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Using DNA to program the self-assembly of colloidal nanoparticles and microparticles

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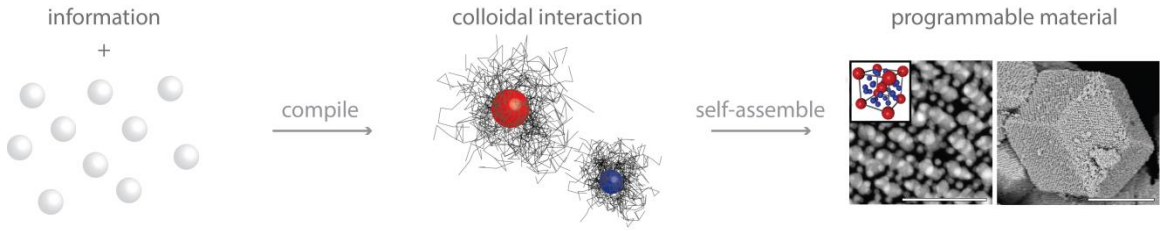
Preface

Deoxyribonucleic acid (DNA) is not just the stuff of our genetic code; it is also a means to design self-assembling materials. Grafting DNA onto nano- and microparticles can, in principle, "program" them with information telling them exactly how to self-assemble. Although fully programmable assembly has not yet been realized, the groundwork has been laid: with an understanding of how specific interparticle attractions arise from DNA hybridization, we can now make systems that reliably assemble in and out of equilibrium. We discuss these advances and design rules that will allow us to control—and ultimately program—the assembly of new materials.



Box 1: Self-assembly is a process by which a system of disordered components spontaneously assembles into an ordered pattern or structure without human intervention. In programmable self-assembly, information is added to the system in order to direct assembly toward a prescribed structure or behavior. In a sense, this information is compiled into a set of local interactions, which then execute the self-assembly of the target structure. The information can take many forms, such as the DNA sequences or component shapes, as discussed in the text. The micrographs are adapted from [73] and [75].

Introduction

Compared to what nature builds using self-assembly, the structures humans can make by mixing together synthetic components seem downright primitive. Nature builds structural components (proteins), catalysts (RNA and enzymes), responsive containers (clathrin cages), and even entire organisms (viruses) through processes that appear to be—from the fact that the same structures can be produced *in vitro*, in the absence of any external energy input—guided by the minimization of free energy[1]. Although there are many beautiful examples of self-assembly in peptides[2,3], polymers[4–7], and colloids[8,9], most synthetic mixtures cannot be targeted toward the wide range of structures and behaviors seen in nature. Indeed, most synthetic self-assembling systems cannot be targeted *at all*, because the interactions that drive the assembly cannot be precisely engineered and are not understood quantitatively.

There is one exception: DNA. It may seem strange to call DNA a "synthetic" system. It is, after all, the stuff of our genetic code, and its double helical structure (Figure 1a) is the key to how it replicates[10]. But the same double helix can be used to assemble structures that have no natural counterparts. In the field known as DNA nanotechnology[11], complex, three-dimensional (3D) nanostructures are routinely assembled by linking together DNA oligonucleotides with "sticky ends"[12], short single-stranded domains that hang from double helices. The sequences of these oligonucleotides can be controlled because they are synthesized directly, one nucleotide at a time.

In contrast to genetic DNA, these synthetic DNA sequences generally do not code for proteins. They do contain information, as we shall discuss, but that information encodes the strengths and specificity of the interactions that guide self-assembly. With the aid of thermodynamic models of nucleic acid hybridization, those interactions can be engineered. That quantitative link between sequence and interactions, combined with the specificity of Watson-Crick base pairing, is why DNA is so widely used in self-assembly.

Synthetic DNA nanostructures now far exceed what nature has built with DNA, at least in terms of their structural complexity (in nature, the primary function of DNA appears to be information storage, not building nanostructures). DNA has been used to build 2D crystals[13], nanotubes[14,15], 3D periodic arrays[16], and many other periodic and aperiodic structures made using DNA bricks[17,18] (Figure 1b) or origami[19–23] (Figure 1c). DNA can even be used to duplicate the functional complexity of biological and biochemical systems: reaction networks, catalysts, logic switches and circuits have all been demonstrated in systems made entirely of DNA[24,25].

But there are limits to the materials that can be made using DNA. DNA itself has no remarkable electrical, optical, or thermal properties, and although the price of synthetic oligonucleotides has decreased dramatically over time, it still remains costly to produce anything larger than laboratory-scale quantities of DNA-based materials[26]. Hence building materials with DNA requires the integration of other components.

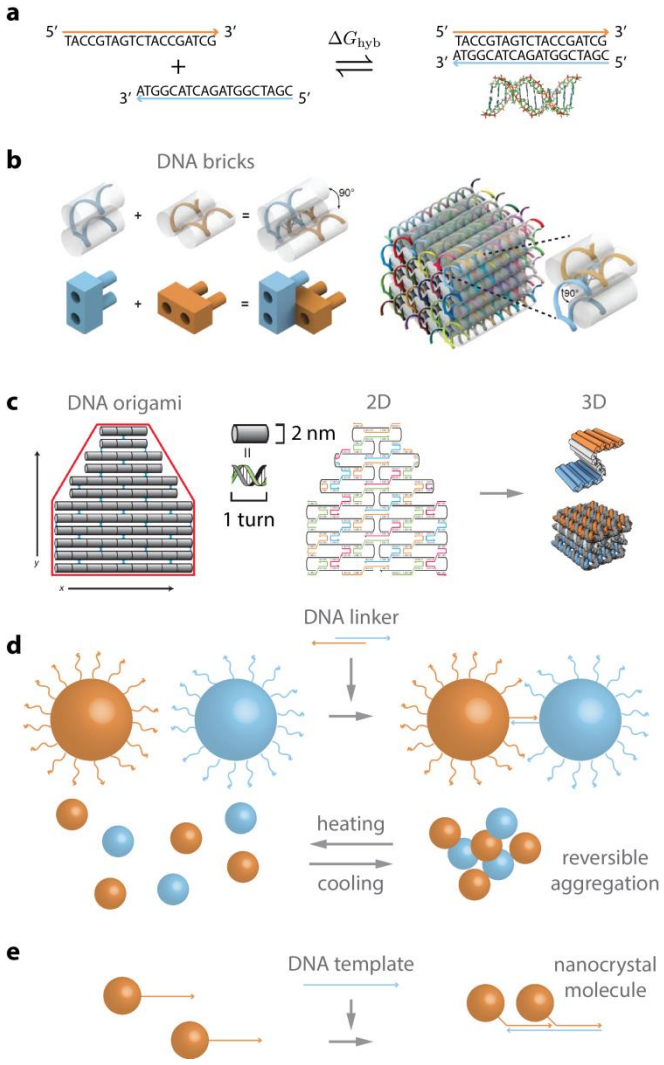


Figure 1. Examples of **DNA-mediated self-assembly.** (a) A single strand of DNA consists of a linear sequence of nucleotides with an associated direction (5’ to 3’). Single strands having complementary sequences can hybridize to form double-stranded DNA (reproduced from Wikipedia), with a change in free energy that can be predicted accurately from their base sequences and thus controlled. The base pairing process occurs in an antiparallel fashion. This simple principle has been used to fabricate materials made entirely of DNA with nanometer-scale precision, including a 3D molecular canvas made of single-stranded DNA bricks (reproduced from [17]) (b), and intricate 2D and 3D structures made by folding a viral genome using single-stranded DNA staples (diagrams reproduced from [19] and [21]) (c). Hybridization can also direct the assembly of DNA-grafted particles, but in a much cruder fashion. (d) In 1996, Mirkin *et al.*[27] showed that uniformly grafted particles could aggregate in a temperature-dependent way. (e) Early experiments by Alivisatos *et al.*[28] showed that monofunctional nanoparticles could be assembled together into “nanocrystal molecules.”

In this review, we examine how colloidal particles, including both nano- and micro-particles, can be integrated with DNA to build materials. The origin of this field lies in two papers published in *Nature* in 1996, both of which were inspired by the earlier work of Seeman and others at the forefront of DNA nanotechnology. In one paper, Mirkin and coworkers[27] showed that spherical gold nanoparticles (around 13 nm in diameter) with grafted DNA oligonucleotides could be made to aggregate reversibly with temperature, owing to the formation and melting of DNA bridges between particles (Figure 1d). The simultaneously-published paper by Alivisatos and coworkers[28] showed that small gold clusters (1-2 nm), each bearing a single DNA strand, could be assembled into dimers and trimers, which the authors termed "nanocrystal molecules" (Figure 1e).

The questions and ideas raised in these papers still echo today. Mirkin and coworkers sought to use DNA to make bulk materials. They realized that interfacing DNA with nanoparticles could produce larger structures at lower cost than could approaches based on pure DNA building blocks: the DNA directs the bulk structure, while the particles confer upon it unique electrical, optical, or structural properties. Although the community still grapples with the issues of cost and mass production today, our control over structure has advanced rapidly. Whereas the first structures were nonequilibrium, disordered gels, it is now possible to make equilibrium crystals of DNA-coated nano- and microparticles. This work has been aided by statistical mechanical models of the interactions between DNA-coated particles. We will discuss these models and the experimental advances enabling equilibrium self-assembly of bulk materials.

Meanwhile, Alivisatos and coworkers sought to control the structure of finite (as opposed to bulk) systems of particles by using multiple DNA sequences. They called these sequences "codons," harkening back to the biological role of DNA and at the same time presaging the use of these sequences in "programmable self-assembly"[29]. "Programmable" refers to the ability to prescribe an outcome—a structure or dynamical response—of a self-assembly experiment by adding information to the system, such as the sequences of the DNA strands. The connection between information, computation, and self-assembly, which is central to DNA nanotechnology[30,31], has recently begun to be explored in DNA-directed assembly of nanoparticles. We will discuss how concepts from DNA nanotechnology can be integrated with our understanding of DNA-mediated interactions to make fully programmable grafted particle systems.

Although our review will survey territory similar to that covered by other recent reviews[32–35], our aims are different. We seek to describe the concepts that link together the seemingly disparate fields of DNA nanotechnology, DNA-mediated assembly of nanoparticles, and DNA-mediated assembly of microparticles. By comparing and contrasting results across a wide range of length scales, from the molecular level to nanometers and micrometers, we uncover common physical principles. These principles are the basis of design rules that tell us how to assemble—and program the assembly of—new materials.

DNA-mediated interactions

Examining such a broad range of length scales necessitates a coarse-grained view of the interactions between the objects that are assembling, whether they are microparticles, nanoparticles, or complexes consisting of only DNA strands. In this section, we show how the molecular-level details can be subsumed into effective (or coarse-grained) interactions between components. Later, we show how these interactions govern the assembly of particles both in and out of equilibrium.

DNA-grafted particles can be prepared through a number of synthetic routes. At the nanoscale, DNA oligonucleotides are often attached to gold nanoparticles through a thiol functional group[27,36]. At the microscale, oligonucleotides can be attached to polymer or silica particles through ligand-receptor binding[37,38], physical grafting[39], or covalent attachment[40,41].

Regardless of the synthetic method, the particles attract one another through the formation of DNA "bridges." A bridge forms when a strand grafted to one particle directly hybridizes to a strand grafted to a second particle[37,42], or when the two grafted strands hybridize with a third strand called a "linker"[43,44] (Figure 2a). The physical model that we shall describe is agnostic toward the type of bridge, though we note that the linker approach offers more flexibility, as the interactions can be tuned without resynthesizing the particles (by, for example, adjusting the concentration of linkers).

We shall assume that bridges can form and break on timescales much smaller than the characteristic time of particle diffusion. Although terms like "sticky end" suggest irreversibility, most DNA-directed assembly techniques require the particles to *not* behave as if they were coated in glue. When the bridges are transient, they create a specific effective attraction between the particles. Below, starting from Watson-Crick base pairing, we describe how this effective attraction depends on the DNA sequences and the temperature.

DNA hybridization in solution

A single strand of DNA consists of a linear sequence of four types of nucleotides: adenine (A), thiamine (T), guanine (G), and cytosine (C). When two strands in solution come together, they can hybridize by base pairing (A binds T; C binds G) to form a duplex with a double-helical structure (Figure 1a). The sequence contributes to the thermal stability of this duplex through two interactions: base pairing between complementary strands, and base stacking between adjacent bases within each strand. Base stacking, and not base pairing, is the dominant stabilizing factor in the DNA double helix[45]. As a result, the overall stability of the duplex cannot be determined by simply adding contributions to the free energy from each base pair.

Instead, we must consider the effect of neighboring nucleotides. The nearest-neighbor (NN) model of DNA hybridization predicts the free energy change of hybridization ΔG_{DNA} when two short, complementary oligonucleotides (A and A*) form a duplex AA*. It is based on empirical measurements of free energies for many common hybridization motifs[46,47], such as Watson-Crick base pairs, internal mismatches, dangling ends, loops, bulges, and hairpins. The equilibrium concentration of AA* as a function of temperature

can be predicted analytically from ΔG_{DNA} using a two-state reaction model, A+A*⇄AA*, or more advanced algorithms[48,49], many of which are available as web-based services[50–52]. Because ΔG_{DNA} varies approximately linearly with temperature, the concentration of AA* increases with decreasing temperature, as the entropic penalty of forming a duplex becomes less significant than the enthalpic gain[46,47].

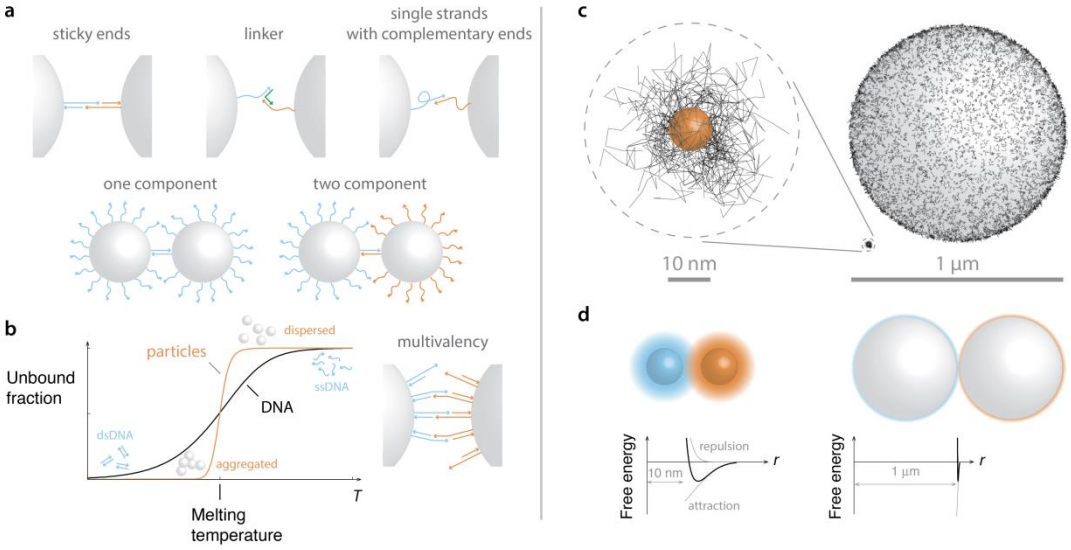


Figure 2. **DNA hybridization induces an effective interaction potential between DNA-grafted particles.** (a) DNA-grafted particles experience an effective attraction due to bridge formation, which can be induced by direct hybridization of grafted double-stranded DNA with dangling "sticky ends," binding of a partially complementary linker strand from solution, or hybridization of grafted single strands having complementary ends. A one-component system consists of particles grafted with the same DNA sequences; a two-component system consists of particles bearing different DNA sequences. (b) Unlike hybridization of DNA in solution, which is a weak function of temperature, a suspension of DNA-grafted particles transitions from a dispersed state to an aggregated one over only a few degrees Centigrade, a result of multivalency in the interactions. The range of the DNA-induced interaction between particles depends on the length of the grafted strands relative to the particle size. (c) For DNA-grafted nanoparticles, the length of the DNA is roughly equivalent to the size of the particles, but for DNA-grafted microparticles it is only about one percent of the particle diameter. As a result, the interaction free energy between DNA-grafted nanoparticles has a relatively soft repulsion and a wide potential minimum, while DNA-grafted microparticles interact more like "sticky spheres" (d).

Interactions between DNA-grafted particles

Hybridization of grafted strands is the basis of the attraction between DNA-grafted particles. Yet the attraction between the particles is different from that between two strands. In experiments one observes that the melting temperature of particles grafted with complementary strands (defined as the temperature where half of the particles are aggregated) can be lower or higher than the melting temperature of the DNA strands in solution[38]. Furthermore, the melting transition of the particles is steeper (Figure 2b), owing to the "multivalency" of the particles—the ability to form more than one DNA bridge at a time (Figure 2b). Because the temperature dependence of the effective attraction is critical to controlling self-assembly, several models have been developed to relate it to both the density of grafted strands, which controls the multivalency, and NN predictions of the hybridization free energy, which controls the strength of each DNA bridge.

A common physical picture links these models together: transient formation of bridges pulls the particles together, while compression of the grafted DNA molecules pushes them apart. Both effects arise from fluctuations at molecular scales. We can therefore think of the particles as interacting through a time-averaged effective potential that, like a Lennard-Jones potential, has a minimum arising from attractive and repulsive components (Figure 2c,d). However, because it averages over many molecular degrees of freedom, the effective potential is not a true potential energy but rather a free energy $F(r)$ that depends on temperature.

Now let us consider a state in which the particles are separated by a distance r and are at constant temperature. The attractive component of the effective potential is $F_a(r) = -k_B T \ln Z(r)$, where $k_B T$ is the thermal energy, and $Z(r)$ is a partition function that accounts for all the possible combinations of DNA bridges, along with their Boltzmann factors. Our reference state is one in which no bridges form. Because there is only one way to form this unbridged state, the probability of observing the system with no bridges is $P_{\text{unbridged}}(r) = 1/Z(r)$. Therefore

$$F_a(r)/k_B T = \ln P_{\text{unbridged}}(r) \qquad (1)$$

If we assume that p, the probability of a bridge forming, is spatially uniform and independent of other bridges forming, then $P_{\text{unbridged}} = (1-p)^N$, where N is the total number of bridges that can form. As temperature decreases, p increases, because the free energy of hybridization ΔG_{DNA} decreases linearly with temperature.

This model explains why the melting transition is so steep: because the particles are multivalent (meaning N is typically much greater than 1), even a small increase in the probability of bridge formation leads to a large decrease in $P_{\text{unbridged}}$. Practically, this means that the attraction between particles varies from negligible to irreversible over a window of only a few degrees Centigrade.

Theory versus experiment

The model described above can be made quantitative by accounting for the density of DNA strands. In 2005, Biancaniello, Kim, and Crocker[43] measured the effective potential between two micrometer-scale particles and modeled their measurements using equation

(1) in the limit of low hybridization yield and large N. To obtain p, they assumed that chemical equilibrium between single strands and duplexes is established locally in space, and they calculated the effective concentration of grafted DNA strands from their surface density, assuming that the strands acted as tethered, freely jointed chains. Although they were able to fit the model to their measurements, the fitted ΔG_{DNA} was much larger than that predicted by the NN model. Also, the predicted temperature dependence, though steep, did not agree quantitatively with experiment. In 2011, Rogers and Crocker[53] refined the model to account for how the concentration of single strands decreases when some of the strands hybridize. Their model quantitatively captured the magnitude and temperature dependence of the measured pair potential. The only inputs to the model were the DNA sequence, persistence length, grafting density, and ionic strength.

Other approaches to modeling DNA-mediated interactions explicitly consider the configurations of the DNA strands. The first such model, developed by Licata and Tkachenko[54] and later tested in experiment by Dreyfus *et al.*[38,55], treated the grafted DNA molecules as tethered rigid rods and ignored any correlations arising from competition between neighboring strands. However, the model did account for how the interaction between the particles is affected by the reduction in configurational entropy of the DNA strands upon binding. Dreyfus *et al.* found that the model was able to reproduce the experimentally-observed steep melting transition, though it did not accurately predict the melting temperature. More complete descriptions of the statistical mechanics of binding between grafted molecules, including the correlations arising from competition for binding, were later developed by Frenkel and collaborators[56–59]. For single-stranded grafted DNA, these models and the continuum approach of Rogers and Crocker agree with each other and with experiment to within the uncertainty of NN estimates of ΔG_{DNA}[60,61]. For other grafted constructs, such or surface-mobile strands[62,63] or the double strands with sticky ends used by Dreyfus *et al.*, the configurational entropy penalty might play a more significant role. However, the various approaches have not yet been tested against direct measurements of the pair interaction in such systems.

Nonetheless, because all of these coarse-grained models predict the same thermal response and interaction potential, we would argue that the interactions between DNA-labeled particles are understood. This understanding allows us to predict the phase behavior[64–67] from experimental variables such as sequence, linker design, grafting density, and particle size. There are some nuances when the size of the particles becomes comparable to the DNA size, in which case the interactions are typically not pairwise additive[57,68,69]. This situation arises for small nanoparticles. Because it is difficult to directly measure the interaction potential in such systems, models are typically tested against experimental measurements of the equilibrium phase diagram. Here the predictions of models that coarse-grain the DNA strands to different extents[57,68,70,71] qualitatively agree with experiment. Quantitative agreement will require further experiments that can better constrain the model parameters. But overall, the current data validate the concept of transient bridging giving rise to effective interactions.

Equilibrium self-assembly

Understanding the effective interactions gives us insights into how to design DNA-grafted systems that can self-assemble in equilibrium. We can see, for example, that temperature control is important: the original demonstrations of DNA-mediated assembly of particles in 1996 produced non-equilibrium aggregates because they were done at temperatures well below the melting point, where the attraction strength is many times $k_B T$. In 2005, Biancaniello *et al.*[43] crystallized DNA-grafted microparticles by incubating the particles for days at a temperature just below the melting point. Similar annealing protocols were used in 2008 by Nykypanchuk *et al.*[42] and Park *et al.*[72] to crystallize DNA-grafted nanoparticles. Recently Auyeung *et al.*[73] showed that slow cooling can be used to make macroscopic single crystals containing more than a million DNA-grafted nanoparticles.

Criteria for equilibrium

Temperature is only one of the variables that need to be controlled to achieve equilibrium self-assembly; it is also necessary to minimize non-specific interactions such as van der Waals forces. Typical assembly experiments use salt concentrations on the order of 100 mM to screen the electrostatic repulsion between DNA phosphate backbones, thereby promoting efficient hybridization. However, such high salt concentrations also screen the electrostatic repulsion that stabilizes the particles against aggregation. Most current experiments involving gold nanoparticles therefore use a protocol developed by Hurst, Lytton-Jean, and Mirkin[36], which gradually increases the surface density of DNA strands through stepwise addition of salt and DNA. This protocol ensures that at any salt concentration there is a sufficiently high grafting density of DNA strands to sterically stabilize the particles.

High grafting density also ensures that the kinetics of binding are sufficiently fast to achieve equilibrium. As we have discussed, equilibrium self-assembly requires the attractions between particles to be a few $k_B T$ deep. However, this condition is necessary but not sufficient. Say, for example, that we have particles with a small number of strands that bind strongly (corresponding to low N and large p in equation (1)). The effective attraction will be weak, but the lifetime of each DNA bond will be large, which can hinder rotational diffusion—and therefore equilibration—of the particles.[74] Equilibration therefore requires particles with a high number of strands that bind weakly. This is why newly developed grafting methods[40,41] aim to achieve high grafting density. In addition, most experiments now use short binding domains (4-6 bases), which ensures that the kinetics of bridge formation and breaking are fast.

Finally, using a flexible tether between the particle surface and the binding domain of the DNA strand can increase the separation between the particle surfaces, thus reducing the van der Waals attraction. Biancaniello *et al.*[43] found that particles with DNA strands attached to a polymer tether were able to crystallize, whereas those in which the DNA was covalently attached to surface functional groups did not. Also, both Nykypanchuk *et al.* and Park *et al.* were able to make crystals only when the sticky ends were separated by a long DNA tether from the particle surfaces.

Crystallization

Most equilibrium experiments on DNA-grafted particles have focused on assembling crystal lattices. Macfarlane and coworkers[75,76] developed a rule, which they called the "complementary contact model," to explain which lattice should form in equilibrium. The rule states that the equilibrium lattice is the one that maximizes the contacts between spheres that have complementary strands. Here we summarize the experiments that have informed this rule, and we explain how the results of these experiments—and the rule itself—can be understood in terms of effective interactions.

First we note that, despite some minor differences, the equilibrium structures formed by nanoscale and microscale particles look strikingly similar. Nykypanchuk *et al.*[42] and Park *et al.*[72] showed that two "species" (which we call A and B) of gold nanoparticles, each of which contains strands with domains complementary to those on the other, crystallize into a cesium chloride (CsCl) lattice (Figure 3). The CsCl structure consists of two interpenetrating simple cubic lattices and is equivalent to a body-centered cubic (BCC) lattice if we do not distinguish the particles. Later, Casey *et al.*[77] showed that the same lattice is formed in a two-component system of polymer particles that are 10-100 times larger than the gold nanoparticles of Nykypanchuk *et al.*[42] and Park *et al.*[72].

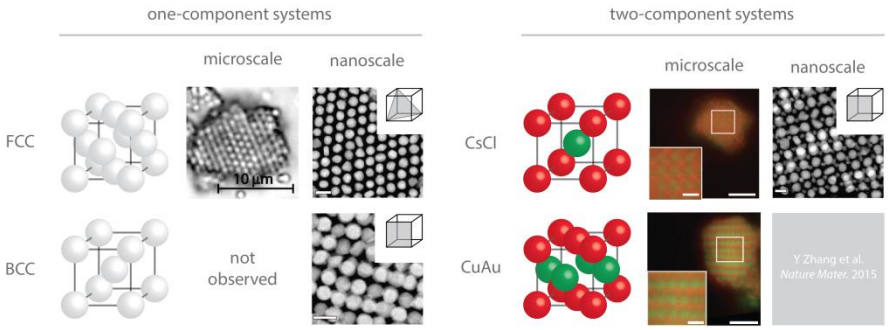


Figure 3. **Equilibrium phase behavior of simple systems is similar at the nanoscale and microscale.** The first successful attempts using DNA to direct the assembly of colloidal crystals resulted in structures with simple ionic and metallic lattices. One-component systems first assembled into face-centered cubic (FCC) crystals, both at the microscale (micrograph reproduced from [43]) and nanoscale[72] (micrograph reproduced from [75]). Two-component systems without self-complementary interactions produced structures with the cesium-chloride (CsCl) lattice at the microscale (micrograph reproduced from [77]) and nanoscale[42,72] (micrograph reproduced from [75]). Increasing the degree of self-complementarity in the two-component systems led to the formation of crystals having the copper-gold (CuAu) symmetry through a diffusionless transformation from CsCl-parent crystals (micrograph reproduced from [77]; see also [148]). A single-component system of nanoparticles grafted with flexible strands self-assembles into a body-centered-cubic (BCC) structure[78] (micrograph reproduced from [75]). The BCC structure has not been observed in DNA-grafted microparticles, likely because the interaction range is short.

Interestingly, the CsCl structure is not the only structure that can form. Another possible equilibrium structure is the copper-gold (CuAu) lattice (Figure 3), in which each particle has eight neighbors of the opposite type, just as in the CsCl lattice (the CuAu structure is equivalent to a face-centered cubic (FCC) lattice if we do not distinguish the particles). Using both theory and experiment, Casey *et al.*[77] incorporated both A-A/B-B attractions and A-B attractions into their system. They found that the CuAu lattice was thermodynamically favored over CsCl for all but the weakest A-A/B-B attraction strengths.

How can we understand the relative stabilities of CuAu and CsCl? A key difference between the two lattices is that in CuAu, each particle has four neighbors of the same type in addition to eight neighbors of the opposite type. So if the A-A/B-B interactions are significant, the complementary contact rule tells us that the CuAu structure should be favored, since it has more interacting pairs of particles.

A wide variety of other crystal structures can be obtained in both one- and two-component systems of particles by varying not only the interaction strengths, but also the particle sizes. Using particles grafted with only a single DNA sequence per species, Macfarlane and coworkers[75,76] assembled eight different crystal structures with symmetries ranging from face-centered cubic (FCC) to Cs_6C_{60}, which has a unit cell containing 23 particles. Later, Wang and coworkers[40] directed the self-assembly of colloidal crystals having many of the same symmetries, but which were composed of polymer spheres 100 times larger. The crystal structures at both of these scales can be explained by the complementary contact rule.

However, there are exceptions to the rule. Scarlett and coworkers[67] found that the CsCl structure is favored over the CuAu structure for small but non-zero (~0.3 k_BT) A-A/B-B attraction strengths, even though CuAu would maximize the number of touching spheres. Also recent work by Thaner and coworkers[78] found that for certain kinds of grafted constructs, BCC (eight nearest neighbors; see Figure 3) is favored over FCC (12 nearest neighbors) in a single-component system of nanoparticles.

These exceptions, and the complementary contact rule itself, can be understood in terms of the effective potential. The equilibrium structure of the entire system (particles and DNA included) is the one that minimizes its free energy $F = U - TS$, where U is the total effective potential—the sum of all the time-averaged interactions. In most cases, F can be minimized by maximizing the number of attractive pair interactions between particles, thus minimizing U. This is the basis of the complementary contact rule.

Exceptions to the rule must involve cases where either the interactions are long-ranged, or where the entropic contribution TS is significant. If the potential is long-ranged, U is no longer proportional to the number of nearest-neighbor pairs. When next-nearest-neighbor interactions are possible, BCC should be favored over FCC[79], perhaps explaining the results of Thaner *et al.* Other exceptions arise when TS is large. Because the configurational entropy of the strands is included in the effective potential—and is therefore part of U, not S—any remaining entropy must come from collective degrees of freedom of the crystal. Thus for certain interaction ranges and strengths the CsCl lattice, which has "soft modes" with high vibrational entropy, should be favored (as Casey, Scarlett and coworkers[67,77] found) over CuAu, which does not.

Contrasting the nanoscale and microscale

Thus far we have emphasized the similarities between the equilibrium self-assembled structures formed by nanoscale and microscale structures. However, there are differences between the two scales that might be important in future experiments and applications.

For example, there are differences in the particle shapes that can be made at different scales. The experiments we have discussed all use spherical particles, but this need not be the case. Gold, the material of choice for DNA-functionalized nanoparticles, can be synthesized in a wide variety of shapes, from nanorods to polyhedra, through control over the growth of different crystal planes[80–84]. At the microscale, most materials that can be functionalized with DNA are amorphous polymers or glasses[41]. Several synthetic techniques have been developed to make nonspherical particles from these materials, including sphere doublets[85], clusters[86,87], silica-coated polyhedra[88,89] as well as spherical particles with indentations[90,91]. Thus at both the nanoscale and the microscale there are many different shapes of particles that can be functionalized with DNA, but the intersection between these two sets of shapes is small.

The material properties—and in particular, the optical properties—of the self-assembled structures will also differ between the two scales. Metallic nanoparticles have plasmonic resonances in the visible and near-infrared part of the spectrum that can be exploited in optical metamaterials[92–95] or light-harvesting structures[96]. Polymer microparticles have Mie resonances in the visible spectrum that are useful for photonic crystals[97] or other strongly scattering materials such as paints and coatings[98,99].

Neither of these differences changes our understanding of how DNA-grafted particles self-assemble. For example, if a particular particle shape could be made at both the nanoscale and microscale, then in general we would expect the equilibrium self-assembled structures to be the same at both scales. However, in certain cases the range of interaction may lead to differences in phase behavior. The range of interaction between two nanoparticles coated with 50-base-long grafted DNA strands is comparable to the diameter of the nanoparticles. In contrast, the range of interaction between two microparticles with the same strands is only about 1% of their diameter (see Figure 2c,d). Therefore a BCC crystal might be stable in a one-component system of nanoparticles but metastable for microparticles[79]. The kinetics of phase transitions should also differ between the two scales, because the critical nucleus size depends on the interaction range[100]. Thus it might be possible to form some crystal phases at the nanoscale but not at the microscale (or *vice versa*), either because the structures are thermodynamically unstable or kinetically inaccessible.

Non-equilibrium self-assembly

As discussed above, achieving equilibrium self-assembly of DNA-grafted particles requires control over the temperature, grafting density, and DNA sequences. But if our goal is to assemble particles *out* of equilibrium, the sensitivity of the interactions to these variables is a useful feature, because it allows us to tune the interaction strengths and kinetics of bridging over a wide range. For example, a small change in temperature changes the strength of the effective attraction by tens or even hundreds of $k_B T$[55].

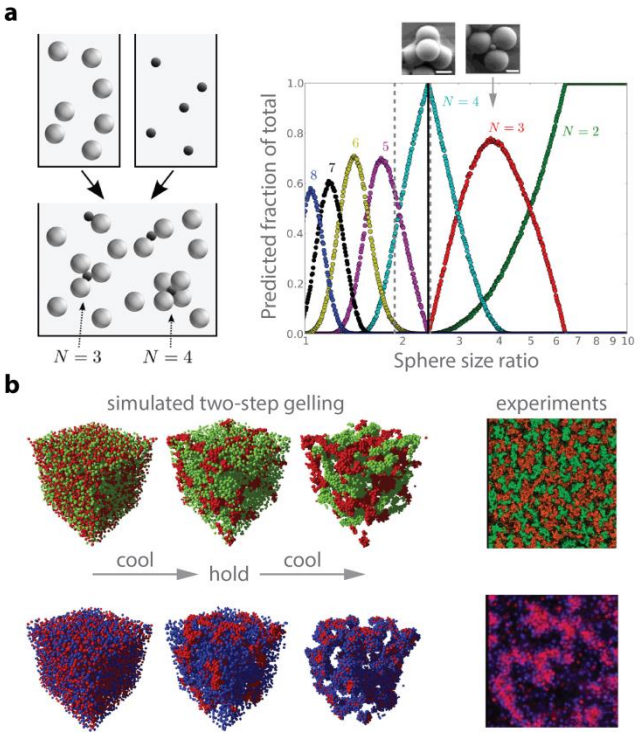


Figure 4. **Non-equilibrium routes to assembly produce colloidal clusters and bicontinuous gels.** (a) Bidisperse mixtures of DNA-grafted particles prepared well below their melting temperature can form finite-sized aggregates: the large particles 'park' on the surface of the smaller ones. The size *N* of the resulting clusters can be tuned by changing the size ratio between the two species (inset shows clusters of size *N*=3 and *N*=4) (plot reproduced from [102]; micrographs from [101]). (b) Colloidal gels having unique topology can be prepared from binary mixtures of DNA-grafted colloidal particles through a hierarchical-assembly approach. First a gel composed of one species of DNA-grafted particles having a high melting temperature is assembled; then the temperature is quenched and a second gel is formed from the second species, which either penetrates the pores of the high-temperature gel (top) or coats its surface (bottom) (reproduced from [105]).

What distinguishes DNA-mediated interactions from other strong interactions such as van der Waals forces is their specificity. With interactions that are both strong and specific, one can control the structure of non-equilibrium aggregates. For example, binary mixtures of DNA-coated particles with strong and specific interactions can be made into clusters with well-defined morphologies (such as tetrahedra) through random aggregation or "parking"[101,102] (Figure 4a). Since the interactions are too strong to allow the system to relax to equilibrium, growth is halted before the system can assemble into a bulk phase. This approach might be useful for making plasmonic clusters for use in optical metamaterials[92,103,104]. DNA can also be used to assemble gels with controlled structure (Figure 4b). Di Michele and coworkers[105] showed that by adjusting the thermal schedule

and relative rates of aggregation between different species of DNA-grafted particles, they could engineer the correlations between particles to yield, for example, bicontinuous gels. The technique of Di Michele *et al.* could be used to make bulk materials with controllable porosity and tortuosity.

Furthermore, at fixed interaction strength, the nucleation, growth, and annealing of self-assembled structures[66,75,106] can be modulated by controlling the kinetics of bridge formation and rupture[74,75]. For example, the kinetics can be tuned to trap compositional defects in a growing crystal. As shown by Park *et al.*[72] in experiments and later by Scarlett *et al.*[67] in simulation, slowly cooling a binary system of DNA-grafted nanoparticles results in compositionally disordered FCC crystals, whereas quenching and annealing the same system leads to the expected CsCl-type crystals. A similar mechanism is thought to explain the hexagonally close-packed (HCP) crystals of DNA-grafted nanoparticles observed by Macfarlane *et al*. They found that decreasing the DNA surface density, and thus slowing the kinetics of bridge formation and breakage near the melting temperature, led to HCP crystals instead of the more thermodynamically-stable FCC[75].

Kinetics also plays an important role in selecting the final self-assembled state when there are several possible degenerate states. Jenkins and coworkers[107] found that hydrodynamic correlations between particles favor specific pathways between crystal structures in systems of DNA-grafted microparticles. They examined an earlier experimental result from Casey *et al.*[77] showing that crystals with the CsCl structure convert to the CuAu structure through a diffusionless transformation, analogous to a Martensitic transformation in metals. The hydrodynamic model of Jenkins *et al.* explains why only the CuAu structure forms out of the myriad possible states with randomly-stacked hexagonal planes.

Towards programmable self-assembly

The examples of equilibrium and non-equilibrium assembly we have seen thus far demonstrate simple *programmable* self-assembly schemes. By "programmable" we mean that information is added to the system to direct the assembly. For instance, in the case of equilibrium crystals in two-component systems, the information is the DNA sequences grafted to the two different species of particles. By analogy to computer programming, the sequences act as "source code" that is "compiled" into a set of interparticle interactions ("machine code") that dictate the structure and function of the assembled material. The assembly is programmable if and only if we understand how the inputs are compiled into interactions. When this is the case—as it is for DNA-grafted particles, because we have a statistical mechanical model of the effective interactions—the experimenter can determine what changes in input will produce the desired output behavior.

But our ability to program the self-assembly of colloidal particles using DNA is still at a primitive level. The number of specific interactions (species) and the resulting crystal structures do not yet go beyond those found in other colloidal systems, such as binary suspensions of oppositely charged colloidal particles[108] or one- and two-component systems of particles interacting through excluded volume[109]. In comparison, DNA

nanotechnology makes use of thousands of different components (unique DNA strands) to create complex self-assembled structures. Furthermore, DNA nanotechnology can produce structures that go far beyond simple periodic arrays[110,111] through algorithmic assembly schemes making use of logic gates that are implemented through DNA sequences[112,113].

How can we extend the programmability of DNA-grafted particles so that they can self-assemble not just into crystals, but into any prescribed structure? In the following subsections, we explore how DNA nanotechnology can help us reach this goal. First, we show how lessons and design rules learned from DNA nanotechnology can be applied to the particle scale; second, we examine how the programmability of DNA-grafted particles can be extended through direct incorporation of DNA nanotechnology constructs.

Specificity for programming structure

The remarkable feature of DNA-mediated interactions is that the number of distinct species in the system can be as large as the total number of "building blocks" (which might be either DNA constructs or DNA-grafted particles). Furthermore, the sequences can be designed to control all the possible interactions between the various species (Figure 5a). With such control, it is possible to program the self-assembly of a target structure by first breaking it down into subunits, then determining how many unique species are needed, and finally mixing various species of building blocks that bind only to their neighbors. In this scheme, each building block is encoded with information about where it must go in the final structure.

Surprisingly, this "maximal specificity" scheme works for assembling complex 3D structures, as has been demonstrated in experiments on DNA bricks[17,18] (Figure 5a). We say "surprisingly" because, as noted by Jacobs, Reinhardt, and Frenkel[114], there are many ways in which such a scheme can go awry. For example, in the presence of any weak non-specific interactions, partially formed target structures can aggregate together. Yet simulations and experiments show that even thousands of distinct DNA bricks can reliably self-assemble into prescribed structures with high yield.

The key to achieving high yield appears to be the annealing protocol, which in experiments is generally tuned empirically. Simulations by Jacobs, Reinhardt, and Frenkel[114,115] show that at high temperatures, there is a free-energy barrier to nucleating a precursor to the target structure. As the temperature is lowered, each nucleated precursor slowly grows until it forms the full target structure. Interestingly, in this scheme, the target need not be the thermodynamically stable product at *any* temperature. Although the precursor to the target must be thermodynamically favored in the nucleation regime, at lower temperature the favorable state is likely an aggregate of these partially-formed targets. Avoiding aggregation requires an annealing protocol that ensures that growth takes place by addition of free building blocks; thus it is crucial to tune the nucleation conditions and cooling schedule.

Can similar schemes be applied to DNA-grafted particles? Simulations have shown that maximally-specific systems of such particles can indeed be programmed to assemble into target structures with high yield. Hormoz and Brenner[116] examined the yield of self-

assembly in the simplest multicomponent colloidal system, isotropic particles of the same size interacting through specific interactions. Their calculations showed that specific, finite sized structures having various degrees of symmetry could be formed with near perfect yield. Moreover, they showed that the yield is maximized when the interactions are maximally specific. Zeravcic, Manoharan and Brenner[117] later examined how different kinds of defects affect the yield and complexity of structures assembled from maximally specific systems. Their simulations showed that arbitrary structures, including a 69-particle model of Big Ben (Figure 5a), could be formed with high yield using this scheme.

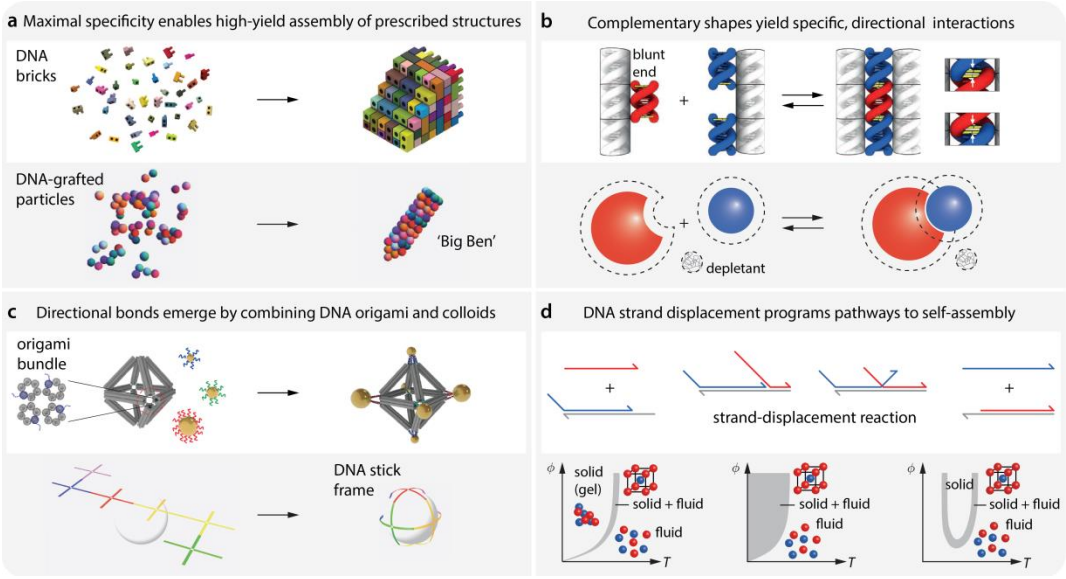


Figure 5: **Forms of information for programming self-assembly.** (a) Specificity: DNA sequences can be used to create orthogonal interactions between many species. By controlling these interactions the experimenter can constrain the allowed contacts between components and dramatically enhance the yield of prescribed structures, as shown in the self-assembly of DNA 'bricks' (adapted from [17]) and in a simulation of the assembly of "Big Ben" from spherical microparticles (adapted from [117]). (b) Shape complementarity: Components with complementary shapes can further constrain the connectivity to yield specific, directional interactions, as seen in blunt-end stacking between DNA bundles (adapted from [130]) and lock and key interactions between colloidal particles. (c) DNA nanostructures: Combining DNA origami with colloidal self-assembly enables the assembly of nanoparticle clusters with prescribed symmetry and handedness through emergent directional interactions (adapted from [131]); wireframe cages could allow similar assembly at the microscale. (d) DNA strand displacement: Programmability can be extended to the thermal response of colloidal phases by modulating the thermodynamics of the bridge-formation process with DNA strand displacement (adapted from [133]).

These simulations avoid the problem of aggregation of nuclei by starting with a system that can form only one copy of the target structure. Thus the assembly can take place under equilibrium conditions at constant temperature. Later simulations by Halverson and Tkachenko[118,119] showed that particles with both specific and directional interactions can assemble into multiple copies of aperiodic target structures in bulk. The directional interactions (which we will have more to say about in the next subsection) can enhance kinetics, because they prevent unwanted metastable configurations from forming. Again, however, these simulations did not examine the nucleation regime; instead they started with a suspension of nuclei whose structures were subsets of the target.

The programmed assembly of DNA bricks provides some important lessons for programming the assembly of DNA-grafted particles. In previous sections we have focused on the conditions for achieving equilibrium assembly or assembly far from equilibrium, but DNA bricks show that it is beneficial to work in between these two extremes, using conditions that shift continuously. Furthermore, the experiments and simulations show that maximal specificity is a design rule for programming the assembly of complex 3D structures.

In one respect, maximal specificity in DNA-grafted particles might be easier to achieve than in DNA bricks. Because the binding between particles is multivalent, a small change in the hybridization free energy can produce a large change in the strength of interaction between two particles. Thus each particle can be synthesized with many different specific interactions through small changes in sequence. This was discussed by Wu and coworkers[120], who argued that microscale particles could be made with more than 40 different orthogonal interactions. However, it remains a challenge to make particles in which all the different interactions have the same temperature dependence.

Programming with directional interactions

Recent synthetic schemes have aimed at producing particles that, like DNA bricks, have both specific and directional interactions (Figure 5b). At the micron-scale, directionality can be enforced by chemically patterning particles with patches of DNA. This approach has been achieved either through direct synthesis[121] or by selective protection and crosslinking of DNA linkers on otherwise isotropically labeled particles[122]. At the nanometer scale, directional interactions can be imprinted by similar methods[123] but can also arise naturally from anisotropy in the particle shape: specific orientations between particles, which typically involve the alignment of crystal facets, can be favored enthalpically if they permit the formation of additional DNA bridges, or entropically if they increase the configurational freedom of the system[124–126].

Experiments have only just begun to explore the variety of structures that can be assembled from these new kinds of particles. These particles cannot yet mimic DNA bricks; there is still no way to make hundreds or thousands of unique species of partices with specific and directional interactions. But there are interesting questions that can be addressed with existing systems and with the help of theory and simulation. For example, given a target structure, what is the smallest degree of specificity and the simplest directional interactions needed for it to assemble in high yield? Resolving such questions

would help establish rules linking the complexity of a structure to the type and amount of information required to program its assembly.

Again, there are lessons to be learned from DNA nanotechnology. A number of recent studies have explored the use of "blunt-end" interactions between double-stranded DNA (dsDNA). These interactions arise from base stacking and not base pairing[127]. Nonetheless, they are highly specific to the shapes of the dsDNA complexes. One geometry involves end-on linkage of shape-compatible, jagged bundles of double helices[127–129], while another involves lateral connection between bundles of double helices with shape-compatible "plugs" and "holes"[130]. In this second case, each plug is formed by a pair of blunt-end-terminated double helices that decorates the lateral face of a bundle, while the corresponding hole is formed by flanking pairs of blunt-end-terminated double helices that decorate the lateral face of the shape-compatible bundle (Figure 5b). This strategy enables construction of an interface that spans a large area and yet is maintained by a modest number of weak interactions. In this way, significant directional control between interaction partners can be achieved, while at the same time the interface can be disrupted with a small and programmable energy input.

Thus, "shape complementarity" can be used in addition to sequence complementarity to program self-assembly. In fact, shape-specific interactions were demonstrated previously in "lock-and-key" colloidal particles. Such particles contain concave regions that can bind to convex regions of other particles in the presence of a depletion force (Figure 5b). The interactions are specific to the curvature: the lock particles and key particles maximize the depletion interaction when they mate. Experiments on blunt-end dsDNA show that this principle can be extended to create multiple orthogonal interactions that can program assembly. Taken together, these results show that it may be possible to make maximally specific DNA-grafted particle systems by using a combination of shape- and sequence complementarity. Using both types of specificity might make it easier to synthesize maximally specific DNA-grated colloid than using either type alone.

DNA nanotechnology meets colloidal self-assembly

Above, we discussed how to transfer knowledge about programmable assembly from DNA nanotechnology to DNA-grafted particles. Here we examine how combining constructs from DNA nanotechnology with colloidal particles can enable assembly schemes that are not easily realizable with either system alone.

For example, DNA origami can behave as a core around which to organize spherical nanoparticle satellites, each isotropically coated with DNA strands[94,95,131] (Figure 5c). For example, Tian and coworkers[131] self-assembled a DNA octahedron with 30-nm edge lengths and unique DNA strands displayed on each of its six vertices, then showed that it could programmably capture gold nanoparticles. Because each satellite can be programmed to display its own unique sequence, these core-satellite assemblages are the closest analogues to DNA bricks at the particle scale. They could be used to assemble open lattices or finite structures with prescribed symmetry and handedness.

Could such schemes be extended to micrometer-scale particles? DNA wireframe cages that can wrap around such particles will be floppy and lack structural integrity unless the edges are made thick, which makes them challenging to assemble. But an interesting

synergy might emerge from the combination of DNA origami and microparticles: a DNA cage wrapped around a microparticle provides addressability to the particle, while the particle could give structure to the cage. One strategy to achieve this assembly is to decorate a DNA stick frame with short single-stranded handles that can link reversibly to complementary strands decorating the colloidal sphere (Figure 5c). In a second step, the terminal branches could be linked by connecting strands. The sphere may help by restricting the fluctuations of the stick frame to its 2D surface.

This concept provides an example of how DNA nanotechnology and colloidal particles might be integrated to yield behavior—in this case, a combination of rigidity and addressability—that is not easily achieved at either scale alone. A related strategy is to use structural DNA nanotechnology to fabricate cavities that are small enough to exclude crowding agents (for example, polyethylene glycols above a certain size). Such cavities could therefore be driven to join with shape-compatible plugs by depletion forces induced by those crowding agents. The plugs and sockets could be implemented on colloidal particles to endow them with lock-and-key interactions.

Programmability need not be limited to the structural domain. Roldán-Vargas and colleagues[132] showed in simulation that competitive binding in a mixture of particles with directional interactions could program the thermal response of gels. Rogers and Manoharan[133] later implemented a similar scheme in experiment using isotropic, DNA-mediated interactions. They used strand displacement[25,134], a tool borrowed from dynamic DNA nanotechnology, to make systems in which soluble single strands can interfere with bridge formation. By controlling the thermodynamics of the strand displacement reactions, they showed that the phase diagram could be programmed in various ways, for example to include low-temperature fluid phases that would crystallize upon heating (Figure 5d). The ability to control the thermal response could prove useful for programming new pathways in self-assembly, which could enable the synthesis of materials that cannot be formed through a single, equilibrium phase transition.

Future work might focus on “active” or dynamic nanosystems that self-organize through energy-dissipating processes—for example, by consuming molecular “fuel.” Such systems have been designed at both the colloidal[135–137] and DNA scale[25,138], but the union of the two scales remains largely unpopulated. The combination of DNA nanotechnology and colloidal particles could be used to produce materials that correct errors during assembly[139,140], move in preprogrammed ways[141,142], or self-replicate[143–145].

Conclusions and perspective

After nearly 20 years of research, the field of DNA-directed colloidal assembly has arrived at a firm footing in terms of chemistry, physics, and engineering: synthesis methods have been refined, models of interactions and collective behavior have been validated, and design rules for equilibrium and non-equilibrium structures have emerged. These design rules are based on our understanding of how the effective interactions between particles emerge from the transient formation of DNA bridges: for example, to achieve equilibrium assembly, we must use short binding sequences and high grafting densities so that the kinetics of particle rearrangement are fast.

New design rules adopted from DNA nanotechnology point the way toward programmable self-assembly of DNA-grafted particles. One such rule is maximal specificity. The most straightforward approach to making maximally specific DNA-grafted colloids will likely involve both sequence-complementarity and shape-complementarity. Another rule, which applies to maximally specific systems, is to control nucleation of target structures and then to prevent aggregation of partially-formed targets by cooling, so that growth of the programmed structures takes place by addition of single particles.

The field is now moving toward integrating other constructs from both structural and dynamic DNA nanotechnology, which should allow us to do more than is possible at either scale alone. One potential area of synergy is making materials. Although there are many ways to assemble colloids, directing the assembly with DNA allows us to decouple the interactions between particles from their composition and to tune the distance between particles independently of their size. These advantages are important for synthesizing materials with unique photonic and plasmonic properties. There are of course constraints on the mass-production of such materials: processing must be done in aqueous media, and a large amount of DNA will be required. But many chemical processes are already moving to "greener" solvents such as water[146], and enzymatic amplification techniques may make mass production of oligonucleotides with specific sequences cost-effective[147].

All of this is to say that the field is poised to grow further. Not only are there enormous possibilities and challenges for applications, but there are also new scientific territories to explore: nonequilibrium assembly, active systems, and the fundamental limits on what can be made through self-assembly. The foundations have been laid; now the fun begins.

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References:

1. Whitesides, G. M., Mathias, J. P. & Seto, C. T. Molecular self-assembly and nanochemistry: a chemical strategy for the synthesis of nanostructures. *Science* **254,** 1312–1319 (1991).
2. Zhang, S. Fabrication of novel biomaterials through molecular self-assembly. *Nat. Biotechnol.* **21,** 1171–1178 (2003).
3. Hernandez-Garcia, A. *et al.* Design and self-assembly of simple coat proteins for artificial viruses. *Nat. Nanotechnol.* **9,** 698–702 (2014).
4. Bita, I. *et al.* Graphoepitaxy of Self-Assembled Block Copolymers on Two-Dimensional Periodic Patterned Templates. *Science* **321,** 939 –943 (2008).
5. Ruiz, R. *et al.* Density Multiplication and Improved Lithography by Directed Block Copolymer Assembly. *Science* **321,** 936 –939 (2008).
6. Mai, Y. & Eisenberg, A. Self-assembly of block copolymers. *Chem. Soc. Rev.* **41,** 5969–5985 (2012).
7. Hu, H., Gopinadhan, M. & Osuji, C. O. Directed self-assembly of block copolymers: a tutorial review of strategies for enabling nanotechnology with. *Soft Matter* **10,** 3867–3889 (2014).
8. Glotzer, S. C. & Solomon, M. J. Anisotropy of building blocks and their assembly into complex structures. *Nat. Mater.* **6,** 557–562 (2007).
9. Sacanna, S., Pine, D. J. & Yi, G.-R. Engineering shape: the novel geometries of colloidal self-assembly. *Soft Matter* **9,** 8096–8106 (2013).
10. Watson, J. D. & Crick, F. H. C. Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. *Nature* **171,** 737–738 (1953).
11. Seeman, N. C. DNA NANOTECHNOLOGY: Novel DNA Constructions. *Annu. Rev. Biophys. Biomol. Struct.* **27,** 225–248 (1998).
12. Seeman, N. C. Nucleic acid junctions and lattices. *J. Theor. Biol.* **99,** 237–247 (1982).

13. Winfree, E., Liu, F., Wenzler, L. A. & Seeman, N. C. Design and self-assembly of two-dimensional DNA crystals. *Nature* **394,** 539–544 (1998).
14. Liu, D., Park, S. H., Reif, J. H. & LaBean, T. H. DNA nanotubes self-assembled from triple-crossover tiles as templates for conductive nanowires. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 717–722 (2004).
15. Rothemund, P. W. K. *et al.* Design and Characterization of Programmable DNA Nanotubes. *J. Am. Chem. Soc.* **126,** 16344–16352 (2004).
16. Zheng, J. *et al.* From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. *Nature* **461,** 74–77 (2009).
17. Ke, Y., Ong, L. L., Shih, W. M. & Yin, P. Three-Dimensional Structures Self-Assembled from DNA Bricks. *Science* **338,** 1177–1183 (2012).
18. Ke, Y. *et al.* DNA brick crystals with prescribed depths. *Nat. Chem.* **6,** 994–1002 (2014).
19. Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. *Nature* **440,** 297–302 (2006).
20. Andersen, E. S. *et al.* Self-assembly of a nanoscale DNA box with a controllable lid. *Nature* **459,** 73–76 (2009).
21. Douglas, S. M. *et al.* Self-assembly of DNA into nanoscale three-dimensional shapes. *Nature* **459,** 414–418 (2009).
22. Han, D. *et al.* DNA Origami with Complex Curvatures in Three-Dimensional Space. *Science* **332,** 342–346 (2011).
23. Han, D. *et al.* DNA Gridiron Nanostructures Based on Four-Arm Junctions. *Science* **339,** 1412–1415 (2013).
24. Zhang, D. Y., Turberfield, A. J., Yurke, B. & Winfree, E. Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA. *Science* **318,** 1121–1125 (2007).

25. Zhang, D. Y. & Seelig, G. Dynamic DNA nanotechnology using strand-displacement reactions. *Nat. Chem.* **3,** 103–113 (2011).
26. Pinheiro, A. V., Han, D., Shih, W. M. & Yan, H. Challenges and opportunities for structural DNA nanotechnology. *Nat. Nanotechnol.* **6,** 763–772 (2011).
27. Mirkin, C. A., Letsinger, R. L., Mucic, R. C. & Storhoff, J. J. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* **382,** 607–609 (1996).
28. Alivisatos, A. P. *et al.* Organization of 'nanocrystal molecules' using DNA. *Nature* **382,** 609–611 (1996).
29. Jones, M. R., Seeman, N. C. & Mirkin, C. A. Programmable materials and the nature of the DNA bond. *Science* **347,** 1260901 (2015).
30. Winfree, E. Algorithmic Self-Assembly of DNA: Theoretical Motivations and 2D Assembly Experiments. *J. Biomol. Struct. Dyn.* **17,** 263–270 (2000).
31. Seeman, N. C. & Lukeman, P. S. Nucleic acid nanostructures: bottom-up control of geometry on the nanoscale. *Rep. Prog. Phys.* **68,** 237 (2005).
32. Geerts, N. & Eiser, E. DNA-functionalized colloids: Physical properties and applications. *Soft Matter* **6,** 4647–4660 (2010).
33. Zhang, X., Wang, R. & Xue, G. Programming macro-materials from DNA-directed self-assembly. *Soft Matter* **11,** 1862–1870 (2015).
34. Theodorakis, P. E., Fytas, N. G., Kahl, G. & Dellago, C. Self-assembly of DNA-functionalized colloids. *Condens. Matter Phys.* **18,** 22801 (2015).
35. Jones, M. R. & Mirkin, C. A. Materials science: Self-assembly gets new direction. *Nature* **491,** 42–43 (2012).
36. Hurst, S. J., Lytton-Jean, A. K. R. & Mirkin, C. A. Maximizing DNA Loading on a Range of Gold Nanoparticle Sizes. *Anal. Chem.* **78,** 8313–8318 (2006).

37. Valignat, M.-P., Theodoly, O., Crocker, J. C., Russel, W. B. & Chaikin, P. M. Reversible self-assembly and directed assembly of DNA-linked micrometer-sized colloids. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 4225–4229 (2005).
38. Dreyfus, R. *et al.* Simple Quantitative Model for the Reversible Association of DNA Coated Colloids. *Phys. Rev. Lett.* **102,** 048301 (2009).
39. Kim, A. J., Manoharan, V. N. & Crocker, J. C. Swelling-based method for preparing stable, functionalized polymer colloids. *J. Am. Chem. Soc.* **127,** 1592–1593 (2005).
40. Wang, Y. *et al.* Crystallization of DNA-coated colloids. *Nat. Commun.* **6,** (2015).
41. Wang, Y. *et al.* Synthetic Strategies Toward DNA-Coated Colloids that Crystallize. *J. Am. Chem. Soc.* **137,** 10760–10766 (2015).
42. Nykypanchuk, D., Maye, M. M., van der Lelie, D. & Gang, O. DNA-guided crystallization of colloidal nanoparticles. *Nature* **451,** 549–552 (2008).
43. Biancaniello, P., Kim, A. & Crocker, J. Colloidal Interactions and Self-Assembly Using DNA Hybridization. *Phys. Rev. Lett.* **94,** 058302 (2005).
44. Xiong, H., van der Lelie, D. & Gang, O. DNA Linker-Mediated Crystallization of Nanocolloids. *J. Am. Chem. Soc.* **130,** 2442–2443 (2008).
45. Yakovchuk, P., Protozanova, E. & Frank-Kamenetskii, M. D. Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. *Nucleic Acids Res.* **34,** 564–574 (2006).
46. SantaLucia, J. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proc. Natl. Acad. Sci.* **95,** 1460–1465 (1998).
47. SantaLucia, J. & Hicks, D. The Thermodynamics of DNA Structural Motifs. *Annu. Rev. Biophys. Biomol. Struct.* **33,** 415–440 (2004).
48. Dirks, R. M. & Pierce, N. A. A partition function algorithm for nucleic acid secondary structure including pseudoknots. *J. Comput. Chem.* **24,** 1664–1677 (2003).

49. Dimitrov, R. A. & Zuker, M. Prediction of Hybridization and Melting for Double-Stranded Nucleic Acids. *Biophys J* **87,** 215–226 (2004).
50. Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **31,** 3406–3415 (2003).
51. Markham, N. R. & Zuker, M. DINAMelt web server for nucleic acid melting prediction. *Nucleic Acids Res.* **33,** W577–W581 (2005).
52. Zadeh, J. N. *et al.* NUPACK: Analysis and design of nucleic acid systems. *J. Comput. Chem.* **32,** 170–173 (2011).
53. Rogers, W. B. & Crocker, J. C. Direct measurements of DNA-mediated colloidal interactions and their quantitative modeling. *Proc. Natl. Acad. Sci.* **108,** 15687 – 15692 (2011).
54. Licata, N. A. & Tkachenko, A. V. Statistical mechanics of DNA-mediated colloidal aggregation. *Phys. Rev. E* **74,** 041408 (2006).
55. Dreyfus, R. *et al.* Aggregation-disaggregation transition of DNA-coated colloids: Experiments and theory. *Phys. Rev. E* **81,** 041404 (2010).
56. Varilly, P., Angioletti-Uberti, S., Mognetti, B. M. & Frenkel, D. A general theory of DNA-mediated and other valence-limited colloidal interactions. *J. Chem. Phys.* **137,** 094108–094108–15 (2012).
57. Mladek, B. M., Fornleitner, J., Martinez-Veracoechea, F. J., Dawid, A. & Frenkel, D. Quantitative Prediction of the Phase Diagram of DNA-Functionalized Nanosized Colloids. *Phys. Rev. Lett.* **108,** 268301 (2012).
58. Angioletti-Uberti, S., Varilly, P., Mognetti, B. M., Tkachenko, A. V. & Frenkel, D. Communication: A simple analytical formula for the free energy of ligand–receptor-mediated interactions. *J. Chem. Phys.* **138,** 021102–021102–4 (2013).
59. Angioletti-Uberti, S., Varilly, P., Mognetti, B. M. & Frenkel, D. Mobile Linkers on DNA-Coated Colloids: Valency without Patches. *Phys. Rev. Lett.* **113,** 128303 (2014).

60. Mognetti, B. M. *et al.* Predicting DNA-mediated colloidal pair interactions. *Proc. Natl. Acad. Sci.* **109,** E378–E379 (2012).

61. Rogers, W. B. & Crocker, J. C. Reply to Mognetti et al.: DNA handshaking interaction data are well described by mean-field and molecular models. *Proc. Natl. Acad. Sci.* **109,** E380–E380 (2012).

62. Feng, L., Pontani, L.-L., Dreyfus, R., Chaikin, P. & Brujic, J. Specificity, flexibility and valence of DNA bonds guide emulsion architecture. *Soft Matter* **9,** 9816–9823 (2013).

63. van der Meulen, S. A. J. & Leunissen, M. E. Solid Colloids with Surface-Mobile DNA Linkers. *J. Am. Chem. Soc.* **135,** 15129–15134 (2013).

64. Tkachenko, A. V. Morphological Diversity of DNA-Colloidal Self-Assembly. *Phys. Rev. Lett.* **89,** 148303 (2002).

65. Xiong, H., van der Lelie, D. & Gang, O. Phase Behavior of Nanoparticles Assembled by DNA Linkers. *Phys. Rev. Lett.* **102,** 015504 (2009).

66. Scarlett, R. T., Crocker, J. C. & Sinno, T. Computational analysis of binary segregation during colloidal crystallization with DNA-mediated interactions. *J. Chem. Phys.* **132,** 234705 (2010).

67. Scarlett, R. T., Ung, M. T., Crocker, J. C. & Sinno, T. A mechanistic view of binary colloidal superlattice formation using DNA-directed interactions. *Soft Matter* **7,** 1912 (2011).

68. Martinez-Veracoechea, F. J., Mladek, B. M., Tkachenko, A. V. & Frenkel, D. Design Rule for Colloidal Crystals of DNA-Functionalized Particles. *Phys. Rev. Lett.* **107,** 045902 (2011).

69. Vial, S., Nykypanchuk, D., Yager, K. G., Tkachenko, A. V. & Gang, O. Linear Mesostructures in DNA–Nanorod Self-Assembly. *ACS Nano* **7,** 5437–5445 (2013).

70. Li, T. I. N. G., Sknepnek, R., Macfarlane, R. J., Mirkin, C. A. & Olvera de la Cruz, M. Modeling the Crystallization of Spherical Nucleic Acid Nanoparticle Conjugates with Molecular Dynamics Simulations. *Nano Lett.* **12,** 2509–2514 (2012).

71. Li, T. I. N. G., Sknepnek, R. & Olvera de la Cruz, M. Thermally Active Hybridization Drives the Crystallization of DNA-Functionalized Nanoparticles. *J. Am. Chem. Soc.* **135,** 8535–8541 (2013).

72. Park, S. Y. *et al.* DNA-programmable nanoparticle crystallization. *Nature* **451,** 553–556 (2008).

73. Auyeung, E. *et al.* DNA-mediated nanoparticle crystallization into Wulff polyhedra. *Nature* **505,** 73–77 (2014).

74. Rogers, W. B., Sinno, T. & Crocker, J. C. Kinetics and non-exponential binding of DNA-coated colloids. *Soft Matter* **9,** 6412–6417 (2013).

75. Macfarlane, R. J. *et al.* Nanoparticle Superlattice Engineering with DNA. *Science* **334,** 204 –208 (2011).

76. Macfarlane, R. J., O'Brien, M. N., Petrosko, S. H. & Mirkin, C. A. Nucleic Acid-Modified Nanostructures as Programmable Atom Equivalents: Forging a New 'Table of Elements'. *Angew. Chem. Int. Ed.* **52,** 5688–5698 (2013).

77. Casey, M. T. *et al.* Driving diffusionless transformations in colloidal crystals using DNA handshaking. *Nat. Commun.* **3,** 1209 (2012).

78. Thaner, R. V. *et al.* Entropy-Driven Crystallization Behavior in DNA-Mediated Nanoparticle Assembly. *Nano Lett.* (2015). doi:10.1021/acs.nanolett.5b02129

66. Haxton, T. K., Hedges, L. O. & Whitelam, S. Crystallization and arrest mechanisms of model colloids. arXiv:1507.06750 [cond-mat][physics:physics] (2015)

80. Grzelczak, M., Pérez-Juste, J., Mulvaney, P. & Liz-Marzán, L. M. Shape control in gold nanoparticle synthesis. *Chem. Soc. Rev.* **37,** 1783–1791 (2008).

81. Li, C. *et al.* High-Yield Synthesis of Single-Crystalline Gold Nano-octahedra. *Angew. Chem.* **119,** 3328–3332 (2007).

82. Kim, D. Y. *et al.* Seed-Mediated Synthesis of Gold Octahedra in High Purity and with Well-Controlled Sizes and Optical Properties. *Chem. – Eur. J.* **17,** 4759–4764 (2011).
83. Hong, S., Shuford, K. L. & Park, S. Shape Transformation of Gold Nanoplates and their Surface Plasmon Characterization: Triangular to Hexagonal Nanoplates. *Chem. Mater.* **23,** 2011–2013 (2011).
84. Lohse, S. E. & Murphy, C. J. The Quest for Shape Control: A History of Gold Nanorod Synthesis. *Chem. Mater.* **25,** 1250–1261 (2013).
85. Kraft, D. J. *et al.* Surface roughness directed self-assembly of patchy particles into colloidal micelles. *Proc. Natl. Acad. Sci.* **109,** 10787–10792 (2012).
86. Manoharan, V. N., Elsesser, M. T. & Pine, D. J. Dense packing and symmetry in small clusters of microspheres. *Science* **301,** 483–487 (2003).
87. Duguet, E., Désert, A., Perro, A. & Ravaine, S. Design and elaboration of colloidal molecules: an overview. *Chem. Soc. Rev.* **40,** 941 (2011).
88. Sindoro, M., Yanai, N., Jee, A.-Y. & Granick, S. Colloidal-Sized Metal–Organic Frameworks: Synthesis and Applications. *Acc. Chem. Res.* **47,** 459–469 (2014).
89. Rossi, L. *et al.* Cubic crystals from cubic colloids. *Soft Matter* **7,** 4139–4142 (2011).
90. Sacanna, S., Irvine, W. T. M., Chaikin, P. M. & Pine, D. J. Lock and key colloids. *Nature* **464,** 575 (2010).
91. Sacanna, S. *et al.* Shaping colloids for self-assembly. *Nat. Commun.* **4,** 1688 (2013).
92. Fan, J. A. *et al.* Self-Assembled Plasmonic Nanoparticle Clusters. *Science* **328,** 1135–1138 (2010).
93. Fan, J. A. *et al.* DNA-Enabled Self-Assembly of Plasmonic Nanoclusters. *Nano Lett.* **11,** 4859–4864 (2011).
94. Kuzyk, A. *et al.* DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response. *Nature* **483,** 311–314 (2012).

95. Kuzyk, A. *et al.* Reconfigurable 3D plasmonic metamolecules. *Nat. Mater.* (2014). doi:10.1038/nmat4031

96. Sun, D. *et al.* Light-Harvesting Nanoparticle Core–Shell Clusters with Controllable Optical Output. *ACS Nano* **9,** 5657–5665 (2015).

97. Kruglova, O., Demeyer, P.-J., Zhong, K., Zhou, Y. & Clays, K. Wonders of colloidal assembly. *Soft Matter* **9,** 9072–9087 (2013).

98. Hallam, B. T., Hiorns, A. G. & Vukusic, P. Developing optical efficiency through optimized coating structure: biomimetic inspiration from white beetles. *Appl. Opt.* **48,** 3243–3249 (2009).

99. Wiersma, D. S. Disordered photonics. *Nat. Photonics* **7,** 188–196 (2013).

100. Anderson, V. J. & Lekkerkerker, H. N. W. Insights into phase transition kinetics from colloid science. *Nature* **416,** 811–815 (2002).

101. Soto, C. M., Srinivasan, A. & Ratna, B. R. Controlled Assembly of Mesoscale Structures Using DNA as Molecular Bridges. *J. Am. Chem. Soc.* **124,** 8508–8509 (2002).

102. Schade, N. B. *et al.* Tetrahedral colloidal clusters from random parking of bidisperse spheres. *Phys. Rev. Lett.* **110,** 148303 (2013).

103. Urzhumov, Y. A. *et al.* Plasmonic nanoclusters: a path towards negative-index metafluids. *Opt. Express* **15,** 14129–14145 (2007).

104. Alu, A. & Engheta, N. The quest for magnetic plasmons at optical frequencies. *Opt. Express* **17,** 5723–5730 (2009).

105. Di Michele, L. *et al.* Multistep kinetic self-assembly of DNA-coated colloids. *Nat. Commun.* **4,** (2013).

106. Kim, A. J., Scarlett, R., Biancaniello, P. L., Sinno, T. & Crocker, J. C. Probing interfacial equilibration in microsphere crystals formed by DNA-directed assembly. *Nat. Mater.* **8,** 52–55 (2009).

107. Jenkins, I. C., Casey, M. T., McGinley, J. T., Crocker, J. C. & Sinno, T. Hydrodynamics selects the pathway for displacive transformations in DNA-linked colloidal crystallites. *Proc. Natl. Acad. Sci.* **111,** 4803–4808 (2014).
108. Demirörs, A. F. *et al.* Long-Ranged Oppositely Charged Interactions for Designing New Types of Colloidal Clusters. *Phys. Rev. X* **5,** 021012 (2015).
109. Shevchenko, E. V., Talapin, D. V., Kotov, N. A., O'Brien, S. & Murray, C. B. Structural diversity in binary nanoparticle superlattices. *Nature* **439,** 55–59 (2006).
110. Mao, C., LaBean, T. H., Reif, J. H. & Seeman, N. C. Logical computation using algorithmic self-assembly of DNA triple-crossover molecules. *Nature* **407,** 493–496 (2000).
111. Rothemund, P. W. K., Papadakis, N. & Winfree, E. Algorithmic Self-Assembly of DNA Sierpinski Triangles. *PLoS Biol.* **2,** e424 (2004).
112. Seelig, G., Soloveichik, D., Zhang, D. Y. & Winfree, E. Enzyme-Free Nucleic Acid Logic Circuits. *Science* **314,** 1585–1588 (2006).
113. Qian, L. & Winfree, E. Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades. *Science* **332,** 1196–1201 (2011).
114. Jacobs, W. M., Reinhardt, A. & Frenkel, D. Rational design of self-assembly pathways for complex multicomponent structures. *Proc. Natl. Acad. Sci.* **112,** 6313–6318 (2015).
115. Reinhardt, A. & Frenkel, D. Numerical Evidence for Nucleated Self-Assembly of DNA Brick Structures. *Phys. Rev. Lett.* **112,** 238103 (2014).
116. Hormoz, S. & Brenner, M. P. Design principles for self-assembly with short-range interactions. *Proc. Natl. Acad. Sci.* **108,** 5193 –5198 (2011).
117. Zeravcic, Z., Manoharan, V. N. & Brenner, M. P. Size limits of self-assembled colloidal structures made using specific interactions. *Proc. Natl. Acad. Sci.* **111,** 15918–15923 (2014).

118. Tkachenko, A. V. Theory of Programmable Hierarchic Self-Assembly. *Phys. Rev. Lett.* **106,** 255501 (2011).

119. Halverson, J. D. & Tkachenko, A. V. DNA-programmed mesoscopic architecture. *Phys. Rev. E* **87,** 062310 (2013).

120. Wu, K.-T. *et al.* Polygamous particles. *Proc. Natl. Acad. Sci.* **109,** 18731–18736 (2012).

121. Wang, Y. *et al.* Colloids with valence and specific directional bonding. *Nature* **491,** 51–55 (2012).

122. Feng, L., Dreyfus, R., Sha, R., Seeman, N. C. & Chaikin, P. M. DNA Patchy Particles. *Adv. Mater.* **25,** 2779–2783 (2013).

123. Maye, M. M., Nykypanchuk, D., Cuisinier, M., van der Lelie, D. & Gang, O. Stepwise surface encoding for high-throughput assembly of nanoclusters. *Nat. Mater.* **8,** 388–391 (2009).

124. Jones, M. R. *et al.* DNA-nanoparticle superlattices formed from anisotropic building blocks. *Nat. Mater.* **9,** 913–917 (2010).

125. Lu, F., Yager, K. G., Zhang, Y., Xin, H. & Gang, O. Superlattices assembled through shape-induced directional binding. *Nat. Commun.* **6,** (2015).

126. O'Brien, M. N., Jones, M. R., Lee, B. & Mirkin, C. A. Anisotropic nanoparticle complementarity in DNA-mediated co-crystallization. *Nat. Mater.* **14,** 833–839 (2015).

127. Woo, S. & Rothemund, P. W. K. Programmable molecular recognition based on the geometry of DNA nanostructures. *Nat. Chem.* **3,** 620–627 (2011).

128. Endo, M., Sugita, T., Katsuda, Y., Hidaka, K. & Sugiyama, H. Programmed-Assembly System Using DNA Jigsaw Pieces. *Chem. - Eur. J.* **16,** 5362–5368 (2010).

129. Rajendran, A., Endo, M., Katsuda, Y., Hidaka, K. & Sugiyama, H. Programmed Two-Dimensional Self-Assembly of Multiple DNA Origami Jigsaw Pieces. *ACS Nano* **5,** 665–671 (2011).

130. Gerling, T., Wagenbauer, K. F., Neuner, A. M. & Dietz, H. Dynamic DNA devices and assemblies formed by shape-complementary, non–base pairing 3D components. *Science* **347,** 1446–1452 (2015).

131. Tian, Y. *et al.* Prescribed nanoparticle cluster architectures and low-dimensional arrays built using octahedral DNA origami frames. *Nat. Nanotechnol.* **10,** 637–644 (2015).

132. Roldán-Vargas, S., Smallenburg, F., Kob, W. & Sciortino, F. Gelling by Heating. *Sci. Rep.* **3,** (2013).

133. Rogers, W. B. & Manoharan, V. N. Programming colloidal phase transitions with DNA strand displacement. *Science* **347,** 639–642 (2015).

134. Zhang, D. Y. & Winfree, E. Control of DNA Strand Displacement Kinetics Using Toehold Exchange. *J. Am. Chem. Soc.* **131,** 17303–17314 (2009).

135. Golestanian, R., Liverpool, T. B. & Ajdari, A. Propulsion of a Molecular Machine by Asymmetric Distribution of Reaction Products. *Phys. Rev. Lett.* **94,** 220801 (2005).

136. Wang, W., Duan, W., Ahmed, S., Mallouk, T. E. & Sen, A. Small power: Autonomous nano- and micromotors propelled by self-generated gradients. *Nano Today* **8,** 531–554 (2013).

137. Keber, F. C. *et al.* Topology and dynamics of active nematic vesicles. *Science* **345,** 1135–1139 (2014).

138. Yurke, B., Turberfield, A. J., Mills, A. P., Simmel, F. C. & Neumann, J. L. A DNA-fuelled molecular machine made of DNA. *Nature* **406,** 605–608 (2000).

139. Winfree, E. & Bekbolatov, R. in *DNA Computing* (eds. Chen, J. & Reif, J.) 126–144 (Springer Berlin Heidelberg, 2003). at <http://link.springer.com/chapter/10.1007/978-3-540-24628-2_13>

140. Murugan, A., Huse, D. A. & Leibler, S. Discriminatory Proofreading Regimes in Nonequilibrium Systems. *Phys. Rev. X* **4,** 021016 (2014).

141. Shin, J.-S. & Pierce, N. A. A Synthetic DNA Walker for Molecular Transport. *J. Am. Chem. Soc.* **126,** 10834–10835 (2004).

142. Lund, K. *et al.* Molecular robots guided by prescriptive landscapes. *Nature* **465,** 206–210 (2010).

143. Leunissen, M. E. *et al.* Towards self-replicating materials of DNA-functionalized colloids. *Soft Matter* **5,** 2422–2430 (2009).

144. Wang, T. *et al.* Self-replication of information-bearing nanoscale patterns. *Nature* **478,** 225–228 (2011).

145. Zeravcic, Z. & Brenner, M. P. Self-replicating colloidal clusters. *Proc. Natl. Acad. Sci.* **111,** 1748–1753 (2014).

146. Li, C.-J. & Trost, B. M. Green chemistry for chemical synthesis. *Proc. Natl. Acad. Sci.* **105,** 13197–13202 (2008).

147. Kosuri, S. & Church, G. M. Large-scale de novo DNA synthesis: technologies and applications. *Nat. Methods* **11,** 499–507 (2014).

148. Zhang, Y. *et al.* Selective transformations between nanoparticle superlattices via the reprogramming of DNA-mediated interactions. *Nat. Mater.* **14,** 840–847 (2015).