DNA Strand Displacement driven Molecular Additive Manufacturing (DSD-MAM)

Contract Number: EE0008310
Dana-Farber Cancer Institute/University of Oxford
Project Period: 2018 June 1 – 2021 November 30

William Shih, PhD, Dana-Farber Cancer Institute (Prime)
Andrew Turberfield, PhD, University of Oxford (Sub-contract)

U.S. DOE Advanced Manufacturing Office Program Review Meeting
Washington, D.C.
Wed 2019 June 12

One of five coordinated 1465 FOA projects in Atomically Precise Manufacturing

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Overview

Timeline

- Projected Issue date: 2018 June
- Projected End date: 2022 November
- Project 29% complete

Budget

	FY 17 Costs	FY 18 Costs	FY 19 Costs	Total Planned Funding (FY 18-Project End Date)
DOE Funded	_	87K	347K	1215K
Project Cost Share	_	21K	89K	313K

Barriers

- Unprecedented integration of three independently moving layers of DNA origami to achieve 2D controllable motion

Partners

- Sub-award to Andrew Turberfield at University of Oxford, Dept. of Physics

AMO MYPP Connection

- Will enable rapid prototyping for development of membranes and catalysts, as well as of high strength-to-weight materials
- Molecular 2D and 3D printers are a broadly applicable platform technology (5.4.3)

Project Objectives

PROBLEM Develop a pathway to scalable integrated nanosystems for atomically precise manufacturing (APM). Currently there is not even a single positionally controlled molecular printer in existence. Furthermore, printing custom molecules one-by-one would be too slow for most applications. Therefore massively parallel molecular printers would be required in these cases.

RELEVANCE Assembly to atomic-level specification will deliver qualitatively new functionalities and low-variability, ultra-high performance, and will enable tools and processes that dramatically reduce the energy and materials costs of manufacture.

PROJECT GOAL Self-assemble molecular 2D printers from DNA. Self-assembly provides a route to **scalable** APM. Self-assembled molecular printers will provide **rapid-prototyping** capability for useful materials, e.g. membranes and catalysts.

POTENTIAL BENEFITS Success will initiate a bootstrapping cascade that will lead to APM as a practical manufacturing technology. This will improve the energy and material efficiency, productivity, and competitiveness of manufacturers across the industrial sector, in accordance with the AMO mission.

Technical Innovation

IMPACT ON FUTURE MANUFACTURING Potential applications: photovoltaics; photosynthetic and fuel cells; thermoelectrics and anisotropic heat spreaders; solid-state lighting; molecular electronic and plasmonic circuits; selectively permeable membranes; self-repairing materials with high strength-to-weight and fracture resistance.

CURRENT PRACTICE DNA walkers that move in 1D have been previously demonstrated. However, controllable motion in 2D has not yet been accomplished.

INNOVATION We will use the rapidly developing 'DNA origami' self-assembly technique to create the required molecular machine tools. Novel functionalities of these nanomachines will include the following: nanometer-precision 'stepper' positioning mechanisms based on multivalent interactions for high cooperativity and greater robustness; integration of three independently moving layers of DNA origami to achieve 2D controllable motion; integration of spatial positioning with deposition functionality.

Technical Innovation

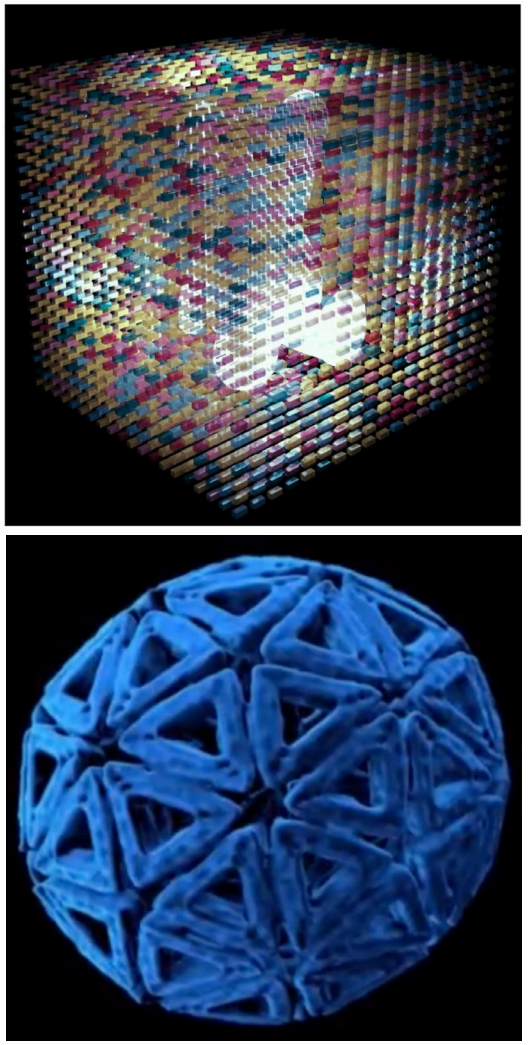
Why APM via structural DNA nanotechnology is realistic for scaling up for manufacturing for R&D purposes?

Recent demonstration of 100x reduced cost of manufacture of DNA nanostructures: DNA origami now can be produced at $180 per gram via bacterial production in a fermenter (Praetorius F, Biotechnological mass production of DNA origami. *Nature* 552, 84, 2017).

Recent demonstration of assembly of gigadalton-scale DNA nanostructures (cf. ribosomes are megadalton-scale)

Ong L et al, Programmable self-assembly of three-dimensional nanostructures from 10,000 unique components. *Nature* 552, 72, 2017.

Gigadalton-scale shape-programmable DNA assemblies. *Nature* 552, 82, 2017.



Hendrik Dietz, TedxTUMSalon 2018

Note: past and current basic-science support (i.e. low TRL) from NIH, NSF, ONR to William Shih for development of DNA origami

Technical Approach

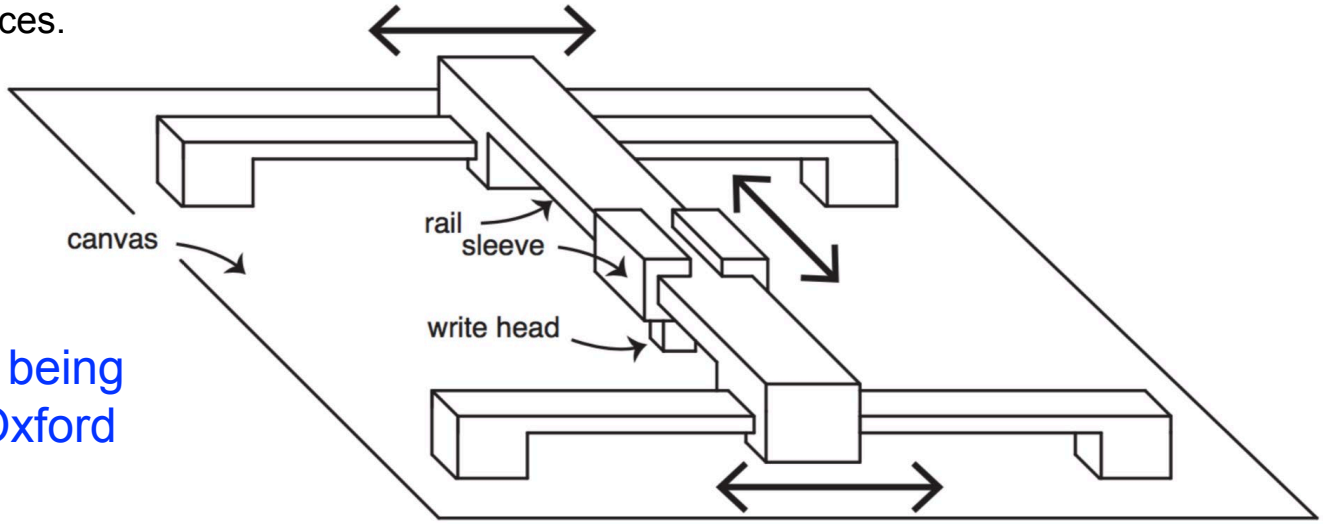
Participant roles and responsibilities

William Shih, PhD, Dana-Farber Cancer Institute: Principal Investigator; pioneer in 3D DNA origami; Project Lead; responsible for implementing the 'stack' architecture; 2017 Foresight Institute Feynman Prize in Experimental Nanotechnology; 2018 Rozenberg Tulip Award in DNA Computing

Andrew Turberfield, PhD, University of Oxford (sub-contract): co-Investigator; pioneer in DNA walkers; responsible for implementing the 'wrap' architecture

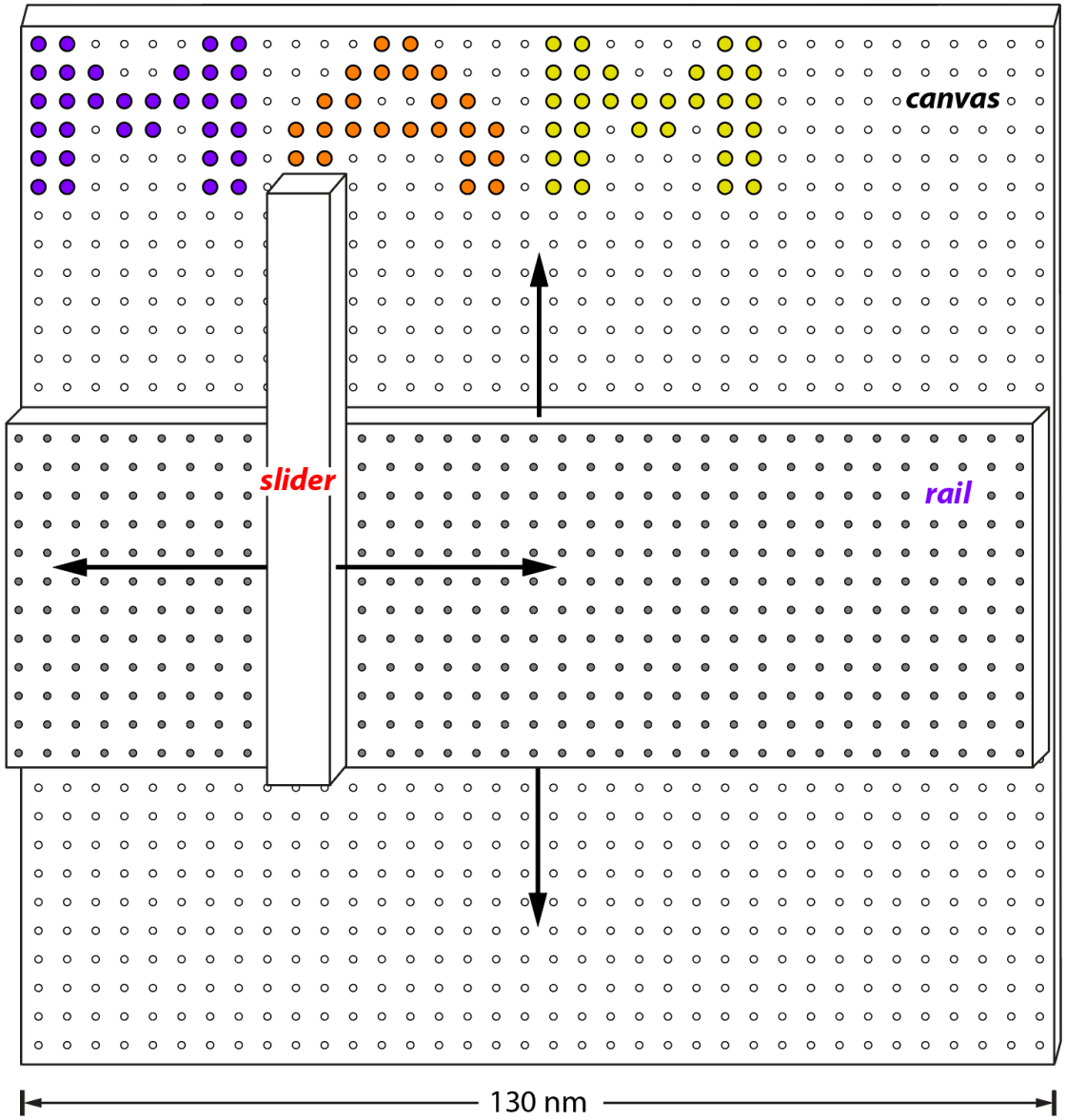
Project risks and unknowns Many of the key technologies required for success of our proposed approach already have been demonstrated. What has never been shown before is the control of multisite interactions at interfaces between DNA-origami surfaces as in the 'stack' and 'wrap' designs. Anticipated failure/low- performance modes include sliders that don't move for one or more cycles or else fall off their rails, sliders that exhibit high positional variance at each register, and printheads that exhibit pixel-writing failures (e.g. missing pixels, unwanted pixels) despite positioning within specifications. Our objective is to develop strategies for minimizing the fraction of DSD-MAM nanosystems that exhibit these kinds of suboptimal performances.

'wrap' architecture being developed by U. Oxford



Technical Approach

'stack' architecture being developed by Dana-Farber Cancer Institute



Stepper motors move **3.5 nm** per transition in response to externally triggered pulses of short DNA strands.

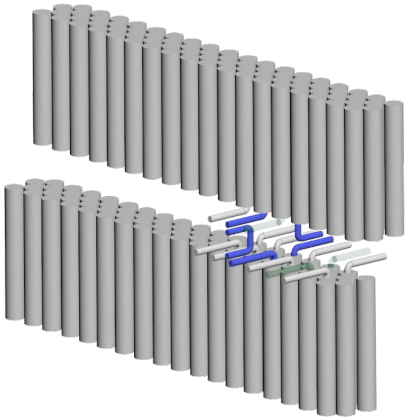
DNA-origami **slider** steps to the left and right on DNA-origami **rail**.

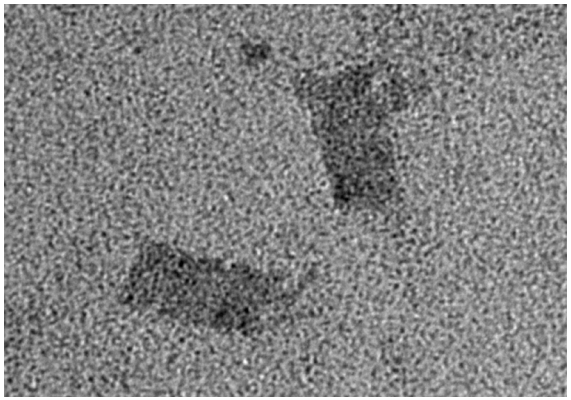
DNA-origami **rail** steps up and down on DNA-origami **canvas.**

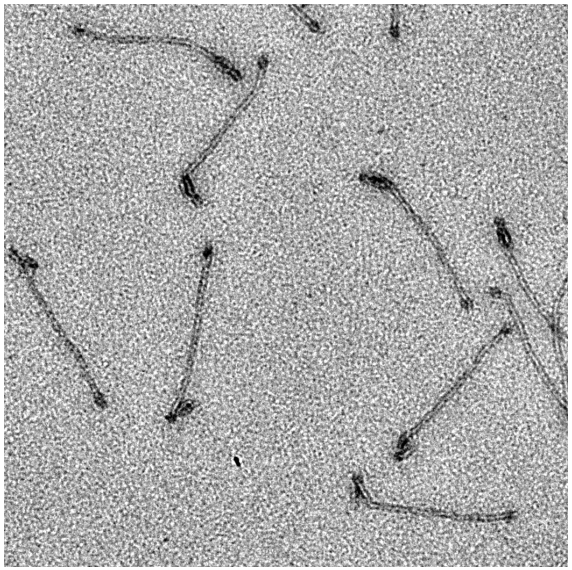
Results and Accomplishments

MONTHS 1–12 We have demonstrated assembly and TEM validation of 1D stepper nanomotor components with two different architectures (‘stack’ and versus ‘wrap’). Specifically, we have recovered purified DNA-origami sliders and rails of the two architectures at >25% yield with respect to input scaffold molecules.

Stack architecture







Wrap architecture

Remaining Milestones

MONTHS 13–14 We will demonstrate full assembly and operation, via agarose gel and TEM analysis, of 1D stepper nanomotors with two different architectures (‘stack’ and versus ‘wrap’). Nanomotors will be controlled in bulk solution by manual pipetting steps. We expect that at least one architecture can be actuated in one dimension with >95% yield per driven step by the end of Period 1.

MONTHS 15–28 We will demonstrate assembly and TEM validation of 2D stepper nanomotors with ‘stack’ and ‘wrap’ architectures. As with the 1D steppers, the 2D nanomotors will be controlled in bulk solution by manual pipetting steps. We expect at least one architecture can be actuated in two dimensions with >95% yield per driven step by the end of Period 2.

MONTHS 29–42 We will demonstrate 2D printing on the canvases, catalyzed by positional control of the writehead. We expect at least one architecture can be patterned with >80% occupancy per designed site by the end of Period 3, as assessed by TEM. We also will demonstrate surface immobilization and microfluidic actuation of 1D and 2D stepper nanomotors. DNA-PAINT super-resolution fluorescence microscopy will be used to monitor stepping of the nanomotors in real-time.

Transition (beyond DOE assistance)

1. Immediate Transition to Early (high premium) Commercial Markets
   - **R&D Tools**: far greater throughput than AFM or single-molecule optical trapping, especially for biophysical investigations; conversely, advantages for molecular printers over conventional DNA origami include faster prototyping, faster dynamic rearrangement of patterns, and the ability to respond with feedback
   - **Therapeutics**: rapid prototyping of novel macromolecules
2. Eventual Transition to multiple manufacturing applications.
   - **Energy conversion**: photovoltaics; photosynthetic and fuel cells; thermoelectrics and anisotropic heat spreaders; solid-state lighting
   - **Information technology**: molecular electronic and plasmonic circuits, quantum computing
   - **Separations**: selectively permeable membranes
   - **Materials**: self-repairing materials with high strength-to-weight and fracture resistance