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S-Layer Proteins for Assembling Ordered Nanoparticle Arrays

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1 INTRODUCTION

The current challenge in the development of the next generation of nanoscale systems will require entirely new materials, fabrication technologies, and devices. The ultimately high requirements in the synthesis of molecular functional units, in particular of nanoelectronic devices, can only be met when novel concepts based on state-of-the-art top-down lithographic procedures are combined with bottom-up approaches using principles learned from nature. Thus, the aim of current research is the development of tools and techniques for a novel combined top-down/bottom-up fabrication technology for nanostructures with critical dimensions below 100 nm that can be used in a flexible way for a variety of devices and architectures.

The bioinspired synthesis of inorganic materials, such as metallic or semiconducting nanoparticles, has already attracted much attention over the last two decades. In particular, the broad base of knowledge about the binding of biological molecules has paved the way for investigating the potential of S-layer proteins (Sleytr and Beveridge 1999) and their self-assembly products as catalysts, templates, and scaffolds for the generation of ordered nanoparticle arrays (Sleytr, Egelseer, Pum, and Schuster 2004; Sára, Pum, Schuster, and Sleytr 2005; Sleytr, Sára, Pum, and Schuster 2005).

2 DESCRIPTION OF S-LAYERS

Crystalline bacterial surface layer (S-layer) proteins represent the outermost cell envelope component of a broad spectrum of bacteria and archaea (Fig. 6.1A) (Sleytr and Beveridge 1999; Sleytr, Egelseer, Pum, and Schuster 2004; Sára, Pum, Schuster, and Sleytr 2005; Sleytr, Sára, Pum, and Schuster 2005). S-layers are monomolecular arrays composed of a single protein or glycoprotein species ($M_w 40-200$ kDa) and exhibit oblique (p1, p2), square (p4), or hexagonal (p3, p6) lattice symmetry with unit cell dimensions in the range of 3 to 30 nm. Depending on the lattice symmetry one morphological unit (=unit cell) consists of one, two, three, four, or six identical S-layer proteins. S-layers are highly porous protein lattices (30–70% porosity) with pores of uniform size and morphology in the 2- to 8-nm range (Fig. 6.1B). S-layers are generally 5 to 10 nm thick.

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S-layers are also highly anisotropic structures with respect to their physicochemical surface properties. Generally, in Bacillacaea the outer S-layer face is less corrugated than the inner one and bears no net charges, whereas the inner face is either net positively or negatively charged. Functional groups (e.g., carboxyl, amino, or hydroxyl groups) or genetically incorporated functional domains (e.g., streptavidin sites for biotin binding, fused with the self-assembling part of the protein) are repeated with the periodicity of the S-layer lattice at a distance resembling the lattice constants, leading to regular arrays of bound functional molecules or nanoparticles. Studies on the structure–function relationship of different S-layers from Bacillaceae revealed the existence of specific (lectin type) binding domains on the N-terminal part of S-layer proteins for heteropolysaccharides (secondary cell wall polymers; SCWPs) covalently linked to the peptidoglycan matrix of the cell wall (Sára 2001; Sleytr, Sára, Mader, Schuster, and Unger 2001).



FIG. 6.1. **A.** Transmission electron microscopical image of a freeze-etched and metal (Pt/C) shadowed preparation of a bacterial cell. The S-layer shows hexagonal lattice symmetry. Bar, 100 nm. **B.** Digital image reconstructions of a scanning force microscopical image of an S-layer protein monolayer reassembled on a silicon surface. The S-layer lattice shows square (p4) lattice symmetry. Bar, 10 nm.

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One of the most fascinating properties of isolated S-layer proteins is their capability to form free floating self-assembly products in solution (e.g., flat sheets, cylinders) (Sleytr and Messner 1989), to recrystallize into extended monomolecular layers on solid supports (Pum and Sleytr 1995; Györvary, Stein, Pum, and Sleytr 2003), at the air-water interface, and on lipid films (Schuster and Sleytr 2000) and to cover liposomes (Küpcü, Sára, and Sleytr 1995; Mader, Küpcü, Sleytr, and Sára 2000) and nanocapsules (Toca-Herrera, Krastev, Bosio, Küpcü, Pum, Fery, Sára, and Sleytr 2005). Depending on the S-layer protein species used and the environmental conditions, double layers in back-to-back orientation may be formed. The reassembly occurs after removal of the disrupting agent used in the dissolution and isolation procedure. In general, a complete disintegration of S-layer lattices in the constituent (glyco)protein subunits on bacterial cells can be achieved using high concentrations of chaotropic agents (e.g., guanidine hydrochloride, urea), by lowering or raising the pH, or by applying metal-chelating agents (e.g., EDTA, EGTA) or cation substitution. The formation of self-assembled arrays is only determined by the amino acid sequence of the polypeptide chains and consequently the tertiary structure of the S-layer protein species. In various S-layer proteins from Bacillacaea it was shown that significant portions of the C- or N-terminal part can be deleted without losing the capability of the subunits for lattice formation (Jarosch, Egelseer, Huber, Moll, Mattanovich, Sleytr, and Sára 2001).

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3 METHODS, MATERIALS, AND RESULTS

3.1 NANOPARTICLE FORMATION BY SELF-ASSEMBLY ON S-LAYER PATTERNED SUBSTRATES

The first report on the use of S-layers as lithographic templates was published by Douglas and Clark almost two decades ago (Douglas, Clark, and Rothschild 1986). In a three-step process, S-layer fragments from *Sulfolobus acidocaldarius* were deposited onto a smooth 20-nm thick carbon coated substrate, metal coated by evaporation with tungsten/wolfram (Ta/W), and then ion milled. This S-layer shows hexagonal lattice symmetry with a center-to-center spacing of the morphological units of 22 nm, a thickness of 10 nm, and pores 5 nm in diameter. The thickness of the metal film was ~1.2 nm. Under ion milling this composite protein–metal structure exhibited differential metal removal leading to 15-nm sized holes periodically arranged in a hexagonal lattice resembling the lattice geometry of the underlying S-layer lattice. Because of the shadowing angle of 40° from the normal to the substrate the coated unmilled S-layers exhibited thicker metal deposits along the edges of the hexagonally symmetric holes. The thinnest metal thickness was

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found in the S-layer holes shadowed by their front edges relative to the incident beam. Subsequent ion milling (normal incidence, 30 s) removed the metal in the holes first and led to a film of nearly uniform thickness perforated by a periodic array of oval holes and metal strips running along the most heavily coated molecular rows. Although significant fluctuations in the shape and size of the holes in the metal film were observed, this first approach laid the foundation for using S-layer as patterning elements for generating ordered nanostructured materials. Later on, in a basically similar approach, this group used fast-atom beam milling to fabricate 10nm diameter sized holes in a 3.5-nm-thick titanium oxide (TiO₂) layer (Douglas, Devaud, and Clark 1992). Scanning force microscopy was used to study the surface profile of these protein–metal structures and the patterning of the supporting substrate.

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A decade later, Douglas and co-workers made use of the S-layer based lithographic approach described in the preceding to fabricate a precisely ordered and located lattice of 5-nm diameter titanium nanoclusters (Winningham, Gillis, Choutov, Martin, Moore, and Douglas 1998). The crystalline S-layer protein fragments $(1-2\mu m \text{ in extent})$ were deposited onto a hydrophilic silicon oxide surface and coated with a thin layer of titanium by evaporation (thickness ~ 1.2 nm). Subsequently, the titanium film was oxidized in air (thickness of the TiO₂ layer 3.5 nm) exhibiting 6-nm diameter metallized pores. Low energy electron enhanced etching (LE4) in a DC hydrogen plasma was used for etching such a thin delicate mask since only LE4 avoided the damage commonly inflicted by standard ion bombardment. The isotropic etching widened the holes to a diameter of 18 nm and transferred their pattern into the silicon. After etching, the mask was completely removed and the patterned surface oxidized in oxygen plasma. After deposition of a further (1.2-nm) titanium layer an ordered array of 5-nm sized metal nanoclusters was formed at the etched hole positions. This superlattice of titanium nanoclusters resembled the lattice geometry of the underlying S-layer patches.

The lithographic approach of using S-layers as nanometric masks was also exploited to fabricate magnetic nanostructures. The hexagonally packed intermediate (HPI) S-layer of *Deinococcus radiodurans* was used for patterning ferromagnetic films (Panhorst, Brückl, Kiefer, Reiss, Santarius, and Guckenberger 2001). HPI displays hexagonal (p6) lattice symmetry with a center-to-center spacing of 18 nm, a thickness of 6.5 nm, and 6-nm wide pores. Accordingly, a hexagonal pattern of uniform 10-nm sized dots and a lattice spacing of 18 nm was fabricated from 2.5-nm thick sputter coated Co, FeCo, Fe, CoNi, and NiFe films. These patterns occurred after dry etching with Ar ions. The most critical parameter for a successful formation of nanodot arrays were etching time, energy, and density of the Ar ions.

Recently, regular arrays of nanometer sized magnetic dots were also generated using the S-layer of *Sulfolobus acidocaldarius* as templates (Malkinski, Camley,

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Celinski, Winningham, Whipple, and Douglas 2003). In this work, a thin layer of chromium (Cr) was deposited onto 1- to 2-µm sized S-layer fragments from *Sulfolobus acidocaldarius* at an angle of 60°. The topography of the protein lattice produced a shadow at each pore leading to an ordered array of holes in the Cr layer. Subsequently, the pattern of this ordered array was transferred into the silicon by plasma treatment yielding an identical pattern of ~4-nm sized holes in the silicon subtrate. An assembly of Fe/Pd dots with an average dot size of 10 nm in diameter, 6.5 nm in height and a lattice spacing of 22 nm (according to the lattice parameters of the underlying S-layer) was fabricated by molecular beam epitaxy. The dots consisted of a sandwich of four 1-nm thick Fe and 0.4-nm thick Pd layers in which the terminating Pd layer was 1.5 nm thick. Finally, after the metal deposition the protein layer was removed. The magnetic properties of these dot arrays were different from those of equivalent continuous films (Malkinski, Camley, Celinski, Winningham, Whipple, and Douglas 2003).

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In addition to the described thermal evaporation techniques cross-beam pulsed laser deposition was used to transfer the nanomorphology of S-layers into metallic (Pt/C) overlayers (Gorbunov, Mertig, Kirsch, Eichler, Pompe, and Engelhardt 1997; Neubauer, Pentzien, Reetz, Kautek, Pum, and Sleytr 1997). The observed surface corrugations of the metallized S-layer sheets showed higher corrugations compared to the uncoated sample. Thus it was concluded that the protein layer had been mechanically stabilized by the metal film.

3.2 WET CHEMICAL SYNTHESIS OF NANOPARTICLES

Based on the investigation of mineral formation by bacteria in natural environments (Douglas and Beveridge 1998), S-layer lattices can be used in wet chemical processes for the precipitation of metal ions from solution, as well (Sleytr, Bayley, Sára, Breitwieser, Küpcü, Mader, Weigert, Unger, Messner, Jahn-Schmid, Schuster, Pum, Douglas, Clark, Moore, Winningham, Levy, Frithsen, Pankovc, Beagle, Gillis, Choutov, and Martin 1997). In this approach self-assembled S-layer structures were exposed to metal-salt solutions followed by slow reaction with a reducing agent such as hydrogen sulfide (H_2S). Nanoparticle superlattices were formed according to the lattice spacing and symmetry of the underlying S-layer. Furthermore, since the precipitation of the metals was confined to the pores of the S-layer, the nanoparticles also resembled the morphology of the pores.

The first example exploiting this technique was the precipitation of cadmium sulfide (CdS) on S-layer lattices composed of the S-layer protein from *Geobacillus stearothermophilus* NRS 2004/3a variant 1 (NRS), and the S-layer protein of *Bacillus sphaericus* CCM2177 (SbpA) (Shenton, Pum, Sleytr, and Mann 1997). NRS reassembles into mono- and double-layered self-assembly products in solu-

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tion and into monolayers on solid supports (Sára, Pum, Küpcü, Messner, and Sleytr 1994). NRS shows oblique (p1) lattice symmetry with unit cell dimensions of a = 9.8 nm, b = 7.5 nm, and a base angle of 80° . The thickness of this S-layer is 4.5 nm and pores show a diameter of 4 to 5 nm. SbpA forms monolayer selfassembly products in suspension and on solid supports (Györvary, Stein, Pum, and Sleytr 2003). The S-layer exhibits square lattice symmetry with a lattice constant of 13.1 nm, and a thickness of the S-layer of 8 nm. Pores in this S-layer are 4 to 5 nm wide. After incubation of the S-layer self-assembly products with a CdCl, solution for several hours the hydrated samples were exposed to $H_{a}S$ for at least 1 to 2 days. The generated CdS nanoparticles were 4 to 5 nm in size and their superlattices resembled the oblique lattice symmetry of SbsB, or the square lattice symmetry of SbpA, respectively (Shenton, Pum, Sleytr, and Mann 1997). In addition, mineralization of the dispersed nanostructures formed in suspension of NRS produced characteristic stripe patterns of organized CdS nanoparticles. The most common stripe pattern consisted of fringes 16 nm in width and 32 nm spaced apart. They were aligned parallel to the longer unit cell vector of the underlying S-layer lattice. These stripe patterns were Moiré fringes, which originated from the superposition of two oblique CdS/S-layer lattices aligned in back-to-back orientation along the longer base vector. In this particular arrangement, the two associated S-layers were in perfect register every fourth lattice row.

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Further on, a superlattice of 4- to 5-nm sized gold particles was formed by using SbpA (with previously induced thiol groups) as template for the precipitation of a tetrachloroauric (III) acid (HAuCl₄) solution (Fig. 6.2) (Dieluweit, Pum, and Sleytr 1998). Gold nanoparticles were formed either by reduction of the metal salt with H₂S or under the electron beam in a transmission electron microscope. The latter approach is technologically important because it allows the definition of areas in which nanoparticles are eventually formed (Dieluweit, Pum, D) and Sleytr 1998; Wahl, Mertig, Raff, Selenska-Pobell, and Pompe 2001). As determined by electron diffraction the gold nanoparticles were crystalline but their ensemble was not crystallographically aligned. Later on, the wet chemical approach was used in the formation of Pd- (salt: PdCl₂), Ni- (NiSO₄), Pt- (KPtCl₆), Pb- (Pb(NO₃)₂), and Fe- (KFe(CN)₆) nanoparticle arrays (unpublished results). Recently, small spot X-ray photoelectron emission spectroscopy (XPS) was used to characterize the elemental composition of the nanoclusters. XPS demonstrated that they consisted primarily of elemental gold (Dieluweit, Pum, Sleytr, and Kautek 2005).

In a similar approach arrays of platinum nanoparticles were fabricated on the S-layer of *Sporosarcina ureae* (Mertig, Kirsch, Pompe, and Engelhardt 1999). *Sp. ureae* exhibits square (p4) lattice symmetry with a lattice spacing of 13.2 nm. Platinum cluster deposition was achieved by precipitation of platinum from K_2PtCl_4 solution and subsequent reduction with NaN₃. Transmission electron microscopical investigations revealed the formation of well-separated metal clusters with an average diameter of 1.9 ± 0.6 nm. Seven clusters per unit cell were formed. They were found in

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FIG. 6.2. **A.** Schematic drawing of the S-layer templated wet chemical synthesis of gold nanoparticles. The S-layer is incubated with a tetrachloroauric (III) acid solution. **B.** Upon irradiation with an electron beam a superlattice of gold nanoparticles with square lattice symmetry is formed. The nanoparticles resemble the morphology of the S-layer pores (inset). Bars, 50 nm. (Reprinted from ref. 26, with permission from Elsevier.).

the pores and indentations in the S-layer lattice. Moreover, UV-VIS spectroscopy was able to stress the role of the S-layer in the process of cluster deposition as templates with a very high density of specific affinity sites in which nucleation takes place. In subsequent work, the formation of palladium nanoparticles on the S-layer of Bacillus sphaericus NCTC 9602 and of Sp. ureae was shown as well (Pompe, Mertig, Kirsch, Wahl, Ciachi, Richter, Seidel, and Vinzelberg 1999; Mertig, Wahl, Lehmann, Simon, and Pompe 2001; Wahl, Engelhardt, Pompe, and Mertig 2005; Hüttl, Ullrich, Wolf, Kirchner, Mertig, and Pompe 2006). The S-layer or B. sphaericus NCTC 9602 shows square (p4) lattice symmetry with a lattice constant of 12.5 nm. Palladium cluster deposition was achieved after activation of the S-layer with K₂PdCl₂. Upon electron irradiation in the TEM 5- to 7-nm sized metallic nanoparticles forming regular arrays resembling the lattice geometry of the S-layer were formed. However, nanoparticle formation was only observed in flattened S-layer cylinders in which Pt and Pd complexes were physically accumulated because of the particular geometry of the resulting S-layer double layer. In addition to the precipitation of nanoparticles for applications in molecular electronics, it must be noted here that recent investigations

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of the electronic structure of the S-layer of *B. sphaericus* NCTC 9602 revealed a semiconductor-like behavior with an energy gap value of ~3.0eV and the Fermi energy close to the bottom of the lowest unoccupied molecular orbital (LUMO) (Vyalikh, Danzenbächer, Mertig, Kirchner, Pompe, Dedkov, and Molodtsov 2004).

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Electrodeposition through crystalline S-layer patches deposited on ultrathin AuPd films draped across the imaging windows of gold TEM grids has also proved to be a valuable method for nanofabrication of inorganic materials (Allred, Sarikaya, Baneyx, and Schwartz 2005). Contrary to shadowing techniques, material growth proceeds from the substrate outward without the need of entirely open regions. Thus, it offers the unique prospect of fabricating nanopatterned materials through multilayered crystalline protein patches. The S-layer of *Deinococcus radiodurans* was used for making cuprous oxide (Cu₂O), nickel, platinum, palladium, and cobalt nanostructured materials on electrically conducting substrates (Allred, Sarikaya, Baneyx, and Schwartz 2005).

Although native S-layers have clearly demonstrated the presence and availability of functional sites for the precipitation of metal ions, a much more controlled and specific way of making highly ordered nanoparticle arrays uses genetic approaches for the construction of chimeric S-layer fusion proteins incorporating unique polypeptides that have been demonstrated to be responsible for biomineralization processes (Naik, Stringer, Agarwal, Jones, and Stone 2002; Naik, Jones, Murray, McAuliffe, Vaia, and Stone 2004). The precipitation of metal ions or binding of metal nanoparticles (see the following) is then confined to specific and precisely localized positions in the S-layer lattice. Currently several silver and cobalt precipitating peptides are under investigation (Naik, Stringer, Agarwal, Jones, and Stone 2002; Naik, Jones, Murray, McAuliffe, Vaia, and Stone 2004). First results are promising and have demonstrated the feasibility to genetically engineer S-layer fusion proteins incorporating metal binding peptides capable of forming monolayers on technologically important substrates such as silicon, glass, gold, or polymeric surfaces. In particular, the possibility of using S-layer streptavidin fusion proteins as patterning elements for the immobilization of biotinylated functional molecules recently has been demonstrated (Moll, Huber, Schlegel, Pum, Sleytr, and Sára 2002; Huber, Liu, Egelseer, Moll, Knoll, Sleytr, and Sára 2006).

3.3 BINDING OF PREFORMED NANOPARTICLES

Although wet chemical methods lead to crystalline arrays of nanoparticles with spacing in register with the underlying S-layer lattice, they do not allow us to precisely control particle size and hence the contact distances of neighboring particle surfaces, both of which are important for studying and exploiting quantum phenomena.

Thus, the binding of preformed, often core-shell, nanoparticles into regular arrays on S-layers has significant advantages for the development of nanoscale electronic devices. Based on the work on binding biomolecules, such as enzymes or antibodies, onto S-layers (Slevtr, Egelseer, Pum, and Schuster 2004; Sára, Pum, Schuster, and Sleytr 2005; Sleytr, Sára, Pum, and Schuster 2005), it has already been demonstrated that metallic and semiconducting nanoparticles can be bound in regular arrangements on S-layers. This is because contrary to conventional carriers, in which location, local density, and orientation of functional groups are only approximately known, with S-layers the properties of a single constituent unit are replicated with the periodicity of the lattice and thus define the characteristics of the whole two-dimensional array. The pattern of bound molecules frequently reflects the lattice symmetry, size of the morphological units, and physicochemical properties of the array. Specific binding of molecules on S-layer lattices may be induced by different noncovalent forces. For example, the distribution of net negatively charged domains on S-layers could be visualized by electron microscopical methods after labelling with positively charged topographical markers, such as polycationic ferritin (PCF; diameter, 12nm) (Fig. 6.3). The regular arrangement of free carboxylic acid groups on the hexagonal S-layer lattice from Thermoproteus tenax was clearly demonstrated in this way (Messner, Pum, Sára, Stetter, and Sleytr 1986).

Recently, gold and amino functionalized CdSe had been bound onto S-layer protein monolayers and self-assembly products of SbpA (Fig. 6.4) (Györvary, Schroedter, Talapin, Weller, Pum, and Sleytr 2004). SbpA monolayers recrystallized on hydrophobic silicon surfaces expose the outer S-layer face toward the

FIG. 6.3. Transmission electron microscopic image of a freeze-dried and metal (Pt/C) shadowed preparation of a bacterial cell wall sacculus onto which polycationic ferritin molecules (PCF; diameter 12 nm) had been bound in an ordered hexagonal arrangement. Bar, 100 nm.

FIG. 6.4. Transmission electron microscopic image of preformed gold nanoparticles (diameter 5 nm) bound on an S-layer with square (p4) lattice symmetry. The superlattice of the gold nanoparticles resembles the lattice geometry of the underlying S-layer. Bar, 50 nm.





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environment. Amino-functionalized 4-nm sized CdSe particle were bound at 1-ethyl-3,3'(dimethylaminopropyl) carbodiimide (EDC) activated carboxyl groups at the outer S-layer face in register with the underlying square S-layer lattice. On hydrophilic silicon surfaces SbpA forms double layers where the inner S-layer surfaces are facing each other and thus, again, expose their outer S-layer face towards the environment. The inner face is only accessible where the double layers are incomplete. Citrate stabilized negatively charged gold nanoparticles of 5 nm in diameter were bound by electrostatic interactions at the inner S-layer face forming extended superlattices (Györvary, Schroedter, Talapin, Weller, Pum, and Sleytr 2004).

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The hexagonally packed intermediate (HPI) S-layer of *Deinococcus radiodurans* was used for the self-assembly of preformed gold nanoparticles into superlattices commensurate with the underlying S-layer lattice (Hall, Shenton, Engelhardt, and Mann 2001; Bergkvist, Mark, Yang, Angert, and Batt 2004). Each hexamer is in the form of a hollow cone-shaped protrusion with a positively charged central channel. High-resolution TEM studies showed that negatively charged monodisperse gold nanoparticles with mean sizes of ~8 nm and ~5 nm, respectively, were electrostatically bound at these sites forming micrometer sized crystalline domains. Additional experiments with 20-nm sized positively charged as well as with 5-nm sized negatively charged gold nanoparticles demonstrated that superlattices were not formed and thus not templated by the S-layer. The nanoparticles were either negatively charged because of surface citrate ions or positively charged because of surface coating with poly-L-lysine.

A major breakthrough in the regular binding of metallic and semiconducting nanoparticles was achieved by the successful design and expression of S-layer-streptavidin fusion proteins which allowed a specific binding of biotinylated ferritin molecules into regular arrays (Fig. 6.5) (Moll, Huber, Schlegel, Pum, Sleytr, and Sára 2002; Huber, Liu, Egelseer, Moll, Knoll, Sleytr, and Sára 2006).

The fusion proteins had the inherent ability to self-assemble into monomolecular protein lattices. The fusion proteins and streptavidin were produced independently in *Escherichia coli*, isolated, purified, and mixed to refold into heterotetramers of 1:3 stoichiometry. Self-assembled chimeric S-layers could be formed in suspension, on liposomes, silicon wafers, and accessory cell wall polymer–containing cell wall fragments. The two-dimensional protein crystals displayed streptavidin in defined repetitive spacing, and they were capable of binding D-biotin and biotinylated proteins, in particular ferritin. Further on, it could be demonstrated that all fused streptavidin functionalities had the same position and orientation within the unit cell and were exposed. Such chimeric S-layer protein lattices can be used as self-assembling nanopatterned molecular affinity matrices capable to arrange biotinylated compounds in ordered arrays on surfaces (Fig. 6.6). In addition, it has application potential as a functional coat of liposomes when a spherical arrangement of nanoparticles is required.

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FIG. 6.5. **A.** Digital image reconstructions of transmission electron microscopical images of negatively stained preparations of native SbsB S-layer protein (*A*) and Streptavidin – SbsB S-layer fusion protein (*B*). The region of highest protein mass in the SbsB lattice is marked in (*A*) and (*B*) by corresponding arrows while the second arrow in (*B*) points toward the additional streptavidin mass. Bars, 10 nm. **C.** Bound biotinylated ferrtin molecules are reflecting the geometry of the underlying SbsB S-layer lattice. The vector pair indicates the orientation of the oblique (p1) lattice. Bar, 100 nm. (Reprinted from ref. 38, with permission from the National Academy of Sciences USA.).



FIG. 6.6. Schematic drawing of S-layer fusion proteins with p1 lattice symmetry. All fused functionalities (e.g., streptavidin) shown as knights here exhibit the same position and orientation within the S-layer lattice.

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4 CONCLUSIONS

In summary, these experiments have clearly shown that S-layers are perfectly suited to control the formation of nanoparticle arrays, either by direct precipitation from the vapour or liquid phase, or by binding preformed nanoparticles. The S-layer approach provides for the first time a biologically based fabrication technology for the self-assembly of molecular catalysts, templates, and scaffolds for the generation of ordered large-scale nanoparticle arrays for applications in electronic or optic devices.

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