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Fabrication of Massive Sheets of Single Layer Patterned Arrays Using Lipid Directed Reengineered Phi29 Motor Dodecamer

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The ability to synthesize patterned arrays in a controllable fashion is of extensive interest for nanotechnology. Most of the current micro- and nanofabrication approaches use a variety of robust materials to generate morphologies with typical geometrical shapes such as chip, pillars, bar, or pyramids. Superlattices have been fabricated by various physical or chemical methods including deposition, donor/receptor interaction, self-assembly, complementation, colloidal crystallization, replica, cross-linking, nanoimprint lithography, or patterned etch pits. The construction of lattices that mimic the structural complexity of biological structures would be very intriguing and challenging. In nanotechnology, a nanomachine is a mechanical or electromechanical device with nanometer size dimensions. Considerable efforts have been focused on the research and development of nanomachines and their potential applications in medical related fields. One promising avenue is to construct synthetic nanomachines that mimic the powerful natural bio-nanomachines and to incorporate them into traditional nanotechnological applications. Living systems manufacture a large variety of nanomachines made of protein, DNA, and RNA with atomic precision, including motors, arrays, pumps, membrane cores, and valves. The structural and conformational complexity of biological molecules brings about new avenues and challenges to experimental approaches at the bio/nano interface. Efficient and reproducible transfer of such 2D crystalline protein films onto solid substrates would have substantial implications in the design of nanotechnological devices. Similarly, protein adsorption on solid substrates with predictable self-assembly patterns is another valuable tool for nanopatterning. The use of DNA, RNA, and protein as building blocks in nanotechnology has an advantage over the chemical materials in their eligibility for site-directed modification and specific conjugation with defined stoichiometry. In addition, they can self-assemble and be connected directly to bio-

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logical molecules. Self-assembly of molecules on a surface can be a simple, versatile, and high-volume production approach for the construction of biological arrays. The ability to replicate biological shapes with nanoscale precision could have profound implications in tissue engineering, cell scaffolding, drug delivery, sensors, imaging, nanotechnology, and nanomedicine.

One particularly attractive candidate found in the viral DNA-packaging machinery, from which both protein and RNA bio-nanocomponents may be harvested, is the Bacillus subtilis bacteriophage phi29 DNA-packaging motor. This powerful motor comprises a portal vertex—a 12-subunit gp10 (dodecamer protein, also called as connector), pRNA, gp16, and ATP—that provides the chemical energy required for DNA packaging. These components can be combined in vitro to assemble one of the most powerful nanomachines constructed to date.

The class of dodecamer (connector) proteins which form varieties of portal vertex shares little sequence homology among different viruses, but the resulted portal vertex has considerable morphological similarity. Information from Cryo-EM and X-ray crystallographic studies revealed the portal vertex is a 12-fold symmetric dodecamer with a truncated cone shape about 7.5 nm long and with a diameter of 6.8 nm at the narrow end (N-terminus) and 13.8 nm at the wide end (C-terminus). The central channel has a diameter of 3.6 nm. The wide end of the dodecamer is embedded in the procapsid shell, while the narrow end of the dodecamer, protruding out of the procapsid, is the foothold for pRNA binding.

Previous work has shown that the wild-type dodecamer can be used to form arrays with a mixture of single, double, or multiple layered structures. Multilayer arrays could be easily produced from ordered aggregates of portal vertex. However, multilayer arrays are of limited use for nanotechnological applications such as replica, which demand uniform, single layer biomolecular arrays. It has been found that some multilayer crystals can be converted to single layer in solution over a period of a few weeks by gradually changing the salt concentration. However, such a step is time-consuming and only generates small sheets as a mixture with single, double, and multilayers. Due to the thinness and frangibility, purification of the single layer from the mixture is almost impossible.

In this study, we demonstrate that we can easily produce huge two-dimensional single layer arrays of terminus-modified portal vertex using a lipid

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**Figure 1.** Multilayer versus single layer sheet arrays of phi29 motor dodecamer. (A) Side view of a multiple layer dodecamer array showing the horizontal face-up and face-down arrangements and the vertical head-to-tail alignment which leads to multiple layers overlap. (B) Side view of a single layer dodecamer array displaying an alternating face-up and face-down arrangement. (C) Native phi29 motor dodecamer (inset) assembled into ordered multiple layer structures as shown by negative-stain electron micrograph. (D) The negative-stain electron micrograph of reengineered phi29 motor dodecamer (inset) arrays shows that a single layer sheet was formed. (E) Projection density map of the single layer of motor dodecamers and the Fourier transform (inset). The unit cell is rectangular, with a lattice constant of ~20 nm. The alternate orientations of the dodecamer can be observed. (F) AFM image of N-strep dodecamer arrays and a line scan across crystalline area with lattice defects (inset). The height difference between the top dodecamer layer and mica surface is ~7.5 nm, which corresponds to a single dodecamer layer.
ARTICLE

RESULTS AND DISCUSSION

Approaches to Redirect Formation of Multilayer Structures into Single Layer Patterned Arrays. The formation of multilayer arrays is driven by two distinct protein interaction mechanisms. First, horizontal side-by-side interactions between individual dodecamers allow for the extension and growth of a two-dimensional layer. Second, interactions between the narrow and the wide ends of dodecamer molecules promote the buildup of multiple layers vertically (Figure 1A). To facilitate the formation of a single layer and prevent the continuous growth of multiple layers, a short peptide sequence was introduced either into the gp10 N- or C-terminus, located at the narrow and wide end of the dodecamer, respectively (Figure 1B).

Strep-Tag Extension of the C- or N-Terminus Favors the Assembly of Dodecamer Sheets in Solution. The phi29 portal vertex is a truncated cone-shaped structure having the gp10 N- and C-terminus located at the narrow and wide end, respectively. Fusion of a simple 22 amino acid Strep-tag to the N-terminus of the portal vertex did not interfere with the assembly of the quaternary dodecamer structure. After expression in E. coli cells, the recombinant gp10 assembled into dodecamer particles with similar shape to the native portal vertex as shown by TEM (data not shown). Additionally, the Strep-tag extension facilitates purification of the dodecamer protein with high yield and homogeneity. It has been previously reported that two-dimensional dodecamer arrays could be grown in solution from concentrated native dodecamer (connector) solution following several weeks of incubation under a defined ionic strength gradient of the buffer. However, without this precise chemical treatment, the native portal vertex has the tendency to form patches of multiple layers. Figure 1A illustrates a multiple layer structure of native dodecamers. The individual dodecamers exhibit both lateral side-by-side and vertical head-to-tail interactions. We have used a reengineered motor dodecamer for the self-assembly of single layer dodecamer sheets (schematically shown in Figure 1B).

Arrays constructed from both native (Figure 1C inset) and reengineered dodecamer (Figure 1D inset) and imaged by TEM are shown in Figure 1C,D. As previously reported, the unmodified dodecamer generated multiple overlapping layers with tetragonal symmetry (Figure 1C). The different shades of gray represent the different layers formed and overlapping. The extent to which different layers overlap cannot be controlled, and thus it is difficult to reproduce the same multilayer structure. Interestingly, the reengineered dodecamer with the added N-terminal extension self-assembled into huge flat sheets that piled into three-dimensional stacks (Figure 1D). Such stacks are different from 3D crystals, of which the stacks are governed by specific interaction between different layers. However, in these stacks, the sheets arrange in random orientation (Figure 2), suggesting that each sheet formed or grew independently. It is understandable that, without support, the fragile sheet of the thin layer could not stand alone (see next section for formation of single layer sheets on supporting lipid monolayer). From the EM images, it appeared that the size of the center channel was smaller (compare Figure 1C and D). This is possibly due to partial filling-up of the edge of the channel by the extended peptide at the C-terminus.

The arrangement of the individual dodecamers was analyzed using statistical classification and averaging (Figure 1E inset). The truncated cone structure of a single dodecamer enables us to distinguish between the face-up and face-down orientations. The corresponding projection density map shows the alternating face-up and face-down arrangement of dodecamers (Figure 1E). The unit cell is a square with a dimension of ~20 nm. Due to the alternating orientations of the motor, the central face-up dodecamer displays a larger diameter (corresponding to the wider C-terminal end) than the surrounding face-down dodecamers located in each corner of the square. The alternative face-up and -down data agree with the previous studies on EM imaging, X-ray crystallography, topological analysis, structure projection of the 2D crystals, 3D reconstruction, and computer modeling of the dodecamers. The individual 12 subunits can also be observed. Formation of single layer arrays was further confirmed by AFM imaging. Freshly cleaved muscovite mica was used as an alternative substrate. Figure 1F shows a typical monolayer as template. The method is simple and reproducible.

Figure 2. Negative-stain electron micrographs and corresponding fast Fourier transform (FFT) of two-layer patterned sheets of N-strep dodecamer. (A) Self-assembled huge flat sheets piled into 3D stacks. (B) Representative fast Fourier transformation. (C) The red and blue circles in the image suggested two layers sheets slightly arranged in different angle, suggesting that each sheet formed or grew independently and stacked together.

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AFM image of crystalline N-strep dodecamer single layers imaged in tapping mode in liquid. Patches of crystalline areas with submicrometer size can be observed. The large scan image shows a high surface coverage of the protein layer with only small imperfections. A line scan across the single layer sample (Figure 1F inset) indicates that the single layer thickness is \( \sim 7.5 \text{ nm} \), which is in excellent agreement with the height of the motor dodecamer determined from the three-dimensional crystal structures. Similar single layer arrays were also produced from dodecamers of gp10 with a peptide extension at the C-terminus which serves as a barrier for the vertical interactions (data not shown).

**Formation of Single Layer Dodecamer Arrays Directed by Lipid Monolayers.** Nanotechnological applications of arrays require the assembly of homogeneous broad and wide-ranging flat sheets. Due to the flexible and fragile nature of proteins, it would be desirable to employ a biological template to direct the assembly of single layer arrays. To explore this possibility, thin layers of biotinylated lipid mixtures were used to direct the assembly of N-strep dodecamers which carry a Strep-tag at the N-terminus of each gp10 subunit. The N-strep dodecamer was preincubated with streptavidin before the lipids were spread. Arrays were grown in situ on lipid monolayers at the air–water interface and then transferred on carbon-coated TEM grids. Saturated dipalmitoyl fatty acid chains (C16) of biotinylated DPPE were mixed with unsaturated phosphatidylcholine fatty acids of egg PC (C18) in a 1:3 (w/w) ratio and spread at the liquid–air interface. Dodecamers were attached to the lipid surface via specific biotin–streptavidin interactions (Figure 3A). Initially, the N-strep-tagged dodecamer bound to streptavidin is randomly oriented in solution. After the lipid mixture containing the biotinylated lipid is applied on the water surface, the N-strep dodecamer/streptavidin complex binds specifically to the biotinylated lipid. The arrangement of the individual dodecamers is dictated by the intermolecular protein contacts and protein–lipid interactions. The distance between subsequent dodecamers is governed by the intrinsic nature of the protein which exhibits strong side-by-side interactions. Protein–protein, protein–lipid, and lipid–lipid interactions contribute to the single layer arrangement dodecamers in the lipid matrix. The unsaturated diluting lipid egg PC provides fluidity and flexibility to the lipid monolayer. The bound protein is carried by the biotinylated lipid through the lipid matrix which confers to the translational and rotational freedom required for the nucleation and growth of crystalline patches. After overnight incubation, the single layer was transferred to a hydrophobic grid and imaged by TEM (Figure 3B). The Fourier transform and the corresponding projection density map of the negatively stained electron micrograph (Figure 3C and D, respectively) revealed tetragonally packed 2D dodecamer crystals with a unit cell size of \( \sim 18 \times 18 \text{ nm}^2 \). The unit cell dimensions are in close agreement with those previously measured on two-dimensional phi29 dodecamer crystals by TEM.

**Single Layer Dodecamer Arrays Visualized by AFM.** Hydrophilic bare mica was used as alternative surface for the assembly and adsorption of single layers of the reengineered dodecamer with either N- and C-strep modified dodecamers. High-resolution images of the patterned surface are shown in Figure 4. The N-strep dodecamers self-assembled into a parallelogram lattice (Figure 4A). Cross-sections along the x (Figure 4B) and y (Figure 4C) directions of the crystalline areas indicated unit cell dimensions of \( \sim 16 \) and \( \sim 13 \text{ nm} \) in the x and y directions,
The angle between the x and y axis has been calculated to be ~71°. Even though the tetragonal arrangement previously observed was maintained, the slightly different and unequal unit cell dimensions suggest that the packaging unit of this type of crystal might be slightly different from that of the lipid-directed N-strep 2D crystal. The crystal lattice in Figure 4 is different from those in Figures 1–3 concerning the angle of the pattern. While asking whether the difference observed in the lattices was the consequence directly related to the mutation of the protein is very intriguing, still little is known. A rectangular lattice with unit cell dimensions of 18 × 18 nm² has been observed for the C-strep mutant. The dodecamer orientation in the self-assembled layer on the mica surface was similar to that in the three-dimensional crystal and generated face-up and face-down arrangements. While occasionally the low force applied for imaging was sufficient to image what appeared to be the narrow ends of the dodecamer, most of the time we could only visualize the wide dodecamer domains due to the nature of tip–sample interactions in AFM imaging.

Mica has more than 10 different phases concerning the surface lattice. The lattices of mica and dodecamer are of a different order of magnitude, in which mica is calculated about 6 Å compared to the protein with a unit cell in the regime of about 16–18 nm. In this AFM imaging, it is not clear whether the mica surface lattice played a role here in organizing the pattern of the proteins array, and whether the mica surface lattice and the protein crystal lattice are relevant or in a good match. However, previous studies have shown that the purified native dodecamer self-assembled into tetragonal arrays in solution without the mica support, guiding of these nanoparticles by the mica surface lattice to form the pattern in this report might not be necessary. Instead, the pattern of the lattice might have been dictated by the intrinsic property of the mutant dodecamer structure.

**CONCLUSIONS**

A short Strep-tag sequence modification of the N- or C-terminus of the phi29 portal vertex facilitates its purification with high yield and homogeneity. The modification did not interfere with the dodecamer assembly and function. The mutant protein exhibited favorable lateral interactions and led to the formation of large dodecamer sheets. In solution, the 2D dodecamer arrays interacted vertically to pile up into 3D stacks of protein sheets as revealed by TEM imaging. Large single layer sheets of highly ordered array have been constructed using a supporting lipid monolayer.

**METHODS**

**Reengineering of Phi29 Motor Dodecamer.** Two clones of portal vertex protein were engineered by attaching a Strep-tag II (W9H-PQE) to either the N-terminus or the C-terminus of each gp10 subunit. Cloning methods of the N-strep and C-strep dodecamer have been described previously.

**Purification of the Reengineered Dodecamer.** A column packed with 1 mL of Strep-Tactin sepharose resin (IBA, St. Louis, MO) was equilibrated with 10 column bed volumes of buffer W (100 mM Tris-HCl, pH 8.0, 500 mM NaCl, 1 mM EDTA, 15% glycerol). After lysis of E. coli cells containing the reengineered gp10, the lysate was clarified and the supernatant was loaded onto the column, followed by washing with buffer W, the protein was eluted with buffer E (500 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, 100 mM Tris-HCl, pH 8.0, 15% glycerol).

**Assembly of Dodecamer Arrays.** Two approaches were used to construct dodecamer arrays: (1) self-assembly from concentrated solutions of purified native and N-strep or C-Strep motor dodecamer and (2) lipid-directed assembly of single layer dodecamer arrays. The schematic illustrations of multilayer arrays or single layer patterned sheets are shown in Figure 1A,B.

**Self-Assembled Dodecamer Arrays.** Concentrated solutions of purified, reengineered C- and N-strep dodecamer in buffer (100 mM Tris-HCl, 0.5 M NaCl, 1 mM EDTA, 0.02% sodium azide 15% glycerol, pH 8.0) were stored at −20 °C. Protein solutions dialyzed and diluted if necessary to a stock of 1 mg/mL were kept at 4 °C for a few days and used for the construction of two-dimensional arrays. A 1:35 dilution of the protein stock solution in imaging buffer (10 mM Tris-HCl, pH 8.0, 500 mM KCI) was applied on freshly cleaved mica. The sample was placed in a humidified, closed Petri dish to avoid drying out. Following 2 h incubation at room temperature, the sample was rinsed with imaging buffer and kept at 4 °C overnight. The sample was allowed to reach room temperature prior to AFM imaging.

**Lipid-Directed Single Layer Dodecamer Arrays.** Two-dimensional dodecamer arrays were grown at the liquid–lipid interface as previously reported by Sun et al. The N-strep dodecamer at a concentration of 0.1 mg/mL was incubated in buffer (50 mM...
Tris-HCl, 100 mM NaCl, 20 mM MgCl₂, pH 8) at room temperature with a 6-fold excess of streptavidin (Sigma). A volume of 15 μL of the N-strep dodacemer bound to streptavidin was placed into a custom-designed Teflon well of 4 mm in width and 1 mm in depth. The lipid mixture of 30 μg/mL biotin-cap-DPPE and 90 μg/mL egg phosphatidylcholine (Avanti Lipids, AL) was prepared in chloroform, and 0.3 μL of the lipid mixture was layered on top of the protein solution and incubated overnight at 4 °C in a humidified chamber.

**Imaging of Single Layer Arrays by Transmission Electron Microscopy (TEM).** Samples were prepared by applying the protein stock solution on hydrophilic glow discharged carbon grids that were negatively stained with 1% uranyl acetate (UA) or 2% (w/v) ammonium molybdate. The two-dimensional arrays grown on the lipid matrix were transferred to a hydrophobic carbon-coated copper grid with minimal dis-charge, washed with distilled water, and negatively stained with an aqueous solution of 1% UA and imaged by TEM. The images were acquired at 45000× magnification on a Philips CM121 TEM operated at 80 kV acceleration voltages and equipped with a CCD camera (Gatan, Inc., PA). The reengineered phi29 motor dodecamer arrays were applied on glow-discharged carbon-coated grids, washed with distilled water and negatively stained 2% ammonium molybdate. The grids were transferred into a JEOL-2100F TEM operated at 120 kV, and the lipid images were acquired at 40000× magnification on a 4k × 4k CCD camera (TVIPS, Germany). Image processing, structural determination, and three-dimensional reconstruction (3D) were carried out by the electron crystallographic method using CRISP software package.3 The two-dimensional (2D) projection map of the dodecamer array was generated using the EMAN software as described elsewhere.4

**Imaging of Single Layer Arrays by AFM.** Special care has been taken to maintain the sample under buffer at all times. Samples were allowed to reach room temperature before being imaged by AFM. The self-assembled dodecamer arrays were imaged in liquid tapping mode using a Nanoscope III multimode instrument (Veeco/Digital Instruments, Santa Barbara, California) equipped with a 130 m μm scanner (j scanner). Tapping in liquid was performed in a buffer droplet in a tapping-mode liquid cell without an O-ring seal. Scanning was performed using narrow-legged cantilevers (OMCL-TR40PSA, Olympus Ltd., Tokyo, Japan) with oxide sharpened Si3N4 tips. The V-shaped cantilevers had a length of 100 μm and a nominal spring constant of 80 pN/ nm. Cantilevers were driven at the resonance frequencies of 8.4 ± 0.5 kHz with piezo drive amplitudes of 50–100 mV, resulting in cantilever amplitudes of ~0.5 V. Scanning was performed at speeds between 1 and 2 Hz. Image processing (2nd order flattening) and data analysis was done with the Nanoscope software version 5.12r5.

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**REFERENCES AND NOTES**

1. Moll, D.; Huber, C.; Schlegel, B.; Pum, D.; Sleytr, U. B.; Sara, M. S-Layer-Streptavidin Fusion Proteins as Template for Nanopatterned Molecular Arrays. Proc. Natl. Acad. Sci. U.S.A. 2002, 99, 14646–14651.
2. Aldaye, F. A.; Palmer, A. L.; Sieiman, H. F. Assembling Materials with DNA as the Guide. Science 2008, 321, 1795–1799.
3. Lubrich, D.; Bath, J.; Turberfeld, A. J. Templated Self-Assembly of Wedge-Shaped DNA Arrays. Tetrahedron 2008, 64, 8530–8534.
4. Seeman, N. C.; Belcher, A. M. Emulating Biology: Building Nanostructures from the Bottom Up. Proc. Natl. Acad. Sci. U.S.A. 2002, 99, 6451–6455.
5. Shu, D.; Moll, D.; Deng, Z.; Mao, C.; Guo, P. Bottom-Up Assembly of RNA Arrays and Superstructures as Potential Parts in Nanotechnology. Nano Lett. 2004, 4, 1717–1724.
6. Colvin, V. L.; Goldstein, A. N.; Allvisatos, A. P. Semiconductor Nanocrystals Covalently Bound to Metal Surfaces with Self-Assembled Monolayers. J. Am. Chem. Soc. 1992, 114, 5221–5230.
7. Dabbousi, B. O.; Murray, C. B.; Rubner, M. F.; Bawendi, M. G. Langmuir–Blodgett Manipulation of Size Selected CdSe Nanocrystals. Chem. Mater. 1994, 6, 216–219.
8. Whetten, R. L.; Khoury, J. T.; Alvarez, M. M.; Murthy, S.; Vezmar, I.; Wang, Z. L.; Stephens, P. W.; Cleveland, C. L.; Luedtke, W. D.; Landman, U. Nanocrystal Gold Molecules. Adv. Mater. 1994, 8, 428–433.
9. Otero, R.; Ecija, D.; Fernandez, G.; Gallego, J. M.; Sanchez, L.; Martin, N.; Miranda, R. An Organic Donor/Receptor Lateral Superlattice at the Nanoscale. Nano Lett. 2007, 7, 2602–2607.
10. Zheng, N. F.; Bu, X. H.; Feng, P. Y. Self-Assembly of Novel Dye Molecules and [Cd₉(Sph)₉]³⁻³ Cube Clusters into Three-Dimensional Photoluminescent Superlattice. J. Am. Chem. Soc. 2002, 124, 9688–9689.
11. Song, Q.; Ding, Y.; Wang, Z. L.; Zhang, Z. J. Formation of Orientation-Ordered Superlattices of Magnetite Magnetic Nanocrystals from Shape-Segregated Self-Assemblies. J. Phys. Chem. B 2006, 110, 25547–25550.
12. Lee, S. W.; Mao, C.; Flynn, C. E.; Belcher, A. M. Ordering of Quantum Dots Using Genetically Engineered Viruses. Science 2002, 296, 892–895.
13. Dujardin, E.; Peet, C.; Stubbs, G.; Culver, J. N.; Mann, S. Organization of Metallic Nanoparticles Using Tobacco Mosaic Virus Templates. Nano Lett. 2003, 3, 413–417.
14. Mirkin, C. A.; Letsinger, R. L.; Mudic, R. C.; Storhoff, J. J. A DNA Based Method for Rationally Assembling Nanoparticles into Macroscopic Materials. Nature 1996, 382, 607–609.
15. Alivisatos, A. P.; Johnson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. Organization of Nanocrystal Molecules Using DNA. Nature 1996, 382, 609–611.
16. Mbindyo, J. K. N.; Reis, B. D.; Martin, B. R.; Keating, C. D.; Natan, M. J.; Mallouk, T. E. DNA-Directed Assembly of Gold Nanowires on Complementary Surfaces. Adv. Mater. 2001, 13, 249–254.
17. vanBlaaderen, A.; Ruel, R.; Withius, P. Template-Directed Colloidal Crystalization. Nature 1997, 385, 321–324.
18. Vossmeyer, T. et al. A Double Diamond Superlattice Built Up of Cd₁₋ₓSₓ(CH₃CH₂OH)₂ Clusters. Science 1995, 267, 1476–1479.
19. Motte, L.; Billoudet, F.; Lacaze, E.; Pileni, M. Self-Organization of Size-Selected Nanoparticles into Three Dimensional Superlattices. Adv. Mater. 1996, 8, 1018–1020.
20. Murray, C. B.; Kagan, C. R.; Bawendi, M. G. Self-Organization of CdSe Nanocrystals into 3-Dimensional Quantum Dot Super Lattices. Science 1995, 270, 1335–1338.
21. Nykypanchuk, D.; Maye, M. M.; van der Lele, D.; Gang, O. DNA-Guided Crystalization of Colloidal Nanoparticles. Nature 2008, 451, 549–552.
22. Gates, B. D.; Xu, Q.; Stewart, M.; Ryan, D.; Willson, C. G.; Whitesides, G. M. New Approaches to Nanofabrication: Molding, Printing, and Other Techniques. Chem. Rev. 2005, 105, 1171–1196.
23. Brust, M.; Bethall, D.; Schiffrin, D. J.; Kieley, C. J. Novel Gold Dithiol Nanonetworks with Non-Metallic Electronic Properties. Adv. Mater. 1995, 7, 795–797.
24. Andres, R. P.; Bielefeld, J. D.; Henderson, J. J.; Janes, D. B.; Kolagunta, V. R.; Kubiak, C. P.; Mahoney, W. J.; Osifchin, R. G. Self-Assembly of a 2-Dimensional Superlattice of Molecularly Linked Metal Clusters. Science 1996, 273, 1690–1693.
25. Jung, G. Y.; Johnston-Halperin, E.; Wu, W.; Yu, Z. N.; Wang, S. Y.; Tong, W. M.; Li, Z. Y.; Green, J. E.; Sheriff, B. A.; Boukai, A.; et al. Circuit Fabrication at 17 nm Half-Pitch by Nanoimprint Lithography. Nano Lett. 2006, 6, 351–354.

www.acsnano.org
26. Heath, J. R.; Williams, R. S.; Shiang, J. J.; Wind, S. J.; Chu, J.; Demirc, C.; Chen, W.; Stanis, C. L.; Bucchignano, J. J. Spatially Confined Chemistry: Fabrication of Ge Quantum Dot Arrays. J. Phys. Chem. 1996, 100, 3144–3149.

27. Cui, Y.; Lieber, C. M. Functional Nanoscale Electronic Devices Assembled Using Silicon Nanowire Building Blocks. Science 2001, 291, 851–853.

28. Craighead, H. G. Nanoelectromechanical Systems. Science 2000, 290, 1532–1536.

29. Grigorov, D. N.; Moll, W.; Hall, J.; Guo, P. Bionanomotors. Encycl. Nanosci. Nanotechnol. 2003, 1, 361–374.

30. Hess, H.; Vogel, V. Molecular Shuttles Based on Motor Proteins: Active Transport in Synthetic Environments. J. Biotechnol. 2001, 82, 67–85.

31. Sára, M.; Pum, D.; Schuster, B.; Sleytr, U. B. S-Layers as Patterned Elements for Application in Nanobiotechnology. J. Nanosci. Nanotechnol. 2005, 5, 1939–1953.

32. Deng, Z.; Lee, S. H.; Mao, C. DNA as Nanoscale Building Blocks. J. Nanosci. Nanotechnol. 2005, 5, 1954–1963.

33. Guo, P. RNA Nanotechnology: Engineering, Assembly and Applications in Detection, Gene Delivery and Therapy. J. Nanosci. Nanotechnol. 2005, 5, 1964–1982.

34. Channon, K.; Bromley, E. H. C.; Woolfson, D. N. Synthetic Biology through Biomolecular Design and Engineering. Curr. Opin. Struct. Biol. 2008, 18, 491–498.

35. Ulijn, R. V.; Smith, A. M. Designing Peptide Based Nanomaterials. Chem Soc Rev. 2008, 37, 664–675.

36. Katz, E.; Willner, I. Integrated Nanoparticle-Biomolecule Hybrid Systems: Synthesis, Properties, and Applications. Angew. Chem., Int. Ed. 2004, 43, 6042–6108.

37. Chan, Y. N. C.; Schrock, R. R.; Cohen, R. E. Synthesis of Single Silver Nanoclusters within Spherical Micromdomains in Block Copolymer Films. J. Am. Chem. Soc. 1992, 114, 7295–7296.

38. Sleytr, U. B.; Carrascosa, J. L. Three-Dimensional Reconstruction of The Bacillus subtilis Ring Assembly in Protein/RNA Interactions Revealed by Nucleic Acids Res. 2006, 34, 519–538.

39. Afonin, K. A.; Cieply, D. J.; Leontis, N. B. Specific RNA Self-Assembly with Minimal Paraeptic Motifs. J. Am. Chem. Soc. 2008, 130, 93–102.

40. Sleytr, U. B.; Cieply, D. J.; Leontis, N. B. Specific RNA Self-Assembly with Minimal Paraeptic Motifs. J. Am. Chem. Soc. 2008, 130, 93–102.

41. Afonin, K. A.; Cieply, D. J.; Leontis, N. B. Specific RNA Self-Assembly with Minimal Paraeptic Motifs. J. Am. Chem. Soc. 2008, 130, 93–102.

42. Sleytr, U. B.; Carrascosa, J. L. Detailed Structure of the Bacteriophage T3 Head-To-Tail Connector. J. Mol. Biol. 1986, 198, 155–165.

43. Valpuesta, J. M.; Sousa, N.; Carrascosa, J. L. Structural Analysis of the Bacteriophage T3 head-to-tail Connector. J. Mol. Biol. 1999, 289, 1355–1365.

44. Valpuesta, J. M.; Sousa, N.; Carrascosa, J. L. Three-Dimensional Structure of a DNA Translocating Machine: the High-Order Architecture of a DNA Translocating Machine. J. Mol. Biol. 2001, 314, 543–551.

45. Valpuesta, J. M.; Sousa, N.; Carrascosa, J. L. Three-Dimensional Structure of a DNA Translocating Machine: the High-Order Architecture of a DNA Translocating Machine. J. Mol. Biol. 2001, 314, 543–551.

46. Valpuesta, J. M.; Carrascosa, J. L. Structural Analysis of the Bacteriophage T3 head-to-tail Connector. J. Mol. Biol. 1999, 289, 1355–1365.

47. Valpuesta, J. M.; Carrascosa, J. L. Three-Dimensional Structure of a DNA Translocating Machine at 10 Å Resolution. Structure 1999, 7, 286–299.

48. Guo, P.; Erdos, S.; Anderson, D. A Small Viral RNA is Required for In Vitro Packaging of Bacteriophage Phi29 DNA. Science 1987, 236, 690–694.

49. Guo, P.; Petersen, C.; Anderson, D. Prohead and DNA-Gp3-Dependent ATPase Activity of the DNA Packaging Protein Gp16 of Bacteriophage φ29. J. Mol. Biol. 1987, 197, 229–236.

50. Sleytr, U. B.; Carrascosa, J. L. Three-Dimensional Reconstruction of The Bacillus subtilis Ring Assembly in Protein/RNA Interactions Revealed by Nucleic Acids Res. 2006, 34, 519–538.

51. Valpuesta, J. M.; Sousa, N.; Carrascosa, J. L. Three-Dimensional Structure of a DNA Translocating Machine: the High-Order Architecture of a DNA Translocating Machine. J. Mol. Biol. 2001, 314, 543–551.

52. Valpuesta, J. M.; Carrascosa, J. L. Three-Dimensional Structure of a DNA Translocating Machine at 10 Å Resolution. Structure 1999, 7, 286–299.

53. Badasso, M. O.; Leiman, P. G.; Tao, Y.; He, Y.; Badasso, M. O.; Jardine, P. J.; Anderson, D. L.; Rossmann, M. G. Structure Determination of the Head-Tail Connector of Bacteriophage Phi29. Acta Crystallogr., Sect. D 2000, 56, 1178–1182.

54. Xiao, F.; Zhang, H.; Guo, P. Novel Mechanism of Hexamer Ring Assembly in Protein/RNA Interactions Revealed by Single Molecule Imaging. Nucleic Acids Res. 2008, 36, 6620–6632.

55. Xiao, F.; Zhang, H.; Guo, P. Novel Mechanism of Hexamer Ring Assembly in Protein/RNA Interactions Revealed by Single Molecule Imaging. Nucleic Acids Res. 2008, 36, 6620–6632.

56. Afonin, K. A.; Cieply, D. J.; Leontis, N. B. Specific RNA Self-Assembly with Minimal Paraeptic Motifs. J. Am. Chem. Soc. 2008, 130, 93–102.

57. Valpuesta, J. M.; Carrascosa, J. L. Three-Dimensional Structure of a DNA Translocating Machine at 10 Å Resolution. Structure 1999, 7, 286–299.

58. Xiao, F.; Moll, D.; Guo, P. Binding of pRNA to the N-Terminal 14 Amino Acids of Connector Protein of Bacterial Virus Phi29. J. Mol. Biol. 2008, 380, 2640–2649.

59. Xiao, F.; Zhang, H.; Guo, P. Novel Mechanism of Hexamer Ring Assembly in Protein/RNA Interactions Revealed by Single Molecule Imaging. Nucleic Acids Res. 2008, 36, 6620–6632.

60. Afonin, K. A.; Cieply, D. J.; Leontis, N. B. Specific RNA Self-Assembly with Minimal Paraeptic Motifs. J. Am. Chem. Soc. 2008, 130, 93–102.

61. Xiao, F.; Zhang, H.; Guo, P. Novel Mechanism of Hexamer Ring Assembly in Protein/RNA Interactions Revealed by Single Molecule Imaging. Nucleic Acids Res. 2008, 36, 6620–6632.
69. Jimenez, J.; Santisteban, A.; Carazo, J. M.; Carrascosa, J. L. Computer Graphic Display Method for Visualizing Three-Dimensional Biological Structures. Science 1986, 232, 1113–1115.

70. Cai, Y.; Xiao, F.; Guo, P. N- or C- Terminal Alterations of Motor Protein Gp10 of Bacterial Virus Phi29 on Procapsid Assembly, pRNA Binding and DNA Packaging. Nanomedicine 2008, 4, 8–18.

71. Sun, J.; DuFort, C.; Daniel, M. C.; Murali, A.; Chen, C.; Gopinath, K.; Stein, B.; De, M.; Rotello, V. M.; Holzenburg, A.; et al. Core-Controlled Polymorphism in Virus-Like Particles. Proc. Natl. Acad. Sci. U.S.A. 2007, 104, 1354–1359.

72. Sun, J. C.; Duffy, K. E.; Ranjith-Kumar, C. T.; Xiong, J.; Lamb, R. J.; Santos, J.; Masarapu, H.; Cunningham, M.; Holzenburg, A.; Sarisky, R. T.; et al. Structural and Functional Analyses of the Human Toll-Like Receptor 3—Role of Glycosylation. J. Biol. Chem. 2006, 281, 11144–11151.

73. Hovmoller, S. CRISP-Crystallographic Image Processing on a Personal Computer. Ultramicroscopy 1992, 40, 181–182.

74. Ludtke, S. J.; Baldwin, P. R.; Chiu, W. EMAN: Semiautomated Software for High-Resolution Single-Particle Reconstructions. J. Struct. Biol. 1999, 128, 82–97.