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Self-assembled two-dimensional protein arrays in bionanotechnology: from S-layers to designed lattices

François Baneyx and James F Matthaei

Although the crystalline S-layer arrays that form the exoskeleton of many archaea and bacteria have been studied for decades, a long-awaited crystal structure coupled with a growing understanding of the S-layer assembly process are injecting new excitement in the field. The trend is amplified by computational strategies that allow for *in silico* design of protein building blocks capable of self-assembling into 2D lattices and other prescribed quaternary structures. We review these and other recent developments toward achieving unparalleled control over the geometry, chemistry and function of protein-based 2D objects from the nanoscale to the mesoscale.

Addresses
Department of Chemical Engineering, University of Washington, Box 351750, Seattle, WA 98195-1750, USA

Corresponding authors: Baneyx, François (baneyx@uw.edu)

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Introduction

Biological building blocks that self-assemble into predetermined supramolecular structures are of considerable interest in bionanotechnology where an ability to control shape, size, geometry and surface chemistry is crucial to the production of advanced materials with tailored properties. Predictive control of shape has been particularly effective with nucleic acids where a variety of one, two and three-dimensional (3D) nanostructures have been produced via strand exchange and DNA Origami technologies [1]. Peptides and peptoids (polymers whose constituent monomers resemble amino acids but have side chains appended to the amide nitrogen rather than to the α carbon) have also been engineered to assemble into 2D structures [2,3,4]. Compared to these molecules, proteins offer a richer and more versatile structural, chemical and functional palette that can be further expanded through rational design, selection and directed evolution.

Two-dimensional (2D) protein arrays are of particular interest in bionanotechnology because they allow for the high-density display of peptides and proteins in sensor, diagnostic and vaccine applications. They also enable the periodic organization (or templating) of inorganic particles with nanoscale control of position for plasmonic, opto-electronic, magnetic and catalytic applications. In nature, 2D protein arrays are only found in the purple membrane patches of *Halobacteria* species [5], and the surface (S-) layer exoskeleton of nearly all archaea and many bacteria [6]. Here we will not discuss the purple membrane — a crystalline assembly consisting of trimers of bacteriorhodopsin tightly packed in a lipid-containing hexagonal array — because its structure and potential for optical applications have been reviewed elsewhere [7,8]. Instead, we focus this review on recent developments in our understanding of S-layer structure-function relationship and on progress in the computational design of entirely new kinds of protein arrays.

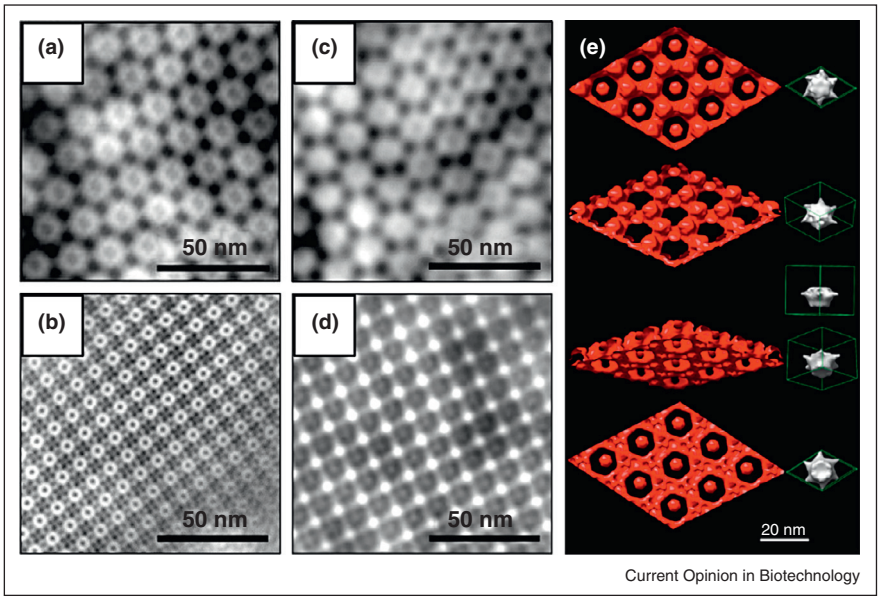
S-layer structure

S-layers are monomolecular lattices of (glyco)proteins that encapsulate certain bacteria and archaea and connect to the cell surface through one or several N-terminal glycan-binding domains. Their function ranges from protective coating, cell adhesion, surface recognition, molecular sieving and ion trapping, to scaffolding for enzymes and virulence factors [6,9]. S-layers are 5-to-20 nm thick in bacteria and up to 70-nm thick in archaea. They have a smooth, hydrophobic outer surface with net neutral charge and a corrugated inner surface that tends to be hydrophilic and carries either a net negative or positive charge [10]. Individual S-layer proteins have molecular masses between 40 and 200-kDa and form morphological units composed of one, two, three, four or six subunits which assemble with oblique (p1, p2), square (p4), or hexagonal (p3, p6) 2D rotational symmetries (Fig. 1a,b) [10]. Center-to-center unit spacing ranges between ≈5 and 30 nm and two or more classes of 2-to-6 nm pores typically perforate the array.

Technological uses

Crystalline patches of S-layer proteins can be stripped from bacteria and archaea via detergent extraction, or by using other agents that disrupt their interaction with the cell wall, and directly used for practical applications [11]. In some cases, S-layer proteins can be expressed in heterologous hosts, such as *Escherichia coli*, unfolded by GuHCl or urea treatment, and re-assembled by dilution or dialysis [6]. Recrystallization from unfolded subunits is most reliably performed at the air-water interface using a Langmuir–Blodgett trough, but is also possible on the surface of zwitterionic lipids and certain technologically relevant

Fig. 1



TEM images of negatively stained *D. radiodurans* **(a)** and *S. ureae* **(b)** S-layers and of the corresponding electrodeposited Cu_2O films (**c,d**). **(e)** TEM-based 3D reconstruction of nanostructured Cu_2O (red). Four different angles are shown along with a protein unit cell (right). Panel **(e)** is reprinted with permission from Ref. [15].

substrates such as silicon, carbon and metals. Reassembled S-layers are a mosaic of well-ordered domains that range in size from about 100 nm to 1–2 μm. However, interdomain dislocations and gaps are not uncommon. The reassembly process is influenced by protein concentration, buffer composition, identity of the surface or interface onto which the array is reassembled, and nature and concentration of added divalent cations, which can induce reassembly transitions from sheets, to cylinders, to morphologically poorly defined structures [12].

S-layers have been evaluated for a myriad of applications including: ultrafiltration membranes; drug delivery systems; scaffolds for immunogen displays; and substrates for the spatial organization of functional molecules, metals, and semiconducting nanoparticles (for recent reviews, see Refs. [13•,9,11]). Because they have a large void content (30–70% porosity), S-layers can also be used to template the synthesis of inorganic structures conformational to the geometry of the pores. Examples include the precipitation of CdS nanoparticles within the pores of the *Bacillus stearothermophilus* S-layer via solution chemistry [14] and work from our own laboratories showing that the *Deinococcus radiodurans* and *Sporosarcina ureae* S-layers can be used as a resist to template the electrodeposition of a broad range of materials including Pt, Ni, Co and Cu_2O (Fig. 1c–e) [15,16].

Although such inorganic nanostructures hold promise for catalysis and opto-electronics applications, they are difficult to produce at large scales with the current S-layer 'patch' technology. Furthermore, the presence of two or more types of pores in an array means that structures of distinct size and shape are also generated. This could be an enormous advantage if one could fill them with two separate materials. However, achieving this — let alone mineralizing crystalline materials within the confined environment provided by the pores — has so far proven elusive. Other S-layer idiosyncrasies (e.g. the fact that inner and outer faces have different topography and chemistry) may complicate the control of surface interactions and interfere with in-registry stacking, thus making the production of 3D structures challenging.

Insights from the SbsB crystal structure

Although Baumeister and coworkers produced outstanding low-resolution TEM reconstructions of S-layers in the mid-eighties [17,18], X-ray crystallography attempts have long been thwarted by the difficulty of obtaining 3D crystals from proteins that evolved to self-assemble into 2D lattices [19••]. As a result, topological information has been obtained the hard way. For instance, in a bid to identify surface-exposed amino acids in the 98-kDa *Geobacillus stearothermophilus* SbsB protein, Howorka and coworkers created 75 cysteine substitution mutants and screened the solvent accessibility of these residues in both the monomeric, dimeric and assembled forms of the protein [20,21]. This heroic effort established that amongst 23 residues that were highly accessible in the monomer, 8 were interfacial,