Communication

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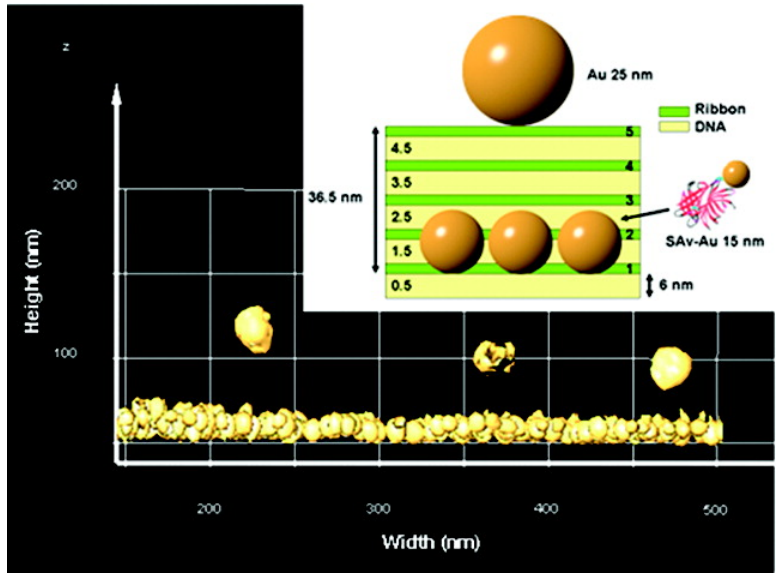

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Electron Tomography Shows Molecular Anchoring Within a Layer-by-Layer Film

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The fabrication of materials with precisely defined structure and composition is of paramount importance in biomedical engineering. The Layer-by-Layer (LbL) self-assembly technique[1] is a frequently used coating technique to produce biomaterial coatings composed of thin organic layers.[2] Many biocompatible LbL coatings are produced with the aim to deliver DNA, peptides, proteins, or drugs,[3] either by slow diffusion or by biological degradation of the film.[4,5] In many cases, bioactive molecules are part of an elaborate and complicated chain of events, in which their presence is only beneficial within certain time frames. Premature diffusion or ill defined positioning might result in release at unwanted time points resulting in a negative effect. It is therefore imperative to obtain precise control over not only their diffusion rate but also their positioning inside the film.

For this reason it is of interest to entrap or anchor such bioactive agents in a predetermined layer or section of these films. However, in most LbL films diffusion of the constituent components leads to distribution of materials over the different deposited layers[6] while included additives diffuse out of the film prematurely in the washing steps employed during the film buildup.

Here we build an LbL coating from alternating layers of DNA and self-assembled ribbons of a bis-urea amphiphile in which biotinylated molecules are anchored through molecular recognition. This approach prevents diffusion of the components within the film and allows us to entrap the bioactive molecules in a precisely determined layer of the coating.

Electron tomography (ET) has been employed for 3D imaging at the nanoscale in both materials science[7] and life sciences.[8] Low temperature, low-dose ET (generally referred to as cryo-electron tomography) allows the nanometer scale imaging of soft, electron beam sensitive samples. Although it has been recognized as a strong and emerging technique in biology, it is still virtually unexplored for the analysis of samples from synthetic origin.[9] Here we use ET to demonstrate with nanometer precision the location of gold-labeled streptavidin bound to the biotin molecules incorporated in our LbL films

The LbL films were prepared from DNA and aggregates of self-organizing ammonium surfactant **1**. In an earlier report we demonstrated that this ammonium bis-ureido surfactant (**1**) forms well-defined highly ordered ribbon-like bilayer aggregates in water, which can be functionalized using the specific molecular recognition of biotinylated molecules containing the same bis-urea moieties (**2**).[10] In the present work we show that the incorporation of these preformed functionalized ribbons allows the positioning of gold-labeled streptavidin in a predetermined layer of the LbL films.

Multilayer [ribbon/DNA] films were first deposited on quartz substrates, using alternatingly an aqueous 1 mg/mL aggregate suspension of **1** and an aqueous 1 mg/mL DNA solution. During buildup of the film, the 260 nm UV absorption of DNA showed a linear increase in intensity (see Supporting Information), which indicated that an equal amount of DNA had been deposited with each additional step.

The surface topology of the multilayer films was analyzed using atomic force microscopy (AFM) after the deposition of each individual layer up to 6 double layers. For ribbon-terminated layers, the contours of individual ribbons could be clearly distinguished (Figure 1b), whereas DNA-terminated layers showed contours of a patch-like topology (Figure 1c). However, small height differences originating from ribbons in the layer underlying the DNA patches could be observed, as well as an imprint of the underlying DNA patches in the ribbons in the top layer. To more accurately determine the surface morphology of the film, the amplitude images were inspected. Features that are present at the surface appear more "sharp" in the amplitude image compared to features originating from underlying structures, which are dampened. In the amplitude images it can be clearly seen that the ribbons appear more "sharp" on ribbon-terminated layers (Figure 1d) and that DNA patches are more pronounced on the DNA-terminated layers (Figure 1e). These results indicate that indeed during the LbL process the DNA and ribbon aggregates do not mix but form separate layers and also that the last deposited layer is indeed the top layer.

However, from these results it is not evident that the ordering of the film is preserved in the underlying layers, which is necessary for molecular anchoring in a predetermined layer. To demonstrate the molecular anchoring of the biotin−streptavidin combination within an LbL film, we prepared such films on a carbon coated TEM grid and investigated them using low temperature, low-dose ET. The first deposited layer consisted of preformed ribbons composed of a mixture of **1** and **2** that were subsequently incubated

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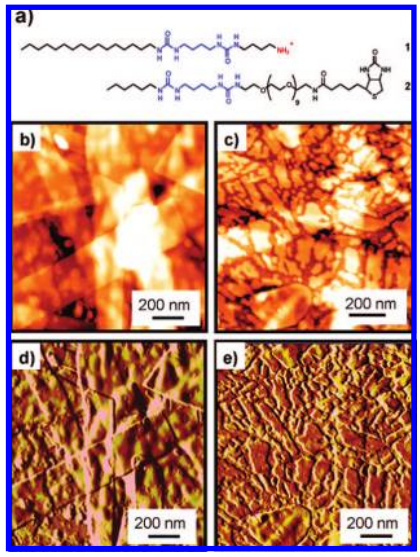

Figure 1. (a) Surfactant **1** and biotinylated molecular anchor **2**. (b) Characteristic height image of a ribbon-terminated layer showing ribbons covering the surface. (c) Same as (b) of a DNA-terminated layer showing patch-like morphology. (d−e) Amplitude images of (b) and (c).

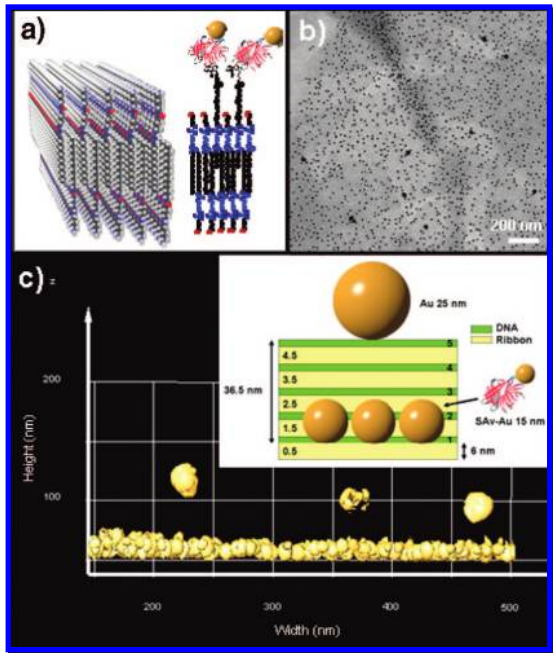

Figure 2. (a) 3D image of the proposed molecular packing within the ribbon structure (POV-ray software) and schematic representation of gold-labeled streptavidin bound to the pendant biotin groups of **2** incorporated into the ribbon structure. (b) TEM images of the gold-labeled 5 double-layer LbL film deposited on a TEM grid. (c) Isosurface presentation of the 3D reconstructed volume of the film in (b), highlighting the gold particles. Grid bars represent 50 nm. Inset: Schematic representation of the LbL film and position of the gold conjugates; the pendant biotin functionality is present on the outside of the ribbon aggregates; therefore the SAv-Au particles are presented on top of the first functionalized layer.

with gold (15 nm)-labeled streptavidin (Figure 2a).[10] Next, the LbL process was continued with DNA and ribbons consisting of pure **1** until the final film thickness of 5 double layers was reached. Finally 25 nm gold particles were deposited on top of the film. The concentration of the larger particles was low as these can obscure the underlying layers in the TEM images. Low magnification TEM images showed overlapping ribbons, of which a significant proportion (i.e., those located in the first layer) was decorated with 15 nm gold particles (Figure 2b).

To determine the distances of the different gold particles with respect to each other, ET was applied. A tilt series of 91 images between +66° and −66° was recorded. The images were recorded at 106 K using low-dose conditions to prevent degradation of the film by the electron beam. Reconstruction of the tilt series to a 3D volume indeed confirmed that the 15 nm gold particles formed a uniform layer at the bottom of the film, with the 25 nm gold particles situated 30−50 nm above this layer (Figure 2c). It is important to note that the variation in this distance is due to the variation in the film thickness, a consequence of the inhomogeneous structure of the ribbon-based layers, which determines the position of the 25 nm gold markers, and not to the variation in position of the anchored 15 nm streptavidin-bound particles.

These results show that indeed the biotin-containing layer had not mixed with the rest of the film, confirming that bioactive molecules can be deposited at a predefined position within the film using this strategy and that the anchoring is strong enough to withstand the many sequential washing and film deposition steps. Taking into account a bilayer thickness of 6 nm for the ribbons and a 2.5 nm diameter for the DNA, the expected theoretical distance of 36.5 nm (for a linear, nondiffuse and layered system) between the two types of gold markers corresponds well to the ∼40 nm average of the observed range.

In summary, it is possible to anchor functional molecules of biological origin into a single specific layer of a multilayer DNA-surfactant film with nanometer precision. The bioactive functionality remained at its predetermined position through the whole film buildup procedure, as a consequence of the supramolecular interactions between the bis-urea units present in the surfactant aggregates. The 3D images underline the potential of electron tomography in applications at the interface of materials science and life science.

Supporting Information Available: Experimental details, spectroscopic data and movie of the tilt series, reconstruction and surface rendering of the 3D tomographic volume. This material is available free of charge via the Internet at http://pubs.acs.org.

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