Improved Isolation of SlaA and SlaB S-layer proteins in *Sulfolobus acidocaldarius*

Pierre Simonin, Carine Lombard, Arnaud Huguet, Adrienne Kish

► To cite this version:

Pierre Simonin, Carine Lombard, Arnaud Huguet, Adrienne Kish. Improved Isolation of SlaA and SlaB S-layer proteins in *Sulfolobus acidocaldarius*. *Extremophiles*, 2020, 24, pp.673-680. 10.1007/s00792-020-01179-9 . mnhn-02862458

HAL Id: mnhn-02862458

<https://hal-mnhn.archives-ouvertes.fr/mnhn-02862458>

Submitted on 17 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title: Improved Isolation of SlaA and SlaB S-layer Proteins in *Sulfolobus acidocaldarius*

Authors and Affiliations: Pierre Simonin¹, Carine Lombard¹, Arnaud Huguet², and Adrienne Kish^{1*}

¹Muséum national d'Histoire naturelle, CNRS, Unité Molécules de Communication et Adaptation des Microorganismes UMR7245 MCAM, CP54, 57 rue Cuvier, 75005 Paris, France

²Sorbonne University, CNRS, EPHE, UMR 7619 METIS, 4, place Jussieu, Paris 75005, France

*Corresponding Author: Adrienne Kish, email: adrienne.kish@mnhn.fr

List of Abbreviations:

SDS = sodium dodecyl sulfate

SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis

HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

EDTA = Ethylenediaminetetraacetic acid

MeOH = Methanol

DCM = Dichloromethane

HPLC–APCI–MS = high performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

GDGT = glycerol dialkyl glycerol tetraethers

Pre-Print prior to peer review - see final version doi:10.1007/s00792-020-01179-9

Abstract:

The *Sulfolobus acidocaldarius* S-layer is composed of two main proteins: SlaA, which forms the ordered structure of the S-layer matrix, and SlaB, which supports and anchors the S-layer in the tetraether lipid membrane. Large batch-scale methods using starting culture volumes of 20-70 L have been developed for the isolation of the SlaA protein from the rest of the cell envelope. These methods rely on the thermotolerance of SlaA and its high resistance to detergents. SlaB, however, remains strongly associated in a large molecular weight aggregate with other cell envelope components, even after removal of the remaining cellular contents. Here we demonstrate a protocol for benchtop-scale (1-2 L) purification of S-layer proteins. Using this protocol, we were also able to identify for the first time the tetraether lipids strongly attached to SlaB.

Keywords: S-layer, cell wall, *Sulfolobus acidocaldarius*, Archaea, protein purification, SlaA, SlaB

Introduction:

The cell walls of extremophilic microorganisms provides both vital protection from potentially damaging agents, as well as an interface for interactions with both other organisms and abiotic elements in the external environment. Cell wall composition varies between different taxonomic groups, but most major phylogenetic groups of bacteria and archaea include cell walls containing proteinaceous surface layers (or S-layers) (Zhu et al. 2017). S-layers can in fact be the sole component of the cell wall for many extremophilic archaea.

S-layers are a self-assembling matrix of repeated protein monomers, often bearing post-translational modifications such as glycosylations, that can modify the interactions between cell surfaces and their surrounding biotic and abiotic environment. The symmetry of the S-layer is determined by the number of monomers composing the repeated structural unit: oblique (P2), square (P4), or hexagonal (P3 or P6). S-layer proteins are one of the most abundant cellular proteins, composing in excess of 10 % of total protein (Sleytr et al. 2011).

S-layers are known to have roles in structural support, cell adhesion, and virulence (Engelhardt 2007a). They have been shown to play key roles in the survival of extremophiles under stress conditions, including salinity and osmotic stress (Engelhardt 2007b; Guan et al. 2012; Im et al. 2013), metal stress (Chandramohan et al. 2018), radionuclides (Merroun et al. 2005; Reitz et al. 2015), thermal stress and mechanical stress (Mader et al. 1999) as well as enhancing UV resistance (Farci et al. 2017). S-layers have been harnessed for biotechnology applications ranging from materials science, to metal biomining and bioremediation, to drug delivery systems (Sleytr et al. 2011; Bartolomé et al. 2012; Sleytr et al. 2014; Ucisik et al. 2015).

S-layer proteins show little sequence conservation between the Bacteria and Archaea. Even within the Archaea there is wide variance in the S-layer protein primary sequences and post-translational modifications, as well as S-layer matrix symmetries, structures, and interactions with the cell membrane and other cell envelope components (Albers and Meyer 2011; Rodrigues-Oliveira et al. 2017). No complete crystal structure has been resolved to date for archaeal S-layer proteins. The lack of conservation inhibits the development of "universal" S-layer tools, such as universal S-layer recognition antibodies. Studies of S-layer proteins must therefore be tailored to each taxonomic group, often employing model species.

The hyperthermoacidophilic *Sulfolobus acidocaldarius* has served as a key model for studying the S-layers of archaea since its isolation in 1972 (Brock et al. 1972). Like other *Sulfolobus* species, the S-layers of *S. acidocaldarius* are composed of monomers of the SlaA protein self-assembled into a hexagonal array with threefold (P3) symmetry (Lembcke et al. 1991), and anchored into a tetraether monolayer cell membrane by the SlaB protein proteins (Veith et al. 2009; Gambelli et al. 2019). Both SlaA and SlaB are known to be N-glycosylated (Peyfoon et al. 2010; Guan et al. 2016). While *Sulfolobus* species share the same the basic S-layer structure, the primary sequence of SlaA and SlaB share only 24-25 % identify with other species such as *S. islandicus* and *S. solfataricus* {Gambelli:2019hd}. The S-layer proteins of *S. acidocaldarius* also show far greater stability against detergents than other *Sulfolobus* species. While *Sulfolobus solfataricus* and *Sulfolobus brierleyi* are only mildly resistant to hot detergent (SDS) treatments, *S. acidocaldarius* was shown to be highly resistant (König and Stetter 1986). Treatment with room temperature 10 % SDS resulted in S-layer sheets, but the *S. acidocaldarius* S-layer proteins remained insoluble up to treatment with 10 % SDS at 90 °C (Weiss 1974).

Many aspects of the S-layer structure and function can be studied using whole cells, however isolation and purification of the S-layer proteins is needed to separate the roles of SlaA and SlaB from those of the other cell envelope components. Despite the simplistic structure of the *Sulfolobus* cell envelope, isolation of the S-layer proteins is complicated by the fact that after an initial cell disruption and removal of the cellular contents, S-layer-containing cell-free sacculi retain the same morphology as whole cells. These sacculi or "ghosts" can contain the complete cell envelope (cell membrane, SlaA, SlaB), or only the cell wall (S-layer only, no cell membrane).

Early extractions of the *S. acidocaldarius* cell envelope lysed cells using either a French press, or detergents (0.15 % SDS with 10 mM EDTA) to aid in destabilizing the cell envelope (Weiss 1974; Michel et al. 1980). Grogan (1989) developed a generalized *Sulfolobus* S-layer purification protocol using 0.4 % N-lauryl sarcosine in buffer (pH 5) to lyse cells and DNase treatment to reduce the lysate viscosity, followed by repeated cycles of treatment with the same solution heated to 56 °C and centrifugation to remove cellular debris, eventually producing a whitish upper phase containing the S-layer proteins overlaying the brownish cell pellet. The higher resistance of *S. acidocaldarius* S-layer proteins to detergent treatments compared to other *Sulfolobus* species were exploited to permitted better separation of the S-layer proteins from the underlying cell membrane. Lipid removal was found to be more effective using SDS rather than Triton X-100. Using large batches (over 20 L) of cell cultures for extractions, it was found that following an initial cell lysis, repeated hot (60 °C) SDS treatments could be employed to separate *S. acidocaldarius* S-layers from other cellular proteins (Michel et al. 1980). SDS was subsequently removed by 6-8 washes in deionized H₂O (dH₂O). This protocol became the standard for *S. acidocaldarius* S-layer extractions.

However, the composition of the protein extracts (SlaA, SlaB) varied between labs. Using the same protocol and the same strain of *S. acidocaldarius*, one group reported obtaining S-layer sacculi composed of both SlaA and SlaB proteins (Michel et al. 1980), while others reported obtaining pure SlaA "ghosts" retaining cell-type morphology (Selenska-Pobel et al. 2011; Reitz et al. 2015). The quantity of cell culture used for extraction also affects the quality of the extracts. Following the same protocol, but using only 1 L of exponential-phase culture instead of large batch cultures (20-70 L), we obtained S-layer ghosts with both SlaA and B proteins rather than pure SlaA (Kish et al. 2016).

The inability to properly down-scaling the S-layer extraction protocol to benchtop scale (1-2 L) cultures, as well as the difficulties encountered isolating SlaB, likely due to the strong association of SlaB with so-far unidentified molecules in the cell envelope, inhibit routine studies of S-layer protein structure and function.

Here we present a method for a benchtop-scale purification of SlaA and SlaB proteins, as well as characterization of the molecules strongly associated with the SlaB anchor protein.

Methods and Results:

The purification of SlaA and SlaB proteins followed three main steps: preparation of cell envelope extracts, separation of SlaA and SlaB, and finally removal of membrane lipids from SlaB.

Culture growth and preparation of S-layer-enriched cell envelope extracts

Sulfolobus acidocaldarius DSM 639 cells were cultivated in Brock's medium (Brock et al. 1972) pH 3.5 supplemented with 0.1% yeast extract and 0.2% D-saccharose at 80 °C with agitation (170 rpm) in 1 L Erlenmeyer flasks with specialty-made extended glass aeration necks in a shaking oil bath until mid-exponential growth phase ($OD_{600nm}=0.5$). The cells were harvested by centrifugation (8000 x g, 10 min), and the cell pellets resulting from roughly 1 L of culture were flash frozen in liquid nitrogen and stored at -80 °C until use for S-layer protein extraction.

To purify the S-layer proteins, we first isolated the S-layer containing cell envelope using a modified version of the protocol of Reitz et al. (Reitz et al. 2015), adapted to benchtop-scale volumes (1-2 L cell culture at $OD_{600nm}=0.5$) rather than bioreactor-scale volumes (70 L) used in previous studies. The cell pellet was gently thawed on ice and resuspended in HEPES buffer (10 mM, pH 7) at 4 °C containing 2 mM EDTA to inhibit metalloproteases as well as chelate any cations attached to anionic head of the tetraether membrane (Pineda De Castro et al. 2016), and 1 mM Pefabloc SC to inhibit serine proteases (Fusi et al. 1991). Preliminary disruption of cell membranes and solubilization of proteins was achieved using a denaturing, anionic detergent (0.15 % SDS). DNA was digested by addition of 100 µg/µl DNase I and 4 mM MgCl₂ for 1 h at 37 °C to reduce the viscosity of the cell lysate. Further solubilization of the cell lysate and cell membrane was then performed by overnight incubation in 2 % SDS at room temperature with gentle agitation (50 rpm). Insoluble S-layer components were separated from the rest of the solubilized cell lysate by centrifugation (40 000 x g, 45 min). The white upper diffuse layer containing the cell envelope was removed from the more compact, dark beige phase underneath, resuspended in the same buffer, and centrifuged as before. The SDS was then removed from the resulting cell layer extracts by eight washes in dH₂O (centrifugation at 40 000 x g, 45 min between each wash step), and the protein components of extracts analyzed by SDS-PAGE.

This enriched S-layer protein sample obtained by hot SDS extraction was then analyzed by SDS-PAGE. Solubilization of the proteins prior to electrophoretic migration was accomplished by incubation in 20 mM sodium carbonate buffer (pH 10) at 65 °C for 30 min. Laemmli buffer was then added and the proteins denatured at 95 °C for 5 min prior to separation by SDS-PAGE (10 % gel), and visualized by Coomassie staining. The only two bands visible (apparent masses near 120 kDa and 55 kDa) corresponded to the previously established apparent molecular masses for polypeptides determined by mass spectrometry to be SlaA and SlaB, respectively (Kish et al. 2016) (see Figure 1). More SlaA was isolated than SlaB, which is coherent with the model for *Sulfolobus* S-layer structure, with six SlaA

proteins composing the hexameric S-layer array anchored by three SlaB proteins (Veith et al. 2009).

After removal of the supernatant, the resulting pellet contains two differently colored phases: a lower, darker beige phase containing cell lysate and a whitish upper phase containing the cell envelope. Previous protocols manually separated the whitish phase from the rest of the pellet (Reitz et al. 2015). However, in practice obtaining a cell pellet sufficiently large to easily manually separate the whitish phase from the darker phase is only possible when starting from batch-scale culture volumes, such as the 70 L bioreactor used by Reitz et al. (Reitz et al. 2015), which are not readily available. To separate the SlaA and SlaB proteins, we instead used size exclusion chromatography.

Separation of SlaA and SlaB by size exclusion chromatography

The S-layer suspension in 20 mM Na-carbonate buffer (pH 10) was injected into a Superdex 200 (GE Healthcare) size exclusion chromatography column. Figure 2 shows the presence of two peaks: the first is an exclusion peak (panel A; peak 1), whereas the second one corresponds to purified SlaA monomers (panel A; peak 2). The purified SlaA monomers were collected. In order to ensure that no SlaA monomers were mixed with SlaB proteins in the excluded fraction, this fraction was reinjected and separated by gel filtration (Figure 2B; peak 1'). The resulting fractions from both injections were stored at 4 °C. Separation of the isolated proteins by SDS-PAGE (10 % gel; see Figure 3) revealed the expected apparent molecular masses for purified SlaB and SlaA proteins in the first and second fractions, respectively.

While this shows that the SlaA and SlaB proteins were successfully separated from all other proteins, the chromatogram for the elution of the SlaB protein corresponds to a larger mass than would be expected for SlaB monomers. We hypothesized that SlaB monomers were aggregating, either due to hydrophobic interactions between SlaB proteins, or due to the presence of membrane tetraether lipids still associated with the SlaB protein. To test the hypothesis that lipids were remaining in association with SlaB even after treatment with 2% SDS, we subjected the enriched SlaB fraction to lipid extraction.

Extraction and identification of membrane lipids associated with SlaB

The enriched SlaB suspension collected from the size exclusion chromatography column (Figure 2B, peak 1') was dried using a rotary evaporator. Lipid extraction from the dried sample was performed using a modified Bligh and Dyer method based on Huguet et al. (2013). Briefly, samples were resuspended in an extraction mix of methanol (MeOH):Dichloromethane (DCM):Buffer (either phosphate buffer (50 mM, pH 7.4) or Na-carbonate buffer (40mM, pH 10)) (2:1:0.8 v/v/v), and placed in an ultrasonic bath (Branson 3510) for 10 min. The MeOH:DCM:Buffer (Phosphate or Na-carbonate) volume ratio was then adjusted to 1:1:0.9, followed by centrifugation (500 x g, 10 min), and collection of the extracts in a separation funnel. The DCM layer containing the lipids was separated from the MeOH: Buffer (Phosphate or Na-carbonate) layer and then extracted three more times with DCM, with the resulting DCM fractions combined and dried over Na₂SO₄. For extraction of lipids from the enriched SlaB suspension, Na-carbonate (40 mM, pH 10) was used.

The lipid extract was then re-dissolved in 200 µL heptane and centrifuged using an Eppendorf Mini Spin centrifuge (1 min, 7000 rpm). The supernatant was collected and analyzed using high performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (HPLC–APCI–MS). Tetraether analyses were performed as

recently described by Huguet et al. (2019). Briefly, tetraether separation was achieved with two silica columns in tandem (150 mm × 2.1 mm, 1.9 μm, Thermo Finnigan; USA) thermostated at 40 °C. Injection volume was 30 μl. GDGTs were first eluted isocratically with 82 % A/18 % B for 25 min (A = hexane, B = hexane/isopropanol 9/1, v/v). The following linear gradient was subsequently used: 82 % A/18 % B to 65 % A/35 % B in 25 min, followed by 65 % A/35 % B to 100 % B, maintained for 10 min and then back to 82 % A/18 % B in 10 min, maintained for 30 min. The flow rate was set at 0.2 ml/min.

HPLC-APCI-MS analysis enabled detection of two tetraether lipids in the sample of purified SlaB proteins, identified on the chromatogram as two peaks with mass to charge ratios (m/z) of 1296 at 10.7 min, and 1294 at 11.4 min (Figure 4A). These masses correspond to the cyclopentane ring-bearing glycerol dialkyl glycerol tetraethers (GDGTs) GDGT-3 and GDGT-4, respectively (Hopmans et al. 2000; Zeng et al. 2019).

To ensure that no other lipids remained attached to the SlaB proteins after lipid extraction, the SlaB proteins obtained after solvent-based lipid extraction were first applied to a Superdex 200 (GE Healthcare) size exclusion chromatography column. The resulting protein fraction was then subjected to a second round of solvent-based lipid extraction using the same protocol as previously, using phosphate buffer (50 mM, pH 7.4) instead of Na-carbonate buffer, after which no lipids were detected (Figure 4B) with a minimum detection limit of 1 ppb.

Having identified that GDGTs were in fact associated with SlaB after hot SDS treatment and size exclusion chromatography, we sought to remove these lipids from the original SlaB protein aggregate obtained after hot SDS treatment. This enriched SlaB protein solution (Figure 2B, peak 1') was first solubilized in an equal volume of 20 mM Na-carbonate buffer (pH 10) and then treated it with 1 % CHAPS for 30 min at 37 °C with agitation. To check whether this treatment was sufficient for removal of the GDGTs associated with SlaB, the CHAPS-treated solution was first applied to a Superdex 200 size exclusion chromatography column to isolate the SlaB proteins which were excluded from the column, followed by lipid extraction following the same modified Bligh and Dyer method described above using phosphate buffer (50 mM, pH 7.4). HPLC-APCI-MS analyses failed to detect any tetraether lipids in association with SlaB. The exclusion of SlaB proteins from the Superdex 200 resin indicates that despite the removal of residual GDGTs by CHAPS following hot SDS isolation of the S-layer proteins, the SlaB proteins remained associated in an aggregate mass.

Taken together, these results suggest that the larger mass observed during elution for purified SlaB was due to both strong association with GDGTs and likely auto-aggregation of hydrophobic, N-terminal transmembrane domain of the SlaB protein (Veith et al. 2009).

Discussion :

Here we present a method for complete isolation of SlaA from SlaB, starting from bench-scale culture volumes. This method improves upon existing protocols by allowing for reduced quantities of cell cultures to be used. In our study, the SlaA was found to have an apparent molecular mass of 120 kDa, despite the calculated molecular mass of 151 kDa based on its primary sequence (Claus et al. 2005). Previous studies have observed a range of apparent molecular weights for SlaA from *S. acidocaldarius* DSM 639 up to 170 kDa (Michel et al. 1980; Grogan 1989; Selenska-Pobell et al. 2011). Some early studies have even

shown two high-molecular weight bands for the SlaA protein (140 kDa and 170 kDa) that were determined by their amino acid composition to be the same SlaA protein (Michel et al. 1980). This difference in the electrophoretic mobility of *S. acidocaldarius* S-layer proteins has been attributed to protein glycosylations, as well as their low solubility in acidic or neutral hot SDS (Grogan 1996). In addition, S-layer proteins remain insoluble in the neutral pH Laemmli buffer containing SDS, and so prior to electrophoretic migration, S-layer proteins must be solubilized in a heated alkaline buffer (Grogan 1989; Selenska-Pobell et al. 2011; Guan et al. 2016). S-layer proteins from other archaea have shown altered electrophoretic migration depending on culturing conditions including temperature, pH, and protein maturation events (Konrad and Eichler 2002; Claus et al. 2005). While the reasons for these differences in electrophoretic mobility are still unknown, attention must therefore be paid when comparing results between studies using different strains of the same organism, as well as the culturing and protein solubilization conditions.

This protocol allowed us to identify for the first time that the molecules aggregating with SlaB in strong association are GDGTs. The interaction between SlaB S-layer anchor protein and membrane tetraether lipids was shown to be highly resistant, even to heat and detergent treatments. Less SlaB is present than SlaA, even in whole cells, coherent with the current model for *Sulfolobus* S-layer structure with twice as much SlaA as SlaB (Veith et al. 2009). There is likely some loss of SlaB during the initial steps of the extraction protocol due to strong attachment to membrane lipids. The final SlaB extract contained no other proteins detectable by SDS-PAGE or mass spectrometry, and no other lipids detectable using our HPLC-MS methods. Despite this, the SlaB proteins still eluted from the size exclusion chromatography column in the exclusion peak. This suggests that SlaB proteins strongly associate with one another, likely due to interactions between monomers, likely due to hydrophobic interactions between their N-terminal transmembrane domains.

Very little is currently known about the role of SlaB. The SlaB anchor protein of *S. solfataricus* are predicted by its primary structure to be shorter than that of *S. acidocaldarius*, containing fewer beta sandwich domains (Veith et al. 2009). Correspondingly, the pseudo-periplasmic space created by the SlaB protein varies in height between the Sulfolobales, from 18 nm in *Sulfolobus shibatae* (Baumeister et al. 1989) to 27 nm in *S. acidocaldarius* (Kish et al. 2016). A recent study of Δ *slaB* mutants in *Sulfolobus islandicus* suggests that the S-layer anchoring role of SlaB can be partially complemented by another S-layer associated protein, designated M164_1049 (Zhang et al. 2019). Another recent study in *Sulfolobus solfataricus* used CRISPR-mediated silencing of gene encoding SlaB to show that in addition to its anchoring role, SlaB is important in virus susceptibility, cell morphology, and cell division (Zink et al. 2019).

These findings allow deeper study of the individual components of the essential cell envelope structure. This is of particular importance given the current absence of tertiary structures for archaeal S-layers, with the sole exception of the C-terminal domain of the *Methanosarcina acetivorans* S-layer protein (Arbing et al. 2012). Extracted S-layers have been used in a range of biotechnology applications, from nanomaterials and bioremediation applications, to immobilized biocatalysts, biochips, and drug delivery (Selenska-Pobell et al. 2011; Bartolomé et al. 2012; Sleytr et al. 2014). Historically, S-layer protein purity is often verified in relation to other proteins by SDS-PAGE. Our results show the importance of analyzing for any attached lipids to ensure protein purity prior to any sensitive downstream applications. Taken together, the findings of this study will permit greater precision when isolating and studying

the S-layer components of Archaea, including their self-assembly, and interactions with other macromolecules as well as ions and molecules in the extracellular environment.

Acknowledgments. This project was supported by grants to A. Kish from the CNRS MITI program Xlife, and the MNHN (AVIV "Projet Fédérateur"). The authors wish to thank Christelle Anquetil for help with HPLC-MS analysis of GDGTs.

References:

Albers S-V, Meyer BH (2011) The archaeal cell envelope. *Nat Rev Micro* 9:414–426. doi: 10.1038/nrmicro2576

Arbing MA, Chan S, Shin A, et al (2012) Structure of the surface layer of the methanogenic archaean *Methanosarcina acetivorans*. *Proc Natl Acad Sci USA* 109:11812–11817. doi: 10.1073/pnas.1120595109

Bartolomé J, Bartolomé J, Bartolomé F, et al (2012) Strong paramagnetism of gold nanoparticles deposited on a *Sulfolobus acidocaldarius* S layer. *Phys Rev Lett* 109:247203. doi: 10.1103/PhysRevLett.109.247203

Baumeister W, Wildhaber I, Phipps BM (1989) Principles of organization in eubacterial and archaeobacterial surface proteins. 35:215–227.

Brock TD, Brock KM, Belly RT, Weiss RL (1972) *Sulfolobus*: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. *Arch Mikrobiol* 84:54–68.

Chandramohan A, Duprat E, Remusat L, et al (2018) Novel Mechanism for Surface Layer Shedding and Regenerating in Bacteria Exposed to Metal-Contaminated Conditions. 9:3210. doi: 10.3389/fmicb.2018.03210

Claus H, Akça E, Debaerdemaeker T, et al (2005) Molecular organization of selected prokaryotic S-layer proteins. 5:731–743. doi: 10.1139/w05-093

Engelhardt H (2007a) Are S layers exoskeletons? The basic function of protein surface layers revisited. *J Structural Biology* 160:115–124. doi: 10.1016/j.jsb.2007.08.003

Engelhardt H (2007b) Mechanism of osmoprotection by archaeal S-layers: a theoretical study. *J Structural Biology* 160:190–199. doi: 10.1016/j.jsb.2007.08.004

Farci D, Slayov C, Piano D. 2017. Coexisting properties of thermostability and ultraviolet radiation resistance in the main S-layer complex of *Deinococcus radiodurans*. *Photochem Photobiol Sci* 17:1–8. doi: 10.1039/c7pp00240h

Fusi P, Villa M, Burlini N, et al (1991) Intracellular proteases from the extremely thermophilic archaeobacterium *Sulfolobus solfataricus*. *Experientia* 47:1057–1060. doi: 10.1007/BF01923341

Gambelli L, Meyer BH, McLaren M, Sanders K, Quax TEF, Gold VAM, Albers S-V, Daum B. 2019. Architecture and modular assembly of *Sulfolobus* S-layers revealed by electron cryotomography. *Proc Natl Acad Sci USA* 8:1–9. doi: 10.1073/pnas.1911262116

- 338 Grogan DW (1989) Phenotypic characterization of the archaeobacterial genus *Sulfolobus*:
 339 comparison of five wild-type strains. J Bacteriol 171:6710–6719. doi:
 340 10.1128/jb.171.12.6710-6719.1989
- 341 Grogan DW (1996) Organization and interactions of cell envelope proteins of the extreme
 342 thermoacidophile *Sulfolobus acidocaldarius*. Can J Microbiol 42:1163–1171. doi:
 343 10.1139/m96-148
- 344 Guan Z, Delago A, Nußbaum P, et al (2016) N-glycosylation in the thermoacidophilic
 345 archaeon *Sulfolobus acidocaldarius* involves a short dolichol pyrophosphate carrier.
 346 FEBS Letters 590:3168–3178. doi: 10.1002/1873-3468.12341
- 347 Guan Z, Naparstek S, Calo D, Eichler J (2012) Protein glycosylation as an adaptive response
 348 in Archaea: growth at different salt concentrations leads to alterations in *Haloferax*
 349 *volcanii* S-layer glycoprotein N-glycosylation. Environmental Microbiology 14:743–753.
 350 doi: 10.1111/j.1462-2920.2011.02625.x
- 351 Hopmans EC, Schouten S, Pancost RD, et al (2000) Analysis of intact tetraether lipids in
 352 archaeal cell material and sediments by high performance liquid
 353 chromatography/atmospheric pressure chemical ionization mass spectrometry. Rapid
 354 Commun Mass Spectrom 14:585–589. doi: 10.1002/(SICI)1097-
 355 0231(20000415)14:7<585::AID-RCM913>3.0.CO;2-N
- 356 Huguet A, Coffinet S, Roussel A, et al (2019) Evaluation of 3-hydroxy fatty acids as a pH
 357 and temperature proxy in soils from temperate and tropical altitudinal gradients. Organic
 358 Geochemistry 129:1–13. doi: 10.1016/j.orggeochem.2019.01.002
- 359 Huguet A, Fosse C, Laggoun-Défarge F, et al (2013) Effects of a short-term experimental
 360 microclimate warming on the abundance and distribution of branched GDGTs in a
 361 French peatland. Geochimica et Cosmochimica Acta 105:294–315. doi:
 362 10.1016/j.gca.2012.11.037
- 363 Im S, Joe M, Kim D, et al (2013) Transcriptome analysis of salt-stressed *Deinococcus*
 364 *radiodurans* and characterization of salt-sensitive mutants. Res Microbiol 164:923–932.
 365 doi: 10.1016/j.resmic.2013.07.005
- 366 Kish A, Miot J, Lombard C, et al (2016) Preservation of Archaeal Surface Layer Structure
 367 During Mineralization. Sci Rep 6:26152–10. doi: 10.1038/srep26152
- 368 Konrad Z, Eichler J (2002) Lipid modification of proteins in Archaea: attachment of a
 369 mevalonic acid-based lipid moiety to the surface-layer glycoprotein of *Haloferax*
 370 *volcanii* follows protein translocation. Biochem J 366:959–964. doi:
 371 10.1042/BJ20020757
- 372 König H, Stetter KO (1986) Studies on archaeobacterial S-layers. Syst Appl Microbiol 7:300–
 373 309. doi: 10.1016/S0723-2020(86)80023-6
- 374 Lembcke G, Dürr R, Hegerl R, Baumeister W (1991) Image analysis and processing of an
 375 imperfect two-dimensional crystal: the surface layer of the archaeobacterium *Sulfolobus*
 376 *acidocaldarius* re-investigated. J Microsc 161:263–278. doi: 10.1111/j.1365-
 377 2818.1991.tb03089.x

- 378 Mader C, Küpcü S, Sara M, Sleytr UB (1999) Stabilizing effect of an S-layer on liposomes
379 towards thermal or mechanical stress. BBA Biomembranes 1418:106–116.
- 380 Merroun ML, Raff J, Rossberg A, et al (2005) Complexation of uranium by cells and S-layer
381 sheets of *Bacillus sphaericus* JG-A12. Appl Environ Microbiol 71:5532–5543. doi:
382 10.1128/AEM.71.9.5532-5543.2005
- 383 Michel H, Neugebauer DC, Oesterhelt D (1980) The 2-D Crystalline Cell Wall of *Sulfolobus*
384 *acidocaldarius*: Structure, Solubilization, and Reassembly. In: Electron Microscopy at
385 Molecular Dimensions. Springer, Berlin, Heidelberg, Berlin, Heidelberg, pp 27–35
- 386 Peyfoon E, Meyer B, Hitchen PG, et al (2010) The S-layer glycoprotein of the crenarchaeote
387 *Sulfolobus acidocaldarius* is glycosylated at multiple sites with chitobiose-linked N-
388 glycans. Archaea. doi: 10.1155/2010/754101
- 389 Pineda De Castro LF, Dopson M, Friedman R (2016) Biological Membranes in Extreme
390 Conditions: Anionic Tetraether Lipid Membranes and Their Interactions with Sodium
391 and Potassium. J Phys Chem B 120:10628–10634. doi: 10.1021/acs.jpcb.6b06206
- 392 Reitz T, Rossberg A, Barkleit A, et al (2015) Spectroscopic study on uranyl carboxylate
393 complexes formed at the surface layer of *Sulfolobus acidocaldarius*. Dalton Trans
394 44:2684–2692. doi: 10.1039/c4dt02555e
- 395 Rodrigues-Oliveira T, Belmok A, Vasconcellos D, et al (2017) Archaeal S-Layers: Overview
396 and Current State of the Art. 8:2597. doi: 10.3389/fmicb.2017.02597
- 397 Selenska-Pobel S, Reitz T, Schöneman R, et al (2011) Magnetic Au nanoparticles on archaeal
398 S-Layer ghosts as templates. Nanomaterials and Nanotechnology 1:8–16.
- 399 Sleytr UB, Schuster B, Egelseer EM, et al (2011) Nanobiotechnology with S-layer proteins as
400 building blocks. Prog Mol Biol Transl Sci 103:277–352. doi: 10.1016/B978-0-12-
401 415906-8.00003-0
- 402 Sleytr UB, Schuster B, Egelseer EM, Pum D (2014) S-layers: principles and applications.
403 FEMS Microbiol Rev 38:823–864. doi: 10.1111/1574-6976.12063
- 404 Ucisik MH, Sleytr UB, Schuster B (2015) Emulsomes meet S-layer proteins: an emerging
405 targeted drug delivery system. Curr Pharm Biotechnol 16:392–405.
- 406 Veith A, Klingl A, Zolghadr B, et al (2009) *Acidianus*, *Sulfolobus* and *Metallosphaera*
407 surface layers: structure, composition and gene expression. Mol Microbiol 73:58–72. doi:
408 10.1111/j.1365-2958.2009.06746.x
- 409 Weiss RL (1974) Subunit cell wall of *Sulfolobus acidocaldarius*. J Bacteriol 118:275–284.
- 410 Zeng Z, Liu X-L, Farley KR, et al (2019) GDGT cyclization proteins identify the dominant
411 archaeal sources of tetraether lipids in the ocean. Proc Natl Acad Sci USA
412 12:201909306–7. doi: 10.1073/pnas.1909306116
- 413 Zhang C, Wipfler RL, Li Y, et al (2019) Cell Structure Changes in the Hyperthermophilic
414 Crenarchaeon *Sulfolobus islandicus* Lacking the S-Layer. doi: 10.1128/mBio.01589-19

- 415 Zhu C, Guo G, Zhang F, et al (2017) Diversity in S-layers. Prog Biophys Mol Biol 123:1–15.
416 doi: 10.1016/j.pbiomolbio.2016.08.002
- 417 Zink IA, Pfeifer K, Wimmer E, et al (2019) CRISPR-mediated gene silencing reveals
418 involvement of the archaeal S-layer in cell division and virus infection. Nat Commun
419 10:4797–14. doi: 10.1038/s41467-019-12745-x

Pre-Print prior to peer review - see final version doi:10.1007/s00792-020-01179-9

Figures:

Figure 1. SDS-PAGE of extracted S-layer ghosts with two bands with apparent molecular masses around 120 kDa (SlaA) and 55 kDa (SlaB).

Figure 2. Separation of SlaA and SlaB protein by gel filtration chromatography. After injection of the enriched S-layer protein sample obtained by hot SDS extraction (**panel A**) we obtain two peaks; the peak 1 is an exclusion peak containing SlaB, whereas the peak 2 corresponds to purified SlaA monomers. To make sure that no SlaA monomers were mixed with SlaB proteins in the excluded fraction, this fraction was then reinjected and separated by

gel filtration (**panel B**). The resulting exclusion peak exclusion (peak 1') shows that no additional SlaA monomers were mixed in with the SlaB.

Figure 3. SDS-PAGE of fractions (1') and (2), resulting from the first and second injections into the gel filtration column, respectively. The bands correspond to the expected apparent molecular masses of SlaA and SlaB, showing that each protein extract is now free of other polypeptides.

446 Figure 4. HPLC/MS identification of tetraether lipids present in samples of purified SlaB
447 before (**panel A**) and after (**panel B**) lipid extraction. The chromatograms show the absolute
448 intensity as a function of retention time.

Pre-Print prior to peer review - see final version doi:10.1007/s00792-020-01179-9