

Supporting information

Mussel foot protein-1 (mcfp-1) interaction with titania surfaces

Dong Soo Hwang^{*a†}, Matthew J. Harrington^{b†}, Qingye Lu^c, Admir Masic^b, Hongbo Zeng^{*c}, J. Herbert Waite^{*d}

^aPOSTECH Ocean Science and Technology Institute, Pohang University of Science and Technology, Pohang 790784

^bDepartment of Biomaterials, Max Planck Institute for Colloids and Interfaces, 14424 Potsdam-Golm, Germany

^cDepartment of Chemical Engineering, University of Alberta, Canada

^dMaterials Research Laboratory, University of California, Santa Barbara, California 93106

Supporting Information Table of Contents

Page	Content
1	Experimental materials and methods
2	Figure S1. AFM tapping mode image of a mica supports TiO ₂ surface in dry
3	Table S1. Spectral assignments for dopa-TiO ₂ resonance Raman peaks in comparison with similar complexes
3	Supplementary references

Experimental materials and methods

Mcfp-1 purification

Mcfp-1 was purified from frozen *Mytilus californinus* feet according to published procedures.^[1] Purified mcfp-1 was treated with 1 mM EDTA in 5% acetic acid and polished with C8 reverse phase column to prevent residual metal ion contamination. Sample purity was assessed by acid urea polyacrylamide gel electrophoresis, amino acid analysis, and MALDI-TOF mass spectrometry. The DOPA in purified mcfp-1 was ~13 mol% by amino acid analysis after a 1h hydrolysis in 6N HCl at 158 °C. Purified samples were freeze-dried and resuspended in a solution of 0.1 M acetic acid, pH 3.0 and thereafter divided into convenient aliquot volumes for storage in aluminum foil-covered vials at -80 °C prior to testing. Thin layer of TiO₂ (~15 nm) were deposited onto mica by e-beam evaporation (PVD-75, Kurt J. Lesker) at 0.05-0.1 nm/s with 1.5×10^{-5} Torr of O₂ and (2-8) × 10⁻⁶ Torr of H₂O. The root mean square (rms) roughness values of mica and TiO₂ determined by AFM (DI, Santa Barbara, CA, USA) with a AFM image (5 μm * 5 μm) was about 0.16 and 0.8 nm, respectively.

SFA experiment

The normal force-distance profiles and adhesion forces of the mcfp-1 were determined using a surface forces apparatus (SFA) in a configuration reported previously.^[2] The smooth and chemically inert surfaces of mica were used as the substrate surfaces for depositing thin films of the protein used in the experiment. Two thin mica sheets (thickness 1-5 μm) were glued onto two cylindrical silica disks (radius $R=2$ cm). For mcfp-1 coating on the surfaces (mica and mica supported TiO₂), 100 μL of mcfp-1 solution (10 μg/mL) diluted in 0.1 M acetic acid (pH~3.0) was dropped onto one the surfaces, and the surfaces are incubated until protein adsorption reaches equilibrium (20 min); then the surfaces are washed more than five times with a buffer that is appropriate for the specific experiment. The thickness of adsorbed mcfp-1 film on the surfaces measured by hardwall distance was constant (8 ~10 nm). Since the mcfp-1 film thickness was less than the hydrodynamic diameter of mcfp-1 (~16 nm), we considered the mcfp-1 film as a monomolecular layers. Higher protein concentration (over ~10 μg/mL) or excessive incubation time with protein solution to surfaces (over ~20 min), generally lead to multi-layer deposition of protein and make the experimental results unreliable. One coated and one uncoated target surface (asymmetric configuration) were then mounted in the SFA chamber in a crossed-cylinder geometry, which roughly corresponds to a sphere of radius R approaching a flat surface based on the "Derjaguin approximation". The measured adhesion or "pull-off" force F_{ad} is related to the adhesion energy per unit area E_{ad} by $E_{ad} = F_{ad}/1.5 \pi R$ (used in this study) for soft deformable surfaces with strong adhesive contact.^[3] A constant rate of approach and separation (5~6 nm/sec) were used for each force run. The experiments were conducted at room temperature (20 °C).

Resonance Raman Spectroscopy

Raman spectra were collected from the surface of samples with a confocal Raman microscope (alpha300; WITec) equipped with a Nikon objective (100X) and using a laser excitation wavelength of 532 nm. Spectra were acquired with a CCD camera (DV401-BV; Andor) behind a spectrometer (UHTS 300; WITec) with a spectral resolution of 3 cm⁻¹. Samples were prepared by incubating protein on TiO₂ surfaces as described in the SFA experimental section. Protein solutions of pH3 and pH5 were incubated for ~20 min after which the surfaces were washed with their respective solutions and dried prior to measurement. Spectra from bare mica, TiO₂ coated mica, and protein incubated on a bare mica surface were acquired as controls. Protein coated samples were sensitive to burning by the laser beam; therefore, laser power was restricted to 10–20 mW and only short integration times of 0.3 s were used for all measurements. The ScanCtrlSpectroscopyPlus software (version 1.38, Witec) was used for measurement and data analysis. Each collected spectrum consisted of 100 accumulations of a 0.3 s integration time. For each sample, three spectra were collected from different regions and averaged. Averaged spectra were smoothed with a Savitzky-Golay smoothing filter. To observe the resonance peaks more clearly in spectra from protein coated samples, the combined mica/TiO₂ background spectra was subtracted.

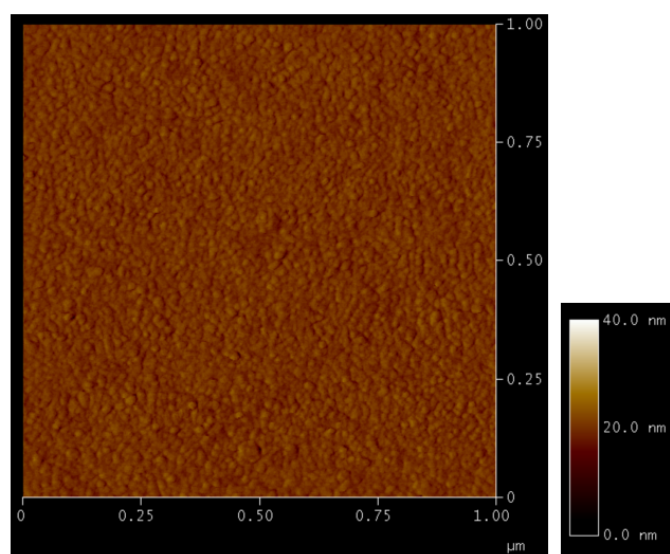


Figure S1. AFM tapping mode image of a mica supported TiO_2 surface in dry.

Mcfp-1 TiO ₂ (pH3)	Mcfp-1 TiO ₂ (pH5)	^b Byssus coating	^c Mefp-1 Fe ³⁺	^d (NH ₄) ₂ [Ti(cat) ₃] •2H ₂ O	^d catechol- anatase	^{e,f} assignments
518	536	550	531	505		δ,ν-chelate CT
594	591	605	591	591		ν Me-O ₃
645	639	639	638	646		ν Me-O ₄
836	836	828	815			ring breathing
			1152	1153	1155	δCH
1274	1271	1271	1274	1270	1261	ν C-O
1332	1332	1326	1326	1338	1329	ν CC
1439	1456	1426	1426	1475		
1487	1487	1475	1491	1490	1483	ν CC + δCH

Ref: ^aPresent study ^bHarrington et al ^cTaylor et al ^dLana-Villarreal et al ^eMichaud-Soret et al ^fOhhrstroëm et al

Table S1. Spectral assignments for DOPA-TiO₂ resonance Raman peaks in comparison with similar complexes

Supplementary references

1. Q. Lu, D. S. Hwang, Y. Liu, H. Zeng, *Biomaterials* **2012**, 33, 1903-11
2. a) C. A. Helm, W. Knoll, J. N. Israelachvili, *Proc Natl Acad Sci U S A* **2007**, 88, 8169-73; b) K. L. Johnson, K. Kendall, A. D. Roberts, *Proc. Royal Soc. London Series a-Math. and Physical Sci.* **1971**, 324, 301.
3. a) Q. Lin, D. Gourdon, C. Sun, N. Holten-Anderson, T. H. Anderson, J. H. Waite, J. N. Israelachvili, *Proc Natl Acad Sci U S A*, **2007**, 104, 3782-6; b) T. H. Anderson, J. Yu, A. Estrada, M. U. Hammer, J. H. Waite, J. N. Israelachvili, *Adv Func Mater*, **2010**, 20, 4196-4205.
4. M. J. Harrington, A. Masic, N. Holten-Anderson, J. H. Waite, P. Fratzl *Science* **2010**, 328, 216-20.
5. S. W. Taylor, B. D. Chase, M. H. Emptage, M. J. Nelson, J. H. Waite, *Inorg Chem* **1996**, 35, 7572-7.
6. T. Lana-Villarreal, A. Rodes, J. M. Perez, R. Gomez, *J Am Chem Soc* **2005**, 127, 12601-11.
7. I. Michaud-Soret, K. K. Anderson, L. Que Jr., J. Haavik, *Biochemistry* **1995**, 34, 5504-10.
8. L. Ohhrstroëm, I. Michaud-Soret, *J Am Chem Soc* **1996**, 118, 328304.