Hybrid polyelectrolyte/bacterial protein (nano)biomimetic surfaces: a model to study bacterial surface layer recovery and bacterial affinity

Mihaela Delcea[1], Dietmar Pum[2], Rumen Krastev[3], Uwe B. Sleytr[2] and José Luis Toca-Herrera[1]

[1]Biosurfaces Unit, CICbiomaGUNE, Paseo Miramón 182, Parque Tecnológico de San Sebastián, 20009 San Sebastián (Gipuzkoa), Spain
[2]Center for Nanobiotechnology, BOKU, Gregor-Mendel Str. 33, 1180 Vienna, Austria
[3]Max-Planck Institute for Colloids and Interfaces, Am Muehlenberg 1, 14420 Potsdam, Germany
jltocaherrera@cicbiomagune.es

Robust (nano)biomimetic surfaces that mimic the behaviour of cell membranes can be built by combining polyelectrolyte multilayer (PEM) deposition and bacterial surface layer technology [1]. This symbiosis can provide a variety of model systems and applications such as, artificial cells and biosensor development.

Polyelectrolytes are used as a cushion for crystalline bacterial cell surface layers (S-layers), replacing the secondary cell wall polymer from bacteria. S-layers are one of the most common cell envelope components of prokaryotic organisms, composed of a single (glyco)protein, representing the simplest biological membrane developed during evolution [2]. Isolated S-layer subunits have the ability to self-assemble into 2-D crystalline structures at the air-water interface [3], on lipid films [4], on liposomes [5], on solid support [6], on solid supports coated with polyelectrolytes and on hollow polyelectrolyte microcapsules [1]. The study of the stability of S-layers is relevant in microbiology and nanotechnology since they play among others, a protective role and can serve as a (nano)matrix for bio-immobilization.

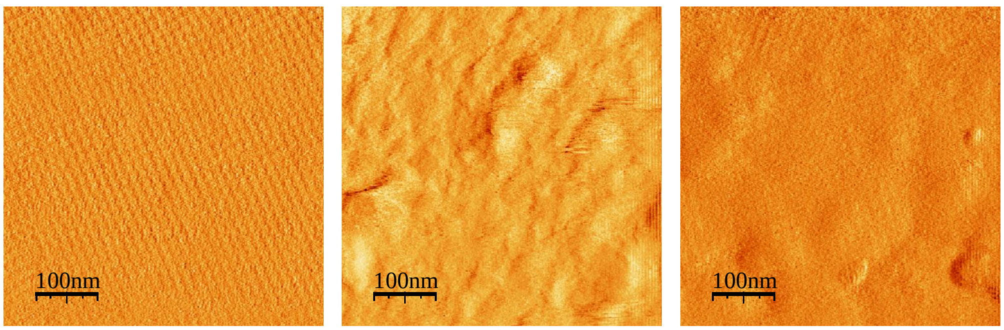
Our studies show that recrystallized S-layers (from *Bacillus sphaericus*) preserve their crystalline structure for three ethanol/water mixtures (20%, 40% and 60% ethanol). After exposing the S-layer surface to an ethanol/water mixture 80:20 (v/v), the 2-D crystalline structure was lost. Treatment with recrystallization buffer led to a recovering of the crystalline structure, showing that the S-layer surface can be denatured and renaturated in a switched on-off process (see Figure 1). This fact could not be observed when the S-layer was exposed at different pH solutions and thermally treated. It was found that the 2-D crystalline structure was lost at pH 3 and at 55ºC.

After studying the chemical and thermal stability of recrystallized S-layers on PEM, a novel hybrid sandwich-like supramolecular structure (polyelectrolyte/S-layer/polyelectrolyte/S-layer) was built. The supramolecular construct permitted to elucidate the bacterial affinity for different polyelectrolytes (Figure 2). It was found that only positive polyelectrolytes could adsorb on the exposed S-layer surface. These results open a possibility to build functional hybrid supramolecular structures with potential applications in nanobiotechnology, and give insight about bacterial adhesion.

References:

[1] J. L. Toca-Herrera, R. Krastev, V. Bosio, S. Küpcü, D. Pum, A. Fery, M. Sara and U. B. Sleytr, *Small*, **1** (2005) 339.
[2] U. B. Sleytr, M. Sara, D. Pum, B. Schuster, *Progress in Surface Science*, **68** (2001) 231.
[3] D. Pum and U. B. Sleytr, *Landesr Academic Press*, Austin, TX, (1996) 175.
[4] B. Schuster and U. B. Sleytr, *Reviews in Molecular Biotechnology*, **74** (2000) 233.
[5] S. Küpcü, M. Sara, U. B. Sleytr, *Biochim. Biophys. Acta*, **1235** (1995) 263.
[6] D. Pum and U. B. Sleytr, *Thin Solid Films*, **244** (1994) 882.

Figures:



a b c

Figure 1. Atomic force microscopy deflection images of S-layers recrystallized on PEM. a) S-layer just as it was formed; b) S-layer treated with EtOH/H2O 80/20 (v/v); c) Recovered S-layer after treatment with recrystallization buffer (S-layer protein renaturation).

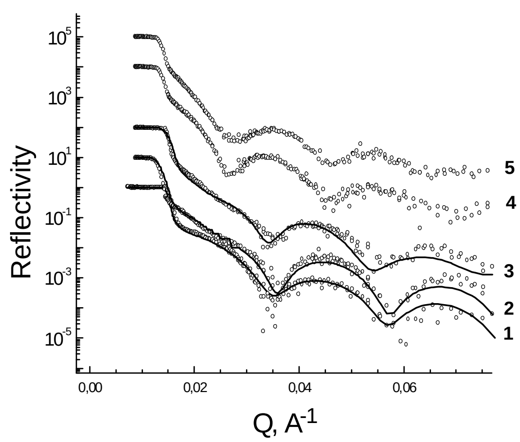


Figure 2. Neutron reflectivity as a function of Q for the following macromolecular structures: 1-Si/PE/PSS-terminated, 2- Si/PE/PSS/S-layer, 3- Si/PE/PSS/S-layer/PAH, 4- Si/PE/PSS/S-layer/$(PAH/PSS)_2$ and 5-Si/PE/PSS/S-layer/$(PAH/PSS)_2$/S-layer. Curves 2 and 3 show respectively the formation of the S-layer on PSS and the affinity of PAH to the S-layer. Note that curves 4 and 5 are similar, meaning that no homogenous S-layer was formed. PE=polyelectrolyte; PSS= Poly(sodium 4-styrenesulfonate); PAH= Poly(allylamine hydrochloride).