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Fabrication of highly oriented nanocluster arrays by biomolecular templating

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Abstract. The formation of highly oriented cluster arrays by chemical deposition of platinum onto a regular 2D protein template is studied. The crystalline bacterial surface layer (S layer) of *Sporosarcina ureae* exhibiting a p4 symmetry with a lattice spacing of 13.2 nm is used as the protein template. Cluster deposition onto the S layer is achieved by chemical reduction of a platinum salt. The growth kinetics of the clusters has been examined by UV–VIS spectrometry. Transmission electron microscopy reveals the formation of well separated metal clusters with an average diameter of 1.9 ± 0.6 nm. The lateral cluster arrangement is defined by the underlying protein crystal. We observe 7 cluster sites per unit cell corresponding to a density of 4×10^{12} cm^{-2}. They are located in the nano-sized pores and gaps of the crystalline S layer.

PACS. 82.30.N Cluster formation in chemical reactions – 68.65.+g Low dimensional structures: superlattices – 87.15 Biopolymers

1 Introduction

There is growing interest in the development of lithographic methods applicable to pattern inorganic material at the nanometer length scale for use in electronic, optical and chemical devices. A novel approach for the fabrication of molecularly engineered nanostructures is the method of biomolecular templating [1–4]. It takes advantage of the well defined structural, physical and chemical properties of self-assembled biological macromolecular complexes. These are used as templates for the build-up of thoroughly predefined inorganic nanostructures. Thus, the biomolecular approach allows to address fundamental issues of nanometer fabrication and supra-molecular engineering.

Biomolecular templating focuses mainly on two different aspects: Firstly, the geometrical shape of the template is employed for the fabrication of artificial nanostructures such as metallic nanowires, e.g. by coating the specimens with thin metal films [2, 5–7]. Secondly, large macromolecular assemblies which are built from identical subunits possess a regular structure. The periodicity arises from the self organization of biomolecules into ordered or even crystalline arrays. Usually this denotes a precise spatial modulation of physico-chemical surface properties of the template, that can be used to accomplish site-specific chemistry [3, 8]. In contrast to serial lithographic methods such as electron beam lithography, biomolecular templating provides the advantage of parallel fabrication and parallel manipulation of material at the nanometer length scale. Parallel manipulation by post-processing of a thin Ta-W film deposited onto a 2D protein template was first demonstrated by Douglas *et al.* [1]. The metal film was treated by ion milling leading to a regular array of holes in the film. They found that the topology of the pattern is determined by the geometry of the template used and that the pattern of the deposited layer was caused by the intrinsic spatial inhomogeneity of the structure of the underlying protein monolayer. The protein template they used was the bacterial surface layer (S layer) of *Sulfolobus acidocaldarius*.

S layers are regular protein crystals which form the outermost cell envelope component of many prokaryotes in almost all phylogenetic branches of bacteria and archaea [9, 10]. S layers exhibit different kinds of lattice symmetry (p1, p2, p3, p4, and p6) with spacings between the centers of neighbouring morphological units in the range of 3 nm to 30 nm. S layers are typically 5 nm to 15 nm thick and possess pores of individual but identical size and morphology with diameters in the range between 2 nm and 6 nm. The possibility to reconstitute isolated S layer subunits *in vitro* into two-dimensional arrays with perfect uniformity over large areas on solid surfaces or at liquid-air interfaces [11] makes them an almost ideal biological template for supra-molecular engineering.

The present report is the first study of the process of chemical deposition of nanometer-sized metallic Pt clusters onto a bacterial S layer. We used the S layer of *Sporos-*

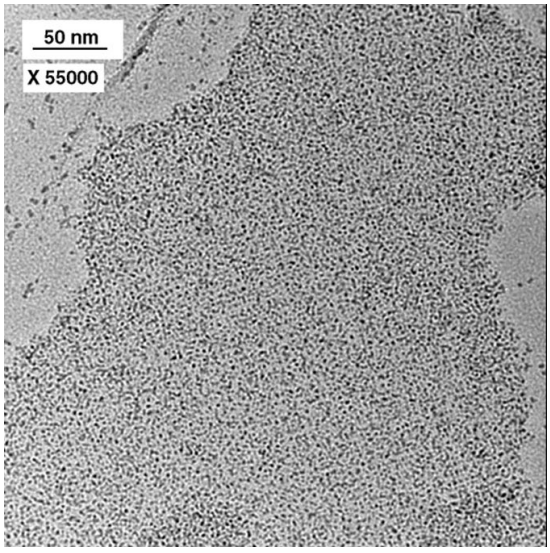

Fig. 1. TEM micrograph of platinum clusters chemically deposited onto an S layer sheet of *Sp. ureae*.

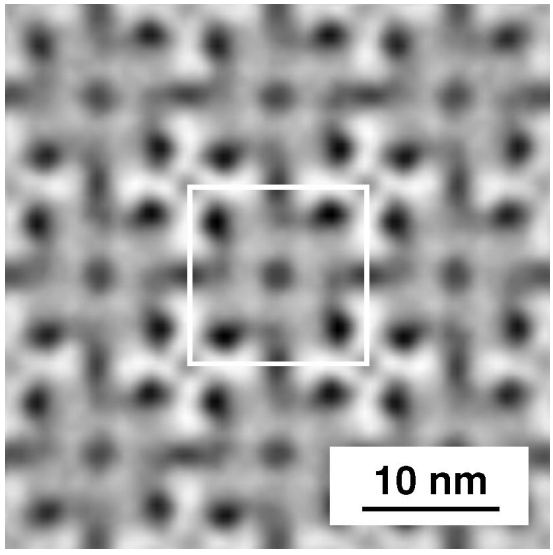

Fig. 2. Spatial distribution of metal clusters (black) on the protein crystal as revealed by image processing. The unit cell of the cluster lattice is marked and possesses a size of 13.2 nm × 13.2 nm.

arcina ureae which has a p4 symmetry, a lattice constant of 13.2 nm, and a complex pattern of pores and gaps that are ≈ 2 nm wide [12]. From investigations with the method of pulsed laser deposition [13], it is known that the *Sp. ureae* S layer exhibits several well defined physicochemical affinity sites for platinum per unit cell. These sites are decorated with metal particles deposited from the gas phase [14].

2 Experimental

2.1 Preparation of the S layer

The S layer sheets were isolated from *Sp. ureae* cells. The conditions of cell cultivation and the S layer preparation are described elsewhere [12]. Isolated S layer was resuspended in a buffer solution consisting of 50 mM sodium phosphate pH 7.8, 1 mM $MgCl_2$, and 0.02% NaN_3. The protein had a final concentration of ≈ 2 mg/ml and was stored at 4 °C.

2.2 Activation of the biological template

The S layer surface was activated by molecular deposition of Pt from a metal salt solution [6, 7]. A sample of 16.5 ml S layer suspension was treated with 1 ml of a 3 mM K_2PtCl_4 solution at 37 °C. Before use, the K_2PtCl_4 solution was aged at ambient temperature for at least one day to allow the $[PtCl_4]^{2-}$ complex to aquate yielding an increase of the reduction potential [15]. After ≈ 30 min of activation the S layer suspension turned from colorless to light yellow getting darker with increasing reaction time, and thereby indicating metal deposition on the protein template. NaN_3 acts as reducing agent here. The activation kinetics were studied by recording the optical absorption of the solution at a wavelength of 600 nm with an Unicam 5600 UV-VIS spectrometer(Cambridge, UK).

2.3 Transmission electron microscopy

TEM investigations were carried out using a Philips CM 200 with LaB_6 cathode at 200 kV while a Philips CM 200 equipped with field emission cathode was employed for high-resolution work (HRTEM). A droplet of the activated S layer suspension was placed on carbon-coated copper grids for ≈ 1 min, excess water removed with filter paper, and the specimen air-dried. Native S layers preparations were negatively stained with 1.5% sodium phosphotungstate. Activated S layers were not stained in addition. The chemical composition of metal clusters formed on the S layer surface during activation was determined by EDX analysis. In order to reconstruct the spatial distribution of metal clusters on the S layer template, digitized TEM micrographs were analyzed applying Fourier methods and correlation averaging [16]. The distribution of cluster diameters was determined by analyzing about 30 000 metal clusters using the image analyzer QUANTIMET 570 (Leica, Bensheim, Germany).

3 Results and discussion

The chemical deposition of platinum onto the S layer of *Sp. ureae* leads to the formation of highly ordered arrays of metal clusters. Figure 1 shows a TEM micrograph of a S layer sheet activated for 24 h. Well-separated, nearly spherical particles (dark) are aligned along the crystalline structure of the protein template reproducing the tetragonal symmetry of the S layer. The corresponding spatial distribution of clusters is illustrated in Fig. 2. It

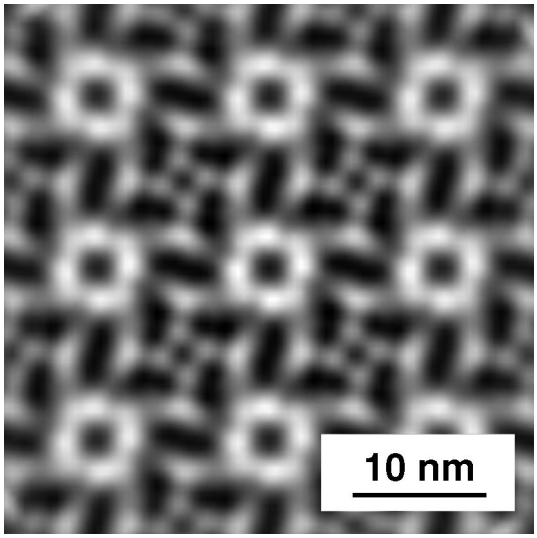

Fig. 3. Projection map of the negatively stained native S layer of *Sp. ureae* obtained after correlation averaging with a resolution of 1.7 nm. Protein appears white, the heavily stain-filled pores appear dark.

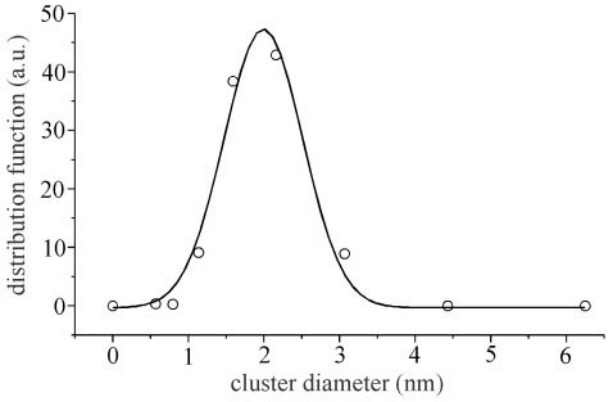

Fig. 4. Diameter distribution function of platinum clusters deposited on *Sp. ureae* S layers.

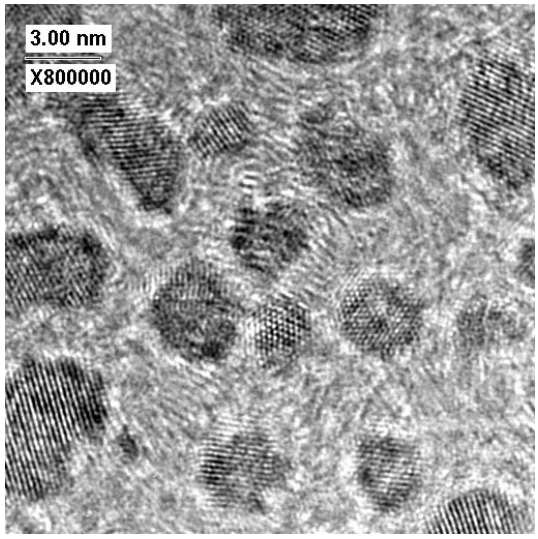

Fig. 5. HRTEM micrograph of the activated S layer template.

was calculated by averaging over many unit cells whose positions had been determined by correlation methods, using a Fourier-filtered TEM image as a reference [17]. Figure 2 shows that the deposited clusters are periodically arrayed. The unit cell of the cluster lattice is quadratic with a size of 13.2 nm × 13.2 nm. This value is in good agreement with the S layer lattice constants of 12.8 nm [18] and 12.9 nm [12] determined in previous studies. Each unit cell contains 7 clusters of about equal size, corresponding to an area density of 4×10^{12} cm^{-2}.

In order to identify the location of the metal clusters on the template we compared our results with images of native S layer preparations, non-activated but negatively stained with phospho-tungstate. Figure 3 shows the averaged projection structure of the native S layer obtained from TEM micrographs of negatively stained specimens outlining the protein mass distribution in the unit cells. Figures 2 and 3 illustrate that the cluster arrays formed by metal deposition from the liquid phase exhibit the same symmetry properties and prefer distinct sites of the protein template. Recently, a similar observation has been reported with the chemical deposition of CdS clusters onto another S layer [19]. However, we can clearly identify the central pores and gaps of the protein structure as specific Pt cluster deposition sites.

In addition to the spatial distribution of the clusters, their diameter distribution was determined by analyzing a series of TEM micrographs using the QUANTIMET image analyzing system. Figure 4 shows the calculated diameter distribution, possessing a maximum at 1.9 nm. This value is in good agreement with the apparent diameter of the S layer pores. Furthermore, the diameter distribution is relatively narrow; the full width at half maximum of the distribution function is only 1.2 nm. The latter observations suggest, that not only the position of Pt particles but also cluster growth is controlled by the protein template. Similar results have been reported for the synthesis of inorganic clusters in bicontinuous cubic phase lipid microstructures [20, 21].

The metallic character of the nano-sized clusters grown on the S layer surface can be demonstrated by HRTEM investigations. Figure 5 shows deposited clusters imaged at 200 kV. The lattice fringes of the clusters with a lattice constant of 0.39 nm are clearly resolved, identifying a pure metallic Pt phase. In addition, the purity of the deposited Pt particles was verified by EDX analysis.

In order to gain more insight into the process of chemical cluster deposition onto the biological template and, especially, to investigate the role of the S layer in the activation reaction, we studied the nucleation and growth kinetics of the clusters by optical spectrometry. UV-VIS spectrometry is a method commonly used for the characterization of Pt soles [15]. In the wavelength region from 500 nm to 700 nm the measured extinction is dominated by the nonspecific absorption of the formed Pt particles [22]. Figure 6 shows the time dependence of the absorbance at a wavelength of 600 nm for solutions with and without pro-

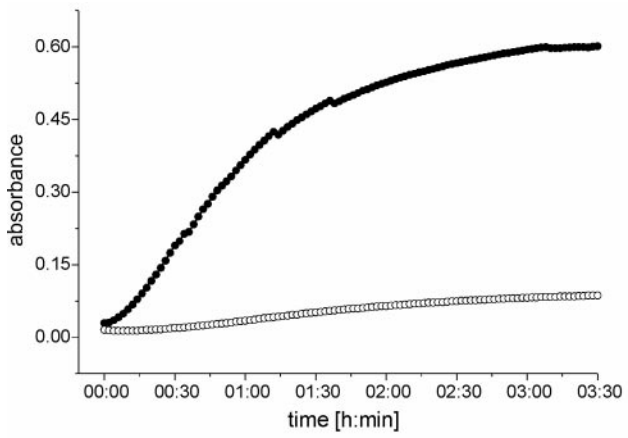

Fig. 6. Time dependence of the adsorbance at 600 nm for the K_2PtCl_4 activation reaction with (●) and without (○) S layer protein.

tein which both contained the same compounds (sodium phosphate, $MgCl_2$ and NaN_3) in identical concentrations. In both cases we observed the development of Pt clusters, however with distinctly different reaction kinetics. In presence of protein, cluster formation is strongly accelerated compared to the reaction in pure buffer solution. This behaviour differs completely from the chemical deposition of Pt at the surface of microtubuli, where the reaction kinetics with and without protein are similar [7]. The result clearly shows that the crystalline surface layer plays an active role in the process of cluster deposition. The S layer serves as a template with a very high density of specific affinity sites where heterogeneous nucleation of clusters takes place. In contrast to that, formation of clusters in the pure buffer solution is a result of homogeneous nucleation. Here the critical radius necessary for particle growth is much larger than in case of heterogeneous nucleation, leading to a longer lag phase for cluster formation.

In TEM investigations we found that the activation process without S layers present but under similar conditions leads to the formation of Pt particles with typical diameters between 20 nm and 30 nm. On the contrary, formation of larger particles due to coagulation of small clusters was not observed with S layers, even not in the final stage of S layer activation. Thus we can conclude that cluster coagulation is strongly suppressed by interaction between the particles and the protein surface, again corroborating the view that specific protein sites are responsible for platinum deposition.

4 Conclusions

The chemical deposition of metallic platinum onto the S layer of *Sp. ureae* yields the formation of stable 2D cluster arrays. Heterogeneous nucleation of clusters takes place at specific affinity sites on the S layer surface. It was found that the position of the formed metal particles as well as their size is determined by a template controlled cluster growth. Since S layers are two-dimensional protein crystals, the resulting cluster arrays are highly ordered and the deposited metal particles are periodically arranged at the nanometer length scale. The method of biomolecular templating described here can be considered as parallel processing of identical nanostructures, performing both their fabrication and manipulation in one step.

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