DNA-mediated nanoparticle crystallization into Wulff polyhedra

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Crystallization is a fundamental and ubiquitous process much studied over the centuries. But although the crystallization of atoms is fairly well understood1,2, it remains challenging to predict reliably the outcome of molecular crystallization processes that are complicated by various molecular interactions and solvent involvement. This difficulty also applies to nanoparticles: high-quality three-dimensional crystals3-12 are mostly produced using drying and sedimentation techniques that are often impossible to rationalize and control to give a desired crystal symmetry, lattice spacing and habit (crystal shape). In principle, DNA-mediated assembly of nanoparticles offers an ideal opportunity for studying nanoparticle crystallization13-17: a well-defined set of rules have been developed to target desired lattice symmetries and lattice constants18-19, and the occurrence of features such as grain boundaries and twinning in DNA superlattices and traditional crystals comprised of molecular or atomic building blocks suggests that similar principles govern their crystallization. But the presence of charged biomolecules, interparticle spacings of tens of nanometres, and the realization so far of only polycrystalline DNA-interconnected nanoparticle superlattices, all suggest that DNA-guided crystallization may differ from traditional crystal growth. Here we show that very slow cooling, over several days, of solutions of complementary-DNA-modified nanoparticles through the melting temperature of the system gives the thermodynamic product with a specific and uniform crystal habit. We find that DNA hybridization can direct nanoparticle assembly along a pathway that mimics atomic crystallization.

The crystallization of nanoparticles mediated by DNA typically involves initial assembly of a disordered aggregate, which upon thermal annealing slightly below its melting temperature transforms into an ordered superlattice (Fig. 1a, blue arrows)12. Transmission electron microscopy (TEM) images and the presence of rings in the small-angle X-ray scattering (SAXS) data show that all superlattices formed using this approach thus far are polycrystalline, with ordered micrometre-sized domains randomly oriented with respect to one another20. Considering that traditional crystallization techniques for atoms and molecules typically rely on slow cooling through the melting temperature20, we hypothesized that such a slow cooling approach applied to DNA-based assembly strategies might yield faceted crystals. DNA-functionalized nanoparticle solutions were therefore heated to above the melting temperature of the DNA links designed to connect particles and then slowly cooled to room temperature (Fig. 1a, red arrows), in a process which typically took two to three days to complete. A key parameter known to the researcher before doing the experiments is the aggregate melting temperature, which is well defined and directly correlated with the nucleic acid sequences used for assembly12.

The slow cooling of the combination of two sets of gold nanoparticles functionalized with complementary DNA linker strands produces superlattices with the expected body-centred cubic (b.c.c.) packing when using 20-nm gold nanoparticles and CsCl packing when using 20-nm and 15-nm gold nanoparticles, as confirmed by the radially averaged one-dimensional SAXS data (Fig. 1b, (i) and (ii)). To enable direct visualization, the superlattices were also stabilized by embedding in silica21. TEM images of these structures reveal uniform crystals with square- and hexagonal-shaped domains for both the b.c.c. (Fig. 1c, (i) and (ii)) and the CsCl (Fig. 1c, (iii) and (iv)) particle packing symmetries, whereas scanning electron microscopy (SEM) allows us to observe surface features and the overall crystal habit (Fig. 1d). Evidently, the slow-cooling process enables DNA-driven assembly and crystallization that favours faceted rhombic dodecahedron microcrystals over the polycrystalline assemblies obtained by annealing below the melting temperature. Although single-crystal formation by annealing below the melting temperature may in principle be possible, the kinetics of reorganization from an irregularly shaped crystal into a well-defined microcrystal are likely to be too slow to be observed experimentally.

SEM images of the microcrystals in different orientations on the substrate are all consistent with rhombic dodecahedron formation (Fig. 2a). Closer inspection of one of the crystals (Fig. 2a, bottom) reveals extraordinarily well ordered nanoparticles at the surface, as well as the presence of common surface defects including ‘particle adatoms’ (a surface defect in which an atom is adsorbed on the surface of a crystal plane) and step edges. The nanoparticle orientation is consistent with a crystal that is enclosed by (110) planes, as expected for rhombic dodecahedra, and which is also the closest-packed plane in a b.c.c. unit cell. A tilting experiment was conducted in the TEM on a single microcrystal to observe the different morphologies that are consistent with a rhombic dodecahedron crystal habit. It is important to note that although these microcrystals exhibited a wide size distribution (Fig. 2c), faceted crystals were the predominant product of slow cooling, and no shapes other than rhombic dodecahedron microcrystals were observed.

In contrast to several prior examples of microcrystals grown from nanoparticle building blocks, in which the overall crystal shape was largely dependent on factors including nanoparticle size and length of the ligand shell3,4,5,6,7,8,9,10,11, the shape of the microcrystals we report here was fairly independent of such parameters: microcrystals made from 5-nm, 10-nm and 20-nm gold nanoparticles all exhibit overall rhombic dodecahedron shapes and b.c.c. packing with lattice parameters of 25.7 nm, 29.1 nm and 39.5 nm, respectively (compare Fig. 3a, b and d). Oligonucleotide length was kept constant in these experiments. Furthermore, rhombic dodecahedron microcrystals were observed for a binary system consisting of 20-nm and 15-nm gold nanoparticles arranged in a CsCl lattice symmetry (Fig. 3c). Thus, we conclude that the rhombic dodecahedron is the thermodynamically most favourable crystal shape for this system over a range of particle sizes and inter-particle distances. Furthermore, molecular dynamics simulations on a colloid model predicted a rhombic dodecahedron equilibrium crystal structure, fully consistent with experimental observations (Fig. 3e).
The formation of rhombic dodecahedron microcrystals from a b.c.c. packing of nanoparticles can be rationalized in terms of the surface energy of the exposed facets. Rhombic dodecahedra are enclosed by (110) facets, which is the closest-packed plane for a b.c.c. or CsCl lattice. When using the standard broken-bond model approximation for surface energy, exposing the closest-packed plane is thermodynamically favoured: it requires breaking the smallest number of particle-to-particle interactions per unit area and thus exposes the lowest-surface-energy facet. From this model, the relative surface energies for b.c.c. metal facets should exhibit a ratio of \( \gamma_{(110)} : \gamma_{(111)} : \gamma_{(100)} = 1:1.22:1.41 \). Similarly, the relative surface energies for face-centred cubic (f.c.c.) metal facets should be \( \gamma_{(111)} : \gamma_{(100)} : \gamma_{(110)} = 1:1.15:1.22 \). These calculations thus predict the Wulff polyhedron, the equilibrium crystal structure, to be a rhombic dodecahedron enclosed by (110) facets for a b.c.c. metal and a truncated octahedron enclosed by (111) and (100) facets for a f.c.c. metal.

In many systems the expected Wulff polyhedron is not always formed, and the validity of the assumptions and approximations made must be analysed for each individual case. We therefore calculated actual surface energy values for our DNA–nanoparticle system using recently developed molecular dynamics simulations that accurately predict the crystallization behaviour of DNA-assembled nanoparticles (see Supplementary Information). In these calculations, the surface energy is defined as the excess energy at the surface of a material.
Figure 3 | Rhombic dodecahedron microcrystals with varying unit cell compositions. a-c, TEM and SEM images of rhombic dodecahedron microcrystals synthesized from a b.c.c. lattice of 5-nm gold nanoparticles (scale bars, left to right, are 1 μm, 1 μm and 2 μm) (a), from a b.c.c. lattice of 10-nm gold nanoparticles (scale bars, left to right, are 1 μm, 2 μm and 4 μm) (b) and from a CsCl lattice of 20-nm and 15-nm gold nanoparticles (scale bars, left to right, are 0.5 μm, 1 μm and 2 μm) (c). d, SAXS data for a b.c.c. crystal made from 5-nm (black trace), 10-nm (red trace), and 20-nm (blue trace) gold nanoparticles. First-order scattering peak $q_0$ and corresponding lattice parameter values are indicated next to the respective scattering pattern for each crystal. e, Molecular dynamics simulation of a binary set of particles exhibiting interactions modelled for the DNA–gold nanoparticle system produces a rhombic dodecahedron microcrystal that is consistent with experimental observations.
compared to the energy of the bulk system. To calculate $\gamma$, periodic boundary conditions were removed from the modelled bulk crystal along the $z$-axis to expose the facet of interest on two sides (Fig. 4). The energy of the bulk crystal $E_{\text{bulk}}$ was subtracted from the energy of the exposed facet $E_{\text{surface exposed}}$, and then divided by twice the area (because two surfaces were exposed and the surface charge density is close to zero) to give the surface energy $\gamma$ of the exposed facet.

Table 1 summarizes absolute surface-energy values for the facets of a b.c.c. crystal and a f.c.c. crystal consisting of DNA-assembled nanoparticles calculated using this model, with the binding strength of complementary sticky ends scaled to 42.3 kJ mol$^{-1}$ (ref. 27) to match the strength of the DNA sequences (TTCCTT) used in our experiments. For the b.c.c. system, the calculated ratios of $\gamma_{(100)}:\gamma_{(110)} = 1.46 \pm 0.02$ and $\gamma_{(111)}:\gamma_{(110)} = 1.24 \pm 0.02$ are in good agreement with the theoretically predicted ratio described above. Evidently, the observation of uniform rhombic dodecahedron crystals from a b.c.c. arrangement of nanoparticles follows the crystallization behaviour expected for a b.c.c. arrangement of atoms$^{28}$. The expected Wulff polyhedron was observed for the b.c.c. nanoparticle system, but no truncated octahedra or other uniform shapes were observed in either experiment or simulation among the faceted crystals obtained for the f.c.c. system. This is probably because the surface energies of the two most stable surfaces in a f.c.c. crystal are too close in energy (predicted and calculated ratio $\gamma_{(100)}:\gamma_{(111)} = 1.15$) for one to be favoured predominantly over the other (Table 1). Furthermore, the SAXS data for the f.c.c. crystals showed evidence of stacking faults and twinning in the lattice structure, defects which may have prevented the formation of uniform crystal shapes (a more in-depth discussion of the f.c.c. system can be found in the Supplementary Information).

Nonetheless, the consistency between the experimental observations and the simulation results provides convincing evidence that the broken-bond approximation used for describing surface energy and crystal growth for atomic systems can similarly be used to describe the crystallization of nanoparticles using DNA interactions; and, hence, that the DNA-guided assembly of nanoparticles provides a nanometre-scale analogue to the crystallization behaviour exhibited by atomic crystals.

The experimental observation of the Wulff equilibrium crystal structure, coupled with computational models, demonstrates the utility of DNA for controlling not only the recognition properties and surface energy of individual nanoparticles, but also the surface energies of the macroscopic nanoparticle assembly in such way that a specific structure can be deliberately programmed and realized in the laboratory. The challenge now for both the experimental and theoretical communities is to build on the principles we have described here to identify and synthesize crystal habits that maximize surface-energy differences, and to create single microcrystals with useful properties that may find practical use such as in photonics and catalytic applications.

### Table 1: Surface energy values calculated for DNA–gold nanoparticle superlattices

<table>
<thead>
<tr>
<th>Structure</th>
<th>System</th>
<th>Relaxed first-neighbour distance (nm)</th>
<th>Lattice constant (nm)</th>
<th>$\gamma_{(100)}$ (mJ m$^{-2}$)</th>
<th>$\gamma_{(110)}$ (mJ m$^{-2}$)</th>
<th>$\gamma_{(111)}$ (mJ m$^{-2}$)</th>
<th>Ratios of surface energies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body-centred cubic</td>
<td>20-nm gold nanoparticle, 150 strands, 18 bp</td>
<td>34.0</td>
<td>39.3 (simulation) 39.5 (experiment)</td>
<td>0.548 $\pm$ 0.005</td>
<td>0.375 $\pm$ 0.005</td>
<td>0.464 $\pm$ 0.003</td>
<td>(110):(100):(111) $\approx$ 1:1.46 $\pm$ 0.02:1.24 $\pm$ 0.02</td>
</tr>
<tr>
<td>Face-centred cubic</td>
<td>20-nm gold nanoparticle, 150 strands, 43 bp</td>
<td>47.8</td>
<td>67.4 (experiment)</td>
<td>0.094 $\pm$ 0.006</td>
<td>0.104 $\pm$ 0.002</td>
<td>0.082 $\pm$ 0.005</td>
<td>(110):(100):(111) $\approx$ 1:1.15 $\pm$ 0.10:1.27 $\pm$ 0.08</td>
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Surface-energy values consider only the contribution of DNA hybridization. As a reference, the surface energy of water is 72 mJ m$^{-2}$. bp, number of DNA base pairs. The stated values and errors indicate the average and associated standard deviation of approximately five independent simulation runs with random initial conditions. The relaxed lattice parameter from simulation is shown as the topmost value; the lattice parameter from experiment is shown as the lowermost value.

**METHODS SUMMARY**

All oligonucleotides used in this work were synthesized on a solid-support MM48 synthesizer using reagents purchased from Glen Research. Sequences can be found in the Supplementary Information. Nanoparticles were functionalized and assembled according to published literature protocols. After particle assembly, slow cooling to room temperature was conducted in a temperature cycler (Life Technologies) at a rate of 0.01 °C min$^{-1}$ unless otherwise specified. Superlattices were characterized by synchrotron SAXS experiments conducted at the Advanced Photon Source at Argonne National Laboratory. Superlattices were transferred to the solid state using a silica embedding method for visualization by TEM (Hitachi HD2300) and SEM (Hitachi SU8030). To reproduce the shapes with molecular dynamics simulations, a colloidal model was validated by computing the interaction potential with simulations$^{46,47}$ with explicit DNA chains and simulations were performed with the LAMMPS package (available at http://lammps.sandia.gov/).

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Supplementary Videos for a visualization of the simulated formation of microcrystals from a b.c.c. and a f.c.c. system of interacting particles modelled as a single bead. To estimate surface-energy values, a scale-accurate coarse-grained model was used\textsuperscript{24} and molecular dynamics simulations were performed on the HOOMD-Blue package (available at http://codeblue.umich.edu/hoomd-blue). More molecular dynamics simulation details and assumptions can be found in the Supplementary Information.

Received 9 July; accepted 27 September 2013. Published online 27 November 2013.


Supplementary Information is available in the online version of the paper.

Acknowledgements C.A.M. and M.O.d.l.C. acknowledge support from the following awards: the Air Force Office of Scientific Research (AFOSR) Multidisciplinary University Research Initiative (MURI) FA9550-11-0-0275, the National Science Foundation Materials Research Science and Engineering Center programme DMR-1121262 at the Materials Research Center of Northwestern University, and the Non-equilibrium Energy Research Center (NERC), an Energy Frontier Research Center funded by the Department of Energy (DoE), Office of Science, Office of Basic Energy Sciences under Award DE-SC0000989. E.A. acknowledges a National Defense Science and Engineering Graduate (NDSEG) Fellowship (number 32 CFR 168a). T.L. acknowledges a Ryan Fellowship from Northwestern University. T.I.N.G.L. thanks S. Dhakal and K. Kohstedt for sharing scripts on the colloid model and S. Patala and J. Zwankken for discussions. SANS experiments were carried out at the DuPont–Northwestern–Dow Collaborative Access Team beam line at the Advanced Photon Source (APS) at Argonne National Laboratory, and use of the APS was supported by the DoE (DE-AC02-06CH11357). The electron microscopy work was performed at the Electron Probe Instrumentation Center of the NU Atomic and Nanoscale Characterization Experimental Center at Northwestern University. The computational work was performed using the TARDIS computer cluster supported by the US Department of Defense National Security Science and Engineering Faculty Fellowship (number FA9550-10-1-0167). T.L. acknowledges Award DE-SC0000989. E.A. acknowledges a National Defense Science and Engineering Graduate (NDSEG) Fellowship (number 32 CFR 168a). T.L. acknowledges a Ryan Fellowship from Northwestern University. T.I.N.G.L. thanks S. Dhakal and K. Kohstedt for sharing scripts on the colloid model and S. Patala and J. Zwankken for discussions. SANS experiments were carried out at the DuPont–Northwestern–Dow Collaborative Access Team beam line at the Advanced Photon Source (APS) at Argonne National Laboratory, and use of the APS was supported by the DoE (DE-AC02-06CH11357). The electron microscopy work was performed at the Electron Probe Instrumentation Center of the NU Atomic and Nanoscale Characterization Experimental Center at Northwestern University. The computational work was performed using the TARDIS computer cluster supported by the US Department of Defense National Security Science and Engineering Faculty Fellowship (number FA9550-10-1-0167).

Author Contributions E.A., T.I.N.G.L., A.J.S., M.O.d.l.C. and C.A.M. designed experiments and analysed data. E.A. collected and analysed data for electron microscopy and X-ray studies. T.I.N.G.L. and M.O.d.l.C. wrote the theoretical model and the simulation details found in the Supplementary Information. E.A. and C.A.M. wrote the manuscript. A.J.S., A.L.S. and B.P. prepared samples and collected electron microscopy data.

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