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Self-assembly of nanoparticles into biomimetic capsid-like nanoshells

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Abstract

Nanoscale compartments are one of the foundational elements of living systems. Capsids, carboxysomes, exosomes, vacuoles, and other nanoshells easily self-assemble from biomolecules, *e.g.* lipids or proteins, but not from inorganic nanomaterials due to difficulties with replication of spherical tiling. Here we show that stabilizer-free polydispersed inorganic nanoparticles can spontaneously organize into nanoshells. The association of water-soluble CdS nanoparticles into self-limited spherical capsules is the result of scale-modified electrostatic, dispersion, and other colloidal forces. They cannot be described by DLVO theory whereas molecular dynamics simulations with combined atomistic and coarse-grained description of the system from 81

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Author contributions:

M.Y. performed the experiments, conceived DLVO theory model, and analyzed the data. H. C. and P.K. conceived the Gauss model and performed MD simulations. G. Z. and P. Z. carried out and analyzed TEM tomography study. J.H.B. contributed the dynamic light scattering experiments and calculations of the surface potential/charge of NPs. N.A.K. conceived the project and designed the study. M.Y., H.C., P.K., G.Z., P.Z., and N.A.K. co-wrote the paper.

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nanoparticles reveal the emergence of nanoshells and their stabilization mechanism. Morphology of the simulated assemblies formed under different conditions matched nearly perfectly the TEM tomography data. This study bridges the gap between biological and inorganic self-assembling nanosystems and conceptualizes a new pathway to spontaneous compartmentalization for a wide range of natural and man-made inorganic nanoparticles.

INTRODUCTION:

A simple yet versatile geometry of nanoshells is at the foundation of their many unique physical, chemical, and biological phenomena. Nanoshells with sub-nanometer pores are frequently used in nature to sustain gradients of pressure, pH, and ion concentration. They also allow compartmentalization of chemical components in liquids providing reaction control, selective transport, protection, and reconfigurable sites for homogeneous catalysis. Biomolecular nanoshells are typically formed by the self-assembly of tens to a few thousands of organic structural units that are either highly anisotropic, *e.g.* phospholipids forming membrane in vacuoles, transport vesicles etc, or bound together by highly specific lock-and-key interactions found, for instance, between structural proteins of viral capsids,¹ cellular vesicles,² exosomes,³ carboxysomes,⁴ and bacterial bubble compartments.⁵

Inorganic nanoshells are being actively studied for their unique optical,⁶ mechanical,⁷ and catalytic⁸ characteristics that often originate in shell-confined quantum mechanical effects. Templating composite films on spherical particles affords making artificial nanoshells with diameters ranging from several hundreds of nanometers to several micrometers.⁹ Kirkendall effect,¹⁰ nonequivalent stoichiometry reactions,¹¹ and Oswald ripening¹² lead to the formation of 10–200 nm nanoshells by chemical transformation of solid NPs using mismatches between ion exchange/diffusion rates for different ions.

The chemical methods laid in the foundation of their formation are conceptually different from those utilized by nature based on self-assembly of bioorganic units with specific molecular geometries. Besides technological significance of inorganic nanoshells, their spontaneous formation from simple inorganic units can shed the light on the genesis of compartmentalized structures in nature and perhaps, life itself. However, it may appear fundamentally prohibited without atomically precise spherical tiling of the structural units or highly anisotropic molecular building organic blocks. Compared to proteins, lipids or polymers,¹³ inorganic nanoscale building blocks will impart new properties and functions to nanoshells. For instance, controllable porosity, stability and reconfigurable geometry of the biomimetic nanoshells would be essential for homogeneous catalysis of “difficult” reactions and structurally responsive systems. Self-assembly of inorganic shells from NPs will also be insightful for self-healing and biomineralization processes leading to nanoshell formation in living organisms.¹⁴

The studies carried out over the past decade indicated that inorganic NPs display distinct ability to self-organize into terminal (supraparticles,¹⁵ nanostars, *etc*) and extended structures (chains,¹⁶ sheets,¹⁷ superlattices^{18,19} *etc.*), but they were not observed to form shells or other open structures or spontaneously formed compartments. Here we demonstrate that simple CdS NPs do produce porous nanoshells and elaborate the mechanism of their

formation. Their assembly from simple structural units without pronounced hydrophobic/hydrophilic anisotropy, lock-and-key interaction, or molecular tiling between the constituents is attributed to the specific interplay of repulsive and attractive forces between NPs, ions and solvent, favoring exclusion of NPs from the center of these assemblies. Despite the generality of approaches and forces involved, the nanoshell formation cannot be fully rationalized based on the traditional colloidal theories. However, it can be understood using molecular dynamics (MD) simulations with atomistic description of NPs, surface ligands, counterions, and solvent molecules. Progressive coarse-graining of NP interactions from fully atomistic MD with *ab initio* calculation of atomic charges to partially atomistic description with Hamaker approximation of core-core dispersion forces allows one to balance accuracy of structural description and effective experimental time window to reveal conditions necessary for nanoshells to form.

RESULTS:

Constituent nanoparticles.

CdS NPs were used in this study as a model structural unit known to be capable of self-organization; these NPs are representative of other semiconductor nanocolloids in respect to the size, shapes, and physical/chemical properties. Nanoshells with diameters of 22 ± 4 nm (Fig. 1a,b) formed during the combined process of synthesis and assembly reminiscent of the protein-assisted self-assembly processes.¹⁹ The pH of the reaction media and final nanoshell dispersion was controlled by 0.01 M NaOH solution and in most experimental series was 9.5. Importantly, no extraneous organic molecules typically coating NP surfaces were used. This structural characteristic is attractive not only by simplicity of the synthetic protocol but it was also essential for this study because layer(s) of organic molecules on the NP surface strongly affect their interactions. Their presence would also alter the generality of the conclusions and modeling strategies.

Nanoshell formation and structure:

Individual NPs, as assembly unit of the nanoshells, can be identified in the transmission electron microscopy (TEM) images by the presence of crystalline interfaces visible at high magnification. Their lattice spacing of 0.34 nm corresponds to the (111) crystal planes (Fig. 1c,d) and is typical for cubic CdS. Oriented attachment observed for other NPs^{16,20,21} did not contribute to the spherical tiling of NPs because it occurs for CdS at a much slower rate than for CdTe¹⁶ or PbSe²² and for symmetry considerations. The wall thickness of these nanoshells was 3–5 nm and was identical to the dimensions of the NP “building blocks” (Supplementary Fig. 3a).

Room-temperature and cryo-TEM tomography of rapidly frozen samples captured the assemblies in their native hydrated state (Fig. 2a–d) and confirmed the formation of nanoshells. The three-dimensional (3D) arrangement of the constituent units can be accurately described by this technique making possible direct juxtaposition of the microscopy data with computer modeling.²³ Multiplicity of NPs in the nanoshells and long time required for dynamic NPs systems to reconfigure make this system particularly difficult to simulate compared with other studies (Supplementary Note 1). The wall thickness of ~4–

5 nm and the diameter of ~10–15 nm for the nanoshells observed from cryo-TEM tomography were consistent with the data in Fig. 1. The fact that the shells were composed of NPs without any special geometrical fit to each other was evident in these images and could be further highlighted by the 3D surface rendering of the nanoshell with identified CdS domains (Fig. 2e, the slice view of the raw reconstruction can be found in Supplementary Figs. 4 and 5). TEM imaging demonstrates that a nanoshell with a diameter of 20 nm accommodated *ca.* 90 NPs. The presence of nanoscale pores with a diameter of *ca.* 2 nm between the constituent NPs can be observed (Fig. 2c,d).

TEM images taken in the course of the nanoshell synthesis/assembly revealed intermediate geometrical shapes leading eventually to the formation of nanoshells. After 5 min, NPs form small arc-shaped agglomerates (Fig. 3a,b). After 10 min, they transform into incomplete crescent-like formations (Fig. 3c,d). The closing of the shells occurs in the subsequent 20 min of the assembly (Fig. 3e,f). Again, no preferred contact surfaces, geometrically matching shapes, or substantially larger agglomerates -- chains,¹⁶ sheets,¹⁷ ribbons,²⁴ *etc.* that were observed in similar systems -- can be identified at any stage of the process. Nanoshells and some individual NPs were exclusive products of this process with nanoshells representing over 90 % of NPs.

Assembly conditions on nanoshell structure:

The self-organization of NPs into nanoshells was found to be pH-dependent. At pH 8.2 and 7.2, nanoshells with diameters of 16 ± 2 nm containing *ca.* 58 NPs (Supplementary Fig. 6) and 11 ± 2 nm containing *ca.* 22 NPs (Supplementary Fig. 7) were observed, respectively. In each case the number of NPs in and the size of the nanoshells were smaller than those formed at pH 9.5 with diameters of 22 ± 4 nm (Fig. 1). Statistical analysis showed that the sizes of constituent NPs captured at the earlier stage of assembly were similar under different pH conditions: 4 ± 0.8 nm for pH 9.5, 4 ± 0.8 nm for pH 8.2 and 4 ± 0.7 nm for pH 7.2 (Supplementary Fig. 3.). Solid supraparticles containing ~ 20 structural units, that could be described as imploded shells appeared when the assembly process was carried out at pH 5 (Supplementary Fig. 8a–c). At pH 4.3, predominantly single NPs were present (Supplementary Fig. 8d–f).

Increase of pH coincides with the increase of zeta potential of the nanoshells, ζ . At pH 7.2, we observed nanoshells with $\zeta = -11 \pm 1$ mV. This electrokinetic potential can be related to total surface charge of $Q_{cds} = -7.2 \times 10^{-19}$ C, equivalent to ~4 – 5 electrons per nanoshell (See Supplementary Methods, the model used here is derived based on classical colloidal theories for solid spheres. While providing some assessment of particle charge, its limitations should also be considered). Since there are ~22 CdS NPs forming the nanoshell, the average charge on the individual NPs approximates to $q = -0.2e$. Similarly, at pH 8.2 and pH 9.5, nanoshells displayed $\zeta = -30 \pm 2$ mV and -50 ± 2 mV, respectively, which corresponds to a total surface charge of $Q_{cds} = -3.7 \times 10^{-18}$ C or -8.9×10^{-18} C, equivalent to ~ 23 or 55 electrons per nanoshell (Supplementary Methods) or an average charge of $q \approx -0.4e$ or $-0.6e$ per NP (about half of NPs are single charged). As one would expect, the increase of average NP charge leads to the increase of nanoshell size and electrostatic

repulsion between the “building blocks”, enhanced by electroosmosis, causes the transition of the assembly pattern from cavity-less supraparticles^{15,25} to nanoshells.

The essential role of the electrostatic interactions in the emergence of the nanoshell geometry of the assemblies can also be inferred from the effect of ionic strength on screening of these interactions. We tested such effect at the condition of pH 9.5 which normally produced large cavities. When the ionic strength was increased from 0.8 mM to 5.8 mM, instead of nanoshells, we observed small 11 ± 2 nm NP clusters (Supplementary Fig. 9a–c). They are still porous, similar to nanoshells (Supplementary Fig. 9b), which indicates their origin to be capsular shrinkage. A further increase of ionic strength to 10.8 mM turned most of the NPs into more compact structures with even smaller diameters (7 ± 2 nm) (Supplementary Fig. 9d–f); electrostatic repulsion screened by increased ionic strength is not strong enough for the stabilization of central cavities, resulting in denser assemblies and in agreement with the pH influence on the formation of nanoshells and previous observations of supraparticles.^{15,25}

DISCUSSION:

Modeling of the nanoshells at several levels of theory and simulations help understanding the mechanisms responsible for their formation. The self-assembly of NPs is controlled by vdW and electrostatic interactions, with contributions of hydrogen bonding, entropic forces, and dipolar interactions.¹⁷ Uniformity of the nanoshell diameter and wall thickness is indicative of the self-limiting growth mode typical for terminal self-assembled structures previously observed for cavity-less supraparticles.^{15,26} One can hypothesize, however, that the absence of the surface layer decreases inter-NP gaps and can strengthen both repulsion and attraction simultaneously. The former effect favors open porous structures, while the latter causes the nanoshell assembly without the spherical tiling.

While some aspects of the supraparticle-to-nanoshell transition can be heuristically explained with classical electrostatics using Gauss’s Law and formulae for spherical capacitors, these models are not quite suitable to self-assembled NP structures. In order to describe the NP-NP forces in water, it is necessary to use Derjaguin-Landau-Vervey-Overbeek (DLVO) theory. Using the approximations traditional for colloids, thermodynamically stable nanoshells will form when the vectorial sum of repulsive and attractive forces associated with each spherical NP in the nanoshell (Supplementary Fig. 2a,b) is zero. Due to the spherical symmetry of the nanoshells, one can calculate the balance of forces, taking, for instance, a NP located at the selected pole. As one could expect, N is dependent on q but the overall dependence is opposite to the one observed experimentally (Supplementary Fig. 2c). Such model also predicts that the increase of ionic strength, I , should lead to larger nanoshells, which is again inconsistent with our observations (Supplementary Fig. 2d). Such predictions may seem counterintuitive in light of shorter distances between NPs. However, the result originates in large part from the fact that the medium is described in DLVO as a continuum dielectric and account for the variable dielectric constant around NPs is needed.²⁷ Also, smaller shells include smaller number of NPs and thus have lower integral pair-wise Coulombic repulsion within the DLVO theory. Modification of the repulsive or attractive components of DLVO making, for instance, NP

carry special patches or involve additional forces, can potentially “fix” the incorrectly predicted trend. Although for some NPs, these assumptions could be helpful, we have no evidence of their validity for NPs used here. Moreover, the DLVO model also restricts the building blocks to spheres and uses highly simplified interactions that are difficult to justify for NPs.²⁸

The variety and complexity of NP-NP interactions in the experimentally observed nanoshells can be accounted for by atomistic MD simulations. Considering the ever-increasing computational power and the improvements in 3D resolution of the nanoscale structures enabled by TEM (Fig. 2), the combination of these methods represents a powerful toolbox for the understanding of fast atom-scale reorganization of single NPs (Supplementary Note 1). However, as we can see from the data below, there remain difficult fundamental problems associated with utilization of these tools for self-assembly of multiple NPs.

Based on the previous studies by Vossmeier et al.,²⁹ the CdS NPs were modeled as tetrahedrons built from atoms of Cd and S in atomically accurate crystal lattice. Since NPs forming the nanoshells in Fig. 1–3 are not made with any special surface ligands, the surface of NP is modeled as simple chemical groups typical for aqueous media, *e.g.*, H-, OH- and H₂O—with corresponding charges. At the same time, the exact distributions of these groups over the NP surfaces are not known, and their coordination with atoms of the NP core is expected to be dynamic being dependent on the local conditions.

Starting from fully atomistic model complemented with quantum mechanical *ab initio* calculations, three MD models of NP surfaces were developed with different degrees of approximations negotiating two contrarian necessities: the atomic accuracy of NP description and the timescale of self-assembly processes. In this respect, all these models are complementary to each other offering different insights into the behavior of the very complex system. Water and counterions solvating the NPs are included explicitly at atomistic level in all MD simulations.

In **Model 1**, the description of NP core and NP surfaces is fully atomistic with atomic charges calculated at *ab initio* level (Fig. 4d and Supplementary Fig. 10). NPs have a 2.4 nm core with a shape of truncated tetrahedron that closely matched the constituent NPs. The NP surfaces are coordinated by H₂O, OH⁻, and H⁺ typical for a wide range of pH conditions. The atomic charges of a family of relevant CdS clusters in water were calculated with Gaussian at the MP2 level, using 6–31G basis set for H, O, S atoms, and LanL2DZ pseudo-potential for Cd atoms. The same *ab initio* calculations were performed for the NP facets, edges, and vertices. The results of both sets of calculations are consistent with each other and summarized in Supplementary Fig. 4.

A net charge on NPs can be varied with the composition of the ligand shell and was chosen to be $q = 0.3e$, $0.9e$, and $1.34e$. The net NP charges match to the experimental values estimated from the zeta-potential measurements for different pH conditions, namely pH 7.2, 8.2, and 9.5.

Model 1 affords detailed evaluation of the NP-NP processes at the interface, however, the computational cost of such model is high. Thus, the accessible experimental timescales of

the multiparticle system are short and did not allow reaching fully equilibrated nanoshells and necessitated consideration of other models. In **Model 2**, the NP surface carried a fixed ratio mixture of hydroxyl-derived surface ligands groups typical for high pH: CdOH^+ , Cd(OH)^-_3 , and Cd(OH)_2 . Mulliken charges were obtained by *ab initio* calculations using a restricted Hartree-Fock functions and a 3–21G basis set in TeraChem. The atomic charges of the O and H atoms for surface ligands were calculated by *ab initio* at the MP2 level of CHARMM force field. In the initial trials we found that Coulombic interatomic attraction between the ligands at the NP-NP interfaces results in strong bonding and sluggish reconfiguration kinetics. The collective nature and rigidity of NP cores further contributed to the strength of these inter-NP bonds which is essential for the understanding of the mechanism of their formation. To access longer self-assembly times, however, the atomic charges from the ligands were placed on the NP cores to give an effective charge on NP of $q = 0e, 2e, 4e$, and $8e$ that is higher than in **Model 1**. These values of NP charges are similar, however, to those determined experimentally for CdTe NPs carrying thioglycolic acid surface ligands.³⁰ While having an empirical and coarse-grained component, this approach facilitated reconfiguration dynamics of NP structural units in the shell and allowed the simulations to address self-assembly phenomena with large number of NPs while retaining largely atomistic description for the system.

Finally, in **Model 3**, a bare CdS core with the size and geometry identical to **Model 2** but without any surface ligands. Furthermore, the core-to-core vdW attraction was calculated using the Hamaker approximation typical for DLVO theory (Supplementary Methods) to accelerate the calculations. Additionally, this approach also mitigated intrinsic problem with many commonly used MD force-fields. The calculation of the interatomic vdW forces is cut-off at a specific distance which is typically as short as 1 nm. This approximation is acceptable for molecular system but is not suitable for multi-NP assemblies.

Model 1 simulations showed that the NPs become interconnected through multiple hydrogen bonds. In ~65 ns long simulations of 40 randomly dispersed NPs, the formation of small clusters, short arc-shaped chains, branched structures were observed (Fig. 4a–c) with the tendency to form more extended assemblies with increasing $q = 0.3e, 0.9e$, and $1.35e$. These self-assembled structures are analogous to the intermediate agglomerates that were observed during the experimental self-assembly of nanoshells (Fig. 3a–d).

81 NPs were pre-assembled then into nanoshells with a radius of ~6 nm. Upon equilibration for ~23 – 29 ns, the NPs reorganization led to a different and more stable conformation of the nanoshell without any trend to fall apart. Closer view at the intermolecular contacts between NPs reveals an extended network of hydrogen bonding between the NPs (Fig. 4e). Comparison of the TEM data in Fig. 1, tomography data in Fig. 2, and simulation data in Fig. 4 indicates that the atomistic MD models give a semi-quantitative agreement with the experiments.

We also monitored the total number of water molecules inside these nanoshells. It increased slightly and stabilized for $q = 0.3e$ and $0.6e$, while not being able to reach the equilibrium for $q = 0.9e$ (Fig. 4f). The ‘swelling’ of the nanoshells leading to the larger size of the cavities at a higher q , agrees with experiment (see above). The influx of water inside the

nanoshells also indicates that osmotic pressure in addition to vdW, electrostatic interactions, and hydrogen bonding is one of the significant reasons for transition from supraparticles to nanoshells.

In **Model 2**, compact nanoshells were preassembled from NPs with an artificial pore in the shell. The latter was made to accelerate exchange of water and ions to further study the osmotic pressure role that could not be fully explored with **Model 1** due to shortness of effective time within the system clock. In a ~85 ns simulation, we observed a continuous reorganization of NPs in the pre-assembled large nanoshells with extra amount of water. Importantly, they underwent slow “deflations” (Fig. 5a–d) unlike data in 4f clearly indicating the tendency to produce terminal structures of specific size. Eventually, the diameter of nanoshells plateaued and the net flow of water and ions in/out the nanoshell approached zero (Fig. 5e–f) reaching a meta-stable state. Similar to **Model 1**, the final nanoshell size in **Model 2** grew with q , in agreement with experiments (Fig. 5e–f). Evaluation of the flux of water molecules and counter ions during the equilibration of the nanoshells (Supplementary Fig. 11c) confirmed that osmotic pressure takes part in the stabilization of the cavity between NPs.

Being a hybrid coarse-grained model with some atomistic components, **Model 3** displayed even faster reconfiguration kinetics than other models. **Model 3** clearly underscores the significance of multiple interfacial hydrogen bonds between the NPs that effectively counteract osmotic pressure and electrostatic repulsion defying the need for spherical tiling possible for organic macromolecules. The NP-NP interfaces in these simulations do not have this attractive component and thus, one can observe partial deconstruction of the nanoshell for effective charges smaller than in **Model 2** (Fig. 5g–j). Visualization of the dynamic behavior of these assemblies also becomes possible in **Model 3**.

Conclusions:

CdS NPs were experimentally observed to spontaneously assemble into stable nanoshells. These nanoshells exemplify terminal formation of self-organized nanostructures from inorganic “building blocks”. Atomistic MD simulations represent an important tool in deciphering the mechanisms of self-organization phenomena when purposefully combined with experiment and TEM imaging. However, limitations with timescale accessible in such calculations necessitate further work on developing atomistic force-fields for the needs of NP community. The geometrical shape of nanoshells from NPs and their pH-induced behavior can be paralleled to that of some viral capsids³¹ and other self-organized nanoshells without atomically sophisticated lock-and-key protein complexes or lipid-like biomacromolecules. The simplicity of their formation and porous morphology facilitate their utilization for compartmentalization of chemical process and open the road to further studies of relationship between nanoparticle assemblies and early living systems³² as well as different biomimetic approaches to catalysis,³³ cascade reactions,³⁴ drug delivery,³⁵ and optical materials.¹⁰

METHODS

Preparation of nanoshells and their characterizations:

Nanoshells were obtained by using 10^{-2} M $\text{Cd}(\text{ClO}_4)_2$ and 8×10^{-3} M thioacetamide solutions as reactants and 10^{-2} M NaOH solution to adjust the pH of the system. Typically, 2.5 ml thioacetamide solution was added into 45 ml DI water, followed by the addition of NaOH to control the pH. 1 ml $\text{Cd}(\text{ClO}_4)_2$ solution was further added into the above solution to initiate the formation and assembly of CdS NPs into nanoshells. TEM images were obtained on a JEOL 3011 high-resolution electron microscope. Zeta potential data were acquired by Zetasizer Nano ZS from Malvern Instruments (United Kingdom). Typically, disposable polystyrene cuvettes were filled with sample solutions which can be put in the instrument's laser path. The system was equilibrated for two minutes to reach a stable temperature before each measurement consisting of twelve runs. Smoluchowski approximation was used when converting electrophoretic mobility into zeta potential based on the Henry equation. No stable electrokinetic peaks for the dispersions with pH under 4.3 can be obtained due to small particle size and instrumental limitations of dynamic light scattering.

The electron tomography studies at room and cryogenic temperatures were carried out on a Tecnai F20 electron microscope (FEI Corporation, Hillsboro, OR) equipped with a Gatan $4\text{K} \times 4\text{K}$ CCD camera and a field emission gun (FEG) operating at 200 kV. At room temperature, a series of 2D projection images were recorded at a nominal magnification of 80,000 by tilting the specimen from -62° to 68° in increments of $2^\circ < 30^\circ$ and $1^\circ > 30^\circ$. Images were recorded at an underfocus value around $1\text{ }\mu\text{m}$. Colloidal gold 10 nm NPs were used as fiducial markers to aid tracking during data collection and image alignment during reconstruction. For cryo-electron tomography, $1.5\text{ }\mu\text{l}$ of a Au NP dispersion were applied to the carbon side of glow discharged perforated R2/2 Quantifoil grids and quickly mixed with $4.5\text{ }\mu\text{l}$ CdS nanoshell solution before plunge-freezing using a manual gravity plunger. Tomography tilt series were recorded at a nominal magnification of 80,000 by tilting the specimen from -61° to 61° in increments of $3^\circ < 33^\circ$ and $2^\circ > 33^\circ$. Images were recorded at an underfocus value around $1\text{ }\mu\text{m}$. For 3D reconstruction of the tilt projection series, we followed the protocols typically used in the field of cryo TEM tomography of soft matter³⁶ and specifically, a simultaneous iterative reconstruction technique (SIRT) algorithm, implanted in program TOMO3D, was allowed to run 50 iterations to calculate a three-dimensional density maps from the bin 2 tilt series.³⁷ The pixel size for tomographic reconstruction was $2.8\text{ }\text{\AA}$. A section of $268 \times 326 \times 131$ pixels encompassing the entire single nanoshell was cut from the entire data set. The pixel array was denoised with $k = 0.6$ for total of eight iterations using the nonlinear anisotropic diffusion, edge enhancing algorithm, available in IMOD according to the technique described previously.^{38,39} The surface rendering was generated using the UCSF Chimera software package⁴⁰ with a threshold set to 4.6 and 2.8 sigma for the room temperature and cryo tomograms, respectively. "Segment Map" tool in UCSF Chimera performed watershed segmentation of NP domains with smoothing step of four and step size of one voxel. A total of 17 regions of interconnected NPs were segmented from a single nanoshell section of the nanoshell in Fig. 2. The wall thickness and diameter was measured from the central tomographic slice of the 3D nanoshell

raw volume in IMOD, instead of surface rendering. The pore size was measured using same method, but at the top slice.

MD Simulations:

Three computational models were used in MD simulations of the self-assembly process of CdS NPs represented as truncated tetrahedrons with a side of 2.4 nm, F43m crystal lattice with a lattice constant $a = 0.582$ nm. (Supplementary Fig. 1). In **Model 1**, atomic charges of the NPs were assigned with reference to Mulliken charges obtained from *ab initio* calculations performed for small CdS clusters representing NP surfaces and cores (Supplementary Fig. 10) in an implicit water environment using Gaussian software package, at the MP2 level with 6–31G basis set for H, O, S atoms, and LanL2DZ pseudo-potential for Cd atoms. In **Models 2 and 3**, the calculations were performed for the entire NP core in an implicit water environment using GPU-powered Terachem software package (Supplementary Methods), at the restricted Hartree-Fock level with 3–21G basis set.

The nanoshells solvated in water were pre-assembled from 81 NPs on a sphere with a radius of ~6 nm. Na^+ counter ions were added to the system to neutralize the NP charges. Partial charges of ions and TIP3P water molecules were assigned based on the CHARMM36 force field. The MD simulations were performed using NAMD software package and the results were visualized with VMD software package. The systems with periodic boundary conditions were simulated in an isothermal-isobaric (NPT) ensemble at $T = 300$ K, maintained by the Langevin dynamics with a damping coefficient of $\gamma_{\text{Lang}} = 0.01 \text{ ps}^{-1}$, and $P = 1$ bar. Long range electrostatic interactions were evaluated using a particle mesh Ewald (PME) summation. Hydrophobic interactions and hydrogen bonding are taken into account through atomistic description of the system. The Gilbert–Johnson–Keerthi (GJK) algorithm was used to determine the number of water molecules and ions inside the nanoshell.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Nanoshells spontaneously form from “naked” polydispersed inorganic nanoparticles. (a) A TEM image of CdS nanoshells (22 ± 4 nm) obtained at pH 9.5. These nanoshells share similarities with capsids but are consisted of small inorganic NPs (3–5 nm) without apparent structural anisotropy, precise molecular tiling and specific lock-and-key interaction. (b) A high-resolution TEM image of a CdS nanoshell clearly showing the packing of constituent NPs on the surface and the thickness of the nanoshell corresponding to a monolayer of NPs. (c) A high-resolution TEM image of several nanoshells showing crystal lattices which can be assigned to (111) planes of cubic CdS. (d) The enlarged high-resolution image of the area in the white rectangle in (c).

Figure 2. Three-dimensional structure of nanoshells from CdS NPs obtained by TEM tomography.

(a, c) Surface and (b, d) cross-section of the nanoshell (a, b) at room temperature and (c, d) cryo-conditions at 77 K, capturing the structural information of assemblies in their native environment and showing the porous nature of nanoshells. (e, center) Three-dimensional surface rendering of the nanoshell with different colored NP domains without any special geometric fit.

Figure 3. Temporal progression and intermediate stages of nanoshell assembly demonstrating the gradual transition from NPs to nanoshells.

Representative TEM images of CdS nanoshells obtained after the assembly at pH 9.5 for (a-b) 5 min showing the presence of individual NPs with the emergence of embryonic forms of nanoshells, for (c-d) 10 min characteristic of arc-shaped assemblies as an intermediate precursor for nanoshells and for (e-f) 20 min producing almost complete shells.

Figure 4. Model 1 MD simulation of NP self-organization with high atomic precision but short effective assembly times.

(a-c) Snapshots taken after ~ 65 ns simulations of assemblies of NPs carrying $q = 0.3e$, $0.9e$, and $1.34e$. Yellow-green units represent individual NPs. Other colors denote assembled NP particles with different degree of connectivity. Sodium counterions are shown as yellow spheres. (d) Atomic charges on the NP surface and in the cores were calculated with *ab initio* methods using small atomic clusters. (e) Snapshot after ~ 22 ns equilibration for pre-assembled from NPs carrying $q = 0.9e$. Inset shows an enlarged view of the highlighted region; atomistic description of both the surface and media are to be noted. (f) The plots for number of water molecules inside the nanoshells with $q = 0.3e$, $0.6e$, and $0.9e$. Stabilization of the cavity size for $q = 0.3e$ and $0.6e$ is indicative of stabilization of the shell in a (local) thermodynamic minimum.

Figure 5. Model 2 and Model 3 MD simulation of NP self-organization with progressive coarse graining and longer effective assembly times.

(a–d) Surface representation of preassembled nanoshells taken from simulations (translucent green) after ~1 ns equilibration and (red) after ~85 ns equilibration, for NPs in **Model 2** carrying net effective charges of $q = 0e$, $2e$, $4e$, and $8e$, from left to right, respectively. The yellow lines mark the artificially formed fixed nanoshell opening made to accelerate the exchange of water and ions and observe longer effective assembly times. The lower images show the corresponding cross-sections of the nanoshells. Sodium counter ions are shown by yellow spheres. The similarity with TEM images in Figure 1 and tomography data in Figure 2 can be noted. (e) The plots for number of water molecules and (f) percent of ions inside the nanoshells in **Model 2**. Gradual stabilization of the number of water molecules and, thus the cavity size is observed. See Supplementary Fig. 11 for the distribution of these ions. (g–h) Surface of **Model 3** nanoshells for NPs with $q = 0e$, $0.6e$, $1e$, and $2e$, from left to right, respectively. The translucent green shades show the initial pre-assembled nanoshells, while

the red images show simulated structures were taken from ~33 ns simulations. Destabilization of the nanoshells with higher q can be observed.

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