S-layers: principles and applications

Uwe B. Sleytr, Bernhard Schuster, Eva-Maria Egelseer & Dietmar Pum

1Institute of Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Vienna, Austria; and 2Institute of Synthetic Biology, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

Correspondence: Uwe B. Sleytr, Institute of Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Muthgasse 11, 1190 Vienna, Austria. Tel.: +43 1 47654 2201; fax: +43 1 4789112; e-mail: uwe.sleytr@boku.ac.at

Received 3 September 2013; revised 10 January 2014; accepted 13 January 2014. Final version published online 24 February 2014.

DOI: 10.1111/1574-6976.12063

Editor: Mecky Pohlschroder

Keywords

crystalline cell surface layers (S-layers); bacterial surface layers; self-assembly; nanobiotechnology; biomimetics; synthetic biology.

Abstract

Monomolecular arrays of protein or glycoprotein subunits forming surface layers (S-layers) are one of the most commonly observed prokaryotic cell envelope components. S-layers are generally the most abundantly expressed proteins, have been observed in species of nearly every taxonomical group of walled bacteria, and represent an almost universal feature of archaeal envelopes. The isoporous lattices completely covering the cell surface provide organisms with various selection advantages including functioning as protective coats, molecular sieves and ion traps, as structures involved in surface recognition and cell adhesion, and as antifouling layers. S-layers are also identified to contribute to virulence when present as a structural component of pathogens. In Archaea, most of which possess S-layers as exclusive wall component, they are involved in determining cell shape and cell division. Studies on structure, chemistry, genetics, assembly, function, and evolutionary relationship of S-layers revealed considerable application potential in (nano)biotechnology, biomimetics, biomedicine, and synthetic biology.

Introduction

With the exception of those prokaryotic organisms which have developed strategies to live under very specialized and frequently extreme environmental conditions in which monocultures are feasible, most organisms have to survive in highly competitive habitats in very complex microbiomes. Consequently, the diversity observed in the molecular architecture of bacterial and archaeal cell envelopes, particularly the structure of the outermost boundary layers, reflects evolutionary adaptations of the organism to specific environmental and ecological conditions.

Among the most commonly observed prokaryotic cell surface structures are two-dimensional arrays of proteinaceous subunits forming surface layers (termed S-layers) on prokaryotic cells (Sleytr, 1976; Sleytr et al., 1988b; Table 1). Since the first ‘macromolecular monolayer’ described by Houwink and Le Poole (1952; Houwink, 1953) in the cell wall of a Spirillum sp., S-layers have now been identified in hundreds of different species of almost every taxonomic group of walled Bacteria and are an almost universal feature of Archaea (Fig. 1; for compilation see, Messner & Sleytr, 1992; Sleytr et al., 1996a, 1999, 2002; Claus et al., 2005; König et al., 2010; Messner et al., 2010; Albers & Meyer, 2011; Hynonen & Palva, 2013).

Because S-layer proteins account for approximately ten percent of cellular proteins in Archaea and Bacteria, they represent interesting model systems for studying the processes involved in the synthesis, secretion, and assembly of extracellular proteins. Moreover, as the biomass of prokaryotic organisms surpasses the biomass of eukaryotic organism (Whitman et al., 1998), S-layer proteins can be considered as one of the most abundant biopolymers on our planet. S-layers also represent the simplest biological protein or glycoprotein membranes developed during evolution (Sleytr, 1975). They are generally composed of a single molecular species endowed with the ability to assemble on the cell surface into closed regular arrays occupying a low free-energy arrangement. Studies on the in vivo morphogenesis of S-layers demonstrated that at high growth rates, approximately 500 subunits per second must be synthesized, translocated to the cell surface, and incorporated into the existing S-layer lattice (Sleytr & Messner, 1983; Sleytr & Beveridge, 1999). It is now evident that...
S-layers as metabolic expensive products can provide organisms with an advantage of selection in quite diverse habitats. Although a considerable amount of knowledge has accumulated on the structure, assembly, chemistry, and genetics of S-layers, relatively little firm data are available about their specific biological functions (Sleytr et al., 2002, 2007b; Hynonen & Palva, 2013). It is now recognized that they can function as protective coats, molecular sieves, molecule and ion traps, promoters for cell adhesion, immunomodulators, surface recognition, antifouling coat-

| Year     | Milestone                                                                 | Reference                     |
|----------|---------------------------------------------------------------------------|-------------------------------|
| 1953     | First evidence of a monomolecular array in a bacterial cell wall fragment | Houwink (1953)                |
| 1968     | Evidence that coherent monomolecular arrays are located on the surface of the cell envelope of intact Gram-positive and Gram-negative bacteria using freeze-etching techniques | Remsen et al. (1968) and Sleytr et al. (1968) |
| 1969     | Description of in vitro assembly of S-layer proteins                      | Brinton et al. (1969)         |
| 1971     | First evidence for a function: S-layer as protective coat against the bacterial parasite Bdellovibrio bacteriovorus | Buckmire (1971)               |
| 1975     | Studies on the self-assembly and homologous and heterologous reattachment of S-layer proteins on cell envelopes of Gram-positive bacteria | Sleytr (1975)                 |
| 1976     | Evidence for glycosylation of archaeal S-layer proteins                    | Mescher & Strominger (1976)   |
| 1976     | Evidence for glycosylation of bacterial S-layer proteins                   | Sleytr & Thorne (1976)        |
| 1986/1987| First nanobiotechnological application of S-layer proteins: Use of S-layer lattices for the production of ultrafiltration membranes with defined molecular sieving properties | Sleytr & Sára (1986), Sára & Sleytr (1987c) and Sleytr & Sára (1988) |
| 1986/1991| S-layers involved in morphogenesis and cell division in archaea           | Messner et al. (1986b) and Pum et al. (1991) |
| 1986     | First sequenced S-layer protein gen                                        | Tsuboi et al. (1986)          |
| 1989/1991| S-layers as combined carrier/adjuvants for conjugated vaccines            | Sleytr et al. (1989, 1991)    |
| 1994     | Proposing S-layer-induced nanopatterned fluidity in lipid films (termed semi-fluid lipid membrane model) | Pum & Sleytr (1994)           |
| 1997/1998| First biomimetic approach copying the supramolecular building principle of archaeal cell envelopes to generate (functional) phospho- and ether lipid membranes | Schuster et al. (1997, 1998a, b) |
| 2002     | First monomeric and oligomeric functional S-layer fusion proteins capable to assemble into ordered arrays | Breitwieser et al. (2002) and Moll et al. (2002) |
| 1999/2002| Surface display of foreign epitopes of SLH domain and whole S-layer protein | Mesnage et al. (1999a) and Avall-Jäskeläinen et al. (2002) |
| 2002/2008| First atomic structures of archaeal and bacterial S-layer protein domains obtained from X-ray studies (2.4 Å resolution) | Jing et al. (2002) and Pavkov et al. (2008) |

The terminology ‘S-layer’ (surface layer) was introduced 1976 (Sleytr, 1976) and generally accepted at the ‘First International Workshop on Crystalline Bacterial Cell Surface Layers’ in Vienna, Austria (August 1984). At the ‘EMBO Workshop on Crystalline Bacterial Cell Surface Layers (S-layers)’ (August 31 to September 2, 1987, Vienna, Austria), S-layers were defined as: ‘Two-dimensional arrays of proteinaceous subunits forming surface layers on prokaryotic cells’ (Sleytr et al., 1988b).
ings, and virulence factors in pathogenic organisms. In those Archaea that possess S-layers as the exclusive enve-lope component external to the cytoplasmic membrane, the lattice is involved in the determination of cell shape and as a structure aiding in the cell division process.

The wealth of information accumulated on the general principles of S-layers led to a broad spectrum of applications. Most relevant for applied S-layer research is the capability of isolated S-layer (glyco)proteins to assemble in defined orientations into crystalline arrays in suspension or on suitable surfaces or interfaces (Sleytr et al., 1999, 2005; Pum et al., 2006, 2010). As S-layers are periodic structures, they exhibit repetitive identical physicochemical properties down to the subnanometer scale and possess pores identical in size and morphology. Most important, properties of S-layer proteins can be changed by chemical modifications and genetic engineering. It is now evident that S-layers also represent a unique structural basis and patterning element for generating complex supramolecular assemblies involving all relevant ‘building blocks’ such as proteins, lipids, glycans, and nucleic acids (Egelseer et al., 2008; Schuster & Sleytr, 2009b; Egelseer et al., 2010; Sleytr et al., 2010; Ilk et al., 2011a; Sleytr et al., 2011, 2013).

Occurrence, location, and structure

The location and ultrastructure of S-layers of a great number of Bacteria and Archaea have been studied by electron microscopy of thin-sectioned, freeze-etched, freeze-dried and shadowed, negatively stained or frozen hydrated preparations (Thornley et al., 1974; Sleytr & Glauert, 1975; Sleytr, 1978; Sleytr & Messner, 1983; Baumeister & Engelhardt, 1987; Sleytr et al., 1988a; Beveridge, 1994; Sleytr et al., 1996a; Pavkov-Keller et al., 2011). More recently, atomic force microscopy (AFM) has become an important method for characterizing S-layer lattices (Müller et al., 1996, 1999; Ebner et al., 2006; Tang et al., 2007; Moreno-Flores et al., 2008; Chung et al., 2010; López et al., 2011). In most Archaea, S-layers represent the only wall component outside the plasma membrane (Fig. 2a and b). Only a few Archaea possess a rigid wall layer (e.g. pseudomurein in methano-genic Archaea) as intermediate layer between the cytoplasmic membrane and the S-layer (Fig. 2c; Claus & König, 2010; Albers & Meyer, 2011). In Gram-positive Bacteria, S-layers are attached to the rigid peptidoglycan-containing layer (Fig. 2d), while in the more complex Gram-nega-tive bacterial cell envelope, the S-layer adheres to the lipopolysaccharide of the outer membrane (Fig. 2e).

The most useful electron microscopy preparation technique for identifying S-layers on a particular organism is freeze-etching of intact cells (Fig. 1). S-layers completely cover the cell surface during all stages of cell growth and division (Sleytr & Glauert, 1975; Sleytr, 1978; Sleytr & Messner, 1989; Messner & Sleytr, 1991b; Pum et al., 1991; Sleytr et al., 1999; Rachel, 2010). For some organisms, two superimposed S-layer lattices composed of different proteins have been observed (Sleytr & Messner, 1983; Sleytr et al., 1996b). Mono-molecular arrays of proteinaceous subunits have also been observed in prokaryotic sheaths (Beveridge & Graham, 1991; Albers & Meyer, 2011).
Symmetry axis:
- Two-fold
- Four-fold
- Three-fold
- Six-fold

Fig. 3. Schematic drawing of the different S-layer lattice types, their base vectors, the unit cell (shaded in gray), and the corresponding symmetry axis. The proteins at one morphological unit are shown in red.

S-layer lattices generally exhibit oblique (p1, p2), square (p4), or hexagonal (p3, p6) space group symmetry (Fig. 3) with center-to-center spacings of the morphological units of 4–35 nm (Beveridge, 1994; Sleytr & Beveridge, 1999; Sleytr et al., 1999, 2002; Albers & Meyer, 2011; Pavkov-Keller et al., 2011). Depending on the lattice type, the morphological units consist of one, two, three, four, or six monomers, respectively (Fig. 3). Bacterial S-layers are generally 5–10 nm thick, whereas archaeal S-layers frequently exhibit a much thicker 'mushroom-like structure' with pillar-like domains anchored to the plasma membrane (Baumeister & Engelhardt, 1987; Albers & Meyer, 2011). Bacterial S-layers reveal a rather smooth outer and a more corrugated inner surface. S-layers represent highly porous protein lattices (30–70% porosity) with pores of uniform size and morphology in the 2–8 nm range. Many S-layers possess two or even more distinct classes of pores (Sleytr & Beveridge, 1999; Sleytr et al., 1999, 2002; Albers & Meyer, 2011; Pavkov-Keller et al., 2011).

Isolation and chemistry

In both Archaea and Bacteria, S-layer lattices differ considerably in their susceptibility to isolation from the supporting envelope structure and disruption into monomeric subunits. Generally, S-layers are isolated from cell wall fragments which were obtained by breaking up the cells and removing the content including the cytoplasmic membrane by addition of hydrogen bond-breaking agents [e.g. guanidine hydrochloride (GHCl) or urea] (Sleytr & Messner, 1983; Schuster et al., 2005; Schuster & Sleytr, 2013), trichloroacetic acid (Nußer et al., 1988), detergents, or cation substitution (e.g. Na⁺ or Li⁺, replacing Ca²⁺; Koval & Murray, 1984; Lortal et al., 1992, 1993), and ethylene diamine tetraacetic acid (EDTA; Cline et al., 1989). In certain cases, even washing cells with deionized water can lead to a dissociation of the S-layer lattice (Kosma et al., 1995). The various extraction and disintegration experiments revealed that the intersubunit bonds in the S-layer lattices are stronger than those binding the crystalline arrays to the supporting envelope layer (Sleytr & Glauber, 1976; Sleytr & Beveridge, 1999). This characteristic property is seen as a major requirement for continuous recrystallization of the lattice into a low free-energy arrangement during cell growth and division. Some archaean S-layers have shown to be highly resistant to common denaturizing agents (Beveridge, 1994). Special isolation procedures are required for S-layers in Archaea where they are associated with the cytoplasmic membrane (Nußer et al., 1988; König et al., 2004; Rachel, 2010). With many solubilized S-layers, it has been demonstrated that isolated subunits reassemble into lattices identical to those observed on intact cells upon removal of the disrupting agent (see also section ‘Assembly and morphogenesis’).

Chemical and genetical analysis of many S-layers has revealed a similar overall composition. They are generally composed of a single protein or glycoprotein species with molecular masses ranging from 40 to 170 kDa (Sleytr et al., 1993a, 2002; Avall-Jääskeläinen & Palva, 2005; Claus et al., 2005; Messner et al., 2010). Most S-layers of Bacteria are composed of weakly acidic proteins or glyco-
proteins, contain 40–60% hydrophobic amino acids, and possess few or no sulfur-containing amino acids. The pI values of the proteins range from 4 to 6. For some Archaea (e.g. Methanothermus fervidus) and in Lactobacillli, however, pIs of the S-layer proteins between 8 and 10 have been determined. Comparative studies on S-layer genes of organisms from different taxonomic affiliations revealed that homologies between nonrelated organisms are low despite the fact that their amino acid composition shows no significant difference. Nevertheless, it is quite obvious that common structural principles must exist in S-layer proteins (e.g. the ability to form intersubunit bonds and to self-assemble into monomolecular arrays, the formation of hydrophilic pores with low unspecific adsorption, and the interaction with underlying cell envelope components).

A few post-translational modifications are known to occur in S-layer proteins, including cleavage of carboxy- or amino terminal fragments, protein phosphorylation, and protein glycosylation of amino acid residues. The latter is a remarkable characteristic of many archaeal and some bacterial S-layer proteins. In fact, S-layer proteins were the first glycoproteins detected in prokaryotes (Mescher & Strominger, 1976; Sleytr & Thorne, 1976) and still are among the best-studied examples of glycosylated prokaryotic proteins (Sára et al., 1989; Sumper et al., 1990; Konrad & Eichler, 2002; Sleytr et al., 2002; Eichler & Adams, 2005; Messner et al., 2008, 2009; Albers & Meyer, 2011; Eichler & Maupin-Furlow, 2013). The glycan chain and linkages of bacterial and archaeal glycoproteins are significantly different from those of eukaryotes (Sleytr et al., 2002; Schäffer & Messner, 2004; Messner et al., 2009). The Halobacterium salinarum S-layer glycoprotein was the first noneukaryotic protein shown to be N-glycosylated (Mescher & Strominger, 1976). Most archaeal S-layer glycoprotein glycans consist of only short heteropolysaccharides, usually not built of repeating units. Moreover, the predominant linkage types are N-glycosidic bonds where the glycan moieties are covalently linked to select asparagine residues of the target protein. Although most S-layer proteins are either N- or O-glycosylated, in few cases both modifications can be present on one protein at the same time (Albers & Meyer, 2011; Eichler & Maupin-Furlow, 2013). An example constitutes the well-characterized Halobacterium volcanii S-layer glycoprotein contains both N- and O-linked glycans (Sumper et al., 1990).

As mentioned before, at the same time when glycosylation on haloarchaea was reported for the first time, glycosylation of S-layer proteins from the Bacteria Thermoanaerobacter thermohydrosulphuricus and Thermoanaerobacter thermosaccharolyticum was discovered (Sleytr & Thorne, 1976; Table 1). Since then, S-layer glycoproteins from several other bacteria have been extensively studied, leading to the awareness of the wide distribution of S-layer glycoproteins among Bacteria.

In Bacteria, N-glycosylation is considered to be a rare event and is represented mainly in Campylobacter spp., Helicobacter spp., and Desulphovibrio spp. (Stimson et al., 1995). Thus, N-glycosylation apparently is far more common in archaea than in bacteria. The degree of glycosylation of bacterial S-layer proteins, that is, the covalent O-glycosidic linkage of glycan moieties to select serine, threonine, and tyrosine residues, varies generally between 2% and 10% (w/w), and the S-layer proteins are typically multiple glycosylated (Messner et al., 2009, 2013).

Moreover, the lipid carrier molecules also differ between Archaea and Bacteria. In Archaea, isoprene-based lipids such as dolichol phosphate and dolichol pyrophosphate play essential roles in the N-glycosylation process by delivering their bound glycan cargo to selected protein targets. In Bacteria, however, undecaprenol pyrophosphate is recognized to play an essential role in S-layer protein O-glycosylation. To sum up, S-layer glycoproteins are among the best-studied examples of glycosylated prokaryotic proteins (Eichler & Adams, 2005; Messner et al., 2008, 2009; Albers & Meyer, 2011). Hence, a more detailed and comprehensive summary cannot be given and is out of the scope of this review. Nevertheless, at this point, we would like to refer to several recent reviews providing deeper information on the glycosylation process in general (Albers & Meyer, 2011; Eichler & Maupin-Furlow, 2013; Messner et al., 2013).

Recently, yet another post-translational modification of S-layer glycoproteins was reported. It could be demonstrated that a subset of secreted euryarchaeal proteins, including the S-layer glycoprotein, is processed and covalently linked to membrane-embedded lipids involving membrane-spanning enzymes referred to as archaeosortases (Szabo & Pohlschroder, 2012; Eichler & Maupin-Furlow, 2013). A distinctive subfamily of the archaeosortase/exosortase superfamily is designated archaeosoratase A (ArtA) because of its restriction to the Archaea and its remote homology to exosortase. To examine the role of ArtA from Haloferax volcanii for the first time in vivo, homologous recombination to construct deletion strains that lack the artA gene was performed (Abdul Halim et al., 2013). Comparison of wild-type and the artA mutant strains resulted in multiple biological phenotypes including alteration in cell shape and the S-layer. These results clearly demonstrated in vivo that, as predicted by in silico work (Haft et al., 2012), the C-terminal hydrophobic transmembrane segment of H. volcanii S-layer glycoprotein is processed by the ArtA. Because the C-terminal tripartite structure consisting of a signature motif, a transmembrane alpha helix domain, and a cluster
of basic residues, which is recognized by archaeosortases, is highly conserved in a large number of euryarchaeal S-layer glycoproteins (all of which possess an archaeosortase), it is very likely that this proposed lipid-anchoring mechanism is a broadly conserved euryarchaeal surface-anchoring mechanism. Most relevant for in vivo membrane function and for nanobiotechnological applications (see also section ‘S-layer supported functional lipid membranes’), this mechanism provides a membrane anchor for S-layer glycoproteins withoutstuffing the lipid membrane with hydrophobic C-terminal transmembrane domains. Most recent studies even indicated that the S-layer lattice of H. volcanii is composed of two S-layer glycoprotein populations. One type of glycoprotein population is anchored to the membrane via the C-terminal hydrophobic transmembrane domain (Fig 2a), while the other one is lipid-modified for enabling membrane association in an EDTA-sensitive manner (Fig 2b; Kandiba et al., 2013). Presently, however, little is known of how these protein-processing events affect S-layer behavior or architecture. Nevertheless, considering that S-layer lattices assembled on lipid membranes (see section ‘S-layer protein–lipid interaction’) induce a nanopatterned fluidity of constituent membrane lipids, the supramolecular concept involving two types of S-layer membrane anchoring mechanisms should provide organisms with more flexibility to adapt to changes in environmental conditions (e.g. temperature dependent membrane properties).

**Assembly and morphogenesis**

**Assembly in vivo**

Numerous in vitro and in vivo studies have been performed to elucidate the dynamic process of the incorporation and reassembly of new subunits into (closed) S-layer lattices during cell growth. The only requirement for maintaining highly ordered monomolecular arrays with no gaps on a growing cell surface is a continuous synthesis of a surplus of subunits and their translocation to sites of lattice growth. In most organisms, the rate of synthesis of a surplus of subunits and their translocation with no gaps on a growing cell surface is a continuous process for maintaining highly ordered monomolecular arrays of S-layer lattices during cell growth. The only requirement for such an orientation resides in the proteins themselves and is not affected by the support (Sleytr, 1975, 1976). For this purpose, two strains of taxonomically closely related hyperthermophilic Bacteria were used (Thermoanaerobacter thermosaccarolyticum and Thermoaerobacter thermohydrodsulfuricum) showing square and hexagonal lattice symmetries, respectively. Upon dialysis of the disrupting agent (urea or GHCl, respectively), the isolated S-layer proteins of both organisms reassembled into regular arrays on the cell walls from which they had been removed. Contrary to the large regular arrays on intact cells, the crystalline patches were much smaller. An unexpected finding was that the S-layer proteins from one organism could attach to cell walls of the other one and form their patterns again. In addition, when a mixture of both S-layer protein species (with square and hexagonal lattice symmetry, respectively) was supplied, small arrays of both types were formed. These observations clearly demonstrated that the information for the dynamic lattice formation and orientation resides in the proteins themselves and is not affected by the support (Sleytr, 1975, 1976).

With the exception of selected methanogenic Archaea (e.g. M. fervidus) where the S-layer is located on the surface of a rigid pseudomurein sacculus (nußer et al., 1988; Fig. 2c) in most Archaea, S-layers assemble as exclusive
wall component in close association with the plasma membrane and consequently have been connected with a cell shape maintaining role (Mescher & Strominger, 1976; Messner et al., 1986b; Pum et al., 1991; Claus & König, 2010; Albers & Meyer, 2011). Analysis of cell morphology and lattice fault distribution provided strong evidence that the S-layers lattice is not only involved in cell shape maintenance but must also be involved in cell fission (Harris & Scriven, 1970, 1971; Nabarro & Harris, 1971; Harris, 1975, 1978). Thermoproteus tenax, an extremely thermophilic archaeon, has a cylindrical shape with constant diameter, but is variable in length (Messner et al., 1986b; Wildhaber & Baumeister, 1987). While no dislocations could be observed on the hexagonal array covering the cylindrical part, six wedge disclinations could be visualized on each hemispherical cap. Thus, it was concluded that the elongation of the cylindrical part of the cell only requires insertion of S-layer subunits at these distinct lattice faults (Messner et al., 1986b). More detailed studies on the involvement of an S-layer in cell morphology and division has been reported for Methanococcus voltae (Pum et al., 1991). The hexagonal S-layer of this highly lobed organism forms a porous but strongly interconnected network. In freeze-etched preparations of intact cells, numerous pentagons and heptagons could be detected in the hexagonal array. Complementary pairs of pentagons and heptagons were identified as the termination points of edge dislocations acting as sites for the incorporation of new morphological units into the lattice and as initiation points for the cell division process. In addition, the analysis of the number and distribution of lattice faults confirmed that the S-layer continuously recrystallizes during cell growth, maintaining an equilibrium of lowest free energy (Pum et al., 1991; Sleytr et al., 2005). The tension within the S-layer lattice is generated by the growth of the underlying protoplast and plasma membrane.

**Assembly in vitro**

The capability of isolated S-layer proteins to assemble into two-dimensional arrays in vivo and in vitro is one of their key properties exploited in basic and application-oriented research. It occurs upon dialysis of the disrupting agents as described before (Fig. 4). The formation of the 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 (Sleytr, 1975). As S-layer proteins have a high proportion of nonpolar amino acids, most likely, hydrophobic interactions are involved in the initial stage of the assembly process. Some S-layers are stabilized by divalent cations, such as Ca$^{2+}$ (Pum & Sleytr, 1994, 1995b; Norville et al., 2007; Baranova et al., 2012) and in the case of extremely halophiles by Mg$^{2+}$ (Mescher & Strominger, 1976; Cline et al., 1989; Eichler et al., 2010) interacting with acidic amino acids. Studies on the distribution of functional groups on the surface have shown that free carboxylic acid groups and amino groups are arranged in close proximity and thus contribute to the cohesion of the proteins by electrostatic interactions (Sára & Sleytr, 1987a; Sára et al., 1988a; Pum et al., 1989; Györváry et al., 2004). S-layer proteins are noncovalently linked to each other and, in the case of their adhesion to supporting structures (e.g. silicon, metal or polymeric solid surfaces, or lipid membranes) differing combinations of weak bonds (hydrophobic bonds, ionic bonds involving divalent cations or direct interaction of polar groups, and hydrogen bonds), are responsible for the structural integrity as well. Nevertheless, disintegration and reassembly experiments led to the conclusion that the bonds holding the S-layer proteins together must be much stronger than those binding them to the support (Sleytr, 1975, 1976, 1978). Once formed, S-layer proteins were never observed to leave the lattice, and thus, it was concluded that lattice growth is irreversible and no S-layer protein turnover occurs. The reason for this irreversibility may be that after the addition of the 'last' protein monomer to the (incomplete) morphological unit, this monomer is locked into place and now has a low probability of leaving (Chung et al., 2010; Comolli et al., 2013) because this final conformational arrangement in 'confinement' constitutes the state of lowest free energy (Chung et al., 2010).

![Fig. 4. Schematic drawing of the reassembly of isolated S-layer proteins](https://academic.oup.com/femsre/article-abstract/38/5/823/494887)

---

S-layer (glyco)proteins

FEMS Microbiol Rev 38 (2014) 823–864

© 2014 The Authors. FEMS Microbiology Reviews published by John Wiley & Sons Ltd on behalf of Federation of European Microbiological Societies.
Reassembly in solution

Self-assembly products are formed in solution during the dialysis of the disrupting agent against selected buffer solutions (ionic strength and pH). Monitoring the time course of self-assembly by light scattering yielded multi-phasic kinetics with a rapid initial phase and slow consecutive processes of higher than second order (Jaenicke et al., 1985). The rapid phase may be attributed to the formation of oligomeric precursors. Concentration-dependent light scattering measurements gave evidence for a ‘critical concentration’ of association, suggesting that patches of 12–16 proteins are formed and recrystallize into the final (native) S-layer structure.

Depending on the morphology and bonding properties of the S-layer proteins, either flat sheets or open-ended cylinders are formed (Messner et al., 1986a; Sleytr et al., 1999, 2005; Bobeth et al., 2011; Sleytr et al., 2011; Shin et al., 2013). The self-assembly products may be composed of mono- or double layers. In addition, it was also observed that closed vesicles may be formed by S-layer proteins recrystallizing in hexagonal lattice symmetry (Sleytr, 1976; Sleytr et al., 2005). In some cases, it was possible to control the self-assembly routes by changing the environmental parameters such as pH, or ionic content and strength of the subphase. In this context, one of the most detailed studied S-layer self-assembly systems is the one of *G. stearothermophilus* strain NRS 2004/3a (Messner et al., 1986a). This S-layer composed of glycoproteins exhibits oblique (p2) lattice symmetry and can be extracted from the peptidoglycan by high molar GHCl. Upon dialysis, the isolated proteins assembled into both flat and cylindrical mono- and double-layer self-assembly products. Depending on the salt concentration during dialysis and dialysis duration, different self-assembly structures were formed. Generally, the presence of low concentration of bivalent cations (e.g. Ca$^{2+}$) led to the formation of a mixture of highly defined sheets and cylindrical self-assembly products.

Reassembly at interfaces

Crystal growth at interfaces (e.g. solid supports, air–water interface or lipid membranes) is initiated simultaneously at many randomly distributed nucleation points and proceeds in plane until the crystalline domains meet, thus leading to a closed, coherent mosaic of individual, several micro meters large S-layer domains (Pum & Sleytr, 1995a, b; Győrvary et al., 2003; Sleytr et al., 2005). The growth of extended S-layers domains is favored at low monomer concentrations due to the corresponding low number of nucleation sites. The individual, dual domains are mono crystalline and separated by grain boundaries.

In a recently carried out detailed study using *in situ* AFM, it was shown that the reassembly of SbpA S-layer proteins from *Lysinibacillus sphaericus* CCM2177 on mica does not necessarily follow the classical pathway of crystal growth. Instead, a kinetic trap keeping the system at a higher-energy, long-lived transient state may hinder the reassembly into extended matrices (Shin et al., 2012). Over time, finally the trapped state transforms into a stable, low energy state. Careful analysis of the time and temperature dependence of formation and transformation yielded an energy difference by only 1.6 kJ mol$^{-1}$ (or 0.7 kT). But the energy barrier to transform into the final low energy state is 38 times higher (61 kJ mol$^{-1}$).

The formation of coherent crystalline domains depends on the S-layer protein species used, the environmental conditions of the subphase, such as ionic content and strength, but, in particular, on the surface properties of the interface. While the reassembly of S-layer proteins at the air–water interface and at planar lipid films is well defined (Pum et al., 1993; Pum & Sleytr, 1994; Weygand et al., 1999, 2000, 2002), the deliberate modification of the surface properties of a solid support allows to specifically control the reassembly process (Pum & Sleytr, 1995a; Sleytr et al., 1999; Győrvary et al., 2003; Comolli et al., 2013). For example, the S-layer protein SbpA, which is currently one of the most detailed studied S-layer proteins for functionalizing solid supports, forms monolayers on hydrophobic and double layers on hydrophilic silicon supports (Győrvary et al., 2003; Moreno-Flores et al., 2008). In addition, in comparison with hydrophilic surfaces, the layer formation is much faster on hydrophobic supports starting from many different nucleation sites and thus leading to a mosaic of small crystalline domains (2D powder). Along this line, the importance of the interplay between hydrophobic and hydrophilic regions was studied in detail by reassembling the S-layer protein SbpA on self-assembled monolayers (SAMs) on gold composed of disulfides with different end groups [hydroxyl (OH) vs. methyl (CH$_3$) groups] and lengths of the individual methylene chains (Moreno-Flores et al., 2008). The formation of monolayers was observed when the hydrophobic end groups (CH$_3$) surmounted the hydrophilic (OH) ones. On the contrary, double S-layers were formed when hydrophilic (OH) groups superseded the hydrophobic (CH$_3$) end groups. The threshold for the transition between native and non-native S-layer parameters was four methylene groups. Finally, it must be noted that different lattice constants were observed on the two surfaces.

SAMs were also used to study the influence of the introduced surface chemistry (López et al., 2011). The SAMs
carried CH₃, OH, carboxylic acid (COOH), or mannose (C₆H₁₂O₆), respectively, as terminating functional groups. It was confirmed that electrostatic interactions (COOH functional groups) induce a faster adsorption than hydrophobic (CH₃ groups) or hydrophilic (OH groups) interactions – as already shown for the reattachment on the bacterial cell (Sleytr, 1975; Mader et al., 2004) and at liposomes (Küpcü et al., 1995b; Mader et al., 1999, 2000), polyelectrolyte nanocapsules (Toca-Herrera et al., 2005), and emulsomes (Ucisik et al., 2013b).

As required by technological demands, a great variety of supports, differing in their physico-chemical properties, are currently investigated. Silicon and metal surfaces are exploited for applications in nano-electronics, glasses in nano-optics, and polymeric surfaces, such as epoxy-based negative photoresists (e.g. SU-8), in microfluidics (Picher et al., 2013). For example, silanization with either aminopropyltriethoxysilane or octadecyltrimethoxysilane is often used in such applications to render the properties of a glass surface hydrophobic (Lopez et al., 2010). AFM and Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) studies demonstrated that the S-layer protein SbpA adsorbs on aminopropyltriethoxysilane- and octadecyltrimethoxysilane-modified surfaces much faster than on the native silicon dioxide rendered hydrophilic by plasma treatment (Lopez et al., 2010). AFM measurements showed that the crystalline domains were much smaller on silanized substrates compared with hydrophilic silicon dioxide ones. The protein adsorption was diffusion controlled up to a threshold concentration of 0.05 mg mL⁻¹ SbpA for silanized substrates and 0.07 mg mL⁻¹ SbpA for silicon dioxide.

Finally, the reassembly of S-layer proteins at the air/water interface and on lipid films, and the handling of such layers by standard Langmuir Blodgett (LB) techniques, opened a broad spectrum of applications in basic and applied membrane research (Scherer & Sleytr, 2000, 2009b) (see section ‘S-layer supported functional lipid membranes’). However, with respect to the reassembly of S-layer proteins per se at such interfaces, a detailed study using the S-layer protein SbpA as model system had been carried out on solid-supported lipid bilayers (Chung et al., 2010). The reassembly of the square lattice followed a multistage, nonclassical pathway in which monomers, with extended conformation, first formed a mobile adsorbed phase from which they condensed into amorphous clusters. In a subsequent phase transition, the S-layer proteins folded into clusters of compact tetramers. In the following, crystal growth proceeded by the formation of new tetramers exclusively at cluster edges. Further studies will show how this information on S-layer recrystallization on ‘model’ supports will help to understand the in vivo assembly process in detail.

**Genetics, domains, and biosynthesis**

In the mid-eighties, first reports on cloning and sequencing of S-layer genes were published. The first complete S-layer gene sequence was that for the outer wall protein (OWP) from *Bacillus brevis* 47 (Tsuboi et al., 1986). To date, the search term ‘S-layer protein’ yields more than 4000 hits in the nucleotide database of NCBI because numerous S-layer genes from *Archaea* and *Bacteria* have been sequenced and cloned (Sleytr et al., 1999; Akca et al., 2002; Sleytr et al., 2002; Avall-Jääskeläinen & Palva, 2005; Messner et al., 2010).

With the accumulation of S-layer gene sequences, screening for putative sequence identities became possible. Although S-layer proteins show low homology on the sequence level, common structural organization principles have been identified. The elucidation of the structure–function relationship of distinct segments of S-layer proteins started with the production of N- and C-terminally truncated forms which were used for recrystallization and binding studies (Jarosch et al., 2001; Ilk et al., 2002; Huber et al., 2005). Thereby, it turned out that S-layer proteins exhibit mostly two separated morphological regions: one responsible for cell wall binding and the other required for self-assembly. The position of the cell wall-anchoring region within the protein can vary between bacterial species. Studies on a great variety of S-layer proteins from *Bacillaceae* revealed the existence of specific binding domains on the N-terminal part for sugar polymers, so-called secondary cell wall polymers (SCWPs), which are covalently linked to the peptidoglycan of the cell wall (Egelseer et al., 2010).

This specific molecular interaction is often mediated by a recurring structural motif of approximately 55 amino acids, which is mostly found in triplicate at the N-terminus of S-layer proteins. These so-called S-layer homology (SLH) motifs are involved in cell wall anchoring of S-layer proteins by recognizing a distinct type of SCWP, which carries pyruvic acid residues (Ries et al., 1997; Lemaire et al., 1998; Chauvaux et al., 1999; Ilk et al., 1999; Mesnage et al., 1999b, 2000; Cava et al., 2004; Mader et al., 2004; Rünzler et al., 2004; Huber et al., 2005). The need for pyruvylation was confirmed by the construction of knock-out mutants in *Bacillus anthracis* and *Thermus thermophilus* (Mesnage et al., 2000; Cava et al., 2004) as well as by surface plasmon resonance (SPR) spectroscopy using the S-layer protein rSbsB of *G. stearothermophilus* PV72/p2 and the corresponding SCWP (Petersen et al., 2008) as binding partners (Mader et al., 2004). For SbsB, the exclusive and complete
responsibility of a functional domain formed by the three SLH motifs for SCWP recognition could be confirmed, whereas for SbpA, the S-layer protein of *L. sphaericus* CCM 2177, an additional 58-amino acid-long SLH-like motif is required (Mader *et al.*, 2004; Huber *et al.*, 2005). The strong correlation between the existence of SLH motifs and the presence of the gene for the pyruvyltransferase CsaB was demonstrated once again in a very recent study (Pleschberger *et al.*, 2013). Sequencing of 8004 bp in the S'-upstream region of the S-layer gene *sbpA* led to the identification of a novel gene cluster comprising five open reading frames (ORFs) which encode proteins involved in cell wall metabolism of *L. sphaericus* CCM 2177. The two ORFs encoding the autolysin rAbpA and the pyruvyl transferase rCsaB were cloned and expressed in *Escherichia coli*, and the recombinantly produced proteins were characterized regarding their secondary structure and their enzymatic activity (Pleschberger *et al.*, 2013).

Recently, the role of the three SLH motifs in the glycosylated S-layer protein SpaA of *Paenibacillus alvei* CCM 2051T was analyzed by site-directed mutagenesis and visualization by *in vivo* studies using homologous expression as well as *in vitro* binding assays (Janesch *et al.*, 2013). It was demonstrated that the SLH motifs of SpaA are sufficient for *in vivo* cell surface display of foreign proteins at the cell surface of *P. alvei*. Furthermore, it was shown that in *P. alvei*, SLH domains have a dual-recognition function, one for the SCWP and one for the peptidoglycan, and that cell wall anchoring of SpaA is not a prerequisite for glycosylation. The coexistence of two N-terminally located binding domains (for SCWP and peptidoglycan) was already described many years ago for the SLH domain carrying S-layer protein SbsB of *G. stearothermophilus* PV72/p2 (Sára *et al.*, 1998).

In contrast, S-layer proteins devoid of SLH motifs are anchored to different types of SCWP via their N- or C-terminal regions. Using affinity studies and SPR spectroscopy, a further main type of binding mechanism was described for *G. stearothermophilus* wild-type strains which involves a nonpyruvylated SCWP containing 2,3-diacetamido-2,3-dideoxynaruronic acid as the negatively charged component and a highly conserved N-terminal region lacking an SLH domain (Egelseer *et al.*, 1998; Schäffer *et al.*, 1999; Jarosch *et al.*, 2000, 2001; Ferner-Ortner *et al.*, 2007).

Among the S-layer proteins from *Lactobacillus*, which are devoid of SLH motifs, the regions important for cell wall binding and self-assembly are quite different. In the S-layer proteins SlpA of *Lactobacillus acidophilus* and CbsA of *Lactobacillus crispatus*, a putative carbohydrate-binding repeat comprising approximately the last 130 C-terminal amino acids has been identified. This is one-third of this S-layer protein and was suggested to be involved in cell wall binding (Smit *et al.*, 2001; Antikainen *et al.*, 2002). The thus far characterized cell wall ligands to which the *Lactobacillus* S-layer attaches include teichoic acids, lipoteichoic acids, and neutral polysaccharides (Avall-Jääskeläinen & Palva, 2005). In contrast, in SlpA of *Lactobacillus brevis* ATCC 8287, the domains responsible for self-assembly (C-terminal) and cell wall binding (N-terminal) are located in a reverse order compared to those in all other *Lactobacillus* S-layer proteins characterized so far (Avall-Jääskeläinen *et al.*, 2008). However, contrary to the *L. acidophilus*-group organisms, the specific cell wall component interacting with the S-layer protein in *L. brevis* ATCC 8287 was shown to be different than (lipo)teichoic acid (Avall-Jääskeläinen *et al.*, 2008).

In Gram-negative bacteria, no general S-layer-anchoring motif has been identified and the S-layer is attached with its N- or C-terminus to the lipopolysaccharide component of the outer membrane (Thomas *et al.*, 1992; Doig *et al.*, 1993; Bingle *et al.*, 1997b). For the *Caulobacter crescentus* S-layer protein RsaA, recrystallization on lipid vesicles was obtained only when the vesicles contained the specific species of *Caulobacter* smooth lipopolysaccharide that previous studies implicated as a requirement for attaching the S-layer to the cell surface (Nomellini *et al.*, 1997). The specific type of phospholipids did not appear critical; phospholipids rather different from those present in *Caulobacter* membranes or archaeal ether lipids worked equally well. However, the source of lipopolysaccharide was critical. Furthermore, efficient recrystallization and long range order could not be obtained with pure protein, although it was apparent that calcium was required for crystallization (Nomellini *et al.*, 1997).

Using selected N- or C-terminally truncated S-layer protein forms as fusion partners for foreign proteins or domains, it turned out that S-layer proteins are able to assemble into geometrically highly defined layers while incorporating a segment that has never participated in lattice formation. To date, a great variety of functional, chimeric S-layer proteins is available (Fig. 5, Table 2 and section ‘S-layers as matrix for functional molecules and nanoparticles’).

Although considerable knowledge has already been experimentally accumulated on the structure, biochemistry, assembly characteristics, and genetics of S-layer proteins, no structural model at atomic resolution was available for quite a while. Therefore, neither their tertiary structure nor exact amino acid or domain allocations in the lattices were known. A first tertiary structure prediction for an S-layer protein (SbsB from *G. stearothermophilus* PV72/p2; Sára *et al.*, 1996b) was obtained by molecular
For the simulation of the folding, SbsB was divided into eight structurally independent domains: three domains at the N-terminus and five domains at the C-terminus. The N-terminal domains consisted mainly of α-helices and the C-terminal ones of β-sheets. The obtained tertiary structure of SbsB showed that the N-terminus of SbsB₁–207 consists of six α-helices that are linked by turns and coils. According to secondary structure predictions and sequence similarity searches, SbsB has three SLH domains with every domain made up of two α-helices, respectively. The C-terminus of SbsB accounts for the main part of the protein. Molecular dynamics (MD) simulations performed for 30 ns (in vacuum) finally led to three main domains at the C-terminal end. The first domain is linear (aa₂₀₈–aa₄₈₆) and connects the N-terminus with the L-shaped part of the C-terminus, which is made up of the other two domains (aa₄₈₇–aa₇₅₅ and aa₇₅₆–aa₉₂₅) which are fibronectin type III and Ig-like group 2 domains, respectively. Later on, the same theoretical approach was used to predict the 3D structure of SbpA from *L. sphaericus* CCM2177 (Horejsi et al., 2011b).

The first high-resolution structure of a domain of a bacterial S-layer protein was obtained from X-ray studies with an assembly-negative, water-soluble, truncated form of the S-layer protein SbsC of *G. stearothermophilus* ATCC 12980 (2.4 Å resolution; Pavkov et al., 2008). Despite the intrinsic property of S-layer proteins to assemble solely in two dimensions, it turned out that this truncated form is well suited for 3D crystallization studies. The crystal structure of rSbsC₁⁻₈₄₄ (P₂₁ space group symmetry) revealed a novel fold, consisting of six separate domains, which are connected by short flexible linkers. Furthermore, SCWP binding induced considerable stabilization of the N-terminal domain (Pavkov et al., 2008) what was later on accordingly confirmed for SbsB by AFM-based single-molecule spectroscopy (Horejsi et al., 2011a). To complete the structure of the full-length protein, additional soluble constructs containing the crucial domains for self-assembly were cloned, expressed, and purified (Dordic et al., 2012). Currently, rebuilding and refinement of the structure is in progress and upon completion will yield the complete structure of the full-length SbsC protein.

Most recently, the full-length atomistic SbsB structure was solved by the use of nanobody-aided crystallization (Baranova et al., 2012). According to this investigation, SbsB consists of seven domains formed by an amino-terminated cell wall attachment domain (SLH domain) and six consecutive immunoglobulin-like domains organized into a φ-shaped disk-like monomeric unit stabilized by an interdomain Ca²⁺ ion coordination. It is interesting to see that the choice of structurally meaningful parts and domain predictions used in the theoretical approach [described before (Horejsi et al., 2008)] are in very good agreement for the first four domains identified in this high-resolution X-ray work.

However, the first reported crystal structure of an S-layer protein from a bacterial pathogen was described for *Clostridium difficile* (Fagan et al., 2009). This S-layer protein contains a low molecular weight protein (LMW) and a high molecular weight (HMW) partner. Both proteins form a tightly associated noncovalent complex, the H/L complex. The crystal structure of a...
Table 2. Functional recombinant S-layer fusion proteins and their applications (modified after; Sleytr et al., 2011)

| Recombinant S-layer protein | Functionality | Length of function | Application | References |
|-----------------------------|---------------|--------------------|-------------|------------|
| SbpA, SbsB                  | Core streptavidin | 118 aa            | Binding of biotinylated ligands (DNA, protein), Biochip development | Moll et al. (2002) and Huber et al. (2006b) |
| SbpA, SbsC                  | Major birch pollen allergen (Bet v 1) | 116 aa            | Vaccine development, treatment for type 1 allergy | Breitwieser et al. (2002) and Ilk et al. (2002) |
| SbpA                        | *Strept*-tag II, Affinity tag for streptavidin | 9 aa             | Biochip development | Ilk et al. (2002) |
| SbpA                        | ZZ, IgG-binding domain of Protein A | 116 aa            | Extracorporeal blood purification | Völlenkle et al. (2004) |
| SbpA                        | Enhanced green fluorescent protein (EGFP) | 238 aa            | Coating of liposomes, Development of drug and delivery systems | Ilk et al. (2004) |
| SbpA                        | cAb, Heavy chain camel antibody | 117 aa            | Diagnostic systems and sensing layer for label-free detection systems | Pleschberger et al. (2004) |
| SbpA                        | Hyperthermophilic enzyme laminarinase (LamA) | 263 aa            | Immobilized biocatalysts | Tschiggerl et al. (2008b) |
| SbpA                        | Cysteine mutants | 3 aa             | Building of nanoparticle arrays | Badelt-Lichtblau et al. (2009) |
| SbpA, SbsB                  | Mimotope of an Epstein-Barr virus (EBV) epitope (F1) | 20 aa            | Vaccine development | Tschiggerl et al. (2008a) |
| SbpA                        | Mycoplasma tuberculosis antigen (mp64) | 204 aa            | Vaccine development | H. Tschiggerl (pers. commun.) |
| SbpA                        | IgG-Binding domain of Protein G | 110 aa            | Downstream processing | Nano-S Inc. (pers. commun.) |
| SgsE                        | Glucose-1-phosphate thymidylyltransferase (RmlA) | 299 aa            | Immobilized biocatalysts | Schäffer et al. (2007) |
| SgsE                        | Enhanced cyan fluorescent protein (ECFP) | 240 aa            | pH biosensors *in vivo* or *in vitro*, fluorescent markers for drug delivery systems | Kainz et al. (2010a, b) |
| SgsE                        | Enhanced green fluorescent protein (EGFP) | 240 aa            | pH biosensors *in vivo* or *in vitro*, fluorescent markers for drug delivery systems | Kainz et al. (2010a, b) |
| SgsE                        | Yellow fluorescent protein (YFP) | 240 aa            | pH biosensors *in vivo* or *in vitro*, fluorescent markers for drug delivery systems | Kainz et al. (2010a, b) |
| SgsE                        | Monomeric red fluorescent protein (RFP1) | 225 aa            | pH biosensors *in vivo* or *in vitro*, fluorescent markers for drug delivery systems | Kainz et al. (2010a, b) |
| SbpA                        | *Haemophilus influenzae* antigen (Omp26) | 200 aa            | Vaccine development | Riedmann et al. (2003) |
| SbpA                        | Antigenic poliovirus epitope (VP1) | 11 aa             | Development of mucosal vaccines | Avall-Jääskeläinen et al. (2002) |
| SbpA                        | Human c-myc proto-oncogene | 10 aa             | Development of mucosal vaccines | Avall-Jääskeläinen et al. (2002) |
| SbpA                        | Levansucrase of *B. subtilis* | 473 aa            | Vaccine development | Mesnage et al. (1999a) |
| SbpA                        | Tetanus toxin fragment C of *C. tetani* (ToxC) | 451 aa            | Development of live veterinary vaccines | Mesnage et al. (1999c) |
| SbpA                        | Pseudomonas aeruginosa strain K pilin | 12 aa             | Vaccine development | Bingle et al. (1997a) |
| SbpA                        | IHNV glycoprotein | 184 aa            | Development of vaccines against hematopoietic virus infection | Simon et al. (2001) |
| SbpA                        | Beta-1,4-glycanase (Cex) | 485 aa            | Immobilized biocatalysts | Duncan et al. (2005) |
| SbpA                        | IgG-binding domain of Protein G | GB1xs             | Development of immunoactive reagent | Nomellini et al. (2007) |
| SbpA                        | Domain 1 of HIV receptor CD4 MIP1x ligand for HIV coreceptor CCR5 | 81 aa, 70 aa | Anti-HIV microbiode development | Nomellini et al. (2010) |
| SbpA                        | His-tag, Affinity tag | 6 aa             | Bioremediation of heavy metals (Cd) from aqueous systems, bioreactor | Patel et al. (2010) |
| RsaA                        | Protective coat | 6 aa             | Protection against antimicrobial peptide in *Caulobacter crescentus* | Patel et al. (2010) and de la Fuente-Núñez et al. (2012) |

S-layer proteins: SbsB of *Geobacillus stearothermophilus* PV72/p2, SbpA of *Lysinibacillus sphaericus* CCM 2177, SbsC of *Geobacillus stearothermophilus* ATCC 12980, SgsE of *Geobacillus stearothermophilus* NRS 2004/3a, SbsA of *Bacillus stearothermophilus* PV72/p6, SlpA of *Lactobacillus brevis* ATCC 8287, SLH (SLH domain of EA1 or Sap) of *Bacillus anthracis*, RsaA of *Caulobacter crescentus* CB15A.
truncated derivative of the LMW protein was resolved down to 2.4 Å resolution and showed two domains (Fagan et al., 2009).

Furthermore, using X-ray crystallography, it was shown that the three SLH domains from B. anthracis SAP assume the shape of a three-prong spindle (Kern et al., 2011).

The first high-resolution crystal structure of an archaeal S-layer protein (2.3 Å resolution) was obtained for Methanosarcina species (Jing et al., 2002; Arbing et al., 2012) being representative for the structure of a large family of homologous archaeal Methanosarcinaceae proteins. While the S-layer structure reveals a protective, porous barrier, it is interesting to see that β-sandwich folds are structurally homologous to eukaryotic virus envelope proteins, suggesting that Archaea and viruses have found a common solution for protective envelope structures.

S-layer glycoproteins, first described in the 1970s (Table 1), were found in both domains, Archaea and Bacteria (see section ‘Isolation and chemistry’). Typically, bacterial S-layer glycans are O-glycosidically linked to serine, threonine, or tyrosine residues, and they rely on a much wider variety of constituents, linkage types, and structures than their eukaryotic counterparts (Schäffer & Messner, 2004; Eichler & Adams, 2005; Messner et al., 2008, 2009, 2010; Ristl et al., 2011; Eichler & Maupin-Furlow, 2013). In the past few years, substantial progress has been made in describing the archaeal N-glycosylation pathway, where the glycan is linked to asparagine. Interestingly, although Eukarya, Bacteria, and Archaea seem to share certain features in their N-glycosylation pathways, the archaeal pathway is a mosaic of the eukaryal and bacterial systems (Albers & Meyer, 2011). In Archaea and Bacteria, only a single gene product (AglB and PglB, respectively) is needed for the oligosaccharyltransferase reaction, whereas in Eukarya, the oligosaccharyltransferase complex is composed of nine membrane-bound protein subunits (Albers & Meyer, 2011; Eichler & Maupin-Furlow, 2013).

For many years, due to the lack of suitable molecular tools, the understanding of the genetic basis for S-layer protein glycosylation was lagging behind the structural analyses. An important milestone was reached with the identification of the first S-layer glycosylation (sgl) gene cluster in the bacterium G. stearothermophilus NRS 2004/3a (Novotny et al., 2004). Until now, about 15 different S-layer glycoprotein glycan structures have been fully or partially elucidated, and several sgl gene clusters have been identified, sequenced, and characterized (Ristl et al., 2011).

In a proof-of-concept study, the transfer of the Campylobacter jejuni heptasaccharide and the E. coli O7 polysaccharide onto the SgsE S-layer protein of G. stearothermophilus NRS 2004/3a as well as the successful expression of the S-layer neoglycoproteins in E. coli could be demonstrated. The degree of glycosylation of the S-layer neoglycoproteins after purification from the periplasmic fraction reached completeness and electron microscopical investigations revealed that recombinant glycosylation is fully compatible with the S-layer protein self-assembly system (Steiner et al., 2008).

Based on the fact that the two Bacteroidales species Bacteroides fragilis and Tannerella forsythia both have general O-glycosylation systems and share a common glycosylation sequon, a very recent study reports on the successful transfer of the B. fragilis O-glycan onto heterologously expressed T. forsythia proteins and vice versa (Posch et al., 2013). The authors showed that ‘cross-glycosylation’ of proteins in Bacteroidales are feasible, allowing the design of novel glycoproteins. To conclude, the S-layer system is a promising strategy for multivalent glycan display approaches where strict nanometer-scale control over position and orientation of the glycan epitopes is desired.

Functional aspects

When other cell surface components (e.g. capsules, glycoylases) are absent, S-layers as the outermost cell envelope component represent an important interface between the cell and its environment. As S-layer carrying Bacteria and Archaea are ubiquitous in the biosphere, the supramolecular concept of a closed, isoporous protein lattice represent specific adaptations to diverse environmental and ecological conditions. Most important, S-layers are generally part of complex envelope structures (Fig. 2) and consequently should not be considered as isolated layers. Several of the functions assigned to S-layers are still hypothetical and not based on firm experimental data.

Cell shape determination

Based on the fact that in most Archaea, the S-layer is the exclusive cell envelope component outside the cytoplasmic membrane (Albers & Meyer, 2011), it was concluded that S-layers must have a shape determining and maintaining function. This assumption was addressed in detail by studying the role of the S-layer in morphogenesis and cell division of the rod shaped T. tenax and Thermoproteus neutrophilus (Messner et al., 1986b; Wildhaber & Baumeister, 1987) and the lobed archaeon M. sinense as described in section ‘Assembly in vivo’ (Pum et al., 1991).
Surface properties and protective coats

Labeling with charged topographical markers and affinity studies revealed that S-layers from several Bacillus strains do not possess a net negative charge as demonstrated for the underlying peptidoglycan-containing layer or other bacterial surface structures (Sára & Sleytr, 1987a; Gruber & Sleytr, 1991; Sára et al., 1992). In native S-layers, carboxyl groups are neutralized by an equal number of amino groups, leading to a charge neutral outer surface (Weigert & Sára, 1995).

For glycosylated S-layer proteins, the long carbohydrate chains were found to be exposed to the ambient environment (Sára et al., 1988a, 1989; Messner & Sleytr, 1991a). Adsorption studies using whole cells which are completely covered with glycosylated S-layers revealed that they can bind to hydrophilic, hydrophobic, positively, and negatively charged materials to a comparable extent (Sára et al., 1988a). In this context, data obtained on cell surface hydrophobicity of a collection of different Lactobacillus strains with and without S-layers have to be taken into account. Contact angle measurements and AFM revealed that cell surface hydrophobicity changed in response to changes in ionic strength offering this strains a versatile mechanism to adhere to hydrophobic and hydrophilic surfaces (Vadillo-Rodríguez et al., 2004). As Lactobacilli can protect the host against infection by invading pathogens in the upper gastrointestinal tract and vagina, this surface properties are of great interest (Hynonen & Palva, 2013).

Interestingly, cell adhesion of the S-layer carrying strain G. stearothermophilus PV72 was less influenced by the environmental conditions than that of the S-layer-deficient variant T5 (Gruber & Sleytr, 1991). For the latter, hydrophobic interaction chromatography revealed a more pronounced hydrophilic surface. In the case of the pathogenic organism Aeromonas salmonicida, it could be demonstrated that the presence of the S-layer makes the cell surface much more hydrophobic (Trust et al., 1983). S-layers are capable of interacting with particles and materials of different physicochemical properties, thereby favoring adherence of whole cells to solid surfaces. In contrast, S-layers from thermophilic Bacillaceae did not adsorb charged macromolecules on their surface or inside the pores because this would hinder the transport of nutrients and metabolites (Sára & Sleytr, 1987a; Weigert & Sára, 1995). Based on these results, S-layers can be considered as structures with excellent ‘antifouling’ properties.

Furthermore, S-layers have been suggested to fulfill a protective function for the living cells by excluding hostile lytic enzymes such as muramidases and proteases. However, this could only be confirmed for a few examples.

The S-layer from Sporosarcina urea was found to protect the murein sacculus from lysozyme attack, possibly due to the presence of pores smaller than the enzyme molecules (Beveridge, 1979). This is in contrast to S-layer carrying muramidase-resistant strains of mesophilic Bacillus species which were found to have a muramidase-resistant chemically modified peptidoglycan but did not possess pores significantly smaller than strains of thermophilic Bacillaceae which allowed free passage of differently sized muramidases (Sára et al., 1990).

S-layers from Gram-negative Bacteria such as A. salmonicida, Campylobacter fetus, Aquaspirillum serpens, and C. crescentus protect the cells from attack by the bacterial parasite Bdellovibrio bacteriovorus by masking the outer membrane components and receptors, but do not show a protective function against other predators, such as protozoa (Koval, 1993; Beveridge et al., 1997).

More recently experimental data were presented which indicate that the S-layer that covers the outer membrane of C. crescentus is involved in protection against antimicrobial peptides present in the environment (de la Fuente-Núñez et al., 2012).

A quite interesting type of protective function was reported for the S-layer lattice of Synechococcus (Schultze-Lam et al., 1992; Schultze-Lam & Beveridge, 1994b), a cyanobacterium capable of growing in lakes with exceptionally high calcium and sulfate ion concentrations. The hexagonally ordered S-layer lattice functions as a template for fine-grain mineralization and is continuously shed from the cell surface to get rid of mineral depositions thereby maintaining basic vital processes such as growth and division as well as nutrient transport.

S-Layers related to pathogenicity

S-layers can contribute to virulence when they are present as a structural component of the cell envelope of pathogens. Bacillus anthracis, the etiological agent of anthrax, is capable of lethality in both animals and humans and is a biothreat of great concern (Blendon et al., 2002). The surface of B. anthracis, the causative agent of anthrax, is unusually complex: an S-layer is present underneath a hexagonally ordered S-layer lattice functions as a template for fine-grain mineralization and is continuously shed from the cell surface to get rid of mineral depositions thereby maintaining basic vital processes such as growth and division as well as nutrient transport.
S-layer proteins, both carrying three SLH motifs (Ariel et al., 2002). Immune reactivity studies using a truncated S-layer protein form devoid of the SLH moiety indicated that the C-terminal segment contributes significantly to S-layer immunogenicity (Ariel et al., 2002).

In a recent study, single domain antibodies (sdAbs) were isolated using a phage display library prepared from immunized llamas (Walper et al., 2012). Interestingly, the protein target for all six sdAb families was determined to be the S-layer protein EA1, which is present in both vegetative cells and bacterial spores. This research demonstrates the capabilities of these sdAbs and their potential for integration into current and developing assays and biosensors (Walper et al., 2012).

Also recently, evidence was provided that B. anthracis S-layer protein K (BslK), an SLH, and near iron transporter (NEAT) protein are surface localized and bind and transfer heme to iron-regulated surface determinant (Isd) proteins in a rapid, contact-dependent manner (Tarlovsky et al., 2013). This finding suggests that the Isd system can receive heme from multiple inputs and may reflect an adaptation of B. anthracis to changing iron reservoirs during an infection. Understanding the mechanism of heme uptake in pathogenic Bacteria is important for the development of novel therapeutics to prevent and treat bacterial infections (Tarlovsky et al., 2013).

Bacillus cereus G9241 is the causative agent of respiratory anthrax-like disease in humans, which is most frequently observed in welders (Callahan et al., 2008). In a very recent study, Wang et al. (2013) showed that B. cereus G9241 elaborates two S-layer proteins, Sap and EA1, which are conserved relative to the S-layer proteins of B. anthracis but not identical to them. The S-layer and S-layer-associated proteins (BslA and BslO) of B. cereus G9241 are retained in the bacterial envelope in a manner requiring csaB, a gene responsible for adding pyruvic acid residues to the SCWP, whose sequence is virtually identical to that of B. anthracis. The finding that B. cereus G9241 csaB mutants cannot retain S-layer proteins and display a concomitant decrease in virulence suggests that S-layer assembly is important for the pathogenesis of this anthrax-like disease and that S-layers and S-layer-associated proteins have many different functions during infection. One of these functions is the control of the chain length of vegetative forms which represents a mechanism for bacterial escape from opsonophagocytic killing. If bacillus chain length exceeds the size of macrophages or granulocytes, Bacteria cannot be engulfed (Wang et al., 2013).

Clostridium difficile is a frequent cause of severe, recurrent postantibiotic diarrhea and pseudomembranous colitis (Kelly & Lamont, 1998). The C. difficile S-layer is the predominant outer surface component which is involved in pathogen–host interactions critical to pathogenesis. S-layer proteins could mediate the binding to both the intestinal epithelial cells and some components of their extracellular matrix fibers, contributing to further tissue damage (Calabi et al., 2002; Cerqueti et al., 2002). Evidence was provided that the HMW subunit functions as an adhesin which mediates adherence of C. difficile to host cells (Calabi et al., 2002). Ausiello and coworkers demonstrated the ability of C. difficile S-layer proteins to modulate the function of human monocytes and dendritic cells (DC) and to induce inflammatory and regulatory cytokines (Ausiello et al., 2006). Thus, S-layer proteins may fine-tune the equilibrium of Th1/Th2 response and affect antibody responses. Host antibody response plays an important role in protection, in particular IgM anti-S-layer proteins have been associated with a reduced risk of recurrent C. difficile-associated diarrhea in humans (Drudy et al., 2004). In this context, a protective effect of anti-S-layer protein serum has also been observed in a lethal hamster challenge model. The potential mechanism of action of the antisera was shown to be through enhancement of C. difficile phagocytosis (O’Brien et al., 2005). Therefore, the possible use of S-layer proteins in a multicomponent vaccine against C. difficile infections for high-risk patients can be envisaged.

It is now evident that Lactobacilli over a long evolutionary period have colonized the mucosa of the upper gastrointestinal tract and the vagina coexisting in a mutualistic relationship with the host. With L. acidophilus NCFM, it was shown that the S-layer protein interacts with a major receptor on DC and that the bacterial cells regulate dendritic- and T-cell immune functions, suggesting that this probiotic bacterium could directly or indirectly interfere with pathogen-induced effects on the host immune system (Konstantinov et al., 2008). As individual strains of immunomodulatory probiotic Bacteria (e.g. dairy Propionibacteria) possess S-layers (Lortal et al., 1993), it will be interesting to study their importance in more detail (Foligné et al., 2010).

Campylobacter fetus, a spiral Gram-negative bacterium, is a recognized pathogen of cattle and sheep that can also infect humans (Guerrat et al., 1978; Smibert, 1978; Blaser, 1998; Thompson & Blaser, 2000). Campylobacter fetus may be either type A or type B based on serotype, lipopolysaccharide structure, and S-layer protein type (Dubreuil et al., 1990; Blaser et al., 1994; Dworkin et al., 1995). The S-layer...
proteins have been shown to play a critical role in C. fetus virulence by protecting the bacterium from phagocytosis and serum killing (Blaser et al., 1988). Graham and coworkers showed that attachment to extracellular matrix components (EMC) was neither correlated with S-layer expression nor with cell surface hydrophobicity (Graham et al., 2008). However, ligand immunoblots, identified the S-layer protein as a major site of fibronectin binding, and modified ECM binding assays revealed that soluble fibronectin significantly enhanced the attachment of S-layer-expressing C. fetus strains to other ECM components (Graham et al., 2008). For further information on S-layers related to pathogenicity, see section ‘S-layers for vaccine development’.

**S-Layers as molecular sieves and antifouling coating**

To determine the size of pores in S-layer lattices of different Bacillaceae, permeability studies were performed according to the space technique (Scherrer & Gerhard, 1971). For this purpose, native and glutaraldehyde-treated S-layer containers were prepared that resembled the shape of whole bacterial cells (Sára & Sleytr, 1987b). Native S-layer containers were composed of three adjacent layers namely the S-layer, the peptidoglycan-containing layer, and an inner S-layer attached to the inner face of the peptidoglycan layer. The latter was formed upon removal of the plasma membrane out of the pool of S-layer subunits originally entrapped in the peptidoglycan layer (Breitwieser et al., 1992). To distinguish between the molecular sieving properties between the S-layer and the peptidoglycan layer, the peptidoglycan layer was digested with lysozyme. The solutions selected for the molecular sieving measurements were sugars, proteins, and dextrins of increasing molecular weights. It was clearly demonstrated that the S-layer lattices allow free passage for molecules with a molecular weight of up to 30 kD and showed sharp exclusion limits between molecular weights of 30 and 45 kD, suggesting a limiting pore diameter in the range of 3–4.5 nm which resembles the pore dimensions determined by high-resolution transmission electron microscopy (TEM) and AFM (Messner et al., 2010; Pavkov-Keller et al., 2011). Moreover, of great relevance in these studies was the observation that the peptidoglycan layer does not limit the passage of molecules capable of penetrating the S-layer. This information on the structure and function of different S-layers of Bacillaceae makes it unlikely that their S-layers have the potential to function as an effective barrier against lysogenic enzymes (Sára & Sleytr, 1987b). Most important a great variety of permeability studies on S-layers from Bacillaceae demonstrated that the surface and pore areas of the protein meshwork have a very low tendency for unspecific adsorption of (macro)molecules (Sleytr et al., 1986; Sára & Sleytr, 1987c, 1988; Sára & Sleytr, 1993; Sára et al., 1993; Sára et al., 1996a). This characteristic of S-layers is seen essential for maintaining an unhindered exchange of nutrients and metabolites between the cell and its environment. In this context, it is also important to note that the S-layer in Gram-positive Bacteria masks the net negative charge of the peptidoglycan layer which significantly determines interactions between living cells and its environment. The information available even allows the assumption that the S-layer surface and the pore areas have excellent antifouling properties (Sára & Sleytr, 1987b; Picher et al., 2013). With S-layer carrying Lactobacillus strains, it could be demonstrated that variations in cell surface hydrophobicity and cell adhesion to surfaces may vary upon changes in pH and ionic strength of the environment (Vadillo-Rodriguez et al., 2004).

**S-Layers as adhesion zone for exoenzymes**

The bacterial cell wall plays a key role in the exchange of substrates between the bacterium and its surrounding environment. Because S-layer-carrying G. stearothermophilus strains produce large amounts of exoproteins with molecular weights above the exclusion limit of their S-layers, the role of these porous lattices with regard to exoprotein secretion and adhesion came under careful scrutiny. It was also suggested that S-layers from members of the family Bacillaceae could delineate a kind of periplasmic space in cell envelopes of Gram-positive organisms and consequently delay or control the release of exoenzymes (Graham et al., 1991; Breitwieser et al., 1992; Sturm et al., 1993). Binding to the cell surface has also been reported for exoenzymes and exoproteins including the outer layer proteins OlpA and OlpB of Clostridium thermocellum (Fujino et al., 1993; Salamitou et al., 1994a, b; Lemaire et al., 1995) and the extracellular enzymes xylanase XynA, pullulanase AmyB and polygalacturonate hydrolase PglA of Thermoaerobacterium thermosulfurigenes EM1 (Brechtl & Bahl, 1999; May et al., 2006). For the xylanase XynA, evidence could be provided that the C-terminally located SLH motifs (Engelhardt & Peters, 1998) are necessary to anchor the extracellular enzyme to the cell surface and that accessory cell wall polymers and not peptidoglycan functions as the adhesion component in the cell wall (Brechtl & Bahl, 1999).

First studies concerning the importance of the S-layer lattice with regard to exoprotein secretion were carried
out with the exoamylase-producing strain G. stearothermophilus DSM 2358 which indicated the putative role of the S-layer as a adhesion site for a high molecular mass amylase (HMMA; Egelseer et al., 1995). Affinity experiments strongly suggested the presence of a specific recognition mechanism between the amylase molecules and S-layer protein domains either exposed on the outermost surface or inside the pores (Egelseer et al., 1995). For further comparative studies, the closely related S-layer-carrying (S\(^+\)) strain G. stearothermophilus ATCC 12980 which is completely covered by the S-layer protein SbsC and the S-layer-deficient (S\(^-\)) variant thereof was selected as model system (Egelseer et al., 1996). On the genetic level, the S\(^+\) and the S\(^-\) strain showed similarity values of 100%, except that in the S\(^-\) variant, expression of the sbsC gene was found to be inhibited by the insertion of the bacterial insertion sequence (IS) element ISBst12 (Egelseer et al., 2000). On starch medium, both strains of G. stearothermophilus ATCC 12980 secreted two smaller amylases and one HMMA into the culture fluid (Egelseer et al., 1996), but only the latter also remained cell-associated. Using heterologously produced N- or C-terminally truncated SbsC forms and the native HMMA for affinity studies, it turned out that the N-terminal part of SbsC must comprise the binding region for the exoenzyme (Jarosch et al., 2001). After elucidation of the hmma gene sequence, the full-length rHMMA, N- or C-terminal rHMMA truncations, as well as C-terminal rHMMA fragments were heterologously produced (Ferner-Ortner-Bleckmann et al., 2009). The different rHMMA forms were used either for affinity studies with rSbsC, peptidoglycan-containing sacculi, and pure peptidoglycan devoid of SCWP, or for SPR studies using rSbsC\(_{31-443}\) (a truncated rSbsC form comprising the N-terminus) and isolated SCWP. On the basis of all available data, a specific binding region for each of the three cell wall components (rSbsC, SCWP, and peptidoglycan) could be identified in the C-terminal part of the rHMMA, representing the smallest regions necessary for interaction (Ferner-Ortner-Bleckmann et al., 2009).

For G. stearothermophilus wild-type strains, changing environmental conditions led to S-layer variant formation (Sára & Sleytr, 1994; Sára et al., 1996b; Egelseer et al., 2000; Scholz et al., 2000, 2001). During the oxygen-induced switch from the wild-type strain G. stearothermophilus PV72/p2 to the variant PV72/p2, not only the S-layer protein but also the type of SCWP was altered (Sára et al., 1996b). However, in all variants investigated so far, the peptidoglycan-chemotype remained constant. In order to adapt to any change in the composition of the cell wall induced by altered environmental conditions and variant formation, the HMMA evolved a multifunctional binding mechanism that provides the enzyme with a great flexibility.

**S-Layers as template for fine-grain mineralization and bioremediation**

S-layers are a very common surface structure in Bacteria including Cyanobacteria (Smarda et al., 2002). A unique ecological role could be demonstrated for the cyanobacterial S-layer of Synechococcus strain GL24. This bacterium was found to induce mineralization of fine-grain gypsum (CaSO\(_4\)-2H\(_2\)O) and calcite (CaCO\(_3\)) in a fresh water lake (Thompson & Ferris, 1990). The S-layer has a hexagonal monomer arrangement and provides regularly spaced, chemically identical nucleation sites for mineral growth (Schultze-Lam et al., 1992; Schultze-Lam & Beveridge, 1994a; Smarda et al., 2002). Mineral formation begins within the large holes of the array when Ca\(^{2+}\) binds to negatively charged sites on the S-layer protein and is joined by SO\(_4^{2-}\), initiating the formation of a mineral aggregate. Eventually, the S-layer becomes encrusted with mineral and is shed so that cells have a patchy appearance with respect to the location of mineralized portions of their surface. Shedding of S-layer material could be a common process of Bacteria to get rid of mineral deposits on their cell surface thereby maintaining basic vital processes such as growth and division as well as nutrient transport. The natural pH value of the lake (c. pH 7.9) promotes the formation of gypsum, but in the course of seasonal warming, further alkalization in the close microenvironment of each photosynthesizing cell pushes the solid mineral field toward the formation of stable calcite crystals in which the sulfate is replaced by carbonate. Although the involvement of cyanobacteria in the formation of calcium carbonate has been well established, microbial involvement in the formation of other carbonate minerals has not been extensively studied. In an experimental system, it could be demonstrated that Synechococcus mediates a similar sulfate-to-carbonate transformation when Sr\(^{2+}\) is the major divalent cation present, forming celestite and strontianite which were considered to be formed by abiotic mechanisms such as evaporation (Schultze-Lam & Beveridge, 1994b). Due to the difficulty of examining the process of calcite nucleation on natural matrices such as Synechococcus S-layers, a very recent review focused on studies of nucleation at carboxyl-terminated alkane thiol SAM surfaces on noble metal substrates. To some extent, these films provide a mimic of two key features of the Synechococcus S-layer because they are rich in carboxyl groups that can bind Ca\(^{2+}\) and they present an ordered array of such functional groups (De Yoreo et al., 2013). The ability to form fine-grain mineral sediments may be much more common among planktonic prokaryotes endowed with S-layers than can be imagined currently (Kling et al., 2011). Because prokaryotes exist since approximately 3.5 billion...
years, they could have had a major impact on global crust development.

Currently, there is much interest in the synthesis of inorganic materials using biomimetic approaches. Inspired by the process of biomineralization, the potential of S-layer proteins and their self-assembly products as catalysts, templates, and scaffolds for the generation of novel silica architectures was investigated (Göbel et al., 2010). For that purpose, the S-layer protein SbpA of L. sphaericus CCM 2177 was used as organic template for the generation of nanostructured silica. Using tetramethoxysilane (TMOS), TEM investigations showed the formation of a nanostructured silica network resembling the S-layer lattice. QCM-D measurements of silica adsorption demonstrated that a certain amount of negatively charged sites, such as phosphate molecules or activated carboxyl groups, significantly promote the deposition of silica on the S-layer (Göbel et al., 2010). Studying the formation of silicified S-layers may help to develop novel silicon-based materials with enhanced mechanical stability and optical properties (Schuster et al., 2013).

Another current approach considers the use of bacterial S-layers as a potential alternative for bioremediation processes of heavy metals in field. The S-layer of Bacillus sphaericus JG-A12, an isolate from a uranium mining waste pile in Germany, was shown to bind high amounts of toxic metals such as U, Cu, Pd(II), Pt(II), and Au(III) (Pollmann et al., 2006). Furthermore, Velásquez and coworkers determined the tolerance of different Colombian B. sphaericus native strains to different heavy metals and came to the conclusion that their S-layer proteins might have the ability to entrap metallic ions, either on living or dead cells (Velásquez & Dussan, 2009). In 2010, a recombinant bioremediation agent of high efficiency and low cost was developed by inserting a hexa-histidine peptide into a permissive site of the S-layer protein RsaA of the harmless, Gram-negative bacterium C. crescentus in order to remove cadmium from contaminated water samples (Patel et al., 2010). To summarize, these special capabilities of the bacterial cells and their S-layers are highly interesting for the clean-up of contaminated waste waters, for the recovery of precious metals from wastes of the electronic industry, as well as for the production of metal nanoparticles.

Applications

Isoporous ultrafiltration membranes

Information on either the mass distribution in S-layer lattices obtained by high-resolution electron microscopy or the ‘functional pore’ size derived from permeability studies led to the use of isoporous protein lattices for the production of ultrafiltration membranes with very accurate molecular weight cutoffs (Sára & Sleytr, 1986; Sára & Sleytr, 1987b; c; Sára et al., 1988b). S-layer ultrafiltration membranes (SUMs) were produced by depositing S-layer fragments as a coherent layer on microfiltration membranes. The mechanical and chemical stability of their composite structure is subsequently obtained by interand intramolecular cross-linking. The chemical and thermal resistance of these membranes was shown to be comparable to polyamide membranes. The uniformity of functional groups on both the surface and within the pore area of the S-layer lattice could be used for very accurate chemical modifications in the subnanometer range.

SUMs produced with S-layers from Bacillus or Geobacillus strains showed a molecular weight cutoff in the range of 30 to 40 kDa (Sára et al., 1996a; Sleytr et al., 2001). The flux of SUMs ranges from 150 to 250 L m⁻² h⁻¹ when measured at 0.2 MPa with water (Sára & Sleytr, 1988). Surface properties and molecular sieving as well as antifouling characteristics of SUMs were tuned by chemical modifications involving activation of carboxyl groups with carbodiimides and subsequently converting them with differently sized and/or charged nucleophiles (Küpcü et al., 1993; Weigert & Sára, 1995; Sleytr et al., 2001). In this way, depending on the specific separation processes, SUMs can be prepared with different net charges, hydrophilic or hydrophobic surface properties and separation characteristics. Most important for separation processes, in comparison with conventional ultrafiltration membranes produced by amorphous polymers, SUMs revealed an extremely low unspecific protein adsorption (membrane fouling) in buffer solutions. Because of their high stability under shear forces, SUMs have also a broad application potential as a matrix for immobilizing functional molecules (e.g. ligands, enzymes, antibodies and antigens; Weiner et al., 1994a, b; Sleytr et al., 2001, 2002; Sára et al., 2006a, b; Egelseer et al., 2010; Sleytr et al., 2011). More recently, SUMs have been used as supporting and stabilizing structures for functional lipid membranes (see section ‘S-layer supported functional lipid membranes’).

S-Layers as matrix for functional molecules and nanoparticles

Because S-layer lattices are composed of identical protein or glycoprotein species, functional sequences introduced either by chemical modification or genetic engineering must be aligned in exact positions and orientation down to the subnanometer scale (Sleytr et al., 1999, 2001, 2005; Sára et al., 2006a, b; Sleytr et al., 2007a; Egelseer et al., 2010; Sleytr et al., 2011, 2013).

Chemical modification and labeling experiments revealed that S-layer lattices possess a high density of functional
groups on the outermost surface. As in bacterial S-layers, the subunits are linked to each other and to the underlying cell envelope layer by noncovalent interactions, a stable immobilization matrix can only be obtained by cross-linking with glutaraldehyde or other intra- and intermolecular cross-linkers (Sára & Sleytr, 1989; Sára et al., 1993, 1996a). For immobilization of foreign, functional molecules such as enzymes, ligands, antigens, or antibodies, free carboxylic acid groups in the S-layer protein were activated with water-soluble carbodiimide which could then react with free amino groups of the macromolecules leading to stable peptide bonds between the S-layer matrix and the immobilized protein (Sára & Sleytr, 1989; Sleytr et al., 1993b; Weiner et al., 1994a; Küpcü et al., 1995b; Breitwieser et al., 1996; Küpcü et al., 1996). Independent of the type of S-layer protein originating from different Bacillaceae, large enzymes such as invertase, glucose oxidase, glucuronidase, or β-galactosidase formed a monolayer on the outer surface of the S-layer lattice (Neubauer et al., 1993, 1994, 1996; Sára et al., 1996a; Sleytr et al., 2001). The activity of smaller enzymes retained upon immobilization strongly depended on the molecular size of the enzyme, the morphological properties of the S-layer lattice as well as the applied immobilization procedure (Küpcü et al., 1995a). For enzymes such as β-glucosidase with a molecular size slightly above the pore size in the S-layer lattice, an activity loss could be prevented by introducing spacer molecules which increased the distance between the immobilized enzyme and the S-layer lattice (Küpcü et al., 1995b).

Furthermore, a universal biospecific matrix for immunoassays and dipsticks could be generated by immobilizing monolayers of either protein A or streptavidin onto SUMs (Breitwieser et al., 1996). SUM-based dipsticks were used for diagnosis of type I allergies, as well as for quantification of tissue type plasminogen activator (t-PA) and interleukin 8 (IL8; Sleytr & Sára, 1997; Breitwieser et al., 1998; Sleytr et al., 2004). Furthermore, the S-layer lattice was exploited as an immobilization matrix for a dipstick assay developed for prion diagnosis (Völkel et al., 2003). SUMs were also chosen as matrix for an amperometric glucose sensor using glucose oxidase as the biologically active component (Neubauer et al., 1993), and S-layer microparticles obtained by mechanical disruption of whole cells were used for the fabrication of a multienzyme biosensor for sucrose (Neubauer et al., 1994).

Just recently, a lab-on-a-chip containing embedded amperometric sensors that are coated with crystalline monolayers formed by the S-layer protein SbpA of L. sphæricus CCM 2177 was developed to provide a continuous, stable, reliable, and accurate detection of blood glucose (Picher et al., 2013). The key feature of this novel concept is the integration of a uniform bioactive S-layer with improved antifouling properties over conventional antifouling strategies that are capable of preventing blood coagulation along ‘foreign’ lab-on-a-chip surfaces such as glass, polydimethylsiloxane, and metal electrodes. This novel combination of biologically derived nanostructured surfaces with microchip technology constitutes a powerful new tool for multiplexed analysis of complex samples.

Several studies already demonstrated that preformed nanoparticles can be bound in regular distribution on native S-layers (Hall et al., 2001; Bergkvist et al., 2004; Györvary et al., 2004). The pattern of bound molecules and nanoparticles reflected the size of the morphological units, the lattice symmetry, and the physicochemical properties of the array. Using electron microscopical methods, the distribution of net negatively charged domains on S-layers could be visualized after labeling with the positively charged topographical marker polycationic ferritin (Messner et al., 1986b; Sleytr et al., 2001).

Due to the promising results obtained with native S-layers as immobilization matrix, genetic engineering of S-layer proteins was envisaged. On the basis of recrystallization studies and surface accessibility screens with various genetically produced N- and/or C-terminally truncated forms, several bacterial S-layer proteins were selected as fusion partner for the construction of chimeric S-layer proteins. It turned out that S-layer proteins are capable of tolerating fusions with foreign proteins or domains that have never participated in lattice formation while retaining the ability to assemble into geometrically highly defined layers. The general applicability of the ‘S-layer tag’ to any fusion partner led to a high flexibility for variation of the functional groups. To date, a great variety of functional S-layer fusion proteins was cloned and heterologously expressed in E. coli or used for surface display after homologous expression (Engels et al., 2010; Ilk et al., 2011a; Sleytr et al., 2011; Table 2). Using TEM and AFM as well as functional tests, it could be demonstrated that the recrystallization properties conferred by the S-layer protein moiety as well as the functionalities of the fused peptide sequences were retained in all S-layer fusion proteins. Moreover, functional proteins maintain their functionality much better on the S-layer protein matrix in comparison when being directly attached (immobilized) to solid supports.

S-layer fusion proteins incorporating either the sequence of the hypervariable region of heavy chain camel antibodies recognizing lysozyme or the prostate-specific antigen (PSA; Pleschberger et al., 2003, 2004), two copies of the Fc-binding Z-domain, a synthetic analogue of the B-domain of Protein A (Völlenkle et al., 2004), the major birch pollen allergen (Bet v1; Breitwieser et al., 2002; Ilk et al., 2002), fluorescent proteins (Ilk et al., 2004; Kainz et al., 2010a, b), core streptavidin (Moll et al., 2002; Huber et al., 2006a, b), a C-terminally fused cysteine residue for patterning of nanoparticles (Badelt-Lichtblau et al., 2009), or
monomeric and multimeric enzymes from extremophiles (Schäffer et al., 2007; Tschiggerl et al., 2008b; Ferner-Ortner-Bleckmann et al., 2013) were successfully recrystallized on various solid supports (e.g. gold chips, silicon wafers, polystyrene or magnetic beads) or on liposomes (Table 2 and Figs 4 and 5).

Based on the demonstrated suitability of the S-layer protein self-assembly system for covalent enzyme immobilization, genetic approaches were pursued to construct fusion proteins comprising S-layer proteins of Bacillaceae and enzymes from extremophiles for the development of novel biocatalysts (Schäffer et al., 2007; Tschiggerl et al., 2008b; Ferner-Ortner-Bleckmann et al., 2011). Significant advantages for enzyme immobilization by the S-layer self-assembly system over processes based on random immobilization of sole enzymes include the requirement of only a simple, one-step incubation process for site-directed immobilization without preceding surface activation of the support. Moreover, the provision of a cushion for the enzyme through the S-layer moiety of the fusion protein prevents denaturation and consequently loss of enzyme activity upon immobilization. In a recent study, for the first time, self-assembling biocatalysts, consisting of S-layer lattices exhibiting surface exposed active multimeric extremozymes, were produced (Fig. 5; Ferner-Ortner-Bleckmann et al., 2013). The challenging step forward was to use enzymes of extremophiles which are active only in the multimeric state. For proof of concept, the tetrameric enzyme xylose isomerase and the trimeric enzyme carbonic anhydrase were selected and fused via a peptide linker to the C-terminal end of the S-layer protein SbpA of L. sphaericus CCM 2177. The study demonstrated that the outstanding robustness and high stability of multimeric extremozymes could be combined with the unique lattice forming capability and periodicity of bacterial S-layers, thereby providing a matrix for a most accurate spatial presentation of the multimeric enzymes (Ferner-Ortner-Bleckmann et al., 2013).

The design of S-layer/extremozyme fusion proteins was based on a rather similar approach chosen for the construction of S-layer/streptavidin fusion proteins allowing to arrange any biotinylated target into the regular arrays formed by the S-layer (Moll et al., 2002; Huber et al., 2006a, b). Core streptavidin was either fused to N- or C-terminal positions of S-layer proteins. As biologically active streptavidin occurs as tetramer, functional heterotetramers consisting of one chain fusion protein and three chains of core streptavidin were prepared by applying a special refolding procedure (Moll et al., 2002). The lattice formed by the S-layer/streptavidin fusion proteins displayed streptavidin in defined repetitive spacing, capable of binding biotinylated proteins, in particular ferritin (Fig. 5b; Moll et al., 2002).

In a different approach, functionalized monomolecular S-layer lattices formed by the S-layer fusion protein rSbpA/STII/Cys exhibiting highly accessible cysteine residues in a well-defined arrangement on the surface were utilized for the template-assisted patterning of gold nanoparticles (Badelt-Lichtblau et al., 2009).

S-Layers for vaccine development

As surface components frequently mediate specific interactions of a pathogen with its host organism, especially S-layers of pathogenic strains are expected to have an important role in virulence (see also section ‘S-layers related to pathogenicity’). For that purpose, S-layer proteins are prime candidates for vaccine development. To date, current experiments focus on the use of S-layer proteins as attenuated pathogens, as antigen/hapten carrier, as adjuvants, or as part of vaccination vesicles (Sleytr et al., 1991; Messner et al., 1996; Sleytr et al., 2002).

Because a reproducible immobilization of peptide epitopes to common carriers which were used as monomers in solution or as dispersions of unstructured aggregates on aluminum salts could not be achieved (Brown et al., 1993; Powell & Newman, 1995), the use of regularly structured S-layer self-assembly products as immobilization matrices was envisaged. Therefore, several conjugate vaccines with S-layer (glyco)proteins and carbohydrate antigens (poly- and oligosaccharides), hapten or the recombinant birch pollen allergen Bet v1 were produced which showed promising results in vaccination trials (Sleytr et al., 1989; Messner et al., 1992; Malcolm et al., 1993a, b; Smith et al., 1993; Jahn-Schmid et al., 1996a, b; Messner et al., 1996; Jahn-Schmid et al., 1997).

In earlier studies, the crystalline surface-layer glycoproteins of T. thermohydrosulfuricus L111–69, G. stearothermophilus NRS 2004/3a and P. alvei CCM 2051 were used for immobilization of spacer-linked blood group A-trisaccharide and of the spacer-linked, tumor-associated T-disaccharide (Messner et al., 1992). The haptenes were immobilized to either the protein moiety or the glycan chains of the respective S-layer glycoproteins. The resulting conjugates were useful for assessing the application potential of haptenated surface-layer preparations as carrier/adjuvants for the induction of immunity to poorly immunogenic molecules (Messner et al., 1992, 1996).

Immunization of mice with conjugates of oligosacchride hapten and crystalline S-layers primed the animals for a strong, hapten-specific, delayed-type hypersensitivity (DTH) response (Smith et al., 1993). Most important, S-layer conjugates also elicited strong antihapten DTH responses when administered by an oral/nasal route. Apparently, the natural assembly of S-layer proteins into
large, two-dimensional arrays endows them with intrinsic adjuvant properties (Smith et al., 1993).

First studies concerning the applicability of S-layers as vaccine carrier for treatment of type I allergy were carried out using native or cross-linked S-layer self-assembly products and cell wall preparations from L. sphaericus CCM 2177 as well as T. thermohydrosulfuricus L111–69 and L110–69 for immobilization of recombinant major birch pollen allergen Bet v 1 (Jahn-Schmid et al., 1996b). Stimulation of human allergen-specific Th2 lymphocytes with S-layer-conjugated Bet v 1 led to a modulation of the cytokine production pattern from Th2 to Th0/Th1, indicating that S-layers may be suitable carriers for immunotherapeutical vaccines for type 1 hypersensitivity. In a subsequent study, the adjuvant effect of S-layer proteins mediated by IL-12 was demonstrated (Jahn-Schmid et al., 1997). In cultures of peripheral blood mononuclear cells, both S-layer protein and S-layer/Bet v 1 conjugate (but not rBet v 1) stimulated the production of high levels of IL-12, a pivotal mediator of Th1 responses (Jahn-Schmid et al., 1997).

In the following years, chemical coupling procedures were replaced by genetic fusion of the major birch pollen allergen Bet v 1 to bacterial S-layer proteins resulting in recombinant fusion proteins exhibiting reduced allergenicity as well as immunomodulatory capacity (Breitwieser et al., 2002; Ilk et al., 2002; Bohle et al., 2004). This was exemplified by two S-layer/allergen fusion proteins, rSbpA/Bet v 1 and rSbsC/Bet v 1, carrying Bet v 1 at the C-terminus (Breitwieser et al., 2002; Ilk et al., 2002). Immunological studies showed that both fusion proteins displayed strongly reduced IgE binding capacity compared with free rBet v 1 and promoted the induction of allergen-specific Th0/1 cells and regulatory T cells (Bohle et al., 2004; Gerstmayr et al., 2007, 2009). For first studies, expression of the S-layer/allergen fusion proteins was carried out in the Gram-negative expression host E. coli which had to be followed by a very material and time consuming purification procedure to remove the associated endotoxin. In a more recent study, Bacillus subtilis 1012, a Gram-positive, nonpathogenic organism with naturally high secretory capacity, was chosen as host for expression of the pyrogen-free recombinant S-layer/allergen fusion protein rSbpA/Bet v 1 (Ilk et al., 2011b).

Structural and immunological investigations of the obtained fusion protein revealed that rSbpA/Bet v 1 was endotoxin-free and showed excellent recrystallization properties and immune reactivity. To conclude, for the first time, a pyrogen-free recombinant S-layer/allergen fusion protein required for vaccine development was produced using a Gram-positive expression system based on B. subtilis 1012 (Ilk et al., 2011b).

To summarize, S-layer carrier conjugates are superior vaccine carriers because (1) they elicit DTH and immuno-protective antibody responses without the use of extraneous adjuvants, (2) they can be administered by several different immunization routes (intramuscular, subcutaneous, nasal/oral), and they are immunological unique, which means that antibody and delayed-type hypersensitivity responses to each S-layer are specific and not cross-reactive (Malcolm et al., 1993b).

The mechanical and thermal stability of S-layer-coated liposomes (Küpcü et al., 1998; Hianik et al., 1999; Mader et al., 1999) and the possibility for immobilization or entrapping biologically active molecules (Küpcü et al., 1995a; Mader et al., 2000; Krivanek et al., 2002) introduced a broad application potential, particularly as carrier and/or drug delivery and drug-targeting systems in or gene therapy. A very recent study described a novel nanocarrier system comprising lipidic emulsomes and S-layer (fusion) proteins as functionalizing tools coating the surface (Ucisik et al., 2013b). In vitro cell culture studies showed that S-layer coated emulsomes can be taken up by human liver carcinoma cells (HepG2) without any significant cytotoxicity. S-layer coating led to a change in the zeta potential of the emulsomes from positive to negative, thus protecting the cell from oxidative stress and cell membrane damage. By combining the high drug loading capacity of emulsomes with recombinant S-layer technology, new applications for these emulsomes in nanomedicine, especially for drug delivery and targeting, can be envisaged (Ucisik et al., 2013a, b).

Bacillus anthracis spores germinate to vegetative forms in host cells and produce fatal toxins. As previously updated (see section ‘S-layers related to pathogenicity’), the S-layer of B. anthracis is composed of two proteins, EA1 and Sap which comprise 5–10% of total cellular protein (Etienne-Toumelin et al., 1995). In a former study, a recombinant B. anthracis strain was constructed by integrating into the chromosome a translational fusion harboring the DNA fragments encoding the SLH domain of EA1 and tetanus toxin fragment C (ToxC) of Clostridium tetani. The immune response to ToxC was sufficient to protect mice against tetanus toxin challenge and could be tested for the development of new live veterinary vaccines (Mesnage et al., 1999c). In a very recent study, the protective effect of EA1 against anthrax was investigated (Uchida et al., 2012). For that purpose, mice were intranasally immunized with recombinant EA1, followed by a lethal challenge of B. anthracis spores. It could be demonstrated that immunization with EA1 greatly reduced the number of bacteria in infected organs and protected the mice from lethal infection, thus suggesting that EA1 is a novel candidate for an anthrax vaccine.

The display of heterologous proteins on the cell surface of lactic acid bacteria (LAB) is an exciting and emerging research area that holds great promise for the
developments of live vaccine delivery system. In this context, the development of live mucosal vaccines using *Lactobacillus* strains carrying S-layers composed of hybrid proteins on their surface is of great interest (Hynonen & Palva, 2013). Small model peptides have already been displayed in each monomer of the S-layer of *L. brevis* ATCC 8287 and *L. acidophilus* ATCC 4356 by chromosomal integration based on homologous recombination (Avall-Jääskeläinen et al., 2002; Smit et al., 2002). A recent study describes a novel characteristic of the S-layer of *L. acidophilus* ATCC 4365, a GRAS status protein, because it contributes to the pathogen exclusion reported for this probiotic strain (Martínez et al., 2012). The S-layer protein of this strain was shown to bind electrostatically to dendritic cell-specific ICAM-3-grabbing nonintegrin (DCSIGN), a cell surface adhesion factor that enhances viral entry of several virus families including HIV type 1, hepatitis C virus, Ebola virus, cytomegalovirus, Dengue virus, and SARS coronavirus and may therefore be applied as novel antiviral agent (Martínez et al., 2012).

One of the greatest obstacles in developing an effective vaccine against *Aeromonas hydrophila*, an important fish pathogen in aquaculture systems, is its high heterogeneity in nature (Poobalane et al., 2010). The results of the study suggested that the recombinant S-layer protein of *A. hydrophila* could be useful as a vaccine antigen to protect fish against different isolates of this pathogenic bacterium.

The S-layer proteins of *C. difficile* have been shown to be involved in gut colonization and in the adhesion process to the intestinal mucosa. Some years ago, O’Brien and coworkers showed that a passive immunization using anti-S-layer protein antibodies significantly delayed the progress of *C. difficile* infection in a lethal hamster challenge model (O’Brien et al., 2005). In a subsequent study, *C. difficile* S-layer proteins were tested as a vaccine component in a series of immunization and challenge experiments with hamsters. However, none of the regimes tested conferred complete protection of animals and antibody stimulation was variable and generally modest or poor (Ni Eidhin et al., 2008). In a recent, novel approach, the *C. difficile* protease Cwp84, found to be associated with the S-layer proteins, was evaluated as a vaccine antigen. Hamster immunization studies demonstrated that Cwp84 is an attractive component for inclusion in a vaccine to reduce *C. difficile* intestinal colonization in humans, which in turn may diminish the risk of *C. difficile* infection (Pechine et al., 2011).

**S-layer supported functional lipid membranes**

The building principle of S-layer supported lipid membranes (SsLMs) is copied from the supramolecular cell envelope structure of *Archaea* (Fig. 2a and b). It is assumed that the cell envelope structure of *Archaea* is a key prerequisite for these organisms to be able to dwell under extreme environmental conditions such as temperatures up to 120 °C, pH down to 0, high hydrostatic pressure, or high salt concentrations (De Rosa, 1996; Stetter, 1999; Hanford & Peeples, 2002; Albers & Meyer, 2011). Hence, S-layers must therefore integrate the basic functions of mechanical and osmotic cell stabilization (Engelhardt, 2007). As suitable methods for disintegration of archaean S-layer protein lattices and their reassembly into monomolecular arrays on lipid films are not yet available, S-layer proteins from Gram-positive *Bacteria* are used for the generation of SsLMs (Schuster et al., 2008; Schuster & Sleytr, 2009b; Schuster et al., 2010; Sleytr et al., 2011).

Moreover, S-layer proteins or glycoproteins can be utilized as biofunctional surfaces (Küpüçü et al., 1995a; Schuster et al., 2008; Schuster & Sleytr, 2009b; Sleytr & Messner, 2009; Sleytr et al., 2010, 2011; Uçisik et al., 2013b). Disregarding emulsomes, these model lipid membranes consist either of an artificial phospholipid bilayer or a tetratherelipid monolayer which replaces the cytoplasmic membrane and a closely associated bacterial S-layer lattice (Fig. 6). In addition, a second S-layer acting as protective molecular sieve and further stabilizing scaffold and antifouling layer can be recrystallized on the top of the previously generated SsLM (Fig. 6). These features make S-layer lattices to unique supporting architectures resulting in lipid membranes with nanopatterned fluidity and considerably extended longevity (Schuster & Sleytr, 2000; Gufler et al., 2004; Schuster et al., 2004; Schuster & Sleytr, 2006; Schuster & Sleytr, 2009b, 2010; Schuster et al., 2011).
S-layer (glyco)proteins

Schuster et al., 2008; Schuster & Sleytr, 2009b; Schuster et al., 2010).

SsLMs have attracted lively interest because of three main reasons: First, they constitute a versatile biomimetic model to study the characteristics of the archaeal cell envelope by a broad arsenal of surface-sensitive techniques and sophisticated microscopical methods. Second, with SsLMs, surfaces with new properties such as an anti-fouling characteristics for application in material science and nanomedicine can be generated. Third, SsLMs provide an amphiphilic matrix for reconstitution of (trans) membrane proteins (MPs). Hence, SsLMs may be utilized in basic research to characterize MPs. This is of paramount importance as the results of genome mapping showed that approximately one-third of all genes of an organism encode for MPs (Gerstein & Hegyi, 1998; Galdiero et al., 2007, 2010) which are key factors in cell’s metabolism and thus, in health and disease (Viviani et al., 2007). Moreover, MPs constitute preferred targets for pharmaceuticals (at present more than 60% of consumed drugs; Ellis & Smith, 2004). Thus, SsLMs received widespread recognition in drug discovery and protein–ligand screening. In the future, the increased knowledge on MPs might allow to rebuild sensory organs, for example an artificial nose, and are of high interest for the development of biosensors based on MPs (Reimhult & Kumar, 2008; Demarche et al., 2011; Srinivasan & Kumar, 2012; Tiefenauer & Demarche, 2012).

S-layer protein–lipid interaction

Formation of S-layer lattices covering the entire area of lipid films has been observed on zwitterionic phospholipids such as phosphatidyl cholines and in particular phosphatidyl ethanolamines, but not on negatively charged phospholipids (Fig. 4; Pum et al., 1993; Pum & Sleytr, 1994; Diederich et al., 1996; Wetzer et al., 1998). Electrostatic interaction has been figured out to exist between exposed carboxyl groups on the S-layer lattice and zwitterionic or positively charged lipid head groups (Küpçü et al., 1995a; Hirn et al., 1999; Schuster et al., 1999) At least two to three contact points between the S-layer protein and the attached lipid film have been identified (Fig. 6; Wetzer et al., 1998). Hence, < 5% of the lipid molecules of the adjacent monolayer are anchored to these contact points (protein domains) on the S-layer protein. The remaining ≥ 95% lipid molecules may diffuse freely within the membrane between the pillars consisting of anchored lipid molecules (Schuster, 2005; Schuster & Sleytr, 2005, 2006, 2009b). These nanopatterned lipid membranes are also referred to as ‘semi-fluid membranes’ (Pum & Sleytr, 1994) because of its widely retained fluid behavior (Györvary et al., 1999; Hirn et al., 1999). Most important, although peptide side groups of the S-layer protein interpenetrate the phospholipid head group regions almost in its entire depth, no impact on the hydrophobic lipid alkyl chains has been observed (Schuster et al., 1998a, b; Weygand et al., 1999, 2000, 2002; Schuster et al., 2003a). To enhance the stability of the composite SsLMs, head groups of phospholipids have been covalently linked to the S-layer lattice (Schrems et al., 2011a, b). Interestingly, it became evident that in nature, archaeal S-layer proteins are targeted for post-translational modifications such as the addition of a lipid (Kikuchi et al., 1999; Konrad & Eichler, 2002; Szabo & Pohlschroder, 2012; Abdul Halim et al., 2013). Hence, our approach to link lipids covalently to S-layer proteins is a biomimetic one as lipid modifications of S-layer glycoproteins are a general property of, for example, halo- philic Archaea. Lipid modification of the S-layer glycoproteins takes place on the external cell surface that is following protein translocation across the membrane (Konrad & Eichler, 2002; Abdul Halim et al., 2013; Kandiba et al., 2013).

Planar lipid membranes

The mechanical properties of free-standing SsLMs were investigated by applying a hydrostatic pressure (Schuster & Sleytr, 2002a). SsLMs revealed a higher structural integrity when the pressure was applied from the S-layer faced side compared with plain bilayer lipid membranes (BLMs). This result supports the ‘osmoprotecting effect’, one putative biological function of S-layer lattices in Archaea (Engelhardt, 2007).

To increase the mechanical stability and longevity in particular with reconstituted peptides or MPs, the BLMs were attached to porous or solid supports to improve their practical applicability (Castellana & Cremer, 2006; Chan & Boxer, 2007; Knoll et al., 2008; Reimhult & Kumar, 2008; Steinem & Janshoff, 2010).

At solid-supported lipid membranes, the task of the S-layer lattice is, beside to act as stabilizing scaffold, to provide a defined tether layer to decouple the BLM from the (inorganic) support and to generate an ionic reservoir necessary for electrochemical measurements (Schuster & Sleytr, 2000, 2009b). Moreover, the reservoir may be tailored using a mixture from full-length and truncated S-layer proteins (Schuster & Sleytr, 2009a) or by the self-assembly of a thiolated SCWP layer on the gold electrode prior S-layer protein recrystallization (Sleytr et al., 2000, 2006; Schuster & Sleytr, 2009b). A very important feature of supported lipid membranes is to preserve a high degree of mobility of the lipid molecules within the membrane (fluidity) and at the same time exhibiting sound condition of the overall membrane structure (longevity).
Györvary and coworkers compared the mobility of lipid molecules of SsLMs to silane- and dextran-supported phospholipid mono- and bilayers, respectively. Most probably due to the repetitive, nanopatterned local interactions of the S-layer lattice with the lipid head groups, the fluidity of lipids was highest in SsLMs (Györvary et al., 1999). Moreover, the longevity of a tetraether lipid monolayer sandwiched by an S-layer lattice on each side revealed in comparison with other approaches (e.g. tethered membranes, polymer cushion), an exceptional long-term robustness of approximately 1 week (Schuster, 2005; Schuster & Sleytr, 2005, 2006, 2009b). This finding reflects also the optimization of the archaeal cell envelope structure by nature over billions of years.

Lipid membranes generated on a porous support combine the advantage of easy manual handling, individual excess to both membrane surfaces, and possessing an essentially unlimited ionic reservoir on each side of the BLM. The surface properties of porous supports, such as roughness or great variations in pore size, have significantly impaired the stability of attached BLMs (Nikoletis et al., 1999). A straightforward approach is the use of SUMs (see section ‘Isoporous ultrafiltration membranes’) with the S-layer as stabilizing and smoothing biomimetic layer between the lipid membrane and the porous support (Schuster et al., 2001, 2003b; Gufler et al., 2004). Composite SUM-supported BLMs were found to be highly isolating structures with a life time of up to 17 h. The life time could be even significantly increased to approximately 1 day forming an S-layer–lipid membrane–S-layer sandwich-like structure on SUMs (Fig. 6; Schuster et al., 2001, 2003b; Gufler et al., 2004). Hence, the nanopatterned anchoring of lipids is a promising strategy for generating stable and fluid supported lipid membranes.

The most challenging property of model lipid membranes is the feasibility to incorporate membrane-active (antimicrobial) peptides (AMPs; Hancock & Chapple, 1999; Wimley & Hristova, 2011) and more important, the reconstitution of (complex) integral MPs in a functional state (Demarche et al., 2011; Tiefenauer & Demarche, 2012). Table 3 summarizes the functional incorporated AMPs in SsLMs resting on gold electrodes or SUMs (Schuster et al., 1998a, 2003b; Gufler et al., 2004; Schrems et al., 2013).

A recent study showed that the S-layer lattice of C. crescentus hindered positively charged AMPs in reaching its outer membrane (de la Fuente-Núñez et al., 2012). Thus, the protection against AMPs was proposed to be one biological function of S-layer lattices. The staphylococcal proteinaceous α-hemolysin (αHL; $M_w = 33$ kDa) formed lytic pores when added to the lipid-exposed side of an SsLM. However, no pore formation was detected upon addition of αHL monomers to the S-layer-faced side of this SsLM. Therefore, the intrinsic molecular sieving properties of the S-layer lattice did not allow passage of αHL monomers through the S-layer pores toward the lipid membrane which is of biological significance in competitive habitats (Schuster et al., 1998b). In addition, this result confirmed the existence of a closed S-layer lattice without any defects tightly attached to the BLM. Notably, even single pore recordings have been performed.

| Table 3. Summary of membrane-active peptides and transmembrane proteins reconstituted in S-layer supported lipid membranes |
| Membrane-active peptide | Source | Remarks/References | Transmembrane protein | Source | Remarks/References |
|-------------------------|--------|--------------------|-----------------------|--------|--------------------|
| Gramicidin A (gA)       | Bacillus brevis | Linear pentadeca peptide (Schuster et al., 2003b) | α-Hemolysin (αHL) | Exotoxin from Staphylococcus aureus | Pore-forming; homohexamer (Schuster et al., 1998a, 2001; Schuster & Sleytr, 2002b) |
| Alamethicin (Ala)       | Trichoderma viride | Linear, 20 amino acids (Gufler et al., 2004) | Ryanodine receptor 1 (RyR1) | Skeletal muscle cells | Ca$^{2+}$-release channel; homotetramer (Larisch, 2012) |
| Valinomycin (Val)       | Several Streptomyces strains, for example S. tsusimaensis and S. fulvissimus | Cyclic dodecadepsipeptide (Schuster et al., 1998b; Gufler et al., 2004) | Nicotinic acetylcholine receptor (nAChR) | Plasma membranes of neurons; on postsynaptic side of the neuromuscular junction | Ligand gated ion channel, 5 subunits (Keppelinger, 2007; Kiene, 2011) |
| Peptidyl-glycine-Valinomycin (PGLa) analogue | Synthesized via protein chemistry | 20 amino acid; analogue negatively charged (Schrems et al., 2013) | Voltage-dependent anion channel (VDAC) | Located on the outer mitochondrial membrane; produced by cell-free expression Segment forms ion-conducting channel; see nAChR | Voltage gated; porin ion channel monomeric but can cluster (S. Damiati, pers. commun.) |
with αHL-reconstituted SsLMs (Schuster et al., 2001; Schuster & Sleytr, 2002b).

Sulfolobicsins, produced by several Sulfolobus strains, are an example for the interaction of proteinaceous toxins with S-layer proteins in nature. These toxins were found to be associated with cell-derived S-layer-coated vesicles resulting in no release of sulfolobicsins in soluble form into the environment. These enzyme-containing vesicles of Sulfolobus islandicus, for example, were shown to kill cells of other strains of the same species (Prangishvili et al., 2000; O’Connor & Shand, 2002).

Table 3 summarizes the pore-forming αHL (Schuster et al., 1998b; Schuster & Sleytr, 2002b) and channel-forming MPs (Keizer et al., 2007; Keplinger, 2007; Keizer et al., 2008; Kiene, 2011; Larisch, 2012) which have been functionally reconstituted in SsLMs resting on gold electrodes or SUMs. To sum up, the ability to act as biomimetic spacer and scaffold for composite lipid membranes with nonintrusive character on ion channel activity make S-layer proteins attractive for biosensor applications, especially those that enhance the stability of BLMs beyond the use of tethers or polymer supports (Bayley & Cremer, 2001; Sugawara & Hirano, 2005). In future, the ability to reconstitute integral membrane proteins in defined structures on, for example, sensor surfaces is one of the most important concerns in designing biomimetic sensing devices (Nikolelis et al., 1999; Trojanowicz, 2001; Demarche et al., 2011; Jackman et al., 2012; Tiefenauer & Demarche, 2012; Schuster & Sleytr, 2013; Sleytr et al., 2013).

S-layer coated liposomes and emulsomes

Unilamellar liposomes are artificially prepared spherical containers comprising of a phospholipid bilayer shell and an aqueous core (Bangham et al., 1965; Tien & Ottowa-Leitmannova, 2000; Cui et al., 2006). In the latter, biologically active molecules such as hydrophilic drugs can be stored and transported, whereas the lipidic shell can be loaded with hydrophobic drugs. Emulsomes, however, are spherical systems with a solid fat core surrounded by phospholipid mono- and bilayer(s) (Fig. 4; Amselem et al., 1994; Vyas et al., 2006). Hence, emulsomes show a much higher loading capacity for lipophilic drug molecules such as curcumin for targeted drug delivery to fight against cancer and other diseases (Andresen et al., 2005; Vyas et al., 2006; Ucisik et al., 2013a, b). Furthermore, S-layer lattices as envelope structure covering the spherical containers (Fig. 4) constitute biomimetic ‘artificial virus-like particles’ enabling both stabilization of the nanocarriers and presenting addressor molecules in a highly defined orientation and special distribution (Sleytr et al., 2010, 2013).

When recrystallizing isolated S-layer subunits of Bacillaceae such as G. stearothermophilus PV72/p2 on positively charged liposomes, the S-layer is attached by its inner face (bearing a net negative charge) in an orientation identical to the lattice on intact cells (Küpçü et al., 1995a; Mader et al., 1999). Coating of positively charged liposomes or emulsomes with bacterial S-layer (fusion) proteins resulted in inversion of the zeta potential from an initially positive value to a negative one (Mader et al., 1999; Ucisik et al., 2013b). A similar behavior was observed for liposomes coated with S-layer proteins from Lactobacilli (Hollmann et al., 2007).

Mader and coworkers demonstrated a much higher mechanical (shear forces, ultrasonication) and thermal stability for S-layer-coated liposomes compared with plain ones (Küpçü et al., 1998; Mader et al., 1999). This finding supports the notion of the high stability of archaeal cell envelope structures. Moreover, to enhance the stability, the S-layer protein on the liposome can be cross-linked (Schuster et al., 2006). In addition, cross-linking can also be utilized for covalent attachment of biologically relevant macromolecules (Sleytr et al., 2005, 2007a, 2010, 2013). In turn, a layer of intact liposomes can also be reversibly tethered via the specific nickel–His-tag linkage on an S-layer lattice (Keplinger et al., 2009).

S-layer-coated liposomes constitute a versatile matrix for the covalent binding of macromolecules (Küpçü et al., 1995a). Biotinylation of S-layer-coated liposomes resulted in two accessible biotin residues per S-layer subunit for subsequent streptavidin binding (Mader et al., 2000). By this approach, biotinylated ferritin and biotinylated anti-human IgG were attached via streptavidin to S-layer-coated liposomes. The biological activity of bound anti-human IgG was confirmed by ELISA (Mader et al., 2000) and by measuring changes in ultrasound velocity (Krivanek et al., 2002). Moreover, S-layer/streptavidin fusion proteins have been constructed in order to bind up to three biotinylated biomolecules per S-layer subunit in a highly defined orientation and position (Moll et al., 2002).

An interesting approach is the recrystallization of the S-layer-enhanced green fluorescent protein (EGFP) fusion protein on liposomes (Ilk et al., 2004). By this means, the uptake via endocytosis of S-layer/EGFP fusion protein coated liposomes into eukaryotic cells such as HeLa cells could be visualized by the intrinsic EGFP fluorescence. The most interesting advantage can be seen in co-recrystallization of, for example, S-layer/EGFP and S-layer/streptavidin fusion proteins on the same liposome. The uptake of these specially coated liposomes by target cells and the functionality of transported drugs could be investigated simultaneously without the need of any additional labels.
Likewise on liposomes, several wild-type, recombinant, and S-layer fusion proteins formed a closed S-layer lattice covering the entire surface of emulsomes composed of a solid tripalmitin core and a phospholipid shell (UCISIK et al., 2013b). In vitro cell culture studies revealed that S-layer coated emulsomes can be up taken by HepG2 without showing any significant cytotoxicity. The utilization of S-layer fusion proteins equipped in a nanopatterned fashion by identical or diverse functions may lead to attractive nanobiotechnological and nanomedicinal applications, particularly as drug-targeting and delivery systems, as artificial virus envelopes in, for example, medicinal applications and in gene therapy (MADER et al., 2000; PUM et al., 2006; SCHUSTER & SLEYTR, 2009b; UCISIK et al., 2013b). Finally, these biomimetic approaches are exciting examples for synthetic biology mimicking structural and functional aspects of many bacterial and archaean cell envelopes having an S-layer lattice as outermost cell wall component (SLEYTR & BEVERIDGE, 1999; SLEYTR et al., 2002, 2013).

Conclusions and perspectives

Regular arrays of macromolecules were first observed about 60 years ago in electron micrographs of prokaryotic cell wall fragments and were viewed originally as a curiosity. S-layers are now recognized as one of the most common envelope surface structures in Archaea and Bacteria. The widespread occurrence and the high physiological expense of S-layers raise the question of what selection advantage S-layer carrying organisms would have in their natural and frequently highly competitive habitats. In this context, it is interesting to remember that under optimal growth conditions for Bacteria in continuous laboratory cultures, S-layer-deficient mutants, or variants possessing S-layers composed of (glyco)protein subunits with lower molecular mass, frequently outgrow wild-type strains (GRUBER & SLEYTR, 1991; MESSNER & SLEYTR, 1992; SÁRA et al., 1996b; SLEYTR & BEVERIDGE, 1999; EGELSEER et al., 2000). Moreover, if present, S-layers are also part of a more complex supramolecular envelope structure and consequently in functional terms must not be considered as isolated protein lattice. Defined domains of S-layer proteins have been identified as being involved in specific interactions with supporting cell envelope components. As S-layers are highly porous structures, some components of the supporting envelope layers such as side chains of lipopolysaccharides (in Gram-negative bacteria; CHART et al., 1984), or SCWPs (in Gram-positive bacteria), may protrude through the protein meshwork. The latter may explain the phenomenon that in Bacillaceae, the expression of a different S-layer protein on the cell surface is accompanied by a change in the chemical composition of the S-layer-anchoring SCWP (SÁRA et al., 1996b). In functional terms, such complete cell surface modifications could prevent either attachment of specific phages or delay host immune reactions in case of pathogenic organisms.

So far no general biological function has been found, and many of the functions assigned to S-layers still remain hypothetical. As S-layers cover the surface of the whole cell as coherent layers, it has been inferred that many biological functions for the layer may depend on both the completeness of the covering and the structural and physicochemical repetitive uniformity down to the subnanometer scale (SLEYTR et al., 2002).

A striking feature of many S-layers of Bacteria and Archaea is their excellent antifouling property. This unique characteristic was first observed in electron micrographs of freeze-etched preparations (SLEYTR & GLAUERT, 1975; SLEYTR, 1978; SLEYTR & MEssner, 1983; PUM et al., 1991) involving ultrafast (30 000 K s⁻¹) vitrification of intact cells (ROBARDS & SLEYTR, 1985). Even when cells were harvested from complex environments or growth media containing a great variety of macromolecular components, the S-layer lattices were never masked by adsorbed molecules (Fig. 1). In this context, it has to be remembered that not only in fast frozen preparations but also in electron micrographs of negatively stained preparations the individual S-layer proteins show deviations from precise lattice positions so that high-resolution studies require digital image processing to correct the spatial distortions (CROWther & SLEYTR, 1977; SAXTON & BAUMEISTER, 1982; SAXTON et al., 1984; HENDERSON et al., 1986; GIL et al., 2006; PAVKOV-KELLER et al., 2011). Obviously, the different electron microscopic preparation techniques retain deviations from the ideal crystal structure created by thermal lattice vibrations. These specific S-layer properties may additionally influence interactions with molecules in close proximity and consequently the observed antifouling properties. More detailed studies on molecular interactions and permeability using isolated S-layers or S-layer ultrafiltration membranes (see section ‘S-layers as molecular sieves and antifouling coatings’ and ‘Isoporous ultrafiltration membranes’) confirmed that the surface of the lattice in Bacteria is charge neutral, preventing non-specific binding of molecules and pore blocking. Moreover, in Bacillaceae, it was shown that S-layer lattices mask the net negative charge of the peptidoglycan-containing layer (SÁRA & SLEYTR, 1986a; GRUBER & SLEYTR, 1991; WEIGERT & SÁRA, 1995). Data derived from antifouling zwitterionic polymer coatings on composite nanofiltration membranes can lead to the conclusion that likewise the ultra-low fouling properties of S-layers may be affected by their zwitterionic surface properties (JI et al., 2012). In zwitterionic coatings developed for many applications...
that require biofouling resistance, ions alternate perfectly in the subnanometer scale between positive and negative charges preventing adsorption of naturally occurring molecules, particularly proteins. Final proof for this hypothesis will depend on structural information of S-layer lattices at atomic resolution and detailed information on the impact of an S-layer surface on the molecular organization of the adjacent water boundary structure. Most recently, the unique antifouling properties of S-layers were successfully exploited for coating microfluidic channels in lab-on-a-chip devices (Picher et al., 2013; Rothbauer et al., 2013).

In addition to the unique physicochemical surface properties, the repetitive topographical characteristics of S-layers should be considered as relevant features affecting hydrodynamic surface properties of cells. It is tempting to speculate that the defined roughness of S-layer surfaces determines the flow resistance of cells in natural environments. Studies on friction flows of liquids at nanopatterned interfaces have shown that the slippage of fluids at channel boundaries is greatly increased using surfaces that are patterned on the nanometer scale (Cottin-Bizonne et al., 2003). With prokaryotic organisms characterized by a large surface to volume ratio, such effects should be of particular importance.

Considering the combination of antifouling properties and increased slippage, the presence of S-layers may facilitate flagella-driven cell locomotion in natural habitats (e.g. soil, mud, body fluids), thus justifying the energy expense of S-layer protein synthesis. It will be interesting to see whether S-layer glycosylation even amplifies these important boundary conditions and surface properties. We presume that besides the more specific functions identified for S-layers of different organisms (e.g. specific interactions with molecules and cell surfaces as observed in pathogens), these features may turn out to be one of the most general ones among bacterial and archaeal S-layers. Thus, antifouling properties and reduced resistance in cell locomotion might be summed up in an S-layer specific topographical ‘Nano-Lotus-effect’, and we should be encouraged to use different methods in surface sciences for scrutinizing this hypothesis. Experimental approaches may imply established techniques for reassembly of S-layer proteins on solid supports at macroscopic dimensions (see section ‘Reassembly at interfaces’). Biomimetic approaches copying these unique S-layer surface properties could be of great technological relevance.

In those Archaea possessing S-layers as exclusive wall component external to the plasma membrane, there is now strong evidence that the crystalline arrays have general potential as a membrane stabilizing framework determining a nanopatterned fluidity of the lipid components (see section ‘S-layer protein-lipid interactions’) and consequently enabling many species of Archaea to dwell under most extreme environmental conditions including temperatures up to 120 °C. In this context, it is of particular relevance to remember that in H. volcanii S-layer lattices, two glycoprotein populations coexist regarding their association with the plasma membrane. The first presumably corresponds to S-layer glycoprotein anchored to the membrane via the C-terminal transmembrane domain, whereas the other glycoprotein population is lipid-modified (most presumably by archaeatic acid) and associated with the membrane (Kandiba et al., 2013). Variations in the relative distribution of both types of populations should enable rapid changes in the membrane ‘nanopatterned fluidity’ and consequently facilitate adaptation of the organism to environmental changes (see also section ‘Isolation and chemistry’). Furthermore, S-layers as exclusive wall component in Archaea appear to be involved in maintaining of cell shape and in fission processes, but more detailed studies will be required to support this notion. Presumably, many recognized and predicted functions of bacterial S-layers, such as forming a barrier against predators, as molecular sieves that exclude hazardous components and retain useful molecules in the periplasmic space and as a promoter of very specific cell adhesion to surfaces and cells co-exist in different prokaryotic organisms. An important area of future S-layers research will concern their relevance in terms of pathogenicity, immunomodulatory capacity, health beneficial (probiotic) properties (Hynonen & Palva, 2013), and virulence of organisms.

Accumulated data on the structure, chemical composition, assembly, surface, and permeability properties have clearly shown that S-layers are the simplest biological (glyco)protein membranes which have developed during evolution. Most important, S-layer morphogenesis follows the theoretically simplest mechanism for a dynamic process of assembly of a closed container composed of monomolecular arrays of identical macromolecules. As there is no theoretical possibility of forming a closed protein membrane with less redundancy of information, it is also tempting to speculate that a simple protein membrane capable of dynamic growth could have initiated a barrier membrane in an early stage of biological evolution (Sleytr & Plothberger, 1980; Pum et al., 1991; Sleytr & Beveridge, 1999; Sleytr et al., 2002).

Moreover, S-layers have been shown to interact specifically with a great variety of amphiphilic molecules (e.g. phospho- and ether lipids) generating more complex supramolecular membrane structures with potential for high transmembrane selectivity. It is even probable that structure–function relationships between S-layer lattices and virus capsids or animal and human virus envelopes exist (Arbing et al., 2012), and we cannot rule out the
possibility that horizontal gene transfer was relevant during co-evolution of different systems. On the other hand, structures that look alike not necessarily have similar functions.

The characteristic properties of S-layers, particularly their structural and physicochemical uniformity and the spontaneous association of constituent subunits under equilibrium conditions, have led to an astonishing spectrum of applications in nano(bio)technology, synthetic biology, and biomimetics (Sleytr et al., 2001; Sára et al., 2006b; Egelseer et al., 2008; Pum & Sleytr, 2009; Schuster & Sleytr, 2009b; Egelseer et al., 2010; Göbel et al., 2010; Sleytr et al., 2010; Ilk et al., 2011a; Sleytr et al., 2011, 2013). In this context, S-layer-carrying Lactobacilli as food grade and potentially probiotic organisms will gain importance for health-related applications such as live oral vaccines. Furthermore, S-layer proteins of Lactobacilli are excellent candidates as carriers of antigens or other medically important molecules relevant for specific adhesion and immunomodulation (Hynonen & Palva, 2013).

S-layers are now recognized as versatile patterning elements for the generation of complex supramolecular structures involving other molecules such as lipids, proteins, glycans, and nucleic acids as well as inorganic materials (e.g. nanoparticles). An important line of future development concerns the combination of S-layers with planar lipid membranes, liposomes, and emulsomes (Schuster & Sleytr, 2009b; Ferner-Ortner-Bleckmann et al., 2011; Ücisk et al., 2013b). This biomimetic approach, copying the supramolecular principle of cell envelopes of Archaea or envelopes of a great variety of viruses, allows stabilizing functional lipid membranes at the macroscopic scale. Most recently, it could be demonstrated that S-layer stabilized lipid membranes can be functionalized by incorporating membrane proteins exploiting cell-free protein synthesis regimes (E.K. Sinner, B. Schuster, S. Damiati, pers. comm.). This unique possibility of significantly improving stability and life time of functional lipid membranes can also be exploited for a broad spectrum of liposome and emulsion technologies as required for drug-targeting and delivery systems, immunotherapy, and gene therapy (see section ‘S-layer coated liposomes and emulsomes’) and eventually may even serve in the long term as supramolecular concept for generating ‘artificial life’ following bottom-up strategies in synthetic biology.

Many areas of applied S-layer research will particularly be promoted by the construction of S-layer fusion proteins comprising the intrinsic self-assembly domain and a fused functional sequence (Table 2). Another interesting application for S-layers concerns tailored S-layer neoglycoproteins utilizing the recrystallization capability of the S-layer protein for the controlled and periodic surface display of ‘functional’ glycosylation motifs. Applications for S-layer neoglycoproteins concern receptor mimics, vaccine design, diagnostics, and drug delivery exploiting specific carbohydrate recognition (Sleytr et al., 2010). Although up to now the development of applied S-layer research has focused on life sciences, in future non-life science applications (e.g. molecular electronics, nonlinear optics) will gain importance (Shenton et al., 1997; Mertig et al., 1999; Vyalikh et al., 2004; Maslyuk et al., 2008; Pum & Sleytr, 2009; Queitsch et al., 2009; Sleytr et al., 2013).

Acknowledgements

The total number of relevant publications (currently more than 2500) on S-layers far surpasses the number of references that can be cited in this review. We therefore apologize to all those whose particular works were not cited here. Part of this work was funded by AFOSR Agreement Awards Nr. FA9550-09-0342 and FA9550-12-1-0274 (to D.P.), and FA9550-10-1-0223 (to U.B.S.), by the Austrian Science Fund (FWF), project P 20256-B11, and by the Erwin-Schrödinger Society for Nanosciences, Vienna.

References

Abdul Halim MF, Pfeiffer F, Zou J et al. (2013) Haloferax volcanii archaeosortase is required for motility, mating, and C-terminal processing of the S-layer glycoprotein. Mol Microbiol 88: 1164–1175.

Akca E, Claus H, Schultz N, Karbach G, Schlott B, Debaerdemaeker T, Declercq JP & König H (2002) Genes and derived amino acid sequences of S-layer proteins from mesophilic, thermophilic, and extremely thermophilic methanococci. Extremophiles 6: 351–358.

Albers SV & Meyer BH (2011) The archaeal cell envelope. Nat Rev Microbiol 9: 414–426.

Amselem S, Yogeav A, Zawoznik E & Friedman D (1994) Emulsomes, a novel drug delivery technology. Proc Contr Rel Soc 21: 668–669.

Andresen TL, Jensen SS & Jorgensen K (2005) Advanced strategies in liposomal cancer therapy: problems and prospects of active and tumor specific drug release. Prog Lipid Res 44: 68–97.

Antikainen J, Anton L, Sillanpaa J & Korhonen TK (2002) Domains in the S-layer protein CbsA of Lactobacillus crispatus involved in adherence to collagens, laminin and lipoteichoic acids and in self-assembly. Mol Microbiol 46: 381–394.

Arbing MA, Chan S, Shin A, Phan T, Ahn CJ, Rohlin L & Gunsalus RP (2012) Structure of the surface layer of the methanogenic archean Methanosarcina acetivorans. P Natl Acad Sci USA 109: 11812–11817.

Ariel N, Zvi A, Grosfeld H, Gat O, Inbar Y, Velan B, Cohen S & Shafferman A (2002) Search for potential vaccine candidate open reading frames in the Bacillus anthracis...
virulence plasmid pX01: in silico and in vitro screening. Infect Immun 70: 6817–6827.

Ausiello CM, Cerqueti M, Fedele G, Spensi F, Palazzo R, Nasso M, Frezza S & Mastrantonio P (2006) Surface layer proteins from Clostridium difficile induce inflammatory and regulatory cytokines in human monocytes and dendritic cells. Microbes Infect 8: 2640–2646.

Avall-Jääskeläinen S & Palva A (2005) Lactobacillus surface layers and their applications. FEMS Microbiol Rev 29: 511–529.

Avall-Jääskeläinen S, Kylä-Nikkilä K, Kahala M, Miikkulainen-Lahti T & Palva A (2002) Surface display of foreign epitopes on the Lactobacillus brevis S-layer. Appl Environ Microbiol 68: 5943–5951.

Bingle WH, Nomellini JF, Sleytr UB, Moller C, Egelseer EM, Sleytr UB, Pum D & Ilk N (2009) Genetic Engineering of the S-Layer Protein SbpA of Lysinibacillus sphaericus CCM 2177 for the Generation of Functionalized Nanoarrays. Biocatalysis 20: 895–909.

Bingle WH, Nomellini JF & Sleytr UB (1997b) Linker mutagenesis of the Caulobacter crescentus S-layer protein: toward a definition of an N-terminal anchoring region and a C-terminal secretion signal and the potential for heterologous protein secretion. J Bacteriol 179: 601–611.

Blaser MJ (1998) Campylobacter fetus–emerging infection and model system for bacterial pathogenesis at mucosal surfaces. Clin Infect Dis 27: 256–258.

Blaser MJ, Smith PF, Repine JE & Joiner KA (1988) Pathogenesis of Campylobacter fetus infections. Failure of encapsulated Campylobacter fetus to bind C3b explains serum and phagocytosis resistance. J Clin Invest 81: 1434–1444.

Blaser MJ, Wang E, Tummuru MK, Washburn R, Fujimoto S & Labigne A (1994) High-frequency S-layer protein variation in Campylobacter fetus revealed by sapA mutagenesis. Mol Microbiol 14: 453–462.

Bingle WH, Nomellini JF & Sleytr UB (1997a) Cell-surface display of a Pseudomonas aeruginosa strain K pilin peptide within the paracrystalline S-layer of Caulobacter crescentus. Mol Microbiol 26: 277–288.

Bingle WH, Nomellini JF & Sleytr UB (1997b) Cell-surface display of the Lactobacillus brevis S-layer protein: toward a definition of an N-terminal anchoring region and a C-terminal secretion signal and the potential for heterologous protein secretion. J Bacteriol 179: 601–611.

Blaser MJ (1998) Campylobacter fetus–emerging infection and model system for bacterial pathogenesis at mucosal surfaces. Clin Infect Dis 27: 256–258.

Blaser MJ, Smith PF, Repine JE & Joiner KA (1988) Pathogenesis of Campylobacter fetus infections. Failure of encapsulated Campylobacter fetus to bind C3b explains serum and phagocytosis resistance. J Clin Invest 81: 1434–1444.

Blaser MJ, Wang E, Tummuru MK, Washburn R, Fujimoto S & Labigne A (1994) High-frequency S-layer protein variation in Campylobacter fetus revealed by sapA mutagenesis. Mol Microbiol 14: 453–462.

Blende R, Benson JM, DesRoches CM, Pollard WE, Parvanta C & Herrmann MJ (2002) The impact of anthrax attacks on the American public. MedGenMed 4: 1.

Bobeth M, Blecha A, Blüher A, Mertig M, Korkmaz N, Ostermann K, Rödel G & Pompe W (2011) Formation of tubes during self-assembly of bacterial surface layers. Langmuir 27: 15102–15111.

Bohle B, Breitwieser A, Zvolle B, Jahn-Schmid B, Sára M, Sleytr UB & Ebner C (2004) A novel approach to specific allergen treatment: the recombinant fusion protein of a bacterial cell surface (S-layer) protein and the major birch pollen allergen Bet v 1 (rSbsC-Bet v 1) combines reduced allergenicity with immunomodulating capacity. J Immunol 172: 6642–6648.

Brechtle E & Bahl H (1999) In Thermus thermophilus EM1 S-layer homology domains do not attach to peptidoglycan. J Bacteriol 181: 5017–5023.

Breitwieser A, Gruber K & Sleytr UB (1992) Evidence for an S-layer protein pool in the peptidoglycan of Bacillus stearothermophilus. J Bacteriol 174: 8008–8015.

Breitwieser A, Kuptc S, Howorka S, Weigert S, Langer C, Hoffmann-Sommergruber K, Scheiner O, Sleytr UB & Sára M (1996) 2-D protein crystals as an immobilization matrix for producing reaction zones in dipstick-style immunoassays. Biotechniques 21: 918–925.

Breitwieser A, Mader C, Schocher I, Hoffmann-Sommergruber K, Aberer W, Scheiner O, Sleytr UB & Sára M (1998) A novel dipstick developed for rapid Bet v 1-specific IgE detection: recombinant allergen immobilized via a monoclonal antibody to crystalline bacterial cell-surface layers. Allergy 53: 786–793.

Breitwieser A, Egelseer EM, Moll D, Ilk N, Hotzy C, Bohle B, Ebner C, Sleytr UB & Sára M (2002) A recombinant bacterial cell surface (S-layer)-major birch pollen allergen-fusion protein (rSbsC-Bet v 1) maintains the ability to self-assemble into regularly structured monomolecular lattices and the functionality of the allergen. Protein Eng 15: 243–249.

Brinton CC, McNary JC & Carnaham J (1969) Purification and in vitro assembly of a curved network of identical protein subunits from the outer surface of a Bacillus. Bact Proc 48.
Brown F, Dougan G, Hoey EM, Martin S, Rima BK & Trudgett A (1993) Vaccine Design. John Wiley & Sons, England, UK.

Buckmire FLA (1971) A protective role for a cell wall protein layer of Spirillum serpens against infection by Belvellovibrio bacteriovorus. Bact Proe 43.

Calabi E, Calabi F, Phillips AD & Fairweather NF (2002) Binding of Clostridium difficile surface layer proteins to gastrointestinal tissues. Infect Immun 70: 5770–5778.

Callahan C, Castanha ER, Fox KF & Fox A (2008) The Bacillus cereus containing sub-branch most closely related to Bacillus anthuris, have single amino acid substitutions in small acid-soluble proteins, while remaining sub-branches are more variable. Mol Cell Probes 22: 207–211.

Candela T, Mignot T, Hagnerelle X, Haustant M & Fouet A (2004) Binding to pyruvylated compounds as an ancestral mechanism to anchor the outer envelope in primitive bacteria. Mol Microbiol 52: 677–690.

Claus H, Akca E, Debaerdemaeker T, Evrard C, Declercq JP, Cline SW, Lam WL, Charlebois RL, Schalkwyck LC & Doolittle WF (1989) Transformation methods for halophilic archaeabacteria. Can J Microbiol 35: 148–152.

Cohen-Bazire G, Kunisawa R & Pfenning N (1969) Comparative study of the structure of gas vacuoles. J Bacteriol 100: 1049–1061.

Comolli LR, Siegerist CE, Shin SH, Bertozzi C, Regan W, Zettl A & De Yoreo JJ (2013) Conformational transitions at an S-layer growing boundary resolved by cryo-TEM. Angew Chem Int Edit 52: 4829–4832.

Cotton-Bizonne C, Barrat J-L, Bocquet L & Charlaix E (2003) Low-friction flows of liquid at nanopatterned interfaces. Nat Mater 2: 237–240.

Crowther RA & Sleytr UB (1977) An analysis of the fine structure of the surface layers from two strains of Clostridia, including correction for distorted images. J Ultrastruct Res 58: 41–49.

Cui HF, Ye JS, Leitmannova Liu A & Ti Tien H (2006) Lipid Microvesicles: on the Four Decades of Liposome Research. Advances in Planar Lipid Bilayers and Liposomes, Vol. 4 (Leitmannova Liu A & Ti Tien H, eds), pp. 1–48. Academic Press, London.

de la Fuente-Núñez C, Mertens J, Smit J & Hancock REW (2012) Bacterial surface layer protects against antimicrobial peptides. Appl Environ Microbiol 78: 5452–5456.

De Rosa M (1996) Archaeal lipids: structural features and supramolecular organization. Thin Solid Films 284–285: 13–17.

De Yoreo JJ, Chung S & Nielsen MH (2013) The dynamics and energetics of matrix assembly and mineralization. Calcif Tissue Int 93: 316–328.

Demarche S, Sugihara K, Zambelli T, Tiefenauer L & Voros J (2011) Techniques for recording reconstituted ion channels. Analyst 136: 1077–1089.

Diederich A, Sponer C, Pum D, Sleytr UB & Löschke M (1996) Reciprocal influence between the protein and lipid components of a lipid-protein membrane model. Colloids Surf, B 6: 335–346.

Doig P, McCubbin WD, Ray CM & Trust TJ (1993) Distribution of surface-exposed and non-accessible amino acid sequences among the two major structural domains of the S-layer protein of Aeromonas salmonicida. J Mol Biol 233: 753–765.

Dordic A, Egelseer EM, Teszar M, Sleytr UB, Keller W & Pavkov-Keller T (2012) Crystallization of domains involved in self-assembly of the S-layer protein SbsC. Acta Crystallogr Sect F Struct Biol Cryst Commun 68: 1511–1514.

Drudy D, Calabi E, Kyne L, Sougioutzis S, Kelly E, Fairweather N & Kelly CP (2004) Human antibody response to surface layer proteins in Clostridium difficile infection. FEMS Immunol Med Microbiol 41: 237–242.

Dubreuil JD, Kostrzynska M, Austin JW & Trust TJ (1990) Antigenic differences among Campylobacter fetus S-layer proteins. J Bacteriol 172: 5035–5043.

Duncan G, Tarling CA, Bingle WH, Nomellini JP, Yamage M, Dorocicz IR, Withers SG & Smit J (2005) Evaluation of a new system for developing particulate enzymes based on the surface (S)-layer protein (Rsaa) of Caulobacter crescentus: fusion with the beta-1,4-glycanase (Cex) from the cellulolytic bacterium Cellulomonas fimii yields a robust, catalytically active product. Appl Biochem Biotechnol 127: 95–110.
Dworkin J, Tummuru MK & Blaser MJ (1995) Segmental conservation of sapA sequences in type B Campylobacter fetus cells. J Biol Chem 270: 15093–15101.

Ebner A, Kienberger F, Huber C et al. (2006) Atomic-force-microscopy imaging and molecular-recognition-force microscopy of recrystallized heterotetramers comprising an S-layer-streptavidin fusion protein. ChemBioChem: 588–591.

Egelseer E, Schocher I, Sara M & Sleytr UB (1995) The S-layer from Bacillus stearothermophilus DSM 2358 functions as an adhesion site for a high-molecular-weight amylase. J Bacteriol 177: 1444–1451.

Egelseer EM, Schocher I, Sleytr UB & Sara M (1996) Evidence that an N-terminal S-layer protein fragment triggers the release of a cell-associated high-molecular-weight amylase in Bacillus stearothermophilus ATCC 12980. J Bacteriol 188: 5602–5609.

Egelseer EM, Leitner K, Jarosch M, Hotzy C, Zayni S, Sleytr UB & Sara M (1998) The S-layer proteins of two Bacillus stearothermophilus wild strains are bound via their N-terminal region to a secondary cell wall polymer of identical chemical composition. J Bacteriol 180: 1488–1495.

Egelseer EM, Idris R, Jarosch M, Danhorn T, Sleytr UB & Sara M (2000) ISBst12, a novel type of insertion-sequence element causing loss of S-layer-gene expression in Bacillus stearothermophilus ATCC 12980. Microbiology 146( Pt 9): 2175–2183.

Egelseer EM, Sara M, Pum D, Schuster B & Sleytr UB (2008) Genetically engineered S-layer proteins and S-layer-specific heteropolysaccharides as components of a versatile molecular construction kit for applications in nanobiotechnology. NanoBioTechnology (Shoseyov O & Levy I, eds), pp. 55–86. Humana Press, Totowa, NJ.

Egelseer EM, Ilk N, Pum D, Messner P, Schäffer C, Schuster B & Sleytr UB (2010) S-Layers, microbial, biotechnological applications. The Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, Vol. 7 (Flickinger MC, ed.), pp. 4424–4448. John Wiley & Sons, Inc., Hoboken, NJ.

Eichler J & Adams MW (2005) Posttranslational protein modification in Archaea. Microbiol Mol Biol Rev 69: 393–425.

Eichler J & Maupin-Furlow J (2013) Post-translation modification in Archaea: lessons from Haloferax volcanii and other haloarchaea. FEMS Microbiol Rev 37: 583–606.

Eichler J, Abu-Qarr M, Konrad Z, Magidovich H, Plavner N & Yuirst-Deutsch S (2010) The cell envelope of Haloarchaea: staying in shape in a world of salt. Prokaryotic Cell Wall Compounds – Structure and Biochemistry (König H, Claus H & Varma A, eds), pp. 253–270. Springer-Verlag, Berlin.

Ellis C & Smith A (2004) Highlighting the pitfalls and possibilities of drug research. Nat Rev Drug Discov 3: 238–278.

Engelhardt H (2007) Mechanism of osmoprotection by archaeal S-layers: a theoretical study. J Struct Biol 160: 190–199.

Engelhardt H & Peters J (1998) Structural research on surface layers: a focus on stability, surface layer homology domains, and surface layer–cell wall interactions. J Struct Biol 124: 276–302.

Etienne-Toumelin I, Sirard JC, Duflot E, Mock M & Fouet A (1995) Characterization of the Bacillus anthracis S-layer: cloning and sequencing of the structural gene. J Bacteriol 177: 614–620.

Fagan RP, Albesa-Jové D, Qazi O, Svergun DI, Brown KA & Fairweather NF (2009) Structural insights into the molecular organization of the S-layer from Clostridium difficile. Mol Microbiol 71: 1308–1322.

Fernier-Ortner J, Mader C, Ilk N, Sleytr UB & Egelseer EM (2007) High-affinity interaction between the S-layer protein SbsC and the secondary cell wall polymer of Geobacillus stearothermophilus ATCC 12980 determined by surface plasmon resonance technology. J Bacteriol 189: 7154–7158.

Fernier-Ortner-Bleckmann J, Huber-Gries C, Pavkov T, Keller W, Mader C, Ilk N, Sleytr UB & Egelseer EM (2009) The high-molecular-mass amylase (HMMA) of Geobacillus stearothermophilus ATCC 12980 interacts with the cell wall components by virtue of three specific binding regions. Mol Microbiol 72: 1448–1461.

Fernier-Ortner-Bleckmann J, Schrens A, Ilk N, Egelseer EM, Sleytr UB & Schuster B (2011) Multitechnique study on a recombinantly produced Bacillus halodurans lacasse and an S-layer/lacasse fusion protein. Biointerphases 6: 63.

Fernier-Ortner-Bleckmann J, Gelbmann N, Tesarz M, Egelseer EM & Sleytr UB (2013) Surface-layer lattices as patterning element for multimeric extremozymes. Small 9: 3887–3894.

Foligné B, Deutsch S-M, Breton J, Cousin FJ, Dewulf J, Samson M, Pot B & Jan G (2010) Promising immunomodulatory effects of selected strains of dairy propionibacteria as evidenced in vitro and in vivo. Appl Environ Microbiol 76: 8259–8264.

Fujino T, Begeni P & Aubert JP (1993) Organization of a Clostridium thermocellum gene cluster encoding the cellulosomal scaffolding protein CipA and a protein possibly involved in attachment of the cellulosome to the cell surface. J Bacteriol 175: 1891–1899.

Galdiero S, Galdiero M & Pedone C (2007) β-barrel membrane bacterial proteins: structure, function, assembly and interaction with lipids. Curr Protein Pept Sci 8: 63–82.

Galdiero S, Falanga A, Vitiello M, Raiola I, Russo L, Pedone C, Isernia C & Galdiero M (2010) The presence of a single N-terminal histidine residue enhances the fusogenic properties of a membraneotropic peptide derived from herpes simplex virus type 1 glycoprotein H. J Biol Chem 285: 17123–17136.

Gerstein M & Hegyi H (1998) Comparing genomes in terms of protein structure: surveys of a finite parts list. FEMS Microbiol Rev 22: 277–304.
Gerstmayr M, Ilk N, Schabussova I, Jahn-Schmid B, Egelseer EM, Sleytr UB, Ebner C & Bohle B (2007) A novel approach to specific allergy treatment: the recombinant allergen-S-layer fusion protein rSbsC-Bet v 1 matures dendritic cells that prime Th0/Th1 and IL-10-producing regulatory T cells. J Immunol 179: 7270–7275.

Gerstmayr M, Ilk N, Jahn-Schmid B, Sleytr UB & Bohle B (2009) Natural self-assembly of allergen-S-layer fusion proteins is no prerequisite for reduced allergenicity and T cell stimulatory capacity. Int Arch Allergy Immunol 149: 231–238.

Gil D, Carazo JM & Marabini R (2006) On the nature of 2D crystal unbinding. J Struct Biol 156: 546–555.

Göbel C, Schuster B, Baurecht D, Sleytr UB & Pum D (2010) S-layer templated bioinspired synthesis of silica. Colloids Surf B Biointerfaces 75: 565–572.

Graham LL, Beveridge TJ & Nanninga N (1991) Periplasmic space and the concept of the periplasm. Trends Biochem Sci 16: 328–329.

Graham LL, Friel T & Woodman RL (2008) Fibronectin enhances Campylobacter fetus interaction with extracellular matrix components and INT 407 cells. Can J Microbiol 54: 37–47.

Gruber K & Sleytr UB (1988) Localized insertion of new S-layer during growth of Bacillus stearothermophilus strains. Arch Microbiol 149: 485–491.

Gruber K & Sleytr UB (1991) Influence of an S-layer on surface properties of Bacillus stearothermophilus. Arch Microbiol 156: 181–185.

Guerrant RL, Lahita RG, Winn WC Jr & Roberts RB (1978) Campylobacteriosis in man: pathogenic mechanisms and review of 91 bloodstream infections. Am J Med 65: 584–592.

Guiller PC, Pum D, Sleytr UB & Schuster B (2004) Highly robust lipid membranes on crystalline S-layer supports investigated by electrochemical impedance spectroscopy. Biochim Biophys Acta 1661: 154–165.

Györvary E, Wetzker B, Sleytr UB, Sinner A, Offenhäuser A & Knoll W (1999) Lateral diffusion of lipids in silane-, dextran-, and S-layer-supported mono- and bilayers. Langmuir 15: 1337–1347.

Györvary E, Steín O, Pum D & Sleytr UB (2003) Self-assembly and recrystallization of bacterial S-layer proteins at silicon supports imaged in real time by atomic force microscopy. J Microsc 212: 300–306.

Györvary E, Schroeder T, Talapin DV, Weller H, Pum D & Sleytr UB (2004) Formation of nanoparticle arrays on S-layer protein lattices. J Nanosci Nanotechnol 4: 115–120.

Haft DH, Payne SH & Selengut JD (2012) Archaeosortases and exosortases are widely distributed systems linking membrane transit with posttranslational modification. J Bacteriol 194: 36–48.

Hall SR, Shenton W, Engelhardt H & Mann S (2001) Site-specific organization of gold nanoparticles by biomolecular templating. Chemphyschem 3: 184–186.

Hancock REW & Chapple DS (1999) Peptide antibiotics. Antimicrob Agents Chemother 43: 1317–1323.

Hanford MJ & Peeples TL (2002) Archaeal tetraether lipids: unique structures and applications. Appl Biochem Biotechnol 97: 45–62.

Harris WF (1975) Negative wedge disclinations of rotation 2pi radians and topological changes of membraneous systems. Philos Mag 32: 37–41.

Harris WF (1978) Dislocations, disclinations and dispirations: distraction in very naughty crystals. S Afr J Sci 74: 332–338.

Harris WF & Scriven LE (1970) Function of dislocations in cell walls and membranes. Nature 228: 827–829.

Harris WF & Scriven LE (1971) Intrinsic disclinations as dislocation sources and sinks in surface crystals. J Appl Phys 42: 3309–3312.

Henderson R, Baldwin JM, Downing KH, Lepault J & Zemlin F (1986) Structure of purple membrane from Halobacterium halobium: recording, measurement and evaluation of electron micrographs at 3.5 A resolution. Ultramicroscopy 19: 147–178.

Hianik T, Küpcü S, Sleytr UB, Rybar P, Krivanek R & Klaatz U (1999) Interaction of crystalline bacterial cell surface proteins with lipid bilayers in liposomes. Coll Surf A 147: 331–339.

Hirn R, Schuster B, Sleytr UB & Bayerl TM (1999) The effect of S-layer protein adsorption and crystallization on the collective motion of a planar lipid bilayer studied by dynamic light scattering. Biophys J 77: 2066–2074.

Hollmann A, Delfefederco L, Glikmann G, De Antoni G, Semorile L & Disalvo EA (2007) Characterization of liposomes coated with S-layer proteins from Lactobacilli. Biochim Biophys Acta 1768: 393–400.

Holt SC & Leadbetter ER (1969) Comparative ultrastructure of selected aerobic spore forming bacteria: a freeze-etching study. Bacteriol Rev 33: 346–378.

Horejs C, Pum D, Sleytr UB & Tschelissnig R (2008) Structure prediction of an S-layer protein by the mean force method. J Chem Phys 128: 065106.

Horejs C, Pum D, Sleytr UB, Peterlik H, Jungbauer A & Tschelissnig R (2010) Surface layer protein characterization by small angle x-ray scattering and a fractal mean force concept: from protein structure to nanodisk assemblies. J Chem Phys 133: 175102.

Horejs C, Ristl R, Tschelissnig R, Sleytr UB & Pum D (2011a) Single-molecule force spectroscopy reveals the individual mechanical unfolding pathways of a surface layer protein. J Biol Chem 286: 27416–27424.

Houwing AL (1953) A macromolecular monolayer in the cell wall of Spirillum spec. Biochim Biophys Acta 10: 360–366.

Houwing AL & Le Poole JB (1952) Eine Struktur in der Zellmembran einer Bakterie. Physikalische Verhandlungen 3: 98.

Hovmöller S, Sjögren A & Wang DN (1988) The structure of crystalline bacterial surface layers. Prog Biophys Mol Biol 51: 131–163.
Howard LV, Dalton DD & McCoubrey JWK (1982) Expansion of the tetragonally arrayed cell wall protein layer during growth of Bacillus sphaericus. J Bacteriol 149: 748–757.

Huber C, Ilk N, Rünzler D, Egelseer EM, Weigert S, Sleytr UB & Sára M (2005) The three S-layer-like homology motifs of the S-layer protein SbpA of Bacillus sphaericus CCM 2177 are not sufficient for binding to the pyruvylated secondary cell wall polymer. Mol Microbiol 55: 197–205.

Huber C, Egelseer EM, Ilk N, Sleytr UB & Sára M (2006a) S-layer-streptavidin fusion proteins and S-layer-specific heteropolysaccharides as part of a biomolecular construction kit for application in nanobiotechnology. Microelectron Eng 83: 1589–1593.

Huber C, Liu J, Egelseer EM, Moll D, Knoll W, Sleytr UB & Sára M (2006b) Heterotetramers formed by an S-layer-streptavidin fusion protein and core-streptavidin as nanoarrayed template for bioshop development. Small 2: 142–150.

Hynonen U & Palva A (2013) Lactobacillus surface layer proteins: structure, function and applications. Appl Microbiol Biotechnol 97: 5225–5243.

Ilk N, Kosma P, Puchberger M, Egelseer EM, Mayer HF, Sleytr UB & Sára M (1999) Structural and functional analyses of the secondary cell wall polymer of Bacillus sphaericus CCM 2177 that serves as an S-layer-specific anchor. J Bacteriol 181: 7643–7646.

Ilk N, Völlenkle C, Egelseer EM, Breitwieser A, Sleytr UB & Sára M (2002) Molecular characterization of the S-layer gene, sbpA, of Bacillus sphaericus CCM 2177 and production of a functional S-layer fusion protein with the ability to recrystallize in a defined orientation while presenting the fused allergen. Appl Environ Microbiol 68: 3251–3260.

Ilk N, Küpcü S, Moncayo G, Klímt S, Ecker RC, Hofer-Warbinek R, Egelseer EM, Sleytr UB & Sára M (2004) A functional chimaeric S-layer-enhanced green fluorescent protein to follow the uptake of S-layer-coated liposomes into eukaryotic cells. Biochem J 379: 441–448.

Ilk N, Egelseer EM & Sleytr UB (2011a) S-layer fusion proteins-construction principles and applications. Curr Opin Biotechnol 22: 824–831.

Ilk N, Schumi CT, Bohle B, Egelseer EM & Sleytr UB (2011b) Expression of an endotoxin-free S-layer/allergen fusion protein in gram-positive Bacillus subtilis 1012 for the potential application as vaccines for immunotherapy of atopic allergy. Microb Cell Fact 10: 6.

Jackman JA, Knoll W & Cho N-J (2012) Biotechnology applications of tethered lipid bilayer membranes. Materials 5: 2637–2657.

Jaenicke R, Welsch R, Sára M & Sleytr UB (1985) Stability and self-assembly of the S-layer protein of the cell wall of Bacillus stea
thermophilus. Biol Chem Hoppe Seyler 366: 663–670.

Jahn-Schmid B, Messner P, Unger FM, Sleytr UB, Scheiner O & Kraft D (1996a) Toward selective elicitation of TH1-controlled vaccination responses: vaccine applications of bacterial surface layer proteins. J Biotechnol 44: 225–231.

Jahn-Schmid B, Graninger M, Glozik M, Kürpüç S, Ebner C, Unger FM, Sleytr UB & Messner P (1996b) Immunoreactivity of allergen (Bet v 1) conjugated to crystalline bacterial cell surface layers (S-layers). Immunotechnology 2: 103–113.

Jahn-Schmid B, Siemann U, Zanker A, Bohle B, Messner P, Unger FM, Sleytr UB, Scheiner O, Kraft D & Ebner C (1997) Bet v 1, the major birch pollen allergen, conjugated to crystalline bacterial cell surface proteins, expands allergen-specific T cells of the Th1/Th0 phenotype in vitro by induction of IL-12. Int Immunol 9: 1867–1874.

Janesch B, Messner P & Schäffer C (2013) Are the surface layer homology domains essential for cell surface display and glycosylation of the S-layer protein from Pseu
dibacillus alvei CCM 2051T? J Bacteriol 195: 565–575.

Jarosch M, Egelseer EM, Mattanovich D, Sleytr UB & Sára M (2000) S-layer gene sbsC of Bacillus stea
thermophilus ATCC 12980: molecular characterization and heterologous expression in Escherichia coli. Microbiology 146(Pt 2): 273–281.

Jarosch M, Egelseer EM, Huber C, Moll D, Mattanovich D, Sleytr UB & Sára M (2001) Analysis of the structure-function relationship of the S-layer protein SbsC of Bacillus stea
thermophilus ATCC 12980 by producing truncated forms. Microbiology 147: 1353–1363.

Ji Y-L, An Q-F, Zhao Q, Sun W-D, Lee K-R, Chen H-L & Gao C-J (2012) Novel composite nanofiltration membranes containing zwitterions with high permeate flux and improved anti-fouling performance. J Membr Sci 390–391: 243–253.

Jing H, Takagi J, Liu JH, Lindgren S, Zhang RG, Joachimiak A, Wang JH & Springer TA (2002) Archaeal surface layer proteins contain beta propeller, PKD, and beta helix domains and are related to metazoan cell surface proteins. Structure 10: 1453–1464.

Kainz B, Steiner K, Sleytr UB, Pum D & Toca-Herrera JL (2010a) Fluorescence energy transfer in the bi-fluorescent S-layer tandem fusion protein ECFP-SgsE-YFP. J Struct Biol 172: 276–283.

Kainz B, Steiner K, Möller M, Pum D, Schäffer C, Sleytr UB & Toca-Herrera JL (2010b) Absorption, steady-state fluorescence, fluorescence lifetime, and 2D self-assembly properties of engineered fluorescent S-layer fusion proteins of Geobacillus stearothermophilus NRS 2004/3a. Biomacromolecules 11: 207–214.

Kandiba L, Guan Z & Eichler J (2013) Lipid modification gives rise to two distinct Haloferax volcanii S-layer glycoprotein populations. Biochim Biophys Acta 1828: 938–943.

Keizer HM, Dorvel BR, Andersson M & al. (2007) Functional ion channels in tethered bilayer membranes – implications for biosensors. ChemBioChem 8: 1246–1250.

Keizer HM, Andersson M, Chase C, Laratta WP, Proemsy JB, Tabb J, Long JR & Duran RS (2008) Prolonged stochastic
single ion channel recordings in S-layer protein stabilized lipid bilayer membranes. Colloids Surf, B 65: 178–185.
Kelly CP & LaMont JT (1998) Clostridium difficile infection. Annu Rev Med 49: 375–390.
Kepplinger C (2007) Anchoring of lipid assemblies to native and modified S-layer lattices - a nanobiotechnological study. Thesis, University of Natural Resources and Life Sciences, Vienna, Austria.
Kepplinger C, Ilk N, Sleytr UB & Schuster B (2009) Intact attachment of diphytanylglyceryl phosphate to the cell-surface glycoprotein of Halobacterium halobium. J Biol Chem 284: 18011–18016.
Klingl A, Moissl-Eichinger C, Wanner G, Zweck J, Huber H, Thomm M & Rachel R (2011) Analysis of the surface proteins of Acidithiobacillus ferrooxidans strain SPS/5 and the new, pyrite-oxidizing Acidithiobacillus isolate HV2/2, and their possible involvement in pyrite oxidation. Arch Microbiol 193: 867–882.
Knoll W, Naumann R, Friedrich M et al. (2008) Solid supported lipid membranes: new concepts for the biomimetic functionalization of solid surfaces. Biointerphases 3: FA125–FA135.
König H, Claus H & Akca E (2004) Cell wall structure of mesophilic, thermophilic and hyperthermophilic archaea. Origins (Seckbach J, ed.), pp. 283–298. Kluwer, Dordrecht.
König H, Claus H & Varma A (2010) Prokaryotic Cell Wall Compounds – Structure and Biochemistry. Springer, Berlin.
Konrad Z & Eichler J (2002) Lipid modification of proteins in Archaea: attachment of a mevalonic acid-based lipid moiety to the surface-layer glycoprotein of Halofexax volcanii follows protein translocation. Biochem J 366: 959–964.
Konstantinov SR, Smidt H, de Vos WM et al. (2008) S layer protein A of Lactobacillus acidophilus NCFM regulates immature dendritic cell and T cell functions. P Natl Acad Sci USA 105: 19474–19479.
Kosma P, Wugeditsch T, Christian R, Zayni S & Messner P (1995) Glycan structure of a heptose-containing S-layer glycoprotein of Bacillus thermoauerophilus. Glycobiology 5: 791–796.
Koval SF (1993) Predation on bacteria possessing S-layers. Advances on Paracrystalline Bacterial Surface Layers, Vol. 252 (Beveridge TJ & Koval SF, eds), pp. 85–92. Plenum Press, New York.
Koval SF & Murray RG (1984) The isolation of surface array proteins from bacteria. Can J Biochem Cell Biol 62: 1181–1189.
Krivanek R, Rybar P, Kúpcú S, Sleytr UB & Hianik T (2002) Affinity interactions on a liposome surface detected by ultrasound velocimetry. Bioelectrochemistry 55: 57–59.
Kúpcú S, Sára M & Sleytr UB (1993) Influence of covalent attachment of low molecular weight substances on the rejection and adsorption properties of crystalline proteinaceous ultrafiltration membranes. Desalination 90: 65–76.
Kúpcú S, Sára M & Sleytr UB (1995a) Liposomes coated with crystalline bacterial cells surface protein (S-layer) as immobilization structures for macromolecules. Biochim Biophys Acta 1235: 263–269.
Kúpcú S, Mader C & Sára M (1995b) The crystalline cell surface layer of Thermoanaerobacter thermohydrodsulfuricus L111-69 as an immobilization matrix: influence of the morphological properties and the pore size of the matrix on activity loss of covalently bound enzymes. Biotechnol Appl Biochem 21: 275–286.
Kúpcú S, Sleytr UB & Sara M (1996) Two-dimensional paracrystalline glycoprotein S-layers as a novel matrix for the immobilization of human IgG and their use as microparticles in immunoassays. J Immunol Methods 196: 73–84.
Kúpcú S, Lohner K, Mader C & Sleytr UB (1998) Microcalorimetric study on the phase behaviour of S-layer coated liposomes. Mol Membr Biol 15: 69–74.
Larisch V-D (2012) Characterization of the ryanodine receptor 1 in model lipid membranes. Master Thesis, University of Natural Resources and Life Sciences, Vienna, Austria.
Lemaire M, Ohayon H, Gounon P, Fujino T & Beguin P (1995) OlpB, a new outer layer protein of Clostridium thermocellum, and binding of its S-layer-like domains to components of the cell envelope. J Bacteriol 177: 2451–2459.
Lemaire M, Miras I, Gounon P & Beguin P (1998) Identification of a region responsible for binding to the cell wall within the S-layer protein of Clostridium thermocellum. Microbiology 144: 211–217.
Linder MB (2009) Hydrophobins: proteins that self assemble at interfaces. Curr Opin Colloid Interface Sci 14: 356–363.
Lopéz AE, Moreno-Flores S, Pum D, Sleytr UB & Toca-Herrera JL (2011) Surface dependence of protein nanocrystal formation. Small 6: 396–403.
López AE, Pum D, Sleytr UB & Toca-Herrera JL (2011) Influence of surface chemistry and protein concentration on the adsorption rate and S-layer crystal formation. Phys Chem Chem Phys 13: 11905–11913.
Lortal S, van Heijenoort J, Gruber K & Sleytr UB (1992) S-layer of Lactobacillus helveticus ATCC 12046: isolation, chemical characterization and re-formation after extraction with lithium chloride. J Gen Microbiol 138: 611–618.
Lortal S, Rouault A, Cesselin B & Sleytr UB (1993) Paracrystalline surface layers of dairy propionibacteria. Appl Environ Microbiol 59: 2369–2374.
Mader C, Kúpcú S, Sára M & Sleytr UB (1999) Stabilizing effect of an S-layer on liposomes towards thermal or mechanical stress. Biochim Biophys Acta 1418: 106–116.
Mader C, Küpcü S, Sleytr UB & Sára M (2000) S-layer-coated liposomes as a versatile system for entrapping and binding target molecules. *Biochim Biophys Acta* 1463: 142–150.

Mader C, Huber C, Moll D, Sleytr UB & Sára M (2004) Interation of the crystalline bacterial cell surface layer protein SbsB and the secondary cell wall polymer of *Geobacillus stearothermophilus* PV72 assessed by real-time surface plasmon resonance biosensor technology. *J Bacteriol* 186: 1758–1768.

Malcolm AJ, Messner P, Sleytr UB, Smith RH & Unger FM (1993a) Crystalline bacterial cell surface layers (S-layers) as combined carrier/adjuvants for conjugated vaccines. *Immobilized Macromolecules: Application Potentials* (Sleytr UB, Messner P, Pum D & Sára M, eds), pp. 195–207. Springer-Verlag, London, UK.

Malcolm AJ, Best MW, Szarka RJ, Mosleh Z, Unger FM, Messner P & Sleytr UB (1993b) Surface layers of *Bacillus alvei* as a carrier for a *Streptococcus pneumoniae* conjugate vaccine. *Bacterial Paracrystalline Surface Layers*, Vol. 252 (Beveridge J & Koval SF, eds), pp. 219–233. Plenum Press, New York, NY.

Martinez MG, Prado Acosta M, Candurra NA & Ruzal SM (2012) S-layer proteins of *Lactobacillus acidophilus* inhibits JUNV infection. *Biochem Biophys Res Commun* 422: 590–595.

Maslyuk VV, Mertig I, Bredow T, Mertig M, Vyalikh DV & Molodtsov SL (2008) Electronic structure of bacterial surface protein layers. *Phys Rev B* 77: 45419.

May A, Puszthaelyi T, Hoffmann N, Fischer RJ & Bahl H (2006) Mutagenesis of conserved charged amino acids in SLH domains of *Thermoanaerobacterium thermosulfurigenes* EM1 affects attachment to cell wall sacculi. *Arch Microbiol* 185: 263–269.

Mertig M, Kirsch R, Pompe W & Engelhardt H (1999) Fabrication of highly oriented nanocluster arrays by biomolecular templating. *Eur Phys J D* 9: 45–48.

Mescher MF & Strominger JL (1976) Purification and characterization of a prokaryotic glucoprotein from the cell envelope of *Thermoproteus neutrophilus*. *Structure and Biochemistry*, Vol. 125. VCH Publishers, New York, NY.

Mescher MF & Strominger JL (1976) Purification and characterization of a prokaryotic glucoprotein from the cell envelope of *Thermoproteus neutrophilus*. *Structure and Biochemistry*, Vol. 125. VCH Publishers, New York, NY.

Mesner P, Unger FM & Sleytr UB (1992a) Characterization of the ultrastructure and the self-assembly of the surface layer of *Bacillus stearothermophilus* strain NRS 2004/3a. *J Ultrastruct Mol Struct Res* 97: 73–88.

Mesner P, Pum D, Sára M, Stetter KO & Sleytr UB (1986b) Ultrastructure of the cell envelope of *Thermoproteus tenax* and *Thermoproteus neutrophilus*. *J Bacteriol* 166: 1046–1054.

Mesner P, Mazid MA, Unger FM & Sleytr UB (1992) Artificial antigens. Synthetic carbohydrate hapten immobilized on crystalline bacterial surface layer glycoproteins. *Carbohydr Res* 233: 175–184.

Mesner P, Unger FM & Sleytr UB (1996) Vaccine development based on S-layer technology. *Crystalline Bacterial Cell Surface Proteins* (Sleytr UB, Messner P, Pum D & Sára M, eds), pp. 161–173. R.G. Landes, Academic Press, Austin, TX.

Messner P, Steiner K, Zarscher K & Schäffer C (2008) S-layer nanoglycobiochemistry of bacteria. *Carbohydr Res* 343: 1934–1951.

Messner P, Egelseer EM, Sleytr UB & Schäffer C (2009) Bacterial surface layer glycoproteins and ‘non-classical’ secondary cell wall polymers. *Microbial Glycobiology: Structures, Relevance and Applications* (Moran A, Holst O, Brennan PJ & von Itzstein M, eds), pp. 109–128. Elsevier, San Diego.

Messner P, Schäffer C, Egelseer EM & Sleytr UB (2010) Occurrence, structure, chemistry, genetics, morphogenesis, and function of S-layers. *Prokaryotic Cell Wall Compounds – Structure and Biochemistry*, Vol. Chapter 2 (König H, Claus H & Varma A, eds), pp. 53–109. Springer, Heidelberg, Germany.

Messner P, Schäffer C & Kosma P (2013) Bacterial cell-envelope glycoconjugates. *Adv Carbohydr Chem Biochem* 69: 209–272.

Mignot T, Mesnage S, Couture-Tosi E, Mock M & Fouet A (2002) Developmental switch of S-layer protein synthesis in *Bacillus anthracis*. *Mol Microbiol* 43: 1615–1627.
Moll D, Huber C, Schlegel B, Pum D, Sleytr UB & Sára M (2002) S-layer-streptavidin fusion proteins as template for nanopatterned molecular arrays. *P Natl Acad Sci USA* **99**: 14646–14651.

Moreno-Flores S, Kasry A, Butt HJ, Vavilala C, Schmittel M, Pum D, Sleytr UB & Toca-Herrera JL (2008) From native to non-native two-dimensional protein lattices through underlying hydrophilic/hydrophobic nanopatterns. *Angew Chem Int Ed* **47**: 4707–4710.

Müller DJ, Baumeister W & Engel A (1996) Conformational change of the hexagonally packed intermediate layer of *Deinococcus radiodurans* monitored by atomic force microscopy. *J Bacteriol* **178**: 3025–3030.

Müller DJ, Baumeister W & Engel A (1999) Controlled unzipping of a bacterial surface layer with atomic force microscopy. *P Natl Acad Sci USA* **96**: 13170–13174.

Nabarro FRN & Harris WF (1971) Presence and function of disclinations in surface coats of unicellular organisms. *Nature* **232**: 423.

Neubauer A, Pum D & Sleytr UB (1993) An amperometric enzyme sensor for sucrose based on isoporous crystalline protein membranes as immobilization matrix. *Anal Lett* **26**: 1347–1360.

Neubauer A, Hödl C, Pum D & Sleytr UB (1994) A multistep enzyme sensor for sucrose based on S-layer microparticles as immobilization matrix. *Anal Lett* **27**: 849–865.

Neubauer A, Pum D & Sleytr UB (1996) Fibre-optic glucose biosensor using enzyme membranes with 2-D crystalline structure. *Biosens Bioelectron* **11**: 317–325.

Ni Eidhin DB, O’Brien JB, McCabe MS, Athie-Morales V & Kelleher DP (2008) Active immunization of hamsters against *Clostridium difficile* infection using surface layer protein. *FEMS Immunol Med Microbiol* **52**: 207–218.

Niokelis DS, Hianik T & Krull UJ (1999) Biosensors based on thin lipid films and liposomes. *Electroanalysis* **11**: 7–15.

Nomellini JF, Küpcü S, Sleytr UB & Smit J (1997) Factors controlling in vitro recrystallization of the *Caulobacter crescentus* paracrystalline S-layer. *J Bacteriol* **179**: 6349–6354.

Nomellini JF, Duncan G, Dorocic IR & Smit J (2007) S-layer mediated display of the IgG-binding domain of streptococcal Protein G on the surface of *Caulobacter crescentus* – development of an immuno-active reagent. *Appl Environ Microbiol* **73**: 3245–3253.

Nomellini JF, Li C, Lavallee D, Shanina I, Cavacini LA, Horwitz MS & Smit J (2010) Development of an HIV-1 specific mimic using *Caulobacter crescentus* S-layer mediated display of CD4 and MIP1alpha. *PLoS One* **5**: e10366.

Norville JE, Kelly DF, Knight TF Jr, Belcher AM & Walz T (2007) A projection map of the S-layer protein sbpA obtained with trehalose-embedded monolayer crystals. *J Struct Biol* **160**: 313–323.

Novotny R, Pfössl A, Messner P & Schäffer C (2004) Genetic organization of chromosomal S-layer glycan biosynthesis loci of Bacillaceae. *Glycoconjug J* **20**: 435–447.

Nußer E, Hartmann E, Allmeier H, König H, Paul G & Stetter KO (1988) A glycoprotein surface layer covers the pseudomurein sacculus of the extreme thermophile *Methanothermus fervidus*. *Crystalline Bacterial Cell Surface Layers* (Sleytr UB, Messner P, Pum D & Sára M, eds), pp. 21–25. Springer-Verlag, Berlin.

O’Brien JB, McCabe MS, Athie-Morales V, McDonald GS, Ni Eidhin DB & Kelleher DP (2005) Passive immunisation of hamsters against *Clostridium difficile* infection using antibodies to surface layer proteins. *FEMS Microbiol Lett* **246**: 199–205.

O’Connor E & Shand R (2002) Halocins and sulfolobicins: the emerging story of archaeal protein and peptide antibiotics. *J Ind Microbiol Biotechnol* **28**: 23–31.

Patel J, Zhang Q, McKay RM, Vincent R & Xu Z (2010) Genetic engineering of *Caulobacter crescentus* for removal of cadmium from water. *Appl Biochem Biotechnol* **160**: 232–243.

Pavkov T, Egelseer EM, Teszarz M, Svergun DI, Sleytr UB & Keller W (2008) The structure and binding behavior of the bacterial cell surface layer protein SbsC. *Structure* **16**: 1226–1237.

Pavkov-Keller T, Howorka S & Keller W (2011) The structure of bacterial S-layer proteins. *Molecular Assembly in Natural and Engineered Systems*, Vol. 103 (Howorka S, ed.), pp. 73–130. Elsevier Academic Press Inc., Burlington.

Pechine S, Deneve C, Le Monnier A, Hoys S, Janoir C & Collignon A (2011) Immunization of hamsters against *Clostridium difficile* infection using the Cwp84 protease as an antigen. *FEMS Immunol Med Microbiol* **63**: 73–81.

Penfold J, Thomas RK & Shen HH (2012) Adsorption and self-assembly of biosurfactants studied by neutron reflectivity and small angle neutron scattering: glycolipids, lipopeptides and proteins. *Soft Matter* **8**: 578–591.

Petersen BO, Sára M, Mader C et al. (2008) Structural characterization of the acid-degraded secondary cell wall polymer of *Geobacillus stearothermophilus* PV72/p2. *Carbohydr Res* **343**: 1346–1358.

Picher MM, Küpcü S, Huang CJ, Dostalek J, Pum D, Sleytr UB & Erly P (2013) Nanobiotechnology advanced antifouling surfaces for the continuous electrochemical monitoring of glucose in whole blood using a lab-on-a-chip. *Lab Chip* **13**: 1780–1789.

Pleschberger M, Neubauer A, Egelseer EM, Weigert S, Lindner B, Sleytr UB, Muyldermans S & Sára M (2003) Generation of a functional monomolecular protein lattice consisting of an S-layer fusion protein comprising the variable domain of a camel heavy chain antibody. *Bioconjug Chem* **14**: 440–448.

Pleschberger M, Saerens D, Weigert S, Sleytr UB, Muyldermans S, Sára M & Egelseer EM (2004) An S-layer heavy chain camel antibody fusion protein for generation of a nanopatterned sensing layer to detect the prostate-specific antigen by surface plasmon resonance technology. *Bioconjug Chem* **15**: 664–671.

Pleschberger M, Hildner F, Rünzler D, Gelbmann N, Mayer HF, Sleytr UB & Egelseer EM (2013) Identification of a novel gene cluster in the upstream region of the S-layer...
gene sbpA involved in cell wall metabolism of Lysinibacillus sphaericus CCM 2177 and characterization of the recombinantly produced autolysin and pyruvyl transferase. 

Arch Microbiol 195: 323–337.

Pollmann K, Raff J, Merroun M, Fahmy K & Selenska-Pobell S (2006) Metal binding by bacteria from uranium mining waste piles and its technological applications. Biotechnol Adv 24: 58–68.

Poobalane S, Thompson KD, Arlo D, Verjan N, Han HJ, Jeney G, Hirono I, Aoki T & Adams A (2010) Production and efficacy of an Aeromonas hydrophila recombinant S-layer protein vaccine for fish. Vaccine 28: 3540–3547.

Posch G, Andrukhov O, Vinogradov E, Lindner B, Messner P, Holst O & Schäffer C (2013) Structure and immunogenicity of the rough-type lipopolysaccharide from the periodontal pathogen Tannerella forsythia. Clin Vaccine Immunol 20: 945–953.

Powell MF & Newman MJ (1995) Vaccine Design: The Subunit and Adjuvant Approach. Plenum Press, New York, NY.

Prangishvili D, Holz I, Stieger E, Nickell S, Kristjansson JK & Zillig W (2000) Sulfolobocins, specific proteinaceous toxins produced by strains of the extremely thermophilic archaeal genus Sulfolobus. J Bacteriol 182: 2985–2988.

Pum D & Sleytr UB (1994) Large-scale reconstruction of crystalline bacterial surface layer proteins at the air-water interface and on lipids. Thin Solid Films 244: 882–886.

Pum D & Sleytr UB (1995a) Monomolecular reassembly of a crystalline bacterial cell surface layer (S layer) on untreated and modified silicon surfaces. Supramol Sci 2: 193–197.

Pum D & Sleytr UB (1995b) Anisotropic crystal growth of the S-layer of Bacillus sphaericus CCM 2177 at the air/water interface. Colloids Surf A Physicochem Eng Asp 102: 99–104.

Pum D & Sleytr UB (2009) S-layer proteins for assembling ordered nanoparticle arrays. Nanobioelectronics – For Electronics, Biology, and Medicine (Offenhäuser A & Rinaldi R, eds), pp. 167–180. Springer, New York.

Rothbauer M, Kupcu S, Sticker D, Sleytr UB & Ertl P (2013) Biophysical characterization of the entire bacterial surface layer protein SbsB and its two distinct functional domains. J Bacteriol 175: 5296–5303.

Salamitou S, Lemaire M, Fujino T, Ohayon H, Gounon P, Jorgenson J, Chen J, Jonoska N & Rozenberg G, ed.), pp. 6865–6873.

Salamitou S, Raynaud O, Lemaire M, Coughlan M, Beguin P & Aubert JP (1997) Biophysical characterization of the entire bacterial surface layer protein ShsB and its two distinct functional domains. J Biol Chem 272: 5207–5215.

Salamitou S, Raynaud O, Lemaire M, Coughlan M, Beguin P & Aubert JP (1994a) ORF3p, a protein carrying a receptor for the docking sequence borne by the catalytic components of the cellulose. J Bacteriol 176: 2828–2834.

Sára M & Sleytr UB (1987a) Charge distribution on the S-layer of Bacillus stearothermophilus NRS 1536/3c and importance.
of charged groups for morphogenesis and function. J Bacteriol 169: 2804–2809.
Sára M & Sleytr UB (1987b) Molecular sieving through S layers of Bacillus stearothermophilus strains. J Bacteriol 169: 4092–4098.
Sára M & Sleytr UB (1987c) Production and characteristics of ultrafiltration membranes with uniform pores from two-dimensional arrays of proteins. J Membr Sci 33: 27–49.
Sára M & Sleytr UB (1988) Membrane Biotechnology: two-dimensional Protein Crystals for Ultrafiltration Purposes. Biotechnology, Vol. 6b (Rehm HJ, ed.), pp. 615–636. VCH, Weinheim.
Sára M & Sleytr UB (1989) Use of regularly structured bacterial cell envelope layers as matrix for the immobilization of macromolecules. Appl Microbiol Biotechnol 30: 184–189.
Sára M & Sleytr UB (1993) Relevance of charged groups for the integrity of the S-layer from Bacillus coagulans E38-66 and for molecular interactions. J Bacteriol 175: 2248–2254.
Sára M & Sleytr UB (1994) Comparative studies of S-layer proteins from Bacillus stearothermophilus strains expressed during growth in continuous culture under oxygen-limited and non-oxygen-limited conditions. J Bacteriol 176: 7182–7189.
Sára M, Kalsner I & Sleytr UB (1988a) Surface properties from the S-layer of Clostridium thermosaccharolyticum D120-70 and Clostridium thermohydrosulfuricum L111-69. Arch Microbiol 149: 527–533.
Sára M, Manigley C, Wolf G & Sleytr UB (1988b) Isoporous ultrafiltration membranes from bacterial cell envelope layers. J Membr Sci 36: 179–186.
Sára M, Küpcü S & Sleytr UB (1989) Localization of the carbohydrate residue of the S-layer glycoprotein from Clostridium thermohydrosulfuricum L111-69. Arch Microbiol 151: 416–420.
Sára M, Moser-Thier K, Kainz U & Sleytr UB (1990) Characterization of S-layers from mesophilic Bacillaceae and studies on their protective role towards muramidases. Arch Microbiol 153: 209–214.
Sára M, Pum D & Sleytr UB (1992) Permeability and charge-dependent adsorption properties of the S-layer lattice from Bacillus coagulans E38-66. J Bacteriol 174: 3487–3493.
Sára M, Küpcü S, Weiner C, Weigert S & Sleytr UB (1993) S-layers as immobilization and affinity matrices. Advances in Bacterial Paracrystalline Surface Layers, Vol. 252 (Beveridge TJ & Koval SF, eds), pp. 195–204. Plenum Press, New York & London.
Sára M, Küpcü S & Sleytr UB (1996a) Biotechnological applications of S-layers. Crystalline Bacterial Cell Surface Proteins (Sleytr UB, Messner P, Pum D & Sára M, eds), pp. 133–159. R. G. Landes Company and Academic Press, Inc., Austin, TX.
Sára M, Kuen B, Mayer HF, Mandl F, Schuster KC & Sleytr UB (1996b) Dynamics in oxygen-induced changes in S-layer protein synthesis from Bacillus stearothermophilus PV72 and the S-layer-deficient variant T5 in continuous culture and studies of the cell wall composition. J Bacteriol 178: 2108–2117.
Sára M, Egelseer EM, Dekitsch C & Sleytr UB (1998) Identification of two binding domains, one for peptidoglycan and another for a secondary cell wall polymer, on the N-terminal part of the S-layer protein SbsB from Bacillus stearothermophilus PV72/p2. J Bacteriol 180: 6780–6783.
Sára M, Pum D, Huber C, Ilk N, Plescherger M & Sleytr UB (2006a) Nanoscale patterning of S-layer proteins as a natural self-assembly system. Biological and Pharmaceutical Nanomaterials Nanotechnologies for the Life Sciences, Vol. 2 (Kumar C, ed.), pp. 219–252. Wiley-VCH, Weinheim, Germany.
Sára M, Egelseer EM, Huber C, Ilk N, Plescherger M, Pum D & Sleytr UB (2006b) S-layer proteins: potential application in nano(bio)technology. Microbial Bionanotechnology: Biological Self-Assembly Systems and Biopolymer-Based Nanostructures (Bernd Rehm MU, ed.), pp. 307–338. Horizon Bioscience, Palmerston North, New Zealand.
Saxton WO & Baumeister W (1982) The correlation averaging of a regularly arranged bacterial cell envelope protein. J Microsc 127: 127–138.
Saxton WO, Baumeister W & Hahn M (1984) Three-dimensional reconstruction of imperfect two-dimensional crystals. Ultramicroscopy 13: 57–70.
Schäffer C & Messner P (2004) Surface-layer glycoproteins: an example for the diversity of bacterial glycosylation with promising impacts on nanobiotechnology. Glycobiology 14: 31R–42R.
Schäffer C, Kählkig H, Christian R, Schulz G, Zayni S & Messner P (1999) The diacetamidideoxyxuronic-acid-containing glycan chain of Bacillus stearothermophilus NRS 2004/3a represents the secondary cell-wall polymer of wild-type B. stearothermophilus strains. Microbiology 145: 1575–1583.
Schäffer C, Novotny R, Küpcü S, Zayni S, Scheberl A, Friedmann J, Sleytr UB & Messner P (2007) Novel biocatalysts based on S-layer self-assembly of Geobacillus stearothermophilus NRS 2004/3a: a nanobiotechnological approach. Small 3: 1549–1559.
Scherrer R & Gerhard P (1971) Molecular sieving by the Bacillus megatherium cell wall and protoplast. J Bacteriol 107: 718–735.
Scholz H, Hummel S, Witte A, Lubitz W & Kuen B (2000) The transposable element IS4712 prevents S-layer gene (sbsA) expression in Bacillus stearothermophilus and also affects the synthesis of altered surface layer proteins. Arch Microbiol 174: 97–103.
Scholz HC, Riedmann E, Witte A, Lubitz W & Kuen B (2001) S-layer variation in Bacillus stearothermophilus PV72 is based on DNA rearrangements between the chromosome and the naturally occurring megaplasmids. J Bacteriol 183: 1672–1679.
Schrems A, Kibrom A, Küpcü S, Kiene E, Sleytr UB & Schuster B (2011a) Bilayer lipid membrane formation on a chemically modified S-layer lattice. Langmuir 27: 3731–3738.

Schrems A, Larisch VD, Stanetty C, Dutter K, Damiai S, Sleytr UB & Schuster B (2011b) Liposome fusion on proteinaceous S-layer lattices triggered via b-diketone ligand–europium(III) complex formation. Soft Matter 7: 5514–5518.

Schrems A, Larisch VD, Sleytr UB, Hohenegger M, Lohner K & Schuster B (2013) Insertion of an anionic analogue of the antimicrobial peptide PGLa in lipid architectures including S-layer supported lipid bilayers. Curr Nanosci 9: 262–270.

Schultze-Lam S & Beveridge TJ (1994a) Physicochemical characteristics of the mineral-forming S-layer from the cyanobacterium Synechococcus strain GL24. Can J Microbiol 40: 216–233.

Schultze-Lam S & Beveridge TJ (1994b) Nucleation of celestite and strontianite on a cyanobacterial S-layer. Appl Environ Microbiol 60: 447–453.

Schultze-Lam S, Harauz G & Beveridge TJ (1992) Participation of a cyanobacterial S-layer in fine-grain mineral formation. J Bacteriol 174: 7971–7981.

Schuster B (2005) Biomimetic design of nano-patterned membranes. NanoBiotecnology 1: 153–164.

Schuster B & Sleytr UB (2000) S-layer-supported lipid membranes. J Biotechnol 74: 233–254.

Schuster B & Sleytr UB (2002a) The effect of hydrostatic pressure on S-layer-supported lipid membranes. Biochim Biophys Acta 1563: 29–34.

Schuster B & Sleytr UB (2002b) Single channel recordings of alpha-hemolysin reconstituted in S-layer-supported lipid bilayers. Bioelectrochemistry 55: 5–7.

Schuster B & Sleytr UB (2005) 2D-Protein Crystals (S-Layers) as Support for Lipid Membranes. Advances in Planar Lipid Bilayers and Liposomes, Vol. 1 (Tien TH & Ottova A, eds), pp. 247–293. Elsevier Science, Amsterdam, The Netherlands.

Schuster B & Sleytr UB (2006) Biomimetic S-layer supported lipid membranes. Curr Nanosci 2: 143–152.

Schuster B & Sleytr UB (2009a) Tailor-made crystalline structures of truncated S-layer proteins on heteropolysaccharides. Soft Matter 5: 334–341.

Schuster B & Sleytr UB (2009b) Composite S-layer lipid structures. J Struct Biol 168: 207–216.

Schuster B & Sleytr UB (2013) Nanotechnology with S-Layer Proteins. Protein Nanotechnology: Protocols, Instrumentation and Applications, 2nd edn, Vol. 996 (Gerrard JA, ed.), pp. 153–175. Humana Press, Springer Science + Business Media, New York.

Schuster B, Pum D & Sleytr UB (1997) Planar supported lipid membranes. FEMS Microbiol Rev 20: 159–162.

Schuster B, Pum D & Sleytr UB (1998a) Voltage clamp studies on S-layer-supported tetraether lipid membranes. Biochim Biophys Acta 1369: 51–60.

Schuster B, Pum D, Braha O, Bayley H & Sleytr UB (1998b) Self-assembled α-hemolysin pores in an S-layer-supported lipid bilayer. Biochim Biophys Acta 1370: 280–288.

Schuster B, Sleytr UB, Diederich A, Bahr G & Winterhalter M (1999) Probing the stability of S-layer-supported planar lipid membranes. Eur Biophys J 28: 583–590.

Schuster B, Pum D, Sára M, Braha O, Bayley H & Sleytr UB (2001) S-layer ultrafiltration membranes: a new support for stabilizing functionalized lipid membranes. Langmuir 17: 499–503.

Schuster B, Gufler PC, Pum D & Sleytr UB (2003a) Interplay of phospholipase A2 with S-layer-supported lipid monolayers. Langmuir 19: 3393–3397.

Schuster B, Weigert S, Pum D, Sára M & Sleytr UB (2003b) New method for generating tetraether lipid membranes on porous supports. Langmuir 19: 2392–2397.

Schuster B, Gufler PC, Pum D & Sleytr UB (2004) S-layer proteins as supporting scaffolds for functional lipid membranes. IEEE Trans Nanobioscience 3: 16–21.

Schuster B, Györvary E, Pum D & Sleytr UB (2005) Nanotechnology with S-layer proteins. Methods Mol Biol 300: 101–123.

Schuster B, Pum D, Sára M & Sleytr UB (2006) S-Layer proteins as key components of a versatile molecular construction kit for biomedical Nanotechnology. Mini Rev Med Chem 6: 909–920.

Shenton W, Pum D & Sleytr UB (2008) S-layer stabilized lipid membranes. Biointerphases 3: FA3–FA11.

Schuster B, Kepplinger C & Sleytr UB (2010) Biomimetic S-layer stabilized lipid membranes. Biomimetics in Biophysics: Model Systems, Experimental Techniques and Computation (Toca-Herrera JL, ed.), pp. 1–12. Research Signpost, Kerala, India.

Schuster D, Küpcü S, Belton DJ, Perry CC, Stöger-Pollach M, Sleytr UB & Pum D (2013) Construction of silica enhanced S-layer protein cages. Acta Biomater 9: 5689–5697.

Shenton W, Pum D, Sleytr UB & Mann S (1997) Synthesis of CdS superlattices using self-assembled bacterial S-layers. Nature 389: 585–587.

Shin SH, Chung S, Sanii B, Comolli LR, Bertozzi CR & De Yoreo JJ (2012) Direct observation of kinetic traps associated with structural transformations leading to multiple pathways of S-layer assembly. P Natl Acad Sci USA 109: 12968–12973.

Shin S-H, Comolli LR, Tschelielessig R, Wang C, Nam KT, Hexemer A, Siegerist CE, De Yoreo JJ & Bertozzi CR (2013) Self-assembly of S-Bilayers, a step toward expanding the dimensionality of S-layer assemblies. ACS Nano 7: 4946–4953.

Simon B, Nomellini JF, Chiou P, Binale W, Thornton J, Smit J & Ara M & Sleytr UB (2006) S-Layer Stabilized Lipid Membranes. Methods Mol Biol 300: 101–123.

Sleytr UB (1975) Heterologous reattachment of regular arrays of glycoproteins on bacterial surfaces. Nature 257: 400–402.
Sleytr UB (1976) Self-assembly of the hexagonally and tetragonally arranged subunits of bacterial surface layers and their reattachment to cell walls. *J Ultrastruct Res* **55**: 360–377.

Sleytr UB (1978) Regular arrays of macromolecules on bacterial cell walls: structure, chemistry, assembly, and function. *Int Rev Cytol* **53**: 1–62.

Sleytr UB & Beveridge TJ (1999) Bacterial S-layers. *Trends Microbiol* **7**: 253–260.

Sleytr UB & Glaeuer AM (1975) Analysis of regular arrays of subunits on bacterial surfaces: evidence for a dynamic process of assembly. *J Ultrastruct Res* **50**: 103–116.

Sleytr UB & Glaeuer AM (1976) Ultrastructure of the cell walls of two closely related clostridia that possess different regular arrays of surface subunits. *J Bacteriol* **126**: 886–882.

Sleytr UB & Messner P (1983) Crystalline surface layers on bacteria. *Annu Rev Microbiol* **37**: 311–339.

Sleytr UB & Messner P (1989) Self-assembly of crystalline bacterial cell surface layers (S-layers). *Electron Microscopy of Subcellular Dynamics* (Plattner H, ed.), pp. 13–31. CRC Press, Boca Raton, Florida.

Sleytr UB & Messner P (2009) Crystalline bacterial cell surface layers (S-layers). *Encyclopedia of Microbiology*, Vol. 1 (Schaechter M, ed.), pp. 89–98. Academic Press/Elsevier Science, San Diego.

Sleytr UB & Plopperger R (1980) The dynamic process of assembly of two-dimensional arrays of macromolecules on bacterial cell walls. *Electron Microscopy at Molecular Dimensions* (Baumeister W & Vogell W, eds), pp. 36–47. Springer-Verlag, Berlin, Heidelberg, New York.

Sleytr UB & Sára M (1986) Ultrafiltration membranes with uniform pores from crystalline bacterial cell envelope layers. *Appl Microbiol Biotechnol* **25**: 83–90.

Sleytr UB & Sára M (1988) Structure with membranes having continuous pores. US Patent Number 4,752,392.

Sleytr UB & Sára M (1997) Bacterial and archaeal S-layer proteins: structure-function relationships and their biotechnological applications. *Trends Biotechnol* **15**: 20–26.

Sleytr UB & Thorne KJ (1976) Chemical characterization of the regularly arranged surface layers of *Clostridium thermosaccharolyticum* and *Clostridium thermohydrodsulphuricum*. *J Bacteriol* **126**: 377–383.

Sleytr U, Adam H & Klaushofer H (1968) Die Feinstruktur der Zellwandoberflächen von zwei thermophilen Clostridienarten, dargestellt mit Hilfe der Gefrierätztechnik. *Mikroskopie* (Wien) **23**: 1–10.

Sleytr UB, Adams H & Klaushofer H (1969) Die Feinstruktur der Konidien von Aspergillus niger, V. Tiegh., dargestellt mit Hilfe der Gefrierätztechnik. *Mikroskopie* **25**: 320–331.

Sleytr UB, Sára M, Küpcü Z & Messner P (1986) Structural and chemical characterization of S-layers of selected strains of *Bacillus stearothermophilus* and *Desulfotomaculum nigrificans*. *Arch Microbiol* **146**: 19–24.

Sleytr UB, Messner P & Pum D (1988a) Analysis of crystalline bacterial surface layers by freeze-etching, metal shadowing, negative staining and ultrathin sectioning. *Electron Microsc in Microbiology*, Vol. 20 (Mayer F, ed.), pp. 29–60. Academic Press, Ltd., London.

Sleytr UB, Messner P, Pum D & Sára M (1988b) *Crystalline Bacterial Cell Surface Layers*. pp. 193. Springer, Berlin.

Sleytr UB, Mundt W & Messner P (1989) Pharmazeutische Struktur mit an Proteinträgern gebundenen Wirkstoffen. EP O 306 473 B1.

Sleytr UB, Mundt W & Messner P (1991) Immunogenic composition containing ordered carriers. US Patent Nr 5,043,158.

Sleytr UB, Messner P, Pum D & Sára M (1993a) Crystalline bacterial cell surface layers. *Mol Microbiol* **10**: 911–916.

Sleytr UB, Messner P, Pum D & Sára M (1993b) *Immovised Macromolecules: Application Potentials*. Springer-Verlag, London, UK.

Sleytr UB, Messner P, Pum D & Sára M (1996a) Crystalline surface layers on eubacteria and archaeobacteria. *Crystalline Bacterial Cell Surface Proteins* (Sleytr UB, Messner P, Pum D & Sára M, eds), pp. 211–225. R. G. Landes Company and Academic Press, Inc., Austin, TX.

Sleytr UB, Messner P, Pum D & Sára M (1996b) Occurrence, location, ultrastructure and morphogenesis of S layers. *Crystalline Bacterial Cell Surface Proteins (S Layers)* (Sleytr UB, Messner P, Pum D & Sára M, eds), pp. 5–33. R. G. Landes Company and Academic Press, Inc., Austin, TX.

Sleytr UB, Messner P, Pum D & Sára M (1999) Crystalline bacterial cell surface layers (S-layers): from supramolecular cell structure to biomimetics and nanotechnology. *Angew Chem Int Ed* **38**: 1034–1054.

Sleytr UB, Sara M, Mader C, Schuster B & Unger FM (2000) Use of secondary cell wall polymer of procaryotic microorganisms. EP 1285003A1.

Sleytr UB, Pum D, Schuster B & Sára M (2001) Molecular nanotechnology and nanobiotechnology with two-dimensional protein crystals (S-layers). *Nano-Surface Chemistry* (Rosoff M, ed.), pp. 333–389. Marcel Dekker, New York, Basle.

Sleytr UB, Sára M, Pum D, Schuster B, Messner P & Schäffer C (2002) Self-assembly protein systems: microbial S-layers. *Biopolymers Polyamides and Complex Proteinaceous Materials I*, Vol. 1st edn. (Steinbüchel A & Fahnstock SR, eds), pp. 285–338. Wiley-VCH, Weinheim.

Sleytr UB, Pum D, Sára M & Schuster B (2004) Molecular nanotechnology and nanobiotechnology with 2-D protein crystals. *Encyclopedia of Nanoscience and Nanotechnology*, Vol. 5 (Nalwa HS, ed.), pp. 693–702. Academic Press, San Diego.

Sleytr UB, Sára M, Pum D & Schuster B (2005) Crystalline bacterial cell surface layers (S-layers): a versatile self-assembly system. *Supramolecular Polymers* (Ciferri A, ed.), pp. 583–612. Taylor & Francis Group, Boca Raton.

Sleytr UB, Sara M, Mader C, Schuster B & Unger FM (2006) Use of secondary cell wall polymer of procaryotic microorganisms. US Patent Number 7,125,707 B2.
Slettrr UB, Egelsem EM, Ilk N, Pum D & Schuster B (2007a) S-layers as a basic building block in a molecular construction kit. FEBS J 274: 323–334.
Slettrr UB, Huber C, Ilk N, Pum D, Schuster B & Egelsem EM (2007b) S-Layers as a tool kit for nanobiotechnological applications. FEMS Microbiol Lett 267: 131–144.
Slettrr UB, Egelsem EM, Ilk N, Messner P, Schiffer C, Pum D & Schuster B (2010) Nanobiotechnological applications of S-layers. Prokaryotic Cell Wall Compounds – Structure and Biochemistry (König H, Claus H & Varma A, eds), pp. 459–481. Springer, Heidelberg, Germany.
Slettrr UB, Schuster B, Egelsem EM, Pum D, Horejs CM, Tscheliessnig R & Ilk N (2011) Nanobiotechnology with S-layer proteins as building blocks. Progress in Molecular Biology and Translational Science, Molecular Assembly in Natural and Engineered Systems, Vol. 103 (Howorka S, ed.), pp. 277–352. Elsevier Academic Press Inc., Burlington.
Slettrr UB, Pum D, Egelsem EM, Ilk N & Schuster B (2013) S-layer proteins. Handbook of Biofunctional Surfaces (Knoll W, ed.), pp. 507–568. Pan Stanford Publishing, Singapore.
Smarda J, Smajs D, Komraska J & Krzyzanek V (2002) S-layers on cell walls of cyanobacteria. Micron 33: 257–277.
Smibert RM (1978) The genus Campylobacter. Anna Rev Microbiol 32: 673–709.
Smit J & Agabian N (1982) Cell surface patterning and morphogenesis; bioGenesis of a periodic surface array during Caulobacter development. J Cell Biol 95: 41–49.
Smit J & Todd WJ (1986) Colloidal gold labels for immunocytochemical analysis of microbes. Ultrastructure Techniques for Microorganisms (Aldrich HC & Todd WJ, eds), pp. 469–519. Plenum Press, New York.
Smit E, Oling F, Demel R, Martinez B & Pouwels PH (2001) The S-layer protein of Lactobacillus acidophilus ATCC 4356: identification and characterisation of domains responsible for S-protein assembly and cell wall binding. J Mol Biol 305: 245–257.
Smit E, Jager D, Martinez B, Tielen FJ & Pouwels PH (2002) Structural and functional analysis of the S-layer protein crystallisation domain of Lactobacillus acidophilus ATCC 4356: evidence for protein–protein interaction of two subdomains. J Mol Biol 324: 953–964.
Smith RH, Messner P, Lamontagne LR, Slettrr UB & Unger FM (1993) Induction of T-cell immunity to oligosaccharide antigens immobilized on crystalline bacterial surface layers (S-layers). Vaccine 11: 919–924.
Srinivasan N & Kumar S (2012) Ordered and disordered proteins as nanomaterial building blocks. Wiley Interdisc Rev Nanomed Nanobiotechnol 4: 204–218.
Steinem C & Janshoff A (2010) Multicomponent membranes on solid substrates: interfaces for protein binding. Curr Opin Colloid Interface Sci 15: 479–488.
Steiner K, Hanreich A, Kainz B, Hitchen PG, Dell A, Messner P & Schiffer C (2008) Recombinant glycans on an S-layer self-assembly protein: a new dimension for nanopatterned biomaterials. Small 4: 1728–1740.
Stetter KO (1999) Extremophiles and their adaptation to hot environments. FEBS Lett 452: 22–25.
Stimson E, Virji M, Makepeace K et al. (1995) Meningococcal pilin: a glycoprotein substituted with digalactosyl 2,4-diacetamido-2,4,6-trideoxyhexose. Mol Microbiol 17: 1201–1214.
Sturm E, Egelsem E, Sára M & Slettrr UB (1993) Can S-layers of Bacillaceae control the release of their own exoproteins? Advances in Bacterial Paracrystalline Surface Layers, Vol. 252 (Beveridge TJ & Koval SF, eds), pp. 297–301. Plenum Press, New York & London.
Sugawara M & Hirano A (2005) Design and application of planar bilayer lipid membranes containing biological ion channels for chemical sensing. Advances in Planar Lipid Bilayers and Liposomes, Vol. 1 (Ottova A & Tien TH, eds), pp. 221–245. Elsevier Science, Amsterdam, the Netherlands.
Sumper M, Berg E, Mengele R & Strobel I (1990) Primary structure and glycosylation of the S-layer protein of Haloferax volcanii. J Bacteriol 172: 7111–7118.
Szabo Z & Pohlschroder M (2012) Diversity and subcellular distribution of archaeal secreted proteins. Front Microbiol 3: 207.
Tang J, Ebner A, Huber C, Ilk N, Zhu R, Pastushenko V, Sara M & Hinterdorfer P (2007) High resolution atomic force microscopy imaging and single molecule force microscopy studies of S-layer-strep-tag I and S-layer-strep-tag II proteins. Biophys J Suppl S: 513A.
Tarlovsky Y, Fabian M, Solomaha E, Honsa E, Olson JS & Maresso AW (2013) A Bacillus anthracis S-layer homology protein that binds heme and mediates heme delivery to IsdC. J Bacteriol 195: 3503–3511.
Thomas S, Austin JW, McCubbin WD, Kay CM & Trust TJ (1992) Roles of structural domains in the morphology and surface anchoring of the tetragonal paracrystalline array of Aeromonas hydrophila. Biochemical characterization of the major structural domain. J Mol Biol 228: 652–661.
Thompson SA & Blaser MJ (2000) Pathogenesis of Campylobacter fetus infections. Campylobacter (Nachamkin I & Blaser MJ, eds), pp. 321–347. American Society for Microbiology, Washington, DC.
Thompson JB & Ferris FG (1990) Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. Geology 18: 995–998.
Thornley MJ, Glauser AM & Slettrr UB (1974) Structure and assembly of bacterial surface layers composed of regular arrays of subunits. Philos Trans R Soc Lond B Biol Sci 268: 147–153.
Tiefenauer L & Demarche S (2012) Challenges in the development of functional assays of membrane proteins. Materials 5: 2205–2242.
Tien TH & Ottova- Leitmanova A (2000) Membrane Biophysics: As Viewed From Experimental Bilayer Lipid Membranes. Elsevier, Amsterdam.
Toca-Herrera JL, Krastev R, Bosio V, Küpü S, Pum D, Fery A, Sára M & Slettrr UB (2005) Recrystallization of bacterial
S-layers on flat polyelectrolyte surfaces and hollow polyelectrolyte capsules. Small 1: 339–348.
Trojanowicz M (2001) Miniaturized biochemical sensing devices based on planar lipid membranes. Fresenius J Anal Chem 372: 246–260.
Trust TJ, Kay WW & Ishiguro EE (1983) Cell surface hydrophobicity and macrophage association of Aeromonas salmonicida. Curr Microbiol 9: 315–318.
Tschiggerl H, Casey JL, Parisi K, Foley M & Sleytr UB (2008a) Display of a peptide mimotope on a crystalline bacterial cell surface layer (S-layer) lattice for diagnosis of Epstein–Barr virus infection. Bioconjug Chem 19: 860–865.
Tschiggerl H, Breitwieser A, de Roo G, Verwoerd T, Schäffer C & Sleytr UB (2008b) Exploitation of the S-layer self-assembly system for site directed immobilization of enzymes demonstrated for an extremophilic laminarinase from Pyrococcus furiosus. J Biotechnol 133: 403–411.
Tsuboi A, Uchihi R, Tabata R, Takahashi Y, Hashiba H, Sasaki T, Yamagata H, Tsukagoshi N & Udaaka S (1986) Characterization of the genes coding for two major cell wall enzymes demonstrated for an extremophilic laminarinase from Pyrococcus furiosus. J Biotechnol 133: 403–411.
Uchida M, Harada T, Enkhtuya J, Kusumoto A, Kobayashi Y, Chiba S, Uchihashi R, Hashiba H, Sasaki T, Yamagata H, Tsukagoshi N & Udaaka S (1986) Characterization of the genes coding for two major cell wall proteins from protein-producing Bacillus brevis 47: complete nucleotide sequence of the outer wall protein gene. J Bacteriol 168: 365–373.
Uchida M, Harada T, Enkhtuya J, Kusumoto A, Kobayashi Y, Chiba S, Hashiba H, Sasaki T, Yamagata H, Tsukagoshi N & Udaaka S (1986) Characterization of the genes coding for two major cell wall proteins from protein-producing Bacillus brevis 47: complete nucleotide sequence of the outer wall protein gene. J Bacteriol 168: 365–373.
Vadillo-Rodríguez V, Busscher HJ, Norde W, de Vries J & van der Mei HC (2004) Dynamic cell surface hydrophobicity of Lactobacillus strains with and without surface layer proteins. J Bacteriol 186: 6647–6650.
Velasquez L & Dussan J (2009) Biosorption and bioaccumulation of heavy metals on dead and living biomass of Bacillus subtilis var. subtilis. J Hazard Mater 167: 713–716.
Viviani B, Gardoni F & Marinovich M (2007) Cytokines and neuronal ion channels in health and disease. Int Rev Neurol 82: 247–263.
Vökel D, Zimmermann K, Breitwieser A, Pabel S, Glatzel M, Scheiflinger F, Schwarz HP, Sára M, Sleytr UB & Dorner F (2003) Immunohemochronical detection of prion protein on dipsticks prepared with crystalline bacterial cell-surface layers. Transfusion 43: 1677–1682.
Völlenkle C, Weigert S, Ilk N, Egelseer E, Weber V, Loth F, Falkenhausen D, Sleytr UB & Sára M (2004) Construction of a functional S-layer fusion protein comprising an immunoglobulin G-binding domain for development of specific adsorbents for extracorporeal blood purification. Appl Environ Microbiol 70: 1514–1521.
Vyalikh DV, Danzenbächer S, Mertig M, Kirchner A, Pompe W, Dedkov YS & Molodtsov SL (2004) Electronic structure of regular bacterial surface layers. Phys Rev Lett 93: 238103.
Vyas SP, Subhedar R & Jain S (2006) Development and characterization of emulsomes for sustained and targeted delivery of an antiviral agent to liver. J Pharm Pharmacol 58: 321–326.
Walser PA, Anderson GP, Broozog Lee PA, Glaven RH, Liu JL, Bernstein RD, Zabetakis D, Johnson L, Czarnecki JM & Goldman ER (2012) Rugged single domain antibody detection elements for Bacillus anthracis spores and vegetative cells. PLoS One 7: e32801.
Wang YT, Oh SY, Hendrickx AP, Lunderberg JM & Schnecwind O (2013) Bacillus cereus G9241 S-layer assembly contributes to the pathogenesis of anthrax-like disease in mice. J Bacteriol 195: 596–605.
Weigert S & Sára M (1995) Surface modification of an ultrafiltration membrane with crystalline structure and studies on interactions with selected protein molecules. J Membr Biol 147: 143–159.
Weiner C, Sára M & Sleytr UB (1994a) Novel protein A affinity matrix prepared from two-dimensional protein crystals. Biotechnol Bioeng 43: 321–330.
Weiner C, Sára M, Dasgupta G & Sleytr UB (1994b) Affinity cross-flow filtration: purification of IgG with a novel protein A affinity matrix prepared from two-dimensional protein crystals. Biotechnol Bioeng 44: 55–65.
Wetzer B, Pfandler A, Györvary E, Pum D, Löschke M & Sleytr UB (1998) S-Layer reconstitution at phospholipid monolayers. Langmuir 14: 6899–6906.
Weygand M, Wetzer B, Pum D, Sleytr UB, Cuvillier N, Kjaer K, Howes PB & Löschke M (1999) Bacterial S-layer protein coupling to lipids: X-ray reflectivity and grazing incidence diffraction studies. Biophys J 76: 458–468.
Weygand M, Schalke M, Howes PB, Kjaer K, Friedmann J, Wetzer B, Pum D, Sleytr UB & Löschke M (2000) Coupling of protein sheet crystals (S-layers) to phospholipid monolayers. J Mater Chem 10: 141–148.
Weygand M, Kjaer K, Howes PB, Wetzer B, Pum D, Sleytr UB & Löschke M (2002) Structural reorganization of phospholipid headgroups upon recrystallization of an S-layer lattice. J Phys Chem B 106: 5793–5799.
Whitman WB, Coleman DC & Wiebe WJ (1998) Prokaryotes: the unseen majority. P Natl Acad Sci USA 95: 6578–6583.
Wildhaber I & Baumeister W (1987) The cell envelope of Thermoproteus Tenax: three-dimensional structure of the surface layer and its role in shape-maintenance. EMBO J 6: 1475–1480.
Wimley WC & Hristova K (2011) Antimicrobial Peptides; Successes, Challenges and Unanswered Questions. J Membr Biol 239: 27–34.