Functionalization of surfaces with S-layer protein lattices

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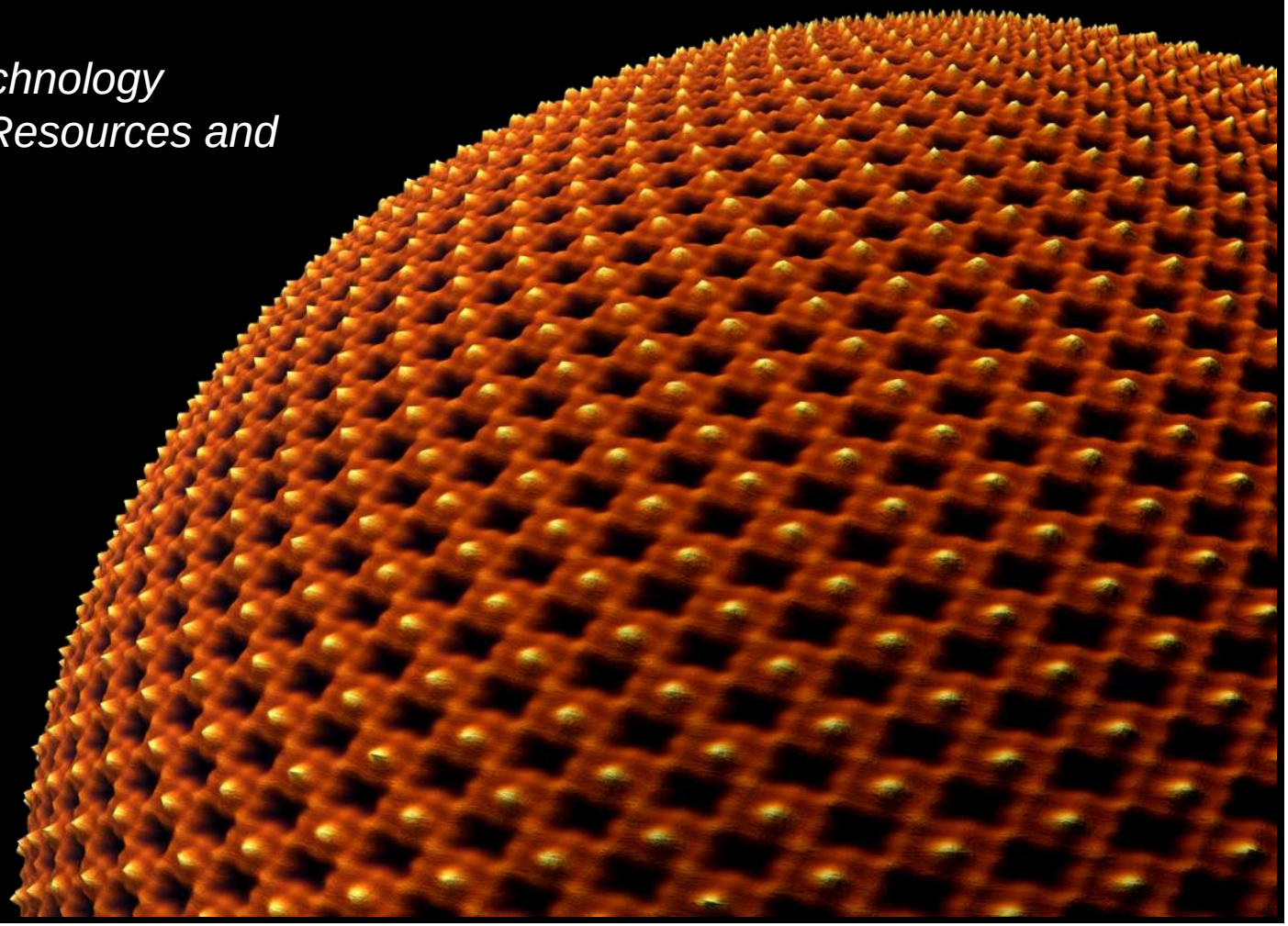
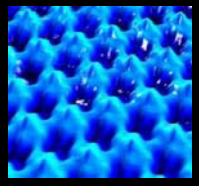
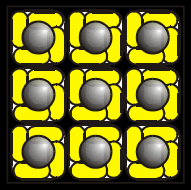


NanoBioEuro 2008

Barcelona

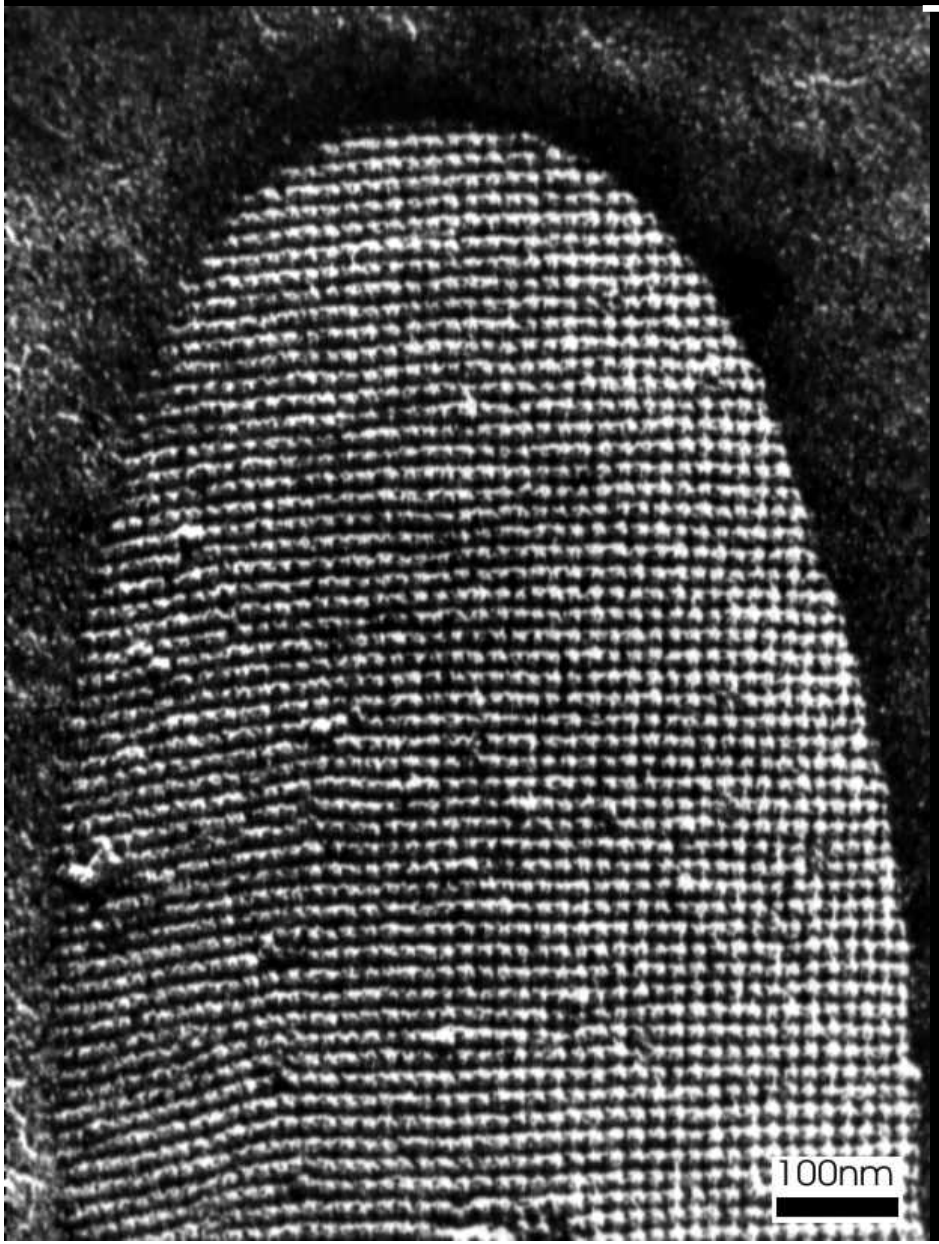
Outline:





- Description of S-layer proteins
- Functional S-layer arrays

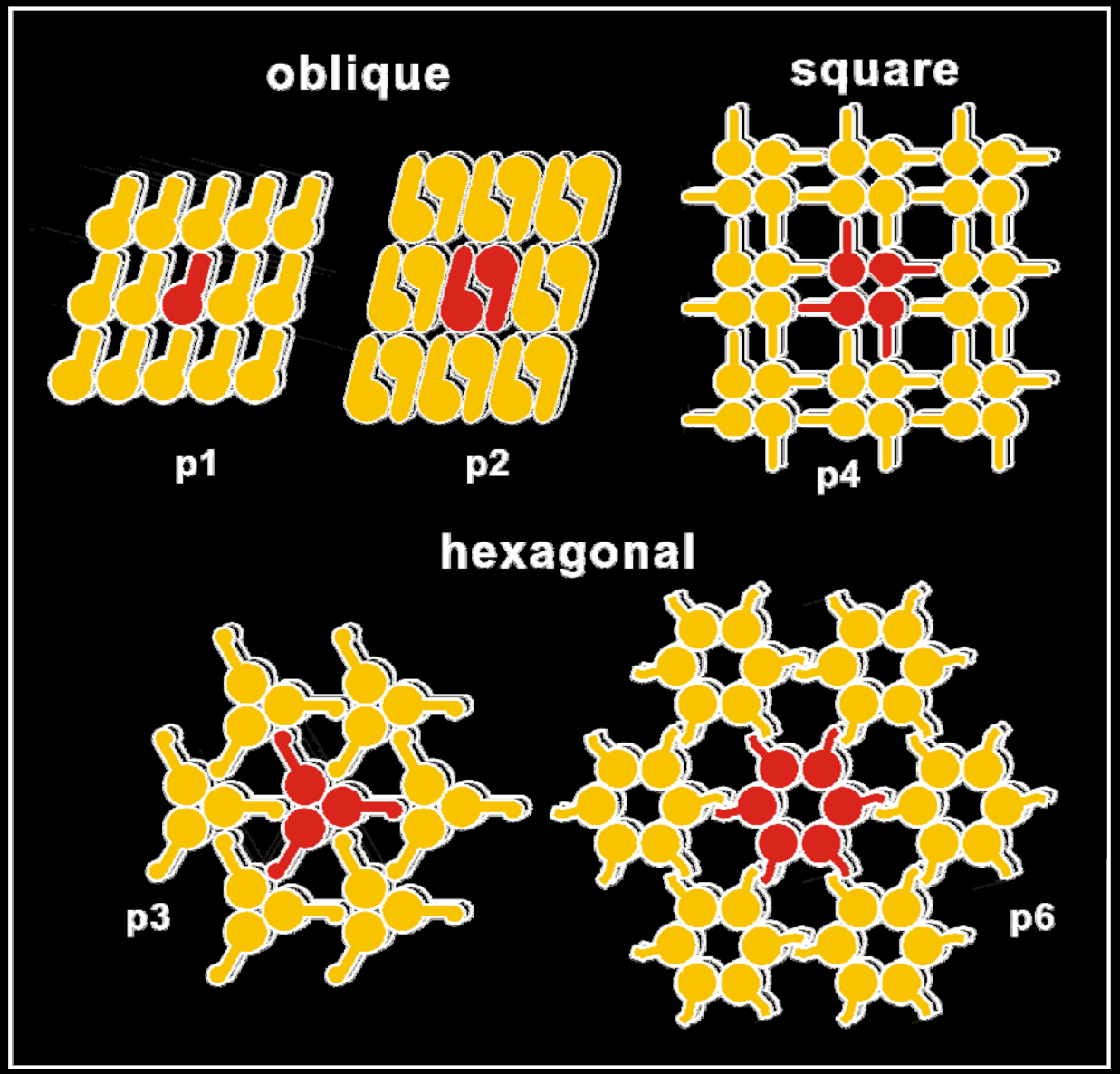
Description of S-layers



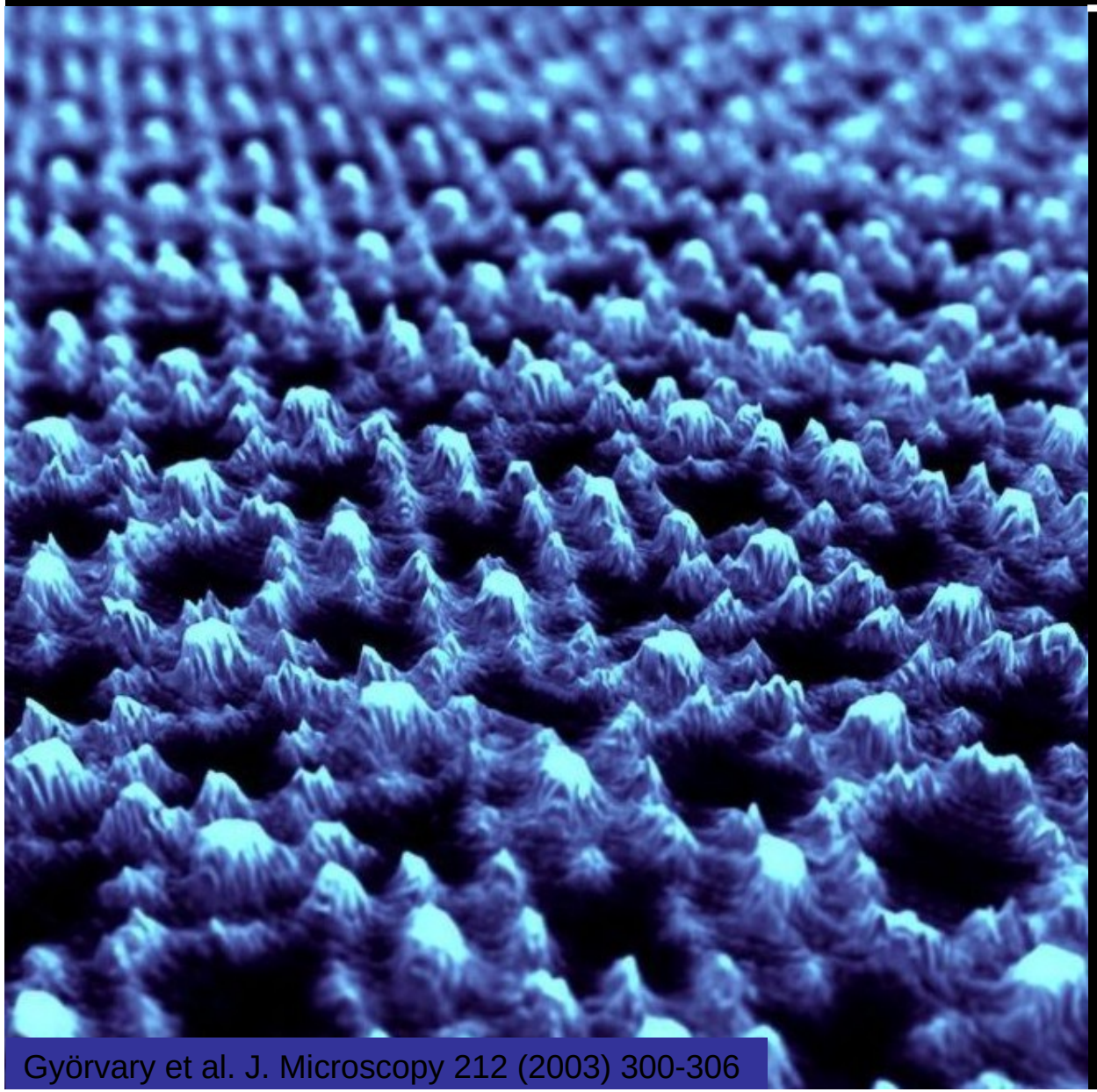
Crystalline bacterial cell surface layer proteins (S-layer proteins) represent the outermost cell envelope component in a broad range of eubacteria and archaea.

TEM of bacterial cells with an S-layer showing square lattice symmetry.

S-layer lattice types



Ultrastructure of S-layers

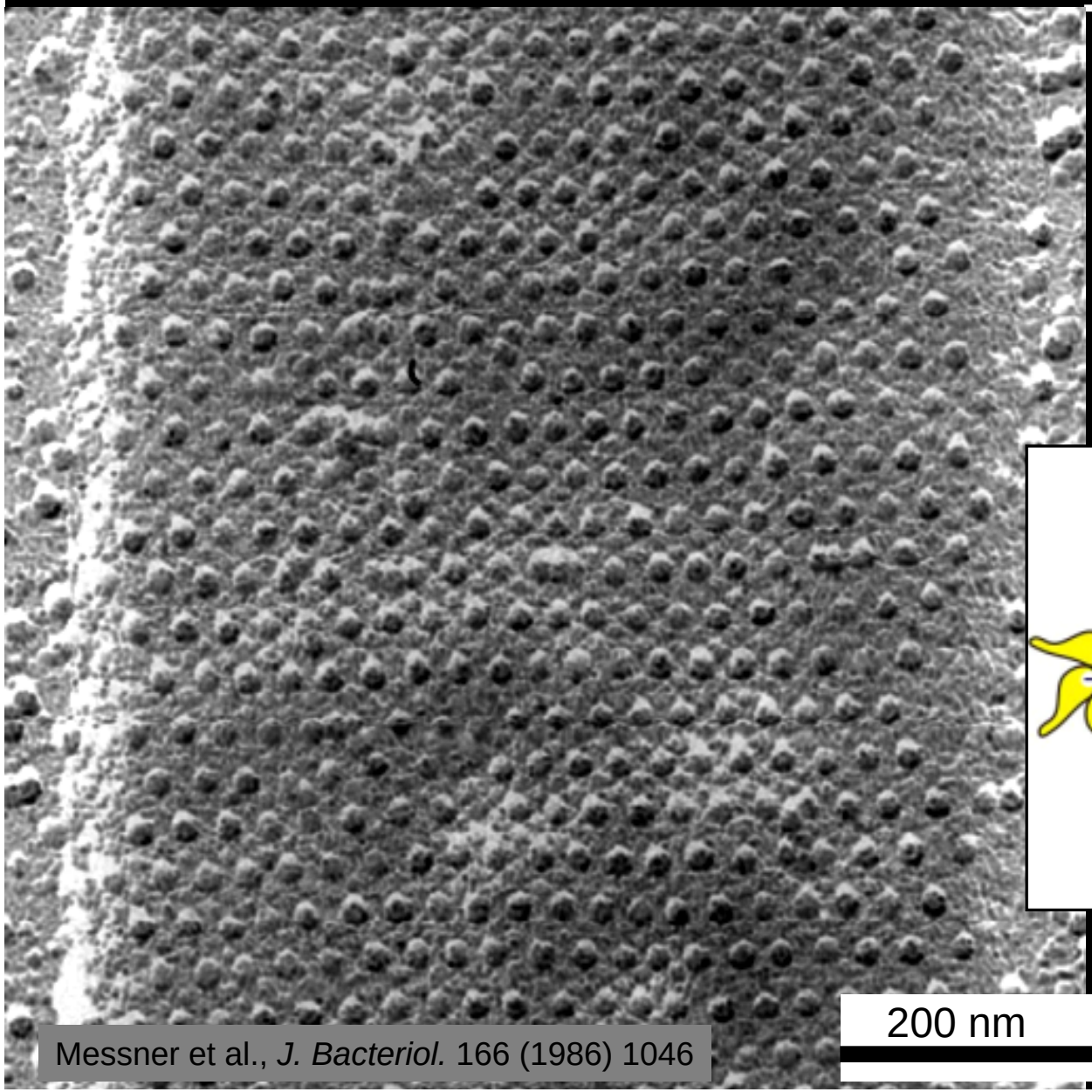


Györvary et al. J. Microscopy 212 (2003) 300-306

- M_r: 40-200 kDa
- Unit cell sizes: 3 – 30 nm
- Thickness: 5 – 10 nm
- Pores are of identical size and morphology

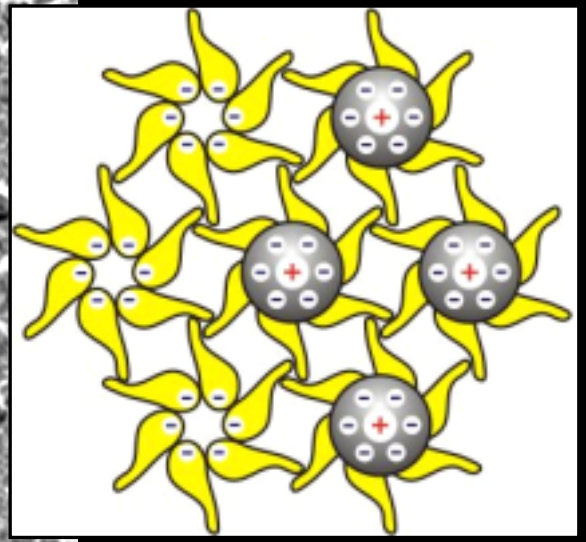
AFM image of an S-layer showing square lattice symmetry (d=13.1nm).

S-layers as patterning elements



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

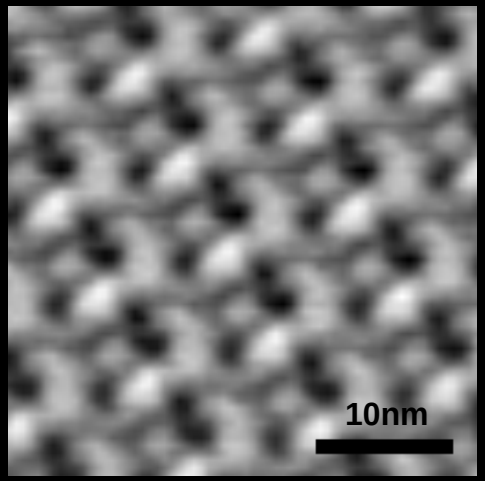
Functional groups and domains are repeated with the periodicity of the lattice

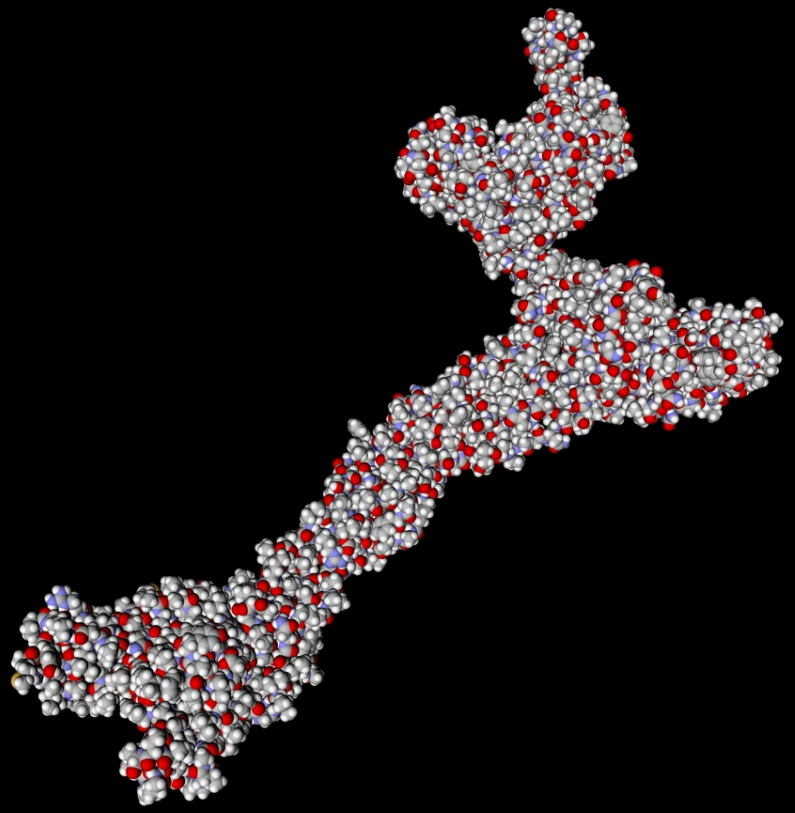


TEM image of PCF molecules bound to an S-layer showing hexagonal lattice symmetry.

3D-structure prediction by molecular modeling

(S-layer protein SbsB from *Geobacillus stearothermophilus* pv72/p2)

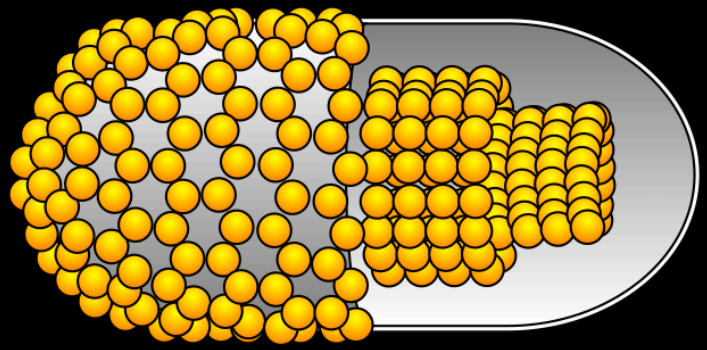




Horejs et al. J. Chem. Phys. 128 (2008) 65106

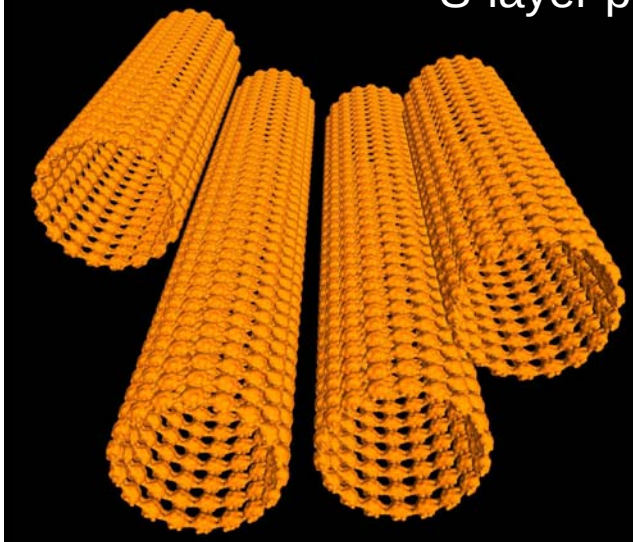
Reassembly of S-layer proteins

bacterial cell

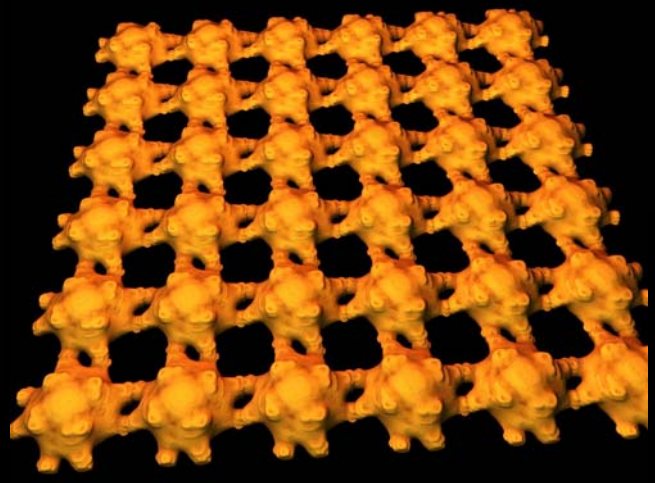


expression host
(e.g. *E.coli*)

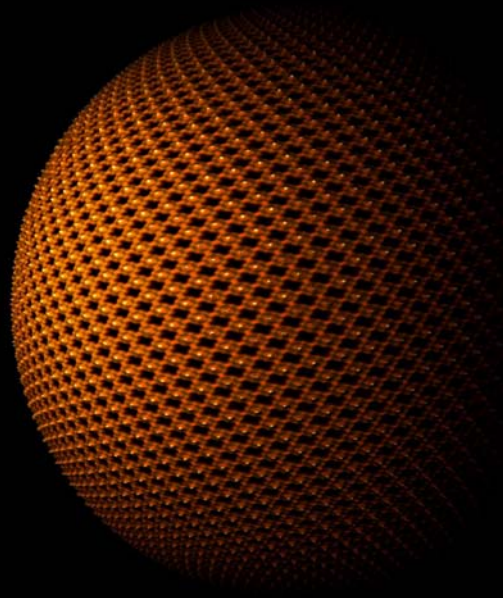
reassembly of native and
recombinantly produced
S-layer proteins



cylindrical self-assembly
products in solution

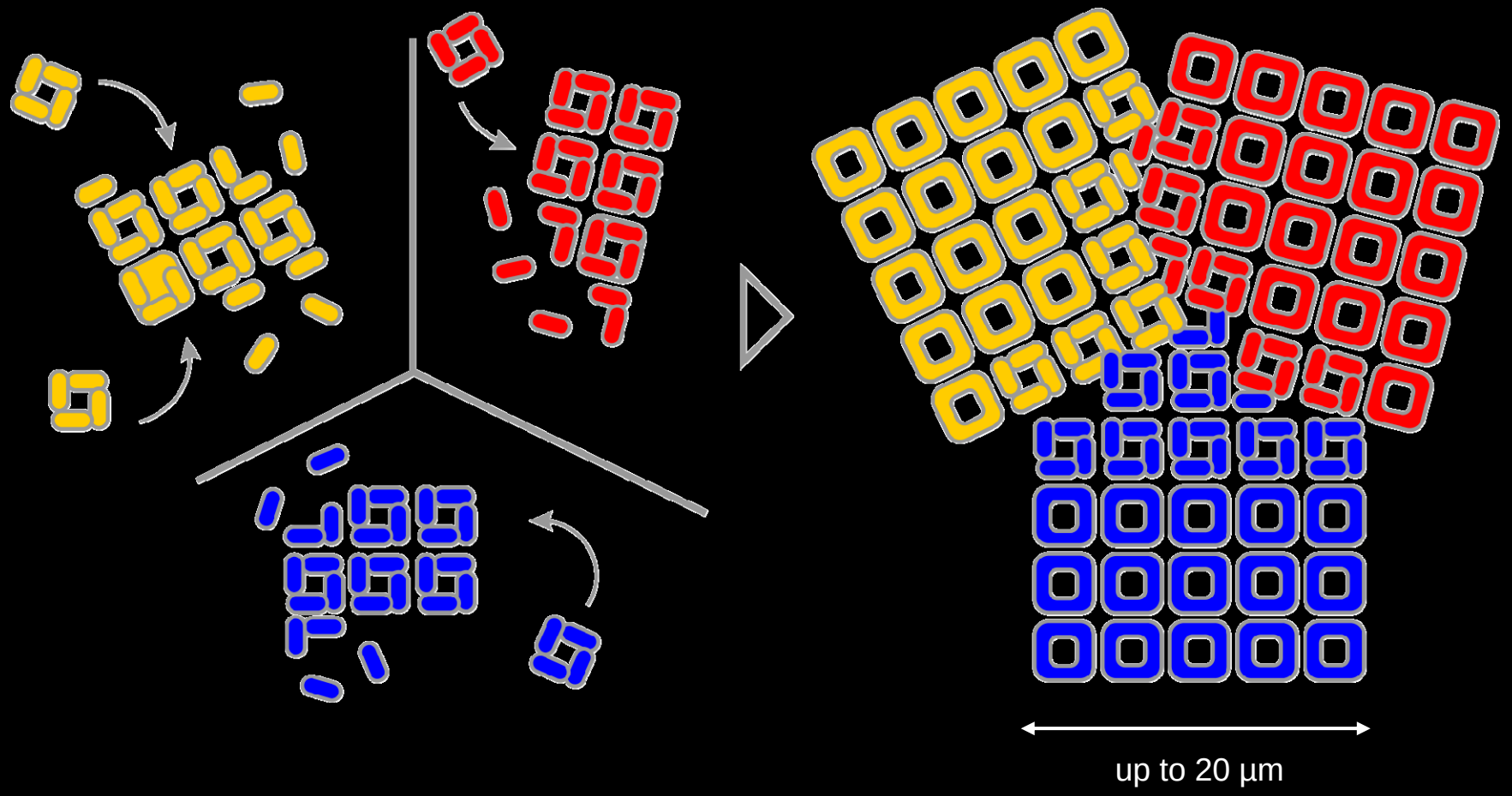


monolayers in solution
and at interfaces



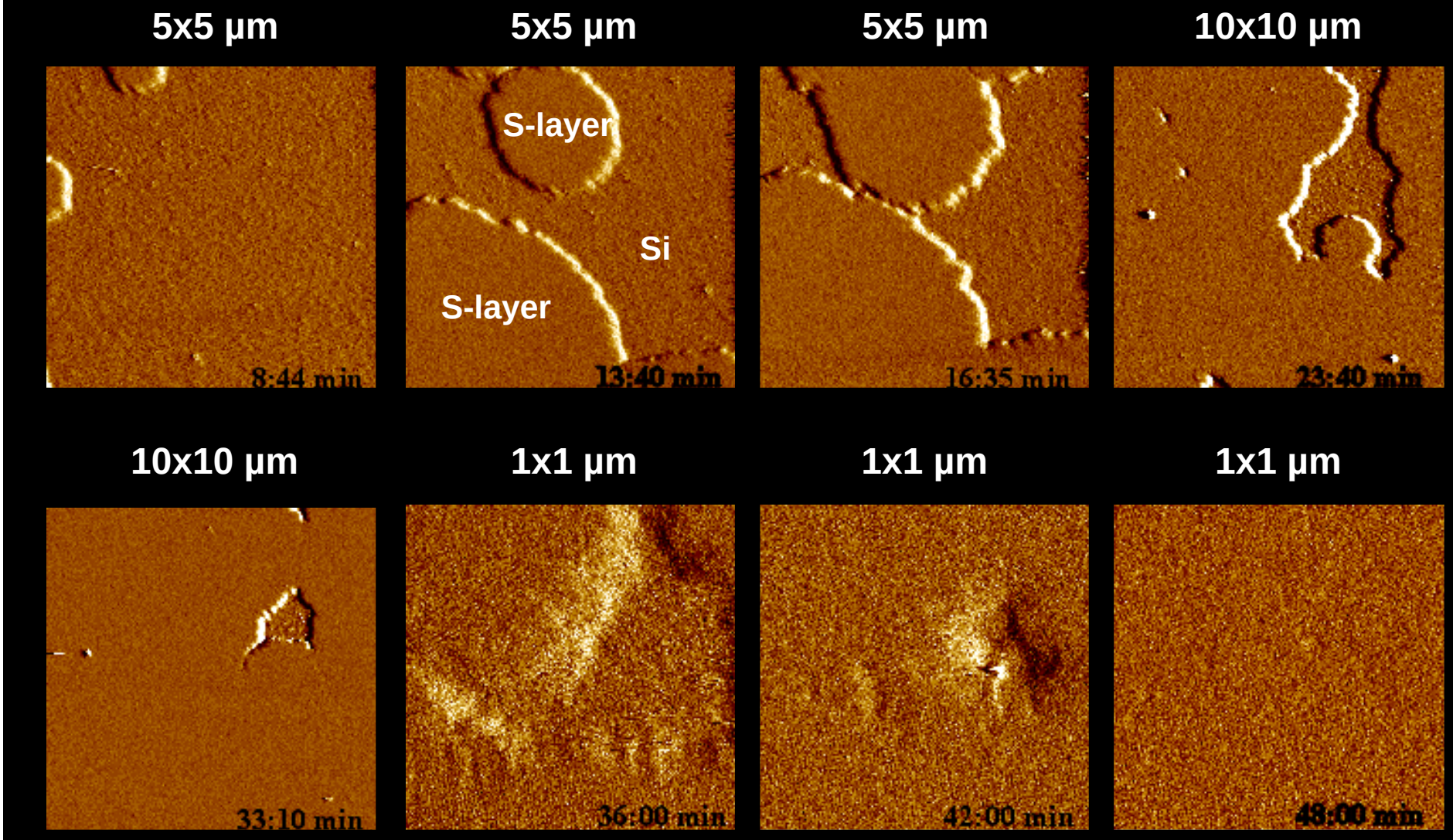
planar lipid membranes,
liposomes and nanocapsules

Crystal growth at interfaces



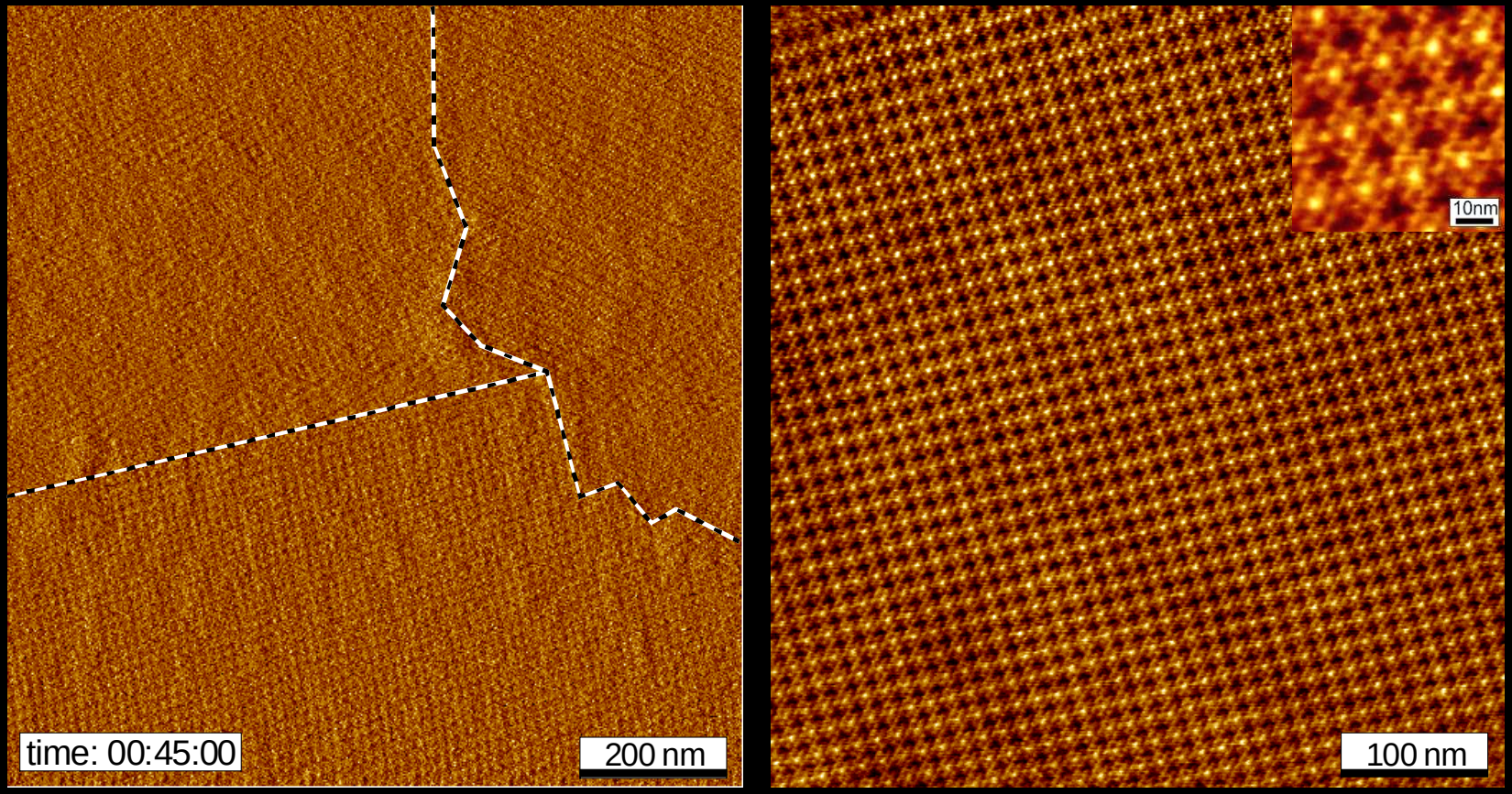
Crystal growth of S-layers on solid supports, at the liquid-air interface or on lipid films.

Crystal growth on silicon



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

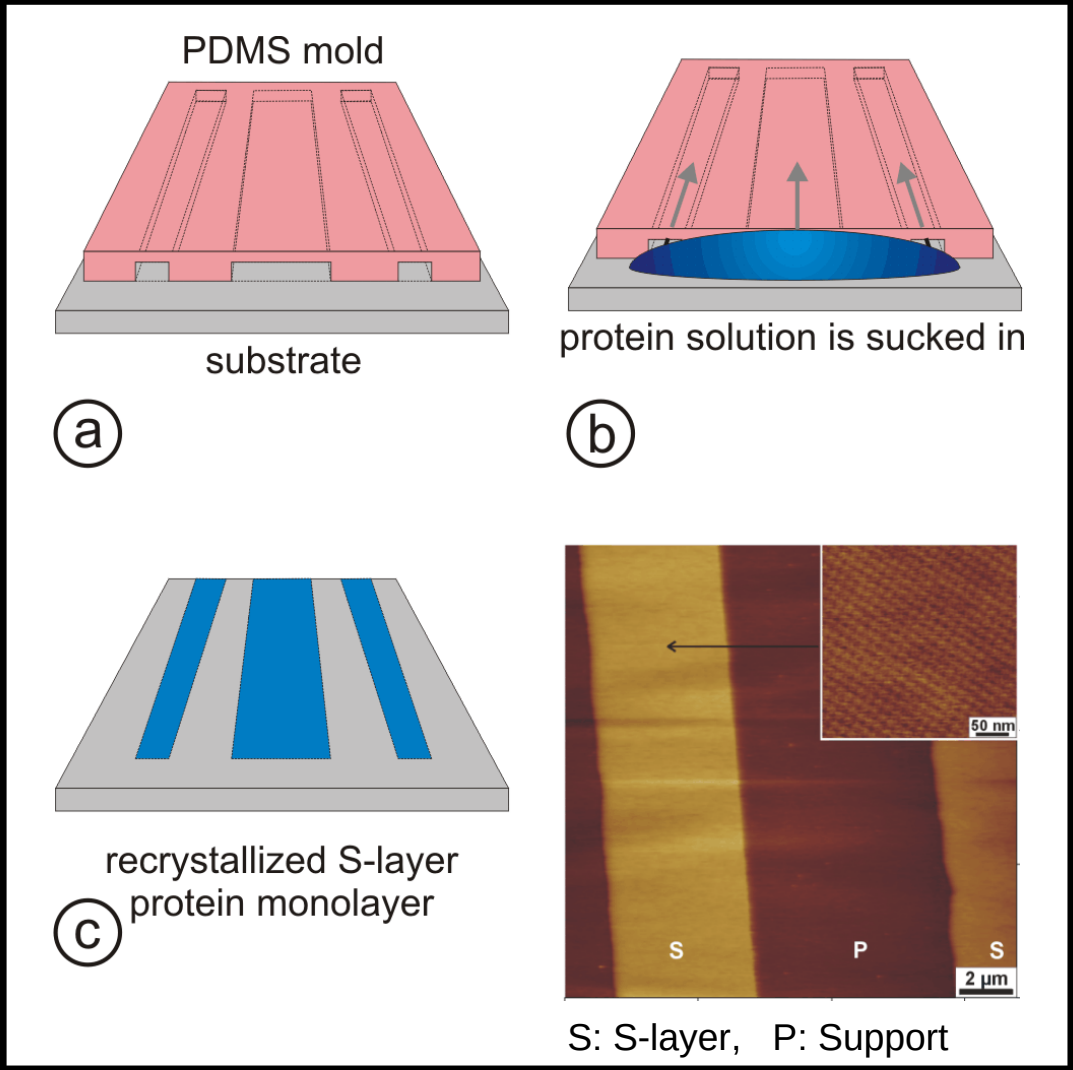
Formation of an S-layer protein monolayer on silicon



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

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

Patterning of S-layers by Soft Lithography (Micromoulding in Capillaries - MIMIC)

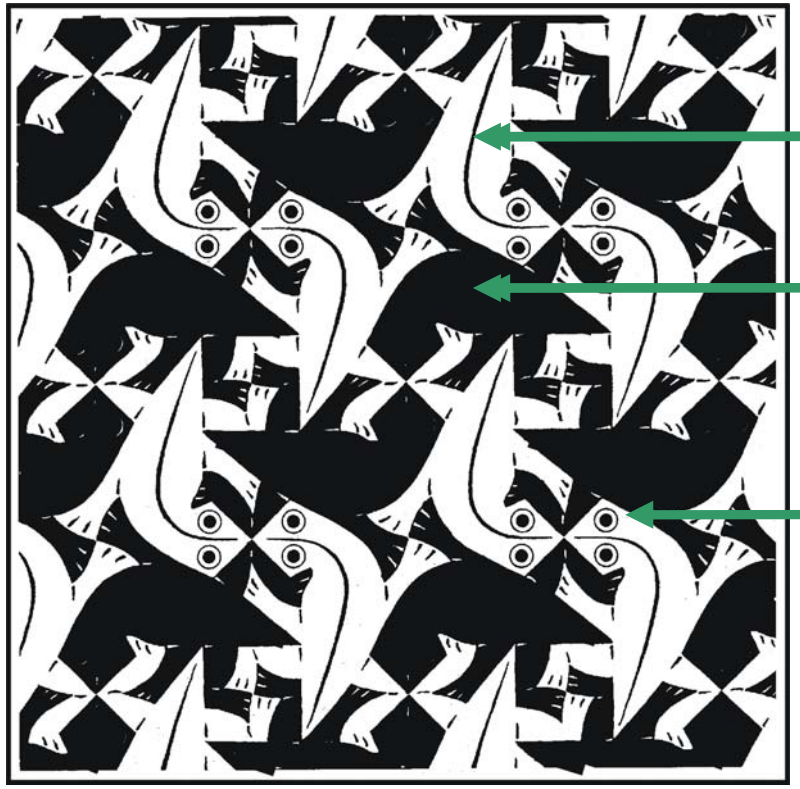


Györvary *et al*., Nanoletters 3 (2003) 315

PDMS = Poly(dimethylsiloxane) (PDMS)

Functional S-layer arrays

- S-layer functionalized solid supports
- S-layer supported lipid membanes
- S-layer coated liposomes



S-layer lattices are composed of identical species of subunits.

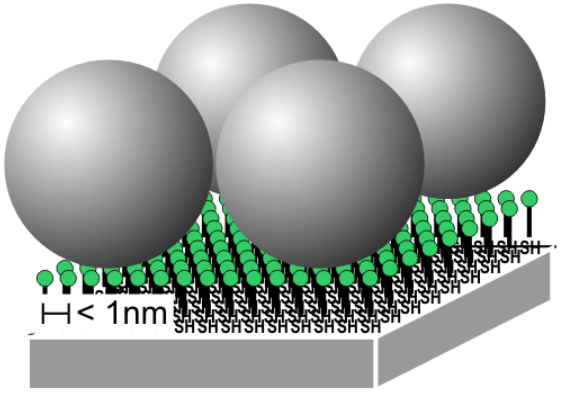
Pores passing through show identical size and morphology.

Functional groups are aligned in well defined positions and show defined orientations.

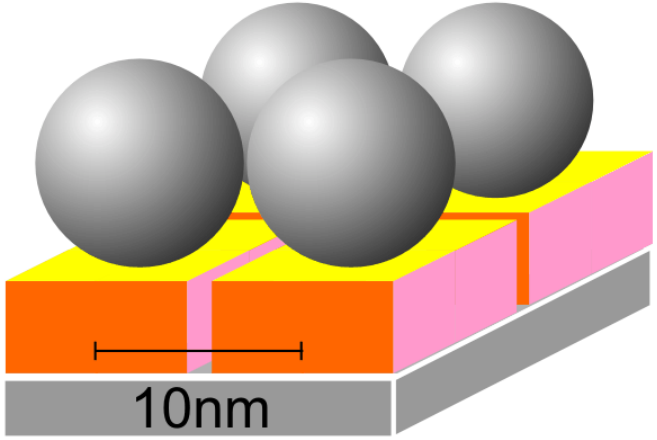
H.C. Escher

Strategies for modification and functionalization of solid supports

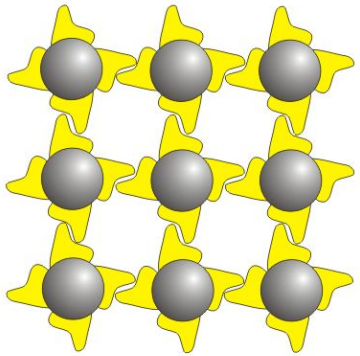
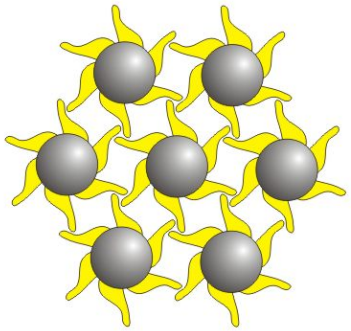
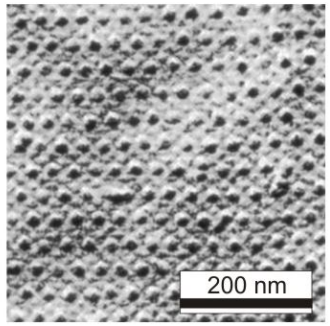
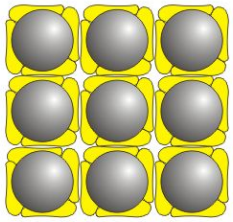
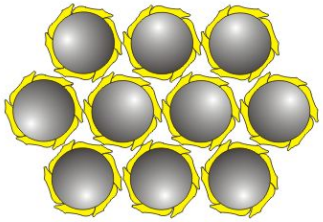
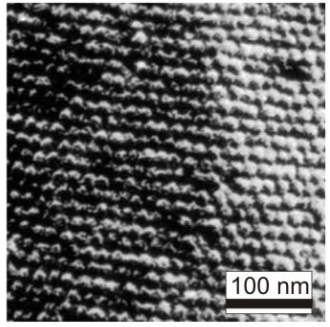
- Self-assembled monolayers (SAMs)



- S-layers lattices



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

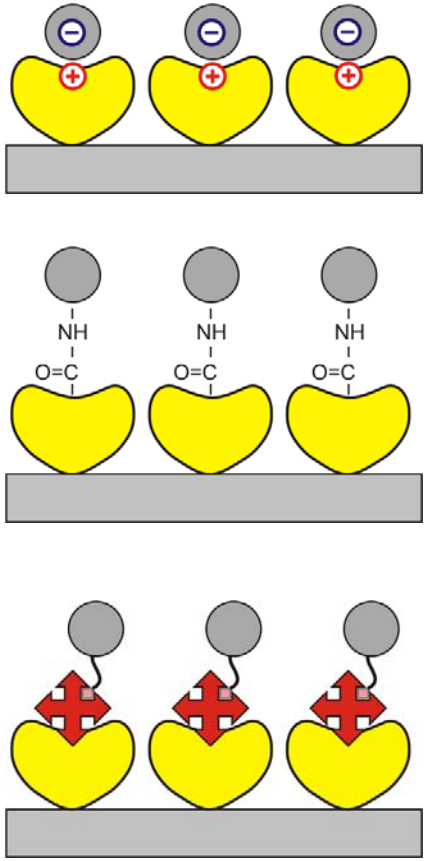
Binding of molecules and nanoparticles on S-layers

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)

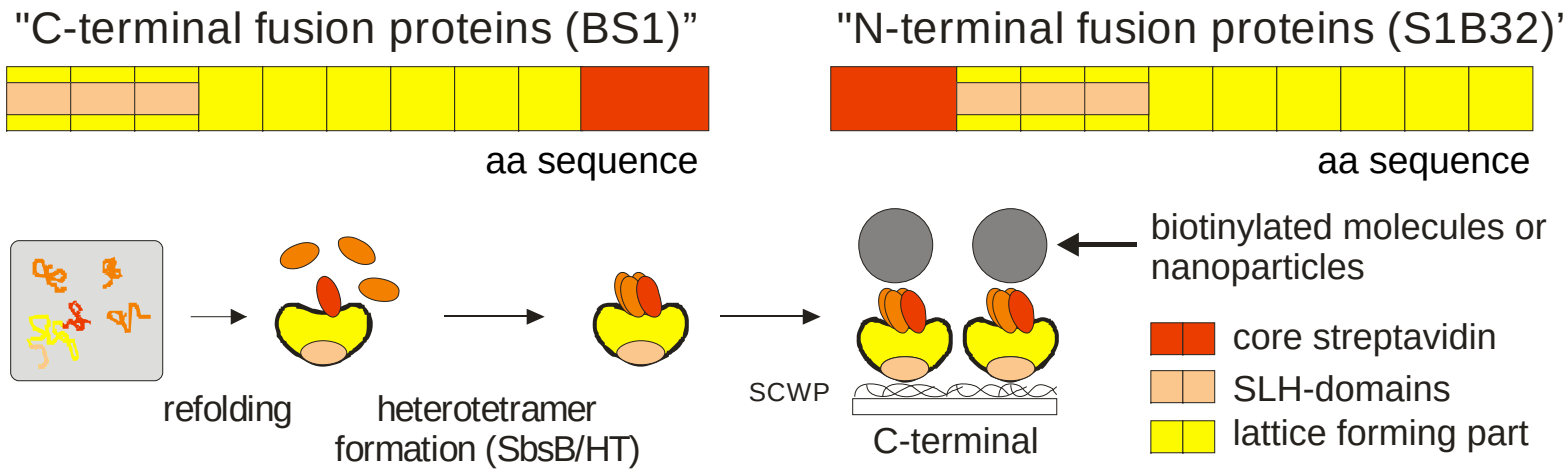
Genetically engineered S-layer protein:

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



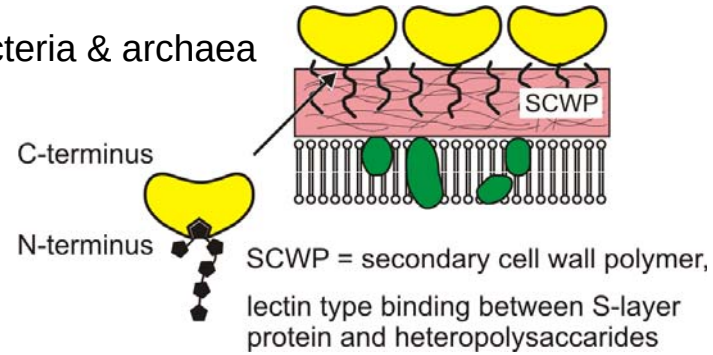
Functional domains (native or genetically introduced) are repeated with the periodicity of the S-layer lattice leading to regular arrays of bound molecules and particles.

Binding of biotinylated molecules and nanoparticles on S-layer streptavidin fusion proteins

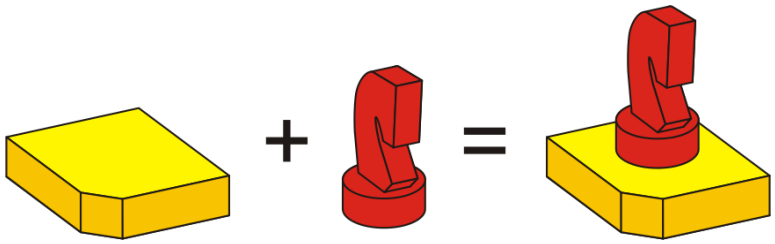


Moll et al., PNAS, 99 (2002) 14646

Gram positive bacteria & archaea

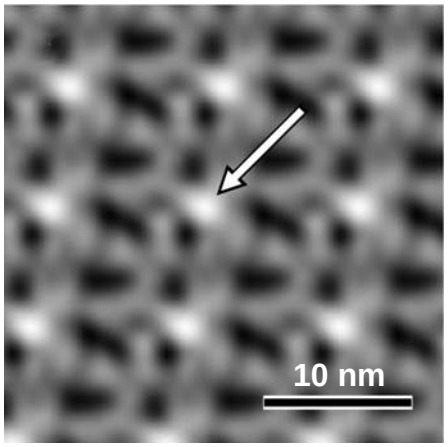


SbsB / Streptavidin fusion proteins

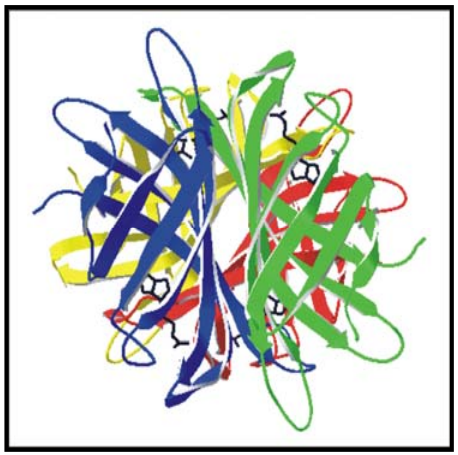


S-layer + function

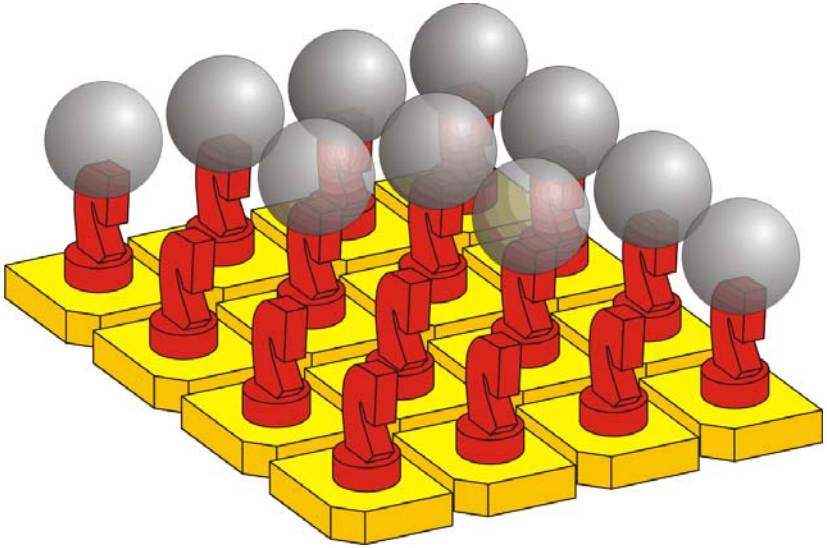
(Enzyme, antibody, antigen, ligand)

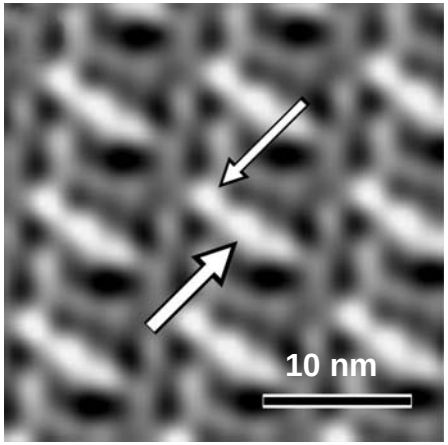


Native S-layer protein

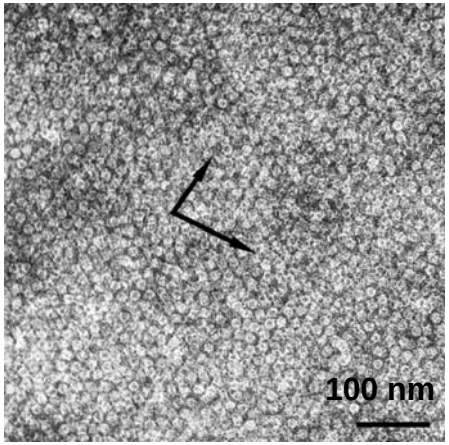


Streptavidin





S-layer fusion protein

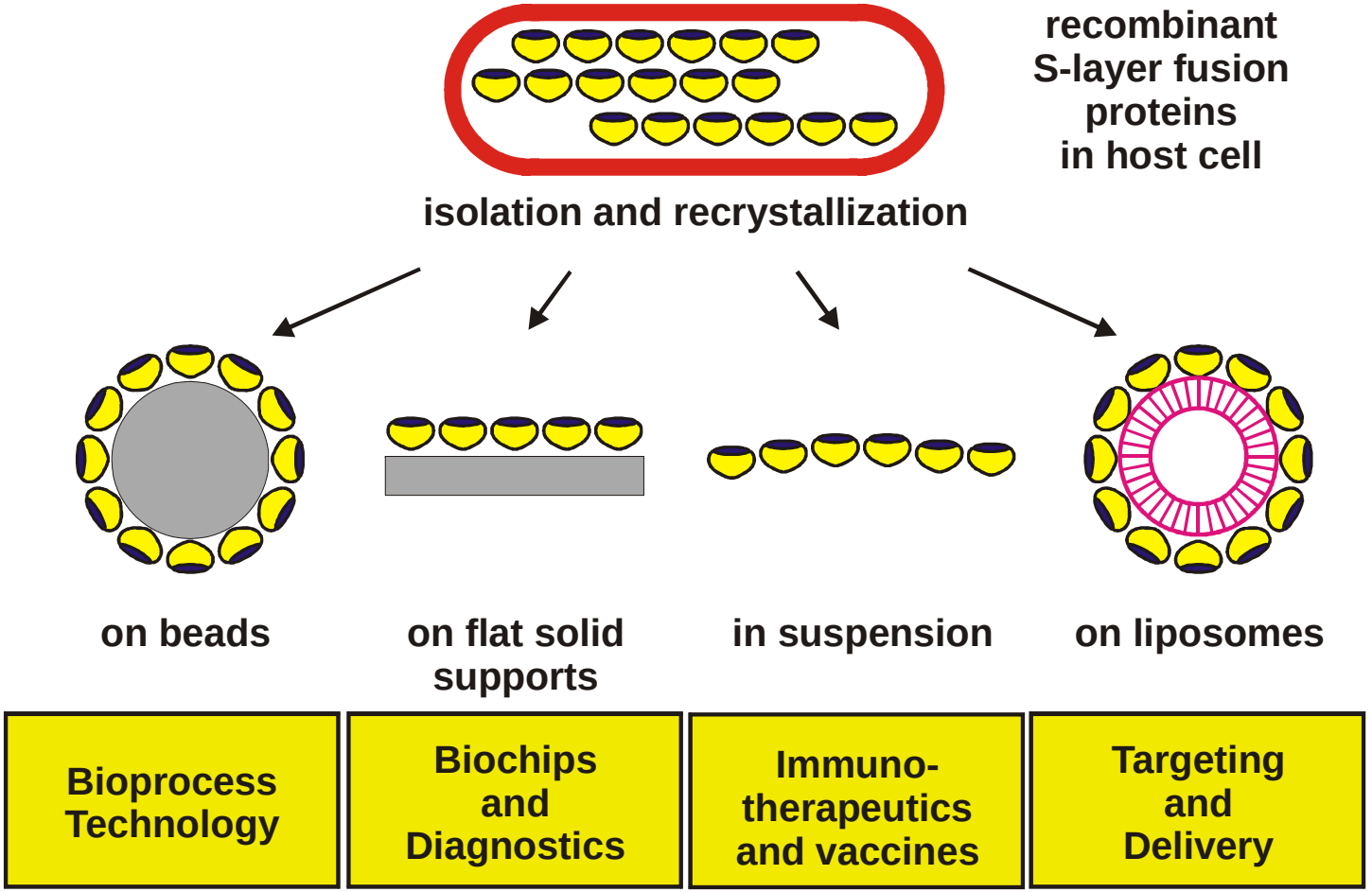


Biotinylated ferritin bound on BS1

S-layer Fusion Proteins

S-layer fusion protein (selected from various constructs)	Length of funct.	Functionality
$rSbsB_{1-889}$ / core streptavidin $rSbpA_{31-1068}$ / core streptavidin	118 aa	Biotin binding
$rSbpA_{31-1068}$ / Bet v1	116 aa	Major birch pollen allergen
$rSbpA_{31-1068}$ / Strep-tag	9 aa	Affinity tag for streptavidin
$rSbpA_{31-1068}$ / ZZ	116 aa	IgG-Binding domain
$rSbpA_{31-1068}$ / GFP	238 aa	Green fluorescent protein
$rSbpA_{31-1068}$ / cAb	117 aa	Heavy chain camel antibody
$rSbpA_{31-1068}$ / LamA $rSgsE_{331-903}$ / RmlA	263 aa 299 aa	(hyper)thermophilic enzymes
$rSbpA_{31-1068}$ / AG4 and AGP35 $rSbpA_{31-1068}$ / CO2P2 $rSbpA_{31-1068}$ / His $rSbpA_{31-1068}$ / rSilC	12 aa 12 aa 6 aa 158 aa	Silver binding Cobalt binding Gold binding Silica & titania binding

Technologies Based on Recombinant S-layer Fusion Proteins

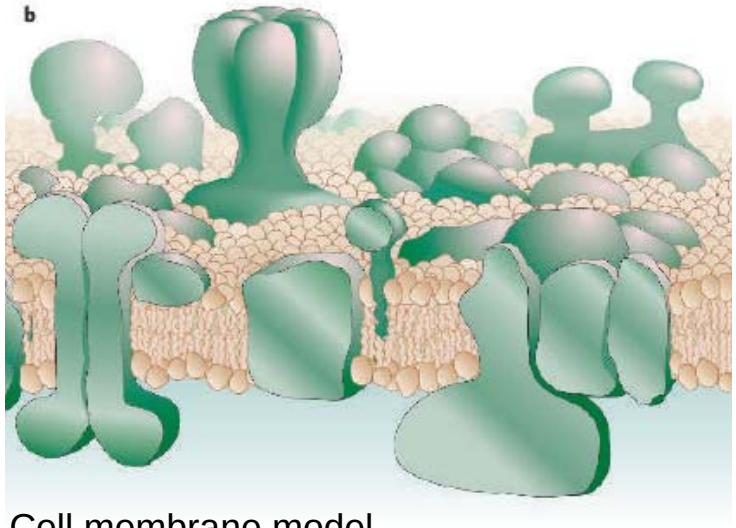


Sleytr *et al.,* 2002, In: A. Steinbüchel, S. Fahnestock (eds.) *Biopolymers*, Vol. 7, Wiley-VCH, Weinheim, Germany.
Sleytr *et al*., 2001, *Progr. Surf. Sci*. 68:231-278
Schuster & Sleytr, 2000 *Rev. Molec. Biotechnol*. 74:233-254

Applications of S-layer Fusion Proteins

- Sensing layers for label free detection systems (SPR, SAW, QCM-D)
- High density affinity coatings (MDS - blood purification)
- Immunogenic and immunomodulating structures (e.g. development of anti-allergic vaccines)
- Stabilization of Langmuir lipid layers
- Functionalization of liposomes or lipid/plasmid particles as targeting and delivery systems
- Immobilizing of enzymes
- Precipitation and binding of nanoparticles

Biomimetic Cell Membranes

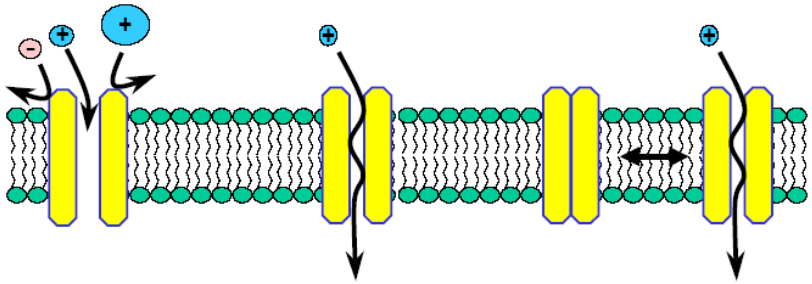


Cell membrane model

Engelman, 2005, *Nature*, 438:578

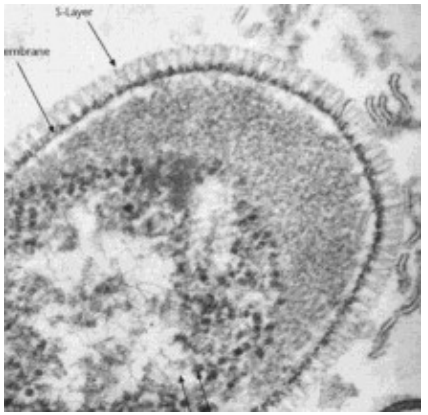
Specific membrane functions

1.selectivity 2.binding 3.opening / closing

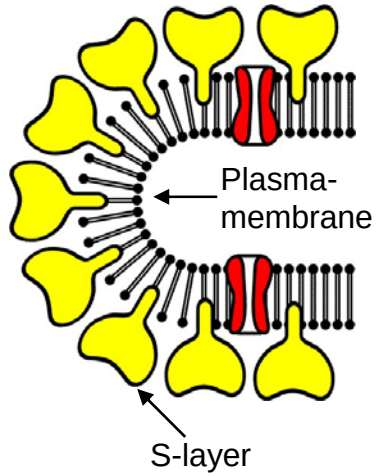


Archaeal cell envelope structure

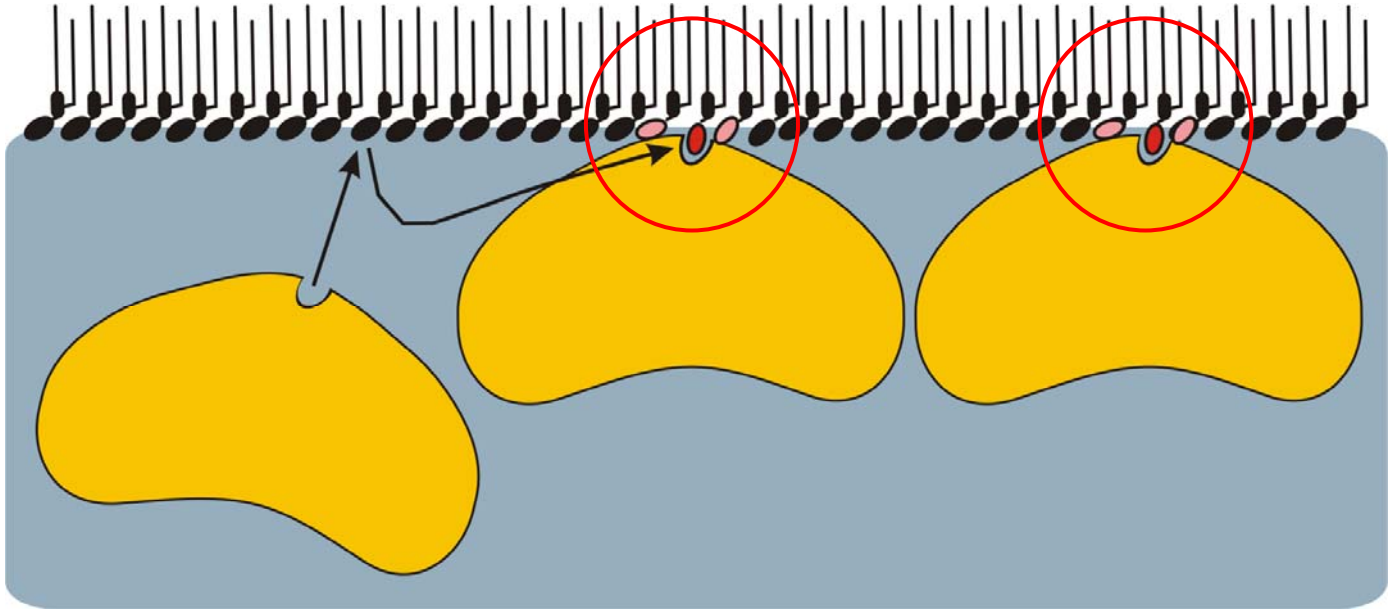
A supramolecular structure optimized in ~ 3,5 billions of years under extreme environmental conditions (120°C, concentrated salt solutions, pH 0, 1100 bar).



TEM of an archaeal cell



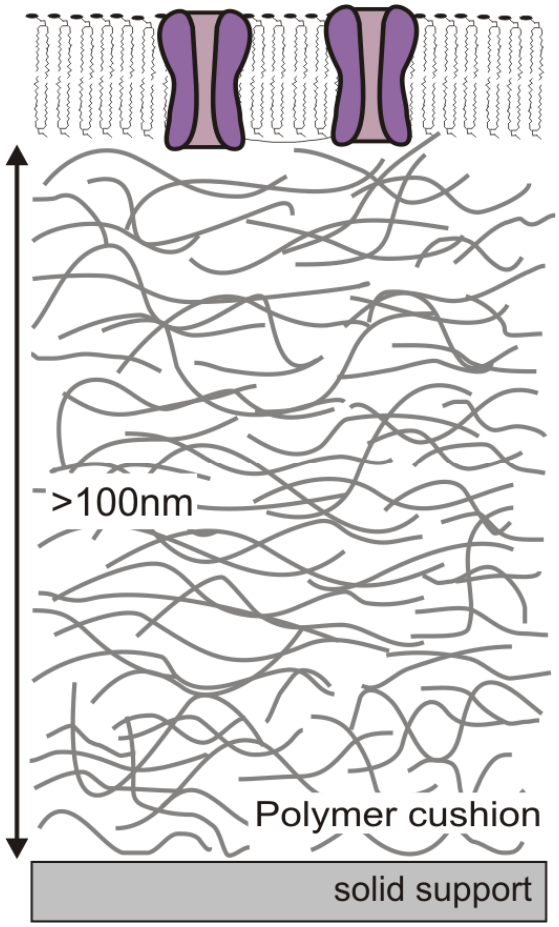
S-layer supported lipid membranes (Semifluid lipid membranes)



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

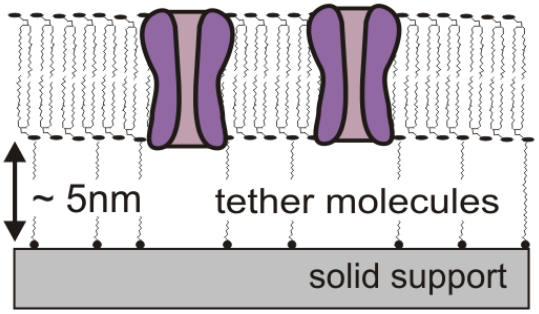
Biomimetic Lipid Membrane (Semifluid Membrane Model)



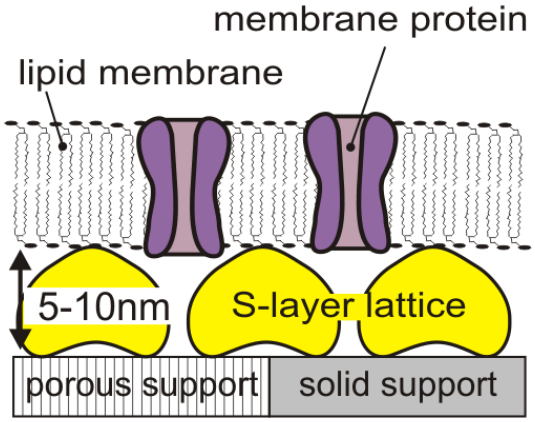
Sackmann, Science 271 (1996) 43

- 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



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

…why stabilized Lipid Model Membranes?

- For long-term studies on functional membrane proteins in lipid membranes:

  ~30 % of all proteins found in various organisms are membrane proteins

  > 50 % of the proteins interact with membranes

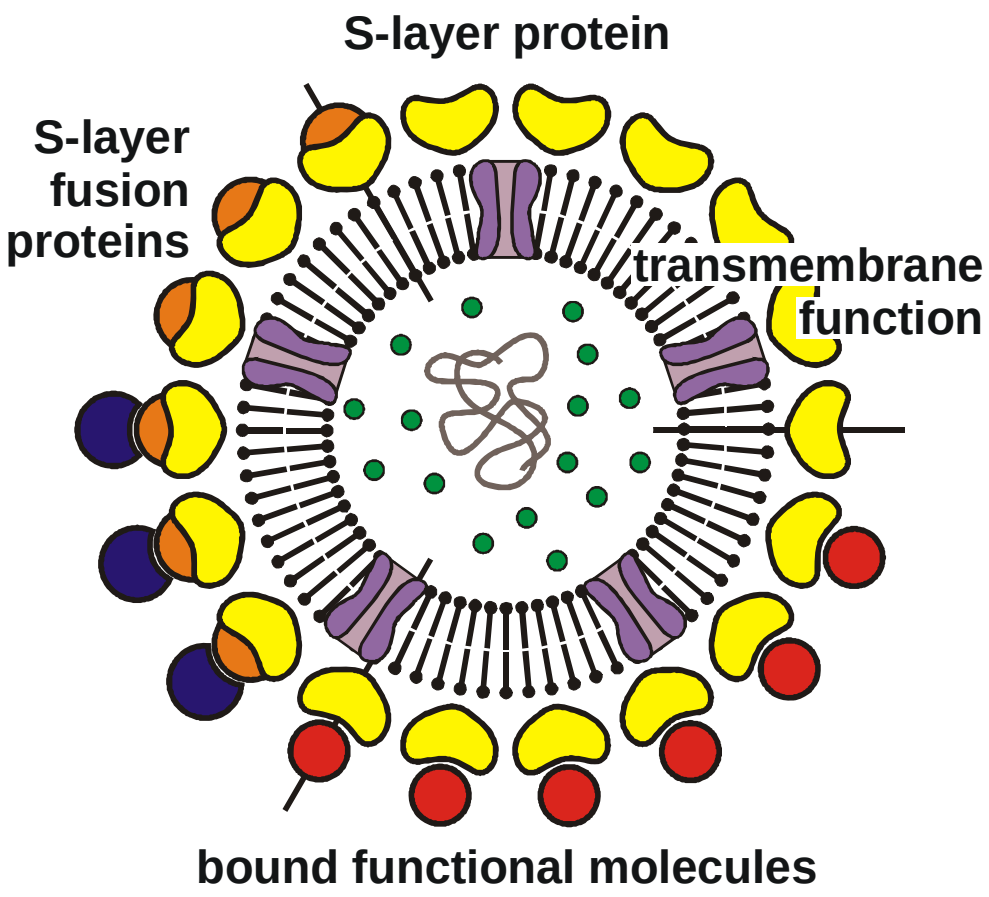
  ~ 15 % of the most sold drugs act on ion channels

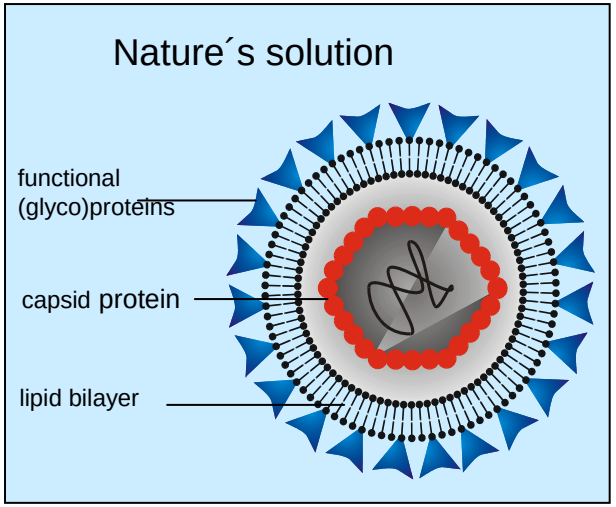
  ~ 60 % of the ethical drugs affect membrane proteins

Application potential of functional lipid membranes:

- Diagnostics
- Linking silicon technology and solid state physics with biological systems (e.g. coupling cells to surfaces)
- Lab-on-a-chip, HTScreening
- Biosensors

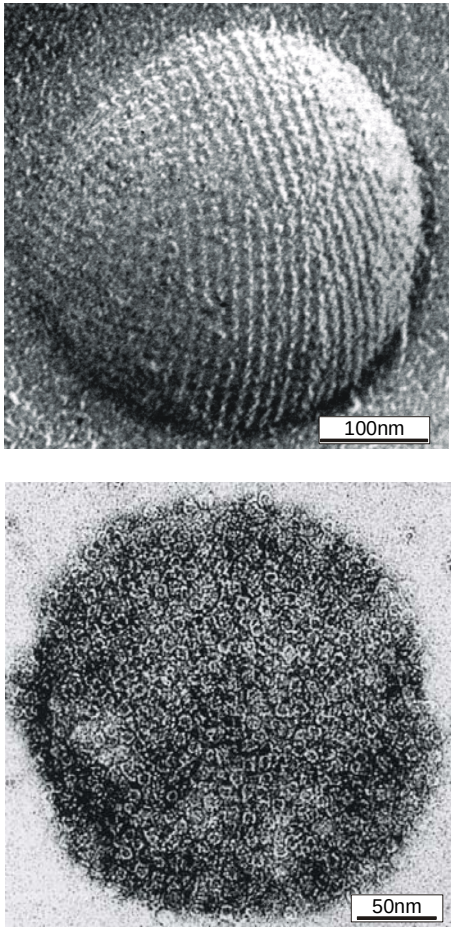
S-layer coated lipid/plasmid transfection particles (Artificial Virus)





"wooden horse" from the 2004 film *Troy*
(R. Burgess, GNU Free Doc License)

Properties of S-layer coated liposomes



What is new in comparison to conventional “naked” liposomes?

- Higher (mechanical and thermial) stability
- Monomolecular coating with functional molecules (e.g. with addressor molecules)
- Possibility for transport of hydrophilic and hydrophobic substances

Potential applications

- Vaccines, immune therapy, drug-targeting and drug-delivery
- Artificial viruses (inclusion of nucleic acid) for gene therapy

Küpcü et al. BBA 1235 (1995) 263
Mader et al. BBA 1418 (1999) 106
Mader et al. BBA 1463 (2000) 142

Summary

Basic research on structure, genetics, chemistry, morphogenesis and function of S-layers has led to a broad spectrum of applications in the Life and Non-life sciences.

The key properties of S-layer proteins for a broad range of applications include their

- ability to self-assemble, and
- to expose functional domains in well defined orientation and position.

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