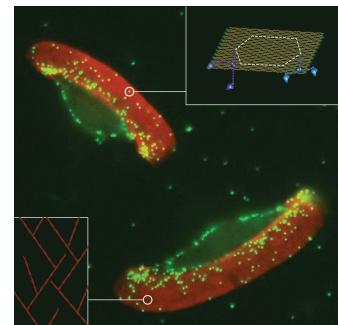
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The emergence of movement patterns, as in stellar streaming, flocks of birds, and schools of fish, is a widespread phenomenon across all scales. In biological environments, the molecular motor myosin teams up to drive cell division, membrane trafficking, and muscle contraction. These cellular functions arise from coordinated interactions of multiple motors tethered to a scaffold with surrounding actin filaments. To examine emergence in collective myosin movement, we engineer two model systems consisting of either rectangular DNA origami scaffolds or DNA nanotubes, patterned with precise control over myosin motor number, type, and spacing.

First, we use flat rectangular DNA origami scaffolds to dissect the sorting of cargo by mixed ensembles of opposite polarity myosin motors, namely myosin V and VI, on a two-dimensional actin network. All mixed-motor scaffolds display solely unidirectional movement. The net directional flux can be finely-tuned by the relative number of myosin V and VI motors on each scaffold. Pairing computation with experimental observations further suggests that motor stall forces are key determinants of the observed competitive outcomes.



Second, we use DNA nanotubes as a platform for engineering artificial thick filaments, as a key component in muscle. Using both myosin V and VI labeled nanotubes, we find that neither myosin density nor spacing has a significant effect on the gliding speed of actin filaments. This observation supports a simple model of myosin ensembles as energy reservoirs that buffer individual stochastic events to bring about smooth continuous motion.

These two model systems provide bottom up platforms for the reconstitution of collective behavior of motor proteins. Beyond the biophysical relevance, our study uncovers elegant engineering principles for designing efficient molecular transporters and force generators on complex landscapes.

## **Plenary Talk 4: Exploring the Space of DNA Signatures**

*Lila Kari*

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Approaches to understanding and utilizing the fundamental properties of bioinformation take many forms, from using DNA to build lattices and polyhedra, to studies of algorithmic DNA self-assembly, to harnessing DNA strand displacement for computations. This talk presents a view of the structural properties of DNA sequences from yet another angle, by proposing the use of graphic signatures of DNA sequences to measure and visualize their interrelationships.

This method starts by computing an “image distance” for each pair of graphic Chaos Game Representations of DNA sequences, and then uses multidimensional scaling to visualize the resulting interrelationships in a two- or three-dimensional space. The result of applying this method to a collection of DNA sequences is an easily interpretable Molecular Distance Map wherein sequences are represented by points in a common Euclidean space, and the spatial distance between any two points reflects the differences in their subsequence composition.

This general-purpose method does not require DNA sequence alignment and can thus be used to compare similar or vastly different DNA sequences, genomic or computer-generated, of the same or different lengths. Our analysis of a dataset of 3,176 complete mitochondrial genomes confirms that these graphic signatures, which reflect the oligomer composition of the originating DNA sequences, can be a source of taxonomic information. This method also correctly finds the mtDNA sequences most closely related to that of the anatomically modern human (the Neanderthal, the Denisovan, and the chimp), and that the sequence most different from it in this dataset belongs to a cucumber.

# Reflections on Tiles (in Self-Assembly)

Jacob Hendricks\*, Matthew J. Patitz\*\*, and Trent A. Rogers\*\*\*

**Abstract.** Tiles in the abstract Tile Assembly Model (aTAM) are not allowed to flip or rotate prior to attachment to an existing assembly. While this assumption is a realistic one for many implementations of DNA-based tiles, for certain implementations of DNA-based building blocks, it is unknown whether or not both of the conditions of this assumption can be physically enforced. When DNA is used as the binding agent, single stranded DNA can be used to prevent relative tile rotation by encoding a direction (north/south or east/west) in the DNA sequence so that only strands with appropriately matching directions are complementary; on the other hand, preventing tiles from flipping may not always be possible, especially if the glues of different sides of a tile are encoded by disjoint DNA complexes. To model self-assembly of particles incapable of preventing such flipping, we define the Reflexive Tile Assembly Model (RTAM), which is obtained from the aTAM by allowing tiles to reflect across their horizontal and/or vertical axes prior to attachment.

We show that the class of directed temperature-1 RTAM systems is not computationally universal, which is conjectured but unproven for the aTAM, and like the aTAM, the RTAM is computationally universal at temperature 2. We then show that at temperature 1, when starting from a single tile seed, the RTAM is capable of assembling  $n \times n$  squares for  $n$  odd using only  $n$  tile types, but incapable of assembling  $n \times n$  squares for  $n$  even. Moreover, we show that  $n$  is a lower bound on the number of tile types needed to assemble  $n \times n$  squares for  $n$  odd in the temperature-1 RTAM. The conjectured lower bound for temperature-1 aTAM systems is  $2n - 1$ . Finally, we give preliminary results toward the classification of which finite connected shapes in  $\mathbb{Z}^2$  can be assembled (strictly or weakly) by a singly seeded (i.e. seed of size 1) RTAM system, including a complete classification of which finite connected shapes may be strictly assembled by a *mismatch-free* singly seeded RTAM system. We also show that arbitrary shapes with scale factor 2 can be assembled in the singly seeded temperature-2 RTAM. These combined results show that the ability of tiles to bind in flipped orientations is sometimes provably limiting, while at other times can provide advantages, and they provide a solid framework for the study of self-assembling systems composed of molecular building blocks unable to enforce the constraints of the aTAM.

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# Optimal program-size complexity for self-assembly at temperature 1 in 3D

David Furcy \*, Samuel Micka \*\*, and Scott M. Summers \*\*\*

**Abstract.** Working in a three-dimensional variant of Winfree’s abstract Tile Assembly Model, we show that, for all  $N \in \mathbb{N}$ , there is a tile set that uniquely self-assembles into an  $N \times N$  square shape at temperature 1 with optimal program-size complexity of  $O(\log N / \log \log N)$  (the program-size complexity, also known as tile complexity, of a shape is the minimum number of unique tile types required to uniquely self-assemble it). Moreover, our construction is “just barely” 3D in the sense that it works even when the placement of tiles is restricted to the  $z = 0$  and  $z = 1$  planes. This result affirmatively answers an open question from Cook, Fu, Schweller (SODA 2011). To achieve this result, we develop a general 3D temperature 1 optimal encoding construction, reminiscent of the 2D temperature 2 optimal encoding construction of Soloveichik and Winfree (SICOMP 2007), and perhaps of independent interest.

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# Molecular Ping-Pong Game of Life on a 2D Origami Array

N. Jonoska<sup>1</sup>, N.C. Seeman<sup>2</sup>

We introduce a model where local control allows oscillating platform attachments/detachments between two molecular floating species [2]. This proposed system consists of a 2D DNA origami array (platform) [3] whose tiles serve as a transmission storage (equipped with “communication” and “identity” strands) and free floating tiles able to attach to their respective “identity” counterparts on the array and transmit signals to its neighboring locations. The communication is achieved through strand displacement mechanism, as originally introduced by Yurke et al. [4]. The array tiles are divided in a checker-board (red/green) fashion such that in an alternate manner, at each cycle, one of the colors receives the floating tiles, attached through the identity strands, and, through communication signals, computes the identity of the next cycle tiles in the other color tiles. We suggest that environmental control of the cycles can be achieved by equipping red (green) identity tiles with different species of dyes or nanoparticles, species whose exposure to appropriate wave length increases local temperature and thereby can prevent attachment or disassociate the appropriate floating tiles from the array. Current experimental findings suggest that hybridization of appropriately labeled single floating origami tiles to a system of duplex origami tiles equipped with labeled single strands can be controlled with cyclic treatments of irradiation [1]. In a similar fashion, cycles of toehold-based chemistry can be envisioned performing the same task.

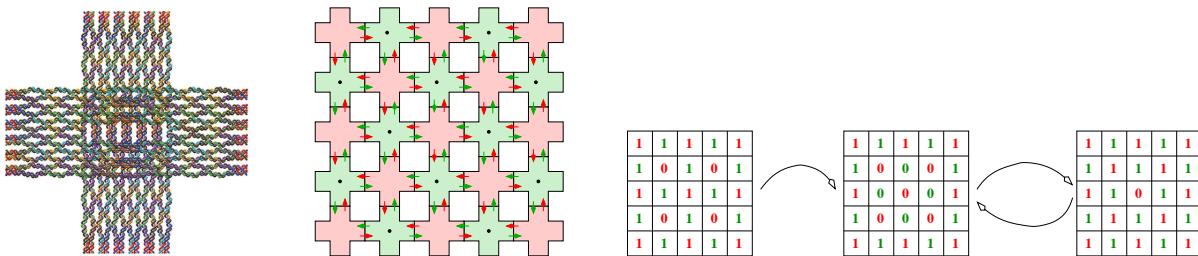


FIGURE 1. (left) Basic DNA Origami Tile. (center) Communication Signals. (right) The arrangement sequence of GoL-like system on a  $5 \times 5$  grid.

We show how algorithmic molecular interactions can control cyclic molecular arrangements by exhibiting a system that can simulate a two-dimensional Game of Life-like (GoL-like) dynamics on a 2D DNA origami array. We also initiate a theoretical model for cooperative molecular interactions on a given platform that allows cycles of programmed molecular arrangements and dynamical information processing to be experimentally feasible; moreover, through the proposed signal transmission and molecular interactions on the predesigned array we show how to implement 2D cellular automata like cyclic arrangements, thereby providing a “modus operandi” for cyclic molecular cooperation.

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# Flipping Tiles: Concentration Independent Coin Flips in Tile Self-Assembly

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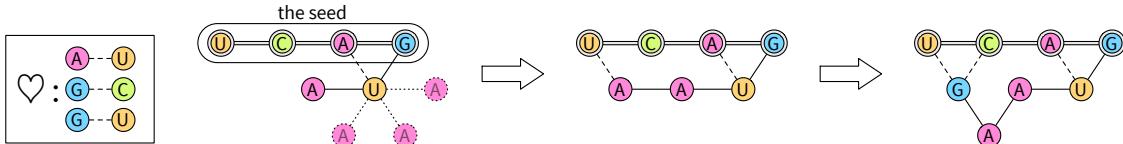
**Abstract.** In this paper we introduce the *robust coin flip* problem in which one must design an abstract tile assembly system (aTAM system) whose terminal assemblies can be partitioned such that the final assembly lies within either partition with exactly probability 1/2, regardless of what relative concentration assignment is given to the tile types of the system. We show that robust coin flipping is possible within the aTAM, and that such systems can guarantee a worst case  $\mathcal{O}(1)$  space usage. As an application, we then combine our coin-flip system with the result of Chandran, Gopalkrishnan, and Reif [3] to show that for any positive integer  $n$ , there exists a  $\mathcal{O}(\log n)$  tile system that assembles a constant-width linear assembly of expected length  $n$  that works for all concentration assignments. We accompany our primary construction with variants that show trade-offs in space complexity, initial seed size, temperature, tile complexity, bias, and extensibility, and also prove some negative results. Further, we consider the harder scenario in which tile concentrations change arbitrarily at each assembly step and show that while this is not solvable in the aTAM, this version of the problem can be solved by more exotic tile assembly models from the literature.

# Efficient Universal Computation by Molecular Co-Transcriptional Folding

Cody Geary, Pierre-Étienne Meunier, Nicolas Schabanel, Shinnosuke Seki

We investigate the algorithmic power of a previously overlooked phenomenon: the fact that molecules fold as they are transcribed (*co-transcriptional folding*). Although our questions are mostly fundamental, the model itself is inspired by the experimental work of Geary, Rothemund, and Andersen (*Science* 345:799-804, 2014), and could be implemented using ssRNA Origami in the future.

We introduce a new theoretical model called *Oritatami* (folding in Japanese). The to-be-folded molecule is modeled as a sequence of beads (its *primary structure*) labelled by an alphabet  $\Sigma$ . Beads *attract* each other according to a symmetric relation  $\heartsuit \subseteq \Sigma \times \Sigma$ ; e.g., in the case of RNA, we may set  $\Sigma = \{A, C, G, U\}$  and  $\heartsuit = \{(A, U), (C, G), (G, U)\}$ . Note that there can be different levels of considering what is abstracted to be a bead, depending on how complex the relation  $\heartsuit$  needs to be for intended purpose. Beads would really represent, as in the real lab world, 5-6nt domains rather than individual nucleotide, and this also realizes many more attraction rules than just A-U, G-C, and G-U. A folded sequence is called a *conformation*. In an Oritatami system, we extend an initial conformation called the *seed* (the *input* in algorithmic terms) by transcribing a primary structure while folding it on the triangular grid so as to form as many bonds as possible according to  $\heartsuit$ . Unlike conventional models of folding like Hydrophobic-Hydrophilic (HP) model, Oritatami does not fold the whole sequence at one time. Instead, it transcribes the next  $\delta_t$  beads of the sequence, considers all possible ways to extend the current conformation by folding them, and determines their locations so as to maximize the number of bonds according to  $\heartsuit$ , where  $\delta_t$  is a system parameter called the *delay time* which acts approximately as a temperature parameter (the larger it is, the less stable is the tail of the folding). For instance, an Oritatami system with delay time 1 settles a bead, once being transcribed, down in the unoccupied positions next to the preceding bead where maximum number of new bonds are formed. Below, we illustrate an Oritatami system with delay time 2 that folds the primary structure UAAG from the placed seed labelled UCAG; note that the locations of the two As change as the transcription goes on.



We study the simulation, prediction, and programming of Oritatami systems. First, we propose an efficient simulation of cyclic tag system (CTS) by an Oritatami system with delay time 3 whose primary structure is periodic. As a corollary, simulating Oritatami systems is **P-hard**. One can determine in polynomial time whether a given Oritatami system folds its sequence in an expected way. As for programming, designing a set  $\heartsuit$  of rules to fold a given sequence into given conformations is proved **NP-hard**. In contrast, if we can choose the sequence, then we can provide an algorithm to design an Oritatami system. The running time of this algorithm is linear in the length of the sequence but exponential in the number of target conformations.

## **Plenary Talk 5: Digital Alchemy for Optimized Self Assembly**

*Sharon Glotzer*

*Departments of Chemical Engineering, Material Science & Engineering, Macromolecular Science & Engineering, and Physics, University of Michigan*

Algorithmic self-assembly generally relies on designing interaction rules so that specific building blocks "fall" into place to assemble a desired target structure. In such cases, the complexity of the interaction ruleset scales with the complexity of the target structure. Crystallographic self-assembly, in contrast, relies on simple, uniform interactions among building blocks (atoms, molecules, colloids), often forming structures of great complexity without the need for interaction specificity. Even excluded volume interactions, in the absence of any other forces, can lead to the entropic assembly of remarkably complex, even aperiodic, ordered structures. In this talk we show how entropic as well as simple isotropic interactions can lead to such complexity, even if the building blocks are identical and relatively simple, and how the choice of "best" shape or interaction ruleset for a target assembly can be formalized through extended ensemble theory and "digital alchemy." By understanding how complexity can be achieved even from simple rules, the lessons we learn suggest that a generalized approach to algorithmic assembly could lead to simplifications in the rules used for generation of complex structures.

# Designs and Algorithms for DNA Folding of Custom 3D Polyhedra

Abdulmelik Mohammed<sup>1</sup>, Eugen Czeizler<sup>1</sup>, Erik Benson<sup>2</sup>, Johan Gardell<sup>2</sup>, Sergej Masich<sup>3</sup>, Björn Höglberg<sup>2</sup>, and Pekka Orponen<sup>1</sup>

We introduce a general algorithmic approach and software pipeline for generating designs of 3D polyhedral DNA origami structures from custom-presented wireframe models [1]. The technique routes the DNA scaffold strand on the mesh of these structures according to a one helix per edge principle, thus enabling the synthesis of large irregular structures with unprecedented complexity.

The design is based on the following restrictions and motivations. First, the meshes should be triangulated to bolster the rigidity of the assembled structures. Second, each edge should be rendered with one double helix for efficient use of DNA base-pairs. Third, the scaffold strand should not cross itself to avoid strand entanglement as well as protrusions due to stacking of crossing helices.

The first requirement amounts to designing a triangulated polygonal model in some 3D design software. To meet the second requirement, the scaffold strand should traverse each edge exactly once, which is possible if and only if the mesh is Eulerian. Since not all polyhedral meshes are Eulerian, we relax the second requirement and add a minimal number of edges to satisfy the condition. Then, scaffold routing becomes equivalent to finding a Chinese Postman Tour [2]. However, a routing based on a CPT can contain scaffold crossings at vertices. To fulfill the third requirement, we note that the scaffold should be routed according to an A-trail, a type of Eulerian trail with restrictions on transitions at vertices based on the order of their incident edges. However, finding A-trails is known to be NP-complete even when restricted to polyhedral graphs with triangular and quadrilaterals faces [3]. On the other hand, Eulerian plane triangulations, which emanate from rigid polyhedral structures in our context, are conjectured to always admit A-trails [4]. This postulate is linked to a 40-year old standing conjecture by Barnette, which states that every bipartite polyhedral graph with three edges per vertex has a Hamiltonian cycle.

Given the general NP-hardness result, we designed and implemented a branch-and-bound algorithm to find A-trails on planar graphs. We systematically structured the search tree and employed a branching order heuristic to considerably reduce the run-time of the algorithm. Coupled with components for mesh modification and planar embedding, our implementation is able to find routes for structured meshes with hundreds of faces within seconds. The use of weighted Eulerian circuits for DNA origami has also been suggested in recent theoretical work [5], proposing the use of TSP solvers for the general task of finding scaffold routings. Our focus is however specifically on non-crossing routings, yielding an effective software pipeline leading from 3D models to strand sequences. A variety of large complex polyhedra designed using this approach have been synthesized and then imaged using Transmission Electron Microscopy and Cryo-Electron Tomography [1].

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# New Geometric Algorithms for Fully Connected Staged Self-Assembly

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We consider *staged self-assembly systems*, in which square-shaped tiles can be added to bins in several stages. Within these bins, the tiles may connect to each other, depending on the *glue types* of their edges. Previous work by Demaine et al. [1] showed that a relatively small number of tile types suffices to produce arbitrary shapes in this model. However, these constructions were only based on a spanning tree of the geometric shape, so they did not produce full connectivity of the underlying grid graph in the case of shapes with holes; designing fully connected assemblies with a polylogarithmic number of stages was left as a major open problem. We resolve this challenge by presenting new systems for staged assembly that produce fully connected polyominoes in  $\mathcal{O}(\log^2 n)$  stages, for various scale factors and temperature  $\tau = 2$  as well as  $\tau = 1$ . Our constructions work even for shapes with holes and uses only a constant number of glues and tiles. Moreover, the underlying approach is more geometric in nature, implying that it promised to be more feasible for shapes with compact geometric description. See the table for a concise summary.

Arbitrary Shapes	Glues	Tiles	Bins	Stages	$\tau$	Scale	Conn.	Planar
Spanning Tree Method [1]	2	16	$\mathcal{O}(\log n)$	$\mathcal{O}(\text{diameter})$	1	1	partial	no
Monotone Shapes [1]	9	$\mathcal{O}(1)$	$\mathcal{O}(n)$	$\mathcal{O}(\log n)$	1	1	full	yes
Hole-Free Shapes [1]	8	$\mathcal{O}(1)$	$\mathcal{O}(N)$	$\mathcal{O}(N)$	1	2	full	no
Shape with holes	7	$\mathcal{O}(1)$	$\mathcal{O}(k)$	$\mathcal{O}(\log^2 n)$	2	3	full	no
Hole-Free Shapes	7	$\mathcal{O}(1)$	$\mathcal{O}(k)$	$\mathcal{O}(\log n)$	2	3	full	no
Hole-Free Shapes	18	$\mathcal{O}(1)$	$\mathcal{O}(k)$	$\mathcal{O}(\log^2 n)$	1	4	full	no
Shape with holes	20	$\mathcal{O}(1)$	$\mathcal{O}(k)$	$\mathcal{O}(\log^2 n)$	1	6	full	no

Table 1: Overview of results. The number of tiles of a shape  $P$  is denoted by  $N \in \mathcal{O}(n^2)$ ,  $n$  is the side length of a smallest bounding square, while  $k$  is the number of vertices of the polyomino, with  $k \in \Omega(1)$  and  $k \in \mathcal{O}(N)$ . An assembly is composed in a planar fashion if all tiles can be attached without making use of the third dimension.

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# Leader Election and Shape Formation with Self-Organizing Programmable Matter

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**Abstract.** In this paper we consider programmable matter consisting of simple computational elements, called *particles*, that can establish and release bonds and can actively move in a self-organized way, and we investigate the feasibility of solving fundamental problems relevant for programmable matter. As a model for such self-organizing particle systems, we will use a generalization of the geometric amoebot model first proposed in [1]. Based on the geometric model, we present efficient local-control algorithms for leader election and line formation requiring only particles with constant size memory, and we also discuss the limitations of solving these problems within the general amoebot model.

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# Coarse-grained modelling of RNA

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We present a nucleotide-level model for RNA, oxRNA [1]. The model is based on the coarse-graining methodology developed for the oxDNA model of DNA [2, 3], which has been successfully applied to a study of a wide range of nanotechnological systems [4]. The oxRNA model is designed to reproduce structural, mechanical and thermodynamic properties of RNA, and the coarse-graining level aims to retain the relevant physics for RNA hybridization and the structure of single- and double-stranded RNA. The model can be used for efficient simulations of the structure of systems with thousands of base pairs, and for the assembly of systems of up to hundreds of base pairs.

We apply the model in a range of nanotechnological and biological settings, such as the folding thermodynamics of a pseudoknot, the formation of a kissing loop complex, the structure of a hexagonal RNA nanoring, and the unzipping of a hairpin motif. Recently, the model has also been extended using Debye-Hückel theory to incorporate effects of different salt concentrations and used to study RNA plectoneme formation at 0.1 M salt [5].

We further use the model to study the thermodynamics and kinetics of an RNA toehold-mediated strand displacement reaction [6]. We obtain the rate of displacement reactions as a function of the length of the toehold and temperature and find that the displacement is faster if the toehold is placed at the 5' end of the substrate and that the displacement slows down with increasing temperature for longer toeholds. Such an effect can be tested experimentally and may provide an additional way to modulate displacement rates, which can be helpful in designing applications of RNA strand displacement to bionanotechnology [7].

The source code implementing oxRNA both on CPU and GPU is available at [dna.physics.ox.ac.uk](http://dna.physics.ox.ac.uk).

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## **Plenary Talk 6: Production of DNA Nanostructures in Bacteria**

*Chris Voigt*

*Department of Biological Engineering, Massachusetts Institute of Technology*

We are developing a basis by which cells can be programmed like robots to perform complex, coordinated tasks for pharmaceutical and industrial applications. We are engineering new sensors that give bacteria the senses of touch, sight, and smell. Genetic circuits — analogous to their electronic counterparts — are built to integrate the signals from the various sensors. Finally, the output of the gene circuits is used to control cellular processes. We are also developing theoretical tools from statistical mechanics and non-linear dynamics to understand how to combine genetic devices and predict their collective behavior. (Research description from the laboratory website)

Guiding systematic improvements of a DNA-actuated enzyme nanoreactor through single molecule analysis

(Manuscript in preparation)

Soma Dhakal<sup>1</sup>, Minghui Liu<sup>2,3</sup>, Matthew R. Adendorff<sup>4</sup>, Adam G. Krieger<sup>1</sup>, Mark Bathe<sup>4</sup>, Hao Yan<sup>2,3</sup>, Nils G. Walter<sup>1,\*</sup>

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Switchable nanomachines provide a platform to control dynamic functional states by altering distances at the nanoscale on demand. Recently, a tweezer-like DNA device was used to control the activity of an enzyme/cofactor pair juxtaposed on the two arms of the tweezer. Initial studies focused on bulk properties of the tweezer-mediated reactions, and hence lacked insight into the mechanism of enzymatic activation. Here, we used site-specific fluorophore labeling of the tweezer to monitor the arm-to-arm distance through single-molecule fluorescence resonance energy transfer (smFRET). The smFRET analysis showed that the tweezer only partially closes in the proposed “closed” state (arm-to-arm distance of ~6.5 nm) and exhibits conformational sub-states. This observation was consistent with the inter-arm distance measured by atomic force microscopy (AFM) of the closed tweezer. Molecular dynamics (MD) simulation showed bending and twisting of the tweezer arms, rationalizing the sub-states. Additionally, smFRET experiments on the isolated Holliday junction (HJ) hinge suggested that the isomer resulting in the tightest closing of the tweezer (isomer-I) is relatively disfavored, further explaining the only partial closing. Realizing the existing deficiencies – incomplete closure upon actuation and conformational heterogeneity, we then used feedback from smFRET, AFM and computational analyses to guide the systematic improvement of such an ad hoc designed DNA tweezer. We rationally improved the closure by increasing the stem length of the DNA hairpin bridging and actuating the tweezer from 3 to 4 base pairs, and by redesigning the Holliday junction(s) to favor optimal isomer-I. The performance of the new tweezer designs was quantitatively assessed by juxtaposing glucose-6-phosphate dehydrogenase (G6pDH) with its cofactor NAD<sup>+</sup> on the tweezer arms and measuring the G6pDH activity through a coupled enzymatic cascade. Using our optimized tweezer, we were able to enhance the bulk activity of G6pDH upon tweezer closure to up to ~12-fold. Further, we developed a single-molecule enzyme assay and show that the individual DNA-actuated enzyme nanoreactors respond to the resulting improved closure and straighter tweezer arms in distinct, predictable ways. Our results suggest that G6pDH stochastically fluctuates between active and inactive states, favoring the active state upon closure of the tweezer. In the future, we anticipate that the improved design strategies developed here will enhance the performance of many other nanodevices such as responsive enzyme nanodevices, biosensing devices, and in production of smart materials.

# Leakless DNA strand displacement systems

Chris Thachuk<sup>1</sup>, Erik Winfree<sup>1</sup>, and David Soloveichik<sup>2</sup>

<sup>1</sup> California Institute of Technology

<sup>2</sup> UCSF Center for Systems and Synthetic Biology

**Abstract.** While current experimental demonstrations have been limited to small computational tasks, DNA strand displacement systems (DSD systems) hold promise for sophisticated information processing within chemical or biological environments. A DSD system encodes designed reactions that are facilitated by three-way or four-way toehold-mediated strand displacement. However, such systems are capable of spurious displacement events that lead to *leak*: incorrect signal production. We have identified sources of leak pathways in typical existing DSD schemes that rely on toehold sequestration and are susceptible to toeless strand displacement (i.e. displacement reactions that occur despite the absence of a toehold). Based on this understanding, we propose a simple, domain-level motif for the design of leak-resistant DSD systems. This motif forms the basis of a number of DSD schemes that do not rely on toehold sequestration alone to prevent spurious displacements. Spurious displacements are still possible in our systems, but require multiple, low probability events to occur. Our schemes can implement combinatorial Boolean logic formulas and can be extended to implement arbitrary chemical reaction networks.

# **Supervised learning in an adaptive DNA strand displacement circuit**

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**Abstract.** The development of DNA circuits capable of adaptive behavior is a key goal in DNA computing, as such systems would have potential applications in long-term monitoring and control of biological and chemical systems. In this paper, we present a framework for adaptive DNA circuits using buffered strand displacement gates, and demonstrate that this framework can implement supervised learning of linear functions. This work highlights the potential of buffered strand displacement as a powerful architecture for implementing adaptive molecular systems.

# Automated Design and Verification of Localized DNA Computation Circuits

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Simple computations can be performed using the interactions between molecules of DNA. These interactions are typically toehold-mediated strand displacement reactions in a well-mixed solution; they are not spatially controlled. Spatial control can be introduced by tethering oligonucleotide reactants to a DNA origami tile. A single-stranded DNA walker can walk down a track of single-stranded anchorages on an origami tile by undergoing a cycle of toehold-mediated branch migration reactions powered by a nicking enzyme. Introducing multiple intersecting tracks on an origami tile enables these walkers to perform Boolean logic by blocking other walkers on tracks that intersect with their own.

The principal type of error observed in these tethered systems is a timing error whereby the walker to be blocked passes through the junction before the blocking walker has arrived to block it. This “missed chance” error can be minimized by understanding the rate at which each walker steps to a new anchorage and then ensuring an appropriate number of anchorages for each track. Probabilistic model checking can determine the shortest length (in number of anchorages) for each track that keeps the missed chance error below a certain threshold. To justify the use of model checking, we show how an intersecting track system can be thought of as a distributed system: Anchorages are networked computers, and the DNA walker is the message that they send to one another. Hence, the track system can be modelled as a stochastic Petri net and the probability of error determined by analyzing the resulting continuous time Markov chain.

To give an example of how these systems can be realized experimentally, we describe a DNA mechanism for a junction anchorage that lies at the intersection between two tracks. The junction is initially in an accept state whereby a walker can step through it and continue walking down its track. If a blocking walker arrives first, however, it switches the junction into a block state where it will trap the other walker on the intersecting track. The theoretical machinery developed in this paper can help design such a system and ensure that it operates with minimal error.

# On low energy barrier folding pathways for nucleic acid sequences

Leigh-Anne Mathieson and Anne Condon

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**Abstract.** Secondary structure folding pathways correspond to the execution of DNA programs such as DNA strand displacement systems. It is helpful to understand the full diversity of features that such pathways can have, when designing novel folding pathways. In this work, we show that properties of folding pathways over a 2-base strand (a strand with either A and T, or C and G, but not all four bases) may be quite different than those over a 4-base alphabet. Our main result is that, for a simple energy model in which each base pair contributes  $-1$ , 2-base sequences of length  $n$  always have a folding pathway of length  $O(n^3)$  with energy barrier at most  $2$ . We provide an efficient algorithm for constructing such a pathway. In contrast, it is unknown whether minimum energy barrier pathways for 4-base sequences can be found efficiently, and such pathways can have barrier  $\Theta(n)$ . We also present several results that show how folding pathways with temporary and/or repeated base pairs can have lower energy barrier than pathways without such base pairs.

# Stochastic Simulation of the Kinetics of Multiple Interacting Nucleic Acid Strands

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<sup>1</sup> California Institute of Technology

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**Abstract.** DNA nanotechnology is an emerging field which utilizes the unique structural properties of nucleic acids in order to build nanoscale devices, such as logic gates, motors, walkers, and algorithmic structures. Predicting the structure and interactions of a DNA device requires effective modeling of both the thermodynamics and the kinetics of the DNA strands within the system. The kinetics of a set of DNA strands can be modeled as a continuous time Markov process through the state space of all secondary structures. The primary means of exploring the kinetics of a DNA system is by simulating trajectories through the state space and aggregating data over many such trajectories. We expand on previous work by extending the thermodynamics and kinetics models to handle multiple strands in a fixed volume, in a way that is consistent with previous models. We developed data structures and algorithms that allow us to take advantage of local properties of secondary structure, improving the efficiency of the simulator so that we can handle reasonably large systems. Finally, we illustrate the simulator's analysis methods on a simple case study.

## Nanoday Talk 1: DNA and Nanotechnology

*Nadrian C. Seeman*

*Department of Chemistry, New York University*

We build branched DNA species joined using Watson-Crick base pairing to produce N-connected objects and lattices. We have used ligation to construct DNA topological targets. We have made robust 2-state and 3-state programmable robotic devices and bipedal walkers. We have constructed a molecular assembly line using a DNA origami layer and three 2-state devices, so that there are eight different states represented by their arrangements. We have self-assembled a 3D crystalline array. We can use crystals with two molecules to control the color of the crystals. Rational design of intermolecular contacts has enabled us to improve crystal resolution to 2.65 Å. We are now doing strand displacement in the crystals to change the color of crystals, thereby making a 3D-based molecular machine. Thus, structural DNA nanotechnology has fulfilled its initial goal of controlling the internal structure of macroscopic constructs in three dimensions.

## **Nanoday Talk 2: Designer DNA Architectures for Programmable Self-Assembly**

*Hao Yan*

*Department of Chemistry and Biochemistry & Biodesign Institute, Arizona State University*

In this talk I will present our efforts in using DNA as an information-coding polymer to program and construct DNA nano-architectures with complex geometrical features. These include single layer curved and complex wireframe DNA origami nanostructures, 2D and 3D lattices etc. Use of designer DNA architectures as molecular scaffolds for directed assembly of interactive biomolecular networks and inorganic nanotrustures will also be discussed.

## Nanoday Talk 3: Programming Functional Structured DNA Assemblies

*Mark Bathe*

*Department of Biological Engineering, Massachusetts Institute of Technology*

Viral capsids and bacterial light-harvesting antennas are two remarkable macromolecular architectures designed through millions of years of natural selection. While genetic engineering offers one approach to re-engineering these highly evolved assemblies for societal purposes, bottom-up self-assembly of structured DNA architectures that can host functional guest molecules offers a complementary biosynthetic approach. Here, I will present our efforts to develop a computational framework to automatically “print” structured DNA assemblies of arbitrary 3D shape at the nanometer scale. Our approach includes atomic-level constraints on the positioning of guest molecules including lipids, peptides, enzymes, RNAs, and chromophores. Applications to the design of custom J-aggregate chromophore assemblies with highly efficient energy transfer rates characteristic of bacterial light-harvesting systems and viral capsids of arbitrary symmetry and size on the ten nanometer scale will be presented.

## **Nanoday Talk 4: Self-Assembly of Polymers and Other Molecules Conjugated to DNA**

*Kurt Gothelf*

*Department of Chemistry, Interdisciplinary Nanoscience Center, Aarhus University*

We are using DNA as a programmable tool for directing the self-assembly of molecules and materials. The unique specificity of DNA interactions and our ability to synthesize artificial functionalized DNA sequences makes it the ideal material for controlling self-assembly and chemical reactions of components attached to DNA sequences. Recently, we applied these methods to DNA templated conjugation of DNA to proteins such as antibodies. In particular we are using DNA origami, large self-assembled DNA structures, as a template for positioning of materials such as organic molecules, dendrimers, and biomolecules. The main focus of the presentation will be on a phenylene vinylene polymer with DNA sequences protruding from each repeat unit and its self-assembly on DNA origami.

## Nanoday Talk 5: The Frame Guided Assembly

*Dongsheng Liu*

*Department of Chemistry, Tsinghua University*

How to precisely control the shape and size of final assemblies, especially using same amphiphilic molecules and under the same environmental conditions, is always a challenge in molecular assembly. Inspired by the cytoskeletal/membrane protein/lipid bilayer system that determines the shape of eukaryotic cells, we proposed and ‘the Frame Guided Assembly’ (FGA) strategy to prepare heterovesicles with programmed geometry and dimensions. This method offers greater control over self-assembly: with same molecular system, the size of final assemblies could be tuned at 1 nm level and their shape could vary from spherical to cubic, and even given sized two dimensional sheets. Most importantly, the principle of the FGA could be applied to various materials such as block copolymers, small molecules including surfactants and lipids, which is a general rule in self-assembly.

## Nanoday Talk 6: Creating Combinatorial Patterns with DNA Origami Arrays

*Lulu Qian*

*Department of Bioengineering, California Institute of Technology*

DNA origami and smaller DNA tiles have been used as scaffolds to create complex patterns for organizing molecules with nanometer precision. Arrays of DNA origami and DNA tiles have been shown to be capable of creating patterns in a larger scale. Here we aim to create a variety of large-scale complex patterns by using a combinatorial approach called Truchet tiling. We developed a symmetric square DNA origami tile that can rotate and attach to one another on all four sides, with which we constructed two-dimensional finite and unbounded arrays. Labeling the origami tile with simple Truchet patterns, we successfully observed a variety of emergent maze-like patterns on the origami arrays. Our approach could be used to better understand the design principles for building robust molecular robots by testing them against increasingly complex environment. It could also potentially be used to efficiently screen functional molecular devices and advance nanoscale fabrication.

## Nanoday Talk 7: Knowledge-Driven Design of Probes and Primers for Nucleic Acid Analysis

*David Yu Zhang*

*Department of Bioengineering, Rice University*

Hybridization of probes and primers to intended nucleic acid target sequences forms the basis of modern nucleic acid detection, sequencing, and quantitation technologies. To date, probe and primer sequence design remains highly empirical, relying on iterative experimental testing and optimization. For complex samples and/or multiplexed analysis, even empirical optimization becomes challenging due to the large number of interactions involved. Our group is focused on the development of novel nucleic acid analysis technologies, using first-principles biophysics approaches. For example, we (1) have developed hybridization probes with median 890-fold single-nucleotide selectivity across 44 cancer driver mutation sequences, (2) are able to precisely tune the selectivity-affinity tradeoff with less than 2% granularity on-the-fly using stoichiometry without resynthesis of new reagents, (3) can detect less than 0.1% single nucleotide variants from 20ng human genomic DNA using quantitative PCR across anneal temperatures spanning 8 °C, (4) can provide qPCR-like log-linear signal responses to target concentration in an enzyme-free setting, and (5) used digital thresholding to provide multiplexed information on multiple target sequences using the same fluorescence channel.

## Nanoday Talk 8: DNA Circuitry and NextGen Sequence Informatics

*Andrew D. Ellington*

*Department of Chemistry, University of Texas at Austin*

DNA nanotechnology has been seen as a means of controlling either structure or information processing. The development of sequence-modulated riboswitches has proved a boon for developing programmable cellular operating systems. While nucleic acid circuitry is operable in cells, there are aspects of the processing of biological information that go beyond the biological systems themselves. The development of NextGen sequencing technologies is rapidly digitizing all of biology. Sequence becomes a means of identification, manipulation, & synthesis of phenotype, both in & out of organisms. DNA nanotechnology & circuitry can potentially cross into this realm too. Our lab has explored the use of DNA circuitry for point-of-care diagnostics applications, but there are perhaps even greater opportunities for information processing at the molecular level prior to or concomitant with sequencing & sequence analysis itself. DNA nanotechnology tools could greatly enable the NextGen revolution.

## Nanoday Talk 9: Synthetic Biology: Reprogramming Life

*James J. Collins*

*Founding Core Faculty Member & Platform Lead, Anticipatory Medical and Cellular Devices,  
Wyss Institute*

*Henri Termeer Professor of Medical Engineering & Science,  
Department of Biological Engineering, Massachusetts Institute of Technology*

Synthetic biology is bringing together engineers, physicists and biologists to model, design and construct biological circuits out of proteins, genes and other bits of DNA, and to use these circuits to rewire and reprogram organisms. These re-engineered organisms are going to change our lives in the coming years, leading to cheaper drugs, rapid diagnostic tests, and synthetic probiotics to treat infections and a range of complex diseases. In this talk, we highlight recent efforts to create synthetic gene networks and programmable cells, and discuss a variety of synthetic biology applications in biotechnology and biomedicine, and biocomputing.

## Nanoday Talk 10: Nucleic Acid-Based Components for Artificial Cells

*Friedrich Simmel*

*Technische Universität München*

Over the past years, DNA nanotechnology and molecular programming have developed successful methodologies for the organization of matter in space and time. The programmable interactions between DNA and RNA molecules can be used to create supramolecular structures, molecular machines and information-processing molecular circuits. Several of these functionalities will now be integrated into more complex systems and encapsulated into cell-scale compartments. Depending on the viewpoint and application, this will ultimately result in soft robotic or artificial cellular systems.

## Nanoday Talk 11: Expansion Microscopy

*Edward Boyden*

*MIT Media Lab and McGovern Institute, Departments of Biological Engineering and Brain and Cognitive Sciences, MIT Center for Neurobiological Engineering, Massachusetts Institute of Technology*

Microscopy has enabled many biological discoveries by optically magnifying images of fixed cells and tissues. We have now discovered that physical magnification of the specimen itself is also possible. By delivering DNA barcode-containing labels to biomolecules in a specimen, synthesizing a swellable polymer network throughout the specimen, enzymatically digesting endogenous biological structure, and dialyzing the sample in water, we can expand cells and tissues by >100x in volume. This process, which we call expansion microscopy (ExM), is isotropic down to the nanoscale, and thus enables scalable nanoscale imaging of large 3-D samples. We are developing strategies for multiplexed readout of anchored DNA barcodes, in order to identify and localize thousands of different kinds of biomolecule, with nanoscale precision, in cells throughout intact tissues- a key step towards understanding the configuration of complex biological systems in normal and disease states.

## **Nanoday Talk 12: DNA Nanoswitches: From Force Spectroscopy to Instrument-Free Interaction Analysis**

*Wesley P. Wong*

*Departments of Biological Chemistry & Molecular Pharmacology and Pediatrics, Harvard Medical School*

*Program in Cellular and Molecular Medicine, Boston Children's Hospital  
Wyss Institute for Biologically Inspired Engineering, Harvard University*

Using DNA self-assembly we have developed a nanoscale mechanical switch for probing molecular interactions. I will discuss how these DNA nanoswitches can be used for a variety of applications, from facilitating massively-parallel single-molecule force measurements on the Centrifuge Force Microscope to enabling instrument-free interaction analysis using a simple gel readout.

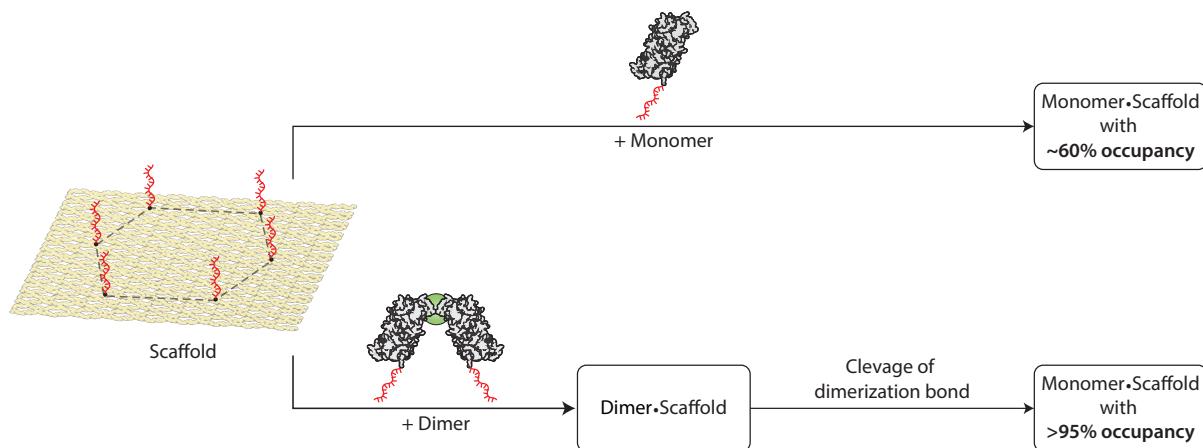
# Using protein dimers to maximize the protein hybridization efficiency with multisite DNA origami scaffolds

Vikash Verma<sup>1</sup>, Leena Mallik<sup>2</sup>, Rizal F. Hariadi<sup>1</sup>, Sivaraj Sivaramakrishnan<sup>1</sup>, Georgios Skiniotis<sup>2</sup>, and Ajit P. Joglekar<sup>1</sup>

<sup>1</sup>Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA

<sup>2</sup>Life Sciences Institute and Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109, USA

DNA origami provides a versatile platform for conducting architecture-function analysis for multi-protein machines. This analysis investigates how the nanoscale organization of multiple copies of a protein affects its overall function. It requires that the copy number of protein molecules bound to an origami scaffold exactly matches the expected number, and that the number is uniform over the entire scaffold population. This requirement is challenging for origami scaffolds with many protein hybridization sites, because it requires the successful completion of multiple, independent hybridization reactions. Here, we show that a cleavable dimerization domain on the hybridizing protein can be used to multiplex hybridization reactions. This strategy yields >95% hybridization efficiency on a 6-site scaffold with low protein concentration and short incubation time. It can also be developed further to enable reliable patterning of a large number of molecules on DNA origami for architecture-function analysis.



# Bandpass Probes for Quantitative Mutation Screening in Nucleic Acid Targets

Zheng (John) Fang<sup>1,2</sup> and David Y. Zhang<sup>1,3</sup>

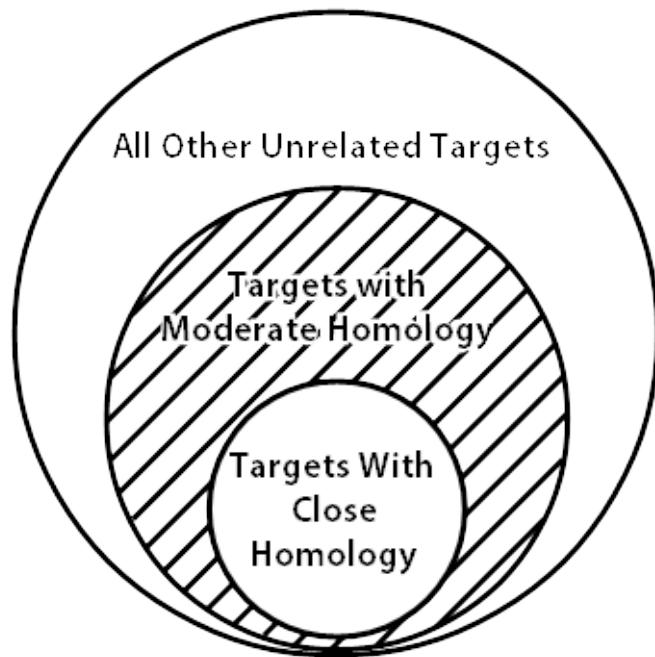
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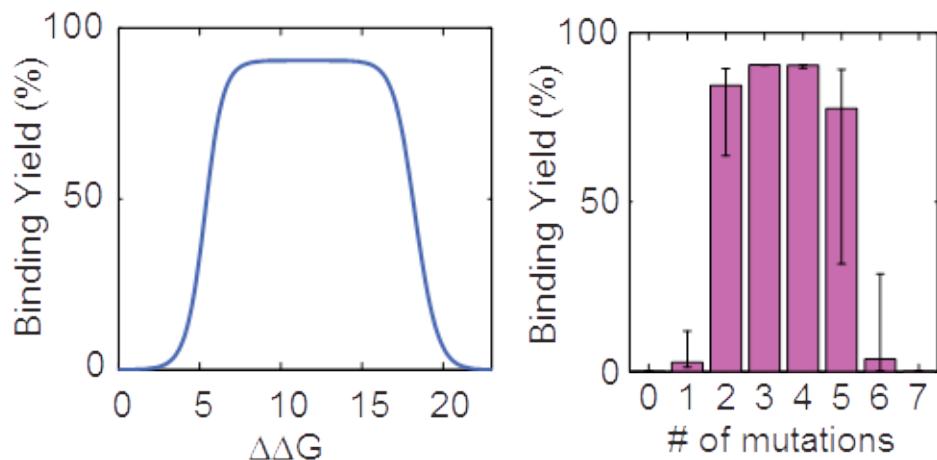
In-vitro detection of mutations in nucleic acid strands have become increasingly specific thanks to the introduction of molecular beacons and toehold probes. However, distinguishing multiple single nucleotide polymorphisms (SNPs) in proximity using hybridization probes can still a challenge. The Bandpass Probes design presented in this poster help discriminate between various levels of homology, with customizable cutoff boundaries designed by the user, as conceptualized in Figure 1.

Based on thermodynamic parameters and nucleic acid hybridization kinetics, it is possible to simulate the real-time interaction of several competing strands in solution. Optimization of this system via experimental and simulations can be used to fine-tune the signal behavior into a bandpass curve with respect to mutation or sequence homology. Our design employs two sets of toehold probes with different hybridization energetics. These probe sets with customizable energetics determine the approximate locations of the low-pass and high-pass cutoffs, hence determining the degree and range of homology that can be detected.

For in-situ hybridization applications, this nucleic acid probe set design can be customized to generate signal only when a certain range of mutation counts appear. As shown in Figure 2, a bandpass probe set have a gradual cutoff, resulting in imperfect effects at the edge of a 2 to 5 SNP bandpass. This information is particularly valuable when targeting genes with high mutation density. Furthermore, the same probe set can be used against different types and quantities of mutations; this allows for a single probe set design to be reused as long as the targeted region of interest and desired bandpass cutoffs do not change.



*Figure 1. Signal interactivity of target against a bandpass probe system. Presence of targets with moderate homology will generate a signal response as shown by the shaded region, while unshaded regions do not. The boundaries of what is considered “moderate homology” is customizable based on the energetics of the probe design.*



*Figure 2. Simulated results of an approximate 2 to 5 SNP bandpass probe set. The left graph shows signal strength with respect to energetics of mutations present in the target DNA strand. The right bar graph show the distribution of signal strength based on 1000 randomized targets with a set number of SNPs.*

## **Robust strand exchange reactions for the sequence-specific, real-time detection of nucleic acid amplicons**

Yu Sherry Jiang<sup>a</sup>, Sanchita Bhadra<sup>b</sup>, Bingling Li<sup>b</sup>, Yan Du<sup>b</sup> and Andrew D. Ellington<sup>b\*</sup>

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Strand exchange reactions are in general highly programmable and thus facilitate the ready development of new and potentially useful nucleic acid circuitry. We have previously shown that strand exchange circuits can be used for signal transduction and background suppression with otherwise noisy isothermal amplification reactions. In particular, loop-mediated isothermal amplification (LAMP) of DNA is a powerful isothermal nucleic acid amplification method for ultra-sensitive nucleic acid diagnostics (it can amplify a few copies of template by  $10^9$  fold in a few hours), but real-time and sequence-specific analysis of the double-stranded concatameric amplicons remains challenging. In order to both improve the specificity of LAMP detection and to make readout simpler and more reliable, we have replaced the intercalating dye typically used for real-time monitoring with a toehold-mediated strand exchange reaction termed one-step strand displacement (OSD), which is essentially the equivalent of a TaqMan probe for an isothermal amplification. Our results showed that LAMP-OSD not only allowed the accurate Yes/No detection of various targets in multiplex analysis, but also demonstrated single nucleotide specificity in Yes/No detection of a melanoma BRAF allele (V600E) in the presence of 20-fold more of the wild-type gene.<sup>1</sup>

LAMP-OSD may be particularly useful for point-of-care (POC) applications, such as detecting the emerging Middle East respiratory syndrome coronavirus (MERS-CoV). Asymmetric five-primer reverse transcription LAMP OSD assays could detect 0.02 to 0.2 plaque forming units (PFU) of MERS-CoV in infected cell culture supernatants within 30 to 50 min and did not cross-react with common human respiratory pathogens.<sup>2</sup> As one more step forward to POC use, we have successfully engineered the system and made the signal readable by an off-the-shelf glucometer.<sup>3</sup>

While OSD probes obviously have analytical advantages in terms of the specificity of detection, into the future they also allow the principles of strand exchange molecular computation to be more generally introduced into molecular diagnostics<sup>4</sup>. Different OSD probes that detected different loops on the same LAMP amplicon have been used as inputs for an AND gate that further decreased false positive detection. Similarly, multiple OSD probes that recognized different amplicons could be used as inputs for an OR gate that was an internal control for accurate quantification. Finally, one of the key issues confronting many molecular diagnostics reactions is multiplexing. By having multiple different inputs funnel through Boolean logic gates, it becomes possible to develop simple outputs based on many inputs, thus bypassing the need for more complicated detection methods.

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# Modular DNA-based Biosensors for Isothermal Detection of Double-Stranded DNA, Oligonucleotides, and Small Molecules

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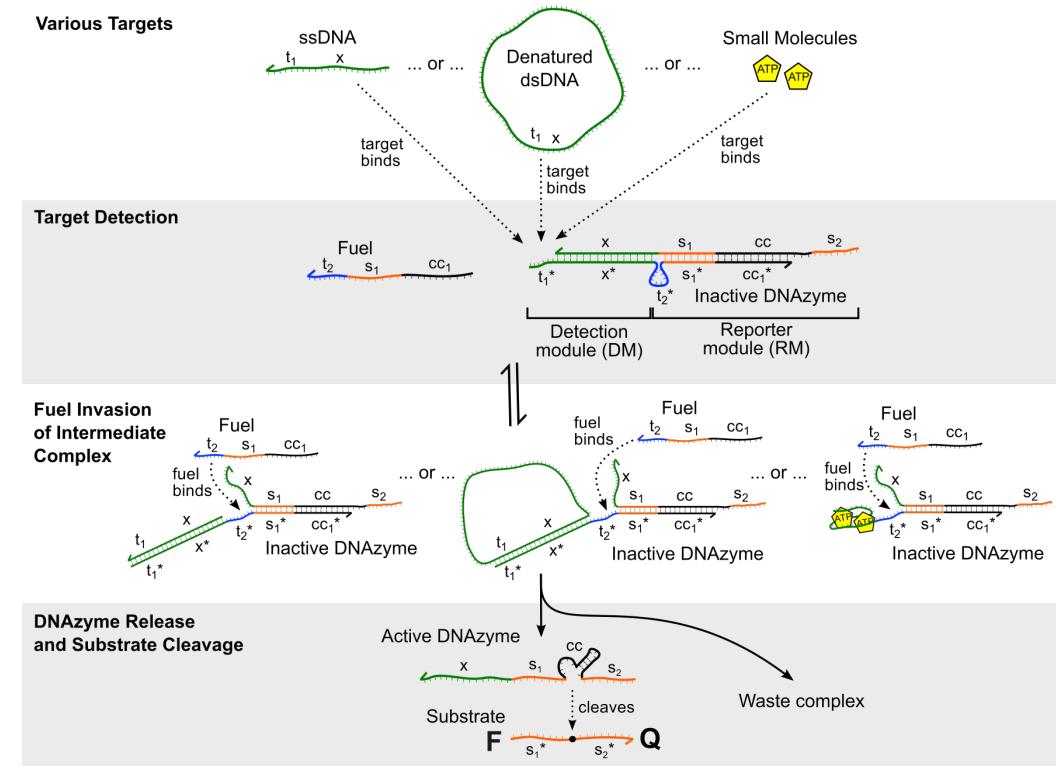
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DNA-based biosensors have great potential for use in developing pathogen detection systems that target genomic signatures or small molecules. A modular, DNA-based biosensor capable of isothermal detection of double-stranded DNA targets, single-stranded oligonucleotides, and small molecules is presented. Biosensors specific to genetic strains were designed to contain independent target detection and reporter modules, enabling rapid design. Plasmid gene variants were detected via a straightforward isothermal denaturation protocol. This isothermal treatment allows detection using hybridization-based biosensors, although topological effects in the denatured dsDNA may limit the availability of the target domain on the denatured plasmid. The sensors were highly specific, even with a randomized DNA background. A limit of detection of  $\sim 15$  pM for single-stranded targets and  $\sim 5$  nM for targets on denatured plasmids was achieved. By incorporating an aptamer sequence in the target module, small molecules were detected using this modular system. In this case, the binding of the partially blocked aptamer to the ligand competes against the hybridization reaction.



**Figure 1.** Sensor activation mechanism in the presence of various target types. Binding of oligonucleotides, or denatured dsDNA, to the detection module by toehold-mediated DNA strand displacement exposed the reporter module toehold. Small molecule targets bound to a structure-switching aptamer in the detection module. In both cases, this allowed the fuel strand to bind and complete displacement of the DNAzyme strand from the complex. The free DNAzyme strand then folded into a catalytically active conformation and generated an amplified fluorescent output by cleaving multiple substrate molecules labeled with a FRET pair.

# Native Characterization of Nucleic Acid Motif Thermodynamics via Noncovalent Catalysis

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Thermodynamics of DNA/RNA motifs are of biological significance and have been studied for decades. The standard model of nucleic acid hybridization thermodynamics is a local model, in which the structure of a nucleic acid complex is dissected into a number of non-overlapping motifs, such as base stacks, bulges, and dangles. Nucleic acid hybridization thermodynamics are calculated by summing  $\Delta G^\circ$  of individual motifs and inaccuracies in calculating approach limit the predictability of oligonucleotide melting temperatures and affinities. Inaccuracies in the  $\Delta G^\circ$  of DNA motifs are compounded in the overall  $\Delta G^\circ$  of hybridization which lead to errors in predicted hybridization affinities and melting temperatures. Currently, the best DNA primer design and folding software exhibit inaccuracy in predicted melting temperature, indicating imprecision even in common motifs such as DNA base stacks and dangles.

Therefore, we introduce a novel method to accurately characterize the  $\Delta G^\circ$  of DNA motifs in native temperature and buffer conditions by observing the relative concentrations of each complex in a given reaction, and fit  $\Delta H^\circ$ ,  $\Delta S^\circ$  of each motif accordingly.

Experiments were designed on the basis of equilibrium built between a duplex carrying dangle motifs and an identical blunt duplex. Two strands with comparable binding affinity are competing with same complementary strand. With the assistance of a catalyst strand, equilibrium is reached in hours.  $\Delta G^\circ$  values of each dangle can be directly calculated from the established equilibrium. In our work, we used electrophoresis as a characterizing approach and used covalently attached fluorophores as a source of imaging signal. Experiments were carried out at different buffer conditions and temperatures.  $\Delta H^\circ$  and  $\Delta S^\circ$  values were fitted from data obtained at different temperatures.

We measured  $\Delta G^\circ$ ,  $\Delta H^\circ$  and  $\Delta S^\circ$  for terminal fluorophores, as well as all 32 single nucleotide dangles and 88 multi-nucleotides dangles covering various lengths and different nearest bases. To minimize uncertainty and inaccuracy of the  $\Delta G^\circ$  values, each group of characterization at different conditions was performed in multiple repeats and verified with an alternate fluorophore. 18 out of 32 single bases dangles exhibit great consistency between two independent approaches. We discovered that multi-nucleotide dangles destabilize DNA duplex as the length increases and the destabilizing effect reaches a plateau when the dangle is 8nt bases or longer.

We believe our method overcomes traditional inaccuracies and is a generalizable technique that can be used to provide an updated database of nucleic acid thermodynamic parameters for more accurate prediction and software design. Based on these data, more accurate biophysical models could be developed to predict the thermodynamics and kinetics of nucleic acid hybridization and folding.

**Key words:** Biophysics Dangle-Thermodynamics DNA-catalyst Electrophoresis  
Fluorescent imaging

# Plasmon Resonance Tuning Using DNA Origami Actuation

Luca Piantanida, Denys Naumenko, Emanuela Torelli, Monica Marini, Dennis M. Bauer, Ljiljana Fruk, Giuseppe Firrao, and Marco Lazzarino

This study is focused on DNA nanotechnology as a tool for nano-bio-objects construction and gold particles functionalization. Gold colloidal nanospheres (AuNPs) functionalized with DNA have been widely employed as functional elements in self assembled nano-architectures. This approach was applied for optical property engineering, plasmonic hot spots in AuNP aggregates, and molecular ruler in AuNP dimers<sup>1</sup> in which the inter-particle gap was controlled with nm precision. The recent advances in DNA biomolecular computing have been the starting point to new software development. By using this new computational platforms is possible to create exceptional DNA self assemble structures, introduced firstly by Paul Rothemund<sup>2</sup> as "DNA origami". The degree of complexity of the structural shape achievable with DNA origami technique make it an excellent tool to create smart DNA scaffolds and dynamic structures<sup>3, 4</sup>. In this work<sup>5</sup> we created hybrid system combining the Localized Surface Plasmon Resonance (LSPR) properties of gold particles with DNA origami structure in order to create hybrid systems. Using this combination we introduced an original approach for plasmon resonance tuning which, in perspective, are the starting point for the development of innovative biosensors. In particular, using a DNA origami actuation to modulate the nanometric separation of two gold nanoparticles, continuous and reversible LSPR tuning has been demonstrated. The actuation mechanism is based on DNA hybridization of three different DNA sequences to induce resonance shift up to 6 nm. Replacing the target DNA sequence the resonance shift could be reversed. The proposed design is suitable to investigate DNA hybridization configurations, or could be used as tunable plasmonic platform for enhanced Raman applications.

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## **Colloidal particles as platforms for DNA nanotechnology**

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### **Track B Abstract:**

We have created 3 open source tools for manipulating DNA and generating DNA-coated hydrogel microparticles. Because DNA is relatively expensive and microparticle substrates are relatively cheap, the use of DNA-coated particles can help make the output of DNA computation scale up. Our poster summarizes our open-source tools and their application in creating DNA-coated hydrogel microparticles for self-assembly and DNA detection.

#### **Part 1. Open Source tools for DNA nanotechnology: PAGE rig, gel scanner, and homogenizer**

Open source tools and methodologies can decrease the cost of DNA nanotechnology. In an era of tight funding, it is imperative to invest every dollar efficiently. Open source methods, 3D printing, and re-purposing commercial electronics can all be used to create powerful instruments. By sharing designs and build instructions over the Internet, off-the-shelf tools and devices can be adapted to enable cutting-edge science. This approach to open source hardware has produced the Rep-Rap 3D printer, Open Thermocycler and similar projects.

We present 3 equipment designs that perform comparably to commercial scientific equipment that cost more than an order of magnitude more. Our gel rig allows for PAGE to be run in the same gel rigs as agarose gels. Our DNA gel scanner costs approximately \$100 but has performance comparable to \$10,000 instruments. Our homogenizer creates DNA-coated hydrogel microspheres and costs less than 10% of a comparable commercial homogenizer. These tools are all applied to the task of generating DNA-coated particles for self-assembly or detection.

#### **Part 2. Generation and application of DNA-functionalized particles**

Microparticles can detect analytes when fluorogenic DNA circuits are immobilized on their surfaces. These circuits can be as simple as one step strand displacement (OSD). These circuits can also execute more sophisticated amplification using DNA catalysis. We describe the use of a DNA walker to catalytically immobilize fluorophores on a microparticle surface.

These DNA-coated hydrogel particles can also self-assemble at centimeter scales. Eventually, the same type of particles may be used to detect nucleic acids within their self-assembled colloidal hydrogel matrix. Applications of these detector particles within a hydrogel/biomaterial matrix may include monitoring secreted signal molecules emitted by entrapped cells.

# DNA Circuits for Analog Computation

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**Abstract:** DNA circuits possess impressive potential to change the future of research and industry in synthetic biology because of their high programmability and versatility. Considerable research has been done to develop reliable and scalable architectures of digital DNA circuits. However, there remains the challenge of constructing practical DNA circuits to perform analog computation. In nature, there are systems that to a large extent work in an analog fashion; for example, analog computation plays a significant role in the functioning of brains and cells. Indeed, we can take careful note of the wide-spread use of analog computational techniques in brains and cells to inspire the design of artificial molecular-scale computational technologies. In particular, DNA-based analog computation can be the key to integration of artificial systems with natural systems that are analog. In analog devices like our DNA-based analog circuits, each basic arithmetic operation is executed by a single corresponding analog gate, whereas conventional DNA Boolean circuits require multiple Boolean gates to execute a single arithmetic operation. Hence our DNA-based analog circuits potentially may require much fewer gates to perform numerical computation compared to their digital counterparts, which means that their operations potentially need less energy and other resources. This property also may make analog circuits attractive in environments that have limited resources, e.g. in living cells or compact synthetic molecular systems. Here, we propose an architecture for the systematic construction of analog DNA circuits. The elementary gates in our analog DNA circuits include addition, truncation, and multiplication gates. The input and output signals of these gates are analog, which means that they are directly encoded (over a given operating range) by the relative concentrations of input and output DNA species respectively, without requiring a threshold for converting to Boolean signals. We provide detailed domain designs of our analog gates, kinetic simulations of our analog gates to demonstrate their expected performance, and we also describe some of our preliminary laboratory experiments demonstrating their operation (our experiments to date are limited to the most challenging gate, namely our analog multiplication gate). Based on these basic analog gates, we describe how analog circuits to compute polynomial functions can be built. Using Taylor Series and Newton Iteration methods, functions, beyond the scope of polynomials, can also be computed by compact DNA analog circuits built upon the proposed architecture. These include approximate computation of multiplicative inverse (and hence division), square root, exponentiation, and logarithm over a given input range.

**Keywords:** DNA circuits; analog computation; DNA self-assembly

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# Continuously Tunable Nucleic Acid Hybridization Probes

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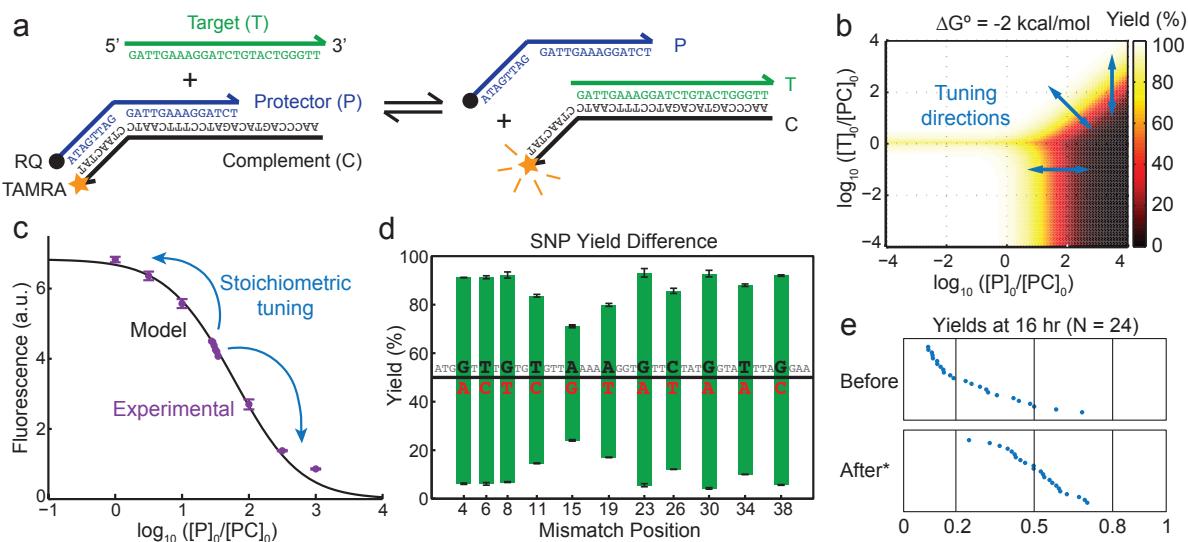
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**Abstract:** *In silico* designed nucleic acid probes and primers often fails to achieve favorable specificity/sensitivity tradeoffs on the first try, and iterative empirical sequence-based optimization is needed, particularly in multiplexed assays. Here, we present a novel, on-the-fly method of tuning probe affinity and selectivity via the stoichiometry of auxiliary species. Using this method, we are able to achieve near-continuous tuning of probe effective free energy (0.02 kcal/mol granularity). As applications, we maximized signal difference for 11 pairs of single nucleotide variants. Using the Nanostring nCounter platform, we have also applied stoichiometric tuning to multiplexed (up to 192-plex) quantitation of RNA from cell lines and FFPE.



**Abstract Figure 1:** **(a)** Schematic of a toehold probe. **(b)** Relationship between hybridization yield and stoichiometry when  $\Delta G^\circ = -2 \text{ kcal/mol}$ . **(c)** Experimental validation of stoichiometric tuning. Each data point (purple) represents the mean of  $n = 3$  and the error bar indicates 1 standard deviation. **(d)** Optimization of yield difference between reference and SNP targets. Each SNP target contains 1 mismatch at the position shown in red. The upper bound of each bar represents the yield of reference target, and the lower bound represents that of the SNP target. The error bar represents 1 standard deviation of  $n = 3$ . **(e)** Stoichiometric tuning of toehold probes on Nanostring nCounter platform in a 24-plex setting.  $[P]_0/[PC]_0$  ratios were simultaneously adjusted to attempt a uniform 50% yield for all 24 species.

# Nature Inspired Computation Design for DNA Circuitry

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In this research, DNA strand displacement is chosen as the main framework to design DNA circuits capable of complex computation mechanism, such as decision making and reinforcement learning mechanism. The goal is to make a DNA-based system that is adaptive and reactive to environmental changes. Our design is based on computational algorithm inspired by natural systems, namely Ant Foraging System. Our circuit design is theoretically feasible in wet-lab experiment by using DNA origami as spatial framework to localize DNA gate complexes. The computation is carried by a walker-strand that traverses along the structure. We verify our design in-silico. The DNA origami can be designed to construct an arbitrary landscape. Ant system algorithm is employed to find the most optimized solution. Future applications include decision making capable machine, and reusable DNA circuits. Figure 1 shows the conceptual design and simulation results.

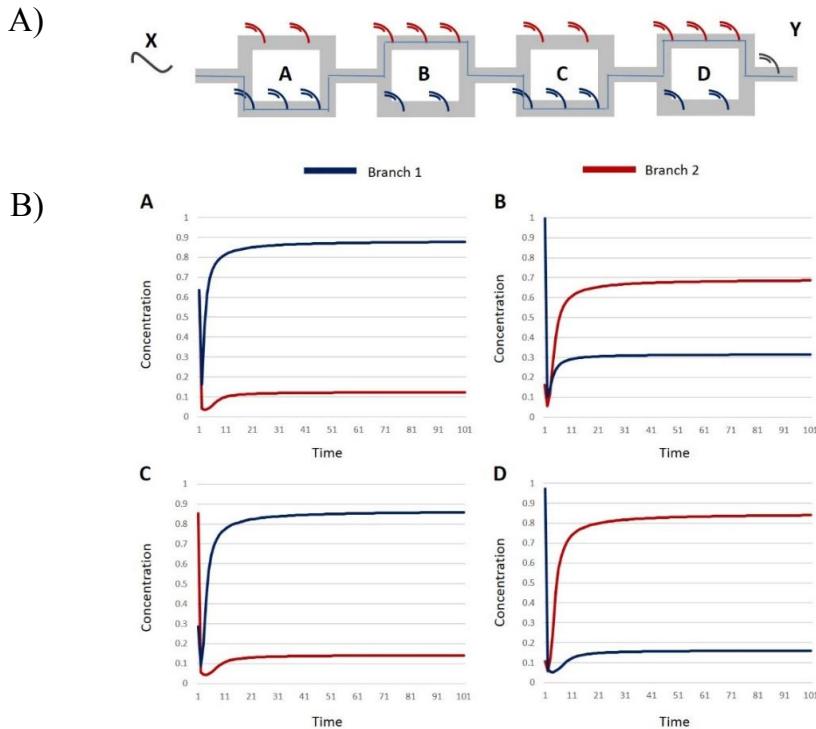


Figure 1: A) Modular designs of the double bridge structure. Red-colored extensions represent a set of gate motifs for the first branch; and blue-colored extensions represent the second branch. Each branch is assigned with arbitrary density of gate numbers (analogous to weight function). Both branches can take input signal X and to transform it into output signal Y after several intermediate reactions, which represents the walking of artificial ant-agent. The double bridge structure, as building blocks, construct a two dimensional landscape consisting of multiple paths with different weight function. The objective of design is to find the most optimum path. Expected solution is represented by the solid line. B) Simulation Results. Our design can infer the most optimum path, which is in this case determined by the density of gates, regardless the initial concentration of the input signals. Each figure shows reaction kinetics of corresponding to each block. Initial concentrations of both input signals in first block are equal, while in other blocks are determined by the final concentration of output signals in their upstream blocks. This design behavior does not depend on the concentration of input signals, but slight amount variation on other species yield slightly different absolute values of output signals concentration. We tune up the simulation parameters by iterated trials, and then choose the best value.

# Encapsulated DNA and Enzyme Reaction-Diffusion Network for Simple Cellular Automaton

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Ken Sugawara<sup>2</sup>, and Satoshi Murata<sup>1</sup>

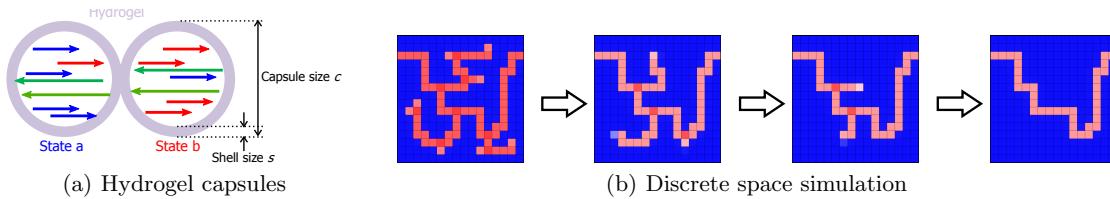
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## 1 Abstract

We introduce a theoretical model of DNA chemical reaction-diffusion network capable of emulating simple cellular automata. The model is based on well-characterized enzymatic bistable switch that was reported to work *in vitro* [1]. Our main purpose is to propose an autonomous, feasible, and macro-scale DNA system that is realistic enough to be implemented experimentally in the future.

As a demonstration, we choose a maze-solving cellular automaton. The key idea to emulate the automaton using chemical reactions is assuming a space discretized by hydrogel capsules which can be regarded as cells (Fig. 1(a)). The capsule is used both to keep the state uniform and control the communication between neighboring capsules.

Simulations based on reaction-diffusion ordinary differential equations were successfully carried out for continuous and discrete space (Fig. 1(b)). The simulation results indicate that our model behaves as expected both in space and time. Further investigation also suggests that more general computation can be performed by changing parameters and initial conditions. Possible applications of this research include pattern formation and a simple computation for the realization of molecular robots [2]. By overcoming some experimental difficulties, we expect that our framework can be a promising candidate to program and implement spatio-temporal chemical reaction systems.



**Fig. 1.** Concept of hydrogel capsules and simulation result. (a) Hydrogel capsules contain DNA strands for the bistable switch. (b) Simulation result of the maze problem is shown. In the figure, both concentration of DNA and time are continuous, while space is discretized.

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# Time-Responsive DNA Circuits for Universal Computation

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## Abstract

Nucleic acids have been shown to develop circuit systems that are predictable, modular, scalable and robust, and several theoretical and experimental designs have been investigated over the last two decades. Nucleic acid circuits have been used to detect the presence of target DNA/RNA molecules, to sense multiple diseases markers, and to investigate cellular pathways in cells. A majority of these approaches are feed-forward and use-once circuits, since the gates/motifs get permanently used up as part of the computation. This paper tackles the issue of building *renewable nanoscale circuits*, thus creating systems that are 1) energy efficient and 2) adaptable to changing inputs to produce the right response. Biochemical circuits are inherently asynchronous, and this necessitates that the circuit be robust to the order of arrival of different reactant species. Our goal is DNA-based logical gates that are *time-responsive*: that (a) ensure that the final output is always correct regardless of differences in the arrival time of different reactant species, and (b) furthermore even if the reactant species (input) change over time, the system modifies its output, and re-computes it based on the new inputs. The property of time-responsiveness appears to be central to self-regulation in cellular organisms. We propose two quite distinct hybridization-reaction systems for time-responsive DNA-based logical gates: (1) one using two-strand motifs, and (2) another using hairpin motifs. We present for both these systems detailed sequence designs and analysis. We also present our preliminary experimental work for the two-strand motif design.

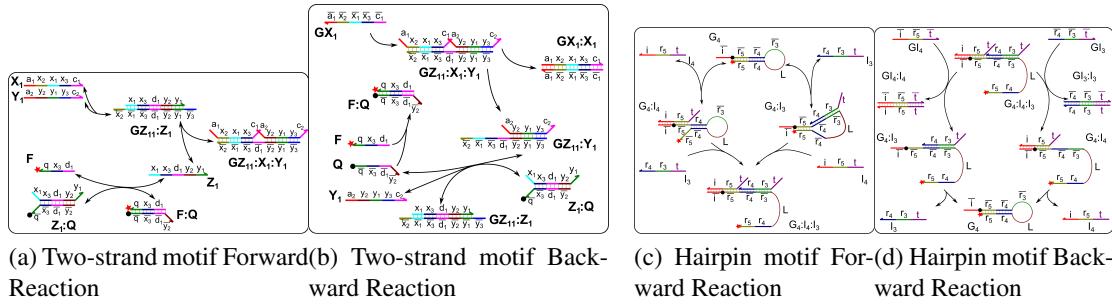


Figure 1: Cooperative Hybridization in Time Responsive Circuits

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# Automated Sequence Analysis for Domain-Level DNA Strand-Displacement Systems

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Recent experiments have demonstrated DNA’s exciting potential for designing and implementing a diverse array of types of biochemical computation. These systems are often designed at the abstract level of domains: functionally distinct, contiguous segments of DNA strands assumed to interact only with their complement. However, the sequences assigned to DNA strands can introduce non-ideal single base pair interactions that disrupt the desired system dynamics. Sequence-level system verification is difficult because the complexity of DNA strand interactions makes direct simulation only possible between small numbers of molecules at a time.

To address this shortcoming of current tools, we have developed a framework for achieving feasible sequence-level verification that uses the domain-level system to guide and interpret sequence-level simulations. This framework makes use of two aspects of the domain-level system: resting sets (that is, sets of conformations that a molecule may quickly convert within but may not quickly leave [1]) and reactions between resting sets. These two concepts encapsulate a simplified description of the domain-level system, and, in principle, verification is possible by examining related system properties, such as conformation probabilities and reaction rates, as determined by corresponding sequence-level data. However, finding a correspondence between data at the sequence level and at the domain level is difficult because of single base pair interactions that occur at the sequence level but are ignored at the domain level. We define a mapping from resting sets to sequence-level secondary structures and from resting-set reactions to sequence-level reaction trajectories that allows this type of sequence-level system verification.

This framework has been implemented in the Python software package KinDA, which integrates existing domain- and sequence-level analysis software tools. The domain-level analysis tool Peppercorn [1] is used to determine a system’s resting sets and resting-set reactions. KinDA then estimates resting-set conformation probabilities based on the frequency with which corresponding sequence-level conformations are sampled, using the Nucleic Acids Package (NUPACK) [2]. In a related process, the stochastic simulator Multistrand [3] produces data on sequence-level reaction trajectories, which are used to estimate rates of the system’s resting-set reactions. These estimates are computed through a combination of Bayesian and maximum likelihood techniques. KinDA is suitable for verifying DNA strand-displacement systems created using the domain-level abstraction after sequence assignment and before direct laboratory testing, or for determining possible reasons for the experimental failure of these systems.

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## Effects of DNA sequences on the performance of logic gate modules

Toshihiro Kojima, Yoko Sakai, Akira Suyama

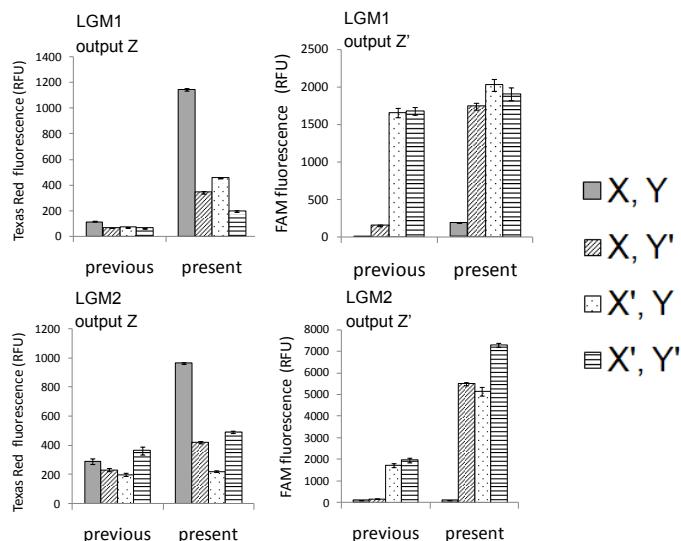
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A molecular circuit for a modular DNA computer generally contains multiple modules of the same function. These identical modules are distinguished from each other on the circuit by means of DNA sequences. If the performance of identical modules is strongly affected by DNA sequences, it becomes difficult to build a large circuit containing many identical modules. Therefore, reaction conditions and sequence-design methods need to be strategized to minimize the influence of DNA sequences on the performance of identical modules.

In this study, we experimentally examined the performance of three logic gate modules (LGMs) to ascertain the conditions and methods that can help minimize the influence of DNA sequences on LGM performance. LGMs are modules for the modular DNA computer, RTRACS<sup>1</sup>, and can perform AND, OR, NAND, NOR, INH (inhibitory) and NINH (NOT inhibitory) operations<sup>2</sup>. The three LGMs we studied—LGM1, LGM2, and LGM3—were prepared for assembling an XOR circuit. The current LGM DNA sequences were different from those of the LGMs we previously used<sup>2</sup>.

We studied LGM performance by measuring output RNA concentrations using molecular beacon probes. The output performance depended on the concentration of primer DNA, P, hybridizing with one of the two RNA inputs of LGM. A primer DNA concentration of 100 nM showed a more successful and sequence-tolerant output performance than did the conditions (10 nM) we used previously<sup>2</sup> (Figure 1). We also examined the output RNA generation step of LGM using gel electrophoresis. Because this step contained amplification reactions, it considerably affected the LGM output performance. LGM3 showed an unpredicted mis-hybridization that decreased its performance. We later found that this mis-hybridization could be predicted in silico by using UNAFold.

The new reaction conditions and DNA sequence design method allow us to develop many LGMs with high output performance. We intend to develop an XOR circuit and other DNA circuits by using our new LGM sets.



**Fig. 1** Concentrations of output RNA ( $Z$  and  $Z'$ ) as a function of input RNA ( $X$ ,  $X'$ ,  $Y$  and  $Y'$ ) combinations of LGM.

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## DNA-Origami Nanotubes: Insights Through Molecular Dynamics Simulations

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Nucleotide based synthetic ion channels provide an important complement to membrane proteins for the design of biomimetic nanopores. However, base-paired self-origami channel structures have not yet been fully characterised, either experimentally or computationally. Here we report all-atom molecular dynamics (MD) simulation studies of a DNA nanotube (DNT).<sup>1</sup> Four independent models have been evaluated to study the stability of a DNT in an aqueous environment, revealing a consistent model of their structural and dynamic properties. At each end of the DNT “clapping” motions were observed. In the central region of the DNT lumen of constant volume filled with water and ions was measured. The influence of DNT structural dynamics on the diffusion of ions and water was analysed. The clapping motions may modulate the intrinsic gating of DNTs when embedded in the membrane, whilst the porous nature of the DNT walls allows lateral leakage of ions and water. These findings provide insights on the intrinsic properties of DNT structures. This in turn prompted us to initiate coarse-grained (MARTINI)<sup>2</sup> simulations to investigate the stability of DNA structures embedded in a lipid bilayer. As an initial proof of principle we modelled a simple 20-meric ds-DNA molecule modified with a central hydrophobic belt of ~24 Å thickness, which approximately corresponds to the hydrophobic core of the phosphatidylcholine bilayer. The lipophilic-DNA is stable and does not significantly perturb the lipid bilayer. In future, coarse-grained MD studies of a DNT embedded in a membrane would allow us to explore longer timescale events, including interactions between the DNT and the lipid bilayer environment. The all-atom MD simulations may be compared with experimental ion-conductance studies, thus informing our understanding of possible gating mechanisms in DNA nanopores. These studies demonstrate that multiscale simulations will provide a valuable approach to investigate properties of novel DNA-based nanopores and their interactions with membrane environments.

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# Homogeneously Multiplexed Quantitation of Nucleic Acid Targets via Digital Thresholding

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(Dated: June 18, 2015)

The detection of DNA sequences is of great importance because of its broad applications in clinical diagnosis and understanding biological function. Multiplexing refers to methods for simultaneous detection of multiple nucleic acid sequences in a single reaction, which can reduce the time, cost, and labor associated with detection. Other than gene chips and beads which are based on surface chemistries, we can use the homogeneous multiplexing of nucleic acid detection. Homogeneous refers to the fact that we do not use spatial separation; all reactions proceed in a well-mixed solution. Current homogenous fluorescence detection uses a fluorescent probe, for example a molecular beacon, to detect a complementary target. This gives an analog fluorescence signal proportional to the target concentration. Readout equipment typically allow limited fluorescence color channels(less than 5-plex), which in turn limits the multiplexing of DNA detection. To overcome the limitation of color channel, we would like to detect different targets with the same fluorophore.

We transform the analog response into digital response. For example, we could design T1 to produce 1 unit of fluorescence signal when its concentration is more than X nM, design T2 to produce 2 unit of fluorescence signal when its concentration is more than Y nM. For this 2-plex digital system, we have 4 possible observed values: 0, 1, 2 and 3 units of fluorescence. This way we can detect 2 targets with the same fluorophore. We can further extend this to detecting 3 or more targets using additional levels of fluorescence to encode the other targets concentration information. Using multiple fluorescence color channels, we can further expand the plex number of our digital detection methods.

In order to achieve this outcome, we design a toehold reporter and a toehold trap directed at one target. The toehold reporter and the target undergo a classic strand displacement reaction with a fluorophore and a quencher on the toehold reporter. When target reacts with the reporter there will be increased fluorescence. The trap is thermodynamically very favorable but without fluorophore and quencher. So the target will first react proportionally with both the trap and the reporter, but the reporter reaction is reversible and re-releases the target, while the trap permanently binds the target. At equilibrium, we will not notice any fluorescence until the trap is used up and the reporter starts to react with the target(shown in Fig.1a). Simulation results shows with appropriate settings(for example in Fig. 1b) the probe set can respond to the target digitally (shown in Fig.1c). Experimental results show when mixing homogenous multiplexing (HM) probe sets HM1 and HM2, they are able to distinguish between 4 outcomes of the two targets (shown in Fig.1d).

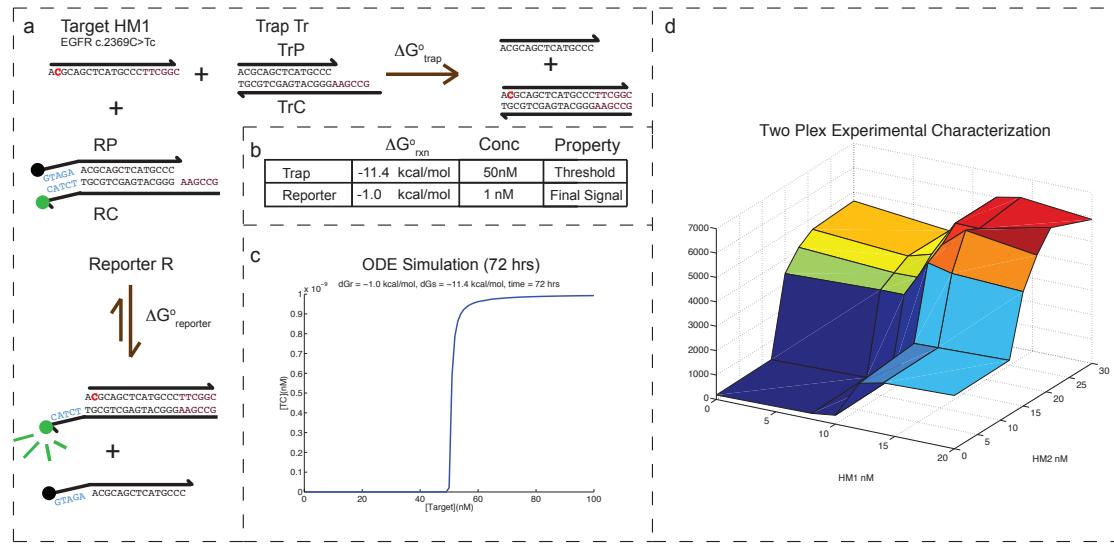


FIG. 1: Design and simulation of homogenous multiplexing probes. (a) Schematic of the multiplexing probe system. (b) Threshold implementation. (c) Computational validation of digital-like signal. (d) Experimental results for 2-plex in single fluorescence channel.

## **Ion-selective and switchable formation of guanine quadruplex structures on DNA origami structures**

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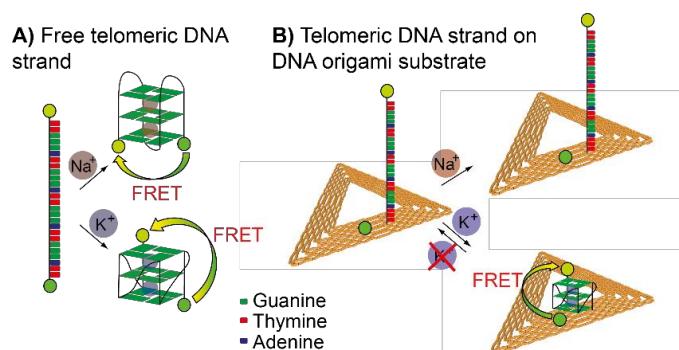
*Piotr J. Cywinski, Research Division Functional Polymer Systems, Fraunhofer Institute for Applied Polymer Research, Potsdam, Germany;*

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DNA origami nanostructures are a versatile tool that can be used to arrange different functionalities with high local control. Additionally, DNA origami structures combine the possibility to carry out measurements in solution and on surfaces for single molecule analysis without changing the nano-environment of the studied molecules.[1] Here, we demonstrate that DNA origami substrates can be used to suppress the formation of specific guanine (G) quadruplex structures from telomeric DNA. The telomere folds into two different G-quadruplex structures in presence of sodium and potassium (Figure 1A). Sodium induces the formation of a basket type G-quadruplex and potassium the formation of a propeller type G-quadruplex.[2] The folding of telomeres into G-quadruplex structures in the presence of monovalent cations (e.g.  $\text{Na}^+$  and  $\text{K}^+$ ) is currently used for the detection of  $\text{K}^+$  ions, however with insufficient selectivity towards  $\text{Na}^+$ .[3] By means of Förster Resonance Energy Transfer (FRET) between two suitable dyes we demonstrate that the formation of G-quadruplexes on DNA origami templates in presence of sodium ions is suppressed due to steric hindrance. Hence, telomeric DNA attached to DNA origami structures represents a highly selective detection tool for potassium ions even in the presence of high sodium concentrations (Figure 1B).[4]

In order to restore the biosensor a reversibly switching detection tool has to be designed. Here, we demonstrate that when a substance with a higher affinity towards potassium is added, the G-quadruplex unfolds and it returns to its starting conformation. Hence, the FRET signal is turned off. The switching process can be repeated several times on DNA origami structures.

Furthermore, DNA origami nanostructures can be used to arrange different organic dyes at different distances to create even more complex FRET systems. Here, we introduced a third dye to create a switchable three color FRET cascade on DNA origami structures, which is selective for potassium ions only.



**Figure 1:** Schematic illustration of studied systems based on FRET measurements. A) Free telomere folds G-quadruplexes in presence of  $\text{Na}^+$  and  $\text{K}^+$ . B) G-quadruplex formation on DNA origami structures is selective for  $\text{K}^+$  only and can be switched on and off several times.

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# Frequency Analysis of DNA Nanostructures through Mass-Weighted Chemical Elastic Network Model

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In our previous study, various DNA hairpin nanostructures (HPs) were synthesized by DNA sequence manipulation in order to use them as biomolecular memory. Also, their vibration characteristics were investigated by the mass-weighted chemical elastic network model (MWCENM) to validate their engineering feasibility [1]. Since MWCENM considers both inertia effect and chemical bond information, compared to other existing ENM methods, it can provide more precise vibration features of DNA HPs including both frequencies and their corresponding mode shapes [2]. In this study, we calculate vibration frequencies of various DNA HPs and compare them with the Raman experiment data. To take into account the experimental setup such as DNA HPs grown up on a substrate, 12 different boundary conditions are applied to DNA HP models when we perform their modal analyses. Although computed frequency values do not match exactly with experiment data due to simplification and approximation in modeling, one can indeed confirm that similar pattern between experiment and simulation enables us to identify vibration mode shape at a particular frequency. Consequently, this study will be a corner stone to build up a direct platform for evaluating dynamics of DNA nanostructures by comparing spectrum data with predicted frequencies using MWCENM.

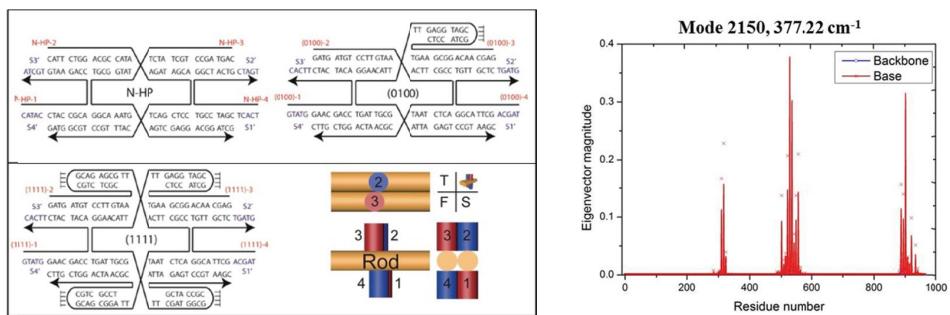


Fig.1. Various DNA HP sequences (left) and vibration intensity of the N-HP structure at Mode 2150, 377.22cm<sup>-1</sup> (right)

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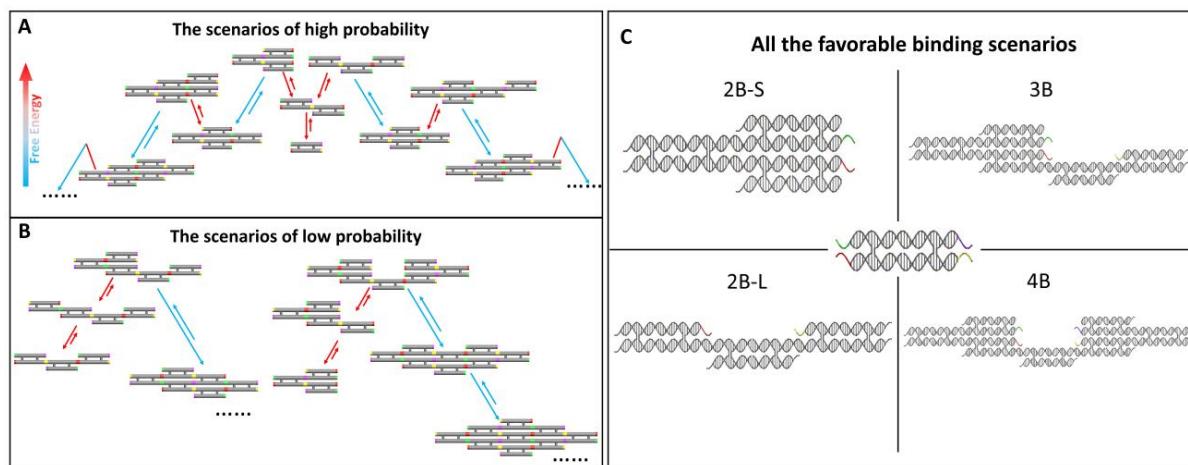
# Thermodynamics and Kinetics of Single DNA Tile Binding During the Nucleation Process of DNA Tile-Assembly

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**ABSTRACT:** Double crossover (DX) DNA tiles have been extensively used as the building blocks in structural DNA nanotechnology for two-dimensional (2D) DNA lattice construction, DNA nanotube engineering and algorithmic DNA self-assembly. However, a comprehensive study of the thermodynamic and kinetics of the association/dissociation of a single DX tile during nucleation process is still lacking. Herein, we used fluorescence based technology to investigate the DX tile binding during the nucleation process in real time, and obtained the thermodynamic and kinetic parameters of the individual tile binding events of 4 different scenarios: 1) tile binding via 2 sticky ends of a shorter distance (2B-S), or 2) a longer distance (2B-L), which are of high probabilities, and 3) tile binding via 3 sticky ends (3B) or 4) via 4 sticky ends (4B) which are the less likely scenarios under low super-saturation conditions. In the high probability scenarios, the free energy change suggests that 2B-L is more favorable than 2B-S. However the binding rate of 2B-S turns out to be approximately 2 times faster than that of 2B-L. In the less likely scenarios, 4B shows more negative free energy change and also faster kinetics in comparison with all the other cases. These results offer experimental evidence that DNA tile differs from the theoretical model “Wang Tile” majorly in kinetics, the former one is anisotropic rather than isotropic. Furthermore, the results shown here suggest further refinement to the kinetic Tile Assembly Model (kTAM), which was employed as the major theory of tile-based assembly. The thermodynamics and kinetics has been applied to control the nucleation process of DNA nanotube formation, the statistic results of nanotube circumference are in consistent with our prediction. The parameters in thermodynamics and kinetics of DX tile binding uncovered from this work would contribute to future improvements and optimizations in dynamic DNA nanotechnology, algorithmic self-assembly system and other nucleic acid based systems.



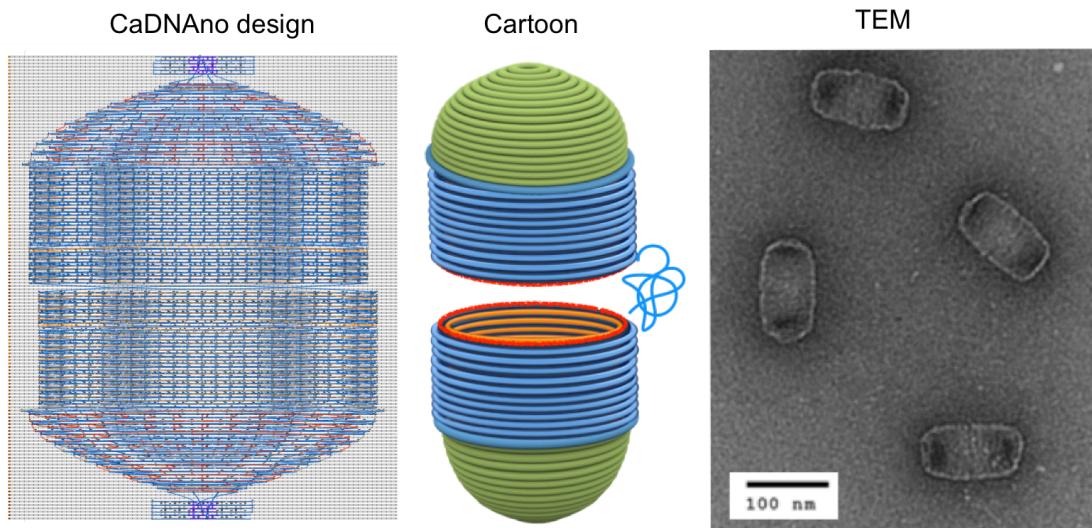
## DNA origami capsules

Maartje M.C. Bastings, Nandhini Ponnuswamy, William M. Shih

In this project, we developed DNA-origami nano-capsules with the aim to develop a DNA based delivery/targeting vehicle and nano-factory. The capsule contains five individual DNA origami units, folded from three separate designs: a dome, and 2 cylindrical units (see figure below). Rotational symmetry around the middle horizontal axis allows for use of the top two units twice. The unique central unit features a pH sensitive interface that allows for pH induced opening of the capsule. Upon sensing a pH of 6 or lower — the pH of endo-lysosomes in cells — the nano-capsule is programmed to open up and release cargo. To achieve this acidic pH trigger, we used the i-motif, which is a cytosine rich sequence that will dissociate from a Watson-Crick base- paired partner strand and then fold up on itself upon protonation of the N-3 of the cytosine ring.

Initial hurdles to overcome are DNA origami stability in the cellular environment and origami uptake through the appropriate internalization pathways. Furthermore, the anchors connecting the cargo to the inner wall of the nano-capsule will be programmed to release below pH 6 as well. The fine-tuning of cargo loading and release is an important focus point for the future success of our capsule.

We hope to use our new DNA capsules as an adjuvant to study pathway specific immune activation, drug delivery and use them as nano-factories for RNA production. Immuno-adjuvants are compounds able to increase or modulate the antigen specific immune response by their ability to activate selectively one of the immune response pathways, Th1 or Th2. The quest to manipulate the immune system to generate optimally effective immunity against different pathogens can be considered the ‘grand challenge’ of modern immunology. By systematic screening of immune-pathway activation of DNA-origamis combined with antigens and danger-signals, we hope to set small steps forward into a better understanding of the complex mechanisms of our immune system.



Controlled translocation of single nanoparticles on lipid membranes confined by DNA origami corrals

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In the biological membrane systems that are heavily occupied by membrane-associated proteins and other biomolecules, the fast encounter of functional proteins in such a crowded environment is essential for the function of the cell. One strategy that is utilized by cells to achieve efficient diffusion in crowded biomembranes, *e.g.* that of the thylakoid, is to form supracomplex to provide space on the membrane for small molecules and proteins to diffuse through. This is particularly important for the diffusion of carriers of high-energy electrons and the constant protein repair processes that facilitate the continuous function of the photosystems. Due to the complexity of these natural biomembranes systems, the construction and investigation of a minimal reconstitution system *in vitro* will help to study the diffusion behavior of nanoscale objects in such sophisticated biological membranes.

Herein, we attempt to reconstitute the confined translocation of particles on lipid membranes confined by DNA origami corrals that is comparable in size to the diffusion paths on the thylakoid membranes. We designed an hn-shaped DNA origami structure with a rectangular inner corral of 40 nm by 20 nm as a confining space on membranes of small unilamellar vesicles. We chose 5 nm gold nanoparticles (AuNPs) as a model to diffuse within the confinement on the lipid membrane. The ssDNA-modified AuNPs were firstly loaded at the “donor” site of the origami corral, and then the AuNP-loaded origami structures were anchored onto the membrane surface mediated by cholesterol-modified DNA (chl-DNA) molecules. After the AuNPs were triggered to release with DNA strand displacement reactions, they remained attached on the membrane by additional chl-DNA anchors and diffused until binding with the “receptor” site on the opposing end of the channel. By TEM observation and FRET measurements we observed that this system showed an effective translocation of the AuNPs within minutes from the donor to the receptor site. To the best of our knowledge, this is the first example that realizes controllable diffusion with confinement on the lipid membranes and will benefit our further efforts to build artificial membrane systems with biological functionalities.

## Gold nanolenses on DNA origami substrates for surface-enhanced Raman scattering

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Surface-enhanced Raman scattering (SERS) exploits the enhancement of electromagnetic fields in close vicinity of plasmonic nanostructures, enabling characterization of analytes at the single-molecule level. The nanometer-scale spatial arrangement of plasmonic metal nanoparticles and analyte molecules has a significant effect on the observed signal enhancements and represents a great challenge in this technique.

In our work DNA origami is used for precise positioning of individual gold nanoparticles (AuNPs) and analyte molecules (Fig. 1). Especially high sensitivities are expected for gold nanolenses (AuNLs), employing defined rows of 3 or more differently-sized AuNPs. We assembled AuNLs consisting of different sets of AuNPs and assessed their potential for SERS measurements. Raman spectra from single AuNLs were measured and signal enhancements in comparison to isolated AuNPs were calculated. In finite difference time domain calculations we estimate the attainable electromagnetic field enhancements in the synthesized systems. Ultimately we aim to develop a versatile platform for various SERS applications.

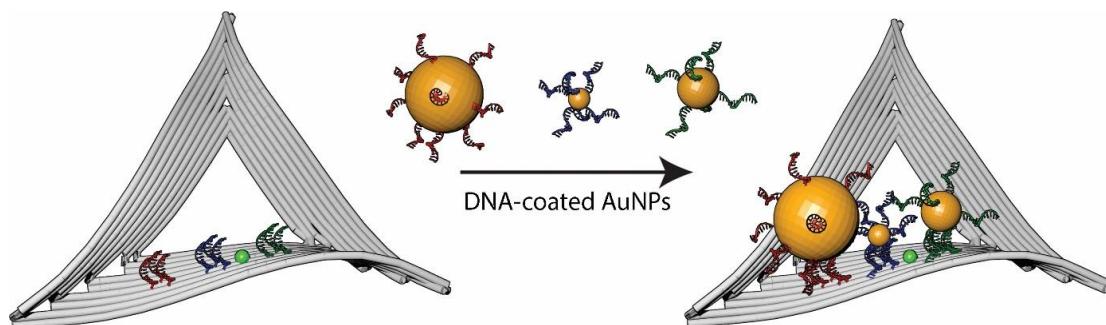


Figure 1: Gold nanoparticles constituting a gold nanolens are assembled by triangular DNA origami scaffold

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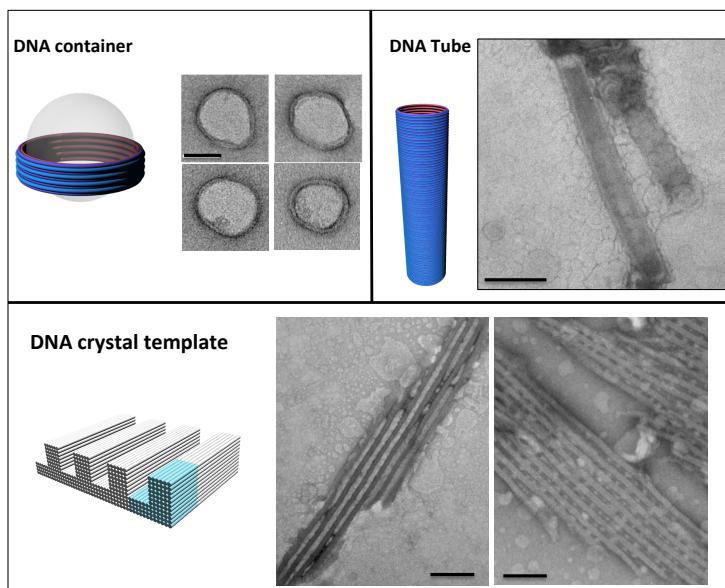
### **DNA Nanostructures Scaffolded Lipid Compartments**

Zhao Zhao<sup>1,3</sup>, Wei Sun<sup>3</sup>, Steve D. Perrault<sup>1,3</sup>, Peng Yin<sup>3</sup> and William M. Shih<sup>1,2,3</sup>

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**Abstract:** Lipid bilayer is one of the most fundamental structures in biology. Different biological functions require specific lipid curvature, size and geometry, so it is important to construct design shaped liposome. Here we present a novel method to reconstitute liposome with controllable geometry within DNA origami cavity. To accomplish this, DNA origami is attached with lipid molecules serving as nucleation sites, which can direct liposome formation while DNA origami serves as boundary cavity. Cylindrical DNA origami has been utilized to produce 75 nm diameter uniform liposomes, and furthermore, polymerized cylinder origami has been tested and tube shaped liposome has been successfully constructed, proved by electron microscopy.

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# PREDICTING ENHANCER REGULATORY OUTPUT USING A SELF-AVOIDING WORMLIKE CHAIN MODEL WITH PROTRUSIONS

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Enhancer-regulated genes are abundant in all organisms<sup>1,2</sup>. Although enhancers have been extensively researched over the past decades, enhancer structure-function relationship is for the most part poorly understood. While it is generally agreed that DNA looping plays a central role in enhancer regulatory output, most enhancer structural features, such as the importance of having several binding sites for a given transcription factor and the functional role of particular arrangements of the binding sites, remain poorly understood. As a result, computationally predicting an enhancer regulatory output function has proven to be difficult<sup>3</sup>.

In order to address this problem, we devised a novel self-avoiding wormlike chain model for enhancers<sup>4</sup>. At its basis, the model relies on numerically generating statistical ensembles of thick chains decorated with adherent protrusions, which are characterized by a particular size, position along the chain, and relative orientation. We employ a weighted biased sampling algorithm<sup>5</sup> to ensure that these ensembles faithfully sample the underlying configurational distribution of the thick chain. Given these ensembles, we are then able to study the effects of the adherent protrusions on the probability of the polymer chain to form a loop, and consequently simulate the effect of DNA-bound transcription factors on regulatory output.

Based on the numerical results we constructed libraries of synthetic bacterial enhancers aimed at testing the predictions of the numerical model and characterized them in a series of *in-vivo* experiments in bacteria. Our combined theoretical and synthetic biology experimental findings suggest that protein excluded-volume inside DNA loops can account for both up-regulating and down-regulating effects in synthetic bacterial enhancers. We found that the nature and magnitude of the regulatory effect is directly influenced by the size of the transcription factor, the number of bound transcription factors and their relative arrangement within the regulatory region. We also show that the magnitude of the effect is directly proportional to the size of the transcription factor and inversely proportional to its distance from the DNA-loop termini. Both the nature of the effect and the magnitude are highly sensitive to the location of the transcription factor binding site within the DNA loop and exhibit an oscillating pattern whose period matches the helical repeat of the dsDNA. Additionally bound transcription factors can either augment or diminish the effect, depending on their relative orientation to the other transcription factors. Consequently, our model introduces a novel physical mechanism, which establishes a basic structure-function relationship for enhancer regulatory output.

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## DNA origami for advanced lithography

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For years, Moore's law has been consistent in predicting the growth rate per square inch on integrated circuits, which has doubled every two years. Unfortunately, this prediction is become less and less reliable due to optical limitations (i.e. diffraction limitation of light) that brakes photolithography resolution improvement at the nanoscale level. In order to continue miniaturization of electronic devices by decreasing CMOS (*Complementary Metal Oxide Semiconductor*) node below 10 nm, DNA self-assembly has been proposed as an alternative to photolithography. In fact, the combination of a nanometric size, an extreme flexibility and an auto base pairing property of the DNA molecule allows the design of scalable architectures with a nanometric precision called DNA origami [1]. These technologies are fast-growing thanks to the low cost of DNA synthesis and the major breakthroughs on software allowing the design of complex DNA shape [2].

Several examples of silicon patterning with DNA origami have been described [3,4]. Despite these progresses, few work has been done on the transfer of DNA patterns in silicon oxide [5]. The transfer process is based on silicon oxide etching with fluoridric acid (HF) using DNA as a mask. According to the humidity level and the temperature of the process chamber, etching occurs either on DNA leading to a negative tone pattern or either outside DNA leading to a positive tone pattern. Although these results have demonstrated the tremendous potential of DNA origami as a lithographic mask, yet the resolution of the technique has to be studied. Moreover, the described etching process needs to be adapted for large-scale processes.

The objective of this research is to study whether transfer of DNA origami patterns can be performed with classical semiconductor etching processes. To do so, we used a 3D DNA origami bearing a sub-20 nm aperture. We showed that these processes, even they are harsh, do not destroy the DNA origami and allow area-selective etching of the silicon oxide substrate with a sub-20 nm resolution.

**Acknowledgment:** The authors thank the CEA through the "Phare Projects" research program (A3DN) for the financial support.

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# Stochastic Analysis of Chemical Reaction Networks Using Linear Noise Approximation

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**Abstract.** Stochastic evolution of Chemical Reactions Networks (CRNs) over time is usually analysed through solving the Chemical Master Equation (CME) or performing extensive simulations. Analysing stochasticity is often needed, particularly when some molecules occur in low numbers. Unfortunately, both approaches become infeasible if the system is complex and/or it cannot be ensured that initial populations are small. We develop a probabilistic logic for CRNs that enables stochastic analysis of the evolution of populations of molecular species. We present an approximate model checking algorithm based on the Linear Noise Approximation (LNA) of the CME, whose computational complexity is independent of the population size of each species and polynomial in the number of different species. The algorithm requires the solution of first order polynomial differential equations. We prove that our approach is valid for any CRN close enough to the thermodynamical limit. However, we show on some case studies that it can still provide good approximation even for low molecule counts. Our approach enables rigorous analysis of CRNs that are not analyzable by solving the CME, but are far from the deterministic limit. Moreover, it can be used for a fast approximate stochastic characterization of a CRN.

# Markov Chain Computations using Molecular Reactions

Sayed Ahmad Salehi, Keshab K. Parhi, Marc D. Riedel

*Abstract*—Markov chains can be used for modeling numerous computing systems and processing applications including signal processing, stochastic and biological processes. Therefore, any systematic method developed for synthesizing Markov chains using DNA strand displacement reactions can be used to implement these applications using DNA. This paper, for the first time, describes a general approach to implement any first-order Markov chain using molecular reactions in general and DNA in particular.

Markov chain is composed of two parts: a set of *states*,  $S=\{s_1, s_2, \dots\}$ , and *state transitions*,  $T_{ij}$ , which represent transitions from state  $i$  to state  $j$ . In the proposed method these parts are mapped to a molecular system as follows. Each *state* in Markov chain is represented by a unique molecular type, referred as *data molecule*. *Data molecule* for each state is distinguishable from molecules corresponding to other states. For example molecules of  $D_i$  represent state  $s_i$ . Each *state transition* is modeled by a unique molecular type, referred as *control molecule*, and a unique molecular reaction. For example, *state transition*  $T_{ij}$ , is represented by *control molecule* of type  $C_{ij}$  and the molecular reaction  $C_{ij} + D_i \rightarrow C_{ij} + D_j$ . This reaction transfers *data molecules* of state  $s_i$  to the *data molecules* of state  $s_j$ , using (but not changing) the concentration of related *control molecules*  $C_{ij}$ . All reactions are assumed to have the same rate.

Two features of a Markov chain are its starting *state* and the probabilities of each *state transition*. These features determine the initial concentrations of all *data* and *control molecules* in the proposed model. The initial concentrations for all *data* molecular types are zero except for the *data molecule* related to the starting state which should be a nonzero value. A larger initial value for *data molecules* increases the accuracy of computation. The concentrations of *control molecules* are determined according to the transition probabilities of the corresponding *state transitions*. In each *state*,  $s_i$ , the ratio of concentration for a *control molecule*,  $[C_{ij}]$ , over the total concentrations for *control molecules* of all outgoing *state transitions*,  $\sum [C_{ik}]$ , is equal to the transition probability of the related *state transition*,  $p_{ij}$ . In other words, the initial concentration for *control molecules* can be determined by this formula

$$p_{ij} = \frac{[C_{ij}]}{\sum_k [C_{ik}]} \quad \text{for all outgoing state transitions from } s_i.$$

Now we have a system of molecular reactions with defined initial values. The ratios of concentrations for *data molecules* at the equilibrium determine the steady state probabilities for each *state* in the Markov chain. In order to analyze our proposed method we synthesize a set of molecular reactions for the Gambler's ruin problem as a simple instance of Markov chain. We analyze and simulate it based on both stochastic chemical and mass-action kinetics models.

In the next step the produced set of molecular reactions is mapped to DNA strand displacement reactions. Since all reactions have two reactants and two products, we design a “*Domain1-toehold1-Domain2-toehold2*” as the DNA strand template for each molecular type in our system. For example for a molecular type  $A$  we have the following DNA strand:



We then simulate the produced DNA strand displacement reactions using Mathematica tool from Caltech. The error in computing the steady-state probabilities of the *states* is shown to be less than 1%. These simulation results validate our proposed model.

For more details the reader is referred to [1].

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# Leakage Reduction by Mismatches and Secondary Structure in a Catalyzed DNA Strand Displacement Reaction

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Catalytic DNA strand displacement systems are set into motion by introducing a unique catalyst sequence to trigger a cascade of toehold-mediated strand displacements. The forward reaction of the metastable system, in the absence of the catalyst, is often referred to as leakage – which limits the system performance. Here, a complete set of base-pair mismatches was examined at key breathing positions between the fuel strand and the substrate complex in a catalytic DNA strand displacement system. Availability was defined as the probability that a base is unpaired at equilibrium and was calculated by NUPACK<sup>1, 2</sup> from the predicted secondary structure ensemble lacking pseudo-knots and non-Watson-Crick interactions. We found changes in availability caused by the secondary structure played a more significant role in the reaction kinetics than mismatch identities. By examining the availability and mutual availability of the fuel and substrate, a semi-quantitative analysis of the leakage rates was provided. By introducing mismatches to the fuel strand, the performance of an already optimized system was improved by a factor of 4. Availability is a novel concept for leakage reduction design in nucleic acid catalytic reactions.

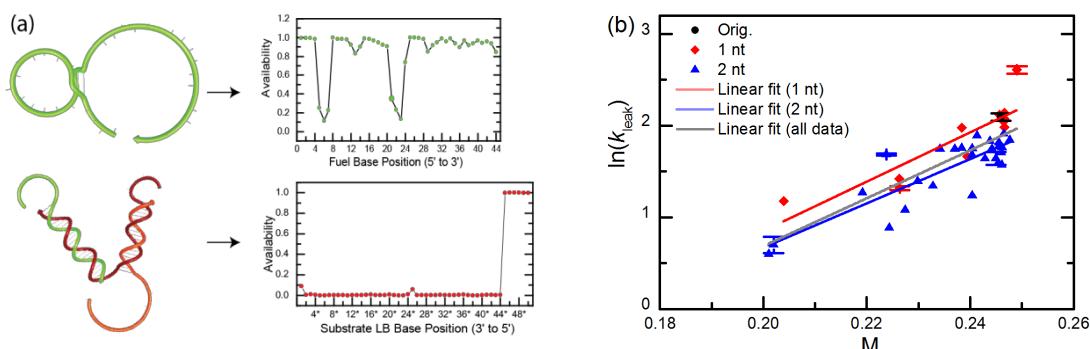


Figure 1: (a) Secondary structures of the fuel and substrate and corresponding base availabilities of the fuel (F) and the linker strand of the substrate (LB). Top left is the secondary structure of the fuel strand and top right is its base availability. Bottom left is the secondary structure of the substrate (the dark red strand is LB, the green strand is OB and the orange strand is SB). Bottom right is the base availability of LB strand. F and LB strands form the waste product. (b) Natural log plot of the leakage rate constant versus the mutual availability between the fuel strand and the LB strand on the substrate – defined as  $M = \sum_i (P_{F(i)} \cdot P_{LB(i)})$ , where  $i$  indexes the base pairs in the F-LB waste product in correct registration. In other words,  $i^*$  is the base position of strand LB that matches the complementary position  $i$  of the fuel strand.  $P_{F(i)}$  is the availability of base  $i$  of the fuel strand and  $P_{LB(i^*)}$  is the availability of base  $i^*$  of the strand LB within the substrate. Representative error bars of select samples are shown, indicating that the scatter of the data is greater than the experimental error. The red, blue, and gray lines are the fits for one-nucleotide (1nt) modifications, two-nucleotide (2nt) modifications, and all modifications combined, respectively. Error bars are shown for select samples whose measurements were repeated to estimate experimental error. Error bars represent the standard deviation of the mean of three samples.

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# Self-avoiding wormlike chain model for double-stranded-DNA loop formation

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We compute the effects of excluded volume on the probability for double-stranded DNA to form a loop. We utilize a Monte Carlo algorithm for generation of large ensembles of self-avoiding wormlike chains, which are used to compute the  $J$  factor for varying length scales. In the entropic regime, we confirm the scaling-theory prediction of a power-law drop off of  $-1.92$ , which is significantly stronger than the  $-1.5$  power law predicted by the non-self-avoiding wormlike chain model. In the elastic regime, we find that the angle-independent end-to-end chain distribution is highly anisotropic. This anisotropy, combined with the excluded volume constraints, leads to an increase in the  $J$  factor of the self-avoiding wormlike chain by about half an order of magnitude relative to its non-self-avoiding counterpart. This increase could partially explain the anomalous results of recent cyclization experiments, in which short dsDNA molecules were found to have an increased propensity to form a loop.

# Combinatorial production of sequenceable, optical DNA origami nanobarcodes for cellular position-tracing in tissue-transcriptomics studies

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One of the current still challenging goals for the single cell transcriptomics field is to introduce the spatial information into the sequencing data, in order to permit the interpretation of cellular gene expression data in the context of the cells' microenvironment (1, 2).

Here we present a method we are developing to combinatorially create a tagging system suitable for tracing the positions of individual cells in tissue samples from RNA-seq data. The tagging system consists of a library of 1024 different fluorescent, geometrically encoded DNA-origami barcodes (3) that are detectable by both fluorescent microscopy and sequencing. This is achieved by encoding the optical code of each barcode into their respective scaffold DNA molecules through cloning (Fig 1.).

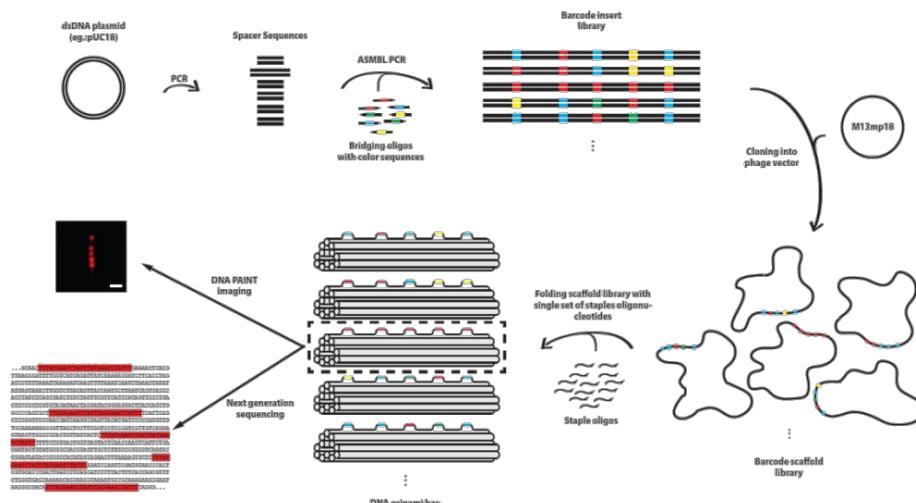


Fig. 1. Schematic representation of the production and detection of the DNA origami nanobarcodes

The technique presented offers to be a multiplexed tagging system that can be constructed without the need of excessive hands-on work, because of the combinatorial nature of its production. Furthermore the detectability of the barcoding system by both super resolution microscopy (e.g.: DNA PAINT, STORM) and next generation sequencing techniques (e.g.: PacBio SRMT, Illumina MiSeq) render the technique a good candidate to couple the spatial information of cells in tissue to their RNA-Seq data through using the system to tag cells in intact tissue samples for a super resolution microscopy mapping step preceding the sequencing.

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# Shaping Assembly: Exploring the Role of Building Block Geometry for Molecular Design

Pablo F. Damasceno & Sharon C. Glotzer

Although largely unexplored, building block's shape corresponds to a major tuning knob in self-assembly [1]. The creation of lock-and-key nanoparticles [2], 3D self-replicators [3], and shape-complementarity-based DNA devices [4] are just a few of the recent works demonstrating how shape's vast parameter space can be explored for complex structure design. In this contribution, we explore the role of building block geometry for self-assembly of Brownian, excluded-volume interacting particles. In particular, we show how intricate structures of unprecedented complexity can be assembled from simple choice of geometrical units, and how the class of structures to be assembled can be predicted based on the proposed theory of Directional Entropic Forces. Finally, we also demonstrate how shape complementarity can be used to promote self-replication of information-bearing strings of building blocks, allowing for an expansion on the information to be transmitted between self-replicated generations. Altogether, those results reveal the advantages of considering the effects of shape when designing structures in the nanoscale.

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# Site-specific attachment of polythiophene to DNA origami

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Conjugated polymers (CPs) feature alternating single and double bonds in their molecular backbone that allow them to conduct. The conductivity (semi-conductive to metallic [1]) and other material properties can be tailored feasibly during the syntheses, which are also less costly than current inorganic semiconductor or metal fabrication. Hence, CPs comprise promising components for future electronic information processing systems. Most research and applications in that direction have focused on the bulk material so far, although the controlled placement of the nanosized single CP molecules would undoubtedly add to the means of micro- and nanocircuit assembly. That imbalance might be not least because single molecule manipulation is still exceptionally demanding and the choice of techniques is limited (mainly AFM, STM). For the alignment of nanosized items, DNA origami [2] has emerged as a valuable tool. However, to the best of our knowledge, it has barely been used to arrange CP molecules [3,4]. This could be due to the challenges of this task, such as first and foremost the insolubility of most CPs in aqueous media, while origami will hardly tolerate organic solvents.

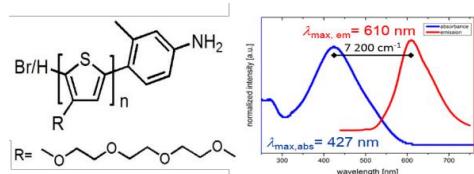


Fig. 1. Formula and linear absorption/steady-state fluorescence emission spectrum of the poly-3-(triethoxymethyl) thiophene synthesized for the coupling to DNA origami.

Here the successful site-specific attachment of a polythiophene (PT) (Fig. 1) to the DNA origami template “tPad” [5] is presented. Step 1 of this 4-step-process was the synthesis of a P3RT-type polythiophene. As the first group, we have chosen this polymer because the PT-specific Kumada catalyst-transfer polycondensation (KCTP) [6] synthesis allowed all the adaptions we set for the material. Firstly, a narrower molecular weight distribution than in [3,4] and a high degree of regioregularity were achieved. Both features are favourable for the conductivity, which currently lies in the semiconductive range for the undoped product. Secondly, in contrast to the previous studies, an intrinsically water-soluble CP could be synthesized by introducing oligo(ethylene oxide) sidechains to each monomer, yielding full compatibility with the DNA origami environment. Thirdly, using the *ex situ* initiated KCTP allowed introducing one functional start group to the PT chains. This was crucial for step 2, the coupling of one oligodeoxynucleotide (ODN) to each chain. Unlike [4], we aimed at a 1-PT-1-ODN conjugate in order to place the PT as site-specifically as possible on the DNA origami. The target conjugate was produced in high yield by strain-promoted alkyne-azide cycloaddition in aqueous solution. This was confirmed by PAGE and HPLC. AFM was used to approve the successful completion of step 3, the synthesis of tPads with specific attachment sites for the conjugate. Regarding step 4, i.e. attaching the conjugate to the tPad by sticky end hybridisation, first experiments have been completed. As observed by AFM, they have resulted in the formation of hybrid structures. Current work focuses on optimizing the attachment yield.

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## Stable DNA Reaction-Diffusion Patterns

Dominic Scalise, John Zenk, Kaiyuan Wang, Ariana Cruz and Rebecca Schulman

Chemical reaction-diffusion patterns are common in biological systems, where they can manipulate the behavior of cells and determine the body plans of some organisms. While synthetic patterning systems exist, it remains challenging to generate time-stable patterns because diffusion tends to mix solutions into homogeneity.

Here we present a simple DNA mechanism for generating temporally stable synthetic reaction-diffusion patterns *in vitro* within agarose gels. Our system consists of two liquid reservoirs at either end of a block of gel, with each reservoir containing a mix of synthetic DNA oligonucleotides. The species from each reservoir diffuse together and meet within the gel, where they react with each other *via* DNA strand-displacement. Two types of reactions are designed: *production* where an output signal strand is released into solution from a precursor complex, and *degradation* where the output is slowly recaptured by a different complex and sequestered into inert waste. The boundary conditions of each species within the gel are maintained by periodically refreshing the liquid reservoirs. We demonstrate the assembly of a set of patterns that are stable on the order of several days, and suggest that by actively maintaining boundary conditions, patterns can persist indefinitely.

Unlike traditional Turing patterns, where the final pattern is largely determined by random fluctuations, here the shape of the stable pattern and the concentrations of a species at each point in the system are determined by simple, well-defined boundary conditions. These features can each be programmed by controlling the concentration of the component species and by designing the reactions between them.

## Direct visualization of a photo-controlled DNA walker on the DNA nanostructure

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A photonic light-driven artificial molecular nanomachine is demonstrated based on DNA origami. A single stranded DNA carrying two pyrene molecules employed as walker is assembled with a rectangular-shaped DNA tile containing four disulfide bond modified stator strands as a linear track.<sup>[1][2]</sup> The walker is located on the surface of DNA tile by hybridizing with stators. The excited pyrene molecules ( $\lambda_{\text{ex}} = 350$  nm) of the walker can induce the cleavage of disulfide bond of associated stator, which will initiate the movement of walker migrating from cleaved stator to the next intact one on the DNA tile consequently. The entire stepping process of the walker is determined by characterizing the distribution ratios of walker-stator duplex at four anchorage sites on the tile. More importantly, the real-time occurred light-fuelled one-step motions of the nanomachine are directly observed during the UV irradiation using high-speed atomic force microscopy (AFM).

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## Lipid-bilayer-assisted two-dimensional self-assembly of DNA origami nanostructures

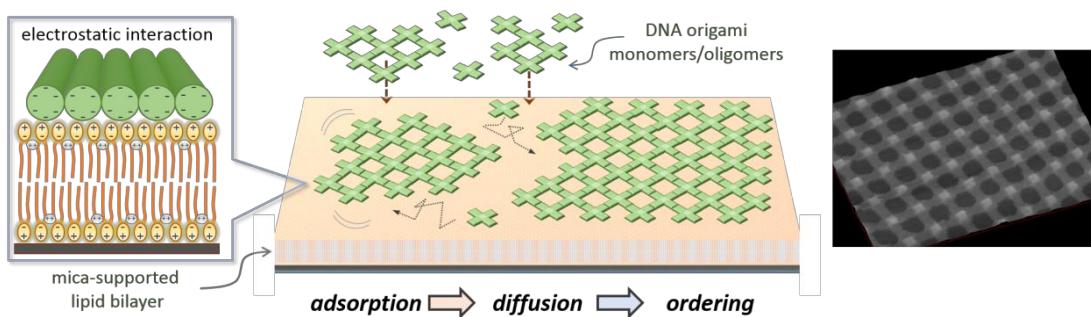
Yuki Suzuki,<sup>†,§</sup> Masayuki Endo,<sup>‡,§</sup> and Hiroshi Sugiyama<sup>†,‡,§</sup>

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Self-assembly is a ubiquitous approach to the design and fabrication of novel supermolecular architectures. Here, we describe a strategy termed ‘lipid-bilayer-assisted self-assembly’ that was used to assemble DNA origami nanostructures into two-dimensional lattices. DNA origami structures were electrostatically adsorbed onto a mica-supported zwitterionic lipid bilayer in the presence of divalent cations. We demonstrated that the bilayer-adsorbed origami units were mobile on the surface and self-assembled into large micrometer-sized lattices in their lateral dimensions. Using high-speed atomic force microscopy (HS-AFM) imaging, a variety of dynamic processes involved in the formation of the lattice, such as fusion, reorganization and defect filling, were successfully visualized. The surface modifiability of the assembled lattice was also demonstrated by *in situ* decoration with streptavidin molecules. Our approach provides a new strategy for preparing versatile scaffolds for nanofabrication and paves the way for organizing functional nanodevices in a micrometer space.



# Multi-Legged DNA-Based Molecular Motors

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## **Abstract**

Natural molecular machines, made of proteins, play a major role in many important biological processes, often with impressive operational yields and speeds<sup>1</sup>. Inspired by biological bipedal motors such as kinesin and with the assistance of single-molecule fluorescence and computer controlled microfluidic<sup>2</sup> device we designed and operated a DNA-based bipedal motor<sup>3</sup> that can stride on a DNA origami track with high operational yield. The reaction yield was 98% per step, which results in overall operational yield of 50% for 36 steps. To the best of our knowledge, this is the highest operational yield achieved by artificial molecular motors so far; however, it is not sufficient for repeatable operation of molecular machines for technological usage, for example, such as molecular assembly line and efficient maneuvering and manipulation of guest molecules. Moreover, this results are for the use of a low concentration "Fuel" strands (200nM) resulting in a slow stepping speed of one step per 1.5 hours. Increasing the fuel concentration to 10uM resulted in a decrease of the overall yield per step, 87%, at one step per 10 minuets rate.

This work focuses on the effort to understand the reasons for the 5-10% walker dissociation, and to increase walker processivity, operational yield and speed. To achieve a basic understanding of the motor's limiting factors, kinetic measurements were made for the different reactions that make up a step, resulting with empiric reaction rates. We later built a kinetic numeric simulation to describe the correlation between each reaction rate and the final stepping yield. One possible solution for the walker dissociation is the multi-leg and multi-foothold approach, in which the walker consists of two pairs of legs and the track consist two rows of footholds. Preliminary results show an increase in yield per reaction of about two times, when operated 10uM fuel concentration. The walker, which consists of four legs, was prepared using "click" chemistry, and the motor, operated by microfluidics, was evaluated using single-molecule fluorescence.

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# Model-based Design of Compliant DNA Origami Mechanisms

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Scaffolded DNA origami is a recently emerging technology that allows the construction of complex nanostructures via molecular self-assembly. This approach has been successfully used to construct complex 2D or 3D static structures. We expand scaffolded DNA origami nanotechnology to design dynamic nanomechanisms by following a design framework that parallels macroscopic mechanism design. First, we design and fabricate the basic building block links and kinematic joints, e.g. revolute (hinge), prismatic, cylindrical, universal, and spherical joint. Second, we assemble multiple links and joints into structures with more complex motion, which we term DNA origami mechanisms (DOM).<sup>1,2</sup>

Recently, we have particularly focused on expanding the design of DOM by introducing concepts of macroscopic design where motion occurs by mechanical deformation of components as opposed to highly flexible hinges (Figure 1). We refer to these structures as compliant DNA origami mechanisms (CDOM).<sup>3,4</sup> The compliant components are designed by placing fewer dsDNA bundles in the component cross-section, which can be easily realized with DNA origami. Furthermore, we have successfully used the classic Euler-Bernoulli beam model and Pseudo-Rigid-Body model to calculate the deformation and energy variation of the compliant components built in CDOM (Figure 2). We have successfully designed and fabricated a compliant hinge joint and compliant bistable mechanism and are currently exploring other folding structures inspired by macroscopic paper origami design motifs. The experimental results, for example the compliant joint angle distributions and the free energy landscape of the four-bar bistable mechanism, which are characterized by a Boltzmann probability distribution, closely match our model predictions. We are further developing novel approaches for actuation of these devices based on DNA strand inputs. In the future, we aim to integrate these machines with molecular computing efforts to enable mechanical nanodevices with improved control and information processing capabilities. In particular the ability to design specific energy barriers as we have demonstrated with a bistable mechanism, may provide a structural mechanism for binary computation.

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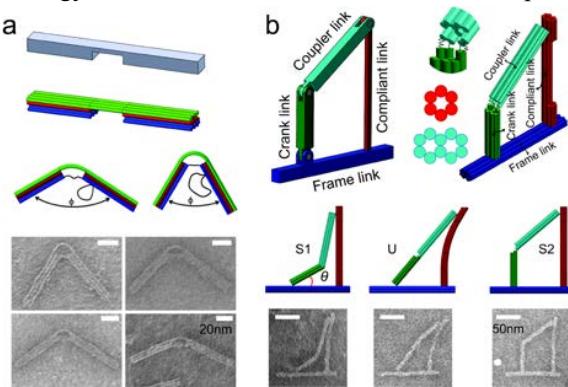


Figure 1. Compliant DNA origami mechanisms. (a) Compliant joint. (b) Compliant four-bar bistable mechanism.

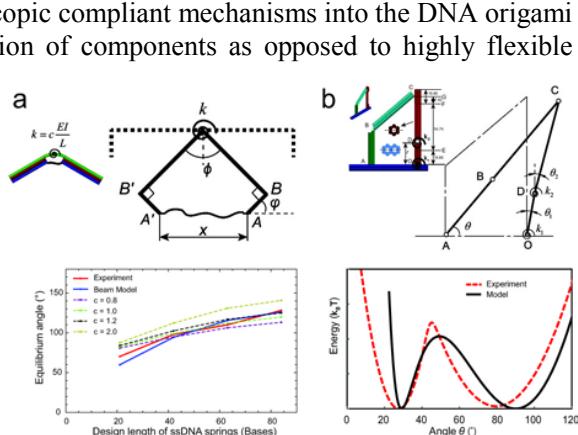


Figure 2. Computational model of compliant DNA origami mechanisms.

## Sticker-based algorithm for addition and its implementation in CUDA\*

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The sticker model [1] was one of the early universal DNA-based computational models. Its operation resembles a register machine, representing a binary-coded number (bitstring) on a partially double-stranded DNA molecule. Computation is carried out through four operations that include the merging/separation of bitstring datasets and the setting/clearing of a bit on every bitstring in a dataset. These simple operations, theoretically allow the construction of an algorithm for every computational problem [5] and thus, the model was initially used in the definition of algorithms for intractable problems. Nevertheless, the exponential growth in the amount of DNA remains as a limiting factor for solving arbitrarily-sized problems. In spite of this and other issues, the high parallelism of DNA-based computing is still an attractive feature, directing the efforts towards the design of a general-purpose molecular supercomputer capable of performing intensive numeric calculations. In this sense, the main challenge consists in the definition of arithmetic algorithms serving as building blocks for these applications.

The present work introduces a sticker-based algorithm for the sum of two  $n$ -bit integers written in two segments within a DNA strand. Using the idea of [2], two input test tubes  $T_c$  and  $T$  are managed to contain strands with and without intermediate carry, respectively. The addition of two integers  $A + B$  proceeds as follows. According to the *Full-Adder* truth table, each bitwise intermediate sum  $o_i = a_i + b_i$  ( $1 \leq i \leq n$ ) consists in an XOR logic operation ( $o_i = a_i \oplus b_i$ ) for strands in  $T$ , and an XNOR ( $o_i = a_i \oplus b_i$ ) for strands in  $T_c$ . Next, strands in  $T$  with  $a_i = b_i = 1$  are sent to  $T_c$ , whereas strands in  $T_c$  having  $a_i = b_i = 0$  are transferred to  $T$ . This process is repeated for the sum  $o_{i+1} = a_{i+1} + b_{i+1}$  of the next bit and so on. The proposed algorithm takes  $5 \text{ tubes} \times 7 \text{ steps} = 35 \text{ tube-steps}$  (metric introduced in [2]) to perform a bitwise addition. Although this result does not outperform the one obtained in [2], it is a significant improvement to the logic gate-based algorithm mentioned in the same paper. Moreover, since a general-purpose molecular computer needs to carry out logical operations, one advantage of logic gate-based arithmetic consists precisely in the reuse of these already defined procedures.

Finally, with the purpose of obtaining computational results, we programmed our algorithm and those presented in [2] and [3], in an in-house parallel implementation of a CUDA-based sticker simulator.

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## Lipid-modified DNA rings on hydrophobic surfaces

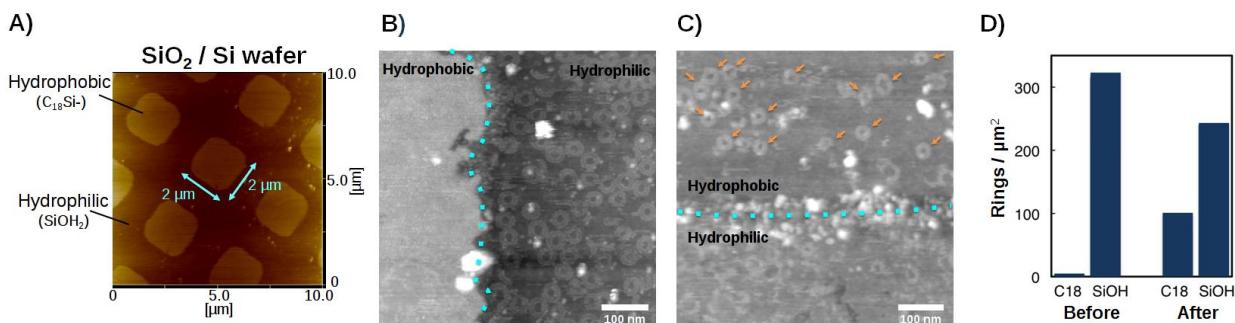
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In this poster presentation we report on the hydrophobic properties of DNA rings [1] modified with cationic lipids ( $2\text{C}_{12}\text{N}^+$ ). DNA rings consist of connected monomers made of two types of single-stranded DNA and their size is about 40nm. Cationic lipids in aqueous solution bind ionically to the phosphates of the DNA backbone and provide a hydrophobic shield for the ring. Unmodified rings in  $\text{Mg}^{2+}$  solution were deposited on a  $\text{SiOH}_2$  surface patterned with hydrophobic regions of  $2\mu\text{m} \times 2\mu\text{m}$ , and remained immobile on the hydrophilic regions. After in-situ lipid-modification of the rings, the modified rings were clearly visualized on the hydrophobic regions by atomic force microscopy (AFM). Quantification of modified rings suggests migration from hydrophilic to hydrophobic regions of the surface. We attribute this behavior to a hydrophobic mechanism driven by the lipid-modification. This mechanism looks promising for reversible hydrophobic actuation since the lipid bind ionically to the backbone of DNA. Switching the binding surface of nanostructures based on the hydrophobic actuation of lipids in solution is promising for the mechanical motion of nanostructures in cargo transportation.



A) AFM image of the substrate with hydrophilic ( $\text{SiOH}_2$ ) and hydrophobic ( $\text{C}_{18}$ ) regions. Before B) and after C) in-situ lipid-modification of DNA rings on the hydrophilic/hydrophobic substrate observed by AFM. D) Number of DNA rings per  $\mu\text{m}^2$  on hydrophilic and hydrophobic regions for before and after lipid-modification (B and C).

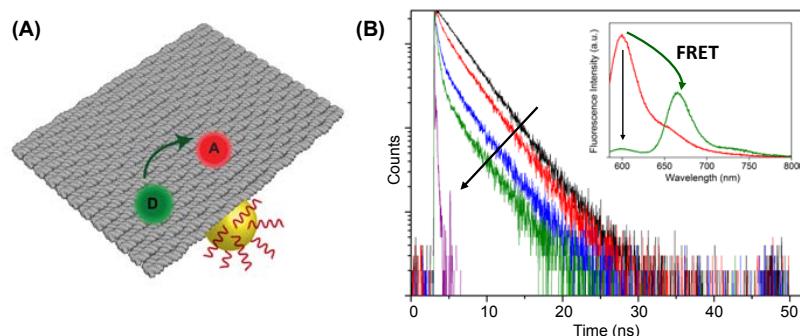
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## Engineering Energy Transfer Rates of Single Fluorophore Molecules close to Gold Nanoparticle using DNA Origami Constructs

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Förster fluorescence resonance energy transfer (FRET) is one of the most popular methods to measure intramolecular distances and conformational changes of single biomolecules. For conventional FRET between organic fluorophores, distance measurement does not easily exceed 8 nm. There is a need to extend this distance for many biochemical and nanophotonic applications and also to understand how external perturbations might influence non-radiative energy transfer. Metal nanoparticles (MNPs) have plasmonic resonance properties that can modify the local electric field and thereby influence the fluorescence emission (enhancement or quenching) of fluorophores.<sup>1</sup> In the presence of a Donor-Acceptor FRET pair, high efficiency of energy transfer is expected to be reached if MNPs and fluorophores are organized in a specific configuration with a high structural control.<sup>2</sup> Here, we used DNA-origami nanostructure<sup>3</sup> as a bread-board to arrange with nanometer precision 5 nm Gold Nanoparticle (AuNP) and a FRET pair (Alexa fluor568 and Atto647N, as donor and acceptor, respectively) at predefined positions on the DNA origami. Fluorescence steady-state and lifetime measurements are employed to study the effect of the AuNP on fluorescence emissions and energy transfer rates by varying the distance of the donor to the AuNP, and maintaining a fixed location of the acceptor. Currently, we are investigating a number of nanoparticle/fluorophore patterns to better understand the interactions between fluorophore molecules and MNPs in order to create an optimal construction for enhancement of energy transfer rates. We demonstrated a significant (up to 3-fold) increase in the FRET rate for large donor-acceptor separations exceeding 7 nm. The experiments are supported by theoretical predictions based on extended Mie theory.



**Figure.** (A) Schematic representation of the assembly of donor-acceptor (denoted D and A, respectively) FRET pair together with AuNP attached to the opposite side of the DNA origami. (B) Time-resolved fluorescence of DNA origamis labelled with single fluorophores, with only donor fluorophore (black), donor with AuNP (red), donor-acceptor with and without AuNP (blue and green, respectively). Inset shows steady state spectra from DNA origamis labeled with only donor (red) and with donor-acceptor (green).

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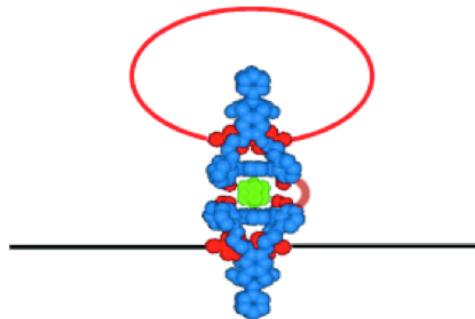
## Design and Construction of a Molecular Turing Machine

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Molecular scale devices capable of performing specific missions are of great interest in nanoscience and pose a fascinating challenge in nanotechnology. Recently, we have developed a catalytic molecular device that can perform processive oxidation on DNA.<sup>1</sup> Inspired by the operation of this Turing-like machine,<sup>2</sup> we are now developing a completely synthetic molecular Turing machine (Figure 1), which is based on a tape-head that can execute actions on a polymer tape according to instructions from an attached ring. The tape head is a double-caged porphyrin macrocycle bridged by a DABCO ligand connecting the metal centers (manganese and zinc) of the two porphyrin cages. One of the cages will contain an interlocked ring compound, i.e., a catenane, which has the ability to shuttle back and forth by external stimuli and to give instructions to the tape. As a tape, polymers will be used containing sites that can be chemically transformed, for example, alkene double bonds in polybutadiene that can be catalytically converted by the manganese porphyrin macrocycle into epoxide functions.<sup>3</sup> The information transfer from one cage to the other can be controlled by molecular recognition effects and positive allosteric conformational changes.<sup>4</sup> Our goal is to use this system for the intelligent tailoring of polymer and to develop a general purpose molecular computer. The synthetic efforts and the progress of developing this molecular Turing machine will be discussed.



**Figure 1:** A molecular device in which information is transferred from a catenane ring (red oval) to a polymer chain (black line).

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# Tile shape as a design parameter for DNA origami tilings

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In traditional DNA tile assembly, small DNA-based building blocks – typically DNA double crossover (DX) tiles – were used for the generation of ordered lattices via self-assembly in two dimensions. The interactions between different tile types were programmed and tuned via sticky end interactions. Implementation of sophisticated assembly rules into these interactions resulted in the formation of algorithmic assemblies such as DNA-based counters, Sierpinski triangles (formed via XOR logic), etc. Using DNA origami structures as tiles, in principle the same strategy can be applied. Compared to DX tiles, however, DNA origami blocks are typically much larger and can be designed with a large diversity of shapes.

Size and shape can be exploited to access additional design parameters for the generation of molecular lattices. Specifically, we here explore tuning parameters such as tile-surface interactions, tile-tile attraction via base-stacking or sticky end interactions, and also repulsive sterical interactions between origami structures. Tile-surface interactions on mica can be tuned electrostatically, e.g., by the addition of monovalent ions such as  $\text{Na}^+$  (figure 1 A). Reduced charge interactions result in higher mobility of the DNA structures and allow the utilization of shape-dependent sterical interactions between the tiles (figure 1 B). In combination with base-stacking in 1D and 2D, and using variously shaped origami tiles, a series of different crystal-like patterns with different lattice types can be produced (figure 1 C and D).

Using several distinct origami structures together can be used for the formation of binary crystals and potentially even non-periodic tiling patterns. Moreover, it is feasible to create patterns consisting of cavities (figure 1 B, C and D), which could be filled by other types of DNA origami tiles in a second assembly step.

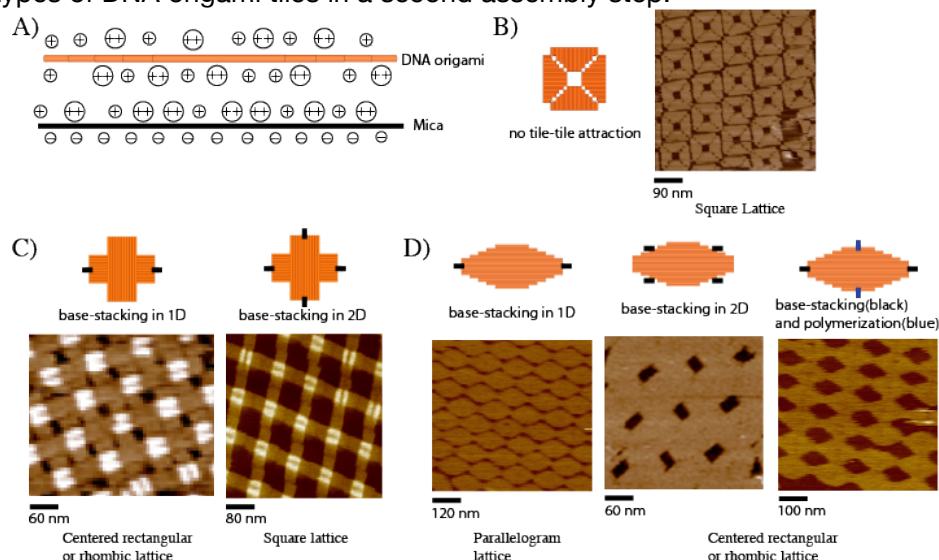


Figure 1: A) Schematic representation of DNA origami-surface interactions B) AFM image of pattern made using square origami with no tile attraction C) AFM images of two different patterns made by cross origami tile using base-stacking in 1D and 2D D) AFM images of three different patterns made using 8-sided polygon, employing 1D and 2D base-stacking (left and middle), and polymerization plus 1D base-stacking (right). Below the AFM images, lattice kind is specified. Black signs in tile models show the active sides for tile-tile interactions.

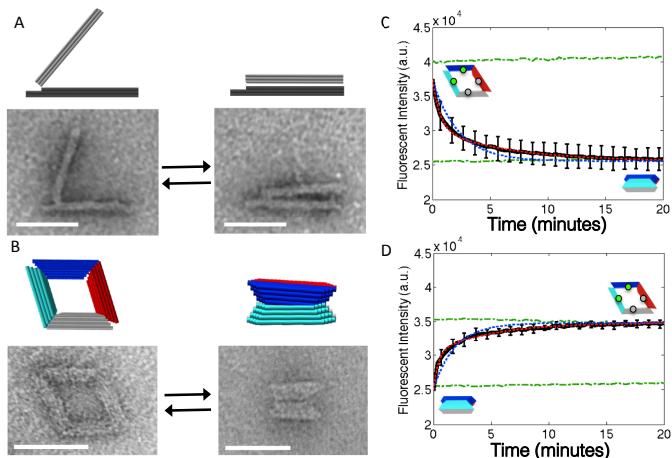
# Rapid Actuation of DNA Origami Mechanisms

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DNA origami enables the precise fabrication of nanoscale geometries. We demonstrate an approach to engineer complex and reversible motion of nanoscale DNA origami machine elements<sup>1</sup>. We developed flexible DNA origami rotational (Fig. 1A) and linear joints that integrate stiff double-stranded DNA components and flexible single-stranded DNA to constrain motion along a single degree of freedom and demonstrate the ability to tune their flexibility and range of motion. Linear motion, which is new to this field, was achieved by folding a tube concentrically around a track, through a novel approach of programming sequential folding of individual components within a structure. In this case the track folds first, and then the tube is constrained to assemble around the track. Multiple joints with 1D motion were then integrated into higher order mechanisms. One mechanism is a crank–slider that couples rotational and linear motion, and the other is a Bennett linkage (Fig. 1B) that moves between a compacted bundle and an expanded frame configuration with a constrained 3D motion path. We have also developed multiple approaches for distributed actuation of these mechanisms using DNA input strands to form new connections distributed throughout the structure to achieve reversible conformational changes. One approach<sup>1</sup> uses stable input strand connections with DNA strand displacement (Fig. 1C-D), and we have recently developed a novel approach for reversible actuation that relies on transient binding of many weak affinity input strands. In this case, the reverse actuation can be achieved simply by removing the input strands from solution. We expect this approach can improve actuation times to second or sub-second timescales because it does not rely on strand displacement. Our results demonstrate programmable motion of 2D and 3D DNA origami mechanisms with steps towards fast mechanical actuation. Since their actuation relies on DNA input strands, these machines could ultimately be integrated with DNA computing to enable control and information processing.



**Figure 1: Actuating rotational motion of DNA origami mechanisms.** (A) Simple rotational motion in 1D using a DNA origami hinge. (B) Four hinges were integrated into a Bennett linkage with complex 3D motion. Our distributed actuation approach using stable connections closes (C) and opens (D) this mechanism on ~minute timescales. Scale bars = 50 nm.

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# **Interactive visualization of RNA folding pathways, with application to the design of molecules with long, diverse folding pathways**

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Elizabeth Sweedyk, and Anne Condon

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**Abstract.** Artificially designed nucleic acid molecules with long, low energy folding pathways have many potential uses, from performing computations with biological material, to having therapeutic applications. However, designing molecules that have predictable folding pathways is challenging. The best currently available tool for examining folding pathways is Multistrand 2.0, a Python package that simulates a DNA or RNA strand folding using a sophisticated and realistic energy model. However, the folding simulated by Multistrand 2.0 is a stochastic process, and thus the simulation often oscillates between a few common strand configurations before a new structure occurs which progresses the pathway. As a result of its realistic and stochastic simulation, Multistrand 2.0 outputs large and often difficult to interpret text files. Although these pathways can be visualized using other tools such as RNAMovies, the output remains difficult to interpret due to the constant oscillation of the pathway. Our software provides users with a simple way of running Multistrand 2.0 which does not require the user to have knowledge of Python, filters out extraneous oscillations from the large output files, and reports summary statistics about the removed loops. We then present interactive visualizations of the filtered pathway and the energies of the structures within the pathway. Researchers can use this software to test and modify new molecular designs until they fold as desired. Additionally, we have analyzed a theoretical RNA molecule designed to take a long, low energy, folding pathway under a simplistic energy model. We discover that it does not take the expected pathway under a realistic energy model, and we propose several modifications to its structure to start the molecular design revision process.

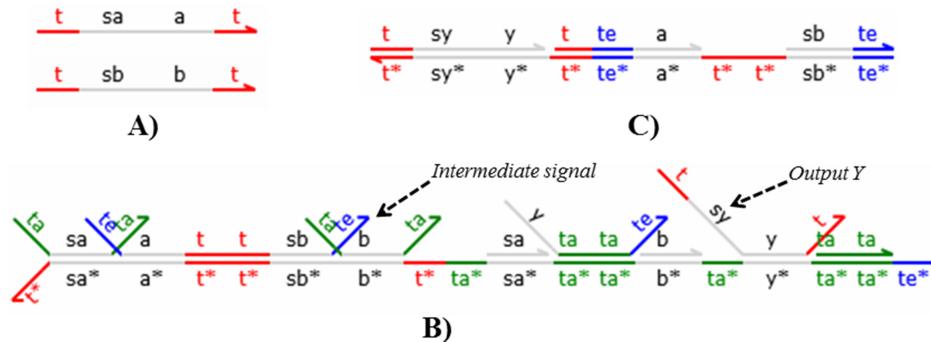
# HALF-ADDER BASED ON DNA STRAND DISPLACEMENT \*

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Strand displacement relies only on the hybridization of complementary nucleotides to displace pre-hybridized partial double-stranded DNA. We present the design of a half-adder circuit using AND and XOR logic gates implemented with strand displacement reactions. Our gates perform logical operations in two stages with an estimated response time of 25 minutes (or less). Unlike previous approaches, the eXclusive-OR function is directly emulated as a single gate, instead of being constructed by means of other basic gates, as for example in [1] and [2]. Design specifications are as follows: input and output signals are represented as single-stranded DNA molecules ( $5' - 3'$  oriented) with *short:long:long:short* (Fig. 1 A)) domain structure. Boolean values are given by the absence (0) or presence (1) of molecules at some concentration. *Toeholds* share the same nucleotide sequence in signals and gates, allowing to perform cascade reactions. Each logic gate is composed of a main complex (Fig. 1 B)) and an auxiliary complex (Fig. 1 C)). Main complexes are partially complementary strands assembled to recognize input variables while an auxiliary complex reacts with an intermediate signal derived from its main counterpart to release or trap the output strand, depending on the truth table of the gate. The feasibility of such designs was demonstrated with stochastic simulations using the Gillespie algorithm [3] implemented in Microsoft Visual DSD software. Computational results showed that the reliability for all tested cases are above 98%. Although these outcomes demonstrate efficient and novel fast responsive DNA gates for the construction of a half-adder, further studies are needed to qualify their performance in cascade and, more conveniently, in laboratory.



**Fig. 1:** Schematic components of XOR gate: A) input variables; B) main complex; C) auxiliary complex.

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# A versatile evolutionary algorithm for generating fit sets of oligonucleotide sequences

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The predictable nature of Watson-Crick base pairing has been the underlying foundation of nucleic acid nanotechnology since its inception in the early 1980's.<sup>1</sup> However, as the size and complexity of nucleic acid based structures and reaction networks has increased, so has the potential for undesired Watson-Crick interactions. Here, we propose a simple yet versatile algorithm for generating fit sets of nucleic acid sequences which conform to an arbitrary domain level design.

The evolutionary algorithm complements state of the art design approaches in three key ways. First, it expands upon existing domain-based approaches by establishing fitness as a function of strand identity (after domains are assembled into a set of strands), as opposed to domain identity (before strands are assembled).<sup>2</sup> Second, it evaluates fitness based solely on Watson-Crick interactions rather than more complex thermodynamic algorithms.<sup>3,4</sup> Finally, the algorithm accepts arbitrary numbers and sizes of domains of either fixed or variable identity – allowing the algorithm to exhibit expanded versatility over traditional Sequence Symmetry Minimization (SSM) approaches.<sup>1</sup>

Execution of the algorithm begins with an initial design provided by the user. The fitness of the design (and all subsequent designs) is evaluated using a metric which penalizes for intra-strand complements, inter-strand complements, and unfit sequence arrangements (such as consecutive bases). Following scoring, the input design becomes the first “mother” generation. From this “mother” design, “daughter” designs are created via the random permutation of one or more domains. All designs are evaluated for fitness, and the fittest design is chosen to be the mother for the next evolutionary cycle. The algorithm proceeds by repeating this cycle a fixed number of times, and ends with reporting the domains and scores of the fittest design identified.

The implementation of our algorithm is a java-based software application named Sequence Evolver (SeqEvo). SeqEvo is a design tool capable of accepting domain level designs as an input and producing sequence level designs as an output. The program was benchmarked on two DNA systems. The first system contained 10 strands, 70 variable bases, and 260 total bases. It took a server utilizing 4 Opteron 6136 processors (32 cores) approximately 20 seconds to select a design with no unintentional complements longer than 3 bases. The second system contained 50 strands, 450 variable bases, and 1640 total bases – and took the same server 36 hours to produce an apt design. These benchmarks suggest that SeqEvo is capable of generating fit sets of oligonucleotide sequences for state of the art sized systems on reasonable timescales.

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# Controlling the Reversible Assembly/Disassembly Process between Components using Molecular Recognition in Molecular Robots

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Biologists believe that the application of regeneration cycle that reverse the signals between assembled and disassembled states extends the lifetime of the cell [1]. The assembly process for creating structures of two and three dimensional deoxyribonucleic acid (DNA) structures has been extensively addressed in many areas of nanotechnology, including applications in diagnostics and transporting molecular payloads to cells [2]. However, the reverse process such as controlling disassembly process has been more difficult to realize [3]. Moreover, the controlled assembly/disassembly of DNA structures in response to chemical or biological stimuli without provision of other materials such as enzymes or changing environmental conditions, that has the potential for applications, cannot be addressed only by currently existing self-assembly approaches.

In this paper, we report a multicomponent assembly and disassembly processes using DNA strand displacement to construct molecular robots. The framework for molecular robotics development designates the components as a part of the robot, which consists of an interface as sensor or actuator. Moreover, assembly and disassembly of the DNA structures were performed in an isothermal chemical reaction using double-stranded DNA as glue code to combine the components. A component is a double-stranded DNA or multi-stranded DNA and implements at least one well-defined interface. In this paper, we used two types of components that each component annealed from two single strand DNA and had 2 interfaces. Furthermore, we designed a new strand displacement schema in double-stranded DNA as glue code. In this schema, displacement domain is a combination of two or more toehold domains. This scheme has the purpose to create flexibility of displacement and toehold domain position in the next strand displacement reaction. As result, the glue code can be as the reversible component assembler and molecular detection.

Molecular recognition is used to control the reversible assembly and disassembly processes of multi components. The proposed molecular recognition model identify not only a single strand DNA but also a micro ribonucleic acid (miRNA) as molecular stimuli in controlling the processes. The process begins from a collection of separate components and glue code, which are unable to form a new structure in the absence of a stimulus signal for assembly. If the stimuli of assembly are detected by the glue code, the assembly process will run to create a new structure or shape. In the experimental work, we used a single strand DNA or miRNA has-mir-21 as stimuli. After the assembly process is completed, the new structure is formed in a stable condition. The disassembly process will run and the shape or structure will be separated into components as same as the initial condition as well as the glue code when the stimulus of disassembly appears. In the experimental work, this process used only a single strand DNA as stimuli. Accordingly, the process will be repeated if there is a stimuli signal. Moreover, it is possible to reuse the component to build different shapes or structures by using different glue code with interface combination. . The assembly and disassembly mechanisms were experimentally demonstrated by gel electrophoresis results, fluorescent and atomic force microscopy observations.

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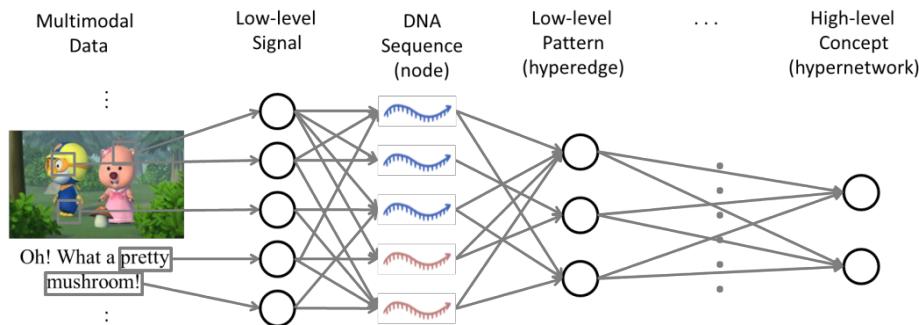
# DNA Encodings for *in Vitro* Molecular Learning of Multimodal Data

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## Abstract

The two characteristics of DNA computing, the ability of performing massively parallel computing and self-assembly between complement sequences, are closely related to the characteristics of the human brain. Inspired by these similarities, the hypernetwork model was developed as an evolving associative memory model that can learn multimodal data in a massively parallel manner [1-4]. *In silico* implementation of the hypernetwork model showed that visual-linguistic concepts could be emerged from watching cartoon videos[5-6]. However, *in vitro* implementation could only be achieved on a small scale and with unimodal data[7], because the number of independent DNA sequences was limited. To learn large scale and multimodal data *in vitro*, a hierarchical method of encoding is needed. In this research, we extracted low-level visual and linguistic signals, and then encoded them into DNA sequences through learning. By further learning processes, high-level visual-linguistic concepts could be evolved from low-level patterns.



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## Acknowledgement

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# Molecular Evolutionary Learning of DNA Hypernetworks for Hand-written Digit Classification

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An intersection between biology and computation is explored by classifying handwritten digits using DNA computing. To do this, we propose employing the Molecular Evolutionary Hypernetwork [1] *in vitro* using specifically encoded DNA as pixels of images. This machine learning algorithm consists of various stages; matching of training or test data with the Hypernetwork (starting with a random single-stranded library of 3-order hyperedges), selection and amplification of similar sequences, isolation, classification, feedback and update of the Hypernetwork used in the next round of training. A key limiting factor of this design is the issue of scalability, as a very large dataset would need to be provided for demonstrating the learning process [2]. To address this, and for the verification of each experimental step, we design, create and test a random single-stranded DNA library using various techniques, which provides a base of larger scale experiments demonstrating molecular learning.

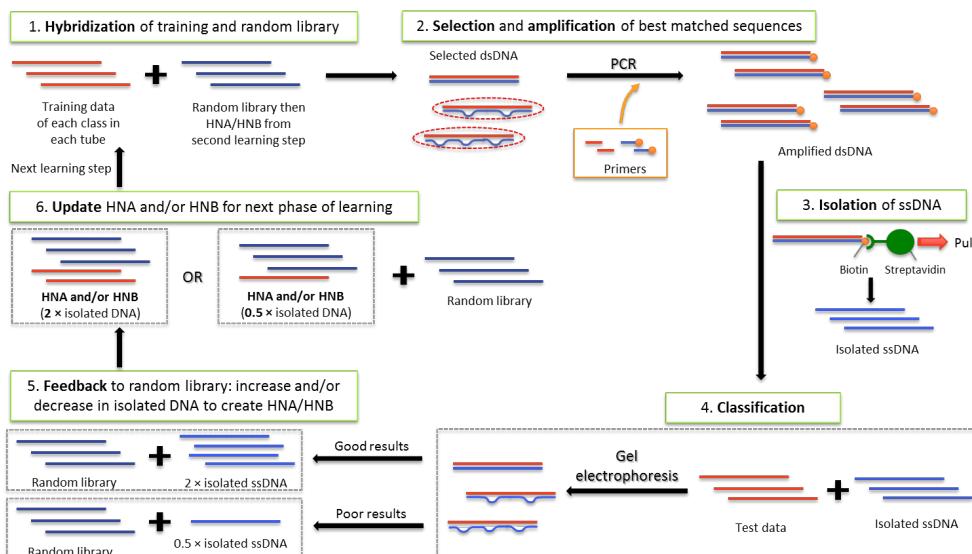


Figure 4. Overall experimental process of the evolutionary molecular Hypernetwork.

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## Reverse-engineering DNA tensegrity triangle crystallization

Evi Stahl and Hendrik Dietz

One key goal of DNA nanotechnology is the bottom-up construction of crystalline host matrices. As proposed originally by Nadrian Seeman, such matrices could enable the diffraction-based structural analysis of guest molecules, such as proteins. In 2009, Seeman reported the first crystal matrices based on a 42-kilodalton DNA triangle (Zheng et al. 2009). These crystals grow at subsaturated concentrations (6  $\mu$ M) from Watson-Crick basepairing unit cells via sticky-end interactions. The Seeman crystals are thus quite distinct from any other biomolecular crystal and represent an attractive, engineerable model system to study the physics of crystallization. The crystals provide a working point for investigating the multidimensional phase space for crystallization. To explore this space, we studied crystal growth while varying several factors, such as solution conditions, the triangle's geometry, its sequence composition, and the strength of sticky-end interactions. We also tested the influence of compositional heterogeneity on the crystallization by titrating the original components against modified ones. We find that the process of crystal formation critically depends on the choice of solution conditions, but is quite robust against a variety of defects and impurities. For example, crystallization still occurs in presence of up to 50% 3'-end truncated strands and in presence of 100% strands with up to four nucleobase-mismatches that alter the geometry of the triangle's core. We used fluorescently labeled oligonucleotides to test whether or not modified 'defective' strands are incorporated into the crystal lattice. Thus we could show that growing crystals co-integrate strands with one-base-long truncations into the crystal lattice, while strands with three-base-long truncations are hardly integrated into the lattice. In the absence of experience with generating crystals from other DNA nanostructures, we hope our studies, once completed, will help identifying design parameters, purity requirements, and solution conditions for the successful crystallization of larger DNA objects.

Zheng, J. et al., 2009. From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. *Nature*, 461(7260), pp.74–77.

## Molecular scale patterning of DNA scaffolds with 3 nm long RecA nucleoprotein filaments

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DNA-facilitated self-assembly has arguably become an attractive route for the creation of novel structures, including functional materials and devices, at nanometer length scales. Sophisticated 2D and 3D DNA nanostructure have been built over the last decade, which could be employed as nano-scale scaffolds to provide the structural framework of novel multicomponent materials. However, to achieve this, technologies which enable accurate positioning of nanoscale functional components onto pre-assembled DNA nanostructures, are required.

Here, we present the use of the protein RecA, a DNA-binding protein involved in the homologous recombination process, as a tool for programmable and highly efficient patterning of DNA scaffolds at sequence-specific locations. RecA proteins polymerize on single-stranded (ss)DNA to form helical nucleoprotein filaments (NPFs) which then self-assemble onto the double-stranded (ds)DNA scaffold at regions homologous to the NPFs' ssDNA sequence i.e. regions with the same or very similar base sequence, and form triple-stranded patterned structures. We demonstrate that RecA-based NPF can be used to form patterned structures anywhere on the DNA scaffold with the precision of single basepairs. We also show that RecA protein-mediated patterning is highly specific, i.e. patterning is limited only to the target location on the dsDNA scaffold leaving the neighbouring regions intact.

NPFs made from 60 nucleotide-long ssDNA are approximately 30 nm in length and were previously shown to pattern DNA scaffolds site-specifically with high efficiency. Multiple locations on the DNA scaffold were patterned concurrently with separations as small as 10 basepairs. However, to enable the creation of novel materials with molecular-scale features, molecular patterning complexes significantly smaller than 10 nm are desired. Here, we also demonstrate the formation of RecA-based NPFs from ssDNA as short as 6 nucleotides, i.e. comprising only two RecA monomers, and hence only 3 nm in length. We show successful site-specific patterning with such truly nanoscale NPFs onto regions of homology located both at the end as well as away from the ends of the dsDNA scaffolds.

These findings position this technique as a valuable nanoscale tool for programmable site-specific self-assembly on DNA-based nanostructures and open up previously inaccessible routes towards the fabrication of DNA-based hybrid devices by self-assembly. We are currently employing this technique to pattern complex branched DNA junctions, with a view to assemble functional nanoscale components.

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## DENA: a Configurable Architecture for Multi-stage DNA Logic Circuit Design

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**Abstract:** In this paper, a configurable architecture is proposed for DNA-based logic circuit design using the micro-fluidic platform. Experimental results show that number of DNA strands and scalability is improved considerably.

**Keywords:** Configurable architecture, DNA computing, Micro-fluidic Biochip.

### 1. INTRODUCTION

DNA computers offer considerable advantages over conventional computers such as massive parallelism, compact and high-capacity storage, nano-scale miniaturization, ultra low-power consumption and self-assembly capability [1]. Efficient DNA computing platforms based on microfluidics are proposed in [2] to implement DNA-based logic gates.

We proposed a configurable architecture to design a general-purpose circuit based on micro-fluidic platform. The proposed architecture can be configured to implement a specific sum-of-product Boolean function on microfluidic tile-based platform. The main contribution of this architecture is able to design a multi-stage logic circuit without DNA strand interfering in a scalable and automatic design flow.

### 2. THE PROPOSED METHODOLOGY

The overall structure of the proposed architecture (Figure 1-a) is an FPGA-induced organization that consists of a two-dimensional array of DNA tile. Inputs and outputs of each DNA tile are DNA strands. Each tile generates the output strands based on the existence of input strands. Output of each tile is fed to the next tile as its input. In the proposed architecture, each DNA tile is realized by a region of a digital micro-fluidic biochip that can be controlled by external electrodes. Therefore, a multi-stage logic function can be implemented on this architecture by assigning the correct value to micro-fluidic electrodes.

Internal structure of a DNA tile is shown in Figure 1-B. Each DNA tile implements a sum-of-

product Boolean function and consists of two 9 sub-block as show in Figure 1-B; one DNA Logic Blocks (DLBs) at cell1 of Figure 1-B, one NOT gate that is implemented as two steps (cells 7 and 9) and one converter at cell 3.

A DLB is a DNA-based 2-level programmable tree that implements a sum-of-product. Basic element of a DLB is COB which is a building block of AND/OR gates (Figure 1-C). In fact, each COB is a syndrome that can be converted to an AND gate and also it can be changed to an OR gate.

NOT gate is implemented in 2 steps(cells 7 and 9 in Figure 1-B) and converter (cell 3 in Figure 1-B) is used to convert the output strands of the cell to a usable format for input of the next cell.

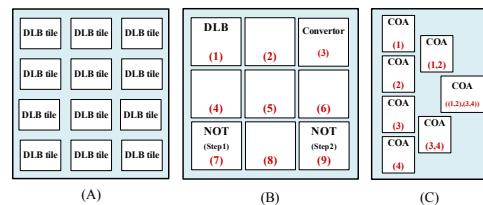


Figure 1. DENA architecture (A) Overall structure (B) a DNA tile(C) internal structure of a DLB

### 3. EXPERIMENTAL RESULTS

We implemented a full adder on the proposed architecture using VisualDSD which is a DNA strand displacement simulator. Experimental results show that the proposed architecture can be used to implement a specific Boolean system, efficiently.

### 4. CONCLUSION

In this paper, a micro-fluidic-based architecture is proposed for DNA logic circuit design that enables automatic design of general-purpose logic circuits with DNA strands. Moreover, each logic circuit can be implemented using a few numbers of orthogonal DNA strands using the proposed architecture.

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# DNA nanotube rings: Race tracks for molecular motors without the finish line

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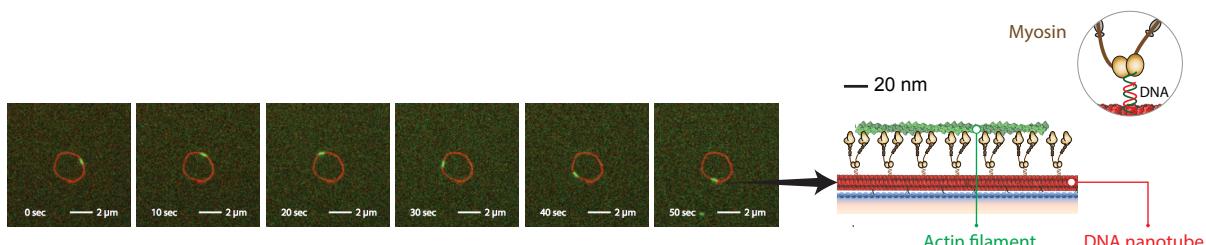
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Muscle contractions, such as those in the beating of the human heart, are enabled by the transduction of chemical energy into mechanical work by myosin motor proteins. In an average human lifetime, a person's heart will beat around 2.5 billion times. Despite the importance of the long-term robustness of myosin activity, biophysical characterizations of myosin motors are carried out over a relatively short timescale ( $\sim 2$  mins). Here, we used a circular DNA nanotube platform to continuously monitor the activity of myosin motor proteins. First, we systematically characterized the ring formation of  $n$ -helix DNA nanotubes with  $n = 4, 6, 8$ , and 10 helix circumferences and  $n, 2n$ , and  $3n$  single-stranded tiles. Nanotubes with a larger circumference have a longer persistence length. We showed that ring formation depends on the persistence length, in accordance with the cyclization of the worm-like chain model developed by Yamakawa and Stockmayer. Next, we showed an actin-myosin interface patterned on a ring shaped DNA nanostructure that allows actin gliding assays to be conducted on a timescale up to  $>10$ -fold longer than traditional gliding assays, as proof of concept of a long timescale characterization method. Finally, we varied the size of our nanotube ring scaffolds to analyze myosin motility as a function of global ring curvature. DNA nanotube rings allow each myosin motor to interact with the same gliding actin filament multiple times, providing a method of assessing the long-term performance of myosin motors. Our circular track may provide an "endless" platform for characterizing the robustness of broad classes of molecular motors.



# Design and characterization of DNA nanostructures based on Polyhedral Meshes

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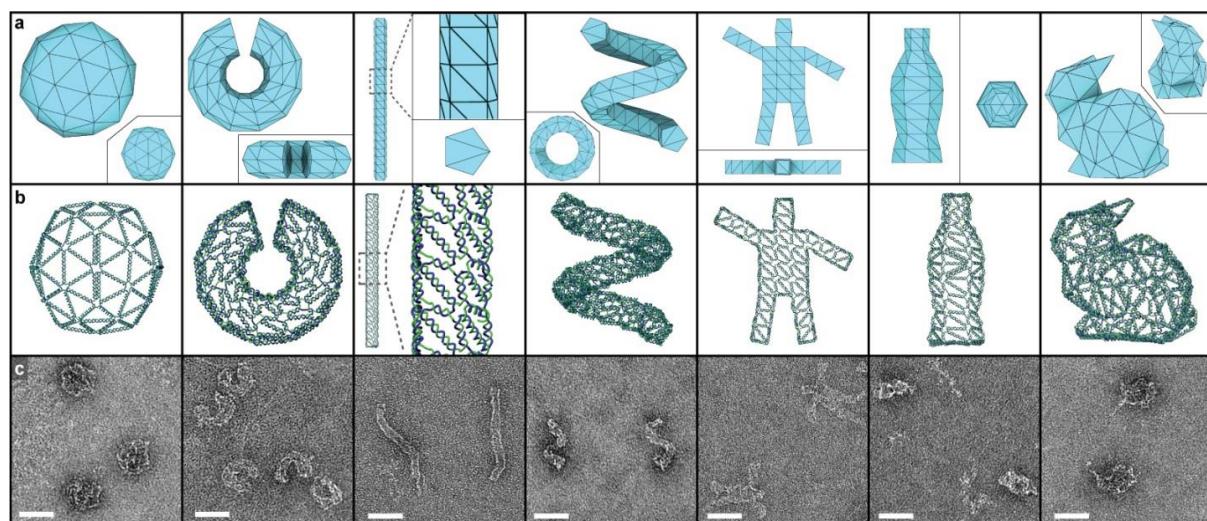
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In the last decade DNA origami has been established as a robust method for production of two and three dimensional objects with nanometer precision. However, the complexity of routing the scaffold strand through every helix of a structure limits the designer to design paradigms where the scaffold routing becomes trivial. In this study, we have developed software tools for finding the scaffold route through arbitrary polyhedral meshes. In addition we have developed vHelix, a plug-in for Autodesk Maya that allows for direct 3D design of DNA nanostructures.

We demonstrate this by producing multiple 2D and 3D structures from polyhedral meshes and characterize them with AFM, TEM and cryo-tomography, revealing both 3D shape and internal mesh features. In addition to the structural capabilities, we demonstrate that this DNA origami design paradigm, in contrast to previous variants, yields structures that are highly stable in salt conditions that are commonly used in biological assays.



**Figure 1.** **a** Mesh models used for structure design. **b** Front view of completed DNA designs from each mesh with single DNA strands rendered as tubes, the staples in blue and scaffold in green. **c** Negative stain TEM micrographs of the assembled structures, scale bars are 50 nm.

# Towards High Throughput Fluorescence-based Force Spectroscopy of Single Molecule Interactions

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While it is generally understood that molecular interactions and the forces which drive them are fundamentally important to biological processes, only in the last few decades have techniques and technologies (such as optical trapping, atomic force microscopy, and magnetic tweezers) been developed capable of probing these interactions. However, the associated instrumentation required for these experiments is often costly, cumbersome, and reliant upon user skill or expertise. Furthermore, the experiments are generally low-throughput, measuring a single interaction at a time. The focus of this study is the development, validation, and application of a nanoscale device for fluorescence-based high throughput single molecule force spectroscopy. Specifically, our goal is to develop a DNA origami device to allow force spectroscopy measurements in single molecular interaction assays on 100s of devices simultaneously using a basic laboratory fluorescence microscope.

The device is tailored to the investigation of the kinetics and mechanical stability of bimolecular (i.e. receptor-ligand) interactions and singular biomolecules in a highly parallel fashion. The nanostructure comprises a stiff platform that contains functionalized attachment points for two biomolecules (a receptor and a ligand), a flexible single stranded DNA (ssDNA) linker that functions as the force probe, and fluorescent molecules to facilitate readout of the binding interaction between the biomolecules under study. A schematic of the device, dubbed the **biomolecular Nano-Angler** (bNA), is presented in Figure 1. The ssDNA force probe acts like a molecular fisherman. The receptor molecule is anchored to a box-like component at the end of the ssDNA linker that acts as a molecular lure. The lure is cast by thermal fluctuations of the ssDNA linker, and when the linker fluctuates far enough, the box can “catch” the corresponding target molecule conjugated to the mainframe of the structure as depicted in

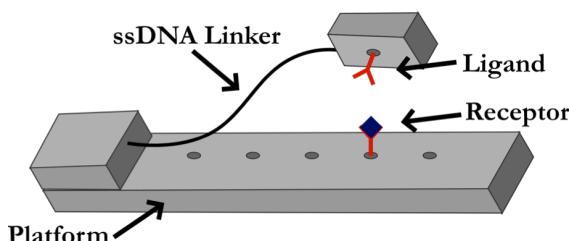


Figure [1]. Illustration of the designed bNA structure at a moderate force binding location. The receptor binding site can be positioned axially along the platform, thereby changing the applied force on the interaction.

This device will enable the investigation of the kinetics and mechanical stability of biomolecular interactions and singular biomolecules in a highly parallel fashion. We have constructed the device and performed proof of principle experiments probing DNA base-pairing interactions. We have demonstrated that the device can facilitate and rupture these interactions with estimated forces ranging from  $\sim 1\text{-}20\text{ pN}$ . We have shown that the fraction of bound interactions decreases with increasing magnitude of force. We are currently developing the single molecule FRET assay and will initially measure the kinetics of previously characterized DNA interactions to verify the behavior of the device. After verifying the functionality, this device will be used to probe the receptor-ligand kinetics of DNA-protein interactions.

Figure 1. Once the interaction is formed the ssDNA linker exerts a constant force on the interaction due to its entropic elasticity. As the binding site of the target molecule is moved down the platform, the amount of ‘stretch’ necessitated by the ssDNA linker increases, thereby increasing the applied force on the interaction. When the interaction ultimately ruptures, the linker also serves to keep the lure molecule in close proximity to the target molecule. Fluorescent molecules integrated into the device at specific locations allow the state of the device (bound vs. unbound) to be measured optically with a Fluorescent Resonance Energy Transfer (FRET) technique.

# Structure-based Design of Scaffolded DNA Origami

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## Abstract

Scaffolded DNA origami is a programmable approach to designing self-assembling materials that form nearly arbitrary geometric shapes with nanometer-level precision. Diverse nanostructures have been synthesized using this approach that employs programmed sequences of synthetic staple strands that hybridize with a long scaffold strand to fold into the target 3D architecture. In current design practice, sequences and topological routing of DNA strands are generated manually using computer-aided design (CAD) software such as caDNAno<sup>1</sup>, Tiamat<sup>2</sup>, or other software. As an alternative approach, here we present an automated sequence design algorithm that uses target 3D CAD shapes to render the locations of four-way junctions and the topological routing of scaffold and staple strands assuming Watson-Crick basepairing. In our approach, the target 3D shape is first idealized using DNA duplexes to obtain the positions and orientations of individual basepairs, including locations of multi-way junctions. Junctions and intervening DNA duplexes are then modeled as vertices and edges, respectively, of an undirected graph in which each path has an associated cost. The optimal topological routing of the scaffold strand is defined as the Eulerian circuit with the minimum cost computed using the Concorde traveling salesman problem (TSP) solver<sup>3,4</sup>. The optimal routing is knot-free and may be compared with alternative routing scenarios to test experimentally their relative impact on folding kinetics. Complementary staple strands are routed following established design principles including preferred lengths and locations of 5'- and 3'-ends<sup>5-9</sup>. Finally, the design algorithm selects nick positions of staple strands based on heuristic design rules to generate staple sequences. We apply the approach to design wireframe-based objects, single-layer objects, and multi-layer solid objects<sup>10,11</sup>. We additionally demonstrate that our algorithm incorporates sequence, topological, and 3D structural design constraints to control the identities and orientations of specific nucleotides for target applications. This approach provides a general algorithmic framework for structure-based automatic sequence design of a wide variety of DNA nanostructures incorporating user-specified geometric and sequence constraints, with application to photonic devices, biological sensors, and probes<sup>12,13</sup>.

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## Poster Abstract

# Detecting and Recovering from Faults in Programmed Molecular Systems

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For reliable operation of programmed molecular systems, the capability to *detect faults* when they occur and to *initiate recovery* is essential. Since a faulty system cannot be depended upon to report its own failure, a monitoring device is needed.

The work presented here describes the design of one such fault protection device, called a *molecular watchdog timer*. A watchdog timer is a standard device used in safety-critical applications to monitor the health of a system and issue an alarm (“bark”) if the monitored system fails. Often, as here, the watchdog timer monitors a system’s health by watching for periodic “heartbeats” from it. If a heartbeat is not received for a set time period, the monitored system is assumed to have failed, and the watchdog timer issues an alarm and/or triggers appropriate recovery actions. Thus, in the design of our molecular watchdog timer, the absence of a heartbeat for a preset time can produce, with arbitrarily high likelihood, a cascading reaction that generates a fluorescent signal visible with FRET.

The molecular watchdog timer itself is comprised of three components: an absence detector, a threshold filter, and a signal amplifier. These components are separately designed as chemical reaction networks. They are verified both individually and when composed using model-based simulation, probabilistic model checking and formal analysis.

To verify its embeddability, the molecular watchdog timer is assigned to monitor the health of a clock (based on the three-species stochastic Lotka-Volterra oscillator) that outputs a periodic heartbeat. Another recovery solution presented here is more demanding but also more appropriate for eventual use in biocompatible environments because it does not require operator intervention. Here, when the molecular watchdog detects the failure of the clock, it handles recovery autonomously by having a repair component reboot the clock.

This design is shown to be realizable at scale. If the “ladders” used as “delays” have size logarithmic in the number of molecules, then the expected time to a false alarm can be as large as desired. A Chernoff bound permits the guarantee that, with arbitrarily high probability, the actual time to a false alarm is nearly as great as the expected time. Moreover, if the heartbeat stops, the alarm species is produced very quickly, so the molecular watchdog timer’s alarm will be triggered.

The molecular watchdog timer offers a simple yet powerful means to prevent faults in programmed molecular systems from leading to interruptions in their reliable operation.

This work is supported in part by NSF Grant 1247051.

# Controlling the Bending and Twist of RNA Assemblies via Artificially Designed Loop-Bulge Kissing Interactions

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**Abstract:** RNA nanotechnology is an excitingly emerging field. Compared to its DNA cousin, RNA nanotechnology has many additional advantages associated with the properties pertaining to RNA, which include the more diverse functionalities, a cornucopia of structural motifs and the ease of production by *in vitro* or *in vivo* transcription and etc. Despite the plethora of structural motifs in naturally occurring RNA folds, most of them suffer from the lack of modularity – their formation may be context-dependent or they may be difficult for generalized reconfiguration, which limits their usage in the design of artificial RNA nanostructures. Herein, we show that an RNA T-shaped junction motif (T-junction) can be formed via the kissing interaction between a hairpin loop and a bulge. This artificially designed motif is very desirable for the RNA nanostructure design because of at least three reasons: (I) this motif can bring two parts (the loop and the bulge) together via Watson-Crick base-pairings, which is essential for the programmable self-assembly; (II) it is formed by paranemic interaction, which eliminates sticky ends and thereby decreases the number of strands necessary; (III) it is branched and therefore suitable for the design of complex nanostructures. Based on this T-junction, we have designed RNA nanostructures self-assembled from C-shaped and Z-shaped tiles folded by only one single stranded RNA. The out-of-plane bending, in-plane bending and twist can be finely controlled by manipulating the component tiles and thereby various 1D, 2D and 3D nanostructures are constructed. Furthermore, these nanostructures can be also formed by a co-transcriptional protocol. This indicates these nanostructures have the potential to be produced, assembled and functional *in vivo*. We believe that this T-junction motif may find broader applications in the field of bionanotechnology and other related fields. Firstly, more complicated geometry and/or topology of RNA assemblies can be constructed using the T-junction-based tiles by virtue of the convenient control of bending and twist. Moreover, there exist similar loop-bulge kissing interactions as essential structural elements in various naturally occurring RNA structures, such as group II intron ribozyme and prohead RNA. Our artificially designed T-junction motif may be useful in re-engineering such functional RNA. In our study, we have constructed various 1D helical structures by twist or a combination of twist and bending. These structures have great potentials as scaffolds for electron crystallography in structural biology.

# Self-Assembly of DNA Graphs and Postman Tours

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DNA graph structures can self-assemble from branched junction molecules to yield solutions to computational problems [1, 2, 4]. For a given graph  $G$ , the self-assembled structure represents a thickened graph  $F(G)$  containing  $G$  as a deformation retract [1]. Through permutation of the edges at one or more vertices, the family of thickened graphs  $T(G)$  of  $G$  can be obtained. Each thickened graph is a topological surface with a set of boundary components  $\delta(F(G))$ . A boundary component  $\sigma \in \delta(F(G))$  tracing every edge of  $F(G)$  at least once and no more than twice is a reporter strand.

Jonoska et al. have proposed studying self-assembly graphs through the boundary components of their thickened graphs, which allows for reading the solutions to computational problems through reporter strands. They showed in [2] that for every multigraph  $G$  there exists a reporter strand. For a given graph  $G$ , a cycle tracing every edge of  $G$  at least once and no more than twice is a postman tour  $\tau$  in  $G$ . We show that for every postman tour  $\tau$  in a connected 3-valent (multi)graph  $G$  there exists a thickened graph  $F(G) \in T(G)$  with a reporter strand  $\sigma$  which contains  $\tau$ . In cases where the postman tour  $\tau$  and the reporter strand  $\sigma$  are non-identical, DNA self-assembly may not be able to replicate the tour without including an extra cycle. Further reduction of the reporter strands to isolate the postman tour contained within  $\sigma$  would yield all non-maximal optimal postman tours of  $G$ .

In [3] the TSP is solved using self-assembly and a weighting method reliant on the melting temperature gradient. Other methods rely on the ratio of A/T to C/G pairs. We consider the application of DNA weighting algorithms such that the weight of the reporter strand identified in the self-assembly is readable.

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## Using DNAzyme to control DNA tiles filling into Origami Frame

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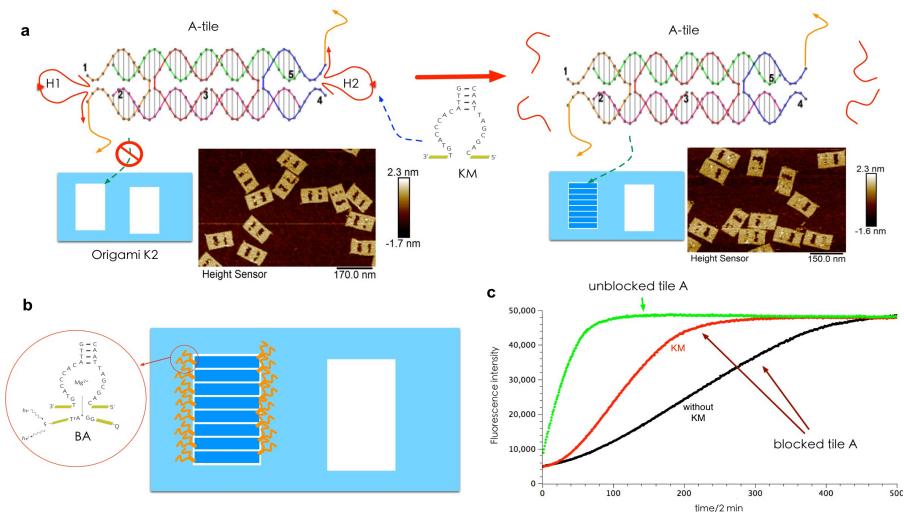
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DNA origami has been proved to be a powerful tool allowing programmable nanostructures assembly based on its well-defined shapes and multi-dimensions. Recently, double-crossover DNA tiles are used to fill into a hollow 2D DNA origami, which provide an efficient method to assemble complex boundary required structures. Here, we present a strategy to control the tile filling process by DNAzyme cutting.

The designed DNA origami frame includes two rectangular holes with pairs of corresponding sticky ends on the inner face. Accordingly, one DNA tile A has four specific sticky ends to hybridize with the sticky ends on origami frame. When mixed together, eight tile A could be incorporated into the left hole (Figure 1a). To control the tile filling process, in our design, the sticky ends of DNA tiles are firstly blocked by two strands H1 and H2 (with the cleavage site of ribonucleobase-containing sequence). Therefore, the sticky ends on tiles are not exposed initially, and thus the tiles are not able to fill into the frames automatically. Only after being cut by DNAzyme KM, the protectors H1 and H2 are separated into two segments, and become easy to depart from the DNA tile, thus the sticky ends are exposed again. In this way, the filling process can be triggered by DNAzyme KM cutting (AFM images in Figure 1a).



**Figure 1.** Control the tile filling process by DNAzyme cutting.

To better detect the filling process, the fluorescent report based on DNAzyme BA is introduced. DNAzyme BA is divided into two subunits, located to the tile sticky ends and inner faces of origami frame, respectively. Thus, only when the tiles incorporate into the frame, the full DNAzyme can be assembled (Figure 1b). It is clear to see that addition of DNAzyme KM can greatly improve the fluorescent signals (Figure 1c).

## Some remarks on the Chemical Reaction Networks implemented by using DNA

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Chemical Reaction Networks, CRNs have been implemented by using DNA [1,2]. Since simple hybridization reactions cannot be cascaded, other reaction mechanisms such as strand displacement reactions [1], DNA polymerization- depolymerization reactions [2] and so on have been used.

In such reactions, intermediate substances play key roles; for example, in implementing the Lotka-Volterra (LV) chemical oscillation, the amplification of the substance  $X_i$  is implemented as  $X_i + G_i \rightarrow O_i$ ,  $O_i + T_i \rightarrow 2X_i$  [1]. In these reactions,  $G_i$  and  $T_i$  are auxiliary substances and they are prepared before the reaction starts ( $G_i$  is called “Gate” and  $T_i$ , “Transfer”, respectively), while  $O_i$  is not prepared and it is produced and consumed in the reactions between the input substance  $X_i$  and these auxiliary substances. We call such intermediate substances intermediators; the intermediators are used in various oscillatory behaviors as the Belousov Zhabotinskii reaction [3], P53 signal transduction system [3], Trytrophic ecological systems [3], genetic network of *Saccharomyces cerevisiae* [4] and so on, and the intermediators regulate oscillatory behaviors [3].

Although intermediators are not used in the formal LV chemical reaction system, which is given as  $X_1 \rightarrow 2X_1$ ,  $X_1 + X_2 \rightarrow 2X_2$ ,  $X_2 \rightarrow$ , that are used in implementing it by using DNA [1]. Mathematical characteristics are different between the; in the formal LV, there are two equilibria and in the LV with intermediators, there is an infinite number of equilibrium points that include equilibria of the formal LV (Fig. 1).

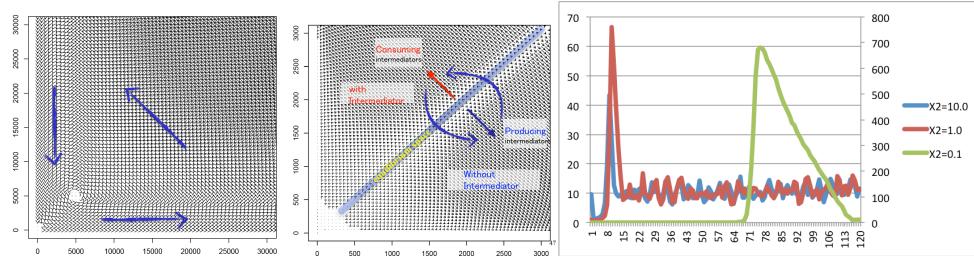


Fig. 1 The vector fields of solution of the formal LV (left), and the LV with intermediators (center), the equilibria of the formal LV are the origin and the center of the white circle, while the equilibria of the LV with intermediators are all the points on the diagonal line (right) amplifying the low concentration of  $X_2$  by using the LV with intermediators, the each line illustrates the time development of the concentration of  $X_2$ ; the initial concentration is 10.0 (blue), 1.0 (red) and 0.1 (green) respectively, where the initial concentration of  $X_1$  is 10.0 (right); the simulations have been done by using a simpler version of Gillespie Algorithm [3,5] and the chemical reactions of the LV implemented by using strand displacement reactions [1].

It has been known that one of the characteristics of dynamical system of the formal LV model (2 species) is periodic, and there are no attractors, and the orbits of oscillations are given by the initial concentration. On the other hand, we found that in the LV with intermediators (implemented by using strand displacement reactions [1]), even if the initial concentrations are different, every orbit is attracted to the same orbit (Fig. 1, right).

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# Intelligent Biodegradable Hydrogels Made of DNA-PEG-DNA Tri-block Copolymers

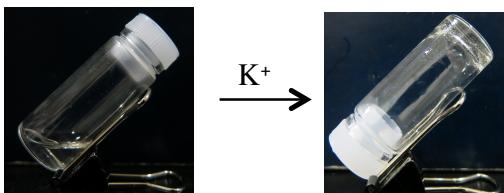
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DNA is a water-soluble and biodegradable polymer, which forms right-handed double helices and carries genetic information in life. Because of its high structure regularity and sequence programmability, DNA has been used as a key component in development of functional nanomaterial.<sup>1</sup> Despite such success in nanoscience, reports on application of DNA to typical material, i.e. hydrogels, are very few, except for some excellent achievements,<sup>2-4</sup> because preparation of DNA in gram scales usually requires unreasonable costs. One solution for this problem is liquid-phase DNA synthesis method developed by Bonora et al.<sup>5,6</sup> They used PEG as soluble polymeric support, which enables coupling reaction of phosphoramidite monomers in liquid phase and can be easily precipitated by ether addition for product separation.

We have applied this system to synthesize DNA-PEG-DNA tri-block copolymer in grams, and successfully prepared biodegradable PEG hydrogels by using higher-order DNA structures, such as G-quadruplexes, for cross-linking points. Aqueous solution of a tri-block copolymer bearing multiple guanosines at the ends, for example, immediately turns into gel upon addition of Na<sup>+</sup> or K<sup>+</sup> ions. Introduction of toehold sequence realized complete control of such sol-gel transition by the addition of complementary DNA strand.



**Figure 1.** K<sup>+</sup>-triggered sol-gel transition of a DNA-PEG-DNA solution.

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## Encoding and Decoding Algorithms for Nucleic Acid Memory (NAM)

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With the ever increasing volume of digital information, it is estimated that the global memory demand will exceed the projected silicon supply by 2040.[1] Critical factors for the information and communication industries are the scaling and energetics of information storage materials. One mechanism to expedite materials discovery for memory applications is to consider unconventional sources that have unique characteristics unavailable in other material systems. Nucleic acids, for example DNA, chemically encode information with a base-4 quaternary code (A,T,C,G). Nucleic Acid Memory (NAM) is a potential alternative to silicon based memory because its volumetric density is 1000 times greater and its energy of operation is 100M times less than flash memory – the industry standard.[1] Rapid progress in DNA synthesis and sequencing has enabled reading and writing of arbitrary digital formats.[2, 3] Although ~100% recovery of encoded information has been demonstrated, the existing algorithms are not universal nor do they compensate for naturally occurring defects. Defects, including the loss of individual bases (letters) and complete sequences (words), are expected during NAM writing (synthesis), reading (sequencing), and storage. Our objective here was to develop efficient and stable error correction algorithms for DNA that: (1) compensate for naturally occurring defects, and (2) account for synthesis constraints such as GC content and sequence repetition. This study is in pursuit of non-biological and nonvolatile NAM applications.

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# An In-vitro Transcriptional Classifier Based on DNA Strand Displacement

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DNA toehold-mediated strand displacement has been demonstrated as an engineering tool to perform complex information processing of nucleic acids. It offers a design architecture that is highly modular and that has been shown to be scalable to circuits with dozens of interconnected components. This technology is particularly attractive in the field of portable medical diagnostics due to its ability to implement detection, embedded analysis and amplification of nucleic acids. However, it is rather challenging to couple DNA strand displacement circuits with native cellular RNA species due to their high degree of secondary structure. Traditionally, highly complex DNA strand displacement cascades have been engineered with sequences that are orthogonal to each in order to avoid cross talk or non-specific hybridization. Here, we present a modular approach to unfold, detect and amplify messenger RNA species or viral RNA independently of secondary structure considerations using a DNA hybridization assay. The resulting output consists of orthogonal DNA sequences able to interact with a downstream strand displacement cascade. We will motivate the implementation of this workflow in the context of building a DNA strand displacement linear classifier circuit to differentiate multiple human cancer cell lines based on their distinct messenger RNA expression profiles.

In order to couple detection of a messenger RNA transcript with a DNA strand displacement cascade, we carry out a competitive hybridization of DNA strands that convert the presence of a given messenger RNA transcript into orthogonal DNA strands. First, we design DNA helper strands that hybridize adjacently to a target region of a messenger RNA transcript and allow for unfolding of the secondary structure. This allows for a targeting probe with a hidden toehold to hybridize to the messenger RNA and release a strand that can trigger a quencher-fluorophore reporter downstream. In the absence of messenger RNA, the targeting probe remains bound to itself and the reporter is not triggered. We demonstrate that this hybridization is facilitated by the application of thermal annealing or sequential addition of Urea and MgCl<sub>2</sub> — which are denaturing and renaturing agents in this context —.

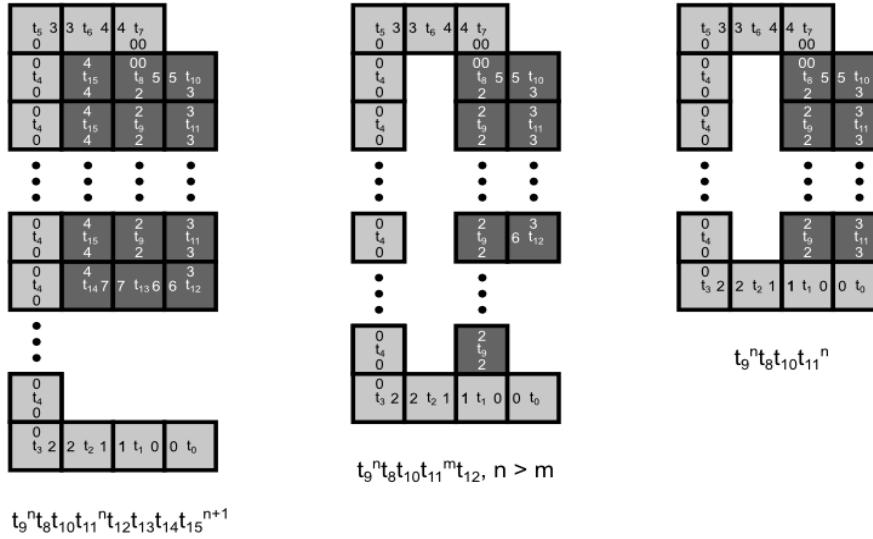
Subsequently, we demonstrate implementation of linear amplification of individual transcripts. First, multiple messenger RNA species are detected using the competitive DNA hybridization method described above. Each messenger RNA transcript can be amplified with a different linear weight by varying the number of targeting probes that hybridize to different regions of a transcript. We demonstrated that addition of each individual probe — with corresponding helper strands — proportionally increases the signal obtained from the reporter. In the context of a transcriptional classifier, this property allows us to assign higher weights to some messenger RNA transcript with respect to others.

Finally, a classifier needs to implement a thresholding function to the sum of the linearly amplified signals. If the net signal after thresholding is positive, a step function produces a binary input in order to indicate that the classifier conditions have been met. In the context of a transcriptional classifier, we will couple detection a linear amplification system as described above with a DNA annihilator gate and a catalytic amplifier, both of which have been previously described in the literature. We expect to engineer a transcriptional classifier cascade such that transcripts that are highly expressed in a cell line of interest will be assigned a positive weight while those that are highly expressed in other cell lines will be assigned a negative weight. Based on the subtraction of amplified positive and negative signals by the annihilator gate, the cellular classifier will be able to identify if the correct gene expression profile is present in solution.

# One-dimensional Noncooperative Tile Assembly with One Dissolvable Glue

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A primary goal of DNA-guided self-assembly is to assemble nanostructures with useful properties that are the product of algorithmically directed interactions between component parts. One desirable outcome of the assembly process is that the assembled structure corresponds exactly to the intended product. Tile models often allow cooperation between two or more tiles to stably bind together with another tile as a powerful mechanism for computation. Meunier et al. demonstrated that cooperation is also necessary for intrinsic universality [1]. The computational power of noncooperative systems is still being explored [2], but these systems have been demonstrated to be more experimentally feasible. In this work, we present a further refinement of the power of noncooperative assembly with mismatches as shown in [3] by demonstrating that there exist inherently one-dimensional assemblies retrievable from 2D assemblies by the use of a single instance of a special dissolvable glue called a **terminal glue**. Upon dissolution, no additional tile attachments are allowed. This refinement of Meunier's use of tree-like structures is consistent with his findings and builds upon [4]. As in [3], regular and some context-free languages can be assembled without the use of this terminal glue. In particular, we show that the language of all palindromes cannot be assembled except in a tree-like fashion. Additionally, we show some non-context free languages can be assembled specifically with the terminal glue. We also present a relation called **mirror concatenation** that defines the group of languages assemblable by noncooperative assembly with mismatches and a terminal glue in the plane. In practice, this work may have some significance as it characterizes those string patterns that would be easy to produce and extract for an assembly reaction mixture.



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## Probabilistic Analysis of Pattern Formation in Tile Self-Assembly

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Self-assembly of nanostructures promises new materials and devices that are manufactured from the bottom up. Frequently, the product of the self-assembly is determined by an algorithm implemented in local interactions between component parts. Through the tile assembly models, most theoretical work has focused on the capabilities and process of deterministic assembly, in which the assembled patterns are specified uniquely by the local interactions among tiles. In this work, the intent is to consider the effects of nondeterminism and errors on the ability of a tiling system to produce the patterns for which it is programmed. Moreover, rather than exclusively focusing on the ability of the tiling system to assemble a single, unique target pattern, multiple target patterns are allowed. This is motivated not only by nature, *i.e.* biological organisms in the same species are not identical, but also material science in which many patterns might produce a desired material property, such as conductivity, plasmonic response, or stiffness. Thus, the notion of probabilistic analysis of pattern formation is introduced in which a nondeterministic self-assembly system produces patterns that may or may not be in its target set. No particular assumption is made on the nature of this nondeterminism, which may result from experimental errors, kinetic effects, or even by design. The assembler is characterized by probabilities that capture its ability to produce the target set or “junk”, *i.e.* patterns that are not and never can become completed patterns in the target set. In the model of directed tile assembly with a locally deterministic tile set, there is no such junk. Nevertheless, junk is a fact of life in experimental implementations, and also possible in nondeterministic assemblers. Therefore, in our model, the quality of the assembler is determined by its ability to produce target patterns with bounded amounts of junk. **Weak** assemblers produce the target patterns, but also lots of junk assemblies that may be substantially different from the targets. **Strong** assemblers produce patterns, all of which are similar to the targets. Assemblers that produce a bounded amount of junk relative to the size of the set of target patterns are **efficient**. Moreover, efficient assemblers can be further characterized by the rates at which target and junk patterns are produced. Therefore, the model provides an analytic framework to classify assemblers in ways that are closer to experimental reality and that might better inform laboratory practice, which heretofore has been based on trial and error.

Strong assemblers not only produce at least a fraction  $\mathbf{p}$  of the target, but every pattern assembled contains at least a fraction  $\mathbf{p}$  of some target pattern. Intuitively, a strong probabilistic assembler has a good “idea” of the properties of target patterns since every individual pattern it assembles is similar to some target pattern in the target set. Moreover, if the assembler also produces a uniform fraction  $\mathbf{q} > 0$  of non-target patterns of arbitrary size, the assembler is efficient. Such efficient assemblers should capture a significant uniform fraction of the target set and should rarely produce patterns not in the target set. (We emphasize that fractions  $\mathbf{p}$  and  $\mathbf{q}$  are relative to the set of target patterns, not assembled patterns.) Given these notions, we show two major results. First, if a set of patterns is probabilistically assembled by any assembler with probability  $\mathbf{p}$  and efficiency  $\mathbf{q}$ , then target patterns are assembled with positive probability at least  $\mathbf{p}/(\mathbf{p}+\mathbf{q})$ , nontarget patterns are assembled with probability at most  $\mathbf{q}/(\mathbf{p}+\mathbf{q})$ , and error patterns must be assembled with a complementary probability within the interval  $[0, 1-\mathbf{p}/(1+\mathbf{q})]$ . We also show that a trichotomy holds for arbitrary assemblers, *i.e.* the rate of error and promising patterns either stabilizes at some constant value, alternates in a periodic fashion, or is at least exponential. Some examples of strong and efficient assembly of 1D and 2D patterns are also provided.

Integrated Amplification and Detection of DNA on A Paper Device  
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Abstract

We report the development of a diagnostics platform that integrates enzyme free amplification and detection of DNA on a paper flow device. Target sequences are first amplified at room temperature via a DNA strand displacement circuit consisting of an amplifier gate and fuel, then flowed through an 8 cm by 3 mm nitrocellulose dipstick where output fluorophore signals are captured by hybridizing to partially double stranded oligos immobilized on the dipstick, and finally imaged by a gel scanner for visualization of signals. The time it takes for the entire process from target amplification to signal visualization using 20 nM of amplifier gate and 40 nM of fuel is less than 2 hours. We observe a 3:1 S/N ratio in paper, and 10:1 S/N ratio when all reactions occur in solution. We find that the increase in leak on paper comes mainly from denatured capture strands possibly caused by the process of being immobilized on nitrocellulose membrane. However, we have demonstrated that this leak can be significantly reduced through a simple modification of the amplifier gate. Without using the leak-reducing amplifier, this setup can detect target concentrations down to 3 nM and yields a 3 fold increase in sensitivity when compared to signal detection coupled to a non-amplifying DNA circuit or through direct reporter hybridization. With the modified amplifier in place, we expect the device capable of reaching sensitivities in the 10-100 pM range with a minimum of 10X gain. Additional work is being conducted on using gold nanoparticles as a substitute for fluorophore readouts, which would allow a colorimetric readout and promises further increases in signal sensitivities. This work could potentially enable the development of rapid and inexpensive platforms for multiplex nucleic acid diagnostics using cascaded DNA circuits.

## Algorithmic Design of Scaffolded DX-Based DNA Origami Cages

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### Abstract

Synthetic DNA can be designed to self-assemble into 2D and 3D objects of many sizes and shapes using  $N$ -way junctions of one or two duplexes per arm.<sup>1</sup> DNA nanocages are a sub-class of closed 3D DNA-based objects that can be designed for use in bionanotechnology and *in vivo* imaging, such as to encapsulate molecules for cellular delivery or to present molecules for cellular recognition.<sup>2</sup> While tile-based self-assembly offers an attractive approach to synthesize these objects,<sup>3</sup> an alternative is to utilize a single scaffold strand as a template that spans the entire structure and hybridizes to complementary staple strands.<sup>4</sup> This approach of scaffolded DNA origami typically offers a high folding yield of the target product with insensitivity to variable staple strand concentrations, which may be difficult to control. However, algorithmic rules for the sequence design of such cages have not yet been explored in detail together with 3D structural and sequence constraints that can be applied during the design process. Here, we investigate various sequence design rules for programming DX-based DNA nanocages,<sup>5</sup> with the ultimate goal of automating the design process to maximize product yield, thermodynamic stability, kinetic rate of assembly, and agreement with target 3D structure for nanocages of any symmetry and size on the 10 to 100 nm scale.<sup>6,7</sup> In our approach, each edge of a given nanocage consists of two duplexes joined with anti-parallel crossovers in order to increase structural rigidity. Unlike cages with one helix per edge, which typically require additional edges to complete an Eulerian circuit,<sup>8</sup> cages with two helices per edge do not require augmentations to route a single scaffold throughout the entire structure. The 3D structure of the target cage provides input to our algorithm that generates the optimal routing of the scaffold strand, the nick and crossover positions of the staple strands, and the sequences of the staples complementary to the prescribed scaffold sequence. Other design considerations we explore include enforcing edge lengths to be multiples of 10.5 base pairs to follow the natural pitch of B-form DNA and constraining the orientation of the major groove to minimize electrostatic repulsion of the DNA backbone.<sup>9</sup> Two experimental techniques are used to test the fidelity of the proposed algorithmic design: gel electrophoresis indicates high folding yields and quantitative PCR indicates high transition temperatures and cooperativity of folding.<sup>10</sup>

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# *De novo* prediction of DNA origami structures through atomistic molecular dynamics simulations

Christopher Maffeo, Jejoong Yoo, and Aleksei Aksimentiev

Since its first demonstration in 2006, the DNA origami method has advanced to encompass self-assembly of complex 3D objects with sub-nanometer precision. Super-resolution optical imaging, FRET and magnetic tweezers have been applied to DNA origami objects to infer information about their *in situ* structure and dynamics. The only experimentally derived atomic-scale model of a DNA origami object was obtained using the cryo-electron microscopy (cryo-EM) method[1], revealing considerable deviation of the *in situ* structure from its idealized design. Recently, we showed that all-atom molecular dynamics (MD) method can be used to obtain realistic atomic-scale models of simple DNA origami structures [2]. However, the accuracy of the MD method for *de novo* prediction of large DNA origami structures has not been assessed.

Here, we report a fully atomistic, explicit solvent simulation of a large DNA origami object used in the cryo-EM reconstruction study. Over the course of the simulation, the DNA origami structure was observed to quickly depart from its initial idealized configuration of parallel helices toward a globally twisted conformation consistent with the cryo-EM reconstruction, see Figure 1. The helices, which were tightly packed at the beginning of the simulation, spread into the characteristic chickenwire configuration. The root mean square deviation between the simulated DNA structure and the pseudo-atomic model based on cryo-EM dropped to a value similar to the reported resolution of the cryo-EM reconstruction, indicating a good agreement between simulated and experimentally-derived structures. The results of our simulations suggest that the MD method can be used to predict the structure of a DNA origami object with accuracy near the resolution of state-of-the-art cryo-electron microscopy. Based on the results of our simulations, we developed a simplified protocol for obtaining realistic, atomic-scale models of large DNA origami objects at a fraction of the computational cost of an explicit solvent MD simulation.

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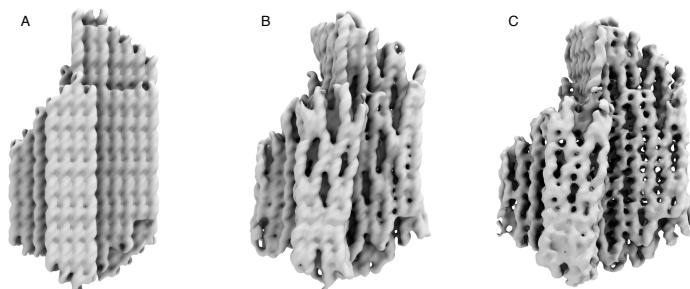


Figure 1: Comparison of the electron density of a DNA origami object obtained through computational and experimental approaches. (A) Electron density corresponding to the idealized design. The atomic coordinates yielding this electron density provided the initial conditions for the MD simulation. (B) Simulated electron density. The electron density was computed directly from the explicit solvent MD trajectory by averaging the density of each atom over the last 20 nanoseconds of the simulation. (C) Experimentally obtained electron density. The electron density was reconstructed from hundreds of experimentally obtained cryo-EM images of the object [1].

# Poster Abstract

## Robust Molecular Finite Automata

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This poster presents a uniform method for translating an arbitrary nondeterministic finite automaton (NFA) into a deterministic mass action chemical reaction network (CRN) that simulates it. The CRN receives its input as a continuous time signal consisting of concentrations of chemical species that vary to represent the NFA's input string in a natural way. The CRN exploits the inherent parallelism of the chemical kinetics to simulate the NFA in real time with a number of chemical species that is linear in the number of states of the NFA. Specifically, if the NFA has  $q$  states,  $s$  symbols, and  $d$  transitions, then our CRN has  $4q + s + 2$  species and  $5q + d$  reactions. The compiler of Chen et al. (2013) would then translate this into a DNA strand displacement network consisting of  $28q + 4d + 2s + 6$  single strands of DNA. Our translation thus appears to make small NFAs implementable in laboratories now and NFAs of modest size implementable in the near future.

Most importantly, our CRN's correct implementation of the NFA is robust with respect to small perturbations of four things, namely, its input signal, the initial concentrations of its species, its output signal (acceptance or rejection of its input string), and the rate constants of its reactions. One key to achieving this robustness is a memory-refresh technique akin to the approximate majority algorithm of Angluin et al. (2007).

In summary we have identified a rich and useful class of computations that can be implemented robustly in the molecular world, i.e., implemented in such a way that they will provably perform correctly, even when crucial parameters are perturbed by small amounts. This robustness is important, because processes that can be implemented, but only with inordinately precise control of parameters, are inherently unreliable and hence inherently unsafe in many envisioned applications. Future research can strive to enhance the robustness here and to extend the class of computations that enjoy it.

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# Strict Self-Assembly of Fractals Using Multiple Hands

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**Abstract.** We consider the problem of the strict self-assembly of infinite fractals within tile self-assembly. In particular, we provide tile assembly algorithms for the assembly of the discrete Sierpinski triangle and the discrete Sierpinski carpet within a class of models we term the *h-handed assembly model* (h-HAM), which generalizes the 2-HAM to allow up to h assemblies to combine in a single assembly step. Despite substantial consideration, no purely growth self-assembly model has yet been shown to strictly assemble an infinite fractal without significant modification to the fractal shape. In this paper we not only achieve this, but in the case of the Sierpinski carpet are able to achieve it within the 2-HAM, one of the most well studied tile assembly models in the literature. We provide a 6-HAM construction for the Sierpinski triangle that works at scale factor 1, 30 tile types, and assembles the fractal in a *near perfect* way in which all intermediate assemblies are finite-sized iterations of the recursive fractal. We further assemble the Sierpinski triangle within the 3-HAM at scale factor 3 and 990 tile types. For the Sierpinski carpet, we present a 2-HAM result that works at scale factor 3 and uses 1,216 tile types. We further include analysis showing that the aTAM is incapable of strictly assembling the non-tree Sierpinski triangle considered in this paper, and that multiple hands are needed for the near-perfect assembly of the Sierpinski triangle and the Sierpinski carpet.

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# Concentration Independent Random Number Generation in Polyomino Tile Self-Assembly

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**Abstract.** We introduce the *robust random number generation* problem wherein, given  $n \in \mathbb{N}$ , one must design a tile assembly system (aTAM or PolyTAM system) whose terminal assemblies can be assigned to one of  $n$  partitions such that the resultant assembly of the system lies within one of the  $n$  partitions with probability  $\frac{1}{n}$  for all concentration distributions of the monomers of the system. This problem is motivated by experimental settings in which concentrations of system monomers cannot be easily controlled. Further, self-assembling an arbitrary range of uniform random states is a key primitive in the implementation of randomized algorithms within the model. The problem can be solved in the aTAM given unbounded space ( $\mathcal{O}(\log n)$  expected space). We conjecture that a guaranteed bounded space solution in the aTAM is impossible; however, we show that this problem is solvable in bounded space in the PolyTAM. We investigate the polyomino size complexity of the problem- that is, how large the individual polyomino monomers must be as a function of  $n$ . Let  $p_n$  be the largest number in the prime factorization of  $n$ . We can solve the problem in  $\mathcal{O}(p_n)$  size complexity in bounded space. Interestingly, given access to one layer of self-assembly in the 3<sup>rd</sup> dimension, we can solve the problem in  $\mathcal{O}(\sqrt{p_n})$  size complexity in bounded space. Continuation of these results include exploration for a  $\mathcal{O}(\sqrt{p_n})$  solution in 2D, determining the tile complexity of the problem (that is, how many unique monomer types are required to solve this problem) and exploration of solving this problem under other models of self-assembly.

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# Multi-Model Cross-Platform Self-Assembly Simulation with VersaTILE

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**Abstract.** Self-assembly is the process by which systems of simple objects autonomously organize through local interactions into larger, more complex objects. VersaTILE is a graphical simulator and tile editor designed to be able to simulate many models of tile-based algorithmic self-assembly. VersaTILE is cross-platform (Java) and currently supports the simulation of the abstract Tile Assembly Model, the dupled Tile Assembly Model, the hexagonal Tile Assembly Model, and the polyomino Tile Assembly Model. It also supports generalized versions of many of the extensions to these models such as probabilistic attachment, temperature programming, concentration programming, negative interactions, a run-time model, and flexible glues. VersaTILE has clean and consistent user-interface that makes it very easy for a user to identify how to use the software. To make things easier, VersaTILE automatically keeps up-to-date and looks the same across all platforms. Users are able to specify custom colors for tiles so that they can quickly get a high-level overview of the state of the tile assembly system. VersaTILE was inspired by other tile-based self-assembly simulators that exist, such as Xgrow and the ISU Tile Assembly Simulator. Simulators for this line of research are important tools for the rapid prototyping and debugging of tile assembly systems. The primary contribution of VersaTILE to this space is that VersaTILE generalizes the self-assembly process and uses polymorphism to allow a developer to make new models by simply constraining or modifying small pieces of the base classes through inheritance. Not only is VersaTILE open-source, but it uses an XML file format to make it simple for others to support or write to the VersaTILE format. Besides being a tool for rapid prototyping and development, VersaTILE is a useful tool for interactive demonstrations as well as education. This poster reviews the major features and functionality of VersaTILE.

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## Dynamic control of DNA Tile Nanotube Nucleation and Elongation

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The cytoskeleton, a versatile dynamic molecular assembly that is ubiquitous in eukaryotic cells, is assembled from a set of semiflexible filaments and protein connectors that organize the filaments into architectures. Dynamic growth, shrinkage and rearrangement of these filaments are driven by the interplay of molecular motors and spatiotemporally controlled factors that affect both the activity of the filament monomers and the proteins that enable filaments to bundle, cap, nucleate and branch. These control “programs” make it possible for the cytoskeleton to organize filaments into structures that separate chromosomes, direct cell motion and produce and reconfigure cellular organelles.

The ability to control analogous, synthetic filamentous architecture and dynamics could enable the design of a new generation of materials with the capacity to dynamically organize into different forms and exert specific mechanical forces. DAE-E tile DNA nanotubes are prime candidates for designing such a system because of their mechanical and structure similarities to cytoskeletal filaments and a growing body of knowledge about their growth dynamics. Currently, DNA nanotube monomers can be activated using strand displacement enabling temporal control of growth [1]. Further, nucleation of these filaments can be controlled using DNA origami nanostructure seeds which function as templates for growth [2], allowing spatial control over nanotube assembly. However, further control over DNA nanotube growth, disassembly and organization is needed to produce integrated programs for assembly and metamorphosis.

Towards this goal, we describe two new mechanisms for controlling DNA nanotube growth and organization. First, we show how capping structures can stop nanotube growth by binding to a nanotube’s terminal end. Second, we show that both this cap and a DNA origami seed can be activated via the addition of a specific DNA strand (Fig. 1). We use fluorescence microscopy and AFM to characterize the structure of the complexes and the dynamics of DNA nanotube nucleation and growth in the presence of activated seeds and caps. Together, control over nanotube nucleation, elongation and growth using DNA strands, possibly outputs from nucleic acid circuits, to control multiple aspects of a self-assembly process and to control DNA nanotube size.

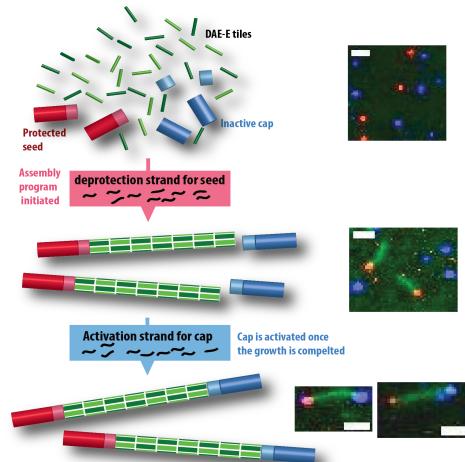


Fig. 1 A schematic of DNA nanotube nucleation, growth and capping along with fluorescence measured images of nanotubes in each stage. Cy3 (green), Atto647 (red) and Atto488 (blue) dyes label DNA nanotube tiles, seeds and caps respectively. Scale bar is 2  $\mu\text{m}$ .

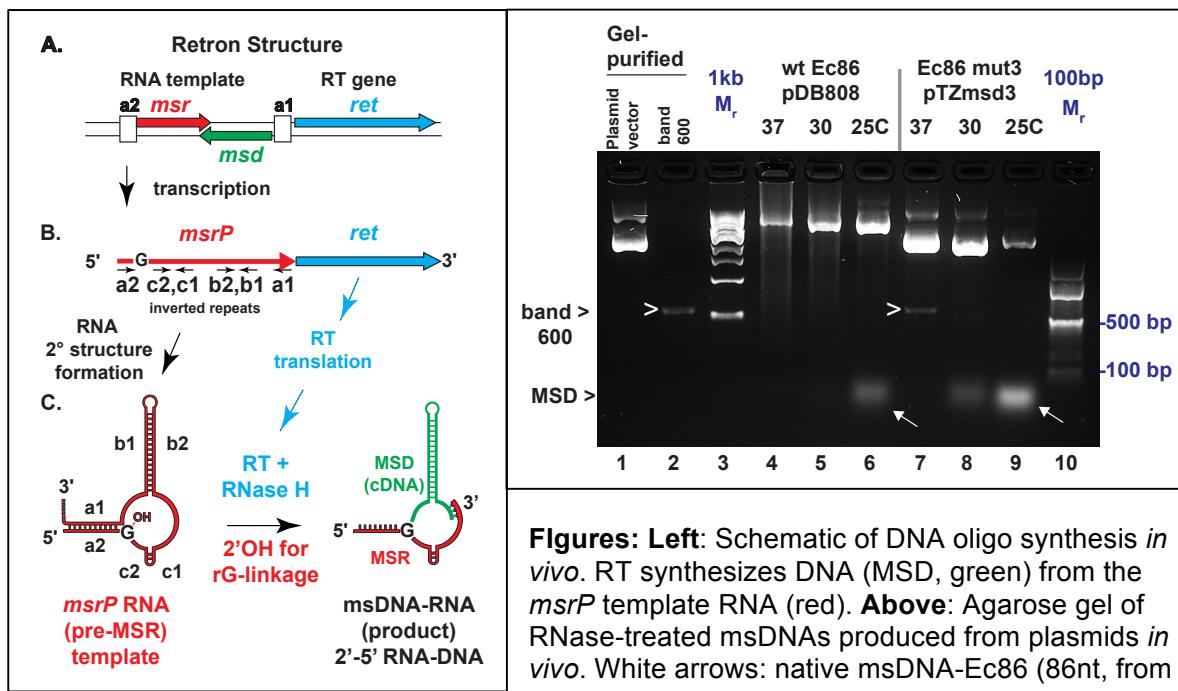
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## Production of DNA Oligonucleotides *in vivo* from an Engineered Bacterial Retron.

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Retrons are natural genetic elements that produce a bacterial reverse transcriptase (RT) that in turn synthesizes stable, multi-copy single-stranded DNA (msDNA) stem-loop molecules within a bacterial cell (*in vivo*).<sup>1</sup> These msDNA stem-loop “oligos” are synthesized as a DNA-RNA copolymer (msDNA-RNA) of ~48-163 nt ssDNA, depending on the retron. The retron expresses a template RNA together with the RT, and the RT specifically binds this RNA and uses it as a template to produce the msDNA<sup>1</sup> (see schematic). The DNA can also be enzymatically detached from its covalently bound RNA component both *in vivo* and *in vitro*, and remains stable *in vivo*.<sup>2,3</sup> In nature a single bacterium can encode two different msDNAs from separate retrons in the same cell.<sup>4</sup> Within certain sequence and structural constraints, msDNA sequences can be modified to produce engineered DNA sequences.<sup>5-7</sup> Here I describe the use of a mutant variant of the *Escherichia coli* retron element Ec86 for the production of synthetic DNA stem-loop sequences *in vivo*. I have designed plasmid vectors that express Ec86 msDNA variants with either partial or full replacement of the native msDNA sequences, and expressed and purified the variant msDNAs via gel electrophoresis. One application of this technology is to encode synthetic DNA “oligos” for self-assembly of DNA nanotechnology elements *in vivo*.



**Figures:** Left: Schematic of DNA oligo synthesis *in vivo*. RT synthesizes DNA (MSD, green) from the msrP template RNA (red). Above: Agarose gel of RNase-treated msDNAs produced from plasmids *in vivo*. White arrows: native msDNA-Ec86 (86nt, from plasmid pDB808) and stem-replaced msDNA-Ec86

mut3 (72 nt, from plasmid pTZmsd3). Band 600 is a retron-plasmid *ori* byproduct (sIDNA).<sup>8</sup>

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## SIMULATION OF A PROGRAMMABLE MOLECULAR COMPUTER \*

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Microfluidic-based molecular computers (MMC) are specific-designed devices that may automatize certain molecular computing paradigms such as the sticker model. The compartmentalization of MMCs began with the design and implementation of the Selection Transfer Module (STM), enabling the capture of sequences with magnetic beads and the ability to achieve optical reprogramming and thus, solving instances of the Max-Clique problem up to 6 nodes [1]. Later, another microfluidic system which consisted of chambers acting as logic gates was reported in [2]. This device may perform the AND operation when chambers are connected in a serial way and the captured sequences are stored (positive selection). On the other hand, if non-captured sequences are stored (negative selection), the OR operation is executed. Recently, a solution to the Minimum Vertex Cover under this architecture was suggested [3]. In the present work, we propose a simulator for the instruction set of such MMC. Our simulator works at a discrete level by calculating the state of DNA molecules during their processing in the MMC, in contrast with others approaches that work at a physical level. Broadly speaking, the components needed for this tool include a template (*i.e.* an input combinatorial library), a set of chambers (where the capture takes place) and a set of memory channels (dedicated to store and redistribute strands). We defined a file format for microfluidic instructions which serves as input to the simulator, performing an instruction cycle analogous to that of transistor computers. The instruction cycle is given by 1) Input: determines whether the input is the template or a memory channel; 2) Control: valves are configured to only use the specified chambers in the instruction; 3) Capture: hybridization may only take place inside the chambers; and 4) Selection: for positive selection, the channels are washed and retained DNA is stored; for negative selection, the content of channels is stored and retained DNA is discarded. Some simulation parameters are required for initialization, as for example, the problem size ( $n$ ), the number of chambers ( $C$ ), the number of memory channels ( $M$ ), the selection type, and the instruction table. Once fulfilled, a combinatorial library of DNA strands (represented as integers) is generated, and each chamber  $c_i$  is initialized. The instruction table is then executed. The simulator was developed in C# using Visual Studio 2010, and the 6-vertices instance of the Minimum Vertex Cover Problem of Zhang *et al.* [3] was coded and tested, generating all the correct answers. In conclusion, we believe that this simulator is a step forward to help with the automatization of molecular computers and to provide an abstraction layer between the molecular algorithm and its physical implementation in a MMC.

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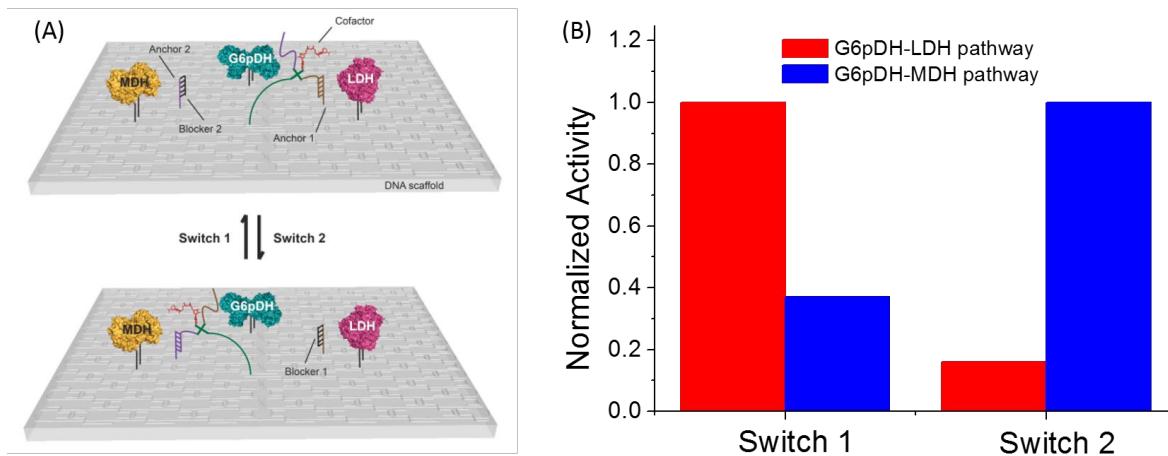
# Directional Regulation of Biochemical Pathways on DNA Nanoscaffolds

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The regulation of biochemical pathways, in which a series of biochemical cascade reactions are catalyzed by multi-enzymes systems, is important to the growth and metabolism of organisms. Here, we take advantage of spatially addressable DNA nanoplatforms to simulate the directional regulation of biochemical pathways in nature. As shown in Figure 1, we constructed a rectangular origami-based multi-enzyme system involving three dehydrogenase enzymes (G6pDH: glucose-6-phosphate dehydrogenase; MDH: malatedehydrogenase enzymes; LDH: lactate dehydrogenase) and a cofactor ( $\text{NAD}^+$ , nicotinamide adenine dinucleotide).According to our previous study<sup>[1]</sup>, a bi-enzyme cascade system achieves high activity when the cofactor is located between the two enzymes, and shows low activity when the cofactor leaves. As a result, we regulate the twobi-enzyme pathways (G6pDH-NAD<sup>+</sup>/NADH-LDH, and G6pDH-NAD<sup>+</sup>/NADH-MDH) by changing the position of the cofactor: in the presence of switch 1, the cofactor will be anchored at the middle position between G6pDH and LDH, thus activating the G6pDH-NAD<sup>+</sup>/NADH-LDH pathway. Similarly, the G6pDH-NAD<sup>+</sup>/NADH-LDHPathway will be blocked, while the G6pDH-NAD<sup>+</sup>/NADH-MDHpathway will be triggered in the presence of switch 2. Our currentexperiment results (Figure 1B) successfully shows the feasibility of this concept. We believe that this concept is useful for the future design of regulatory biological circuits for diagnostic and therapeutic applications.



**Figure 1.** (A) Schematic illustration of the directional regulation of a DNA nanoscaffold based multi-enzyme pathways: by changing the position of cofactor ( $\text{NAD}^+$ ) to anchor 1 or anchor 2, G6pDH- NAD<sup>+</sup>/NADH-LDH pathway or, G6pDH- NAD<sup>+</sup>/NADH-MDH pathway is activated, respectively. (B) The activity of enzyme pathways in the presence of switch 1 or switch 2.

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# Hierarchical Assembly of Three-Dimensional DNA Structures

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We extend the Tile Assembly Model with signal passing capabilities [1, 2] to three dimensions (called here *3D Active TAM*) and present a design for the hierarchical growth of an aperiodic, self-similar, three-dimensional structure. The proposed model extends the 2HAM model, although it can be easily adjusted as an extension of aTAM. The main building block motif is a modified Tensegrity Triangle DNA motif [4] depicted in Figure 1(a). This motif has been used for the design and construction of three dimensional crystals and has shown to build rigid rhomboidal structures [4]. In the 3D Active TAM this motif is modeled with a cube where each face corresponds to one end of one of the duplexes in the triangular motif. Recent experiments have shown that tiles (motifs) with intramolecular strands protecting the sticky ends can control the assembly step and provide cascading process of molecular growth [3]. The 3D Active TAM consists of a set of cubes (as tiles) whose sides consist of active or inactive labels (glues) that can assemble at a given temperature. Due to the size of the motif, the theoretical model allows for at most one label, active or inactive, per side (compared to multiple labels allowed in the two dimensional model). In addition, the inactive label is activated by an attachment of a specific side of the cube, hence there are at most three signals (inactive labels) in a cube.

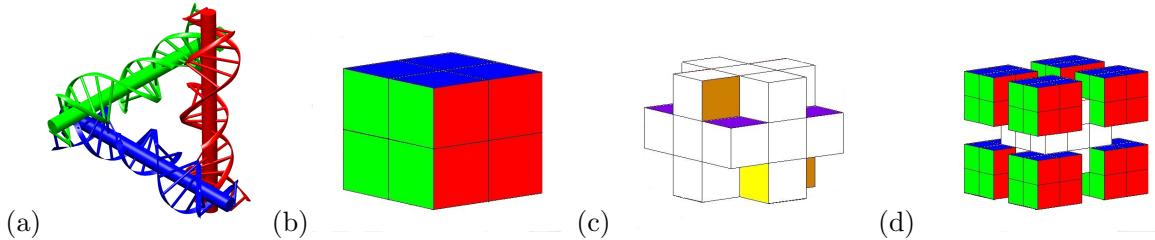


FIGURE 1. (a) Tensegrity triangle motif, (b) basic cube at level 0, (c) level connector, (d) cube structure at level 1. (b) - (d) Hierarchical 3D assembly models generated by a tile assembly simulator (currently in development).

We show a design for the hierarchical growth of a cubic structure within the 3D Active TAM operating at temperature 2. In the first iteration, the tile system produces ‘connector’ structure (Figure 1(b)) and  $2 \times 2 \times 2$  cube structures (Figure 1(c)). Eight of the cubes connect to form a larger  $5 \times 5 \times 5$  cube through the ‘connector’ (Figure 1(d)). The second iteration uses these second level cubes to connect into a larger  $11 \times 11 \times 11$  cube, etc. Placements of four marked cube types in the system within the hierarchically built structure show that the limit assembly is an aperiodic ‘tiling’ of the three dimensional lattice.

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# Rapid Unbiased Transport by a DNA Walker Utilizing Toehold Exchange

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Ever since the step-by-step (hand-over-hand) movement of molecular motors such as dynein, myosin and kinesin super families was mechanistically characterized, attempts have been made to mimic their dynamic behavior in the form of synthetic molecular walkers.<sup>1-6</sup> Several DNA-based molecular walkers have been synthesized, motivated by the long-term goal of controlling molecular transport processes with the programmability and structural robustness of DNA nanotechnology. Previous studies have shown that DNA walkers can walk directionally along a track upon sequential addition of a DNA strand as chemical “fuel”.<sup>2,3</sup> In some studies, more sophisticated tasks were coupled to walker motion, such as templating sequential chemical reactions and assembling gold nanoparticles.<sup>7,8</sup> Despite this progress, the DNA walkers reported so far have been constrained by slow translocation rates, typically on the order of a few nm/min.<sup>4,9</sup> By comparison, natural protein motors have translocation rates of ~1 μm/s under saturating ATP conditions,<sup>10,11</sup> a 3-4 orders of magnitude faster rate. It is desirable to reduce this gap if synthetic DNA walkers are to serve as useful agents of molecular transport.

The translocation rate of many DNA walkers is believed to be limited by slow catalytic steps or slow release of cleavage products. In contrast, the displacement of one strand in a DNA duplex by another can be catalyzed by the nucleation of short single-stranded overhangs, or “toeholds”, a process that can be very rapid when the reagents are present at high concentration.<sup>12</sup> Since DNA scaffolds can be used to generate local effective reagent concentrations in excess of 100 μM in bimolecular reactions, a DNA walker using toehold exchange reactions for locomotion may be able to realize higher translocation rates than any previously reported DNA walkers.

Here we report the design and single-molecule fluorescence resonance energy transfer (smFRET) characterization of a novel class of reversible DNA transporters that utilizes strand displacement mediated by toehold exchange. The fastest rate constant of stepping approaches 1 s<sup>-1</sup>, which is ~100-fold higher than typical DNA-based transporters. We present evidence that the walking occurs by a rapid branch migration step followed by slower dissociation and rebinding of toehold sequences. While branch migration is rapid and may be treated as a rapid equilibrium process, the rate constant of stepping between adjacent track sites is dependent on the length of the associated toeholds.

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## Molecular information processing in micro gel beads with entrapped DNAs

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### Abstract

Spatially distributed DNA devices in compartments which communicate between the compartments extend the possibilities of DNA devices such as drug delivery with molecular information processing. Here, we report molecular communication among spatially distributed DNA devices in multi-compartmental micro gel beads.

We utilize calcium alginate gel as a matrix to distribute DNA devices. We adopted the multi-barrelled capillary method to make multi-compartmental micro gel beads which contains different ingredients in each compartment [1]. The mesh size of the calcium alginate gel is experimentally measured; the mesh size of the gel made of 1.5% sodium alginate gelates in calcium chloride solution is less than 20nm [2]. Based on this, we designed a triangular DNA motif with the side lengths of 20nm which used as an anchor for DNA devices (Fig.1). It has a single stranded part, called domain used as a scaffold to anchor DNA devices.

In order to examine molecular communication between the compartments, we have fabricated micro gel beads with two compartments containing different DNA devices in each compartment (Fig.2). When an input strand initiate toe-hold mediated strand displacement in the upper compartment, an output strand is released and it diffuses into the lower compartment. It triggers the second strand displacement. These reactions are verified by the color change of each hemisphere by using fluorescent microscope. The result shows the cascade between the compartments has been successfully realized. More complex transitions including cascade reaction in three-compartment bead and inter-bead molecular communication will be reported at the conference.

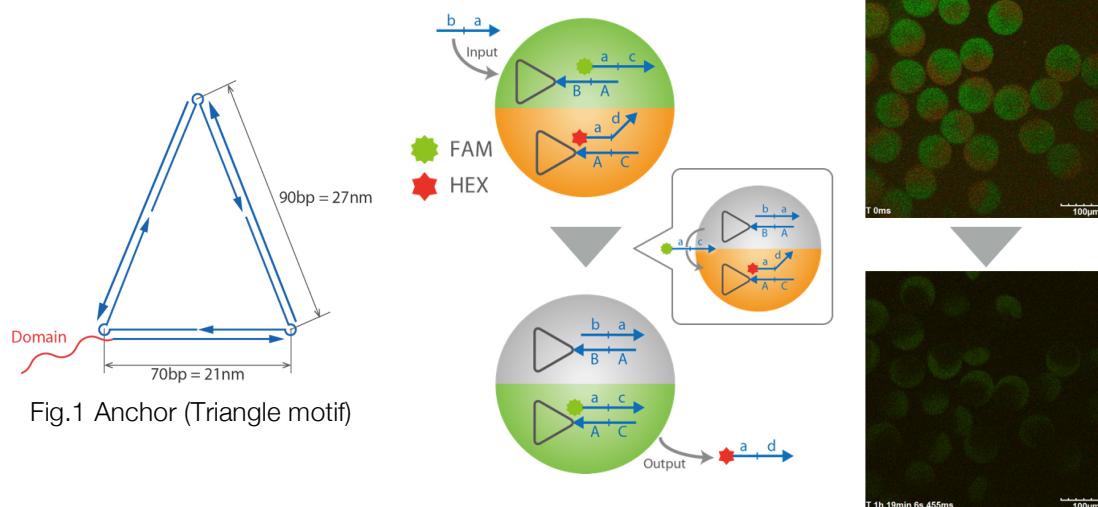


Fig.1 Anchor (Triangle motif)

Fig.2 Cascade reaction in two-compartment micro gel beads

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# An evaluation of “refolded” and “prefolded” strategies with signaling aptamers

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During the past decade aptasensors have been developed based on the notion that aptamer conformations could be stabilized by a ligand following refolding from an inactive conformer.<sup>1</sup> One implementation of this strategy is to denature the aptamer with an antisense oligonucleotide (Figure 1a).<sup>2</sup> A great disadvantage of this strategy is that there is a large kinetic barrier associated with unfolding the aptamer to obtain the active, binding conformation. Indeed, many researchers mischaracterize this as the ligand inducing a conformational change in the aptamer, which it does not. To improve the general methodology for developing aptasensors, we have developed a competition strategy in which an oligonucleotide competes with the ligand for binding to a given aptamer conformation. While the

oligonucleotide can induce conformational change, it must also choose between the aptamer and a molecular beacon (that will in turn generate signal, Figure 1b). Using an anti-ricin aptamer as a starting point, we developed “prefolded” aptasensors with a measured limit of detection (LOD) of 30 nM. No corresponding “refolded” aptasensors were successfully generated, again likely because of the deep kinetic trap that this and other aptamers frequently find themselves in.

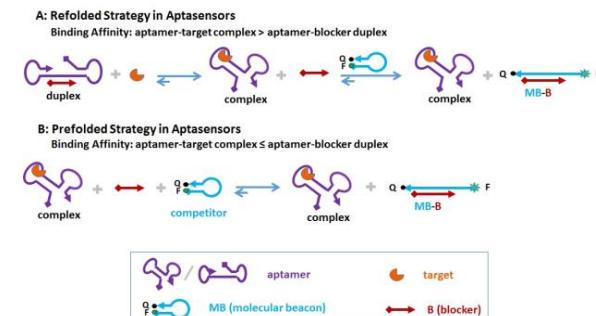


Figure 1. Schematic view of the “Refolded” aptasensor (A) and the present “Prefolded” aptasensor.

There are several advantages to this strategy. First, because of the use of molecular beacons as readouts, it is inherently a ‘signal on’ strategy, improving signal:noise ratios. Many aptasensors in contrast rely on quenching. Second, the blocker and the molecular beacon can be readily manipulated without having to change the sequence or secondary structure of the aptamer itself. The modular nature of the components should generally make sensor design much easier, with the lengths and sequences of blockers being matched to both ligand binding energies and molecular beacon transducers. Moreover, this modularity should allow signals to be transduced into various readouts, including electrochemical detectors. In this regard, the LOD for the “prefolded” strategy proved to be as low as 2.5 nM for RTA detection on an electrochemical platform.

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## Creating combinatorial patterns with DNA origami arrays

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DNA origami (Rothenmund 2006) and smaller DNA tiles (Winfree et. al. 1998) have been used as effective scaffolds to create complex patterns for organizing molecules with nanometer precision but of a limited size. Arrays of DNA origami (Liu et. al. 2011) and DNA tiles have been shown to be capable of creating patterns in a larger scale, either periodically or following a specific set of rules defined by a cellular automaton (Rothenmund et. al. 2004). Here we aim to create a variety of large-scale complex patterns by using a combinatorial approach with a mathematically simple and elegant rule called Truchet tiling. We developed a symmetric square DNA origami tile that can rotate and attach to one another on all four sides, with which we constructed two-dimensional finite and unbounded arrays. Labeling the origami tile with Truchet patterns using double-stranded extensions, we successfully observed a variety of random maze-like patterns on the origami arrays created in one test tube (Figure 1). With a combination of just pi-pi stacking bonds and very short sticky ends, we identified edge designs of the origami tile that simultaneously allow cooperativity and sufficient specificity in tile-tile interactions and yielded robust crystallization with a simple incubation protocol. Origami arrays on the scale of ten by ten microns were reliably generated. The DNA origami arrays with combinatorial patterns could be used to test molecular robots against diverse operating environment with increasing complexity, thus help us to better understand the engineering principles for building robust molecular devices. As combinatorial approaches have been used to substantially accelerate drug discovery (combinatorial organic synthesis), identification of functional nucleic acids (SELEX), and optimization of new materials (combinatorial material screening), our approach could also potentially be used to efficiently screen functional molecular devices and advance nanoscale fabrication that involves the organization of components in molecular electronic, photonic and plasmonic devices.

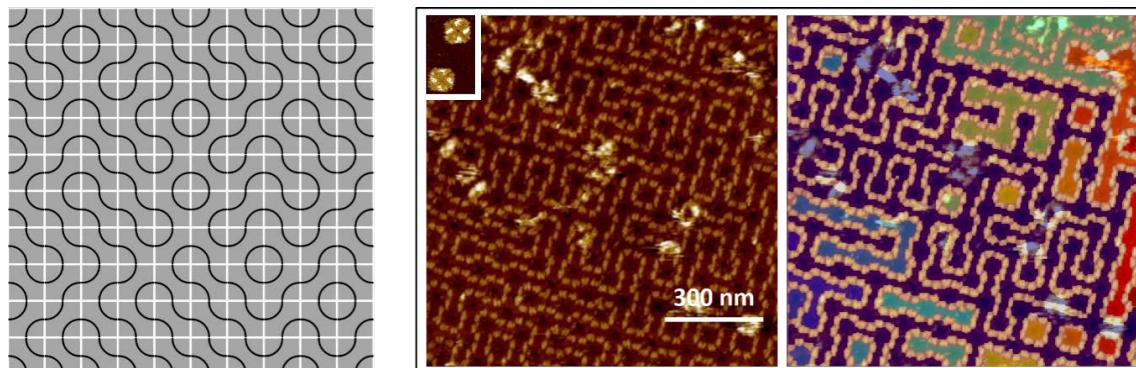


Figure 1. Combinatorial patterns on DNA origami arrays. Computer generated 10 by 10 Truchet array (left) creates a random maze-like pattern. AFM images (right) are colored to show continuous paths that can be explored in the mazes (insert - individual tiles).

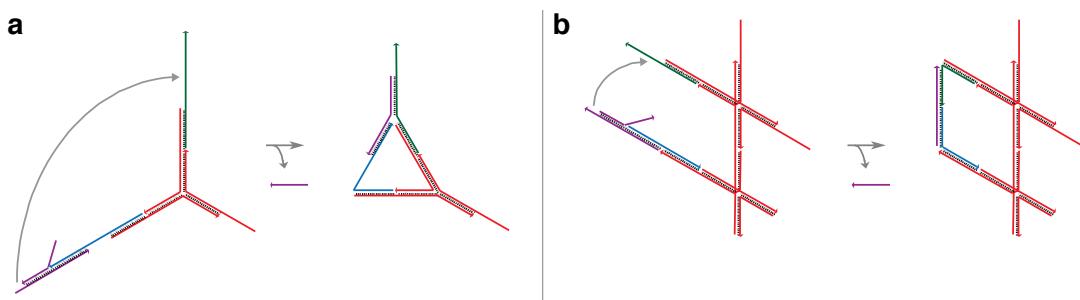
# Paradigms for developmental self-assembly of gridiron structures

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Developmental self-assembly allows the triggered, isothermal self-assembly of DNA into complex geometries using strand displacement reactions. This method uses a prescriptive molecular program to determine not only the final state of the structure, but all the assembly and disassembly pathways connecting the possible intermediates. In previous work, a DNA tetrahedron was synthesized and characterized as a proof-of-principle for the growth of well-defined three-dimensional wireframe structures incorporating ring formation reactions. These ring formations fundamentally involve competition between the desired intramolecular interactions and an undesired intermolecular pathway that leads to the formation of multimers. In particular, the tetrahedron structure was based on three-arm junctions that required a significant change in the junction angle for the ring-formation reaction, a situation that was likely both kinetically and thermodynamically unfavorable. The lower yield of these ring-forming reactions relative to normal assembly reactions is a major limit to the scalability of this method.

Here, theoretical work is presented on motif design for the developmental self-assembly of gridiron wireframe structures based on four-arm Holliday junctions. Four-arm junctions are promising as a primitive for this application because their structure is well defined and their conformational dynamics have been thoroughly studied. Their preference for a stacked structure with two double-helical domains at a  $60^\circ$  angle can be used to devise ring-formation reactions that require little or no conformational change, which may allow improvement of the yield relative to the scheme used in the tetrahedron structure. The successful demonstration of scalable developmental self-assembly would allow structures the size of origami or single-stranded brick structures, but with the advantages of isothermal and temperature-robust assembly, the capability for algorithmic self-assembly and error correction, and the ability for the growth of the structure to respond to intricate combinations of molecular inputs.



**Figure: Comparison of ring formation reactions.** **a**, Ring formation reaction for the tetrahedron system. The toehold and its complement are oriented far from each other at equilibrium, and the central three-arm junction must distort considerably to reach the  $60^\circ$  angle required by the triangular structure. These factors lower both the attempt frequency and the thermodynamic favorability of the desired intramolecular reaction. **b**, Ring formation reaction for the gridiron system. The two Holliday junctions are designed to prefer a conformation where two of the struts are parallel, biasing the structure towards conformations where the toehold and its complement are close to each other. Additionally, completion of the ring formation reaction does not require any distortion of the junctions. Note that the tetrahedron structure requires the central three-arm junction to be flexible, while the gridiron structure makes use of the rigidity of the Holliday junction. All structures in this figure are drawn roughly to scale.

## Electronic control of DNA-based nanoswitches and nanodevices

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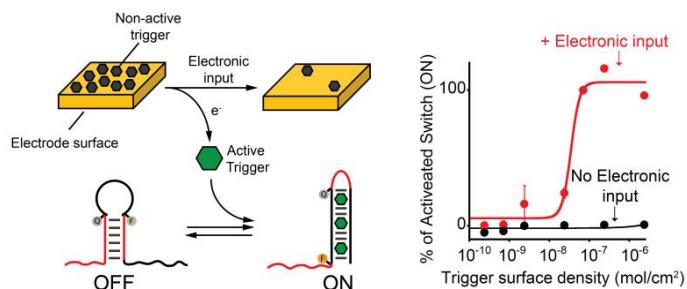
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The growing and exciting field of DNA nanotechnology, where synthetic nucleic acids are rationally engineered and designed to build novel responsive nanomachines or functional nanodevices, represents one of the most interesting examples of bio-inspired technologies. A limitation in the field of DNA nanotechnology is associated with the fact that DNA-based nanodevices or nanostructures can be controlled and regulated only by a restricted class of molecular effectors (i.e. inputs). The need to achieve higher spatio-temporal and hierarchical control of DNA nanodevices is still partially unmet and would benefit from new strategies using external highly controllable inputs.

Since the first revolutionary discoveries of Volta, Faraday and other pioneer electrochemists, the possibility to control redox reactions through an electronic input (applied voltage) has represented one of the major breakthrough in the history of chemistry. However, in the last 30 years electrochemistry, especially in the field of nanotechnology, has been mainly used for sensing or analysis purposes and the many potentialities of this technique have not been utilized.

In response to the above consideration, in this work we demonstrate the possibility to use electronic inputs as a way to rationally control and finely regulate the functionality of different nucleic acid-based nanodevices and nanoswitches. To electronically activate these DNA-based nanodevices we have used as electronic input a voltage potential applied at the surface of an electrode chip coated with a non-active trigger. Such applied potential promotes an electron-transfer reaction at the electrode-solution interphase leading to the release of a molecular input (active trigger) from the electrode surface that ultimately triggers the DNA-based nanodevice (Figure below). To demonstrate the versatility of our approach we used four model DNA-based nanodevices that are representative of a larger class of conformational-switching and DNA-based enzymes. More specifically, we used two conformational-change DNA nanoswitches responding to metal ions ( $Hg(II)$  and  $Ag(I)$ ), a stem-loop DNA molecular beacon responding to a specific DNA sequence and a copper-responsive DNAzyme. With all these systems we demonstrate fine-tuning control by modulation of the electronic input.

The possibility to use electronic inputs as a way to control DNA-based nanodevices appears particularly promising to expand the available toolbox to be used in the field of DNA nanotechnology and can open the future to new and exciting avenues. For example, the possibility to regulate through an external input DNA-based conformational change can be used to introduce additional control over the formation and functionality of DNA nanostructures with an unprecedented spatio-temporal control.



## Hierarchical self-assembly of DNA origami rods

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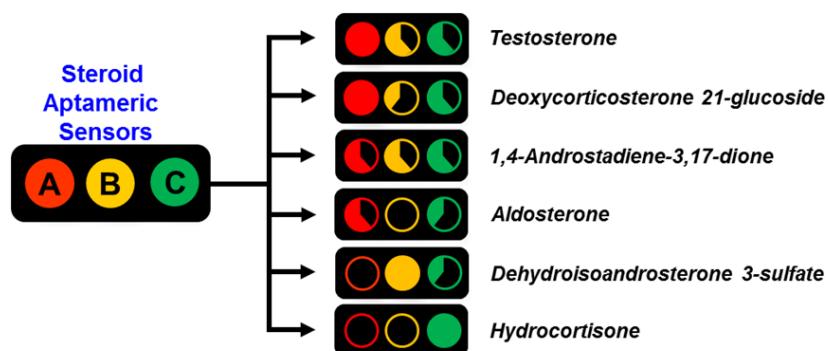
The connection between the macroscopic properties of a self-assembled liquid crystalline structure and the microscopic features of the constituent molecules is the essential theme that permeates the field of liquid crystals. Previous studies have shown that monodisperse rod-like colloids such as filamentous bacteriophage self-assemble into 2D monolayer membranes and 1D twisted ribbons in presence of attractive interactions mediated by non-absorbing polymers. The microscopic properties of the colloidal particles play an important role in determining the physical properties of these mesoscopic assemblages. Using DNA origami six-helix bundles as a new building block for the self-assembly of rod-like colloidal particles, we demonstrate that formation of higher order structures from the assembly of colloidal rods is universal. By tuning the aspect ratio, flexibility and chirality of the DNA origami rods we can control the physical properties of the entire self-assembled structures.

# Multi-Steroid Discrimination with High-Affinity Steroid Aptameric Sensors

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Cross-reactive sensor arrays have become a powerful tool for the identification and classification of analytes based on the formation of distinct patterns generated through interactions with non-specific, but semi-selective receptors [1, 2]. Recently, we found three new steroid aptameric sensors that bind steroids with high-affinity (e.g. nM range) through a simple SELEX method [3]. By applying a structure-switching/fluorescence-dequenching mechanism, we built a cross-reactive array with these aptameric sensors and discriminated six different steroids; deoxycorticosterone 21-glucoside, dehydroisoandrosterone 3-sulfate, hydrocortisone, testosterone, aldosterone, and 1,4-Androstadiene-3,17-dione. The figure below shows the response degree of three individual aptameric sensors to each steroid. Finally, the results show that the powerful discriminatory capacity between structurally similar steroids over full ranges of concentrations from low nM to  $\mu$ M suggest that these aptameric sensors have potential as good classifiers for molecular recognition and use in biomolecular computing [4].



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# Software for the design and analysis of surface chemical reaction networks

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The chemical reaction network (CRN) abstraction has proven to be a powerful formalism for modeling molecular computation, and arbitrary CRNs can in principle be implemented using DNA strand displacement systems (Soloveichik 2009). However, CRNs only model well-mixed systems, which can be difficult to scale because of the increased possibility of unwanted side reactions with increasingly complex systems. The surface chemical reaction network (CRN) is a discrete, asynchronous extension of the chemical reaction network designed to model molecular computation using molecular species tethered to a surface (Qian & Winfree 2014). A surface CRN consists of some network (usually a grid) of sites for molecular species, as well as a set of unimolecular and bimolecular reactions of the same form as those of a classical CRN. Using only these reactions, surface CRNs can be programmed to exhibit a wide range of behaviors, including simulation of Turing machines. Additionally, the local nature of surface CRNs should allow efficient scaling and reduce the impact of unwanted side reactions.

Although relatively simple surface CRN designs can implement powerful algorithms, they can be difficult to verify and debug by hand. To aid in the visualization and verification of surface CRN systems, we have constructed a general-purpose surface CRN simulator. Our simulator is a standalone Python program using an efficient priority queue based Gillespie-like algorithm. The program is capable of simulating arbitrary surface CRN reaction sets and initial states, specified in a simple configuration file. Using the simulator, we have constructed and debugged a number of surface CRN designs using our simulator, including both asynchronous and locally-synchronous Greenberg-Hastings automata, a Turing machine implementing parentheses-matching, and a locally-synchronous majority vote automaton. We also present a set of surface CRN reactions implementing continuously-active logic circuits, which we have used to construct a square-root circuit, a modular binary counter, and an emulator for Conway's game of life. The surface CRN simulator has been used to reveal subtle flaws in early transition rule sets and circuit designs, and corrected versions of the aforementioned systems have been unit-tested using the simulator. Finally, we report a novel logic gate motif in which each gate calculates on two sequential signals from the same line instead of simultaneous signals from distinct lines, and show that this motif is more efficient for implementing some circuits (in particular, for game of life emulation).

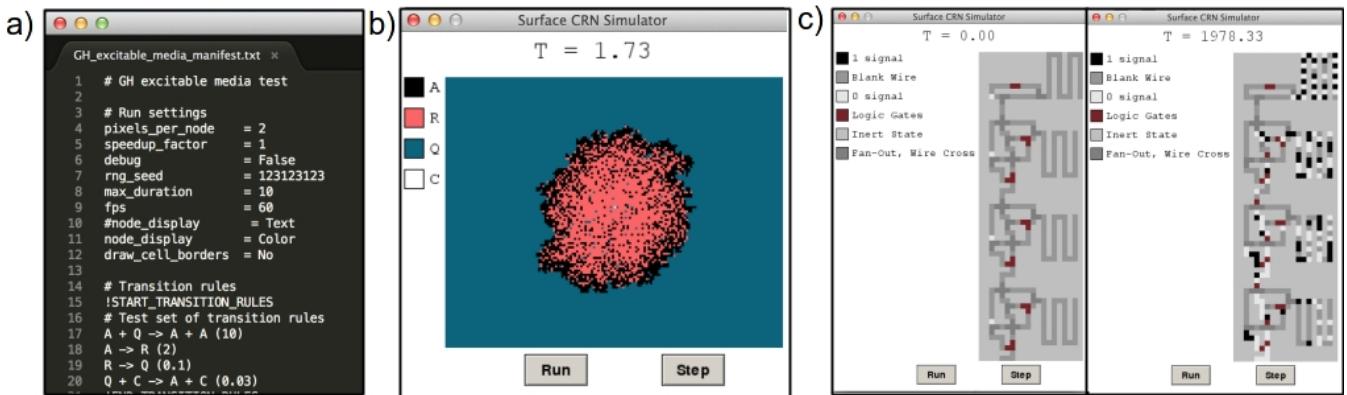


Figure 1: A typical configuration file for the surface CRN simulator (a) and running screenshots of the program simulating an asynchronous Greenberg-Hastings automata (b) and a 4-bit binary counter (c).

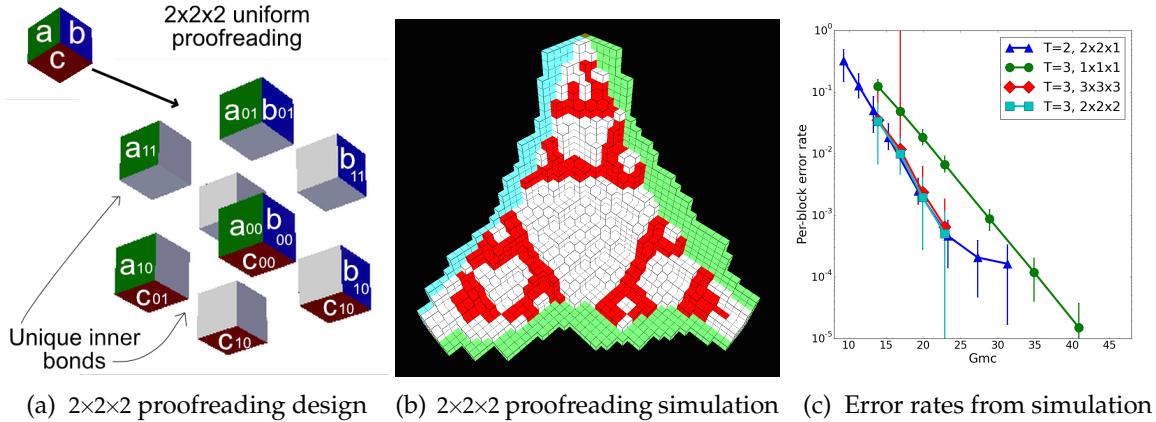
# Proofreading and Self-Healing in a 3D Tile Assembly Model

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The two-dimensional kinetic tile assembly model has been a great resource for studying realistic tile assembly. Much work in the kTAM has been done on transforming tilesets to give them more desirable properties, such as reducing error rates with proofreading techniques or properly regrowing damaged sections with self-healing designs. However, self-assembly in three dimensions offers both more computational power and opportunities to create diverse structures. Here, we present a straightforward extension of the kTAM to three dimensions called kTAM3, and a similar aTAM extension called aTAM3. We develop a simulation tool for 3-dimensional tile assembly based on xgrow that can empirically estimate error rates. We also develop a three-dimensional self-healing transformation and prove its effectiveness in the aTAM3. We examine the effects of  $k \times k \times k$  uniform proofreading on per-block error rates. Transformations allowing a temperature-3 tileset to properly grow at a temperature of 2, and corresponding error rates, are investigated. To estimate error rates from simulations, because one error can increase the probability of future errors, we only consider whether any given assembly has at least one error, and use the estimated probability of having at least one error to estimate the per-block (single tile or proofread block) error rate. It is found that, as in the kTAM, the per-tile error rate of an untransformed tileset is  $\propto e^{-G_{se}}$  where  $G_{mc} = TG_{se} - \log \delta$  where  $\delta$  is the number of competing tile types, which is the same as in the 2D case and is consistent with a theoretical argument.  $2 \times 2 \times 2$  uniform proofreading significantly decreased the error rate by a factor of  $\approx 8$ , and that factor increases as a function of  $G_{se}$ . We observed no further improvement with  $3 \times 3 \times 3$  proofreading. Initial results suggest that three-dimensional proofreading is an effective method of lowering error rates and that the temperature-2 transformation allows a tileset to grow at a lower temperature without irreversibly impacting error rates.

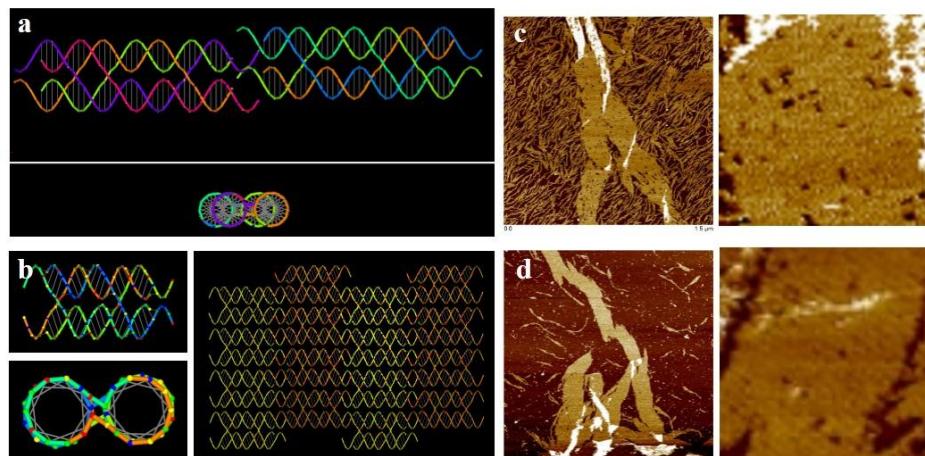


## Self-assembly of RNA and DNA nanoarrays with Paranemic Crossovers

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Nucleic acid molecules are valuable building blocks for constructing multi-dimensional nanostructures. Self-assembly of DNA molecules have provided a highly effective way to construct a wide range of designer nano-scale architectures. However, designing RNA nanostructures with complex higher order structures remains challenging. Here we demonstrate the design of both DNA and RNA nanostructures based on paranemic crossover motifs to create 2D tessellation lattices. Figure 1a illustrates the design of AB-tile system for the DNA PX motifs based on 10.5 bases per turn and Figure 1b illustrates the corrugated design of an RNA motif with 11 bases per turn. With careful consideration of inclination and phases of crossovers, different positions of PX were assigned. By designing appropriate sequence and annealing conditions, these motifs self-assemble into micron sized 2D arrays. The successful growth of self-assembling DNA and RNA nanoarrays based on the paranemic crossover motifs provides new design routes to increase the complexity of designer DNA and RNA nanoarchitectures.



**Figure 1** a. Schematic drawing of the paranemic crossover DNA motifs used to construct self-assembled 2D DNA nanoarrays. b. Schematic drawing of the paranemic crossover RNA motif used to construct self-assembled 2D RNA nanoarrays. c. Corresponding zoom in and zoom out AFM images of the resulting 2D array from the design shown in a. d. Corresponding zoom in and zoom out AFM images of the resulting 2D array from the design shown in b. The zoom in AFM images are 200 nm x 200 nm.

# Characterizing “Leakless Strand Displacement” with Double Long Domains

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The DNA strand displacement reaction has been extensively used in dynamic DNA nanotechnology to build various molecular devices like logic circuits, sensors, amplifiers and motors. However, DNA strand displacement systems are vulnerable to undesired triggering reactions (leak) in the absence of the correct invader strand [1]. Leak critically impedes the application of strand displacement reactions by reducing sensitivity and disrupting desired system behavior. We experimentally investigate a domain-level design to reduce system leakage, starting with the basic “translator” primitive [1] that converts a signal of one sequence into that of another. Unlike the traditional single long domain (SLD) design, which relies on the sequestering of a toehold domain, the double long domain (DLD) design sequesters an additional long domain (the corresponding theory work will be presented as a Track A paper in DNA21 [2]). While kinetic measurements of the leak for the conventional SLD design confirm leak rates of  $2 - 12 \text{ M/s}$ , the leak rate in the DLD design was not measurable. Beyond kinetics, we confirm the theoretical prediction that the amount of leak in the DLD scheme is lower at thermodynamic equilibrium (which puts an upper bound on the *total* leak), and further identify the complex responsible for the leak via PAGE gels. The poster will also focus on systematically exploring different sequences to assess consistency. Our work could offer a new way to improve the robustness of DNA strand displacement, which sets a foundation for future development of dynamic DNA systems.

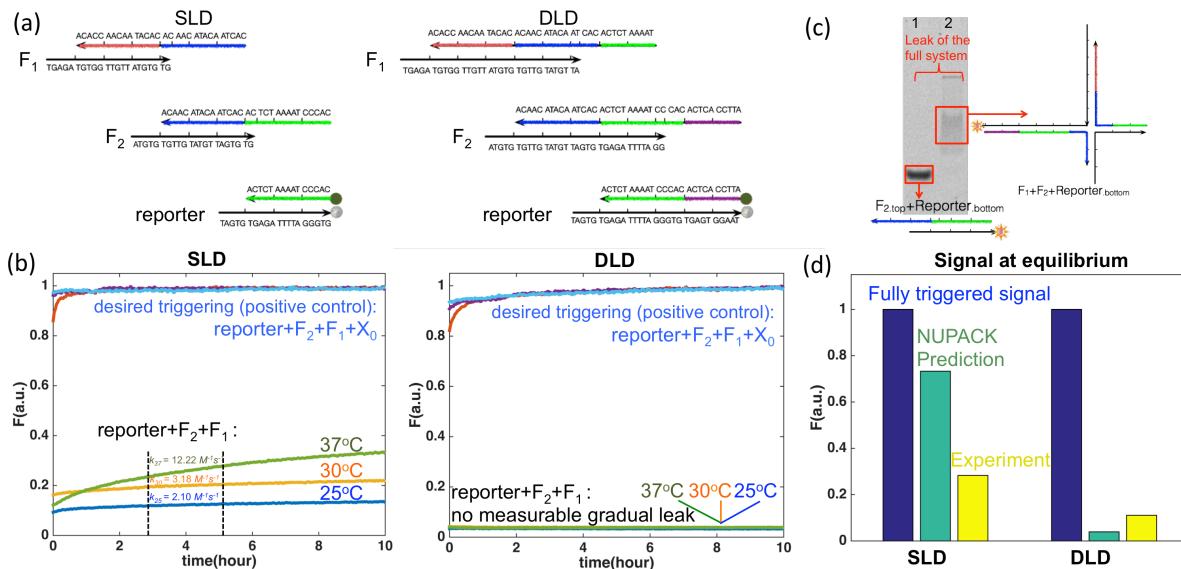


Figure 1. (a) Design and the sequences of SLD and DLD translator schemes. (b) Kinetics of SLD and DLD at different temperatures. (The concentration of reporter is 400 nM. F<sub>1</sub> and F<sub>2</sub> are 350 nM.) (c) PAGE gel (without staining) where only complexes with an unquenched fluorophore are visible. Lane 1: SLD (reporter+F<sub>2</sub>+F<sub>1</sub>) Lane 2: DLD (reporter+F<sub>2</sub>+F<sub>1</sub>). Gel confirms that leak in the DLD system requires the formation of heavy transient complexes as predicted theoretically [2]. (d) Thermodynamic equilibrium of SLD and DLD. (Blue) signal of fully triggered reporter. (Green) fraction of leak predicted by NUPACK. (Yellow) experimental leak fraction after F<sub>1</sub>, F<sub>2</sub>, and reporter are slowly annealed (18 hours).

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Noah Jakimo and Joseph Jacobson  
MIT

### Strand Displacement Activated CRISPR-Cas9 Guide RNA

The transcriptome of a biological cell enables classification of many important cellular properties, such as disease, metabolism and cell type. Therefore, it is desirable to design biomolecular circuitry that can be expressed in the cell to sense, report and respond to transcriptome state. We have tested RNA structures that are functional as guide RNA for Cas9 when bound to a target RNA of interest and otherwise has low background formation into active ribonucleoprotein complexes. The transition between active and inactive forms is achieved through a toehold-gated strand displacement mechanism between domains of the guide RNA structure from *S. pyogenes*. Many desirable responses to detection can be chosen from the ensemble of engineered Cas9 mutants and fusion proteins or programmed into the domains of guide RNA.

## Late-Breaking: First Steps Towards Automated Implementation of Molecular Robot Tasks

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Bar Ilan University, Israel

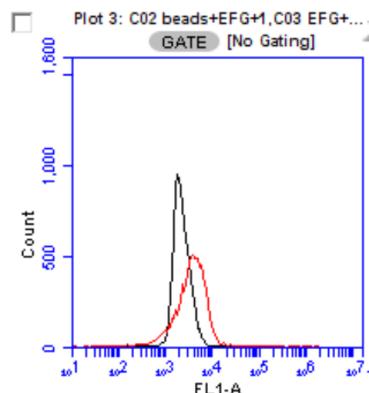
We report late-breaking initial results in our research on automatically generate molecular robot (nanobot) implementations of specific tasks. In particular, we focus on biomolecular robotics tasks combining steps such as fetching molecules, releasing molecules, shielding toxic molecules from exposure to the environment, etc. Existing nanobots able to carry out such tasks, e.g. [1, 2]; however, so far have always had to be manually crafted for each specific task.

We designed—and implemented a compiler for—a domain-specific, rule-based language for describing molecular robotics tasks, as discussed above, without committing to a particular nanobot implementation. The compiler translates programs written in this language, generating an output which consists of recipes for specializing known nanobot designs for the specific task at hand. To do this, the compiler consults a library of known parametric nanobot designs, allowing it to automatically instantiate combinations of nanobots that together carry out a complex task. This separates the design of a task (described by the programs) and the design of nanobots with different properties (in the compiler’s library of parametric nanobots). The compiler can output different combinations of robots which may carry out the task.

We conducted several experiments, and are reporting here on one. The compiler successfully compiled the single-rule program below. Rules are built from conditions which determine *When* and *Until* they are effective, and *Actions* that dictate what to do when the rule is in effect. These allow *Pick*, *Drop*, *Expose* and *Protect* some material in specific location. For example, in the rule below, we want a molecular payload denoted by \$. and normally protected from the environment to be exposed, when in the vicinity of beads marked by DNA strands A, B, and C. The compiler output an implementation using two clamshell [1] robots, each capable of responding to two strands. The first responds to A and B by releasing an intermediate T. The second exposes in response to T and C. The figure measures activity by fluorescence: low or no responses when only the first robot is interacting with beads carrying A, B and C; high response (red line) when the two robots are interacting with the beads.

*An example of a simple task  
(portions removed)*

```
Rule : ExposeDrugAtLoc
{
    Actions: expose ($. @ (A& B& C));
}
```



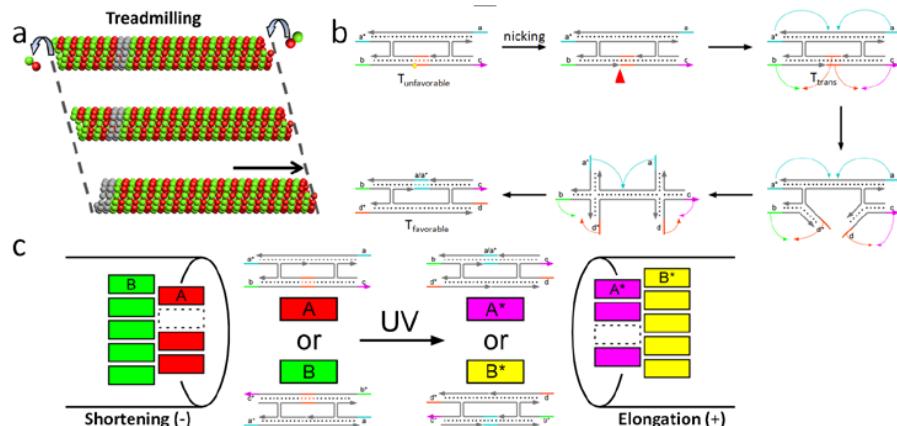
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# DNA Nanotube Treadmilling Driven by Holliday Junction Isomerization

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Although much of our current understanding of self-assembly comes from the examination of static systems, the greatest challenges and opportunities lie in building and studying dynamic systems.<sup>1</sup> One of the most intriguing examples of dynamic self-assembly in living cells is the polymerization of microtubules<sup>2</sup> (Figure 1a). In the study reported here, we employed Holliday junction (HJ) isomerization dynamics as the fueling strategy to generate treadmilling behavior, which refers to the coexistence of assembly at one end and disassembly processes at the other end based on distinct kinetics.<sup>3</sup> Ideally, DNA tiles can be designed such that the rate of assembly is approximately equals to the rate of disassembly, resulting in steady-state treadmilling and fixed length nanotubes. The existence of two populations of HJ stacking conformers showed distribution toward one conformer depending on the junction sequence<sup>4</sup>. By covalently linking two HJs to a double crossover (DX) structure, free energy can be stored in the DX tile with the unfavorable HJ conformer sequence at both crossover junctions. FRET based melting curve measurements indicated that the formation of  $T_{favorable}$  of DX is thermodynamically more favorable than the  $T_{unfavorable}$  (Figure 1b). A photo-cleavable site was embedded between the two crossovers on the  $T_{unfavorable}$  tile, opposite to the central strand loop with a strategically positioned nick point. By UV exposure, cleavage of the strand creates a pair of 5 bp sticky ends between the two crossovers that allow the DX tile reconfiguration from  $T_{unfavorable}$  to  $T_{favorable}$  (Figure 1b). The process of DX tile reconfiguration showed first-order reaction kinetics with an overall reconfiguration rate  $\sim (31.4 \pm 0.3) \times 10^{-3} \text{ s}^{-1}$  (a half decay time  $\sim 30$  second). The new pair of sticky-ends generated from the photo-cleavable site directs the re-polymerization on the opposite end of the Origami seed (Figure 1c). This study will lay the groundwork for building more sophisticated dynamic and developmental self-assembly systems using nucleic-acid building blocks.



**Figure 1.** (a) A schematic of treadmilling: a unidirectional subunit flux (grey) is labeled to illustrate treadmilling; (b) DX tile reconfiguration to display a different set of sticky ends is driven by UV-triggered HJ isomerization; (c) A schematic of the coexistence of assembly and disassembly processes at both ends of a DNA nanotube.

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# A DNA strand displacement circuit compiler demonstrated using unpurified strands

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The success of computer engineering has inspired attempts to use hierarchical and systematic approaches for developing molecular devices with increasing complexity. To enable the design and construction of a wide range of functional molecular systems, we need software tools such as a compiler that can automatically translate high-level functions to low-level molecular implementations and provide models and simulations for predicting and debugging the designed molecular systems. DNA strand displacement is a simple and powerful mechanism for creating novel biochemical circuits, and a few proof-of-principle compilers have been developed for designing such systems. However, there has been little independent experimental validation of such molecular compilers, most of which were developed in parallel with experimental findings. Here we show that a small group of students with minimum prior knowledge and skills, backgrounds ranging from computer science to bioengineering, were taught to use the Seesaw Compiler<sup>[1]</sup> for creating a complex DNA strand displacement circuit with a logic function that they designed (Fig. 1ab). Spending ten weeks with a combination of dry and wet labs for 6 hours each week, the students successfully demonstrated half of the circuit, comprising 54 distinct DNA species (Fig. 1c). Unpurified DNA strands were used here to demonstrate cost- and time- effective experimental procedures. The main challenge that we had to overcome of working with unpurified strands was to infer the effective concentrations of all molecular species from a small set of experiments, and to adjust the circuit design correspondingly. Compared with purified DNA molecules, the speed of computation was decreased and the undesired product was increased, but nonetheless the overall circuit behavior remained qualitatively correct. Our results suggest that compilers could indeed enable researchers with diverse background to easily design and construct molecular devices, and DNA strand displacement circuits in particular are suitable architectures for systematic designs and robust demonstrations.

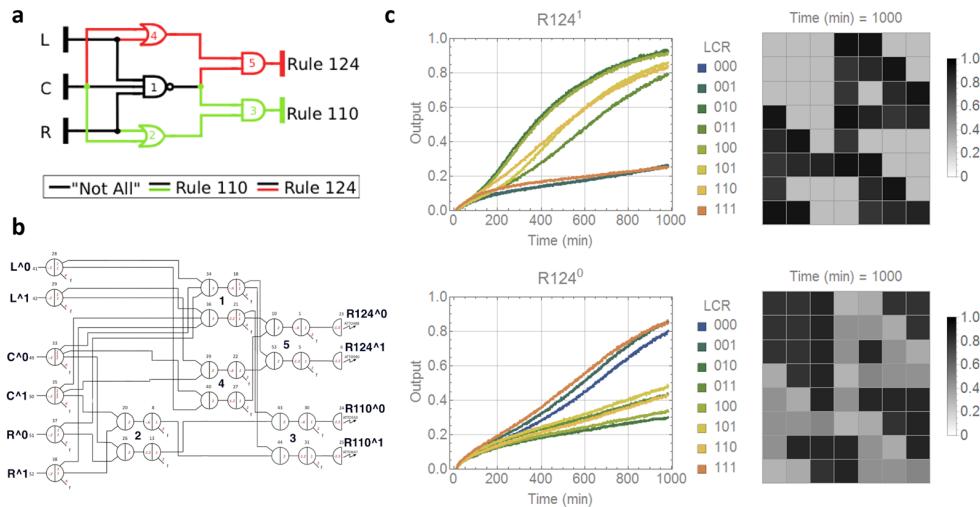


Figure 1. (a) A logic circuit that computes elementary cellular automata rules 124 and 110. (b) The corresponding seesaw DNA circuit implementation. (c) Fluorescence kinetics experiments of the rule 124 sub-circuit. Data at 1000 min was used to plot an array of 7×8 cells that illustrate eight generations for a pair of dual-rail outputs of the rule 124 circuit with starting configuration {0, 0, 0, 1, 0, 0, 0}. Perfect circuit behavior would correspond to an image of a black dog with white background for R124<sup>1</sup> and an inverted image for R124<sup>0</sup>.

[1] <http://www.qianlab.caltech.edu/SeesawCompiler/>

# A Systematic Process for Design and Implementation of Algorithmic DNA Tile Systems

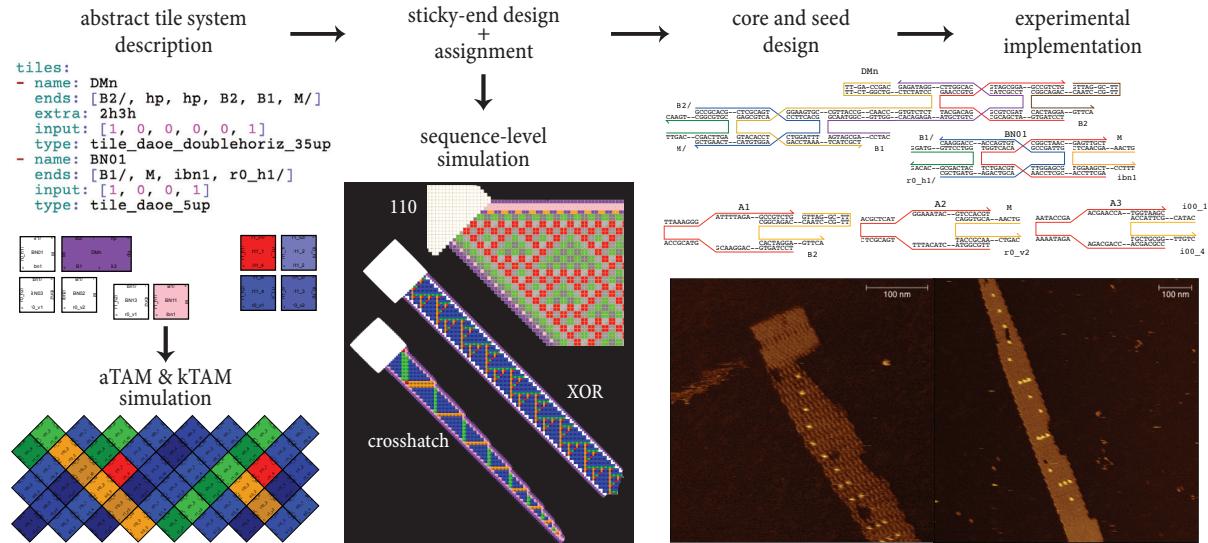
Constantine G Evans,<sup>1,2</sup> Masa Ono,<sup>1</sup> Matthew Cook,<sup>3</sup> and Erik Winfree<sup>1</sup>

<sup>1</sup>*California Institute of Technology*, <sup>2</sup>*Evans Foundation*, and <sup>3</sup>*ETH Zurich*

The development of systematic design and implementation methods, like caDNAno for DNA origami, simplifies the use of DNA nanotechnology, especially for new researchers, and facilitates applications and advances in the field. Combining software packages and experimental protocols developed in prior work, we have developed a process for systematically designing and implementing experimental algorithmic tile systems of double-crossover (DX) tiles from abstract designs.

Our software implements improved versions of our sticky-end sequence design and end assignment algorithms to provide more consistent, optimized tile attachment and detachment kinetics for complex systems, and allows expanded kinetic Tile Assembly Model (kTAM) simulations with rotatable tiles and sequence-dependent sticky-end energetics. Consistent core sequence design is done through improved versions of PepperCompiler and spuriousC, taking into account sticky-end sequences. Along with consistent experimental protocols, the software creates a simple, often one-step pathway from abstract descriptions of tile structures and glues to experimental implementations.

As an application of our process, we have designed a ribbon-growth framework to implement a wide variety of abstract tile assembly systems on a ribbon structure in an experimentally-robust way. The ribbon is origami-nucleated and controls spurious nucleation without a zig-zag design, allowing simple uniform proofreading and faster growth. The width of the ribbon can also be programmably controlled by the logic of internal growth, allowing algorithmic assembly to control the shape of the constructed crystals. We have designed three systems using this ribbon and our software: the “crosshatch,” XOR, and Rule 110 systems, all incorporating uniform proofreading, and have early experimental results with implementations of the crosshatch and XOR systems.



An outline of our software's tile system design process, and some early AFM images of experimental implementations. All diagrams were generated by the software, or through generated Xgrow tile system simulations.

## **3D VISUALIZATION OF CADNANO AND PROTEIN DATA USING A CLOUD STREAMING FRAMEWORK FOR WEB-BASED LARGE MODEL VIEWING**

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3D visualization is an integral component toward understanding the structural and functional complexities involved for biological design in domains such as structural biology, DNA origami, and multi-scale biological systems. As recent structures and designs are becoming increasingly large and more complex, the existing industry standard desktop and web-based applications can easily push beyond the limits of advanced in-core processing and rendering.

We present an extensible, cloud-based framework optimized for visualization of large 3D data in the browser. It is designed to overcome limitations of scalability, capability, accessibility, collaboration, and outreach for biomolecular datasets. As an extension of the visualization framework, Autodesk Research Molecule Viewer demonstrates common usage scenarios for interaction, visualization, and molecular representation of Cadnano design files and Protein Data Bank entries. This data can be fetched from a database, or through local upload, to the cloud for translation into a streamable 3D viewing file type. For example, a Cadnano.json design file can be uploaded, translated, and streamed into the browser for exploration.

The technology leverages a scalable cloud-based translation and streaming pipeline, and extends three.js on WebGL-enabled browsers to support a distributed data model. A root manifest streams in smaller component parts, which render as required, to absolve the need for entire model storage in memory. The resulting Viewer enables rapid loading, viewing, and interaction with increasingly larger and more complex models and cellular environments.

The framework can be extended to include a wide range of large-scale datasets to facilitate and advance biological visualization and design.



# Winner-Take-All Neural Network Computation with a Simple DNA Motif

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DNA is an excellent substrate for autonomous biochemical information processing including logic circuits and neural network computation. A standing challenge is to experimentally demonstrate advanced molecular pattern recognition and signal classification with increasing complexity. A key to this challenge is searching for efficient circuit designs that permit scaling up of synthetic DNA-based neural networks with simple and robust components. Here, using a DNA strand displacement mechanism based on cooperative hybridization (Zhang *JACS* 2010), we extend the previously developed seesaw DNA motif (Qian and Winfree *Science* 2011) with a new two-stranded component that mediates annihilation between a pair of signal species (Fig. 1b). With this extended seesaw motif, we developed a systematic approach for implementing winner-take-all behavior that can be used to construct artificial neural networks (Fig. 1abc). We demonstrated a winner-take-all function with up to three competing input species, varying the concentration of each input from 10% to 90% of a maximum signal level (Fig. 1d). The winner was successfully selected (the corresponding output signal was amplified to approach the maximum signal level) and the other two species were sufficiently inhibited (the corresponding output signals remained low). In the worst case, the difference between the winner species and the strongest competitor was 20%, and the winner species itself was 40% of the maximum signal level. By setting a predetermined maximum signal, this design ensures composability with computation units downstream of the winner-take-all layer. While improved network behavior could be achieved by reducing rate differences among all annihilation reactions, we showed that small rate differences introduced by DNA sequence variations can be tolerated. Our simple model using mass-action kinetics with identical rate constants for all annihilation and amplification pathways was accurate enough to capture the range of the experimental results. Our results suggest that winner-take-all circuits provide an alternative to linear-threshold circuits for implementing DNA-based neural networks. In terms of scalability, it could allow for molecular pattern recognition with fewer components, thanks to the elimination of negative weights and dual-rail logic.

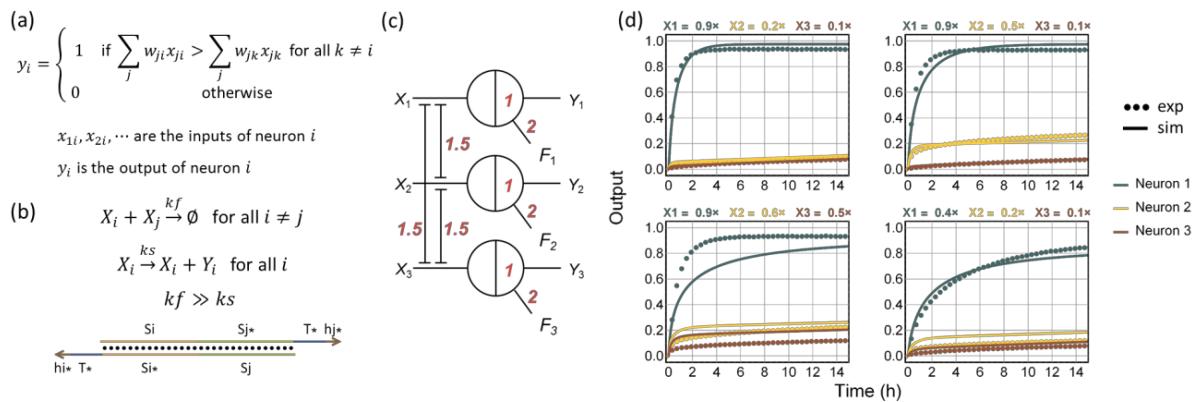


Figure 1. (a) Winner-take-all function as a mathematical model for artificial neural networks. (b) General chemical reaction network and DNA implementation of winner-take-all with extended seesaw DNA motif. (c) Seesaw DNA abstraction diagram for a three input system. (d) Simulations and fluorescence kinetics experiments of a three-input winner-take-all system. Other sets of inputs performed similarly, but slightly slower due to toehold kinetics. Pairwise annihilation of inputs is modeled as a set of biomolecular reactions.  $1\text{x}$  is 50 nM, the maximum signal level.

# Dynamic Self-Assembly of DNA Nanotubes

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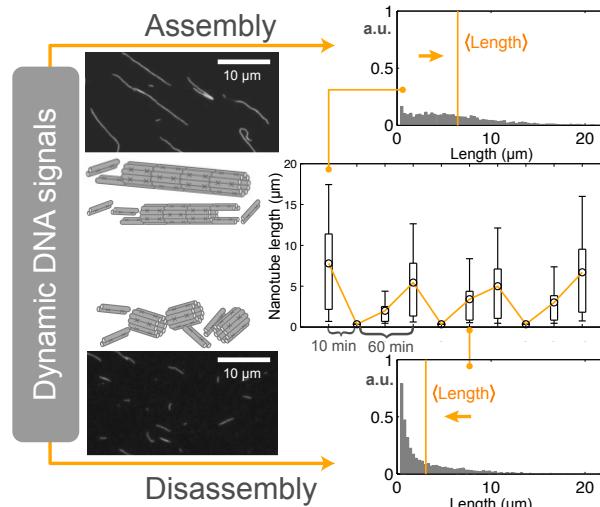
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Biological systems reconfigure their shape at the level of single cells, tissues, and organs, in response to external stimuli, for a variety of purposes such as growth, development, and self-repair. Cell shape reconfiguration is accomplished by directing the spatial organization of molecular materials (for example protein scaffolds in the cytoskeleton) through molecular circuits which sense, process, and transmit environmental information. These circuits are organized in a modular fashion; however, cellular pathways are still too complex to be directly embedded in a synthetic material. An alternative route is offered by nucleic acid nanotechnology: logic and dynamic circuits [1, 2, 3, 4], and a variety of nanostructures [5, 6, 7] have been successfully demonstrated. We aim at directing assembly and disassembly of DNA nanostructures with dynamic DNA inputs and circuits, mimicking the cellular organization of dynamic biomaterials.

Here, we present our results in controlling assembly and breakage of DNA nanotubes assembled from double crossover tiles [8, 5]. Our tiles are designed to include a toehold domain enabling strand invasion at the sticky ends of tiles. Invasion rapidly weakens the self-assembled structure, causing the nanotubes to collapse into smaller fragments. Removal of DNA species used for invasion, through another layer of strand displacement, allows the nanotubes to reassemble isothermally. Our design thus makes it possible to control growth reversibly, unlike previously proposed schemes [9]. We control nanotubes assembly and disassembly with nucleic acid inputs directly added in solution and with transcriptional systems; in particular, we show that nanotube length can be controlled with an autonomous molecular oscillator [3, 10, 11]. We characterize the influence of experimental design parameters such as toehold orientation and length, buffer conditions, and temperature, showing the overall tunability of breakage and reassembly dynamics. We use a variety of experimental assays including optical microscopy and time lapse movies, atomic force microscopy, and gel electrophoresis.



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## "Addressing DNA mediated self-assembly of carbon nanotube transistors"

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The scaling of Moore's law using complementary metal-oxide semiconductor (CMOS) has slowed down in recent years. Carbon nanotubes (CNTs), with their ultra thin bodies and ballistic electronic transport can allow post-silicon scaling for next generation Logic technology. Simulations<sup>1,2</sup> of suitable CNT based device structures for such technology require placement of CNTs at very tight pitch (under 10nm inter CNT distance), to allow for the density scaling and source/drain contact scaling.

Conventional lithography, electron beam or optical, cannot address sub ten nanometer resolution due to material limitations and cost effectiveness. DNA nanotechnology offers a route to bottom-up, parallel self-assembly of CNTs with nanometer precision that can help benchmark logic device design. However, the challenge of self-assembly of DNA-CNTs on technological substrates still remains unresolved. We have, in the past, demonstrated surface-based self-assembly of DNA wrapped CNTs into viable CNT device geometries using DNA nanostructures<sup>3</sup>. In this poster we will present techniques for placement of DNA-CNTs on relevant oxide substrates, with precise orientation and position control, in order to establish their device behavior. Furthermore, first ever fabrication of sub ten nanometer DNA-CNT devices on SiO<sub>2</sub> and HfO<sub>2</sub> along with their device behavior will be discussed.

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