sense that it transforms from a nonmagnetic insulating phase to a magnetic metallic phase under applied pressures on the order of 10 GPa (42–45), which is in contrast to other metallic rare earth hexaborides in which the $f$-electrons order magnetically in the ambient ground state. Our observation of a large three-dimensional conduction electron Fermi surface revealed by quantum oscillations may be related to reports of a residual density of states at the Fermi energy in SmB$_6$, through measurements of heat capacity (23, 46), optical conductivity (47), Raman scattering (48), and neutron scattering (49). Another possibility is that quantum oscillations could arise even in a system with a gap in the excitation spectrum at the Fermi energy, provided that the size of the gap is not much larger than the cyclotron energy (50). Within this scenario, the residual density of states observed at the Fermi energy with complementary measurements and the steep upturn in quantum oscillation amplitude we observe at low temperatures appear challenging to explain.

REFERENCES AND NOTES

Electron tomography is routinely used for 3D analysis of materials (5–9). This method cannot be applied to individual particles in a liquid because it relies on acquisition of images of a single object at many different tilt angles over a period of 2 to 5 hours, assuming the object is static during the entire acquisition. Single-particle cryo-electron microscopy (cryo-EM) is a common method for the determination of 3D structures in biological sciences. The average 3D Coulomb potential map (i.e., density) of a protein is reconstructed from tens of thousands of transmission electron microscopy (TEM) images of randomly oriented copies of the same protein embedded in vitreous ice (10). The unknown 3D projection angles of the images are determined by computational methods (II). Single-particle cryo-EM has succeeded in reconstructing biological molecules with nearly 3 Å resolution (10, 12). A similar approach was recently applied to reconstruct the atomic structure of homogeneous ultrasmall gold clusters (13). However, the single-particle method is not readily applicable to 3D reconstruction of colloidal nanoparticles because of their intrinsic structural inhomogeneity at the atomic level.

TEM has undergone technical improvements in the past decades (5, 14–17). The image resolution has been improved with the introduction of electron lens aberration correctors (15). The development of direct electron detectors has led to improvements in image quality and rapid acquisition of movies that allow for compensation for beam-induced specimen motion, thereby providing a substantial enhancement to single-particle cryo-EM (18).

We present a hybrid method for reconstructing the 3D structures of individual nanoparticles in solution. The method represents a combination of three technological advancements from TEM imaging in biological and materials science: (i) the development of graphene as a covering to hold a liquid in vacuum (the so-called graphene liquid cell, or GLC) that allows atomic-resolution imaging of nanoparticles that move and rotate freely in solution by aberration-corrected TEM (3, 19); (ii) the advent of direct electron detectors, producing movies with millisecond-to-frame time resolution of the rotating nanocrystals (18); and (iii) a theory for ab initio single-particle 3D reconstruction, used to solve the inverse problem of recovering the unknown 3D orientations of the individual noisy nanocrystal projections (10). The resulting hybrid technique, 3D structure identification of nanoparticles by GLC EM (abbreviated as SINGLE), was used to separately reconstruct the 3D structures of two individual Pt nanocrystals in solution.

Pt nanocrystals were chosen because of their high electron scattering strength, because their detailed atomic structure is important for catalysis, and because earlier graphene liquid cell studies have shown that they grow by nanoparticle aggregation, resulting in complex structures that are not possible to determine by any previously developed method. Pt nanocrystals with sub–2 nm diameter were prepared in solution. Two graphene sheets were grown by the chemical vapor deposition method and used to entrap solvated nanocrystals (3). The graphene provides an ultrathin covering of material to maintain liquid conditions in the TEM vacuum and presents an inert surface onto which the nanocrystals do not adsorb. The translational and rotational motions of the particles in liquid pockets with sub–50 nm diameter were imaged in situ using TEAM I, a TEM instrument with geometrical and chromatic aberration correction, operated at 300 kV using a direct electron detector (Fig. 1A). The corrector was set to apply a slightly negative spherical aberration coefficient of about –10 Å. Under these imaging conditions, we produced white atom contrast when using a small underfocus value of 30 to 50 Å.

Movies of the moving particles had a temporal resolution of 50 frames/s, a field of view of 1024 × 1024 pixels, and a Nyquist sampling limit of 0.56 Å. Each movie frame represents a 2D projection of many particles in random orientations. To reconstruct the individual 3D structures, we chose a small region around each single particle of interest in each individual frame to create a set of projections per particle. The 3D orientations of the resulting series of noisy 2D images of a single rotating particle were recorded computationally using an ab initio 3D reconstruction algorithm adapted from one originally developed to recover orientations from cryo-EM images of many identical individual particles (II).

Although the TEM movies contain many particles, not all particles could be used for reconstruction because of overlap with other particles and insufficient rotation. Here, we present the two most reliable 3D reconstructions from a 1561-image series (particle 1 in Fig. 1, B and C, and movie S1) and a 1171-image series (particle 2...
in Fig. 1, D and E, and movie S2). Shown in Fig. 1, B to E, are the EM density maps of the two Pt nanocrystals. The rendered particle volumes are 5300 Å$^3$ (diameter 22 Å) for particle 1 and 4800 Å$^3$ (diameter 20 Å) for particle 2. Along with the direct visualization of the spatial distribution of Pt atomic planes, external and internal structures of the particle are uncovered. Each reconstruction shows three distinct crystal domains in both of the Pt particles. In Fig. 1, B and D, we show views of the EM maps in an orientation that reveals distinct lattice planes of the core domain. Our 3D reconstruction methodology produced reconstructions at near-atomic resolution from relatively small sets of noisy experimental TEM images of nanocrystals in random orientations.

**Fig. 2.** The underlying structural principle of the small Pt nanoparticles studied here. (A) Schematic illustration of the front view of particles 1 and 2 shown in Fig. 1. Both particles are composed of a dense central disc of atomic planes (blue lines) with conical protrusions (orange lines) anchoring on each side of the disc. (B) Cross-sectional view of the EM density map of particle 1 and 2 along the vertical plane. (C) View orthogonal to (A), showing the overlaid lattices. Scale bars, 0.5 nm. (D) Schematic illustration of the top view of particles 1 and 2 shown in (C). (E) Cross-sectional view of the EM density map of particles 1 and 2 along the equatorial plane.

**Fig. 3.** Cross-sectional study of particle 1. (A) 3D density map of particle 1 with color coding to highlight the three sections. Cross sections 1 and 2 are in arbitrary positions near crystal domain interfaces. Scale bar, 0.5 nm. (B) Slab through the 3D reconstruction of particle 1 along the vertical plane, with tentative atomic positions indicated. ABC repeats of (111) planes are visible. (C) Slab along cross section 1. The exposed (100) surface of the fcc Pt crystal is shown consistently in both exposed surfaces. Intersections with (111) planes in the top domain are displayed by red dashed lines. (D) Slab along the equatorial plane of (A) exposes a (110) plane. The intersections with (111) planes are shown by blue dashed lines. Pseudo-atomic structure (right) demonstrates the rotation angle (14°) between the (100) and (110) surfaces from the top and bottom domains, respectively. (E) Slab along cross section 2. The disordered (100) surface of the fcc Pt crystal is shown consistently in both exposed surfaces. Intersections with pseudo (111) planes in the bottom domain are displayed by red dashed lines.
We tested the validity of SINGLE using 1000 multislice TEM simulations of a randomly oriented Pt nanocrystal with dodecahedral symmetry and corresponding size. Reconstructions were obtained from images with a signal-to-noise ratio (SNR) roughly corresponding to that of the experimental images (see fig. S1). This verified that the experimental images can conform to the projection-slice theorem with ideal microscope conditions (20).

To confirm the existence of a projected lattice in the experimental images, we calculated their power spectra, which showed distinct spots along different crystalline zone axes (fig. S2). Closer examination of the power spectra and corresponding orientation coverage throughout the image series revealed that each particle continuously undergoes small local rotations followed by rapid orientation changes, often accompanied by lateral movement. The noise variance in the individual frames of the movie exceeds the signal variance by approximately a factor of 5, despite the strong scattering from the heavy Pt atoms (movies S3 and S4 show 200 raw TEM images). Although sufficiently thin to observe atomic detail, the liquid between the graphene sheets introduces a granular background, making it difficult to distinguish the facets of the nanocrystal in the individual frames. To enhance the image contrast and allow accurate 3D orientation determination, we averaged the image series in sets of five consecutive frames, resulting in a time resolution of ~100 ms. After frame averaging, we carefully scanned the image series to remove a small fraction (~20%) of those averages that did not show any lattice contrast because the particles had moved out of the narrow ideal focal plane of the aberration-corrected microscope.

Initial 3D models were obtained using our recently developed framework (PRIME) for ab initio single-particle 3D reconstruction (11). The standard technique used in biological single-particle cryo-EM (10) assigns each image the single best matching orientation, as determined by correlation matching to a gallery of reference images, obtained by projecting an a priori available 3D reference model. Two fundamental limitations of the standard technique are the bias introduced by the initial 3D model and the lack of mechanisms for modeling the alignment errors when data are noisy and the model is of poor quality. These limitations may be quite substantial when reconstructing nanocrystals, because every particle is different and the particle population cannot be averaged, as in traditional single-particle EM. The individual frames also have a low SNR because of the relatively low per-frame electron dose and because of the granular background introduced by the liquid. PRIME overcomes these drawbacks by using weighted orientation assignment and stochastic optimization for determination of an optimal orientation weight distribution without any a priori information about the nanoparticles.

To initialize the 3D reconstruction process, we assigned the random orientations to images, producing a featureless spherical density map. The random orientations were refined by stochastic optimization of the correlation between the images and reprojections of the density map, using information from 30 Å to 3 Å and a discrete search space of orientations. The resulting initial model had a resolution of 2.5 Å according to the 0.143 Fourier shell correlation (FSC) criterion (21). We extended the PRIME algorithm by introducing a continuous orientation search space and used stochastic optimization to determine a continuous distribution of weights that related the continuous distribution of orientation parameters to the 3D reconstruction. Each round of the PRIME iterative alignment procedure involved determination of orientation weights for all particle images, followed by a weighted 3D reconstruction by direct Fourier reconstruction using a Kaiser-Bessel interpolation kernel. A few hundred iterations were executed, and in every round the FSC was calculated and used to construct a 3D Wiener filter that filtered the map such that the optimal SNR was obtained at the present resolution (22). The resolution of the final refined maps was measured to 2.10 Å (particle 1) and 2.14 Å (particle 2), respectively.

Even though the FSC methodology provides an accurate measure of the resolution when the reference structure used for matching has been appropriately low-pass filtered, it does not ensure against grossly incorrect structures. To validate our structures, we determined the agreement between the individual images used for reconstruction and the corresponding reprojections of the reconstructed 3D map. All image reprojection pairs (fig. S8) were generated for the two reconstructions, and the Fourier ring correlation (FRC) (23) was calculated between all pairs. The average FRC was larger than 0.143 to a resolution of 1 Å and showed a distinct peak spanning the 1 to 2 Å resolution region (fig. S3). This peak is due to the correlation between atomic densities in the reprojections and atomic densities in the images. We concluded that our reconstructions showed excellent agreement with the images. The spatial resolution is higher for particle 1, which has a larger number of frames, indicating that the present resolution can be improved by acquisition of longer movies that cover a wider range of rotational orientations.

We had anticipated that the Pt nanocrystals would have at least twofold rotational symmetry, perhaps even cubic symmetry. Remarkably, the

![Fig. 4. Twist grain boundary free energies as a function of misalignment angle for the (110)-(000) crystal planes of a nanocrystal with 1135 atoms. Shaded regions indicate error bars of 1 and 2 standard deviations. The inset shows a relaxed nanocrystal with an initial misalignment angle of 14°. The non-flat grain boundary is located in the center of the nanocrystal. Images around the perimeter show the two relaxed planes at the grain boundary from the 100 (red) and 110 (white) grains for different misalignment angles.](image)
reconstructions appeared asymmetrical, and attempts to apply C2, C4, C5, and D2 point-group symmetry by aligning the images to the principal symmetry axis and doing symmetric orientation refinement failed to improve the correlation between the reprojections and the images. This suggests that the Pt nanocrystals do not possess icosahedral, cubic, or pentagonal symmetry but are intrinsically asymmetric. Reconstruction by the traditional single-particle cryo-EM technique, using an initial model based on a faceted face-centered cubic (fcc) nanoparticle, would suffer severe initial model bias and would not reproduce the true asymmetrical multidomain structure.

Our reconstructions of particles 1 and 2 revealed different asymmetrical crystal structures with the same underlying principle: a dense central disc of atomic planes (the core) with conical extensions anchored on each side, protruding in opposing and orthogonal directions with respect to the equatorial plane (see schematics in Fig. 2A with the lattice in different crystal domains colored blue and orange). Despite this similarity, the reconstructed 3D structures of the two Pt particles show interesting differences. First, the atomic arrangements on the surfaces observed in the EM density maps of particle 1 and 2 are distinct (Fig. 1C and Fig. 2E). Second, the two particles show different degrees of crystallinity in each domain. Whereas straight [111] crystal planes in three domains of particle 1 are shown throughout the cross-sectional images along the mid-vertical plane (Fig. 2B) and vertical planes at different depths (Fig. 4k), particle 2 shows more disordered internal structures within the domains. Also in particle 1, the conical protrusions have [111] atomic planes tilted with respect to the [111] atomic planes in the core. Particle 2 exhibits protrusions with a larger degree of disorder relative to the well-aligned [111] lattices in the particle 1 protrusions. Views of the reconstructions perpendicular to the equatorial plane (Fig. 2C) and a schematic illustration (Fig. 2D) show the overlaid lattices of the core (blue lines) and protrusions (yellow lines) with different tilting angles for particle 1 (14°) and particle 2 (7°).

The cross-sectional views along the equatorial plane (Fig. 2E) for particles 1 and 2 show similar arrangements of the [111] lattice planes, but cross-sectional images along the horizontal planes below and above the equatorial plane for the two particles (particle 1 in Fig. 3, C to E; particle 2 in Fig. 4S) indicate that the two particles are assembled by multiple domains but in unique geometries. The multiple domains and twisted grain boundaries that are present in the reconstructions are similar to previous observations of Pt nanocrystal growth trajectories, where small particles were observed to join along surfaces with low ligand coverage (9). The multimain arrangement is also supported by other tomography reconstructions of larger Pt nanocrystals containing multiply twinned domains with decahedral symmetry and central screw dislocations (3, 5). Multidomain structures are common in many other colloidal metal nanoparticles, which likely evolve as a result of multiple coalescence events during growth (3, 24–26). Our observation of heterogeneously structured Pt nanoparticles formed in the same solution confirms that individual particles from the same synthesis follow different nucleation and growth trajectories.

Figure 3A presents a 3D density map with colored sections indicating the upper (blue), core (gray), and lower (purple) domains of particle 1. Figure 3B shows a cross section perpendicular to the equatorial plane with the tentative atomic positions (different colored dots in each domain) indicating [111] lattice planes of fcc crystal that have repetition in every three crystal planes in all three crystal domains. Atoms closer to the surface seem to deviate farther from a perfect fcc structure. This is presumably explained by the fact that surface atoms are prone to relax excess free energy because of insufficient coordination and stabilization by surface ligand binding. In addition, the interface between the domains is disordered and not flat—a general consequence of the reduction in surface energies expected for nonscale crystals relative to their bulk counterparts. The locations of three cross sections along horizontal planes (cross section 1, equator, and cross section 2) are indicated in Fig. 3A. Cross sections 1 and 2 are positioned near the interface between the central disk and the upper and lower conical protrusions, respectively. Cross section 1 exposes two facing surfaces from the upper and core domains (blue and gray densities in Fig. 3C, respectively). Cross section 1 shows a (100) surface with red dashed lines that trace the [111] planes exposed on the (100) surface. The cross section at the equator (Fig. 3D) exhibits a (110) surface, and blue dashed lines indicate the [111] planes exposed to the (110) surface. The red and blue lines are mapped onto the pseudo-atomic illustration of (100) and (110) surfaces with the proper orientation (a 14° rotation angle) in the right image of Fig. 3D. At cross section 2, near the interface between the central disk and the lower conical protrusion, a surface structure with pseudo-100 patterns traced by red dotted lines is exposed from the core and lower domains (gray and purple densities in Fig. 3E, respectively), which deviate from the ideal fcc structure. Figure 3 shows that the conical protrusions and the core join along (100) and (110) surfaces with distortions at the interface. Multiple domains merging along the low-index crystal planes, such as (100) and (110), are presumably formed by coalescence events between small particles during the particle growth as a route to minimize excess surface energy. We previously observed a similar scenario: Two small Pt particles join along the [111] surfaces during growth (3). Presumably, coalescence along the low-index surfaces and the consequent evolution of the interface structure are mechanisms by which the nanocrystals reduce free energy.

To examine whether there is a thermodynamic rationale for the multidomain structures that we observed, we computed the free energies for the grain boundary formation and for the ligand-exposed surfaces. This was done using the Frenkel–Ladd method (27) for an embedded atom model of Pt (28), using an appropriate thermodynamics integration path (fig. S6). We found that for low-angle misalignments, like those observed in particle 1 (14° rotation angle between [111] planes exposed on (110) and (100) surfaces from the central disc and upper domain in Fig. 3), the grain boundary free energy for a nanoparticle with 1200 atoms is 0.3 J/m², increasing to 0.55 J/m² for misalignment angles of up to 59° (Fig. 4). Small rotational relaxation of the crystal grains is found to be energetically viable at these interfaces, which may explain the orientation of the side protrusions in particle 1 with respect to its central section.

The surface free energy for the ligand-exposed interfaces was computed to be 2.8 J/m² as averaged over the (100) and (111) surfaces, weighted appropriately for the cuboctahedral shape. The disparity of scales between the grain boundary free energies and the much larger exposed surface free energies confirms that there is a large thermodynamic driving force for coalescence, even when such events result in grain boundary formation. As the free energy gain upon coalescence is much larger than thermal energies, initial aggregates are likely irreversible. The resultant grain boundaries are then kinetically arrested over laboratory time scales (see supplementary materials).

Our results show that the SINGLE methodology can be used to investigate the structural principles underlying the assembly and transient morphology of any stable, small nanoparticle in solution. We envision that SINGLE can be applied directly to in situ 3D structural studies of many other kinds of solvated particles.

REFERENCES AND NOTES
Summer declines in activity and body temperature offer polar bears limited energy savings

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Polar bears (Ursus maritimus) summer on the sea ice or, where it melts, on shore. Although the physiology of “ice” bears in summer is unknown, “shore” bears purportedly minimize energy losses by entering a hibernation-like state when deprived of food. Such a strategy could partially compensate for the loss of on-ice foraging opportunities caused by climate change. However, here we report gradual, moderate declines in activity and body temperature of both shore and ice bears in summer, resembling energy expenditures typical of fasting, nonhibernating mammals. Also, we found that to avoid unsustainable heat loss, the swimming, nearshore bears employed unusual heterothermy of the body core. Thus, although well adapted to seasonal ice melt, polar bears appear susceptible to deleterious declines in body condition during the lengthening period of summer food deprivation.

The current rate of Arctic sea-ice loss, unprecedented in at least the past several thousand years, is outpacing predictions and accelerating (1). This raises concerns about the persistence of polar bears (Ursus maritimus) (2), which hunt on the surface of the sea ice, most successfully between April and July (3), when ringed seals (Pusa hispida) use this substrate for rearing pups and molting (4). Between August and October, hunting can be poor (5) as seals reduce ice surface time (4). Additionally, in about two-thirds of the polar bear range (6), seals become largely pelagic as ice retreats from the continental shelf (7, 8). Some polar bears spend this period on shore, where foraging is also usually limited (9).

To reduce the loss of body condition during summer food deprivation, shore bears purportedly enter a state of lowered activity and resting metabolic rate similar to winter hibernation but without denning (10). This “walking hibernation” could partially compensate for the negative impacts of extended ice melt (11). However, in western Hudson Bay, Canada, shore bears lose body mass at a rate indicative of, rather than hibernation-like, metabolism (12). The physiological state of bears that follow the retreating sea ice into the central Arctic basin in summer is unknown. In addition, recent sea-ice loss may be increasing the frequency of long-distance swims by polar bears (13), during which they risk losing over 10 times more heat than they produce (supplementary text) because their fur loses 90% of its insulation value when wet (14), and their subcutaneous fat does not provide blubber-like insulation (15).

To understand polar bear responses to these challenges of summer ice melt, we investigated activity on shore (2008 and 2009) and on ice (2009) in the Beaufort Sea (Fig. 1) by affixing telemetry transmitters and activity loggers (16) to 25 females (mean age = 10 years ± 1 SE, age range = 4 to 20 years) and one male (age 3). We recorded temperatures of the body core (an index of metabolic rate) (17) and periphery by implanting loggers into the abdomens (core) of 10 bears (nine females, mean age = 11 years ± 2 SE, age range = 3 to 23 years; one male, age 6) and the rumps (periphery) of seven other individuals (six females, mean age = 9 years ± 2 SE, age range = 5 to 20 years; one male, age 2).

Fig. 1. The western Arctic on (A) 11 May 2009 and (B) 31 August 2009. Locations are shown for ice polar bears (blue circles), shore polar bears (red squares), and whale carcasses (triangles). The 300-m depth contour is shown as a dashed line. Sea ice is shown in white.

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References (29–38)
3D structure of individual nanocrystals in solution by electron microscopy
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Supplementary Materials for

3D structure of individual nanocrystals in solution by electron microscopy

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This PDF file includes:
Molecular Dynamics Simulation
Materials and Methods
Figs. S1 to S9
Captions for movies S1 to S4

Other supplementary material for this manuscript includes:
Movies S1 to S4
Captions for Movies S1 to S4

**Movie S1.** 3D EM density map of particle 1.

**Movie S2.** 3D EM density map of particle 2.

**Movie S3.** TEM movie of Pt nanoparticles in GLC. The movie is composed of 200 subsequent non-averaged frames with the full field of view binned by 2.

**Movie S4.** TEM movie of Pt nanoparticles in a small section of the full field of view at full resolution. The section of interest is selected from the lower right quadrant of Movie S3.

**Molecular Dynamics simulation**

The width of a grain boundary can be computed by determining local volumes surrounding atoms in a structure using a Voronoi tessellation (29). For a (110)-(100) grain boundary, similar to that between the upper and core sections of particle 1, this width is around 5 Å (Figure S7 (A) and (B)). Any additional grain boundaries in the nanoparticle that are not overlapping with this interface, as is the case for the three domain structure observed, have additive contributions to the free energy of the nanoparticle. The surface free energy for the ligand-exposed interfaces was computed to be 2.8 J/m² as averaged over the (100) and (111) surfaces, weighted appropriately for the cuboctahedral shape. The disparity of scales between the grain boundary free energies and the much larger exposed surface free energies confirms that there is a large thermodynamic driving force for coalescence, even when such events result in grain boundary formation. For example, aggregating three N = 400 particles into a single N = 1200 crystal is favored by -11.3 kJ/mol for a single crystalline structure and -8.2 kJ/mol for a three-domain-structure, such as those observed here. In both cases, the free energy gain per particle is much larger than typical thermal energies, likely rendering the initial aggregation irreversible. Small relative rotational distortions of the crystal grains are
found to be energetically viable for (110)-(100) interfaces, which may explain their prevalence in our structures. As detailed in the supporting information, similar thermodynamic driving forces exist for aggregation along other low-index crystallographic axes and for other size particles (Figure S7 (C) and S8), consistent with previous observations (Figure S8) (3, 5). For larger nanoparticles, slow surface diffusion and the tight packing of adsorbed ligands are likely to kinetically mediate these large thermodynamic driving forces. However, for smaller nanoparticles that are likely to be involved in aggregation, such steric effects have been shown previously to be less important and do not saturate all potential surface binding sites (4, 30). In all cases, the resultant planar grain boundaries have slow relaxation at room temperature and once formed they are kinetically arrested over experimental timescales.

Materials and Methods

Verification of the 3D reconstruction method with simulated Pt crystal structure
To verify that our 3D reconstruction methodology is capable of producing near-atomic resolution reconstructions, we generated 1000 randomly oriented projection images of a simulated symmetrical Pt nanocrystal with dodecahedral symmetry. The images were generated with multi-slice simulation (31), using the input parameters C₃ of -10 μm, C₅ set to 1.0 mm, a defocus of 37 Å, an objective limit of 20 mrad, a convergence angle of 0.5 mrad, and a defocus spread of 50 Å, and a slice value of 1 Å. The pixel resolution of the simulated images was 0.58 Å to match the pixel resolution of the experimental images. Gaussian noise was added to the images to give a signal-to-noise ratio (SNR) of 0.2, roughly corresponding to the SNR of the individual experimental frames. The simulated images were processed by PRIME exactly as for the experimental images as described above. The final reconstruction had a resolution of 2.5 Å and showed the expected dodecahedral symmetry (Fig. S2). The point group symmetry was not used as prior information—the probabilistic (or weighted) orientation assignment in PRIME automatically identifies the point-group symmetry without it being imposed as a
constraint in the orientation search. The same behavior was observed in the past when processing cryo-EM images of D2 and D7 symmetrical molecules.

**Sample preparation of Pt nanocrystal solution**

Pt particles dispersed in solution for GLC TEM series acquisition can be prepared by two methods. Pt particles can be synthesized and dispersed in solvent prior to the encapsulation in GLC.

Pt nanoparticles with sub-2 nm size are synthesized by following a previously reported method with modifications (32). 10 mL of tetra-n-octylammonium bromide solution (0.05 M, 0.273 g in 10 mL) in toluene is added dropwise into a round-bottomed flask containing an aqueous solution of H₂PtCl₆·6H₂O (0.03 M, 0.00648 g in 3.75 mL H₂O). Then, the mixture is stirred for 10 min with a magnetic stirring bar. An aqueous solution of sodium borohydride (0.4 M, 0.0529 g in 3.5 mL H₂O) is quickly injected into the reaction mixture. The pale-yellow organic phase immediately turns into brown which indicates the formation of Pt nanoparticles. After 30 sec from the injection of sodium borohydride, 51 μL (2.50 × 10⁻⁴ mol) of dodecanethiol is added. The mixture is subsequently stirred for 3 h, followed by separation of the organic phase using a separation funnel. 40 mL of ethanol is added to the organic solution containing the nanoparticles, and the mixture is maintained in a freezer for 1 h to allow precipitation of the particles. The particles are then separated by centrifugation (6000 rpm for 20 min), washed several times with a mixture of ethanol and acetone. As-synthesized Pt particles are soluble in organic solvents for the preparation of a graphene liquid cell. The particle solution is diluted to half of the concentration by using a mixture of o-dichlorobenzene and oleylamine with a volume ratio 20 to 1. The final particle solution in a mixture of o-dichlorobenzene and oleylamine maintains its colloidal stability for liquid phase TEM for longer than 6 months.

**Graphene liquid cell preparation**
Graphene is synthesized by chemical vapour deposition (CVD) on 25 μm thick copper foil (99.8 %, Alfa Aesar, Ward Hill, MA). The copper foil is inserted into a quartz tube and heated to 1,000 °C under a H₂ flow of 10 cm³/min at 150 mTorr. After annealing for 30 min, a gas mixture of 25 cm³/min CH₄ and 10 cm³/min H₂ at 520 mTorr is introduced for 20 min to synthesize graphene. Fast cooling to room temperature with a CH₄ flow of 20 cm³/min under a pressure of 330 mTorr follows.

The synthesized graphene on Cu foil is transferred to Quantifoil holey C TEM grids using a direct transfer method (33). In brief, a graphene-covered Cu foil is placed onto a glass slide and gently flattened by pressing with another glass slide. Graphene on the exposed side is removed by mild O plasma etching. The Quantifoil TEM grid is placed onto a graphene side of Cu foil with the Quantifoil C film side facing the graphene. Then 10.0 μl of isopropyl alcohol is dropped onto the sample to wet the interface, and the sample is dried at 80 °C on a hot plate for 20 min to promote adhesion between the Quantifoil C film and the graphene. The grid is floated on an aqueous solution of 113 mM ammonium persulfate, (NH₄)₂SO₄, to etch the underlying Cu foil and rinsed several times by floating the graphene transferred grid on deionized water. Two graphene coated holey C film TEM grids are prepared using this method. For encapsulation of the liquid sample, 0.5 μl of either Pt growth solution or synthesized Pt particle solution is placed onto one graphene coated TEM grid that is held by self-closing tweezers. A small portion of the edge of the other graphene coated TEM grid is cut by a razor blade to promote a close contact between the two graphene sheets when it is placed on the TEM grid held by the tweezers tip. The partially cut graphene TEM grid is gently placed onto the graphene TEM grid with the sample solution and left in ambient conditions for several hours to promote solvent drying mediated bonding between two graphene surfaces. As-prepared graphene liquid cells are plunged into isopropanol solution to clean off the excess solution and then dried in ambient conditions.

Acquisition of movies of Pt nanoparticles
The images were acquired using TEAM I, an FEI Titan 80/300 transmission electron microscope (TEM) with a post-specimen geometric- and chromatic-aberration corrector at the National Center for Electron Microscopy within The Molecular Foundry. The aberration-corrector was aligned for approximately -10 μm third-order spherical aberration (C₃ or C₃₀) to produce white atom contrast at a small under focus value of 30 – 50 Å (34). The detector used is a prototype of the Gatan K2-IS direct electron detector with 1024 x 1024 pixels with superior contrast transfer and ~50 frames-per-second (fps) acquisition capability (18). Liquid pockets that were used in imaging are in the diameter range of sub-50 nm. It was necessary to sum 5 images to provide sufficient SNR for the reconstruction algorithm.

Molecular dynamics simulation

**Calculation Details** To represent a Pt nanocrystal, we start with an ideal cuboctahedral shape. This shape is predicted from simple thermodynamic arguments that minimize the relative surface free energies given different solvent exposed crystal facets and a size constraint. These equilibrium constructions yield stable cuboctahedral structures for a range of nanocrystal sizes (35). Interactions between Pt atoms are modeled with the embedded atom model (EAM) (36), which is based on a second moment expansion of a tight-binding expression for the energy of a delocalized electronic system. Its functional form can be written as a sum of pair functions and a many body function dependent on the local density

\[
U(\mathbf{r}_N) = \sum_{i<j} \phi(\mathbf{r}_{ij}) + \sum_{\lambda} f \left( \sum_{j} \rho(\mathbf{r}_{ij}) \right)
\]

where \( \phi(\mathbf{r}) \) is a pair function, \( \rho(\mathbf{r}) \) is a local density function and \( f(\rho) \) is the embedding function. The forms of these functions are quintic splines with smooth second derivatives and asymptote to zero to insure non-impulsive forces and typically fit to ab initio data. EAM potentials have been use previously with much success in modeling surface reconstruction and deformation in metallic systems. The potential we adopt for Pt was
developed by Sheng et al., parameterized with first principles data and optimized with a host of experimental reference data including lattice constant and vacancy formation energies (28). Of particular relevance to this study is the accurate recovery of the bulk Young's and shear moduli at low temperature and stacking fault energies.

To compute the grain boundary free energy, we use the Frenkel-Ladd model and construct a thermodynamic integration path that references an ideal harmonic, or Einstein, crystal (27). The schematic path is illustrated in Fig. S7. Specifically, we compute the reversible work to transform a defect-free nanocrystal into one with a symmetric planar twist grain boundary along one of the lower index crystal planes, (100), (111) or (110), characterized by a misalignment angle. We do this in three steps. First, we compute the free energy of the defect-free nanocrystal interacting with the EAM potential relative to a purely harmonic system with the same geometry. We do this by thermodynamic integration,

$$\mathcal{H} = U(r_{h}^{3N}) + (1 - \lambda) \left( \Delta U(r_{p}^{3N}, r_{p}^{3N}) \right) + \lambda \sum_{i} \alpha (r_{i} - r_{p,i})^{2}$$

(2)

where $r_{p}^{3N} = \{r_{p,1} ... r_{p,N}\}$ are the positions of all atoms in the ideal nanocrystal, $\Delta U(r_{p}^{3N}, r_{p}^{3N}) = U(r_{p}^{3N}) - U(r_{p}^{3N})$ and $\lambda$ varies between 0, where the system interacts with the EAM potential, and 1 where the system is composed of non-interacting harmonic oscillators. The free energy, different for adiabatically changing from the system defined by $\lambda = 0$ to $\lambda = 1$, can be calculated from

$$\Delta F_{1,2} = \int_{0}^{1} d\lambda \left( \frac{d\mathcal{H}}{d\lambda} \right)_{\lambda}$$

$$= \int_{0}^{1} d\lambda \left( \sum_{i} \alpha (r_{i} - r_{p,i})^{2} - \Delta U(r_{p}^{3N}, r_{p}^{3N}) \right)_{\lambda}.$$  

(3)

The benefit of doing this is that the free energy for an ideal harmonic system in 3 dimensions is known exactly and is given by
\[
F_2 = U(\tau_p^{3N}) - \frac{3k_BT}{2} N \ln(k_B T \pi / \alpha),
\]

(4)

where \(k_B\) is Boltzmann’s constant and \(T\) is the temperature, here 300 K. Computing the free energy difference between the defect-free nanocrystal and one with a grain boundary for a purely harmonic system with the same number of degrees of freedom and the same spring constants is then,

\[
\Delta F_{2,3} = \Delta U(\tau_d^{3N}, \tau_p^{3N}),
\]

(5)

where \(\tau_d^{3N}\) are the positions of the atoms for a nanoparticle with a grain boundary. Finally the free energy for transforming the nanocrystal with a grain boundary from a harmonic reference to a fully interacting system is the same as Eq. 3,

\[
\Delta F_{3,4} = -\int_0^1 d\lambda \left\{ \sum_i \alpha (\tau_i - \tau_i^h)^2 - \Delta U(\tau_d^{3N}, \tau_h^{3N}) \right\}_{\lambda},
\]

(6)

but with \(\tau_p^{3N}\) replaced by \(\tau_h^{3N}\). The total grain boundary free energy is then given by the sum

\[
\Delta F_{GB} = \Delta F_{3,4} + \Delta F_{2,3} + \Delta F_{1,2}.
\]

(7)

In order to evaluate the integrals in Eq. 3 and 5, we must discretize the path. To do so we follow de Koning and Antonelli by choosing points along the curve defined by

\[
\lambda(\tau_i) = \tau_i^5 (70\tau_i^4 - 315\tau_i^3 + 540\tau_i^2 - 420\tau_i + 126),
\]

(8)

where \(\tau_i = i/p\) is the \(i\)th path of \(p\) total sampling points (37). This places additional sampling on the lower and higher ends of \(\lambda\) where effects due to unconstrained center of mass motion can lead to rapid variations in the integral.

**Symmetric grain free energies** We have computed \(\Delta F_{GB}\) as a function of misalignment angle \(\Theta\), which we denote \(\Delta F(\Theta)\), for the low index crystal planes (100), (111) and (110).
The results of these calculations for a \( N = 1135 \) atom nanoparticle is shown in Fig. S9, where we have normalized each curve by the area of the grain boundary \( Y(\Theta) = \Delta F(\Theta)/A \), in such a way that the asymptotic value for large nanoparticles is consistent with a bulk grain boundary free energy computed with periodic boundary conditions. Each crystal plane has a different symmetry. The (100) surface is four-fold symmetric and as a result \( Y(\Theta) \) has a period of 90°. Approaching 45°, \( Y(\Theta) \) is monotonically increasing and fairly featureless before decaying back to 0 at 90°. The (111) surface is three-fold symmetric and as a result \( Y(\Theta) \) has a period of 120°. The (111) surface is close-packed and as a result the free energy as a function of \( \Theta \) is fairly featureless, reaching a flat profile at 20° before decaying back to 0 at 90°. The maximal value of the (111) surface relative to the (100) surface is greater by a factor of 2, as has been observed previously. At 60°, there is a sharp dip in the free energy, which reflects the formation of a coherently twinned plane common to all FCC lattices, whose energy is much lower than a grain boundary's. The (110) surface is two-fold symmetric and as a result \( Y(\Theta) \) has a period of 180°. This surface exhibits a much richer structure than either of the more closed-packed surfaces as a function of misalignment. Specifically, there are symmetric meta-stable minima at 70° and 110° separated by large free energy barriers. These minima are due to the formation of incoherently twinned planes. Further, the corrugation of the (110) surface results in an overall higher surface free energy relative to the (100) or (111) surfaces. The cusps found in each of these functions are similar as those found in bulk materials (38).

**Asymmetric grain, (110)-(100), free energies** We have also computed \( \Delta F_{\text{GB}} \) for an asymmetric, (110)-(100), grain boundary like that found in our experiment. The results of this are shown in Fig. 6. As an asymmetric grain boundary, even with 14° misalignment between relative [111] surface directions, there is a nonzero free energy of 0.3 J/m². This free energy increases to 0.55 J/m² at relative orientations of 59° before decreasing consistent with the 4-fold symmetry of the (100) surface. These values contain contributions both from in-plane disordered packing as well as surface stresses from the
line defect circumscribed at the boundary. Thus, the packing at the interface determines the overall excess free energy.

**Width of the grain boundary** Using a Voronoi tessellation, the local volume around each atom can be computed. The distribution of volumes cleanly distinguishes between interior and surface atoms. Fig. S8 (A) and (B) show the excess volume is localized near (110)-(100) grain boundary at \( z = 0 \). The width of the interface is \( \sim 5 \) Å as determined by the full-width at half max of a Gaussian fit to the profiles. The localization of excess volume near a grain boundary is a signature of disrupted packing. Moreover, we have found that an additional grain boundary in the nanoparticle, as determined by the excess volume, has additive contributions to the free energy of the nanoparticle.

**Ligand exposed surface free energies** In order to determine the excess surface free energy of a single grain nanocrystal, we analyze the finite size scaling of the free energy of cuboctahedral nanoparticles. This is done with the thermodynamic path in Fig. S7, where only steps 1 and 2 are necessary, with particle sizes between 100 and 30000 atoms. In order to extract the chemical potential and surface free energy of the Pt atoms, we fit the free energy to the form,

\[
F(N) = \mu N + f_s N^{2/3},
\]

where \( \mu \) is the chemical potential and \( f_s \) is the non-extension contribution to the free energy, which is expected to be of the form of an exposed surface tension times a specific area reflective of the cuboctahedral geometry. The data and fit are shown in Fig. S8 (C), where \( f(N) = F(N)/N \). This fit yields a value of \( f_s = 308 \) kJ/mol. Using the values of the maximal coverage and binding energy of oleylamines from the reference (30), we can approximately correct the excess surface energy for ligand coverage, assuming that entropic effects from ligand packing are negligible. This yields \( f_{s,l} = 270 \) kJ/mol.

**Thermodynamic driving force for aggregation** Using the data computed above, the
thermodynamic driving force for aggregation can be determined. This is done by considering an expression for the free energy difference between a nanoparticle of size $N$ and an aggregate made up of a multiple of nanoparticles $N_b$ with $b$ independent grain boundaries. The following generic form follows, assuming a constant geometry,

$$
\Delta F_{\text{agg}} = f_{\text{gb}} \left[ N_b N^{2/3} - (N_b N)^{2/3} \right] - b f_{\text{gb}} (N_b N)^{2/3},
$$

(10)

where $f_{\text{gb}}$ is the grain boundary free energy for a specific crystal direction times a specific area for the grain boundary. In Eq. 10 volume terms proportional to a chemical potential drop out, and we have neglected logarithmic corrections associated with the reduction of entropy due to aggregation. From the free energies above, the maximal grain boundary free energies for symmetric grains are $f_{\text{gb}}^{110} = 38$ kJ/mol, $f_{\text{gb}}^{100} = 22$ kJ/mol, $f_{\text{gb}}^{111} = 12$ kJ/mol, and for the asymmetric (110)-(100) grain is $f_{\text{gb}}^{110-100} = 25$ kJ/mol. With Eq. 10 and the parameters above, the thermodynamic driving force for aggregation can be computed. Generalization for aggregates of non-similar sizes is straightforward. Note that the scaling analysis done here does not require a definite determination of the volume or surface area of the nanocrystal, as typically such measures are ill-defined.
Fig. S1. Validation of the 3D reconstruction methodology using simulated data of a dodecahedral Pt nanoparticle. (A) Reconstruction from images without noise (top panel) and representative images (bottom panel). (B) Reconstruction from images with a SNR of 0.1 (top panel) and representative images (bottom panel).
**Fig. S2.** Power spectra and orientation coverage for particle 1 and 2. Orientation coverage is extracted by the projection orientation vector onto the horizontal plane. Particle 2 series shows better rotational coverage than particle 1 series, which suffers from a missing wedge of information.
Fig. S3. Validation of the 3D reconstruction for particle 1 and particle 2. (A), (B)
Comparison of frame averages (left column) with re-projections of the map (right column) for particle 1 and 2, respectively. (C), (D) The diagrams show the average Fourier Ring Correlation (FRC) between the images and the re-projections for particle 1 and 2, respectively. The FRC is larger than 0.143 to 1 Å resolution and shows a distinct peak spanning the 1-2 Å region. This peak is due to the correlation between atomic densities in the re-projections and atomic densities in the images.
Fig. S4. Slabs through the density maps of (A) particle 1 and (B) the particle 2 along the vertical cross-sections with different depths: behind the mid-plane (left), at the mid-plane (middle), and the front plane (right). Whereas straight lattice planes are clearly seen in multiple domains of particle 1, particle 2 shows more disordered internal structures. It is difficult to specify the exact atomic arrangement, since the current resolution is slightly anisotropic. However, the presence of additional domains and edge dislocations are likely causes of such disordered lattice patterns.
**Fig. S5.** Slab through images of particle 2 along the horizontal cross-sections with different depths: above the equatorial plane (top), at the equatorial plane (middle), and below the equatorial plane (bottom).
Fig. S6. Schematic thermodynamic integration path for computing twist grain boundary free energies characterized by a crystal face and misalignment angle, $\Theta$. 
**Fig. S7.** (A) The average Voronoi cell volume as a function of displacement from the center of the nanocrystal, where the grain boundary originates. Three different color curves represent these distributions for three different initial misalignment angles. (B) Cross-sectional view of the relaxed nanocrystal with an initial misalignment angle 14°. Blue-to-red color gradient scales decreasing Voronoi cell volume from near to away the grain boundary as observed in the plot for 14° in (A). Surface atoms show a high cell volume due to unsaturated coordination. (C) The scaling behavior of the specific free energy per particle computed from thermodynamic integration of a cuboctahedral nanoparticle. The solid red line is a fit to the form in Eq. 9.
Fig. S8. Twist grain boundary free energies as a function of misalignment angle for the (100), (111) and (110) crystal planes of a $N = 1135$ atom nanocrystal. The grain boundaries are located in the center of the nanocrystal. Shaded regions indicate error bars of 1 and 2 standard deviations.