Bioinspired Nanoarchitecture

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We are exploring the use of self-assembling microbial surface-layer ("S-layer") proteins as biological materials in nanofabrication and templating.[1] S-layer protein arrays are 2-D biological nanostructures that represent a surface feature common to almost all archaeal microorganisms, and have so far been identified in hundreds of different species belonging to all major phylogenetic groups of bacteria.[2] S-layer lattices usually display oblique (p1, p2), square (p4), or hexagonal (p3, p6) lattice symmetries and as a result of their crystalline nature they display distinct classes of pores of identical size and morphologies (**Figure 1**). In addition to their highly ordered structure, one of the most interesting properties of S-layers is their ability to recrystallize into monolayer sheets at air-liquid, and liquid-solid interfaces. This reassembly property is one of the key features that make S-layer proteins especially promising as candidates for an emerging class of "biotools" with a broad range of potential applications in nanotechnology.

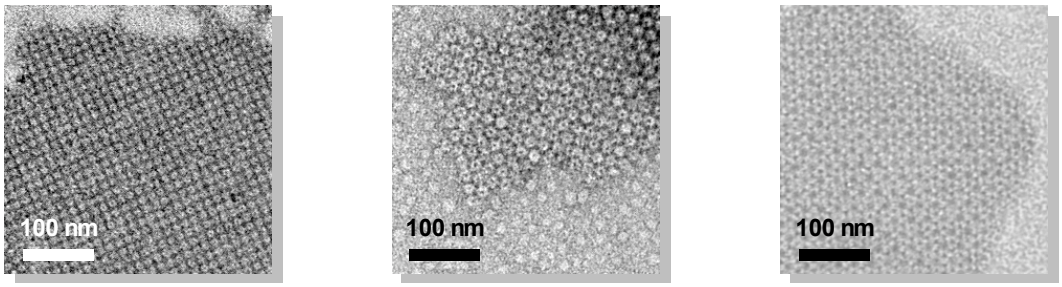

Figure 1. Transmission electron microscopy (TEM) images of isolated S-layer protein structures showing different lattice symmetries and spacings (Λ). The source microorganism is given below each TEM image.

The broad goal of this NIRT research program is to utilize S-layer proteins as a base nanoarchitecture to create unique self-assembled nanostructures by a simultaneous effort along the lines of the following objectives:

1. Conduct molecular investigations of S-layer *in vitro* self-assembly
2. Improve long-range ordered assembly and ligand binding of S-layers using recombinant DNA and chemical approaches
3. Fabricate ordered metallic/semiconductor nanostructured arrays through S-layer biotemplating

We expect that by understanding biological processes at the nanometer scale, and by exploiting the unique ability of these materials to self-assemble and create 2-D structures, we will enhance our knowledge base in not only how biology builds structures but also the fabrication of novel nanoscale devices. In our case, various ordered-array nanostructures can be designed and built through S-layer templates, in which both the size of the individual building blocks and the spacing between them can be controlled in the range of several to tens of nanometers, hardly achievable through other known approaches. This will provide us a unique opportunity to study systematically the collective quantum effects associated with these types of nanostructures. Clearly this effort builds our knowledge on the interfacial area between the organic and inorganic worlds. The NIRT will help to explore this environment which is largely overlooked but is critical to nanotechnology.

Because the *in vitro* reassembly of monomeric proteins is vital for creating functional S-layer-based nanoarchitectures in a "bottom-up" fashion, a deeper comprehension of the phenomenon of protein self-assembly is obviously important. We are therefore utilizing a variety of SbpA S-layer protein variants derived from *Bacillus sphaericus* to investigate the surface reassembly and ligand-

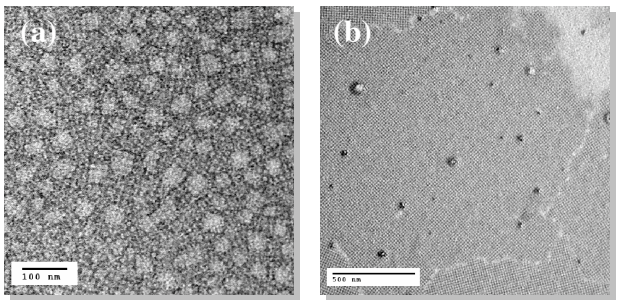

Figure 2. Influence of substrate type on unmodified SbpA S-layer protein reassembly. **(a)** Reassembly on hydrophobic carbon-coated formvar TEM surfaces results in small patches ~100 nm. Scale bar = 100 nm. **(b)** Reassembly on hydrophilic SiO_2-coated TEM surfaces results in micrometer-sized single-crystalline domains. Scale bar = 500 nm.

binding properties of S-layers. Knowledge about the molecular mechanics of reassembly of these proteins can potentially enable us to better control/influence the formation of large-area S-layer lattices. Results obtained in our laboratory have shown that the reassembly of S-layers onto solid surfaces gives different adsorption patterns depending on the physico-chemical characteristics of the supporting substrate. For example, whereas longer-range single-crystal lattices can typically be obtained on hydrophilic surfaces, S-layer reassembly on hydrophobic surfaces tends to result in only random-sized polycrystalline domains (**Figure 2**).

In order to study S-layer recrystallization in greater detail, we have fabricated several gold-patterned silicon test array structures (**Figure 3**) using the advanced electron-beam lithography systems housed within the Cornell Nanoscale Science & Technology Facility (CNF). Through the use of appropriate surface functionalization chemistries, we plan to use these arrays to develop methods for site-specific control over the reassembly process in order to generate large-area (*i.e.* > 1 μm^2) monolayer protein single-crystals that will be useful for biotemplating applications. Towards this goal, we are exploring a number of candidate surface-modification systems, which we have termed AIMs – *A*ssembly *I*nitiating *M*onolayers. One example of a carbohydrate-based biomimetic AIM system which we have successfully designed and synthesized in our laboratory is shown in **Figure 4**. The rationale for designing this particular system stems from previous findings which demonstrate that the S-layer-like homology (SHL) domain of certain S-layer proteins (*e.g.* SbpA *B. sphaericus*) recognizes a negatively charged secondary cell wall polymer (SCWP) with the structure _3)-[4,6-O-(1-carboxyethylidene)] ~0.5-_-D-ManpNAc-(1_4)-_-D-GlcpNAc-(1_ as its binding site.[3] Together, the compounds shown in **Figure 4** are expected to give a mixed self-assembled monolayer (SAM) on gold surfaces. By varying the carbohydrate abundance (*i.e.*, by varying the relative amounts of 1 and 3 in the formation of the mixed SAM) and spatial distribution on the gold surface, we will investigate whether S-layer nucleation is favored at specific regions of our test array substrates.

The nano-sized features, long-range order, and stable binding of nanoparticle arrays biotemplated on S-layers makes them particularly interesting candidates for optical sensor applications such as Surface Plasmon Resonance (SPR) and Surface Enhanced Raman Spectroscopy (SERS), where ordered metal nanoparticles have been shown to lead to dramatically enhanced sensitivity. In this NIRT, S-layer protein lattices isolated from the Gram-positive bacterium *Deinococcus radiodurans* and the acidothermophilic archaeon *Sulfolobus acidocaldarius* were investigated and compared for their ability to biotemplate the formation of self-assembled, ordered arrays of inorganic

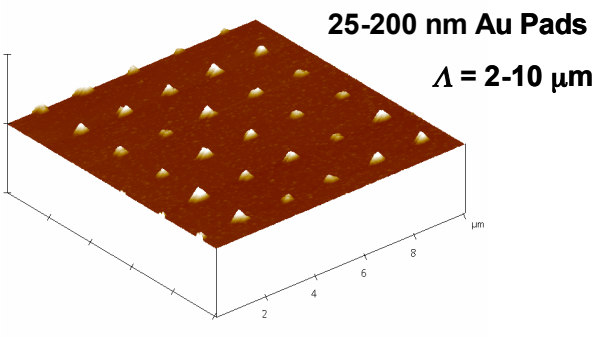

Figure 3. Gold-patterned test array for studies of *in vitro* recrystallization using S-layer proteins. These test structures, patterned *via* electron-beam lithography, contain square features 25-200 nm in

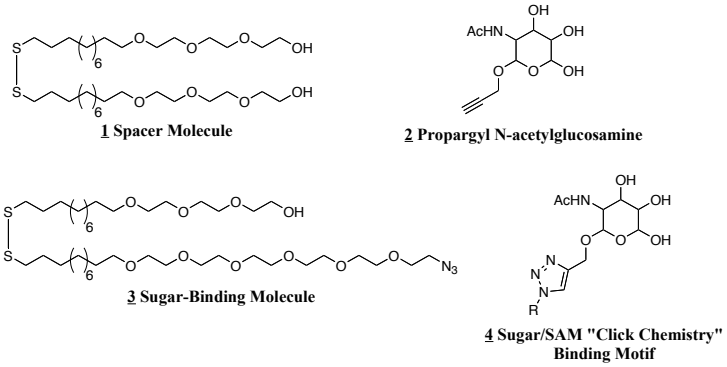

Figure 4. Components of a biomimetic carbohydrate-based self-assembled monolayer system for investigating S-layer reassembly on gold-patterned substrates.

nanoparticles.[4] The nanoparticles employed for these studies included citrate-capped gold nanoparticles and various species of CdSe/ZnS core/shell quantum dots (QDs). The QD nanocrystals were functionalized with different types of thiol ligands (negative- or positive-charged/short- or long-chain length) in order to render them hydrophilic and thus water-soluble. Transmission electron microscopy (TEM), Fourier transform analyses, and pair correlation function (PCF) calculations revealed that ordered nanostructured arrays with a range of spacings (~7-22 nm) and different geometrical arrangements could be fabricated through the use of the two types of S-layers (**Figure 5**). Our results demonstrate that it is possible to exploit the physico-chemical/structural diversity of prokaryotic S-layer scaffolds to vary the morphological patterning of nanoscale metallic and semiconductor nanoparticle arrays.

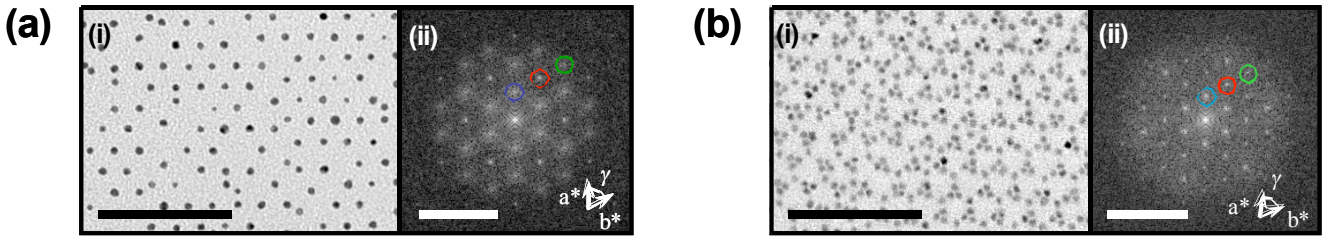

Figure 5. Brightfield TEM images (i) and corresponding 2-D FFT power spectra (ii) of unstained S-layers after incubation in a solution of water-soluble nanoparticles. **(a)** Gold nanoparticles biotemplated on HPI S-layer. **(b)** CdSe/ZnS core-shell quantum dots functionalized with 7-carboxy-1-heptanethiol biotemplated on SAS S-layer. The blue, red, and green circles mark, respectively, representative diffraction spots which can be indexed to the (10), (11), and (12) lattice lines found in a (hypothetical) 2-D hexagonal array structure. For the TEM images, scale bar = 100 nm. For the FFT spectra, scale bar = 0.18 nm^{-1}.

Another intriguing possibility being explored by us is to utilize S-layer-templated metal nanoparticle arrays as catalysts and/or ultra-high resolution etch masks for spatially controlling the fabrication of highly ordered, vertically aligned carbon nanofibers/tubes and silicon (Si) nanopillars. Such types of ordered structures can potentially be used for constructing nanoscale devices, *e.g.* field-emitters, high-spatial resolution intracellular probes, *etc*. In our laboratory, we have successfully employed an inductively-coupled plasma (ICP) $SiCl_4$ etch process to create 100 nm-high silicon nanopillars using an etch mask generated from 5 nm gold nanoparticles adsorbed onto the HPI S-layer (**Figure 6**). These results are promising and suggest that further optimizations of the etch parameters will be necessary in order to maintain a better degree of hexagonal ordering in the nanopillar arrays.

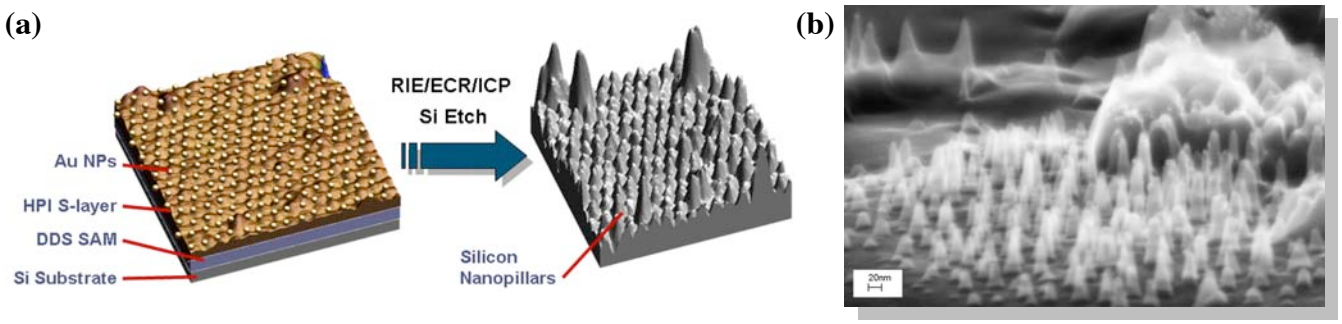

Figure 6. Fabrication of Si nanopillars using Au nanoparticles biotemplated on the HPI S-layer as a nanoetch mask. **(a)** Schematic of the etch process. **(b)** SEM image of nanopillars fabricated using an ICP $SiCl_4$ process.

As part of the NIRT K-12 education outreach effort, a pilot program has been initiated to encourage students to explore both macroscopic and microscopic patterns in nature that display periodic symmetries resembling those found in S-layer protein lattices. Objects with highly periodic features, *e.g.* fractals, are ubiquitous in nature and are actively studied by scientists across many disciplines including physics, chemistry, biology, astronomy, and earth science. We are currently collaborating with high school teachers in the Ithaca district to develop an activity that enables students to do the

same by examining various types of bacterial species having colony morphologies with interesting symmetry properties. Additionally, high-quality microscopes and video capture equipment have been purchased and distributed to our collaborators, allowing their students to examine other types of natural biological/non-biological samples for repeating patterns.

References (10 point font)

[1] For further information about this project please email Carl A. Batt at **<cab10@cornell.edu>**

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[3] Ilk, N.; Kosma, P.; Puchberger, M.; Egelseer, E.M.; Mayer, H.F.; Sleytr, U.B.; Sara, M., J. Bacteriol., 1999, 181, 7643-7646.

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