# STABILITY AND DENATURATION OF RECRYSTALLISED BACTERIAL PROTEIN LAYERS ON HYDROPHILIC SUBSTRATES

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Crystalline monomolecular cell surface layers, S-layers, are one of the most common outermost cell envelope components of the prokaryotic organisms protecting them from competitive habitats. They are composed of a single protein or glycoprotein and exhibit oblique, square or hexagonal lattice symmetry. Since isolated S-protein subunits are able to re-assemble into crystalline arrays on lipid films and polyelectrolytes making biomimetic surfaces, S-layer technology is currently used in nanobiotechnology [1, 2]. An important aspect of the biomimetic surfaces built with S-layers is their stability under extreme solvent conditions or temperature. Chemical (pH, alcohol) and physical (thermal) denaturant conditions have been used to test the stability of recrystallised S-layers on hydrophilic surfaces. Atomic force microscopy (AFM) was used to monitor the loss of stability and the changes in protein layer conformation [3]. Recrystallized bacterial Surface layers from *Bacillus sphaericus* (SbpA) on hydrophilic silicon wafers loses the crystalline structure at 80% ethanol/water mixtures, being the change in structure reversible after treating the surface with buffer solution. SbpA on silicon supports denatures at pH 3 and at 70ºC, being the process irreversible. Cross-linking of SbpA enhances the stability for high ethanol and acidic conditions, but it does not improve thermal stability. Recrystallized SbpA on secondary cell wall polymer (SCWP), a natural environment for the protein layer in bacteria, is more resistant to ethanol and pH exposure than recrystallized SbpA hydrophilic silicon supports. Apart from the biomimetical advantages of using SCWP, this biopolymer is difficult to purify, synthetic polyelectrolytes with similar physical chemical properties can be used. We are currently studying the stability of recrystallised SbpA on poly(styrene)sulfonate. First results showing the loss of crystalline structure when metal-chelator EDTA is present in solution are shown. This work opens the way to understand the denaturing processes taking place on S-layers and extends the knowledge about the phisico-chemical properties of the cell wall of prokaryotic organisms.

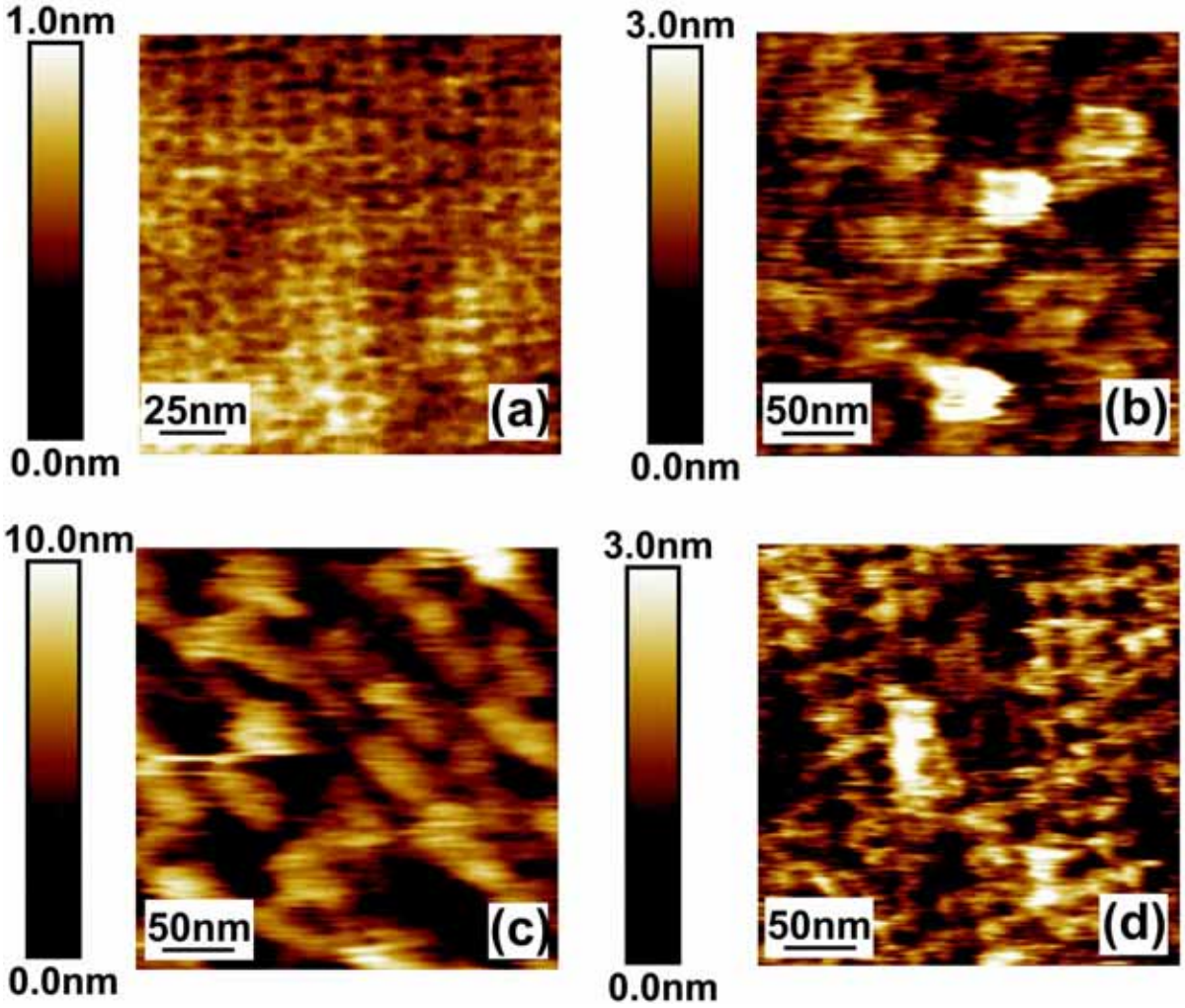


**Figure 1:** AFM deflection images of S-layers on Si wafers showing the distortion of the lattice after the different denaturant processes. a) S-layer square lattice under physiological conditions; b) S-layer after treatment with EtOH:$H_2O$ 80:20 ; c) S-layer after treatment at 70°C for 10min; d) S-layer after treatment with pH=3 buffer. b) and c) lead to total or partial recovery of the lattice, d) leads to irreversible loss.

**References:**

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