Prediction and Control in DNA Nanotechnology

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Abstract

DNA nanotechnology is a rapidly developing field that uses DNA as a building material for nanoscale structures. Key to the field's development has been the ability to accurately describe the behavior of DNA nanostructures using simulations and other modeling techniques. In this review, we present various aspects of prediction and control in DNA nanotechnology, including the various scales of molecular simulation, statistical mechanics, kinetic modeling, continuum mechanics, and other prediction methods. We also address the current uses of artificial intelligence and machine learning in DNA nanotechnology. We discuss how experiments and modeling are synergistically combined to provide control over device behavior, allowing scientists to design molecular structures and dynamic devices with confidence that they will function as intended. Finally, we identify processes and scenarios where DNA nanotechnology lacks sufficient prediction ability and suggest possible solutions to these weak areas.

Keywords: DNA nanotechnology, DNA origami, simulations, molecular dynamics, Artificial Intelligence, machine learning, statistical mechanics, kinetic modeling

Introduction. DNA nanotechnology[1] is a field that uses the canonical base-pairing rules of DNA to rationally program its self-assembly into nanoscale structures. Over the past 40 years of the field's development, DNA nanotechnology has enabled scientists to make advancements in diagnostics,[2] therapeutics,[3] metrology,[4] computation,[5] photonics,[6] and other applications.[7] DNA nanotechnology's best-known design paradigm, DNA origami,[8] is emerging as a highly versatile tool for creating elaborate devices for investigating nanoscale and microscale phenomena, which are leading to applications that would not have been foreseen even a decade ago.

Key to many of these developments has been an increasing capability of prediction and control, which fall largely into the domain of modeling. Every technology which finds its way into long-term use has relied upon the ability to predict behavior given a set of inputs and thus control system behavior by optimizing those inputs. DNA nanotechnology is no different, and scientists can now use it purely as a tool for investigations that have little to do with the technology itself. This has been enabled largely by the advent of modeling techniques which can reliably predict the outcomes of correct DNA structure folding, *e.g.*, oxDNA;[9, 10] availability of various biochemical-biophysical tools for characterizing DNA structure and properties;[11] and our understanding of fundamental processes like toehold-mediated strand displacement,[12] change in form of DNA,[13-15] *etc*. As time goes on and DNA nanotechnology begins to be relied upon for commercial applications in potentially high-cost industries such as healthcare,[16, 17] the value of *quantitative* understanding of DNA nanotechnology will surely increase. However, the field currently lacks a comprehensive description of the available modeling paradigms and opportunities to improve our ability to predict experimental outcomes. In this review, we will describe the various modeling techniques used in the field of DNA nanotechnology and how they are used to predict device behavior, which ultimately affords control over DNA nanotechnology. We will also identify

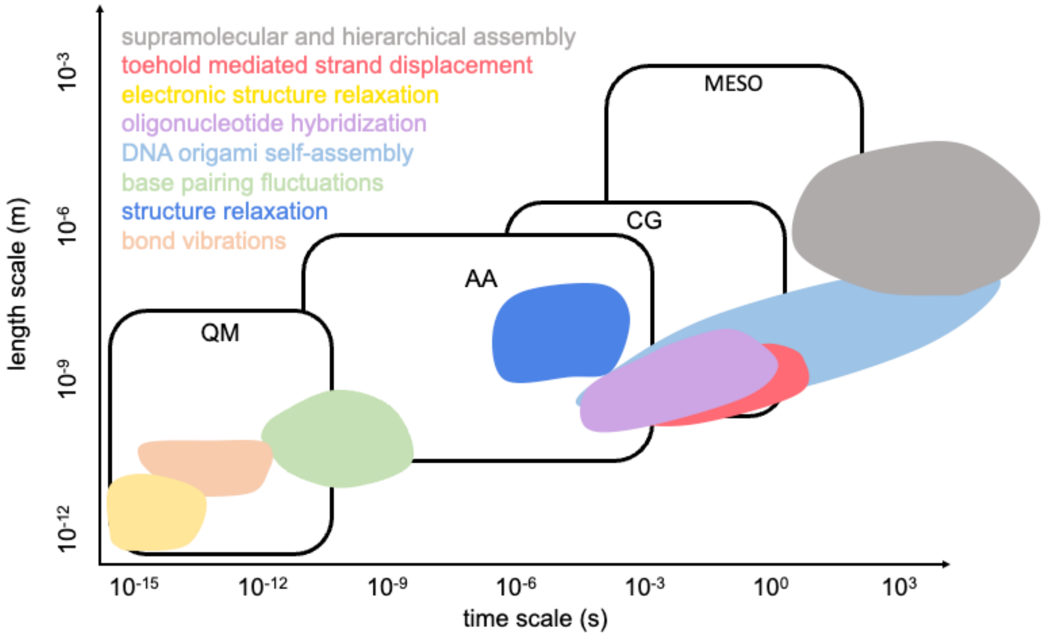

Figure 1: Length and time scales of features and processes in DNA nanotechnology and the length and time scales practically accessible by different modeling techniques, including quantum mechanics calculations ("QM"), all-atom molecular dynamics simulations ("AA"), coarse-grained simulations ("CG"), and mesoscopic modeling ("MESO"). Note that the shape representations for each dynamic process type are not precise but generally capture their range.

underutilized modeling techniques as well as areas where modeling is currently insufficient and therefore hinders the progress of DNA nanotechnology.

The span of length and time scales relevant to DNA nanotechnology is enormous. At the smallest scale, bond vibrations occur on a timescale of femtoseconds. Base-pair fluctuations occur on a timescale of nanoseconds and length scale of Angstroms. Hybridization of tens of nucleotides can take milliseconds for individual hybridization events[18] on partially hybridized strands and seconds to minutes for separated strands to hybridize in bulk solution at physically relevant concentrations.[19] The self-assembly of DNA structures thousands of nucleotides in size takes seconds to thousands of seconds and spans tens to hundreds of nanometers,[20] and hierarchical assembly and larger-scale organization of gigadalton-scale DNA assemblies can take thousands to tens of thousands of seconds and span from hundreds of nanometers to microns.[21-23] To further complicate matters, some components of DNA nanotechnology behave rigidly where they can be modeled as continuum bodies,[24] while others exhibit significant thermally driven stochasticity[25, 26] which is best described by statistical mechanics.

Unsurprisingly, there is not a single modeling technique that can capture the full span of these behaviors, and some compromise must be made between detail / resolution and time / length scale when conducting simulations. In this review, we will categorize modeling techniques by resolution into quantum mechanics, all-atom, coarse-grained, mesoscopic, and continuum resolution models and describe the utility and limitations of each. We will also describe the different simulation techniques available for each of these models, including explicit-solvent molecular dynamics, Brownian dynamics, rigid-body dynamics, finite element analysis, statistical mechanics theory, kinetic modeling, and machine learning to determine experimental or simulation observables; we will also describe existing techniques which span multiple resolution or simulation paradigms. A diagram of where some of these modeling techniques fit into DNA nanotechnology's span of length and time scales can be found in Figure 1. We will describe how simulations and experiments are compared to refine models and improve predictive power. Finally, we will describe grand challenges in the field and propose some solutions to those challenges.

Resolutions of Modeling and Simulation Techniques.

Quantum Mechanics (QM). In QM simulations, the Schrödinger equation is approximately solved for the time-independent many-electron case to obtain the electronic distributions of all species in the system. Density Functional Theory[27] (DFT) is the most common technique used for this purpose. DFT replaces the many body wavefunctions $\Psi(\mathbf{r}_1, \mathbf{r}_2, \ldots, \mathbf{r}_N)$ of a system, where $\mathbf{r}_i$ denotes the Cartesian coordinates of individual bodies, with a single spatial electronic density $n(\mathbf{r})$ which is notably a function of a single set of Cartesian coordinates; the many other properties of the system such as potentials and energy gradients may then be expressed as functionals of

$n(\mathbf{r})$. Interaction forces computed based using this method can also be propagated in time and used to simulate system dynamics in a method known as *ab-initio* molecular dynamics simulations.[28]

The above formalism contains all the nuance of multibody effects, non-spherical interactions, chemical reactivity, and electron transport, so in theory it should accurately reproduce the structural and dynamic behavior of DNA nanodevices. However, the schemes employed to calculate these electronic structures are extremely computationally expensive, so the use of QM in direct simulation of DNA origami devices has been mostly limited to the study of very specific aspects, such as electron transfer in single-and double-stranded DNA,[29-32] the formation of G-quadruplexes,[33] the impact of polarization effects on ionic binding to DNA quadruplexes,[34] and QM studies of basic DNA features like hydrogen bonding,[35] base stacking,[36] and ionic binding in canonically-bound DNA.[37] A less apparent yet more pervasive way that QM simulations have impacted DNA nanotechnology is through their role in parameterizing classical force fields of DNA, ions, and water molecules commonly used in all-atom molecular dynamics (AAMD) simulations.[38] Interestingly, the advent of quantum computing may eventually make it possible to rapidly compute electronic structure and conduct quantum mechanics-based simulations at significantly lower cost, which could open the doors for new modeling paradigms.[39]

All-Atom Molecular Dynamics (AAMD) Simulations. In atomistic models, the electronic degrees of freedom, explicitly treated through QM simulations, are implicitly treated by means of semi-empirical potential energy functions representing the “effective” interatomic interactions between the individual atoms of a molecular system. By propagating the classical Newton’s equations of motion for every atom found in a molecular system of interest, the dynamics of the system can be simulated in physical time. The simplified treatment of interatomic interactions in AAMD simulations allows access to orders of magnitude longer time scales than is possible with QM simulations, although the accuracy of the results obtained is highly dependent on the quality of the imposed interatomic interactions (*i.e.*, the implemented force field). Atomistic models have been utilized to study biological systems for almost 50 years,[40] and the first atomistic simulations of nucleic acid molecules were reported in the early 1980s by Levitt[41] and Tidor *et al.*[42] In these simulations, canonical B-form DNA duplexes of under 20 base pairs were simulated for tens of picoseconds with an implicit treatment of the solvent, leading to highly distorted and unreasonable conformations. Since that time, major advances in algorithms and computing hardware have drastically expanded the accuracy, sizes, and simulation times accessible with atomistic accuracy, offering fundamental insights into the structure and function of both nucleic acids and proteins.

The 1990s saw rapid development in the atomistic modeling of biological systems, as computing power allowed for the inclusion of explicit solvent in AAMD simulations.[43] Different force fields such as AMBER[44] and CHARMM[45, 46] were first developed during this decade, capturing the conformational dynamics of B-form DNA duplexes over longer time scales.[47, 48] However, the time step for numerical integration is still limited to the order of femtoseconds by the extremely fast vibrational frequency of bonds and angles involving hydrogen atoms.[49] Hence, a limitation of AAMD simulations is that trillions of time steps must be integrated to capture many phenomena of biological interest that occur over milliseconds,[50] a number that challenges the capabilities of even the most advanced massively parallel supercomputers (*i.e.*, Anton).[51]

In DNA nanotechnology, AAMD can be used for producing atomically-precise relaxed structures for comparison to experimental characterization techniques like cryo-electron microscopy (cryo-EM);[52] for more detailed mechanistic understanding of the conformations of critical components in larger DNA assemblies; and for quantifying interactions of DNA nanostructures with proteins.[53] Figure 2 presents several notable studies which make use of atomistic modeling and demonstrates a variety of applications of AAMD toward prediction and control in DNA-based systems. The first atomistic simulations of a DNA origami nanostructure were performed by Yoo and Aksimentiev in 2013.[54] By simulating millions of atoms including ions

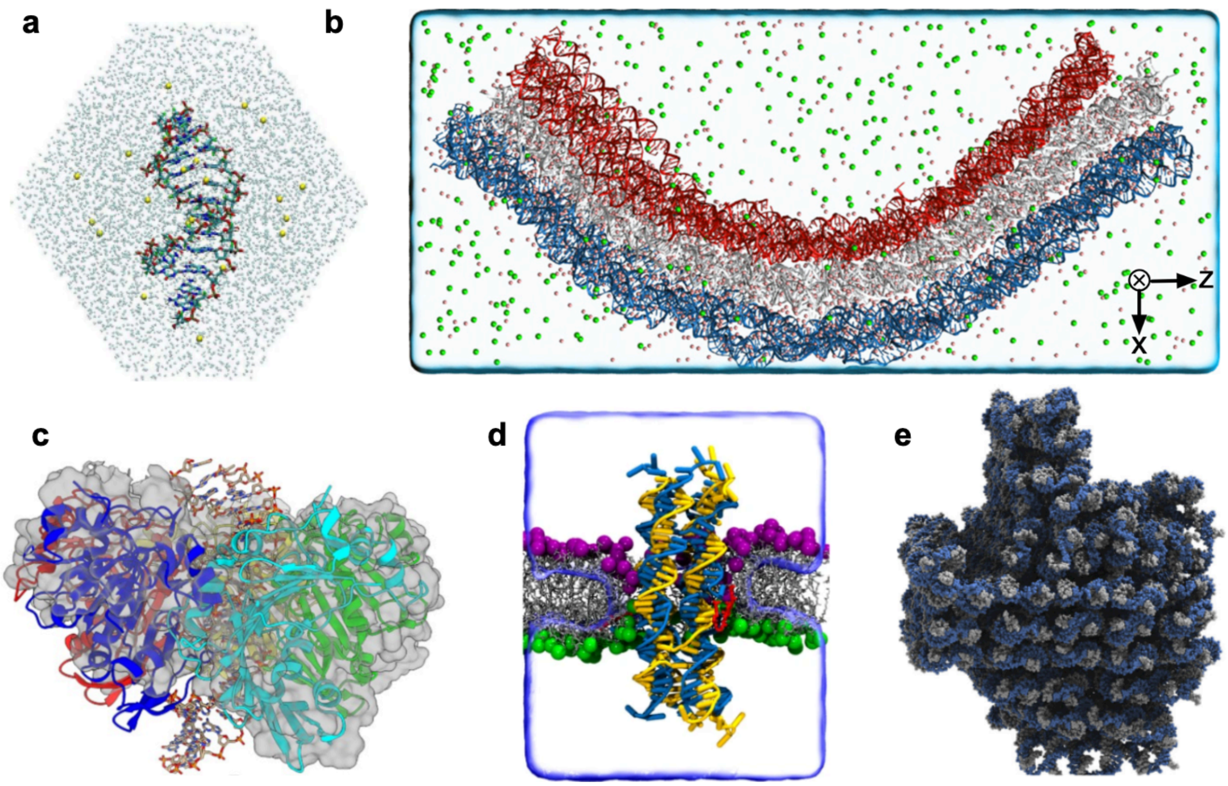

Figure 2: Applications of all-atom molecular dynamics simulations to prediction and control in DNA nanotechnology. The simulations were used for prediction of: (a) base pair and base pair step properties, (b) conformational dynamics of an 18 DNA helix bundle, (c) DNA-protein interactions, (d) cholesterol-mediated stabilization of a DNA nanostructure within a lipid bilayer, and (e) conformation of a DNA nanostructure for comparison to cryo-EM reconstruction. (a) reproduced with permission from ref [55]. Copyright 2009 Oxford University Press. (b) reproduced with permission from ref [54]. Copyright 2013 National Academy of Science. (c) reproduced with permission from ref [56] . Copyright 2021 Oxford University Press. (d) reproduced with permission from ref [57]. Copyright 2018 Springer Nature. (e) reproduced with permission from ref. [58]. Copyright 2012 National Academy of Science.

and water, DNA origami nanostructures approximately 50 nanometers long were simulated for over 100 nanoseconds, revealing significant departure of the simulated nanostructures from their idealized conformations. Explicit treatment of the solvent allows for detailed studies on the effect of the environment in the stability and local conformation of DNA origami nanostructures,[52, 59-63] while also allowing for the characterization of the ionic conductivity of DNA origami constructs such as nanopores.[57, 64-68] Critically, some AAMD force fields can reasonably capture differences in interactions between DNA and monovalent ions like sodium *vs.* divalent ions like magnesium,[69] a feature that is not present in coarser modeling techniques but can be very important to DNA nanostructure function. For example, phenomena such as magnesium-mediated DNA form change may be captured with AAMD simulations[70] but would not be captured with an implicit solvent model which does not account for ionic size, correlation effects, and coordination. Finally, non-canonical DNA motifs which might not be captured using coarse-grained models can often be captured with AAMD.[71] Overall, AAMD is generally not used for simulating entire DNA origami devices. The computational cost of atomistic simulations often restricts their use to studying small DNA origami constructs (such as Seeman J1 sequences[72, 73]), to subsections of DNA devices (where the rest of the DNA origami is fixed[60, 74, 75]), and/or to very short times.[76]

Current force fields have shown great agreement with multiple experimental observations of DNA structure and dynamics, including sequence-dependent conformations,[77-80] deformability,[81, 82] ionic conductivity,[83-85] spectroscopic features,[86-88] and DNA-surface interactions.[89, 90] Force field refinement and creation will remain a process in constant evolution for as long as computer power continues improving and as the ever-growing access to longer time scales exposes the inaccuracies of existing force fields. We can thus expect additional uses of AAMD simulations to arise with the improvement of these models.

Coarse-Grained Simulations. AAMD has two primary limitations when applied to simulations: hydrogen bonds have very fast vibrations, and so simulations conducted with timesteps beyond ~2 femtoseconds will encounter numerical instability and fail; and there may be many atoms in the system, resulting in expensive force calculations and a large amount of computation time for each increment of physical time being simulated. Coarse-grained representations and their corresponding coarse-grained molecular dynamics (CGMD) simulations attempt to circumvent both of these issues by changing the representation of molecules to one that is coarser than an AA representation. Coarse-grained modeling has been covered exhaustively in the past,[91] but we will provide a general overview here.

In this approach, groups of atoms are each considered as a single representative particle. This serves to remove fast vibrational modes from the system, which allows much larger timesteps to be used, and also reduces the quantity of particles in the system, which reduces the computational cost of force calculations at each timestep. In addition, the many degrees of freedom arising from solvent molecules are removed by replacing the explicit solvent from AAMD with a simple implicit solvent representation. There are a few approaches to achieving such coarse graining. Some “top-down” schemes attempt to reproduce experimentally characterized mechanical properties (*e.g.*, bending and torsional persistence length and pitch of DNA) in addition to thermodynamic properties (*e.g.*, melting temperatures) through effective interaction potentials between coarse-grained particles.[10, 92] Other coarse graining techniques may begin with a reference AAMD simulation and attempt to match its equilibrium behaviors, which generally include distance and angle distributions between points in the AA model mapped to the CG particles. These “bottom-up” methods (readers are referred to multiple excellent reviews[93, 94] on this topic) include iterative Boltzmann inversion (IBI),[95, 96] force matching,[97] relative entropy minimization,[98] and others. Machine learning approaches have also been used for bottom-up parametrization.[99]

There have been a few different coarse-grained DNA models developed over the past decade, including Martini[100] and oxDNA,[101] among others.[102, 103] The top-down parameterized oxDNA model has proven to be the most reliable at reproducing the behavior of large DNA nanostructures, and top-down models have generally provided better overall accuracy than bottom-up parameterizations. This is likely because top-down approaches focus on capturing specific characteristics of DNA, *e.g.*, propeller twist, major and minor groove spacing, and persistence length. All of these characteristics are directly and intuitively related to the equilibrated structure, conformations, and other characteristics of DNA nanostructures that scientists desire to predict with simulations. On the other hand, bottom-up parameterization schemes often have a broad set of solutions, and many resulting potentials may be unphysical. For example, DNA can form a variety of structures, but an AA simulation used for parameterization may only capture DNA as a duplex. Bottom-up parameterization based on such simulations would thus not account

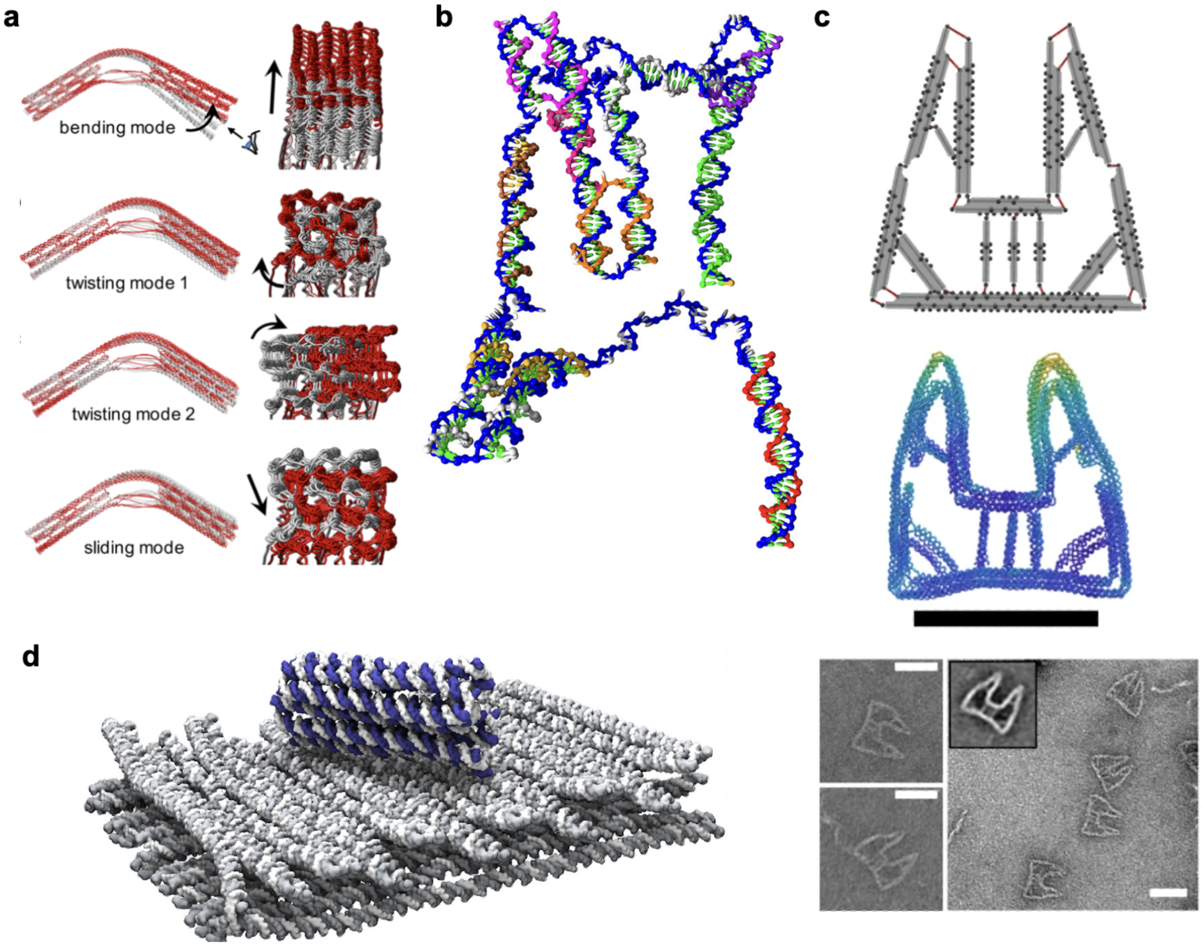

Figure 3: Coarse-grained modeling of DNA nanostructures. (a) Principal component analysis of a large DNA origami hinge. (b) direct simulation of DNA nanostructure self-assembly. (c) Use of coarse-grained modeling as a predictor of the equilibrium shape of a nanopincer. (d) Characterization of a nanorotor made of DNA. (a) reproduced with permission from ref [104]. Copyright 2017 American Chemical Society. (b) reproduced with permission from ref [105]. Copyright 2016 American Chemical Society. (c) reproduced with permission from ref [106] . Copyright 2021 Springer Nature. (d) reproduced with permission from ref [107]. Copyright 2022 Elsevier.

for the myriad other relevant DNA motifs such as Holliday junctions that are fundamental to DNA nanotechnology. Even if AA simulations used for parameterization did include whole DNA nanostructures with all possible motifs, bottom-up schemes with simple descriptions of potentials are not typically designed to handle the interaction complexity of DNA; therefore, the resulting model would be unlikely to correctly capture transitions or behaviors of other structures, or even the structure on which the parametrization was based. To summarize, top-down coarse-graining has provided the most accurate coarse-grained models because it focuses on directly reproducing features that are relevant to experimental observables.

Coarse graining's primary tradeoff is the resulting loss of detail compared to AA models – the particles are less well resolved, and the potentials have been fitted as an approximation to the interactions of many atoms, so they are not perfect and typically replace an anisotropic set of interactions which may be functionally important with a spherical or ellipsoidal approximation. In addition, solvent-specific effects such as the aforementioned ionic effects are lost. However, the smallest modes of motion of AAMD simulations are typically not very important in the context of DNA nanotechnology, and many properties of interest can be reproduced using CG models. oxDNA has played a vital role in probing the equilibrium conformations of structures, stability verification of designs of large DNA nanostructures produced using common design packages,[108-115] structural characteristics of archetypal DNA origami structures,[116] conformations of ssDNA brushes on DNA origamis,[117] basic motion and reconfiguration of dynamic devices,[26, 104, 118-121] and DNA hybridization-based phenomena such as toehold-mediated strand displacement (TMSD).[92, 122-124] The self-assembly of DNA nanostructures has even been directly simulated (**Fig. 3b**).[105] Besides use in self-assembly, all of these examples used CG based simulations to iteratively modify DNA nanostructure designs and ultimately control experimental outcomes without having to repeat experiments. It is further worth noting that this has become a somewhat standard practice that is not often reported in literature; most DNA origami designs that have been fabricated for use in research were first simulated using coarse-grained molecular dynamics and were often modified based upon results from these simulations.

Sometimes, instead of using MD simulations, Monte Carlo (MC) simulations[125] can be used to sample the configurational space. This can be advantageous because this technique may offer larger physical jumps between sampled configurations resulting in more efficient sampling. MC simulations perform a sequence of perturbations of the particle configuration in a stochastic manner that is not associated with time integration and then accept or reject those moves based on the incurred energy change ΔE, typically according to the Metropolis acceptance criterion $P_{acc} = \min(1, \exp(-\Delta E/k_\mathrm{B}T))$. This procedure enables thermodynamically valid sampling such that the average of an observable in the simulation corresponds to the true ensemble average of that observable, which is ideally the expected value of that observable in an experiment. For dense systems like DNA origami, MC move types which move a single particle tend to be inefficient. However, carefully constructed "cluster" move types which perturb groups of particles together may be drastically more efficient than MD. More complicated implementations of MC like virtual move Monte Carlo (VMMC) enable these more sophisticated move types to be used[101] and have been implemented in the oxDNA simulation package. Figure 3 presents a few representative examples where CG simulations have played a role in prediction and nanostructure design.

Mesoscopic Models. Along the same vein but perhaps deserving its own discussion, mesoscopic modeling (*meso* serving to describe an intermediate scale between molecular scale and macroscale systems) is an even coarser way to represent DNA structures. Generally, CG modeling groups a maximum of several atoms per representative particle and intends to use clusters of particles to mimic the behavior of monomers or small molecules; we can draw a distinction here for mesoscopic models, which further coarsen the representation of these systems to the point where the molecular detail is not well defined.

Typically, representative particle size is on the order of a few nanometers or larger and many mesoscopic models are bead-chain models. At this scale, the molecular detail of the system is not of concern and more emphasis is placed on reproducing properties which take place over larger length scales, *e.g.*, persistence length, end to end distance distributions, general shape of nanostructures, *etc.* This can be useful for capturing very coarse behaviors of dynamic nanostructures like the opening and closing of hinges when several devices need to be considered simultaneously. This kind of simulation would be quite expensive to perform using finer models like oxDNA but can be very efficiently performed using coarser representations. With such coarse models, the relaxation timecales of the particles often become shorter than the simulation timestep so that the highly efficient overdamped Langevin dynamics (Brownian dynamics) simulations can be used to describe device motion. Mesoscopic models are also generally good candidates for implementation into lattice models.[126]

While mesoscopic models have been used for studying various DNA-based systems such as chromatin,[127-133] DNA renaturation,[134] DNA motion in microfluidic and nanofluidic systems,[135, 136] and viral DNA packaging,[137] they are generally underutilized in DNA nanotechnology. So far, mesoscopic models have been used in the beginning stages of multiscale models for modeling DNA nanostructures,[138] and patchy mesoscopic representations of DNA building blocks have been used to elucidate the nucleated nature of DNA Brick self-assembly [139, 140] and the self-assembly of DNA tetrahedra into cubic diamond crystals.[141]

Statistical Mechanics Models. Molecular simulation techniques are not generally capable of studying kinetic phenomena such as the folding of large DNA origami or the supramolecular assembly of larger structures from DNA origami tiles. Accessing the long timescales involved in these processes requires more efficient computational techniques and analytical theories based on statistical mechanics.[142] Statistical mechanics uses the statistical behavior of molecular systems (*e.g.*, the Boltzmann relation at constant temperature) to compute the long-timescale or bulk behavior of the system of interest. The ability to analyze systems using statistical mechanics comes from the observation that molecular microstates are Boltzmann distributed, $P_\nu \propto \exp(-\beta E_\nu)$. Here ν is a system microstate, E_ν is its energy, and $\beta \equiv 1/k_\mathrm{B}T$, where k_B is the Boltzmann constant and T is the system temperature. The partition function Q describes the sum of Boltzmann factors of all possible states in the system, or $Q = \sum_\nu e^{-\beta E_\nu}$ in the case of a constant T, constant number of particles, and constant volume (NVT) ensemble. The expectation value of any collective variable can thus be expressed as $\langle X \rangle = \sum_\nu X_\nu P_\nu = \sum_\nu X_\nu e^{-\beta E_\nu}/Q$. Statistical mechanics-based models developed for DNA origami applications to this date have generally addressed one of three objectives: prediction of the conformational dynamics and actuation behavior of dynamic DNA origami nanostructures, characterization of the dynamics of systems involving strand displacement reactions, and prediction of the kinetics and thermodynamics of assembly processes. Besides the general role of statistical mechanics in MD and MC simulations and in CG model development,[143] most existing methods have focused on fitting and predicting the behavior of simple dynamic DNA origami devices like hinges.[24] These systems usually incorporate short single stranded molecules in each arm, referred to as "overhangs". The binding and unbinding of these overhangs, driven by actuation methods such as changes in salt concentration or TMSD, drives conformational changes between system states. Hybridization affinities are additionally controlled by tuning the length, sequence, and location of these strands.[120] The thermodynamic properties of the hinges are then fully described by hinge and overhang dynamics, whose effects can be decoupled. Marras *et al.*[26] and Crocker *et al.*[142] used partition functions to characterize the free energy associated with ion-mediated and temperature-mediated actuation of DNA origami nano-hinges, respectively. These models have shown great agreement with experiments, suggesting notable control over the behavior of dynamic DNA origami nanodevices. However, their applications are limited to a narrow range of devices; theories and models characterizing the long-time behavior of dynamic DNA nanostructures are few and rare.

Kinetic Modeling. Many analysis techniques used in DNA computing and molecular programming, such as chemical reaction networks and automata-theoretic models, have diffused into structural and dynamic DNA nanotechnology, inspiring diverse mechanisms for actuation, communication, and programmability while providing fundamental insights into mechanistic processes such as those involving assembly. DNA origami platforms have been used to study the dynamic behavior of DNA strand displacement (DSD) systems, ensuring the (reaction-limited) spatial locality typical of other computing models.[144-146] While TMSD is often used for actuation,[12, 24, 147] in DSD systems, these reactions are used to execute signal processing and control instructions[146, 148] such as logic gates ,[144] fork and join gates,[149] catalytic gates,[150] neural network computation,[151] and oscillators.[152] Integration of DNA and RNA enzyme strategies has expanded the design toolbox of these nucleic acid circuits,[153-155] allowing for the design of feedback control mechanisms,[156] predator-prey dynamics,[157] and transcriptional oscillators,[158] among other circuit implementations.

The system size that can be solved analytically[148] is limited and has long since been exceeded by the complexity of experimentally implemented circuits. Modeling techniques thus play an important role in determining the state space of DSD systems,[148] estimating signal propagation times,[159, 160] and verifying if the observed behavior of a system corresponds to the behavior predicted from design.[148] Two modeling approaches specifically stand out by virtue of their simplicity: reaction-diffusion equations and Brownian dynamics.[161] The kinetics of a DSD system can be modeled as a continuous time Markov process through the state space of all possible conformations,[159] which can be characterized either by means of mathematical treatments or stochastic simulations (*i.e.*, Monte Carlo Gillespie algorithms). For simple DSD systems based strictly in strand displacement reactions, the different conformations of a system are defined by the state of the strands, and strand displacement reactions occur with an effective rate related to diffusion, hybridization-denaturation rates (typically modeled using specialized software such as Multistrand[159]), and the physical constraints of the system.[146, 160, 162] These three properties can be integrated to obtain the effective strand displacement rates by means of reaction-diffusion equations,[163-165] reactive Brownian dynamics simulations,[146, 162, 166] or analytical theories involving first passage times.[160] Most DSD analysis has now been automated and can be treated computationally using specialized kinetics software such as Visual DSD,[165] KinDA[167] and DyNAMiC Workbench.[168]

Similar modeling strategies have been used to design, program, and optimize the algorithmically directed assembly of DNA origami tiles.[169, 170] A wide variety of DNA origami tile shapes and interactions are possible,[171] offering ways to build large, complex assemblies with nanoscale resolution. Experimentally implemented systems have been regulated by diverse physical processes, including hybridization,[172] strand displacement,[173] shape complementarity,[171, 174] and base stacking.[171, 175] Knowledge of the thermodynamics and kinetics of tile binding can then be used as input for theoretical models at different levels of abstraction, including kinetic models,[170, 176] Monte Carlo models,[177] and chemical reaction networks.[178, 179]

Kinetic modeling has also been used in structural and dynamic DNA nanotechnology systems beyond the context of DSD and tile systems. Many systems are purely reaction-based and so their behavior can be represented using mass-action kinetics, *i.e.*, as a system of ordinary differential equations (ODEs) describing the time evolution of concentration which can be solved using eigenvalue decomposition or ODE solvers. To provide a few examples, kinetic modeling has been used to study individual attachment and detachment processes between separated DNA origami structures demonstrating autonomous regulation behavior;[180, 181] to describe the growth of polythymine brushes onto DNA via a catalytic enzymatic polymerization process;[182]

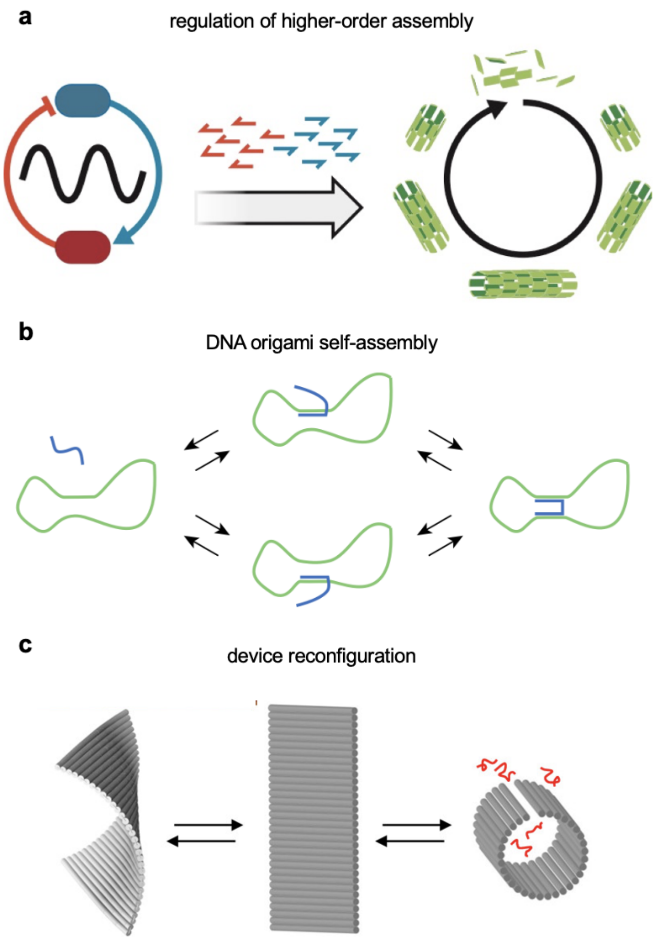

Figure 4: Kinetic modeling in DNA nanotechnology. (a) Kinetic model of higher-order self-assembly and disassembly of DNA Nanotubes. (b) Kinetic model of DNA origami self-assembly. (c) Kinetic model of a reconfigurable DNA origami sheet. (a) reproduced with permission from ref [180]. Copyright 2019 Springer Nature. (b) reproduced with permission from ref [183]. Copyright 2015 AIP Publishing LLC. (c) reproduced with permission from ref [184]. Copyright 2014 American Chemical Society.

Similar strategies to those described for DSD systems have also been successfully implemented to model DNA origami folding[183, 185, 186] and DNA origami tile-tile assembly.[187, 188] Figure 4 depicts a few relevant kinetic systems.

Continuum Modeling. While CGMD and mesoscale simulations are very useful in DNA nanotechnology and offer some of the best value in terms of accuracy vs. computational cost, in some cases it is expedient to use continuum modeling. Continuum modeling assumes that the response of a system to a perturbation can be approximated as a continuous function satisfying macroscopic balance and conservation principles rather than behaving as a set of discrete particles interacting with conservative interaction potentials. When applied to solid mechanics, partial differential equations are often used to solve the spring equation for continuum solids in order to compute the global deformation response to an applied load which is introduced as a boundary condition. When geometry is too complex to analytically solve this equation, the problem can be discretized into a set of small volumetric elements whose displacements are described by the equation $\mathbf{f} = \mathbf{K}\mathbf{u}$, where $\mathbf{f}$ is the set of all forces on connective elements (nodes), $\mathbf{u}$ is the displacement vector of the nodes, and $\mathbf{K}$ is a stiffness matrix which accounts for both compressive/tensile stiffness and bending and torsional stiffness within the element accounting for the intrinsic moduli of the material and moment of inertia of the element shape in the direction of the applied load. Continuity between all elements is assumed through their shared nodes, which converts this partial differential equation problem into a matrix algebra problem which can easily be solved.

A finite element model called CanDo[189] has been developed for continuum analysis of DNA nanostructures; this model treats individual nucleotides as connected continuum nonlinear beams (since DNA's behavior is more complex than the usual, linear macroscopic treatment of most isotropic engineering materials) and computes the equilibrium configuration and fluctuations of the DNA nanostructure being studied. While it does not serve as final validation for structural integrity or stability, CanDo is a good first check for having attained the desired final structure before running a CG simulation, as CanDo can usually predict undesired twisting and bending of structures in a small fraction of the simulation time required by CGMD simulations. This allows experimental groups designing DNA origami to quickly test their designs and return to the drawing board if significant undesired bending or twist are predicted. The more recent SNUPI model[190] improves upon the CanDo model by adding electrostatic interactions and has been shown to work well for computing the equilibrium conformations of hierarchical superstructures consisting of many DNA origami,[191, 192] a task that has proven quite challenging for both AAMD and CGMD simulations. Continuum mechanics provides additional value to DNA nanotechnology by providing practically useful quasi-analytical scaling behavior of new systems. For example, basic solid mechanics including beam bending calculations based on moment of inertia can be used to intuitively explain the relationship between DNA origami cross section and structural persistence length with remarkable agreement.[193] This is useful because as DNA nanotechnology has been advancing in complexity (with nanostructures containing structural elements with many different cross sections being introduced recently[110]), these continuum mechanics assumptions provide basic predictive power for controlling the deformation behavior of these structures and provide quantitative data for comparison of multiple cross sections when they, for example, contain the same overall cross-sectional area but different shapes.

Multiscale and hybrid resolution models. There are some approaches which combine multiple modeling paradigms to address specific limitations of individual techniques. For example, some larger DNA nanostructures may be prohibitively expensive to equilibrate at a coarse-grained resolution. To address this, a model has been developed which performs major equilibration steps like global structure relaxation at a mesoscopic resolution and gradually refines modeling resolution to achieve atomically reasonable equilibrium conformations in just a few minutes.[138] This model has the added benefit of accessing very long dynamic timescales for other purposes such as the simulation of applied electric fields. Other examples of hybrid approaches include a recently developed model which allows CGMD simulations of DNA to be run in the presence of a mesoscale protein representation[194] and, in the area of chromatin modeling, a mesoscale representation of DNA and a coarser representation of nucleosomes with charges represented using a discrete surface charge approach.[128] Another multiscale model used oxDNA to sample the local free energy landscape of DNA Brick self-assembly, then used that data to produce a two-state kinetic rate model of the self-assembly process.[195] Examples of multiscale and hybrid resolution models can be found in Figure 5. Quantum Mechanics / Molecular

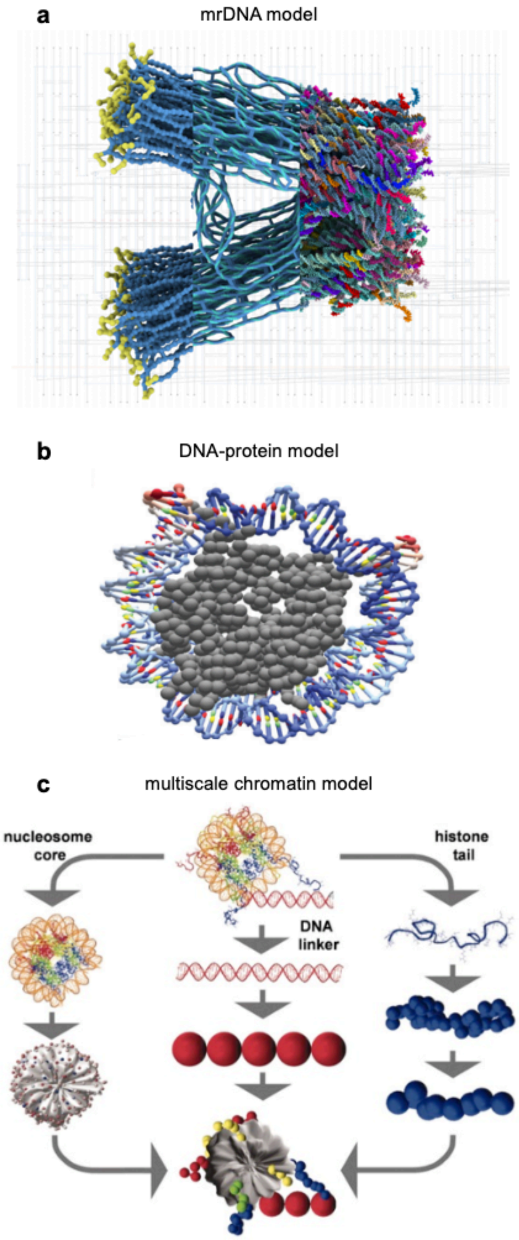

Figure 5: Three multiscale / hybrid scale models used to solve tractability problems in DNA nanotechnology. (a) multiresolution DNA model used for rapid configurational equilibration. (b) hybrid DNA - protein model for simulation of DNA and protein complexes. (c) Hybrid scale nucleosome-DNA model for simulation of chromatin. (a) reproduced with permission from ref [138]. Copyright 2020 Oxford University Press. (b) reproduced with permission from ref [194]. Copyright 2021 The Royal Society of Chemistry. (c) reproduced with permission from ref [133] . Copyright 2006 National Academy of Science.

Mechanics (QM/MM) multiscale models have also been used in limited context to study DNA. QM/MM models apply QM based behavior to reactive species, *e.g.*, those undergoing some kind of chemical reaction, while treating other species using standard AAMD forcefields within the same simulation. This enables timescale access approaching that of AAMD while still capturing the physics required for phenomena like ATP hydrolysis[196] or enzyme activity.[197] In the context of DNA nanotechnology, combinations of QM and MD simulations have already been applied toward the optimization of dye placement[74] to afford control over device signal output and could find future use in photonic systems[198] or systems containing quantum dots.[199-201]

Analysis of simulations

The objective of simulations is typically to produce data that can be compared with an experimental result or another simulation to provide interpretable information about system behavior. However, simply running simulations of DNA nanostructures and attempting to "observe" phenomena by eye is often insufficient to capture interesting phenomenological behavior. Trajectories must be analyzed to remove unimportant degrees of freedom and capture the true mean structure, dynamics, or some other property of the system. We will describe some of the most common analysis performed on simulations.

Mean structure computation and removal of diffusive degrees of freedom. Computation of the mean structure of a DNA nanodevice from dynamic simulations is often a first step in the process of trajectory analysis. Molecular simulations are often conducted on species which are floating in solution; these species correspondingly have six degrees of freedom (three degrees of translation, and three axes of rotation) which are not relevant to the actual intra-device motion. To address this, we may isolate the specific components of the body of molecules that we want to analyze (excluding other bodies which do not participate in or are not relevant to the process under study) and perform a rotational and translational transform to minimize the mean squared distance between the selected particles in frame i and those particles in every other frame of the simulation. This frame *i* is often selected at random but may also be selected to force alignment to some basis, *e.g.*, Cartesian axes. This yields a trajectory of a structure which does not translate or rotate but instead solely performs its own internal motions, which is much easier to analyze. Taking the mean of this modified trajectory provides what is referred to as the “mean structure”, which is the average structure accounting only for internal motions. Examples of where this analysis has been applied include characterizing polythymine brush extent on DNA nanostructures,[117] and capturing the mean structure of a dynamic DNA origami hinge,[120] both of which demonstrated good agreement with experiments.

Principal Component Analysis. To determine the dynamical behavior of structures, one may conduct principal component analysis (PCA).[202] In a general sense, PCA generates a set of orthogonal vectors within a D-dimensional dataset along which the first vector captures the largest deviation in that dataset, the second component produces the second largest deviation in a dataset, *etc.,* until there are no more orthogonal vectors that can be created. This results in $(D-1)$ vectors or *principal components*. In the case of molecular simulations, PCA can be used to compute the combinations of particle motions that produce the largest overall deviation from the structure’s mean. To do this, one can calculate the Cartesian displacement of particles in each frame from the mean structure of an N-particle system, flattening them into a vector of length $3N$ for each frame, computing a covariance matrix of this vector, and computing the eigenvectors and eigenvalues of that covariance matrix, where each eigenvector is a “component” of motion. These components can then be redistributed into a set of 3-dimensional vectors $\mathbf{v}$ each accounting for the motion of a single particle. This is often diagrammatically represented using an image of the mean structure at $\mathbf{c}$ superimposed with a new image of the structure where each particle has been moved by its corresponding 3-dimensional vector to locations $\mathbf{c} \pm \mathbf{v}$ (Figure 3a). With PCA, one can understand how a structure is moving, including determining whether undesired motions are occurring, thereby allowing researchers to better design dynamic devices to have more controlled motions.[104] The recent introduction of the oxView webserver[203] enables these components to be computed easily.

Estimating the mechanical properties of structures. The exact mechanical properties of DNA nanostructures could be of import to future experiments in DNA nanotechnology. Past studies have focused on applying the principles of mechanical engineering beam analysis to DNA nanostructures. The chemistry and biophysics communities typically address the bending stiffness of DNA nanostructures in terms of persistence length l_p, whereas the mechanical engineering community tends to use quantities like Young's modulus E and bending moment of inertia I to describe stiffness. These properties are connected by the relationship $l_p = EI/k_\mathrm{B}T$.[204] The persistence length can be computed using standard methods from MD simulations,[205] providing a quantity which can be compared against experiments.[206] The mechanical stiffness of other bodies like hinges can be determined by calculating hinge angle distributions and computing the slope of the free energy landscape $dF/d\theta$.[24]

Calculating diffusion coefficients. The diffusion of particles in a liquid, which is the typical environment for DNA systems, generally follows Stokes-Einstein behavior,[207] where the translational self-diffusivity D_s is inversely proportional to the particle's hydrodynamic radius. D_s can be ascertained in simulations from the slope of a plot of mean squared displacement as a function of time change t in the long-time limit. These diffusive statistics apply not only to a single isolated particle, but also to the centroid of a group of particles forming a single-stranded oligonucleotide or even an entire DNA origami, although many existing models like oxDNA do not account for hydrodynamic interactions[208] and so simulations using these may be inaccurate for comparison to experimental techniques. AAMD simulations should hypothetically produce accurate diffusion behavior that accounts for hydrodynamics, although this technique suffers from periodic boundary condition artifacts and computational expense.

Enhanced Sampling and Computation of Energy Landscapes. The free energy of systems with respect to some reaction coordinate is often of interest. For example, one might wish to design a DNA hinge with an energy barrier to opening and closing. Describing this energy barrier

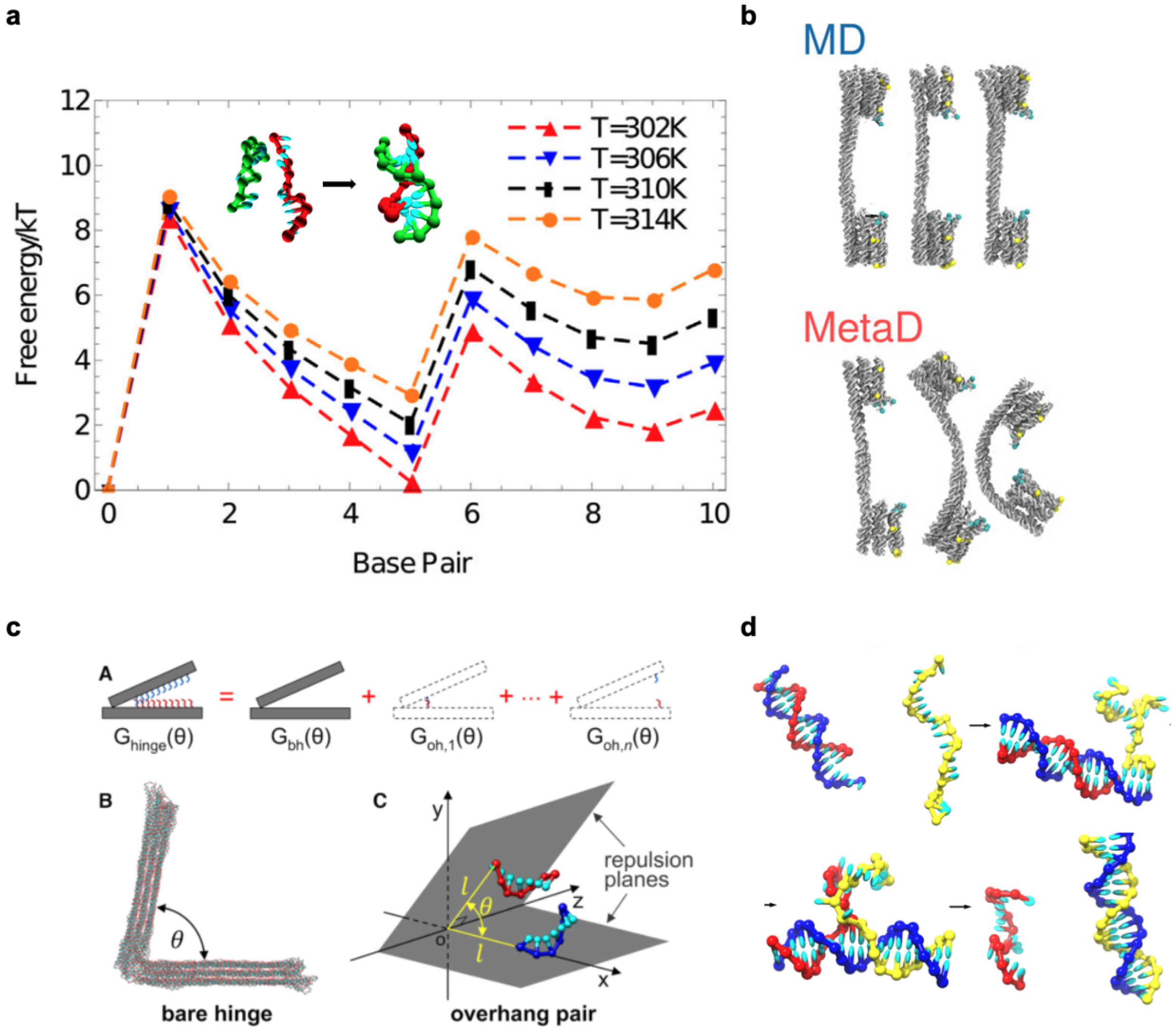

Figure 6: Use of enhanced sampling to predict DNA nanodevice behavior. (a) pseudoknot formation in a dodecamer. (b) metadynamics to improve conformational sampling of a DNA nanostructure. (c) umbrella sampling using hinge angle of a DNA nanostructure as a reaction coordinate. (d) umbrella sampling of a toehold-mediated strand displacement reaction. (a) adapted with permission from ref [101]. Copyright 2021 Frontiers Media S.A. (b) reproduced with permission from ref [209]. Copyright 2022 The Authors. Published by American Chemical Society. (c) reproduced with permission from ref [120] . Copyright 2020 Oxford University Press. (d) adapted with permission from ref [122]. Copyright 2013 Oxford University Press.

and quantifying the timescale of transitions between states would likely involve conducting CGMD simulations of that hinge which sample its full configurational space. However, such simulations might be prohibitively long since the transition time across energetic barriers scales exponentially with barrier height normalized by thermal energy. For this reason, one can turn to enhanced sampling techniques such as umbrella sampling, metadynamics, or various other techniques.[210] Enhanced sampling applies a known bias to a simulation which drives the system being studied out of local energy minima and then uses that bias to determine the free energy landscape along a reaction coordinate. Normally, the Hamiltonian of a system is simply a function their coordinates $\mathbf{r}^N$ and momenta $\mathbf{p}^N$: $H(\mathbf{r}^N\mathbf{p}^N)$. As the most basic example, umbrella sampling modifies the Hamiltonian being used in the simulation to $H' = H(\mathbf{r}^N\mathbf{p}^N) + \frac{1}{2}k_\chi(\chi - \chi_0)^2$ where χ is the current location along the reaction coordinate being considered and χ_0 is some value around which we would like to sample, and k_χ is some stiffness which is selected roughly corresponding to how steep the energy gradient is along the area being sampled. By running simulations with this modified Hamiltonian for many different values of χ_0 such that there is a contiguous set of simulations with significant overlap in the distribution of χ between each, we can effectively force the sampling of the entire energy landscape of interest. These overlapping distributions in χ are then combined using weighted histogram analysis[211] to produce a contiguous free energy landscape across the entire range of χ. This is a simple yet very powerful paradigm which has only recently begun to be exploited for more advanced analysis of DNA nanostructures.[104, 120] Another enhanced sampling technique, metadynamics,[212] defines a reaction coordinate and then begins sampling the energy landscape along that reaction coordinate. As sampling proceeds, artificial potentials (usually Gaussians) are added to the current location being sampled to favor exploring other sections of the energy landscape. This pushes the system out of well-sampled regions and into less well-sampled ones. Once the entire range of interest has been sampled, the artificial potentials are summed and inverted to reveal the true free energy landscape. This technique has been used to enhance the sampling of the configurational space of DNA nanostructures for comparison to experiments involving DNA origami with broad configurational flexibility,[209] but it has also been applied in a variety of biochemistry systems[213-215] and will surely find additional uses. Figure 6 depicts a few studies utilizing enhanced sampling.

Comparing Simulation Results to Experiments

The value of predictive simulation data is somewhat limited unless a sufficiently large body of paired data, consisting of simulations and corroborative experimental data, has been produced to merit confidence that the simulations are accounting for all or nearly all sources of experimental variability. This experimental data may be generated in several ways, from direct imaging with light microscopy to fluorescence spectroscopy to more advanced and well-resolved techniques like cryo-electron microscopy (cryo-EM). Generally, simulations and experiments are compared by defining useful collective variables and then designing schemes for measuring those variables both in simulations and in experiments. The distributions or other features of these collective variables can then be compared between simulation and experiment to determine whether the model is capturing the relevant physics. We previously described the ways that simulations are processed to gather collective variables of interest; in this section, we will describe a few ways in which experiments are used to generate collective variable data which may be mappable to simulations.

Electrophoresis. The most common first step in experimental characterization of a DNA origami nanostructure is gel electrophoresis of the folding products. Since larger DNA species flow more slowly through an agarose gel under an electric field, differently sized species will separate over time where they can be compared to reference DNA ladders. The separation of these species is based upon the general concept of electrophoretic terminal velocity v. The net force F acting on a molecule carrying charge q and being driven by electric field E is given by $F = qE - \gamma v$, where γ is the friction coefficient of the species. Since agarose gel is highly viscous, species rapidly reach terminal velocity so $F = 0$ and thus $v = qE/\gamma$. Since q and γ scale differently with molecule size, larger DNA species tend to have a slower terminal velocity and thus migrate more slowly through the gel, resulting in a band of DNA (identified using a loading dye) which is closer to the well where the sample was loaded than smaller species. For sufficiently long stimulation times, these DNA bands become so well separated that they can be distinguished from each other and individually isolated and further characterized. This technique is primarily comparative, as the ladder and existing known species serve as points of reference. However, this can be useful for identifying bulk aggregation, dimerization, disassembly, and other desired / undesired behavior. Furthermore, bands from these gels may be repurified and assayed directly to further characterize the experimental result. This may serve to corroborate modeling-based predictions of structural stability or instability, or to direct further modeling and simulation to understand the cause of experimental outcomes such as aggregation.

Direct imaging. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) are typically used to provide direct images of DNA nanostructures. AFM works on the principle of mechanical cantilever bending, where a nanoscale silicon cantilever with a sharp tip is dragged or tapped across a nanoscale surface; a laser beam pointed at the top of the cantilever helps to measure the cantilever's angular deflection, from which the height of the tip can be calculated. The height values z measured at each location *x*,*y* scanned using a motorized stage then provides a three-dimensional map of surfaces at very high resolution. TEM works on the principle of an electron source which fires electrons through a specimen and records the un-scattered electron density behind the sample, providing an image of the specimen being sampled. TEM works on the principle of an electron source which fires electrons through a specimen and records the un-scattered electron density behind the sample, providing an image of the specimen being sampled.

This provides a massive amount of useful information that can be compared to simulations, ranging from simple qualitative comparison between images and equilibrium simulations[112] to measuring the extent of polymers grafted to DNA origami for comparison to CGMD simulations[216] or nanoparticles,[217, 218] to measuring the angle distributions of hinge and lever nanodevices and comparing these distributions to simulations.[104] The complexity in this technique arises from measuring collective variables, which must generally be performed manually, although this can be enhanced using artificial intelligence (see below).

Spectroscopy. Spectroscopy is the study of matter's interactions with electromagnetic waves. This is often a coarse but very effective way of observing DNA nanostructures and their behavior. Within spectroscopy is fluorescence, a phenomenon where certain molecular species absorb light at one (often invisible) wavelength and then emit (usually visible) light in their excited state. The emitted light can be used to determine the locations of DNA nanostructure components that have been labeled with fluorescent groups. Fluorescent particle tracking has been used to quantify the energy landscapes of DNA origami devices, revealing free energy landscape features that are corroborated by coarse-grained molecular dynamics simulations.[107] One adjacent example to fluorescence is Förster resonance energy transfer (FRET), a nonradiative dipole-dipole energy transfer phenomenon arising when one molecular species ("donor") is excited. If that species is close to a species which is capable of receiving energy from resonance energy transfer ("acceptor"), the excited donor species will shunt its energy to the acceptor species and excite it, then causing the acceptor to emit its characteristic excitation signal; if the donor is not close to an acceptor, the energy from excitation will be emitted as the donor's signal. When FRET pairs are placed on different parts of a DNA nanostructure, the signal arising from that FRET pair can be used to determine whether those two parts are near each other or far away from each other. While very simple in nature, this is extremely useful as it can indicate open / closed states for hinge and box type structures,[2] or can serve as a signal for whether a certain component has incorporated into or dissociated from a DNA nanostructure.[20] This data is also easy to corroborate with simulations since a simple collective variable can be defined based on the separation of the pair's grafting locations and the system can be predicted using CGMD simulations[219].

Ultraviolet-visible (UV-Vis) spectroscopy measures the absorption of UV and visible spectrum electromagnetic radiation by a sample. DNA's spectral absorption can be used to estimate the concentration of DNA in solution,[220] which can provide information about DNA nanostructure yield. G-quadruplexes also have a distinct spectral absorbance from ssDNA or dsDNA, leading to the use of UV-Vis spectroscopy to quantify the behavior of DNA-based nanoswitches.[221] Finally, UV-Vis spectroscopy has been used to quantify DNA binding onto gold nanoparticles.[222]

As the characteristic energy levels of rotation and vibration of DNA molecules fall in the high gigahertz (GHz) to low terahertz (THz) spectra,[87, 223-227] these wavelengths have also been used to manipulate DNA origami nanostructures, increasing the yield of assembly of DNA origami[228] and allowing for the development of biosensors and antennas with tunable resonance.[229-231] To better understand these devices, computational THz spectroscopy of DNA origami molecules can be performed using specialized software[86, 232, 233] and algorithms[234, 235] together with atomistic simulations.[87, 88] However, such treatment can only be implemented on small molecular systems.

Cryo-Electron Microscopy (Cryo-EM). Cryo-EM[236] vitrifies samples at extremely low temperatures and then uses transmission electron microscopy (TEM) to resolve 2D projections of those samples. Mathematical transformations can be performed on the 2D data to recover 3D structural information.[237] This can be used to resolve the structure or dynamics of proteins or DNA nanostructures in a nearly atomically precise manner. In DNA nanotechnology, cryo-EM reconstructions of DNA origami[52] have been used as validation for new simulation models.[190]

Dynamic light scattering (DLS). DLS uses the autocorrelation of laser light scattering intensity to determine the diffusive properties of the species contained within a sample. It is most often used to obtain the diffusion coefficient of species and to estimate the hydrodynamic radius of those species.[238-240] For a single species in solution, the autocorrelation of scattering intensity decays as a single exponential with a decay constant $\tau = q^2 D$, where q is the wave vector and D is the translational diffusion coefficient. This is relatively straightforward to compare to approximations for the diffusive properties of non-spherical shapes,[241-243] making for a useful point of comparison with the DLS result.

Small angle X-ray scattering (SAXS). This technique is used to determine the sizes and distributions of nanoparticles in solution[239, 244] in addition to their shapes and for distinguishing between different conformational states and resolving their transitions.[245] SAXS functions on the same principle of standard x-ray scattering: when the paths of scattered x-rays differ by a perfect multiple of their wavelength λ, those x-rays collectively exhibit a high intensity from cooperativity, while paths differing by a perfect half multiple $\lambda/2$ are mutually destructive and will produce essentially no scattered signal; SAXS data is typically a plot of scattering intensity as a function of scattering vector q; this data may be interpreted to provide information about particle size and shape which can be compared directly to simulations. While its use has been limited, SAXS promises to resolve similar behavior to that which can be captured using fluorescence, but without the use of bulky and saturation-prone fluorescent molecules.

Less Common Techniques. Some additional experimental characterization techniques applied toward DNA origami include small angle neutron scattering,[246] ion mobility spectrometry – mass spectrometry,[247] and individual particle electron tomography.[248]

Artificial Intelligence and Machine Learning

Artificial Intelligence (AI) and Machine Learning (ML) are somewhat distinct from the rest of the methods covered in this review since they are more general. However, due to the incredible power of inference, ML and AI have seen broad adoption across the domains of physics,[249, 250] biology,[251, 252] and materials science,[253, 254] and discussion of their use in the context of prediction and control in DNA nanotechnology is warranted. AI is a discipline that concerns solving problems that cannot necessarily be addressed procedurally, in a manner inspired by the learning behaviors of intelligent beings like humans. In most cases, AI attempts to establish a mapping relationship between some input and a desired output. This output may be, for example, the correct identification of the subject of an image or an estimation of the probability distribution of some collective variable. This process is often accomplished using feedback,[255-257] where the output is compared to the ground truth (expected outputs); the mapping function is then adjusted until it can reliably reproduce these input-output relationships.

As a subset of AI, ML covers algorithms capable of learning from pre-existing examples to make decisions or predictions by recognizing the underlying patterns of a problem. There are three main categories of ML: supervised learning, unsupervised learning, and reinforcement learning. Supervised learning is commonly adopted when a labeled dataset is available for the model to capture the relationship between feature and label. On the other hand, unsupervised learning is adopted when labeling is physically or economically prohibited, or when the goal is to identify the underlying distribution of data, e.g., using clustering algorithms.[258] Both supervised and unsupervised learning provide data-driven predictions on properties of interest. In the framework of reinforcement learning, the objective would be for an agent to identify an optimal strategy (policy) by maximizing a given reward function. The agent does this by directly interacting with the environment and learning from the received rewards.

To establish and evaluate machine learning models, one can partition an available labeled dataset into training, validation, and isolated testing groups. This partitioning approach allows one to train the model on the training set, tune the hyperparameters (a setting on a machine learning model which determines how the model will learn from data) on the validation set, and evaluate the model performance on the isolated testing set. If accuracy and reliability need to be improved, more statistical methods can be employed, such as cross-validation or nested cross-validation. For how AI and ML work, we refer readers to existing comprehensive literature addressing these topics.[259, 260]

In DNA nanotechnology, AI has been adopted to address emergent topics in the field, including nanostructure annotation, design optimization, and device development. The most straightforward application is to apply AI toward direct imaging data of DNA nanostructures. Correctly quantifying the values of collective variables in DNA nanostructures and annotating AFM imaging usually takes a tremendous amount of human input, where a researcher must manually label hundreds to thousands of data points to establish sufficient statistical power in an experiment. AI promises to enable automated labeling of experimental data in a way that is not only less work but also less error-prone than manual human entry.[261-263] For example, YOLOv5,[264] a type of convolutional neural network which performs extraordinarily well in image recognition tasks, has been implemented in the context of nanostructure detection to avoid laborious manual annotation of AFM images, leading to a much more labor-efficient and statistically robust approach to distinguishing between DNA nanostructures.[265] A deep neural network has also been used to directly improve the resolution of DNA origami AFM images.[266] Similar goals can also be achieved by nanoTRON,[267] an open-source imaging package that performs classification tasks and has been shown to reliably reconstruct nanostructures from super-resolution DNA-PAINT[268] imaging of DNA origami. A more recent study used AI to reduce the amount of sampling required to reconstruct these structures by an order of magnitude,[269] indicating that AI can be used to massively improve throughput in DNA nanotechnology based imaging applications.

Beyond accelerating laborious analysis tasks, AI has also been applied to develop better DNA origami designs. In simulation work, researchers have implemented shape annealing and evolutionary strategies on the oxDNA model and simulation software package[10, 92, 270] to design nanostructures that closely match a desired shape profile.[271] AI has also been applied to provide more accurate prediction of structural properties, *e.g.*, constructing quantum-accurate electron density profiles for DNA nanostructures up to ~225 kDa.[272] Another interesting experimental application of AI to DNA origami is the use of ML to predict and optimize the performance of molecular photonic wires based on their chromophore attachment configuration.[273] eXtreme Gradient Boosting (XGB)[274] has additionally been used to classify the sequence of molecular barcodes for multiplexed detection of biomolecules on surfaces.[275]

Grand Challenges and Opportunities

The past ten years have produced astounding advancements in our ability to understand and optimize the behavior of DNA nanodevices. However, several behaviors of DNA origami are still not under our control. Firstly, the folding of DNA nanostructures and the associated yield of this process are not predictable. While several design and fabrication recipes help to avoid folding problems,[8, 276, 277] they must be developed on a case-by-case basis and do not always guarantee good folding results. Better understanding the folding process will lead to much higher confidence that designs will fold properly, both within the field and when researchers adopt DNA origami as a tool in other fields.

Several properties of correctly assembled DNA nanostructures are also not very predictable, *e.g.*, the interaction of DNA with proteins. Addressing this issue is well underway,[194] but the field still lacks a coarse-grained force field which is capable of accurately capturing the interactions between different peptides and DNA. In addition to proteins, the effect of ions on DNA, specifically multivalent ions, may not be accurately captured with CG modeling. This is likely because CG models tend to treat salt effects using Debye-Hückel theory, which is too simple to address multivalent ionic effects. Currently, multivalent ion effects in the oxDNA model are treated by simply applying an artificially high monovalent ionic concentration. While this functions well in fabrication conditions, it is not clear how it will scale with multivalent ion conditions deviating significantly from these conditions. This may potentially be resolved in the future using more complex CG interactions that account for other factors such as ion type, correlation, and valency in addition to ionic concentration. CG models in adjacent fields like chromatin modeling have successfully reproduced multivalent cation-mediated effects,[278] offering potential solutions to this challenge.

The size of DNA nanostructures, especially those hierarchically assembled from multiple DNA origamis, is increasing at a rapid pace. At the larger length and time scales associated with these structures, existing CG simulation techniques are becoming insufficient to characterize dynamic behavior. Mesoscale models using a coarser representation than the oxDNA model may be useful for this purpose, where capturing transition behavior over microns and seconds may be tractable. The aforementioned mrDNA model[138] may be a good starting framework for this.

Lastly, AI can be used to improve our understanding of several under-addressed topics in DNA nanotechnology. The first is to use AI to understand fundamental processes. For instance, one can exploit the inference power of ML models to predict the transition behavior of dynamic DNA origami devices. This can take place either in a simulation or in experiments for the inference of relevant collective variables, or in identifying interesting behavioral patterns. The second is to exploit AI to improve assembly yields and control defects. Instead of relying on expert intuition to craft assembly rules to obtain desired origami design with sufficient yields, we envision that reinforcement learning or active learning can be well suited for this type of task. The third is to encourage the use of AI in DNA technology by improving data accessibility. Powerful ML models have achieved great success in other research fields, such as Alphafold 2[279] in the protein field, largely thanks to the existence of vast data repositories like the RCSB Protein Data Bank.[280] Yet, the adoption of ML has been relatively slow in DNA nanotechnology because there has not historically been a database or a data-sharing platform for DNA nanostructure data, hindering AI workers from accessing the data and extracting knowledge or building models on it. A promising new resource in the DNA nanotechnology space has been Nanobase, a database and repository for DNA nanostructures.[281] This makes it possible to see the designs used in various publications in the field, with additional data available describing fabrication protocols. This resource could be further leveraged in AI and ML applications and in data harvesting for the enhancement of the field if additional information about these nanostructures was provided, *e.g.*, AFM or TEM images of folding products and standardized and accessible data fields for annealing ramps and salt conditions used in the fabrication of these nanostructures. This could potentially aid in the improvement of our understanding of DNA origami folding and provide a robust body of reference data against which new simulation models may be parameterized. Another way that ML and especially deep learning can contribute is in the development of simulation force fields where AI can provide non-functional parametrization of CG force fields which may be more accurate than functional representations.

Conclusion

In this review, we discussed the broad range of time and length scales over which events in DNA nanotechnology take place and the modeling resolutions used to represent DNA nanostructures at these length and time scales. We then discussed different simulation methods for the prediction and control of DNA nanostructure and device behavior. We described the different experimental characterization techniques that are available to compare to model predictions and how they are used in synergy with modeling for practical device design and fabrication. We then discussed the recent development of AI and ML based tools for DNA nanotechnology. Finally, we discussed the current limitations and gaps in the modeling and simulation space and identified key opportunities in prediction and control that may further aid in the advancement of the field.

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