Self-assembled three-dimensional chiral colloidal architecture

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Although stereochemistry has been a central focus of the molecular sciences since Pasteur, its province has previously been restricted to the nanometric scale. We have programmed the self-assembly of micron-sized colloidal clusters with structural information stemming from a nanometric arrangement. This was done by combining DNA nanotechnology with colloidal science. Using the functional flexibility of DNA origami in conjunction with the structural rigidity of colloidal particles, we demonstrate the parallel self-assembly of three-dimensional microconstructs, evincing highly specific geometry that includes control over position, dihedral angles, and cluster chirality.

Ever since the discovery of colloidal crystals, with their notable opalescence and promise for photonic applications, scientists have aspired to program the self-assembly and self-replication of arbitrary functional materials on the colloidal length scale (1–6). Traditionally, spherical colloids self-assemble through isotropic, nonspecific van der Waals, depletion, and Coulombic forces. Recently, the specific binding of anisotropic particles (7), lock-and-key particles (8), and DNA-coated patchy particles (9) have extended the repertoire of colloidal self-assembly. Although micron-sized colloidal particles are important as models for equilibrium and active systems in physics, chemistry, and biology and for technologies ranging from catalysis to photonics, their available structures are still largely limited to minimal moment geometries—compact structures determined by the geometry of maximally kissing particles, rather than by design (9).

The tools of DNA nanotechnology (10) have been used to actualize a variety of complex structures, including clusters of varied symmetry (11, 12), DNA bricks (13), knots (14, 15), and self-replicating tiles (16). Particularly versatile is DNA origami (17), which can bind and fold programmably in two and three dimensions. DNA origami can also serve as a template—a breadboard for organizing passive (18, 19) and active (20) nanoparticles, proteins (21), and other species (22). The aim of this report is to introduce hybrid DNA origami–colloid structures, extending many of the advances of DNA nanotechnology to the micron regime and beyond.

DNA origami is not naturally commensurate with colloids. The typical M13-based two-dimensional (2D) origami molecule has an area smaller than 90 nm by 90 nm, covering less than 0.3% of the surface of a 1-μm diameter sphere. A functional design requires an extended DNA origami structure that will bind as a flat unit to a colloid, making remote points on its surface individually addressable (Fig. 1). To this end, we designed an elongated, beltlike origami molecule that spans a sizable arc-length of the surface of a micron-sized particle (Fig. 2). Both the length and bending rigidity of the DNA origami are crucial aspects of its design. The DNA origami belt has tailored anisotropic mechanical bending rigidities, ensuring that the belt traces a geodesic on the curved surface of a colloid. We engineered the mechanical rigidity of the DNA origami by using the persistence length of a DNA duplex under standard conditions, $\xi_{\text{duplex}} = 50 \text{ nm}$, and the bending modulus of a beam with rectangular cross section, which is proportional to its thickness cubed times its width. Our beltlike DNA origami is one double helix thick and four double helices wide, and extends 580 nm in length (Fig. 2), with a persistence length of $\xi_{\text{bend}} = 200 \text{ nm}$ perpendicular to the flat face and $\xi_{\text{bend}} = 3200 \text{ nm}$ parallel to the face along the width (Figs. S1 and S2).

To demonstrate that this technology can assemble a truly 3D microstructure, we constructed a DNA origami complex resembling the shape of the letter L. A cross-shaped DNA origami (23) has two sets of horizontal sticky ends complementary to the tips of two different origami belts (Fig. 2, C to E, and figs. S3 to S5). The cross DNA origami fixes the angle between the two belts to be 90°. Once the DNA origami L-belt complex binds to the central colloid, the relative positions of the tips of the L prescribe the binding angle and dihedral angle of the satellite colloids to form an irregular tetrahedron (see tables S1 and S2).

Figure 1 outlines the main steps of the synthesis. A DNA origami L-belt complex, programmed with four sets of sticky-end patches (A, B, C, and D), is mixed with an excess of DNA-coated colloids (24) (step 1). The origami molecule binds to the central colloidal particle (red A) through 40 identical sticky ends, composed of eight nucleotides (nt) each (sequence A), on the underside of the belt. Balancing the binding energy with the belt’s mechanical rigidity resulted in a design with sticky ends that have a melting temperature of 32°C (25). This intermediate-strength binding ensures that, once annealed, the belt lies “flat” along a great circle on the surface of the colloid, and the particle inherits the binding configuration of the remaining patches (see supplementary materials). Top-side patches B, C, and D point away from the central particle and can bind to further satellite DNA-coated colloids. Each patch is made of a specific set of 16 identical 11-nt sticky ends with a melting temperature of 52°C.

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The measured melting temperatures of different parts of the cluster are consistent with the design (tables S3 to S5). In the following steps (2, 3a, and 3b), we added DNA-coated colloids complementary to the remaining patches. In this way, we could prepare asymmetric particle clusters with different colloidal spheres at the apices of a designed irregular tetrahedron, a construction requiring most of the technology needed to make arbitrary free-form structures. Such structures may find use in chiral photonic metamaterials (26, 27) and propeller-based swimmers (28, 29).

To form the irregular tetrahedra, we used a 0.71-μm diameter polymethyl methacrylate (PMMA) central particle (red A’); the satellite

![Diagram](http://science.sciencemag.org/)
particles are a 0.8-μm diameter polystyrene (PS) particle (lime B′), a 0.69-μm diameter PS particle (cyan C′), and a 0.4-μm diameter PS particle (green D′) (Fig. 1, step 3a, and Fig. 3, D and E). In this way, we have prepared so-called Van’t Hoff-LeBel colloids by having each of three “valences” differently occupied (movies S1 to S3). We were able to dictate the chirality of the final cluster by switching the DNA coating of two of the satellite colloids (C′ and D′, green and cyan, steps 3a and 3b in Fig. 1). The clusters that formed have a well-defined binding specificity, binding angles, dihedral angle geometry, and chirality. We measured the angles by embedding the clusters in a polyacrylamide gel and locating the coordinates of their centers in 3D by using confocal microscopy. Confocal images of the tetrahedra show excellent agreement with the designed geometry and chirality (Fig. 3D, Table 1, and movie S4). Using the IUPAC convention, we ascribed the assembled tetrahedron as left-handed: an (S)-colloidal tetrahedron (Fig. S6).

Fig. 4. Equilibrium digital sedimentation of density-matched clusters. A typical purification process of 1-ml suspension produces 0.1 mg of target clusters (over 104 particles) with a 5% yield (see supplementary materials).

(A) The target structure is assembled from particles of different densities: PS (1.05 g/cm3) and PMMA (1.18 g/cm3). The equivalent density of the target cluster is 1.09 g/cm3, which is higher than light water (1.00 g/cm3) but lower than heavy water (1.11 g/cm3). When centrifuged in a tube layered with a density-gradient step, the target cluster stops at the interface of semihave water (1.07 g/cm3) and heavy water and can be readily extracted (fig. S9).

(B) Wide-field fluorescent microscopy image of 10 assembled clusters from an overlaid z-stack (see also movie S3). Inset shows zoom-in of two tetrahedra showing all four particles of each. (C) Relative cluster abundance counted from 200 particles. As high as 70% were tetrahedra. The most frequent impurities, 14%, are clusters with density slightly lower than the target structure. The second most frequent impurities are all the various partially reacted clusters (dimers and trimers), amounting to 8% when combined. The least occurring impurities are the unreacted monomers that started with more than a 10-fold excess and amount to only 7% after separation. Scale bar for (B) is 10 μm (inset 1 μm).

Table 1. Comparison of the design range with the measured geometrical parameters of the angles and dihedral angle of self-assembled clusters. See Fig. 3. The binding-patch size (40 nm) and the polydispersity of the central sphere’s diameter (150 nm) set the design range. The major contribution to the measured angle distribution is the locating error of the 3D confocal image (~50 nm).

<table>
<thead>
<tr>
<th>Angle</th>
<th>Design range</th>
<th>Measured</th>
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<tbody>
<tr>
<td>Trimer (θ)</td>
<td>77°–86°</td>
<td>83° ± 6°</td>
</tr>
<tr>
<td>Lime-red-green (α)</td>
<td>79°–97°</td>
<td>92° ± 18°</td>
</tr>
<tr>
<td>Lime-red-cyan (β)</td>
<td>79°–97°</td>
<td>80° ± 11°</td>
</tr>
<tr>
<td>Dihedral (γ)</td>
<td>85°–105°</td>
<td>99° ± 15°</td>
</tr>
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The enantiomer, an (R)-colloidal tetrahedron, was synthesized by switching the DNA coating of two of the satellite colloids (green C′ and cyan D′, see Fig. 1; step 3b; Fig. 3F; and movie S5). For either enantiomer, all imaged clusters showed their programmed chirality.

The tetrahedron reveal their handedness through depletion-induced stereospecific face recognition. We induced an attractive force between the clusters and a overslip using depletion interactions (8). The free-energy gain from depletion, ΔF, is proportional to the excluded volume gain, V, which in turn is proportional to the sum of the particles’ radii (Ri) of a given face ΔF ≈ k_B T ΔV = k_B T ∑ Ri, where k_B and T are Boltzmann’s constant and absolute temperature (figs. S7 and S8). The face with the three largest spheres (lime-red-cyan) has a depletion free-energy gain that is at least 5k_B T greater than any other face. This increased affinity to the surface makes it 99% more likely to bind. When the tetrahedron’s (R)-enantiomer is depleted to the glass surface, the lime-red-cyan triangle is observed clockwise (Fig. 3G), whereas the (S)-enantiomer shows the counterclockwise arrangement (Fig. 3G, inset), consistent with their handedness.

Using just a single DNA origami belt, we were able to program the self-assembly of colloidal trimers (Fig. 3, A and B, and movie S6). The central colloid was made of a 0.71-μm diameter PMMA particle (red A′), and the two satellite colloids were 0.69-μm diameter PS (green B′ and cyan C′). We controlled the binding angle by choosing the relative placement of the top-side patches along the origami belt. The target angle was 82°. The measured angle (83°) is within the designed range and experimental error (Fig. 3A, Table 1, and supplementary materials). We demonstrated the specificity of the binding by switching a green satellite colloid with an orange particle (C′) (Fig. 3C and movie S7). This makes a micron-sized colloidal cluster with particles of three different species self-assembled with an arbitrary prescribed angle. Micron-sized self-assembly of three different particles with direct control over their binding angle solves an inherent limitation in colloidal self-assembly—programmable anisotropic interactions. Micron-sized self-assembled structures based on wetting (7–9), or crystal symmetry (30, 31), cannot, a priori, offer the direct, designer-prescribed, 3D control of geometry or chirality—control that was only accessible on the nanometric scale (10).

Our structures rely on having only one origami molecule per cluster. We achieve this by saturating the origami with a 5- to 20-fold excess of the central colloid, making the binding of a colloid to more than one origami statistically improbable. When the origami-colloid reaction is complete (see supplementary materials for estimated reaction rates), an excess of unreacted and partially reacted particles remains in the reaction vessel. We developed a purification scheme for the target structure: digital step-gradient centrifugation (Fig. 4). The target cluster is assembled from particles with different densities such that the
buoyancy of the cluster can be matched by tuning the solvent’s density. The particles were made of PS (1.05 g/cm³) and PMMA (1.18 g/cm³), and the dense solvent used was heavy water (D₂O, 1.11 g/cm³) (Fig. 4A). This system offers great flexibility in the choice of the relative particle sizes and can be extended easily to particles of different materials (22, 3D). For the purification process, a tube is layered with heavy water, semi-heavy water, and reaction solution and is centrifuged at 4000g for 20 min, during which a band is formed and then extracted (fig. S9). Microscopy shows up to 70% purity in a single purification step (Fig. 4, B and C, and movie S3).

We have shown that hybrid DNA origami-colloids can self-assemble in three dimensions into micron-sized clusters with prescribed binding angles, dihedral angles, and chiralities. Their basic design can be generalized in several ways, by wrapping a colloid in networks of crosses and squares, to align with neighboring clusters specifically to form either cis, trans, or arbitrary angle bonds. Further adhesive patches can be added with two or more different sticky-end sequences, to either cis, trans, or arbitrary angle bonds. We have also developed a scalable equilibrium purification scheme, applicable to particles of different materials, with which we can separate target structures.

REFERENCES AND NOTES

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SUPPLEMENTARY MATERIALS
www.sciencemag.org/content/358/6336/633/suppl/DC1
Materials and Methods Figs. S1 to S9 Tables S1 to S5 References (32–34) Movies S1 to S7 Data Files S1 to S4
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Precise chiral colloidal assembly

A challenge for particle assembly is to bring different colloids together in a controlled and uniform way that goes beyond making lattice structures. Ben Zion et al. used DNA origami to pattern colloidal particles and assemble them into clusters with controlled chirality and composition. DNA belts wrapped flat along the curvature of a colloidal particle in an L-like shape. This meant that other achiral colloidal particles, each furnished with a specific complementary DNA belt, could only attach in one orientation.

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