S-layer based nanostructures

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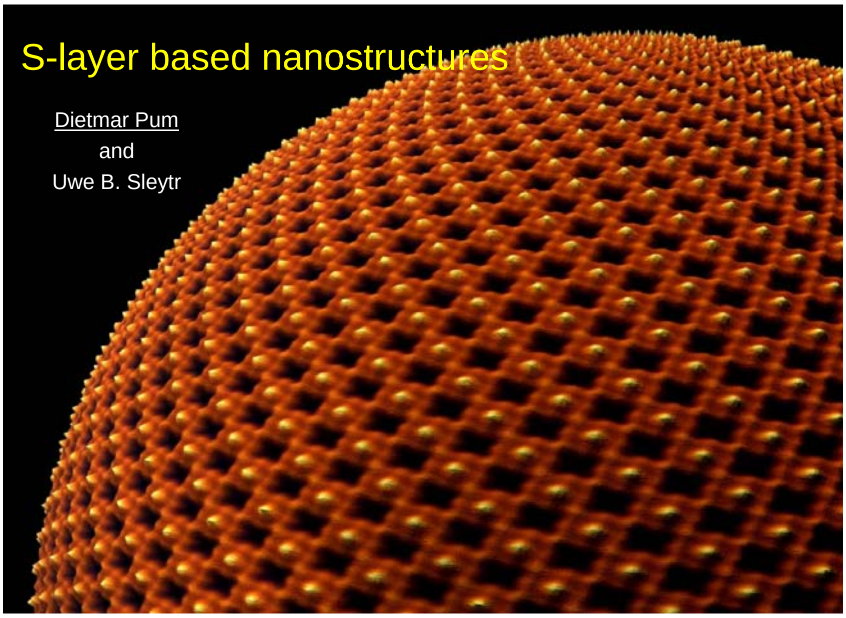
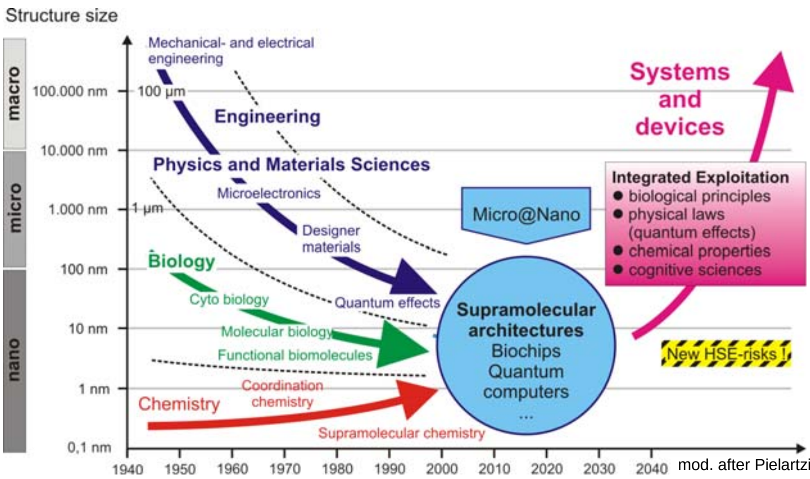
and
Uwe B. Sleytr



Center for NanoBiotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria

Converging Technologies



mod. after Pielartzik, Bayer

NT is not a „rebranding" of older science !
Its influence is revolutionary rather than evolutionary.

Outline

- Development of a (bio)molecular construction kit
- Description of S-layer proteins (basic principles)
- S-layers as nano-scale patterning elements for life- and non- life science applications

Which are the basic building blocks in a biomolecular construction kit

- Biological molecules (e.g. Proteins, Lipids, Glycans, Nucleic acids)
- Chemically or Genetically Modified Molecules
- Chemically Synthesized Molecules

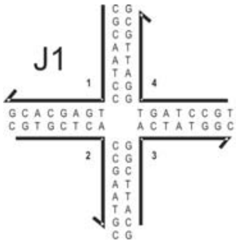
Key capabilities

- Self-assemble
- Molecular recognition
- Adaptable and evolved structure & function
- Dynamic structures

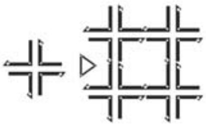
Basic Structures (Patterning Elements) for Generating Complex Supramolecular Structures

- DNA
- Monomolecular crystalline bacterial cell surface layers (S-layers)

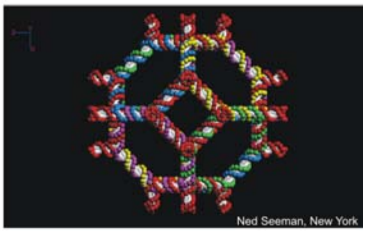
Nanoscale Assembly and Manipulation of Branched DNA (Ned Seeman, NY University)



basic structure: junction J1



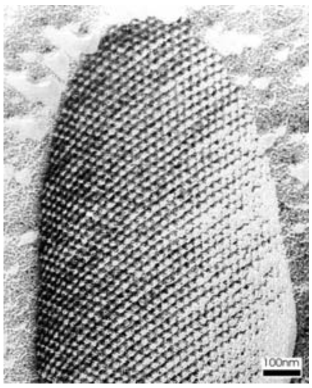
main features of "nanoscale assembly"



http://seemanlab4.chem.nyu.edu/homepage.html

Description of S-layers

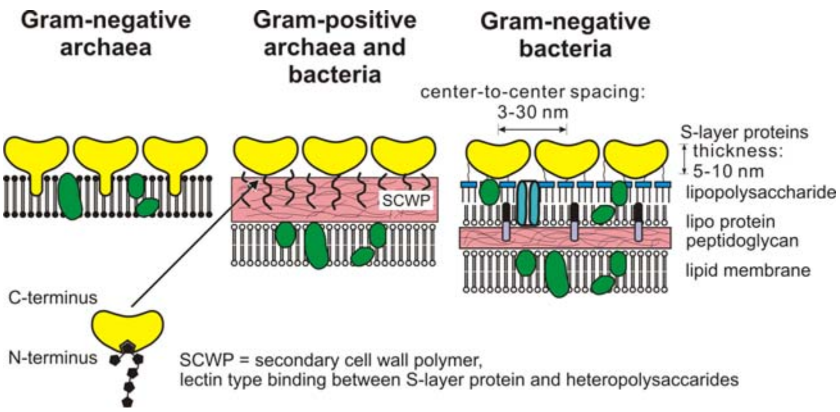
S-layers are crystalline, monomolecular (glyco)protein arrays representing one of the most commonly observed surface structures in eubacteria and archaea.



TEM of freeze etched preparations of bacterial cells with an S-layer showing square (left) and hexagonal (right) lattice symmetry.

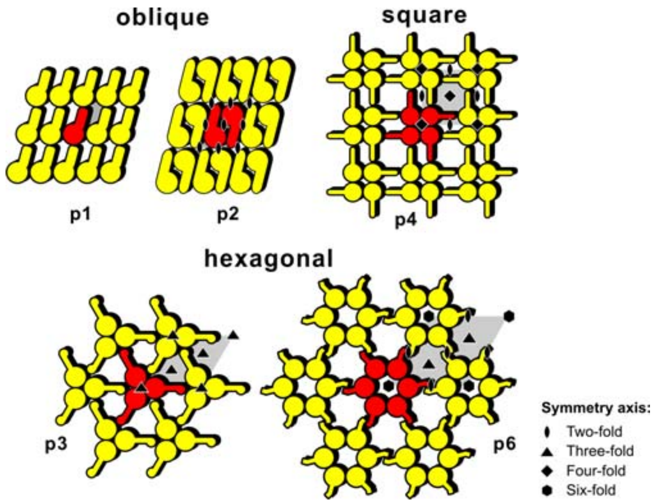
For review see: Sleytr et al., 1999, *Angew. Chemie Int. Ed.,* 38:1034-1054

Prokaryotic cell envelope profiles containing S-layers



S-layer lattice types

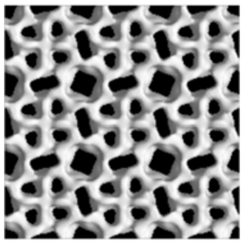
- M_r of constituent subunits: 40-200 kDa
- Center-to-center spacing of morphological units: 3 – 30 nm



Ultrastructure of S-layer proteins

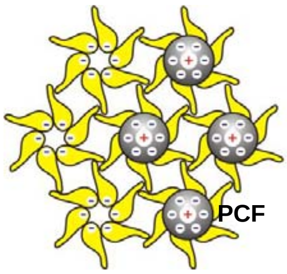
Pores

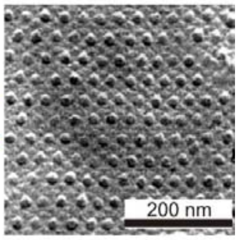
- Isoporous structures (pores are of identical size and morphology)
- Pore diameter: 2 – 8nm
- Porosity: 30% - 70%

Functional groups

- Functional groups (e.g. carboxyic acid or amino groups) are aligned in well defined positions and orientations.

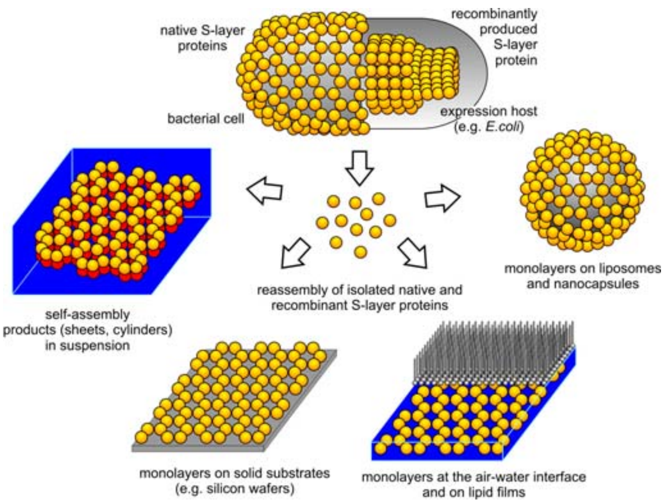




(PCF = polycationic ferritin)

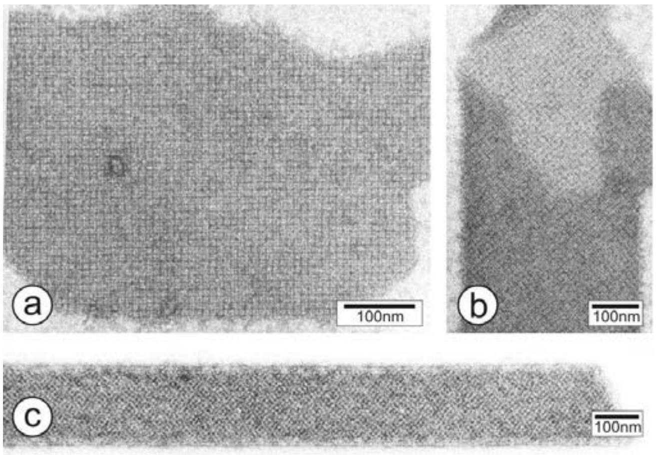
Messner et al., *J. Bacteriol.* 166 (1986) 1046

S-layer reassembly

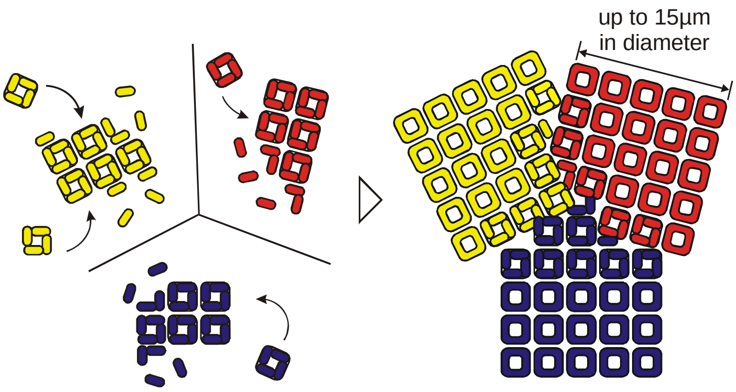


For review see: Sleytr et al., 1999, *Angew. Chemie Int. Ed.,* 38:1034-1054

S-layer self assembly in suspension

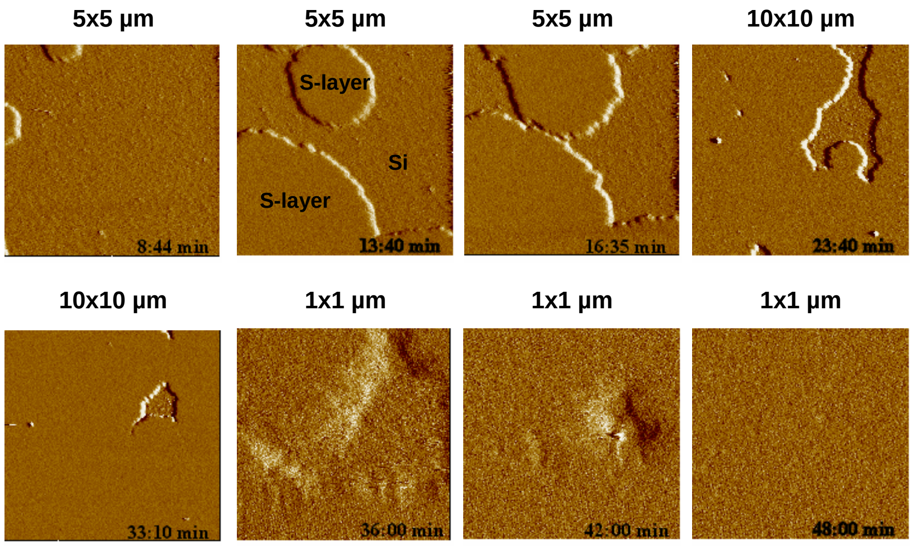


Crystal growth at interfaces



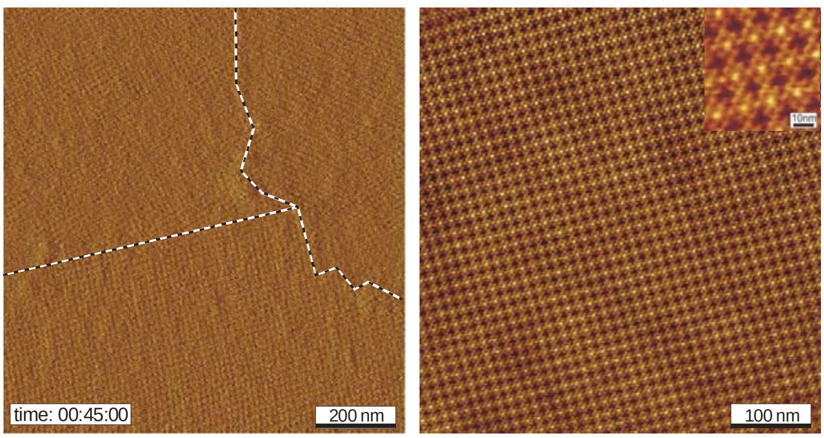
Crystal growth of S-layers on solid supports, at the liquid-air interface or on lipid films.

Crystal growth on silicon



Györvary et al. J. Microscopy 212 (2003) 300-306

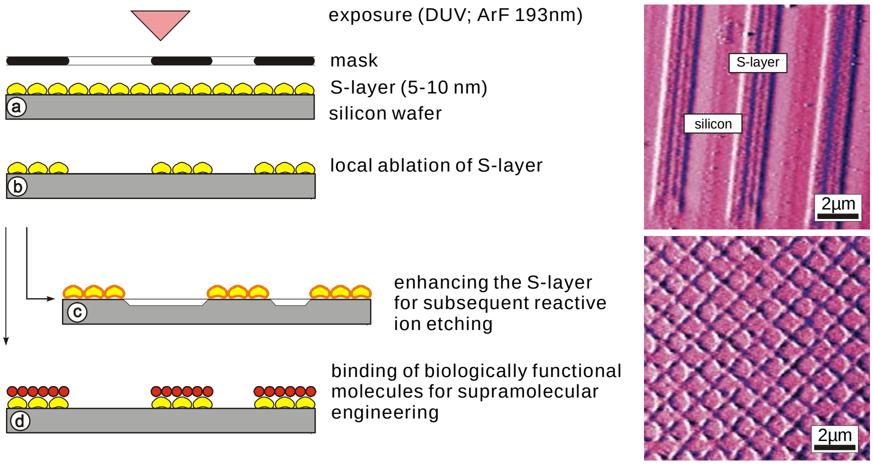
Formation of an S-layer protein monolayer on silicon



AFM images of the recrystallization process of S-layer proteins from *B. sphaericus* CCM2177 on silicon

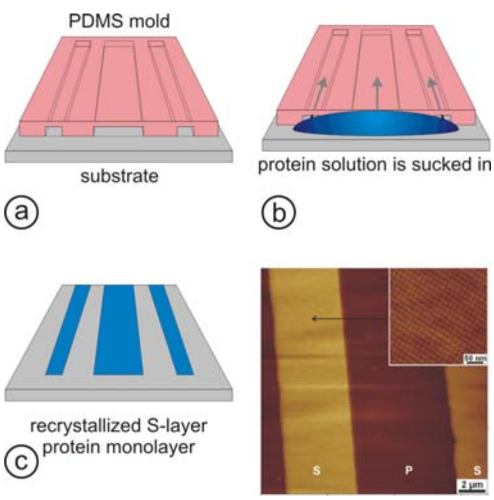
Györvary et al. J. Microscopy 212 (2003) 300-306

Patterning of S-layers by optical lithography



Pum et al., Coll. & Surf. B: Biointerfaces 8 (1997) 157-162

Patterning of S-layers by Soft lithography (Micromoulding in capillaries - MIMIC)



Györvary et al., Nanoletters 3 (2003) 315

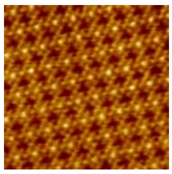
S: S-layer, P: Support

PDMS = Poly(dimethylsiloxane) (PDMS)

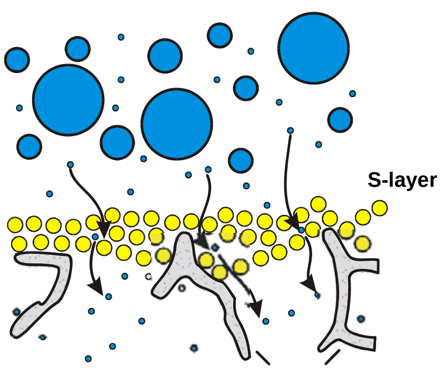
NanoBiotechnologcal applications of functional S-layer arrays

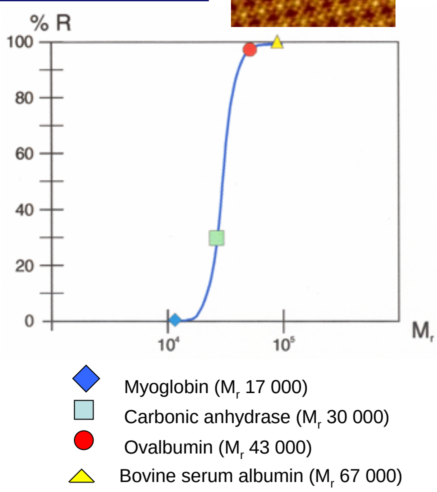
- Ultrafiltration membranes
- Matrices for a well defined binding of functional molecules and nanoparticles (biosensors, nano electronics and optics).
- Supporting structure for functional lipid membranes (planar membranes, liposomes and nanocapsules).
- Drug delivery and drug targeting systems (artificial viruses).

S-layer Ultrafiltration Membranes
(exploiting isoporosity)



S-layer ultrafiltration membrane

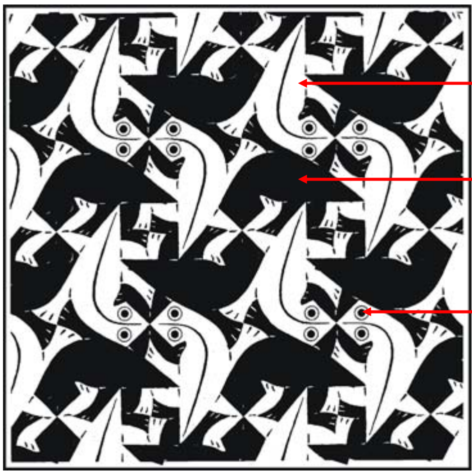




Sára & Sleytr, 1987, *J. Membr. Sci.*, 33:27-49

S-layers as templates for the formation of regularly arranged nanoparticle arrays

Patterning elements for a molecular construction kit



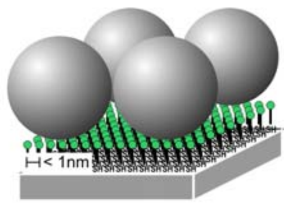
S-layer lattices are composed of a single (glyco)protein subunit

Pores in S-layers have identical size and morphology

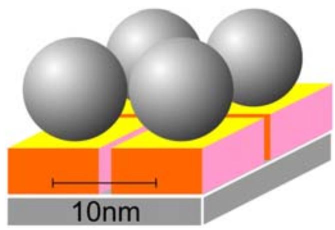
Functional groups on the protein lattices are aligned in well defined positions and orientations

Strategies for modification and functionalization of solid supports

- Self-assembled monolayers (SAMs)



- S-layers lattices



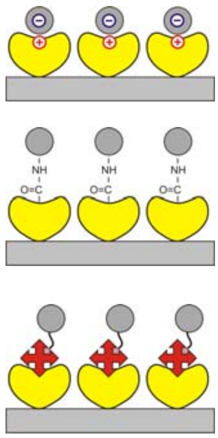
Binding of molecules and nanoparticles on S-layers

Native S-layer protein:

- Binding by surface chemical methods using electrostatic bonds (e.g. free carboxyl groups) covalent bonds (e.g. EDC-activated carboxyl groups)

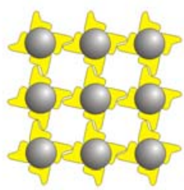
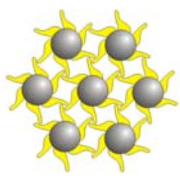
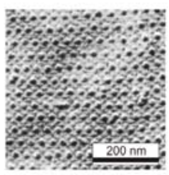
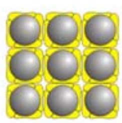
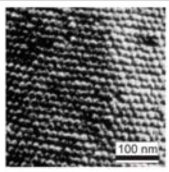
Genetically engineered S-layer protein:

- Design and expression of recombinant S-layer fusion proteins.
- Binding by functional domains on S-layer fusion proteins



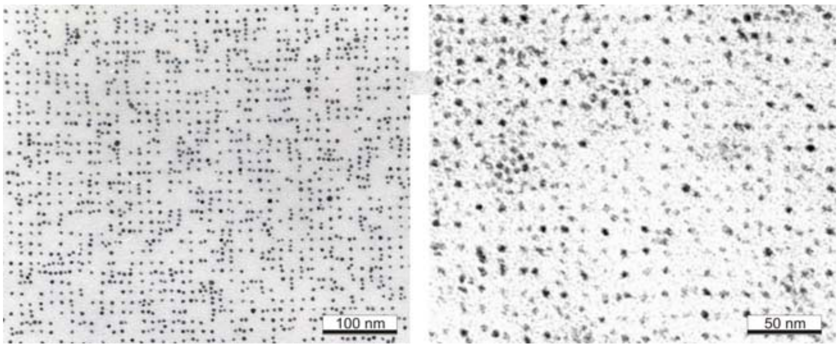
Functional domains (native or genetically introduced) are repeated with the periodicity of the S-layer lattice leading to regular arrays of bound molecules and particles.

Binding of macromolecules and nanoparticles into regular arrays on S-layers

	Lattice symmetry		Examples
Lattice spacing	 square (p4)	 hexagonal (p6)	
			

The superlattice of bound molecules and nanoparticle resembles the lattice parameters of the underlying S-layer

Binding of nanoparticles on S-layers



Au (diameter 5nm) CdSe-NH_2 (diameter 5nm)

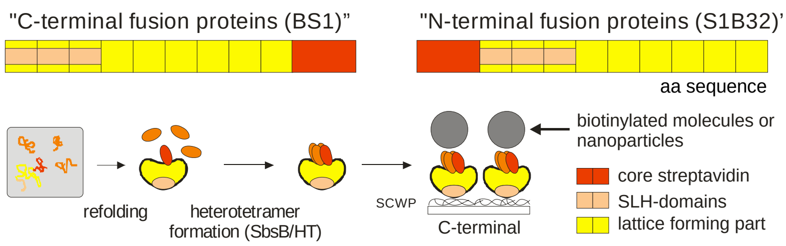
Controlled binding of nanoparticles by functional groups on an S-layer with square lattice symmetry (d=13.1nm)

CdSe particles were kindly provided by A. Schrödter, D. Talapin and H. Weller, University Hamburg

Györvary et al., J. Nanosci. Nanotechnol. 4 (2004) 115

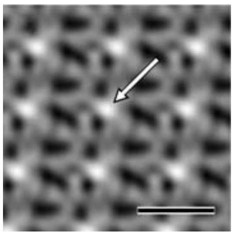
Binding by functional domains on S-layer fusion proteins

SbsB / Streptavidin fusion proteins

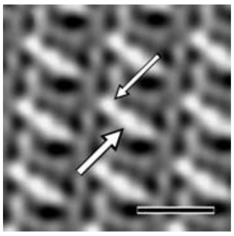


Moll et al., PNAS, 99 (2002) 14646

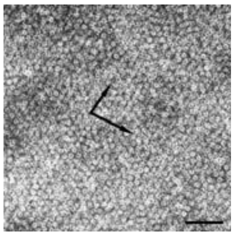
SbsB / Streptavidin fusion proteins



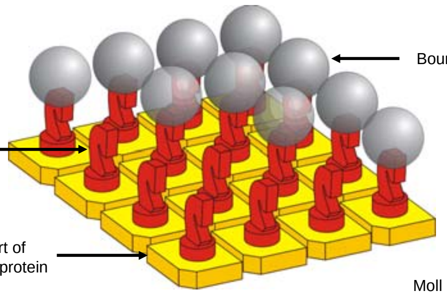
Native SbsB S-layer protein.
Bar, 10nm



SbsB/Stv fusion protein.
Bar, 10nm.



Biotinylated ferritin bound on
BS1. Bar, 100nm.



Moll et al., PNAS, 99 (2002) 14646

S-layer fusion proteins

S-layer fusion protein (selected from various constructs)	Length of funct.	Functionality
rSbsB$_{1\text{-}889}$ / core streptavidin rSbpA$_{31\text{-}1068}$ / core streptavidin	118 aa	Biotin binding
rSbpA$_{31\text{-}1068}$ / Bet v1	116 aa	Major birch pollen allergen
rSbpA$_{31\text{-}1068}$ / Strep-tag	9 aa	Affinity tag for streptavidin
rSbpA$_{31\text{-}1068}$ / ZZ	116 aa	IgG-Binding domain
rSbpA$_{31\text{-}1068}$ / GFP	238 aa	Green fluorescent protein
rSbpA$_{31\text{-}1068}$ / cAb	117 aa	Heavy chain camel antibody
rSbpA$_{31\text{-}1068}$ / AG4 and AGP35 rSbpA$_{31\text{-}1068}$ / CO2P2	12 aa 12 aa	Silver binding peptide Cobalt binding peptide

Mature proteins:
B. sphaericus CCM2177 variant A (SbpA): 1,238 aa
Geobacillus stearothermophilus pv72/p2 (SbsB): 889 aa

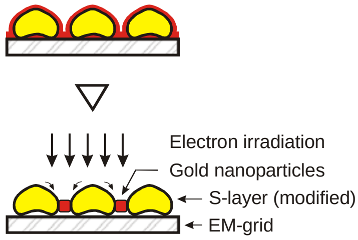
Synthesis of metallic superlattices on S-layers

- Recrystallization of S-layer proteins on a solid substrate
- Optional: Chemical modification of the S-layer proteins (e.g. conversion of free amino groups into thiol groups)
- Incubation with metal solution
- Reduction of the metal salt by:
  - chemical treatment (e.g. H_2S), or
  - electron irradiation
- Materials used:

  Gold, platinum, palladium, nickel, CdS

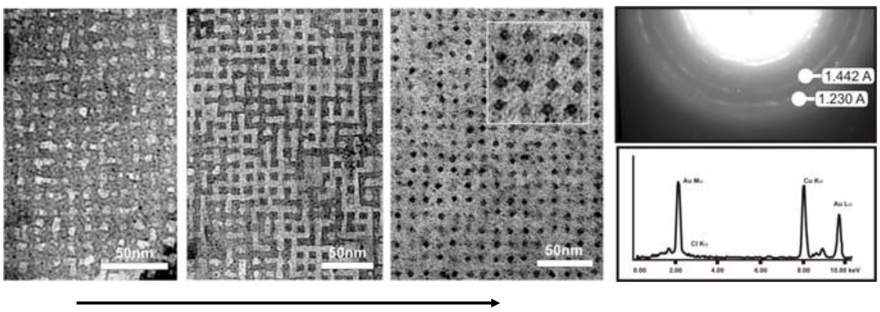
Example:

treatment with tetrachloroauric(III) acid solution



Shenton et al., Nature, 389 (1997) 585
Gorbunov et al., Appl.Surf.Sci. 109/110 (1997) 621
Dieluweit et al., Supramolec. Sci. 5 (1998) 15
Winningham et al., Surf. Sci. 406 (1998) 221
Mertig et al., Eur. Phys. J. D9 (1999) 45

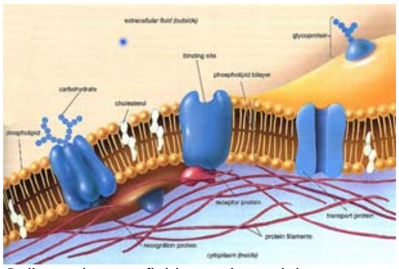
Synthesis of a gold superlattice on an S-layer with square lattice symmetry



increasing electron dose

Dieluweit et al., Supramolec. Sci. 5 (1998) 15

Biomimetic Cell Membranes

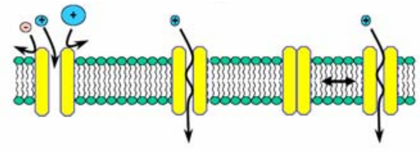


http://sun.menloschool.org/~cweaver

Cell membrane: fluid mosaic model

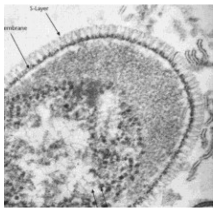
Specific membrane functions

1.selectivity 2.binding 3.opening / closing

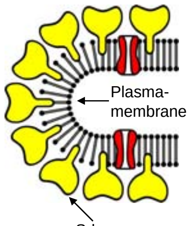


Archaeal cell envelope structure

A supramolecular structure optimized in ~ 3,5 billions of years under extreme environmental conditions (120°C, pH 0, concentrated salt solutions, 1100 bar).



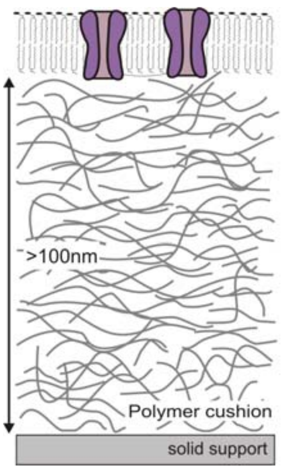
TEM of an archaeal cell



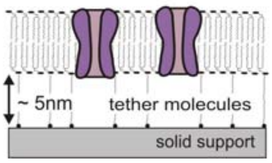
Biomimetic Lipid Membrane (Semifluid Membrane Model)

- S-layer stabilized solid supported lipid membranes
- S-layer acts as stabilizing structure, tethering structure and ionic reservoir
- Applications:

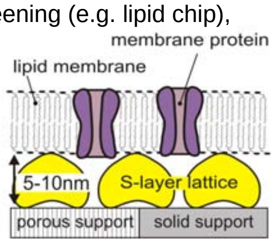
  Membrane Sensors, Lab-on-a-chip devices, High Throughput Screening (e.g. lipid chip), DNA sequencing



Sackmann, Science 271 (1996) 43

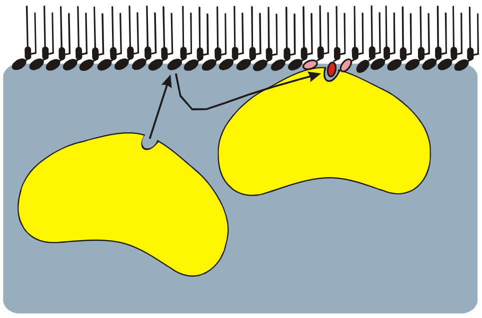


Schiller et al. Angew. Chem. Int. Ed. 42 (2003) 208



Schuster et al., Langmuir 17 (2001) 499
Schuster et al., Langmuir 19 (2003) 2392
Gufler et al. BBA 1661 (2004) 154

S-layer supported lipid membranes (Semifluid lipid membranes)



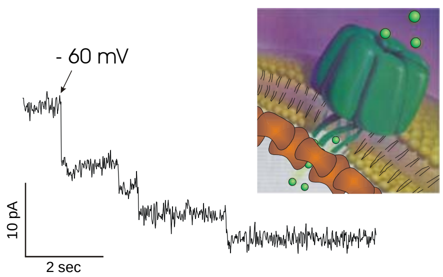
Methods:

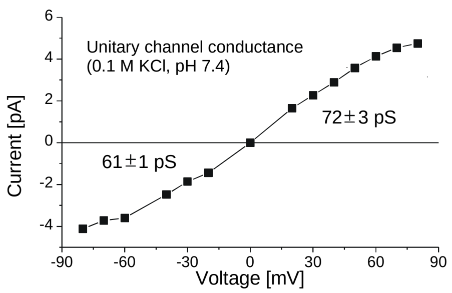
- Grazing-incidence X-ray diffraction
- X-ray and neutron reflectometry
- Infrared spectroscopy (FT-IRRAS)
- FRAP (fluorescence recovery after photo bleaching) with fluorescent lipid probes

Diederich et al., Coll. & Surf. B 6 (1996) 335
Wetzer et al., Langmuir 14 (1998) 6899
Weygand et al., Biophys.J. 76 (1999) 458

Györvary et al., Langmuir 15 (1999) 1337
Weygand et al., J. Mater. Chem. 1 (2000) 141
Weygand et al., J. Phys. Chem. B 106 (2002) 5793

Single α-Hemolysin Channels Reconstituted in S-layer Supported Lipid Bilayers





Schuster *et al.*, 1998, *Biochim. Biophys. Acta Biomembr.*1370:280-288.
Schuster *et al.*, 2001 *Langmuir* 17: 499-503
Schuster *et al.*, 2002, *Bioelectrochem.* 55:5-7
Schuster *et al.*, 2003, *Langmuir* 19:2392-2397

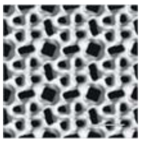
Application potential of S-layer supported lipid membranes

- Exploiting functional lipid membranes at meso- and macroscopic scale (e.g. as required for biosensors or high throughput screening)

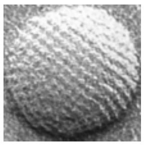
- Linking silicon technology and solid state physics with biological systems (e.g. coupling cells to surfaces)

Summary

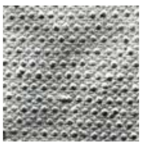
Basic research on structure, genetics, chemistry, morphogenesis and function of S-layers has led to a broad spectrum of applications in molecular nanotechnology and biomimetics:



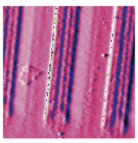
Isoporous ultrafiltration membranes



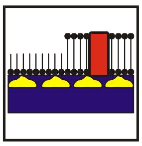
Vaccines, artificial viruses, drug delivery and targeting, gene therapy



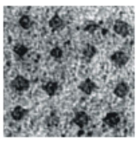
Matrices for controlled immobilization of molecules



Patterning by microlithographic procedures



Biomimetic lipid membranes



Nanoelectronic applications

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