Formation of layered titania and zirconia catalysed by surface-bound silicatein†

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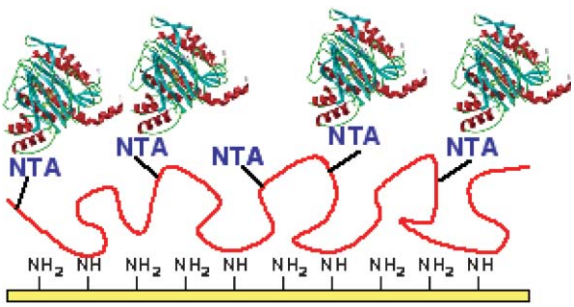
Silicatein immobilised on self-assembled polymer layers using a histidine-tag chelating anchor group retains its hydrolytical activity for the formation of biosilica, and catalyses the formation of layered arrangements of biotitania and biozirconia.

Self-assembled monolayers (SAM) of alkanethiol derivatives on metal electrodes provide numerous possibilities for immobilising proteins.[1] In the past few years, such devices have gained increasing interest in (nano)biotechnology. In particular, the current and potential importance of immobilised enzymes for the design and development of biosensors,[2] bioelectronic systems,[3] or biocatalytic cells[4] has stimulated a large number of experimental studies in this field. Customising the catalytic functions and optimising the efficiencies of these systems represent a challenge in current research as they require a detailed characterization of the structural and functional properties of the active adlayers as well as of the dynamics of the processes involved.

In a preliminary report, we have demonstrated the formation of biosilica catalysed by surface-bound histidine tagged silicatein.[5] Silicatein in sponges has been identified as constituent of biosilica.[6,7] In this contribution we demonstrate that surface bound silicatein is not only capable of polycondensating biosilica, but is also significant for the synthesis of other metal oxides such as TiO_2[8] and ZrO_2 using water stable alkoxide precursors such as [titanium bis-(ammonium-lactato)-dihydroxide],[8] and anionic hexafluorozirconate (ZrF_6^{2-}),[9] resulting in the synthesis of oxide particles with a layered morphology at neutral pH and room temperature. Synthesis of TiO_2 and ZrO_2 proceeds by hydrolysis of suitable metal complexes under extreme pH and temperature conditions. A biological synthesis of titania and zirconia would thus help mitigate the shortcomings of chemical methods.

In order to immobilise silicatein onto surfaces we designed a reactive ester polymer, poly(acetoxime methacrylate), which specifically reacts with primary amines.[10] The gold surfaces were exposed to a cysteamine solution (3 mmol) for 12 h followed by the reaction of the amine group with the reactive polymer. This leads to a reactive thin film bound to cysteamine. The recombinant protein has a short affinity sequence of histidines (histidine-tag) that is capable of binding metal ion complexes such as nitrilo-triacetic acid (NTA). Thus an ω-terminated NTA amine was chemisorbed on top of a soft reactive polymer thin film.[10] The stepwise architecture of the self-assembly and immobilisaton of His-tagged silicatein is shown in Scheme 1. This spacer layer decouples the protein from the surface and thus provides a hydrophilic layer (between the protein and the Au substrate) which establishes a water-containing sub-layer space, which reduces the hydrophobic influence of the metal surface.[11]

The self-assembled monolayer formation of the cysteamine, polymer, NTA and silicatein immobilisation to the gold (111) surface was monitored using the surface sensitive optical technique of surface plasmon resonance (SPR) spectroscopy. The plasmon spectra were recorded against water on bare gold slides, after self-assembly of cysteamine and polymer, NTA and after immobilisation of silicatein on the surfaces by Ni^{2+} coordination. Fig. 1a displays the corresponding SPR spectra of all these experiments and the corresponding minima reflectivity angles. The shifts of the plasmon curves were calculated, corresponding to angular changes of 0.160° (monolayer of cysteamine and polymer on bare gold after quenching all unreacted active ester groups), 0.420° (NTA onto polymer) and 0.70° (silicatein on Ni^{2+} coordinated surface). In a separate experiment immobilisation of silicatein without Ni^{2+} could not be detected, supporting the chelating efficency of histidine tags with Ni^{2+}. These shifts in surface plasmon resonance angle were converted by complex Fresnel calculations to an average thickness of the monolayers of cysteamine and polymer, cysteamine polymer and NTA, and the immobilised silicatein layer



Scheme 1 Immobilisation of silicatein using cysteamine, a reactive ester polymer, and the NTA ligand.

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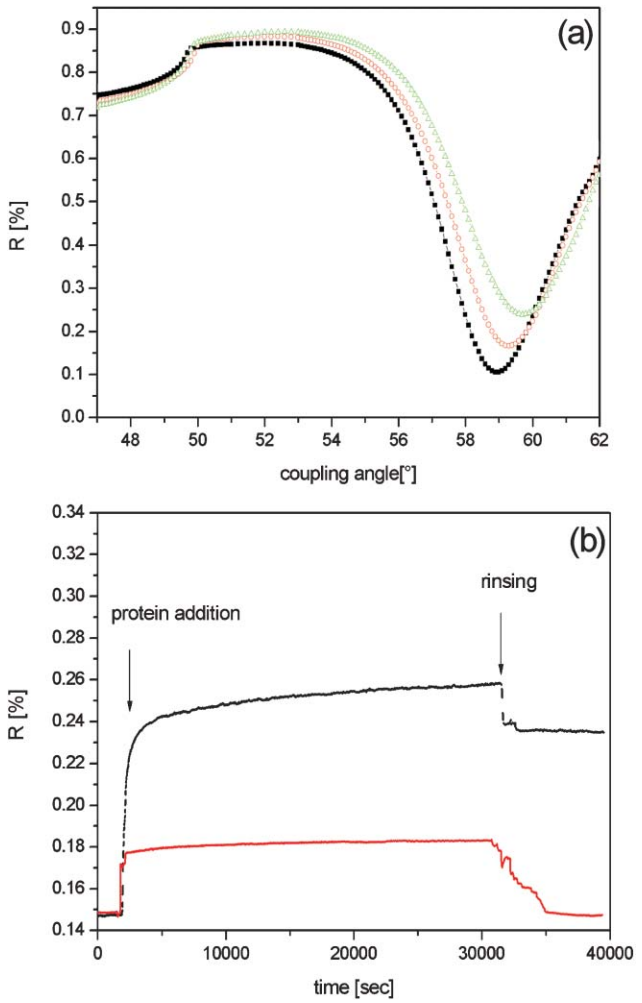

Fig. 1 (a) SPR curves of cysteamine on gold (filled squares), polymer with NTA (open circles) and immobilised silicatein thereon (open triangles). (b) Reflectivity plotted as a function of time in the absence (solid line) and presence (dotted line) of Ni^{2+} indicating the specific binding of the silicatein to the NTA linker.

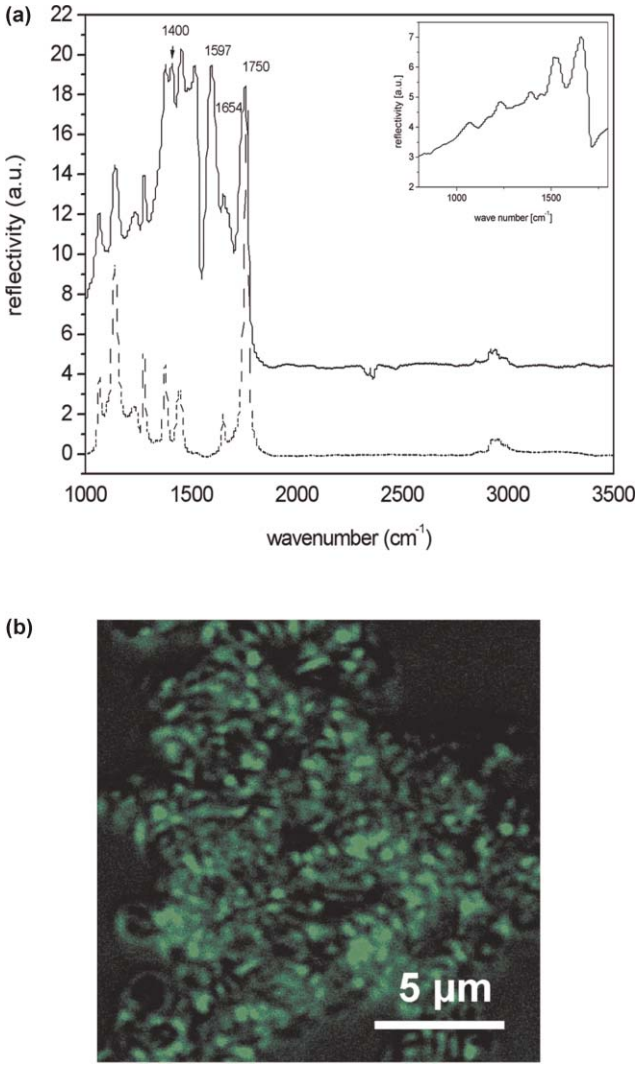

Fig. 2 (a) Surface FT-IR spectra of polymer functionalized gold substrate (dotted line) and covalently bound NTA anchor ligand on top (solid line). The surface FT-IR spectrum of surface bound protein is given in the inset. (b) CLSM image of fluorophore labelled silicatein immobilised on a Au(111) substrate.

assuming a refractive index of $n = 1.5$ for all layers. For the cysteamine polymer we derived a surface thickness of 16 Å, which increased to 32 Å after NTA binding. Subsequent silicatein binding increased the thickness by 34 Å which is close to the theoretical diameter of 30 Å for a protein with a molecular weight of 24000 Da.

Fig. 1b compares the protein immobilisation under specific and non-specific binding conditions. The solid line (lower trace) represents the non-specific binding in the absence of Ni^{2+} ions. After protein addition a slight increase in thickness was observed. However, upon rinsing, any non-specifically bound protein could be washed away. In contrast, in the presence of Ni^{2+} ions chelated to the NTA linker specific binding was achieved (dashed line, upper trace). A strong increase in thickness was observed upon protein addition, which was stable even after thorough rinsing.

The fixation of the reactive polymer was proven by FT-IR reflectometry. Fig. 2a shows the surface FT-IR spectra of the polymer functionalized cysteamine SAM on gold slides (dotted lines) and the polymer-bound NTA ligand (solid line). Clearly, the carbonyl peak of the reactive ester can be seen at 1765 cm^{-1} as well as a vibration of the amide group at 1648 cm^{-1}. After binding the NTA ligand to the polymer layer the polymer layer was rinsed twice with concentrated ammonia solution in order to quench the unreacted acetooxime functional groups. The binding of the NTA ligand is demonstrated by the presence of additional vibrations originating from the symmetric and asymmetric stretch of the carboxylate groups at 1597 and 1400 cm^{-1}.[12] Upon attachment of the protein to the NTA ligand (as shown in the inset of Fig. 2a) the C=O vibrational bands broaden due to the multitude of amide groups present in the surface bound protein.

Binding of silicatein to the surface functionalised with NTA on top of the polymer thin films was also confirmed by confocal laser scanning microscopy (CLSM, Leica TCS SL with an argon laser).[13] Fluorescence of the dye molecules was excited at 488 nm and detected from 504–514 nm using a 20 fold dry objective. The advantage of CLSM is that (i) detection of protein using fluorophore labled antibodies is very specific, *i.e.* this antibody does not bind to any other component, and (ii) a large surface area

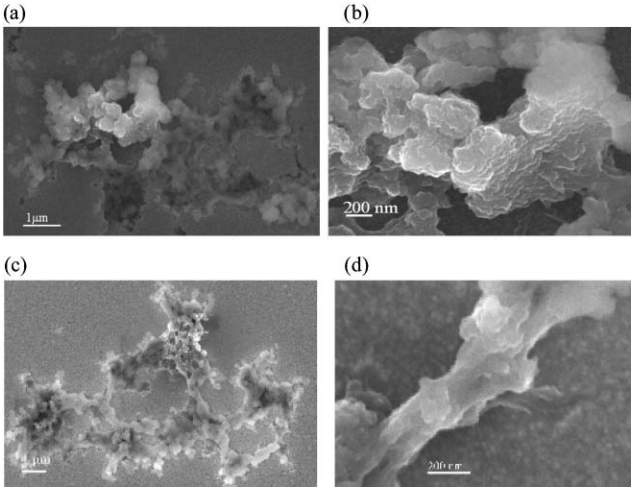

Fig. 3 SEM/HRSEM images of TiO_2 (a) and (c) and ZrO_2 (b) and (d) formed by catalysis using surface-bound silicatein. No observable formation of TiO_2 or ZrO_2 occurred on NTA alkanethiol modified surfaces.

can be seen. Fig. 2b shows the CLSM image of immobilised silicatein after exposing the surface to fluorophore labeled antibodies. The surfaces with immobilised silicatein were reacted with solutions containing PoAb-aSilic; the immunocomplexes were then stained with fluorophore-labeled (Cy3-label) secondary goat anti-rabbit antibodies. The surface bound silicatein strongly reacted with the antibodies. Due to the presence of Cy3, they appeared green and fluoresced at 520 nm using an Olympus AHBT3 light microscope, together with an AH3-RFC reflected light fluorescence attachment. The high magnification image in Fig. 2b indicates that (i) silicatein is immobilised on the SAM, (ii) the surface coverage is large, and (iii) exhibits partly cluster-type adsorption patterns.

In order to confirm the hydrolytic activity of the surface-bound silicatein for the deposition of titania or zirconia, we performed two comparative sets of experiments. One set of experiments used surface-bound silicatein, and a second set of experiments was conducted with non-functionalised gold surfaces. Under these similar sets of conditions for both experiments, we observed the formation of structured titania or zirconia only on silicatein-coated surfaces, as evidenced by the SEM images in Fig. 3a and 3c. Fig. 3b and 3d show HRSEM images, which reveal a layer-like appearance of titania and zirconia nanoparticles with diameters of 50–60 nm using immobilised silicatein. No formation of titania or zirconia was observed, when the precipitation was performed on surfaces modified with polymer NTA only. The surface turned black when exposed to the electron beam in the scanning electron microscope, which indicates the presence of organic material, such as NTA alkanethiol, that decomposes upon exposure to the electron beam. Furthermore, the distribution pattern of TiO_2 and ZrO_2 is reminiscent of that observed for silicatein in Fig. 2.

The formation of titania and zirconia by surface bound protein indicates that the hydrolytic activity of silicatein is quite unspecific, *i.e.* charge density and coordination number of the metal atom seem to be less important (c.n. 4 for Si, 6 for Ti, 6–8 for Zr). Moreover, the active site of the protein immobilized on top of a "polymer cushion" is freely accessible from solution.

There are significant morphological differences between TiO_2 and ZrO_2 resulting from silicatein- and base-catalysed syntheses. The product resulting from the catalysis by surface bound silicatein has a layer-like appearance, whereas the particles resulting from a base catalysis have a more spherical character. These morphological differences may be directly related to the nucleation mechanism occurring during the growth of TiO_2 and ZrO_2. Surface-bound silicatein should exhibit a number of nucleation sites (*e.g.* clusters of contiguous -OH side chains on the silicatein protein upon which the newly formed MO_x (M = Ti, Zr) polyhedra might condense. Such structural features of the surface bound silicatein can act as "electrostatic reliefs" which lower the heterogeneous nucleation barrier while keeping the intrinsically high barrier for homogeneous nucleation (*i.e.* base-catalyzed precipitation from solution) constant. By offering favorable surface sites on which nuclei can form, the surface-bound silicatein (in particular clusters as seen in Fig. 2) may act initially as a template and stabilize smaller nuclei (with lower surface roughness) than those formed by homogeneous nucleation. The potential for a summation of weak interactions (*i.e.*, hydrogen bonding and van der Waals forces) at the interface between the polymer/silicatein layers and the crystallising mineral may even stabilise a layered polymorph of TiO_2. There exists, in fact, a true layered polymorph of TiO_2 ($TiO_2(B)$[14]) which is formed under different (hydrothermal) conditions.

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Notes and references

1 (*a*) K. L. Prime and G. M. Whitesides, *Science*, 1991, **252**, 1164; (*b*) I. Willner and E. Katz, *Angew. Chem., Int. Ed.*, 2000, **38**, 1180.
2 T. Wink, S. J. van Zuilen, A. Bult and W. P. van Bennekom, *Analyst*, 1997, **122**, 43.
3 I. Willner and E. Katz, *Angew. Chem., Int. Ed.*, 1998, **37**, 3723.
4 I. Willner and E. Katz, *New J. Chem.*, 1999, **23**, 481.
5 M. N. Tahir, P. Théato, W. E. G. Müller, H. C. Schröder, A. Janshoff, J. Zhang, J. Huth and W. Tremel, *Chem. Commun.*, 2004, 2849.
6 (*a*) K. Shimizu, J. N. Cha, G. D. Stucky and D. E. Morse, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 6234; (*b*) J. N. Cha, K. Shimizu, Y. Zhou, S. C. Christianssen, B. F. Chmelka, G. D. Stucky and D. E. Morse, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 361.
7 A. Krasko, R. Batel, H. C. Schröder, I. M. Müller and W. E. G. Müller, *Eur. J. Biochem.*, 2000, **267**, 4878.
8 J. L. Sumerel, W. Yang, D. Kisailus, J. C. Weaver, J. H. Choi and D. E. Morse, *Chem. Mater.*, 2003, **15**, 4804.
9 V. Bansal, D. Rautaray, A. Ahmad and M. Sastry, *J. Mater. Chem.*, 2004, **14**, 3303.
10 M. Eberhardt, N. Metz, R. Zentel and P. Theato, *Polym. Prepr.*, 2005, **46**, 1, 100.
11 W. J. Miller and N. L. Abbott, *Langmuir*, 1997, **13**, 7106.
12 N. Wu, L. Fu, M. Su, M. Aslam, K. C. Wong and V. P. Dravid, *Nano Lett.*, 2004, **4**, 383.
13 W. E. G. Müller, M. Rothenberger, A. Boreiko, W. Tremel, A. Reiber and H. C. Schröder, *Cell Tissue Res.*, 2005, **321**, 285.
14 (*a*) M. Tournoux, R. Marchand and L. Brohan, *Prog. Solid State Chem.*, 1986, **17**, 33; (*b*) T. P. Feist and P. K. Davies, *J. Solid State Chem.*, 1992, **101**, 275.