1. Overview & Motivation

Ultra-high density data storage and massively parallel computation are two pressing needs for the semi-conductor and synthetic biology communities, which are realized in this project. Specifically, we explore the use of highly programmable synthetic DNA-based assemblies to both encode and read-out petabyte-scale datasets within sub-millimeter-sized 2D and 3D assemblies to overcome the impending data storage limit of silicon- and magnetic-tape-based data storage technologies[2]. These molecular data stores may be used to report on the results of solution-based molecular computations for application to biomolecular sensing and bioanalysis, including temporal-based recording in environmental sensing. While numerous studies have utilized single-stranded DNA or DNA tiles to demonstrate powerful capability to sense, actuate, and record cellular and environmental cues in programmed DNA-based cascades and 2D assemblies[3-10], encoding such information for optical readout and long-term archival storage has not yet been realized.

Toward this end, programmed 3D DNA-based memory blocks offer unprecedented capabilities for ultra-dense data storage and efficient optical read-out. Specifically, precisely defined 2D and 3D molecular hard-drives can now be engineered at the nanometer-scale by combining top-down design and synthesis of arbitrary complex DNA nanoparticle geometries encoded with unique optical signatures, which, together, offer ultra-dense data storage capabilities orders of magnitude denser than existing solid-state hard drives[2]. This data storage approach is now feasible due to rapid recent progress in DNA origami technology, whereby arbitrary DNA nanoparticle geometries can be designed from the top-down and assembled by annealing a long, single-stranded DNA scaffold with molar excesses of short complementary oligonucleotide staples[12-14]. These staples are composed of sequences that bring scaffold sites distant in sequence space nearby in Euclidean space, programmed by Watson-Crick base pairing and crossover motifs, and can be used with single-stranded extensions to both organize distinct DNA nanoparticles in 3D space, as well as integrate them with optical molecular or nanoscale components for data encoding. The nanoscale precision offered by these assemblies has demonstrated unique capabilities to spatially organize chromophores[15-17], metals[18-23], and semiconductors[24-27], as well as their combinations[22,25,26], attractive for applications in light harvesting[16,28-30], structural biology[31,32], and data storage and molecular computation[33-36].

We combine these unique strengths of DNA origami to build a generalized framework for programmed hierarchical self-assembly of DNA nanoparticles for the coordination of optical elements, nanoparticles (metallic and semiconductor) and fluorophores. In each case, the packaged optical elements are used as information containing media (memory blocks), which are organized in precise hierarchical manners for long-term storage of arbitrary datasets and efficient optical read-out. In Aim 1, we will investigate monomeric block formation, exploring the self-assembly of arbitrary geometric DNA objects with incorporated optical elements that can be manufactured as information carriers, while allowing for superstructure formation through DNA-sequence barcoding. We will explore static assembly of 1D arrays of such ***DNA*** *nanoparticles integrated with* ***M****emory* ***B****locks* (DNAMB) for encoding bitstream information that can be read out by fluorescence and electron microscopy. In Aim 2, we will explore 2D and 3D assembly, investigating techniques to algorithmically assemble and read out digital 2D and 3D information using optical and tomographic methods. In Aim 3, we will use molecular decision computing to assemble distinct, alternative lattices based on specific external signals. These results will offer the ability to encode and decode arbitrary datasets in ultra-dense molecular hard-drives, with environmental sensing and recording.

To achieve this over-arching aim, we combine expertise in molecular computation from Glotzer (University of Michigan) and Bathe (MIT), DNA-based molecular synthesis and self-assembly from Gang (Columbia University) and Bathe, expertise in semi-conductor fabrication and material conversion from Bawendi (MIT) and Gang, and expertise in optical encoding, sensing and analysis from Bawendi and Bathe. Gang is an international leader in DNA nanoparticle and hierarchical nanoparticle assembly. Bathe is a

leader in computational design and self-assembly of complex DNA origami assemblies, as well as fluorescence imaging and analysis. Bawendi is a leader in fluorescent nanoparticle engineering and fabrication, including original pioneering work in semiconductor quantum dot fabrication and interfacing nanoparticles with biological materials. Glotzer is a recognized leader in physics-based modeling of hierarchical self-assembly of nanoparticles. Thus, our team has combined expertise across DNA assembly, nanoparticle design, synthesis, functionalization, nanoscale semiconductor fabrication, and modeling hierarchical assembly to achieve our goal of addressable self-assembly of structured information arrays, or an ultra-dense molecular hard-drive.

Graduate student and postdoctoral mentees will be trained in a highly interdisciplinary manner in biological engineering, chemistry, physics, and materials science, training a new generation of scientists with foundations in biology-inspired design of nanoscale materials for semiconductor research.

2. Previous Work

Results from Prior NSF Support

Oleg Gang (PI) has not received any form of NSF support. Prior joining Columbia University he was at the Brookhaven National Laboratory and his research was supported by the Department of Energy. Gang demonstrated efficient strategies for assembly and characterization of 2D and 3D DNA-driven nanoparticles architectures, both periodic and arbitrary designed.

***Current support*.** Glotzer is lead PI for the DMR Computational and Data-Enabled Science & Engineering project DMR-1409620 "Fast, scalable GPU-enabled software for predictive materials design & discovery," (7/1/2014 – 6/30/2018, $595,880). This award supports the sustained development of the HOOMD-blue Molecular Dynamics simulation package to add capabilities and user interface tools and to optimize the code for new generations of GPU architectures.

***Intellectual Merit*.** Over the 4-year timeframe of that award, Glotzer is expanding the already rich capability set of HOOMD-blue to include discrete-element MD and Monte Carlo algorithms optimized for GPUs. DEM-MD and Hard Particle Monte Carlo (HPMC) were formally released as open-source components of HOOMD-blue in 2016; and bounding volume hierarchy (BVH) neighbor lists were made available in 2015. These modules enabled numerous publications outside of the DMR-1409620 award: A study on clathrate colloidal crystals utilized DEM-MD to model DNA-coated polyhedral nanoparticles[37]; HPMC for orientational order of colloidal discoids[38], quasicrystalline superlattices[39], assemblies of hard cuboctahedra[40], space-tessellating structures[41], and shape-driven solid–solid transitions[42]. BVH neighbor lists enabled studies by others on the axial dispersion of Brownian colloids in microfluidic channels, stratification dynamics, and machine learning for autonomous crystal structure identification.

Connection to current proposal. The CDS&E grant is for software development, specifically focused on development of DEM-MD and MC methods used for rigid bodies. In contrast, the work proposed here will use traditional molecular dynamics methods with force fields, and will not benefit from the new DEM and MC modules. However, improvements made to HOOMD-blue generally under the current grant, including code optimization on new GPU architectures, will certainly benefit the planned studies.

***Broader Impacts*.** Newly developed simulation tools will be of immediate interest to the particle assembly communities. The approaches and tools are transferable and will be of immediate and even broader interest to the materials, engineering, and chemistry communities interested in crystallization of, e.g., atoms, molecules, and proteins, helping researchers to move into an era of predictive materials design. Four peer-reviewed manuscripts have been published in the first two years of the grant.

Moungi Bawendi (co-PI)

***Current support*.** Bawendi is co-PI for the ECCS project ECCS-1449291 "Knowledge-Based Continuous and Scalable Manufacture of Quantum Dots," (9/1/2014 – 8/31/2018, $1,300,000). This award is joint with Prof. H. Kulik and Prof. K. Jensen and supports the modeling and continuous fabrication of quantum dots using microfluidic techniques, in particular for the class of II-V quantum dots based on InP and InAs.

Intellectual Merit. This grant supports research to (1) understand the kinetics of growth of III-V quantum dots (QD) based on InP and InAs using a combination of theory and experiments, (2) improving the quality QD, and (3) provide a paradigm for the manufacture of QDs through the use of continuous flow microfluidics. The Bawendi group has used NMR and UV-Vis to probe the kinetics of QD growth, providing input to the modeling and for microfluidic reactors. Significant progress was avchieved in the quality of large InAs core shell QD, increasing the range of sizes and wavelengths accessible with high photoluminescence quantum yield, and narrowing their fluorescence linewidth. The Bawendi lab has also helped developed a kinetic growth model for the formation of InAs-based QD.

Connection to current proposal. The work on Continuous Manufacturing focuses on the synthesis of III-V quantum dots. In contrast, the work proposed here will focus on the self-assembly of DNA based materials, including the inclusion of pre-formed nanomaterials, which is distinct from the previous work. The synthetic intuition gained from the previous grant will undoubtedly be of use in the present work.

Broader Impacts. The modeling and synthetic routes to high quality QD based on III-V materials are important in the context of LED based lighting and displays, where the QD serve to downshift the blue light form GaN based LED's to from either white light and an RGB display. The work has led to the filing of patent applications. In addition, the outcomes of the project are being incorporated into a 3-week undergraduate laboratory module that centers around QD and their applications (course 5.38, module 10). This module was created by the Bawendi group about 10 years and has been taken since then by undergraduates at MIT as part of the Chemistry laboratory requirement. The work has also generated numerous papers that have been published in journals such as Nano Letters and Nature Communication.

Mark Bathe (co-PI)

Current support. Bathe is lead PI on Designing Materials to Revolutionize and Engineer our Future (DMREF) grant CMMI-1334109 "Computational Design Principles for Functional DNA-Based Materials" ($1,706,468; 01/15/2014-12/31/2017). This grant provides funding for the development of a computational tool to determine optimal design parameters for the synthesis of DNA-based materials.

Intellectual Merit. This grant supports research to develop an experimentally validated computational framework for the prediction of 3D DNA assemblies, and to develop strategies to synthesize DNA architectures based on fundamental physical principles. The Bathe lab extended CanDo to include sequence-based design of programmed DNA assemblies with complex curvature designed off-lattice [43,44]. This was applied to the coarse-grained finite element procedure to predict the 3D structure for arbitrary DNA assemblies[45,46]. The Bathe lab predicted the mechanical properties of DNA origami objects[47], and assembly of hexagon tiles[48]. They developed the top-down computational design procedure DAEDALUS for scaffolded DNA origami nanoparticles[14] and incorporated a complete set of secondary motifs[49].

Connection to current proposal. The completed work is focused on the physics-based modeling and 3D structure prediction of structured DNA assemblies, whereas the proposed work pursues thermodynamic modeling to predict optimal sequence design for high-yield and defect-free folding, first, and self-assembly of DNA origami objects into large-scale 3D extended crystalline materials, second, both of which are distinct from previously funded work.

Broader Impacts. Computational and experimental results have been broadly disseminated worldwide through the publications and online software tools. These tools are used in teaching undergraduate and graduate courses at MIT, as well as by the nanotechnology community in industry (e.g., Eurofins Genomics, Inc.). Because these computational tools dramatically reduce the cost and time related to DNA nanostructure synthesis, they have major impact on a variety of fields. Computational design and analysis tools are disseminated worldwide online using the free web portals CanDo (http://cando-dna-origami.org) and DAEDALUS (http://daedalus-dna-origami.org). Source code from CanDo is available from MIT under academic licensing and DAEDALUS is available under Open Source license. DAEDALUS has additionally been converted from proprietary MATLAB code to Python code for broader dissemination using funds from an NSF-EAGER award to the Bathe and Yan labs.

3. Research Aims

Overview. Structured DNA nanoparticles are capable of nanoscale self-assembly that can be leveraged for mesoscale structural design of digital memory (**Fig. 1**). DNA nanoparticles have also proven to be an excellent scaffold for coordinating metals, fluorophores, and semiconductors, enabling the synthesis and assembly of 1D, 2D, and 3D lattices containing information. Research in this area is at the intersection biological materials with digital components, offering substantial potential for transformative methods in data storage and computation. Here, we apply our technique of designer DNA nanoparticles of different sizes and shapes (**Fig. 1A and B**) to encapsulate information-storing chemical adducts such as metals, fluorophores, and semiconductor quantum dots (**Fig. 1C**). These DNA integrated memory blocks (DNAMB) are *bit package modules* for information storage arrays. DNAMB are then precisely coordinated into superstructures by addressed assembly using unique barcode complementarity. Algorithmic rules-based assembly from vertices and along edges of shaped DNA nanoparticles allows for 1D arrays and 2D lattices to be assembled from the monomeric units. Metallic nanoparticles (NP) including coordinated gold allows for binary data storage and readout by transmission electron microscopy (TEM), whereas fluorescence and quantum dot (QD) assemblies offer increased data density per memory block together with rapid fluorescence microscopy-based readout. Finally, DNA-PAINT[50] offers the ability to encode and read out unique fluorescent barcodes with nearly unlimited potential for memory block density (**Fig. 2**).

To leverage these preceding opportunities for ultra-dense molecular data storage, we focus our

Hierarchy of Information encoding	Parameters	Readout	Density [bit/block]
Gold nanoparticles	NP absence/presence	TEM, X-ray	1
Quantum dots	Color (R, Y, G, B)	SIM	2
Quantum dot packets	Color & intensity (N_R, N_G, N_B, N_Y)	SIM	2–6
Intercalated dyes & Fluorescent metallic clusters	Color, polarization, & intensity	Polarized microscopy	3–7
DNA-PAINT	DNA barcode addressability (1,000+ orthogonal primers)	Super resolution	Encoding dependent

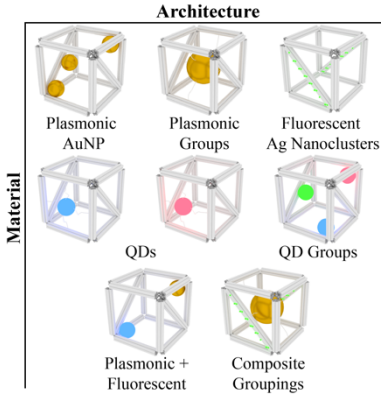

Fig. 2. (Left) An information encoding using different types of nano-objects. (Right) An example of various bit packages using cubic DNA frames and memory blocks of different kinds, as labeled.

investigation on approaches for encoding arbitrary information in static and dynamic arrays built from DNAMB. In Aim 1, we use our top-down structural design to study methods of information encoding by chemical adduct formation, followed by assembly of the DNAMBs in 1D arrays to encode digital information read out by TEM and super-resolution microscopy. In Aim 2 we broaden our encoding strategies to include 2D and 3D lattices from DNAMBs, investigating strategies for the self-assembly of information-storing lattices and their 3D characterization. In Aim 3 we use strategies developed in Aims 1 and 2 on mesoscale assembly combined with molecular computation to enable the formation of distinct memory states in 2D and 3D lattices, memory reprogramming, and erasing.

Aim 1. Programmed molecular memory in static 1D nanoparticle arrays

Overview & Rationale. Rational design at the intersection of biological materials and memory encoding requires a fundamental understanding of monomeric DNA nanoparticles in both their high-throughput addressing, as well as their complexing with memory compounds, and self-assembly into high-density, defect-free arrays. In Sub-aim 1.1, we analyze individual monomer DNAMBs as the foundation of the

DNAMB arrays, assembling a set of DNA particle complexed with either gold nanoparticles (Au NP), or from 1 to 48 fluorophores or QDs. This framework realizes assemblies of data-blocks that can encode from 1 to 14 bits of arbitrary, persistent or long-term memory. In Sub-aim 1.2, we will explore array DNAMB self-assembly using complementary barcode sequences that will bring isolated monomers together into programmed arrays. In Sub-aim 1.3 we will combine the methods from the prior Sub-Aims to assemble 1D arrays that will encode arbitrary digital information as a bit sequence of memory blocks. We plan to achieve in the project an encoding of a song in a cubic micron of assembled DNAMB array.

Sub-aim 1.1. Rational design of monomeric DNA nanoparticles

Overview. Fundamental DNA Memory Blocks (DNAMB) with ability to coordinate information in 2D and 3D space should have addressable interactions and defined shapes. Such parameters are required for building information storage arrays. For dense data storage, DNA nanoparticles must be assembled in high throughput, in high yield, with precise functionalization in defined locations along the edges of the nanoparticle. DNA nanoparticles composed of six-helix-bundle (6HB) edge types allow for several functionalization points along the edges. This enables us to perform unique DNA nanoparticle barcoding as well as internal functionalization with metallic and semiconductor particles for optical data encoding and read-out.

Preliminary Results. The Bathe lab has generated an algorithm for generalized assembly of 6HB edge type wireframe DNA nanoparticles, or so-called *DNA frames*. This algorithm allows for the top down design of nearly any shape, limited only by the DNA scaffold length. The monomeric DNA frames are composed of 6 antiparallel DNA helices per edge organized on a honeycomb lattice for structural rigidity compared with single or double duplex edges, designed with edge lengths that are multiples of 21 base pairs, consistent with the pitch of B-form DNA. Thus, DNA frames can have edge lengths of 42 (14 nm), 63 (21 nm), 84 (28 nm), or more (**Fig. 1A**). Additionally, because this algorithmic sequence design allows for general shapes, nanoparticles can be, for example, tetrahedra, octahedral, pentagonal bipyramids, or any irregular geometries that offer diverse geometric coordinations in 2D and 3D (**Fig. 1B**). This scaffold routing procedure has been validated by TEM and cryo-TEM, showing monodispersed particles with predicted shape from atomic modeling. The Gang lab has shown 6HB and 10HB edge type DNA frames[19,51] and used them for coordination of nanoparticles in chiral and non-chiral arrangements[52] and 3D arrays[19,51]. Finally, the Glotzer lab has developed powerful simulation methodologies to explore self-assembly pathways of structured nanoparticle assemblies[42,53,54]. The Bathe lab and others have shown templating various dyes along the DNA[15], which may be broadened to include unique optical encoding with organic and inorganic dyes and quantum dots developed by the Bawendi lab[55-58]. Taken together, these strategies can be leveraged to encode and organize digital information at the molecular scale.

Proposed Research. We will develop strategies to assemble DNAMB that represent discrete bit-packets of data (**Fig. 3**). DNA nanoparticle monomers will be assembled to have internal single-stranded DNA overhangs for the coordination of memory blocks, and external overhangs for the presentation of unique sequence barcodes. Internal-facing overhangs will guide coordination of memory units (e.g., gold, quantum dots, fluorophores for DNA-PAINT). In addition to organic dyes, we will use fluorescent Ag clusters[59,60], combining a small size (1 nm) and a superior emission (provied by J. Martinez, LANL). Information within DNA frame can be stored in a variety of ways, including simple binary encoding determined by the presence or absence of a heavy metal NP. The presence of fluorescent moieties such as intercalating dyes or QDs allows for additional memory parameters such as optical wavelength encoding (e.g., blue encoding binary 01, red encoding binary 10, green encoding binary 11, etc.). Further, optical memory has the advantage of intensity-encoding, whereby the number and type of fluorophore per individual DNA nanoparticle can be varied to offer encoding of information in both wavelength and intensity. For example, 16 fluorophores per color per nanoparticle would allow up to 2 bytes of information per nanoparticle, compared with 1 bit for a metallic nanoparticle.

In contrast to encoding information in the number of QDs attached to the DNA nanoparticle, the information can alternatively be encoded in the polarization states of the particles[61]. Given the highly programmable nature of DNA nanoparticle frames, fluorescent dyes or Ag clusters organized in specific orientations within DNA frames will create net emission dipoles. The polarization of the emitted fluorescence will then report on the specific orientation of the particle, detected using polarized light microscopy. For example, a DNA cube in 2D exhibits two polarization states depending on its orientation (horizontal or vertical) in the assembly, in addition to the "0" state. DNA nanoparticles with reduced symmetry can encode more polarization states (**Fig. 2**).

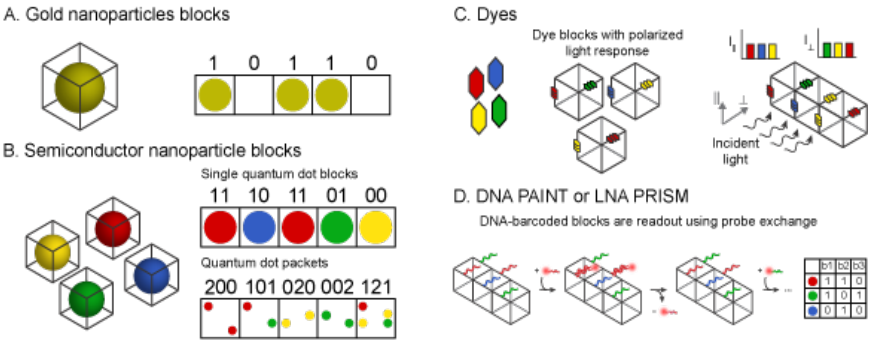

Fig. 3. Encoding strategies using DNA frames (e.g. cube) integrated with Memory Blocks (DNAMB). DNAMB can encode simple binary (A), or, for more information density, QD can be used individually or in clusters (B). Higher information density can be achieved through wavelength, intensity, and polarization using dyes (C). DNA frame arrays can act as a blank canvas for more information packaged and read out by DNA-PAINT (D).

Finally, information can be encoded by adding overhangs (docking strands) with orthogonal barcode sequences on the DNA nanoparticles that can be detected using complementary fluorescently labeled nucleic acid probes. This approach is called DNA-PAINT when low affinity probes are used to achieve super-resolution imaging[50,62], or LNA-PRISM for its diffraction-limited counterpart[11]. The Bathe lab has recently applied LNA-PRISM and DNA-PAINT to achieve 13-channel confocal and 9-channel super-resolution imaging of neuronal synapses[11]. Through orthogonal probe design, each imaging probe can be easily designed to specifically bind only its own docking strand on the DNA nanoparticles. Because the binding of imaging probes to docking strands is reversible, sequential probe exchange can easily be performed by introducing distinct imaging probes, labeled with either identical or distinct fluorophores. Importantly, the probe exchange enables much higher information encoding density due to the large number of orthogonal sequences in contrast to color encoding. For example, 40 bits of information can be encoded into a single DNA nanoparticle when 40 sequences are used. The readout would be performed using 10 rounds of probe exchange, with 4 probes with different colors in each round. We will use structured illumination microscopy (SIM) together with LNA-PRISM to readout of arrays with unit block size ≥100 nm for faster readout. For arrays with unit block size <100 nm, we will employ DNA-PAINT to slow readout the array with higher resolution (~20 nm).

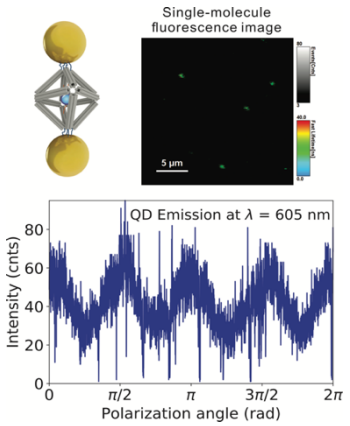

Fig. 4. Polarized emission of QD (blue, top left) inserted in DNA frame and coordinated with Au NP (yellow). Emission intensity (bottom) of imaged (top right) individual clusters (preliminary data).

Characterization of these individual memory blocks will be accomplished by several techniques to ensure precisely defined control necessary for information encoding densities. Initially, the building blocks will be generated to contain Au NP at precise locations internal to the DNA frame and/or at vertices. The presence and absence of the Au NP will be detected by TEM. QD of precise dimensions and color synthesized by the Bawendi lab[55,56] will be embedded on the edges and

vertices of DNA nanostructures as demonstrated similar to the strategies developed by the Gang lab to incorporated metallic nanoparticles[51,63] and others[64]. Moreover, coordinating QD with plasmonic particles using DNA frame allow to modulate an emission polarization, as recently shown by the Gang lab (**Fig. 4**). Dyes can be covalently attached through the DNA backbone using phosphoramidite chemistry, which enables rigid control on the orientation of the dyes relative to the DNA frame[65]. Optical memory encoding will be read out by TEM and super-resolution microscopy on the monomers.

Sub-aim 1.2. Arbitrary lattice assembly of monomeric DNA nanoparticles into 1D arrays

Overview. Programmed assembly of 1D nanoparticle arrays will enable novel biomaterial technologies on the nanometer-to-micrometer-scale. Development of techniques associated with this mesoscale engineering will have impact on light harvesting, energy transport, catalysis, and semiconductor printing. Toward this end, here we investigate the rational design and self-assembly of programmed 1D arrays. Self-assembly from monomeric subunits generated in Sub-aim 1.1 will be explored, with overhangs for complementary association through bridging DNA strands, or by attachment through intermediary adaptor molecules such as gold nanoparticles.

Preliminary Results. Previously, the Bathe lab has assembled 1D tetramer arrays based on tetrahedron monomers, showing extended lattices can be assembled from uniquely addressed components. The assembly was carried out through 2 complementary overhangs per edge that allowed coordination both by attachment, and in the directionality of the attachment. The individual monomers were initially folded separately, and then brought together and annealed below the melting temperature of the monomers, thus allowing for high-yield assembly of tetramer superstructures. Similarly, the Gang lab demonstrated assembly of lD nanoparticle arrays from DNA frames of different geometries, with NP positioning both inside and outside the frame[52,63].

Proposed Research. Each DNA nanoparticle will have an addressable affinity tag, barcoded by DNA sequences derived from a subset of 240,000 orthogonal 16-mer single-stranded DNAs[66], which allows for deterministic self-assembly of (240,000-N)! 1D arrays of length N from a mixture in solution. Short poly-T spacers are used to ensure robust hybridization in solution, with the barcode sequences also orthogonal to the DNA nanoparticle scaffold. New computational sequence design strategies will be developed to convert arbitrary encoded information into barcodes that will be used for deterministic self-assembly into ordered 1D memory arrays that can be optically detected and decoded. High-throughput synthesis of DNAMB will be enabled using automated pipetting and microfluidics, where pre-folded nanoparticles will be complexed with optical memory elements and DNA barcodes prior to 1D array assemly (**Fig. 5**).

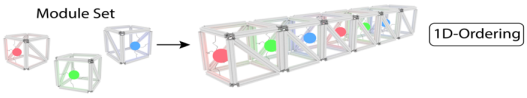

Fig. 5. Assembly of 1D array from DNAMB based on cubic DNA frames and QDs with different emission wavelengths.

Computational sequence design by the Bathe lab will be combined with computational simulation of hierarchical self-assembly by the Glotzer lab to explore optimal strategies for DNA particle size, geometry, and barcode overhang positioning for robust self-assembly. Because distinct object geometries themselves can be used to encode information via fluorescence brightness and polarized emission of the DNA nanoparticle, proof-of-principle for memory encoding in linear strings will proceed by first encoding bitstream data using structural TEM analysis from programmed nanoparticle arrays, followed by their fluorescence read-out in Sub-aim 1.3. Error-rates in 1D arrays will be evaluated for the optimal design of DNA designs to control assembly pathways. This hybrid computational-experimental approach will offer full control over monomer geometry, and control over 1D array assembly.

Sub-aim 1.3. Programmed synthesis and optical read-out of fluorescent 1D arrays

Overview. Building on the self-assembly of DNAMB into 1D arrays using monomers designed in earlier sub-aims, we will apply memory-encoding strategies to the assembly of bitstream data. Assembly strategies developed in Sub-aim 1.2 will be applied to coordinate 1D array generation of memory. Readout of memory arrays will be accomplished by combination of nanoparticle visualization and fluorescence microscopy by the Bathe and Bawendi labs[11,57,67]. Patterned arrays will be decoded to retrieve encoded memory, and arrays will be encapsulated in silica for archival data storage and retrieval.

Preliminary Results. Previously, the Bathe lab has used LNA-PRISM and DNA-PAINT to read-out protein-protein interactions with nanometer-scale precision[11]. Highly specific, orthogonal single-stranded DNA and LNA probes have been designed with ability to detect the corresponding docking strand sequence using either stochastic blinking in DNA-PAINT or diffraction-limited confocal imaging with LNA-PRISM[11]. These docking sequences or barcodes can easily be incorporated into DNA nanoparticle designs as overhangs to encode information. The Bathe lab has also applied silica-encapsulation of structured DNA assemblies[68] (Sub-aim 2.3), offering long-term protection of DNA arrays without deterioration due to oxidation during fluorescence readout.

Proposed Research. We will increase the bit-depth encoded in the 1D linear array from Sub-aim 1.2 by using QD, wherein each color (red, blue, green, and yellow) represents a value placeholder for encoding. QD packets containing variable ratios of the different colors of QDs further increases the bit depth per building blocks (**Fig. 2B**). We will apply methods developed by the Bawendy lab for tailoring QD emission[55,56] and characterizing them within DNA frames and arrays[57,58]. QD packets containing variable ratios of the different colors of quantum dots further increases the bit depth per building block. The information encoded in arrays containing QD will be read out using fluorescence imaging, validated by TEM. Fluorescent single-stranded DNA and LNA barcodes will additionally be used to read-out 1D arrays either with 10-nanometer resolution using PAINT that requires time-lapse, solution-based imaging for super-resolution reconstruction, or 100-nanometer resolution imaging LNA-PRISM and SIM, which can be applied without time-lapse imaging to silica-encapsulated 1D arrays. The encapsulation process secures the bitstream data at high temperatures and harsh environmental conditions while still offering optical read-out. Assembly of linear and branched arrays of up to 100 nanoparticles will allow for micrometer-scale designs encoding for 200-byte reads.

Aim 2. Dense, programmable molecular memory in 2D and 3D bit module lattice assemblies

Overview & Rationale. Nanoparticle self-assembly depends on a balance of interaction forces, entropic effects, and system kinetics[37,53,69-73]. We can leverage these properties to direct self-assembly of shaped DNA nanoparticle into 2D and 3D arrays by controlling the position and valency overhangs that provide connectivity between DNA nanoparticles. Wireframe structure of DNA particle is highly suitable for encapsulation of memory blocks (e.g. Au NP, QDs, fluorescent dyes) and creation of DNAMB, a pixel in 2D or 3D arrays. To achieve information storage capabilities, it is required to investigate how the connectivity properties of DNAMB can be translated into their designed arrangement in the information-storing arrays. To self-assemble these systems into 2D and 3D ultra-dense data blocks, we will investigate the minimum interaction specificity needed to direct self-assembly into high fidelity ordered 2D and 3D arrays. We will also explore information retrieval from these arrays in 2D and 3D within pixels consisting of 1x1, 2x2, 4x4, etc., nanoparticle block arrays. In addition, we will establish methods for generating robust memory arrays that can preserve information under extreme conditions.

Sub-Aim 2.1. Defined 2D and 3D lattice assembly with nanometer-scale precision

Overview. Foundational results of Aim 1 are extended to program 2D and 3D arrays of DNAMB. In this first Sub-aim, we focus on understanding the requirements for assembling ordered arrays from shaped DNA nanoparticles of different geometries and with inter-particle coordination numbers (valency). We will reveal

principles that relate the geometry of DNA particle and the array structure. The over-arching objective is to discover efficient structural motifs for high-density, robust inter-molecular packing with low assembly error, and optimal optical readout. In this Sub-aim, we focus on our ability to assemble ordered 2D and 3D from shaped DNA nanoparticles, while in Sub-aim 2.2 we will extend these approaches for assembly of algorithmic arrays with designed position of information carrying DMAMB.

Preliminary Results. The Gang group has demonstrated the ability to assemble DNA frames containing functional nanoparticles (QD, Au NP) and subsequently read out the positions and structure of the stored functional particles ("bits"). Polyhedral 3D DNA origami frames (e.g., cubes, octahedra, tetrahedra) can be assembled into ordered 1D to 3D arrays with varying lattice symmetries[19,51]. These frames are formed from single-stranded M13 phages folded using DNA origami techniques, and are typically on the order of 15-60nm. Specific structures can be engineered by varying DNA-binding sites on the frames, as discussed in Aim 1. Through careful annealing, highly-ordered 3D architectures containing 10^5-10^6 elements can be formed. We confirmed that the target structure had been formed using SAXS (**Fig. 6**). The Gang group demonstrated the development of SAXS and TEM methods for the detailed characterization of nanoparticle arrays[19,51,74,75].

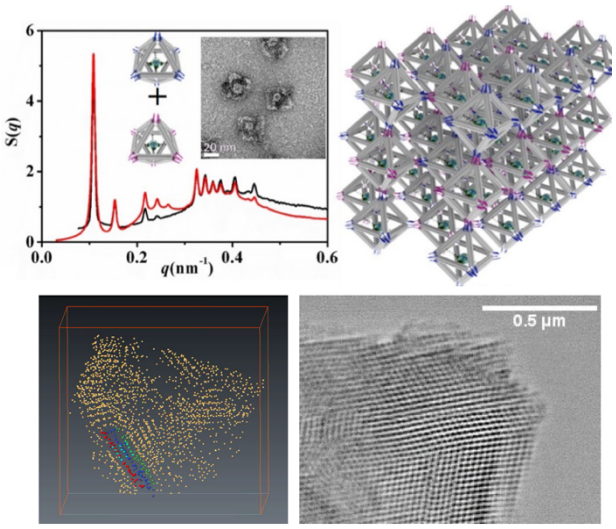

Fig. 6. Assembly of 3D lattices from DNA frames encapsulating QDs, preliminary data. (Top) In-situ structural probing (SAXS) from assembled 3D lattice (right) of QD in DNA octahedra frames (shown by TEM). (Bottom left) 3D cryo-TEM tomographic reconstruction of NP lattice with 2 nm resolution (Right): X-ray tomography of 3D array, one projection is shown, measured at NSLS-II synchrotron with <15 nm resolution.

Functional particles can be placed into the self-assembled lattice either at the stage of individual assembly of DNA frame with nanoparticle, following by a lattice formation, or after the lattice of empty DNA frames has been formed, as shown by preliminary results from the Gang lab. That flexibility potentially allows for enabling write-erase optical arrays, as discussed in Aim 3. We visualized real-space 3D information of the functional particles using Cryo-TEM tomography[76-78] and silicated sample using X-ray tomography[79-81] (**Fig. 6**). The combination of sample-integrated SAXS and local-pixel tomography provide a full quantitative information about array. Using these methods, we can resolve 10 nm nanoparticles, demonstrating feasibility of reading out the information from 3D arrays.

Proposed research. We seek to enable the self-assembly of higher-ordered architectures in 2D and 3D by engineering DNA frame properties and to examine how to control an assembly arrays for DNAMB of different shapes **(Fig. 1)**. We will seek to assemble DNA frames into ordered arrays to explore the leading parameters controlling the arrays formation for DNAMB, synthesized in Aim 1. Toward this end, the Glotzer group will perform predictive modeling to target specific structural assemblies, the Bathe will design DNA polyhedral frames, and the Gang group will conduct assembly and characterization of 2D and 3D structures. Together, these experimental and computational efforts will be used to *inversely* and *rationally* assemble target memory arrays that seek to maximize the density of data with minimum error; the outcome of these studies will be used for assembly information-encoded arrays in Sub-aim 2.2.

Computationally, Glotzer and colleagues will develop a bit module interaction model to study the role of the DNA linkages on DNA cage self-assembly. Specifically, previous work on modeling solid particles with DNA-facilitated attraction[37] will be extended to model the DNA cages that will be experimentally

made by Gang, and it will consider realistic features of nanoparticle systems[82,83]. With this model in place, we can then extend the framework of *digital alchemy*, which treats particle properties as a thermodynamic variable, to particle interactions (here, DNA linkages)[54]. In this way, we can *inversely design* ideal DNA cages (e.g. shape, patchy interactions) that will robustly assemble a target structure. We will seek to balance site specificity without being overly unique—that is, design the *highest information* interactions that will allow for the minimum amount of linkage specificity for directing self-assembly[84,85]. This computational framework for the *inverse design* of bit packages that will assemble a given structure will enable high-throughput screening of particles of interest and serve as the basis for complex hierarchical structure and array assembly in the remainder of Aims 2 and 3.

Experimentally, DNA frames can be varied in shape, size, and DNA linker directionality and specificity. Interparticle binding motifs will be explored to seek optimized motifs for static and dynamic organization, controlling both the rate of self-assembly as well as the ultimate structural fidelity of the assembly by minimizing defects locally. By differentiating inter-DNAMB connectivity using varying DNA frame linking designs, we will investigate distinct assembly pathways. By designing the directional binding of DNA frames, we will vary the anisotropic preferential attachment of new components, leading to control over the ultimate array assembly. The structure formation will be probed by optical methods for 2D arrays by SAXS and TEM and x-ray tomography for 3D arrays.

Sub-Aim 2.2. Hierarchical assembly logic for higher-dimensional information storage

Overview. In Sub-aim 2.1, we explored approaches to assembling DNA frames into target 2D and 3D assemblies. Next, we precisely order "bit modules"—that is, DNA cages carrying functional particles—into arrays of discrete information. Toward this end, we design modules that carry the minimal information needed to reach target arrangements through a combination of particle anisotropy and DNA linkers. DNA

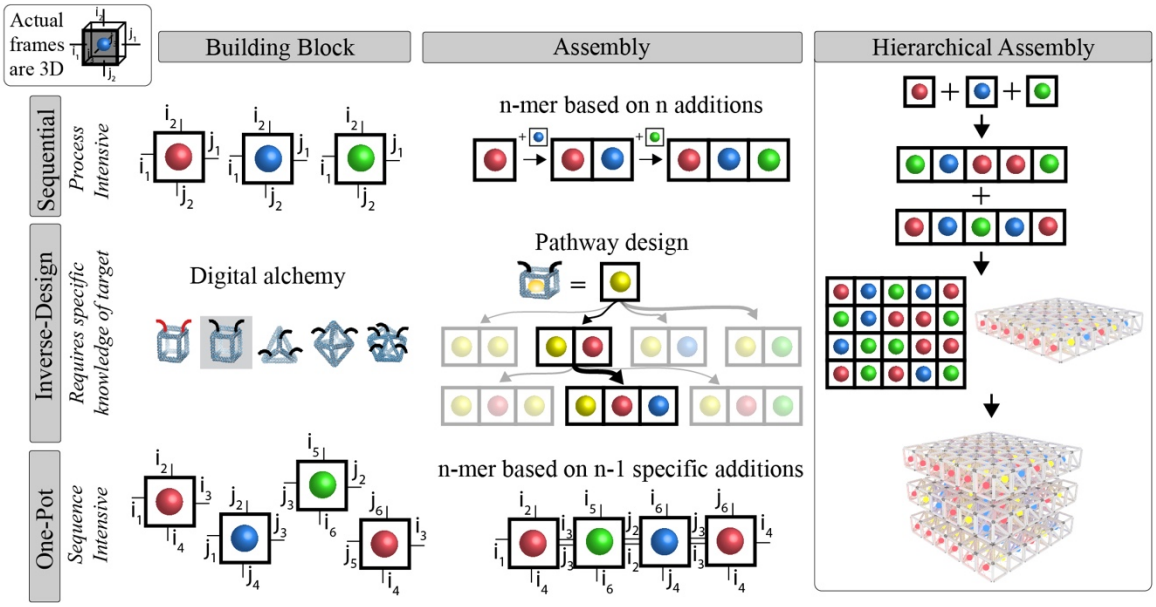

Fig. 7. Strategies for building information encoded 1D, 2D and 3D arrays. Sequential operations are very deterministic and can be carried out by automated robotic equipment, but even so are heavily process intensive and require many individual assembly steps. One-pot systems can be fully computationally defined, though in practice are heavily sequence intensive and be subjected to errors more readily than in molecular-scale systems when accounting for kinetic and thermodynamics of packing larger objects and materials. A hierarchical assembly methodology offers a hybrid approach of both strategies, where a sequential addition of structures preformed in a one-pot setup provide the desired 3D material organization.

computing groups have previously used DNA linkers to self-assemble complex 2D patterns[6] and 3D shapes[9]. Here we extending these approaches to realize hierarchical 3D nanoparticle assembly design so that pixelated images act as dense data storage units. We will explore several complementary approaches to hierarchical assembly engineering, including sequential nanoparticle addition and "one-pot assembly", each of which will be explored together with inverse computational design of self-assembly pathways and particle geometries to achieve a robust assembly of designed arrays. Using the optical characterization strategies from Sub-aim 2.1, we will decode the information encoded in the structure, and probe sources of error and information loss in the self-assembly and read-out processes.

Preliminary results. The Gang lab has hierarchically assembled DNA frames in 1D and 2D using anisotropic, selective binding interactions[63]. By varying the addressable and directional binding properties, it is possible to access a diversity of mesoscale architectures ranging from linear chains to 2D structures and arbitrarily designed mesoscale objects. These mesoscale building blocks can then be tagged with additional DNA linkers for hierarchical nanoparticle assembly.

Proposed research. Assembly of encoded 3D arrays can be approached in two strategies, or a combination thereof (**Fig. 7**). In an entirely "one-pot" assembly of a 3D array, all modules are linked with a large binding sequence set that has been fully computationally defined. Such an approach requires an enormous number of unique binding sequences, and even if fully defined, can run into high error rates when considering the assembly and packing of large (as compared with molecular assembly) and charged modules and/or materials. A second approach using sequential binding based on module groups of similar binding layouts requires less sequence diversity and can be automated using robotic liquid handling. However, this is vastly more process- and time-intensive than one-pot assembly. This approach represents hierarchical assembly, whereby 1D structures ("strings") would be formed from the modules, 2D planes formed from the 1D libraries, and finally 3D encoded arrays from stacking of selected planes. An optimal assembly process that balances fully-encoded organization with direct addition of binding components would offer a hybrid approach of hierarchical assembly with sequential addition of groupings of computationally defined structures. Each of these strategies will be explored in this aim, using a combination of high-throughput, structure-based computational modeling and experiments.

The Glotzer group will extend their digital alchemy framework to probe diverse DNA linkage sequences and conjugation designs to realize specific, targeted inter-particle interactions. In addition, they will explore the roles of these interactions on the kinetics of array assembly to enable *pathway design*[86] into desired arrays while avoiding undesirable "side products". In this way, we will explore computationally the interplay between the two extremes of one-pot and sequential assembly, and identify which combinations provide lowest assembly error while minimizing both assembly time and the number of required unique binding sequences. The Gang group will employ a home-built robotic system for automatic synthesis and assembly of DNAMB; that will allow establishing practical methods for creation of large number of diverse blocks required for the hierarchical assembly. While such approaches have been applied to molecular systems, they have not yet been realized for DNA frames integrating inorganic NP. To implement complementary pathway design strategies, Gang will fabricate DNA frames with thermally differentiated inter-vertex hybridizations to promote highly specific assembly path during thermally-driven self-assembly. For example, DNAMB strings will be assembled at higher temperatures, and planar and 3D arrays assembled at lower and lowest temperatures, respectively. We will use SAXS and tomography methods to reveal the pathway-controlled assembly process. The computational design of frames and pathways will be performed jointly between the Bathe, Glotzer, and Gang labs.

Sub-Aim 2.3. Fabrication of Robust Optically Active Arrays

Overview. Structural DNA nanotechnology provides an impressive control over molecular and nanoscale structure formation. However, the formed structures are not stable over the broad range of conditions and temperatures typically required for device operation; limiting practical applications of methods for

fabrication of information storage materials discussed in Aims 1 and 2. To address this challenge, new methods for the mineralization of DNA nano-architectures with preserved structural organizations are required. Such methods will allow for creating robust optical arrays, in which structures are initially formed from DNA and optical elements, and then encapsulated into an inorganic matrix. Although a number of methods have been published[87-89], a major challenge lies in the difficulty of performing uniform, controllable mineralization that does not disrupt DNA architecture and nanoparticle organization. Moreover, controlling mineralization in 3D is even more challenging because locally precise coating of molecular structures is required. Here, we will develop a biomimetic nanoreactor approach for mineralization of DNA with optically suitable encapsulation, silication. We will study the structural, optical, and thermo-mechanical properties of these materials, including their application to the nanoparticle arrays developed in Aims 1 and 2.

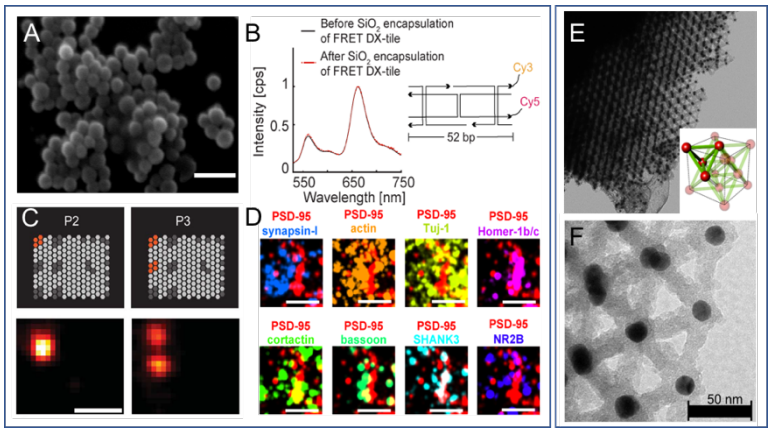

Fig. 8. Silica encapsulation of DNA structures with optical elements. (A) Silica encapsulate DNA assemblies. (B) FRET pair, Cy3 and Cy5, confirm integrity upon encapsulation. (C) Encoding information using DNA-PAINT. Rectangular origami allows 6-bit information per site (top). The corresponding measured images[1] (bottom). (D) Multiplexed DNA-PAINT images of synaptic proteins labeled with 9 different docking sequences[11]. (E) TEM image of silicated 3D lattice array of Au NP assembled with DNA tetrahedra (inset). 3D architecture and order are preserved at temperature >1000 deg.C and pressure > 8GPa (preliminary results from the Gang lab) (F) Uniform coating of DNA by silica. Scale bars: (A) 1000 nm, (B) 50 nm, (C) 500 nm, (E) 50 nm.

Preliminary results. The Bathe lab has developed silica coating that allows encapsulating the entire structure. These particles are formed by a sol-gel process and modified with ammonium functional groups for electrostatic binding of DNA nanostructures. Post-shell growth of another silica layer (**Fig. 8**) protects DNA-based materials from strongly acidic and alkaline environments while maintaining the desired structure as verified by energy transfer measurements (**Fig. 8B**). In contrast, the Gang lab has recently developed a new methodology for the precise coating of DNA bundles by few nm silica a (**Fig. 8E**) using locally confined nanoreactors. This method offers a spatially controlled encapsulation of DNA and a fabrication of full 3D replica of DNA constructs with preserving 3D architecture. Our TEM and SAXS experiment reveal that the structures are stable at the extreme conditions, temperature >1100 deg. C and pressures >8 GPa. The control over the 3D architecture using a lattice engineering approach developed by the Gang group[19,51] allow for the 3D structuring of silica encapsulated DNA constructs.

Proposed research. Inspired by biomineralization, we will apply nanoreactor strategies for coating DNA origami with a uniform mineralized layer. This approach is different from DNA-templating that does not offer spatial control over ionic levels. Recently, the nanoreactor strategy has been successfully used for the fabrication of nearly monodispersed nanospheres or nanorods using the polymer-grafted unimolecular micelles[90] or cellulose nanocrystals[91]. Specifically, DNA origami will first be protected with amphiphilic molecules, such as short lipids, to create DNA-based nanoreactors. The subsequent mineralization will be performed in selected solvent, where the desired ions or precursors will be loaded and concentrated in the nanoreactors. The nucleation will be triggered with specific chemicals, such as reducing agent, and the growth will be controlled with pH and temperature. *Ex-situ* characterization methods will be used to examine the fabricated nano-architectures. However, the nucleation and growth pathways in the

nanoreactor-based synthesis are currently not clear. The confined space with organic skeletons can affect the energy barriers for nucleation and growth of inorganic materials. Here, we will exploit *in-situ* characterization methods, including of liquid-cell TEM, X-ray scattering, and X-ray absorption fine structure, EXAFS. Liquid-cell TEM provides real-space monitoring (including structural and composition analysis) of nucleation and growth with both temporal and spatial resolution.[92] EXAFS probes the fine cluster structures at atomic levels[93], which is strongly valuable for understanding of nucleation processes.

Aim 3. Dynamic Information Storage and Processing

Overview. Algorithmic design rules can govern the formation of 1D, 2D, and 3D structures through different pathways, as described in earlier Aims, which will allow for forming optical arrays and storing of information. In this approach, the binding remains static once a given module set is synthesized and assembled. In contrast, here we investigate the possibility of inducing changes in material organization based either on an input signal or environmental cue, resulting in an altered 2D or 3D nanoparticle organization for optical read-out. Expanding this approach for dynamic control of arrays is beneficial for our ability to (i) *re-write and erase* information and (ii) *converting input chemical and biochemical molecular information into optical read-out information*.

To achieve such a dynamic system, we design bit modules with switchable binding sites, which offers

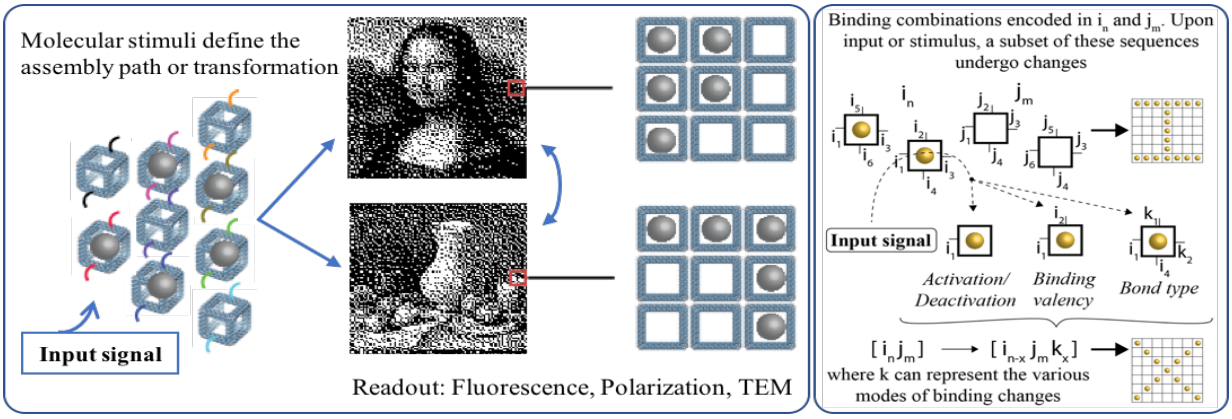

Fig. 9. (Left) Writing dynamic patterns with switchable modules: bonds of modules are activated by a specific input signal (DNA strands) and the resulting output pattern depends on the type and number of activated bonds. (Right**)** Different bond changes can be induced to subsets of binding sequences. A structure defined by binding sequences *i* interacting with *j* under one set of binding rules is induced into transformed state, where *i* and *j* sequences, also must interact with *k* bond switches, where *k* can represent either a new sequence or addition/removal of existing *i* and *j* sequences.

activation of desired bonds of the module or its reprogramming for controlling multiple binding sites of a module (**Fig. 9**). Thus, the formation or transformation of a desired structure will be controlled by programed activation of defined sets of bonds. An alternative approach exploits asymmetric placement of nanoparticles wit hin DNA frames, where the ordering of frames is not altered, but reorientation of frames induces different interactions between the optical memory blocks they contain. These changes in array structure will reflect changes in spectral or polarization read-outs.

Preliminary Results. The Gang lab has synthesized grid-style arrangements of DNA frames, where each frame is individually addressable via its unique DNA sequence that is complementary to a particular particle. Thus, this DNA grid is capable of distributing a given set of nanoparticles into predesignated patterns (**Fig. 10**). Such individual addressability accorded binding allows for changes in particle location based on changes either to the sequences defining frame-frame interactions or frame-particle interactions.

The use of DNA replacement reaction allows for erasing and re-writing the patterns. Gang has previously also demonstrated the reconfigurations of 3D DNA-NP lattices when triggered by specific DNA inputs[94].

Proposed Research. The ability to induce changes in 2D and 3D structural information that are measurable using optical read-out will be explored in three distinct formats, following the concept presented in overview (**Fig. 9**). These formats will provide useful insight into the ability to store and rewrite dynamic information, namely whether reordering the DNA frames within a structural matrix, or the functional materials themselves within the existing structure, represents a more promising approach. Computational simulation of self-assembly and optical read-out by the Glotzer and Bathe groups will offer insight into the optimal encoding and dynamic assembly strategies to pursue experimentally in the Gang and Bawendi groups.

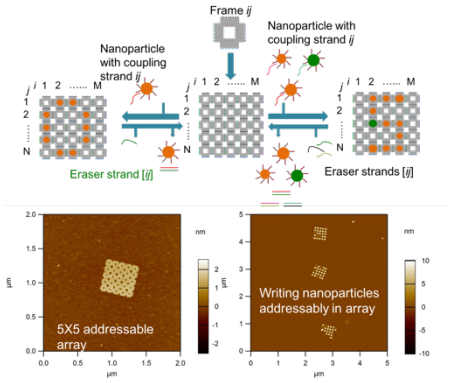

Fig. 10. Addressable DNA arrays from encoded frames *ij* for writing information by optically active nanoparticles. NPs occupy positions on array that matches their encoding. The writing and erasing can be correspondingly controlled by the hybridization and replacement DNA reactions (preliminary data).

In the first approach, frames possessing gold nanoparticles will be designed towards algorithmic assembly as discussed, with additional sequence design applied to a subset of frame overhangs so that they display a different binding sequence upon a stimulus – the first test of which will be a single-stranded DNA input. Such a change affects frame-frame binding. This binding sequence change can be tracked by observing the final structure in AFM – with and without the addition of the input stimulus at the initial stage of frame mixing. Dynamic structural change can be tested by first assembling the frames, and then after the initial structure is formed, adding the input and observing final structure. The second approach entails a material setup similar to the first, but where the changes to binding sequence take place in the internal compartment of the DNA frames. Changes can be tracked as before, with input addition taking place concurrently with frame addition and after frame addition. Both of these approaches will be tested first using the 2D organization present in Figure 10, with these results following work in 3D space.

In the third approach, a frame maintains its position in the overall structure but undergoes a reflection or rotational transformation within that same position in the array. If memory blocks are asymmetrically bound in the frame, their optical properties will be affected. We aim to examine two types of materials, QDs from the Bawendi lab and Au NPs, and two associated optical outputs, fluorescence and polarity (**Fig. 11**). Frames with QDs undergo a reflection transformation and induce a change the fluorescence output which can be tracked. Frames possessing grouping of QD and plasmonic nanoparticles can undergo, upon signal addition, a rotation transformation that will result in the change of the polarization of QD emitted light (see Aim 1.2). Building upon the computational hierarchical assembly models developed in Aim 2, the Glotzer group will seek to develop pathways that allow for transitions between two metastable states. Such transitions opens several intriguing possibilities for computation; design a response behavior[95,96,97] Computational studies using digital alchemy[42] will both

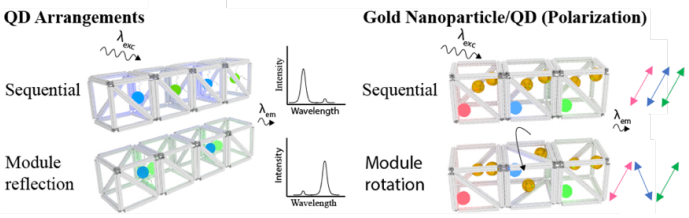

Fig. 11. Exploring different switchable optical modalities for spectral readout: (left) Rotation of DNA frame with QD affects emission spectrum, or (right) polarization of emission.

inform and extend experimental work by the groups of Bathe and Gang on embedding dynamic responses into the bit package system.

4. Management Plan

This research project will be managed and overseen by PI Gang. Gang will organize quarterly super-group meetings by videoconference for all PIs and personnel involved in the project. Graduate students and postdocs working on the project will additionally meet bi-weekly by videoconference to present and share their work related to the project. 2-4 week student and postdoc exchanges between Columbia, MIT, and the University of Michigan will be held to facilitate training across inter-disciplinary lines in biological engineering (MIT/Bathe), computational science (Michigan/Glotzer and MIT/Bathe), and semi-conductor research and engineering (Columbia/Gang and MIT/Bawendi). Online communication and documentation resources including Slack, Trello, and GoogleDocs will additionally be used routinely to coordinate overall workflow between the four PIs and their research groups.

5. Broader Impacts

Training graduate students in an interdisciplinary manner across synthetic biology and semi-conductor research is a central training mission of this proposal. The Gang group is located at the Department of Chemical Engineering and Applied Physics that allows for close interaction between students of different backgrounds. The Bathe and Bawendi labs are located nearby in the Departments of Biological Engineering and Chemistry, respectively, and will interact collaboratively through joint advising of graduate students involved in this project. Similarly, graduate students in the Gang and Glotzer groups will interact with the Biological Engineering environment at MIT through regular visits to MIT and thesis committee meetings that Bathe will participate in. The Gang, Bathe, Bawendi, and Glotzer groups will additionally involve high school and undergraduate students in this research project in a highly inclusive manner to build a diverse community of students, teachers, researchers, scientists, and engineers trained at the interface of semi-conductor research and synthetic biology. Students involved in the research will be encouraged to participate in the BIOMOD (http://biomod.net/) competition held at Harvard University annually, where they will be exposed to diverse synthetic biology research projects and international project teams. Close integration of theory, computational modeling, biological experimental design, data acquisition, and experimental data analysis in the present research program promises to provide a rich, interdisciplinary educational research experience for the graduate students involved. In addition to their normal academic duties, the PIs are active in a broad array of outreach and educational activities.

Outreach to Under-represented Minorities, Women and K-12. All PIs have a track record of, and will continue to engage in, outreach to local minority and female high-school students. For example, Gang has hosted undergrad female students Erica Palm (SBU/Tuffts), Charlotte Kirk (MIT), Dana Capitano (SBU). Gang gave lectures at the SigmaCamp (sigmacamp.org) and SchoolNova (schoolnova.org) to middle- high-school students on DNA nanotechnology. Bathe previously hosted Nikita Kodali (the Middlesex School, Concord, MA) who subsequently attended and graduated near the top of her class at MIT. Bathe additionally hosts a number of undergraduate researchers including Jordan Smith, an under-represented minority student, and computational science high school student Chaoqun Tao (summer 2016), who was co-author on a publication. Bawendi and Glotzer engage in similar outreach activities.

Curricular Developments. Related to the results of this proposal, Bathe collaborates with Dr. Gael McGill (Director of Molecular Visualization at Harvard Medical School) to develop molecular animations and simulations of structured DNA assemblies for the undergraduate Biological Engineering course Thermodynamics of Biomolecular Systems at MIT. During the proposed research, the Bathe lab will continue to promote and support use of its lab's design software related to DNA nanotechnology, CanDo and DAEDALUS, for team use in the BIOMOD competition, as well as other courses internationally related to DNA nanotechnology at the high school and undergraduate levels.