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Formation of micropatterned titania photocatalyst by microcontact printed silicatein on gold surfaces

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Preparation of recombinant Cys-tagged silicatein-α

The forward primer 5'-TGT TGC TGT TGC TGT TGC TGT TGC CCT GAA GCT GTA GAC TGG-3' (8x Cys residues underlined) and the reverse primer 5'-TAG GGT GGG ATA AGA TGC ATC GGT-3' were used to amplify the open reading frame of *Suberites domuncula* mature silicatein-α ($aa_{115-330}$) from a cDNA template (NCBI accession number AJ272013) by polymerase chain reaction (PCR). PCR was carried out at an initial denaturation for 5 min at 95°C, followed by 32 amplification cycles (95°C for 30 s, 56°C for 30 s, and 72°C for 60 s) and a final extension step at 72°C for 10 min. The resulting amplicons were T/A-ligated into the bacterial expression vector pTrcHis2-TOPO TA (Invitrogen, Karlsruhe, Germany), in frame with vector sequences encoding a C-terminal 6x His-tag (Scheme S1). Open reading frames were confirmed, using an automatic DNA sequenator (Li-Cor 4300). Following transformation of TOP10 *Escherichia coli* cells (Invitrogen) with these constructs, recombinant protein expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 24 h. Subsequently, proteins were extracted and purified by nickel-nitrilotriacetic acid (Ni^{2+}-NTA) metal-affinity chromatography.

Scheme S1 Illustration of recombinant Cys-tagged silicatein-α used for microcontact printing. The amino acid residues of the C-terminal Cys-tag and the N-terminal His-tag (for Au-binding and nickel-nitrilotriacetic acid metal-affinity chromatography purification, respectively) are indicated. Drawn not to scale.

(QIAexpress Kit; Qiagen, Hilden, Germany) according to [S1]. Then, protein expression was assessed through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), using Novex protein marker as standard (Invitrogen). After blotting on polyvinylidene fluoride membranes (PVDF; Millipore, Billerica, MA), recombinant silicatein was immunodetected colorimetrically following consecutive incubations with anti-histidine primary antibodies (anti-His; 1:3,000 dilution, 1h; Invitrogen), alkaline phosphatase (AP)-conjugated species-specific secondary antibodies (1:10,000 dilution, 1h; Dianova, Hamburg, Germany), and 4-nitro blue tetrazolium chloride (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) (Invitrogen). Protein sizes were determined with the Precision protein marker (Bio-Rad, München, Germany).

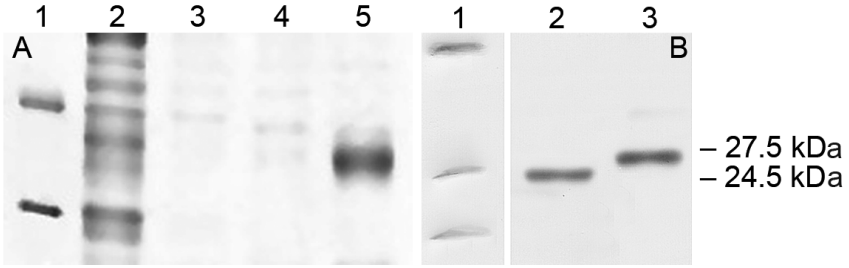

Fig. 1 Expression and purification of recombinant silicatein. After electrophoretic size-separation (SDS-PAGE) and transfer on Western blots, recombinant Cys-tagged silicatein (and silicatein$_{wt}$ for comparison) was detected colorimetrically via anti-histidine antibodies, AP-conjugated secondary antibodies, and NBT/BCIP at the expected size of ca. 27.5 kDa (24.5 kDa). (A) SDS-PAGE: 1) protein marker; 2) crude extract of IPTG-induced, silicatein-expressing *E. coli* strain; 3) flow-through during Ni^{2+}-NTA affinity chromatography purification; 4) wash fraction; 5) elution of recombinant Cys-tagged silicatein from Ni^{2+}-NTA matrix. (B) Western blot: 1) protein marker; 2) silicatein$_{wt}$; 3) Cys-tagged silicatein.

Microcontact printing of silicatein-α on gold substrates and synthesis of TiO_2 micropatterns

For μCP of patterned silicatein on Au, PDMS stamps (AMO GmbH, Aachen, Germany) were cleaned with 2-propanol and, then, inked with the recombinant and refolded protein by dipping into a solution of 5 μg silicatein in 250 μL TBS for 1 min at room temperature (RT). Subsequently, the stamps were dried by removing excess solution with a stream of air before they were placed in conformal contact with the bare gold substrate (Au(111)) for 10 s. Finally, the printed surface was extensively washed with TBS.

For immunodetection of printed silicatein, the Au surfaces were incubated for 90 min (RT) with silicatein specific primary antibodies (diluted 1:1,000 in 15% blocking solution [Roche Applied Science, Mannheim, Germany]),[S2] followed by incubation with Cy3-labeled species-specific secondary antibodies (90 min, RT) (Dianova). For visualization, the samples were inspected by confocal laser scanning microscopy (LSM; Carl Zeiss GmbH, Göttingen, Germany), using the 543 nm line of the helium/neon laser.

To obtain corresponding TiO_2 micropatterns, the printed gold surfaces were incubated in an aqueous solution of 250 μM titanium bis(ammonium lactato)-dihydroxide (TiBALDH; Sigma-Aldrich) for 2 h (RT). Afterwards, the surfaces were washed with water and ethanol to remove unreacted precursor and dried for 4 h at 60°C.

The samples thus obtained were carefully washed, air-dried, and analyzed via scanning electron microscopy (LEO Gemini 1530; Zeiss, Oberkochen; Germany) as well as microRaman spectroscopy (532 nm excitation line; Bruker Senterra Optics; Ettlingen, Germany).

Photocatalytic activity of silicatein-synthesized micropatterned TiO_2 on gold substrates

The photocatalytic activity of the TiO_2 micropattern was tested spectrophotometrically via degradation of methylene blue (MB) as model reaction. Thus, the patterned gold surfaces were

placed within quartz-glass vessels and, then, covered with an aqueous solution of MB (1 x 10^{-5} M). Subsequently, the photoreaction vessels were positioned in a closed cabinet and UV-irradiated (366 nm) at a distance of 20 cm by a 50 W high-pressure mercury lamp. During irradiation, the solution was agitated for up to 1 h. At given time intervals, the absorption was measured at 660 nm with a SmartSpec spectrophotometer (Bio-Rad) and compared to that of the controls (i.e., as-prepared MB solution, Cys-tagged silicatein-printed gold surfaces without titania micropatterns, pristine gold substrate that had been pre-incubated with TiBALDH). Furthermore, photodegradation of MB was analyzed using a gold substrate with printed silicatein and micropatterned TiO_2 that had been calcined (500°C, 3h; Carbolite chamber furnace RHF 1500).

References

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