Supplementary information

to the manuscript

“Protein adsorption on nano-scaled, rippled TiO$_2$ and Si surfaces”

by

Jana Sommerfeld, Jessica Richter, Raphael Niepelt, Stefanie Kosan,
Thomas F. Keller, Klaus D. Jandt, and Carsten Ronning

Experimental procedures

Both TiO$_2$ and Si were sets of commercially available <100> single crystal samples (CrysTec GmbH, Berlin). The ion beam bombardment was performed with Xenon ions in order to exclude any doping effects of the material, as noble gas atoms do form bonds in these materials and tend to diffuse out after irradiation at room temperature. The Xe$^+$ ion energy ranged from 5 – 20 keV in order to vary the wavelength of the created ripples. The angle between the surface and the incident ion beam was 70-72° and typical ion fluences of $10^{16}$ ions/cm$^2$ (fluxes of 1 µA/cm$^2$) were used. The samples were cleaned with ethanol and dried using compressed air before further experiments were performed. As a reference, untreated and therefore single crystalline Si and TiO$_2$ samples were investigated as well.
Fig. 1s: AFM pictures of ripples structures before protein adsorption. a) Si was irradiated with 7 keV Xe ions and an ion fluence of $3 \cdot 10^{16}$ cm$^{-2}$ resulting in an average ripple wavelength of 52 nm. b) The same wavelength was observed for TiO$_2$ that was irradiated with 10 keV Xe ions and an identical ion fluence of $3 \cdot 10^{16}$ cm$^{-2}$.

X-ray photoelectron spectroscopy (XPS) has been performed using a Quantum 2000 (PHI Co., Chanhassen, MN, USA) spectrometer with a focused monochromatic Al K-alpha source (1486.7eV) for excitation and detecting 45° to the surface normal to clarify the possibility of a stoichiometric change.

Contact angle measurements (CAM) have been conducted with a DSA10 drop shape analysis system (Krüss GmbH, Hamburg, Germany), with a 5 µl droplet of Millipore water to check for a possible change of hydrophilicity. The ultrapure Millipore water has a resistance of 18.2 MΩ·cm at 25°C and a total organic carbon (TOC) content of less than 10 ppb.

For the adsorption experiment, human plasma fibrinogen (HPF) was dissolved in phosphate buffered saline (PBS) with a concentration of $c = 10 \mu$g/ml. The HPF solution was heated up to 37°C (human body environment) and a 1 ml droplet was added to each sample in the vial. Then, the samples were stored for 2 hours at 37°C to allow the proteins to adsorb. Subsequently, the protein solution was exchanged in two steps by PBS and Millipore water to avoid the attachment of not adsorbed proteins or
residues. Eventually, atomic force microscopy investigations have been done in air within 24 hours to assure the stability of the proteins. We used a MultiMode AFM by Digital Instruments (Digital Instruments, Santa Barbara, CA) with a Nanoscope IIIa controller and an E Scanner, allowing a maximum scan size of 12 µm and a vertical resolution in the subnanometer range. We used the Micro Cantilever AC 160TC by Atomic Force that provides a tetrahedral Si tip with a radius of less than 7 nm. Moreover, the cantilever was single-side coated with an Al-reflex coating. The referring resonance frequency ranged from 314.5 to 334 kHz and the spring constant varied between 48.9 and 88.8 Nm⁻¹.

**Ripple wavelengths of Si irradiated with Xe as function of ion energy and contact angle measurements**

The wavelengths of all Si samples were determined by fast Fourier transformation (FFT) and plotted in Figure 2s(a), which shows a clear power law dependence with increasing ion energy: the higher the ion energy ε used, the higher the ripple wavelength λ is (λ ~ ε²ᵐ). For Si we obtained an exponent m of 0.5 ± 0.05, which is well in agreement with experimental data already published,¹ obtained from irradiation with argon ions. Contact angle measurements (CAM) of water reveal that compared to non-irradiated and flat Si, the contact angle increases marginally from (84.5 ± 4.6)° to (85.0 ± 2.0)° for flat, irradiated Si. After changing the morphology and thereby increasing the reciprocal wavelength of the created ripples, the contact angle decreases about 25°. Nevertheless this decrease is not unambiguous within the range of error for the rippled Si substrates.

Fig. 2s: a) Wavelength of the formed ripple on Si as a function of the ion energy. The wavelength of the created ripples follows a power law dependence with increasing ion energy. b) Contact angles of water on flat and rippled Si as a function of the reciprocal ripple wavelength. Compared to non-irradiated and flat Si (triangle), the contact angle does not change for flat and irradiated Si. When introducing a curvature (increase of the reciprocal wavelength) the contact angle slightly decreases. No significant change of the contact angle was observed for different wavelengths. The values for rippled Si exhibit larger error bars, because of the smaller extent of the irradiated area in comparison to the flat Si that could be investigated.

**XPS-measurements on non- and Xe-irradiated TiO$_2$ and Si surfaces**

The high resolution spectra we obtained for flat TiO$_2$ before and after the irradiation process are presented in Figure 3s, upper row. For the non-irradiated TiO$_2$ we found the TiO$_2$ related O 1s peak at 529.9 eV$^{2,3}$ that shifts slightly ($\Delta E = 0.3$ eV) to higher binding energies in the case of irradiated TiO$_2$. In addition, we observed a peak at 532.2 eV, originating from atmospheric OH-groups$^3$. Due to an evaluation of the corresponding peak areas, a slight decrease of the oxygen content was determined.

---


for irradiated TiO$_2$, which is in good agreement with Naguib et al.$^4$. One can see clearly the Ti 2p$_{1/2}$ and Ti 2p$_{3/2}$ peaks$^5$ of TiO$_2$ at the binding energy of 458.6 eV and 464.3 eV, respectively, that show no change after the irradiation process. The lower row of Figure 3 represents the results for the O 1s and the Si 2p peak of flat Si with a native SiO$_2$ layer before and after the irradiation process. It ought to be mentioned here, that though the natural SiO$_2$ layer was sputtered away during ion beam bombardment, a new SiO$_2$ layer is formed immediately after the irradiation process after venting the implanter. The O 1s peak shown in Figure 3c is located at a binding energy about 532.5 eV in case of non-irradiated Si, which correlates well with Nefedov et al.$^6$. The peak position shifts only negligibly ($\Delta E < 0.1$ eV) towards higher binding energies after irradiation. Interestingly, we found a slight increase of oxygen after the irradiation process that might be caused by an enhanced oxidation process of Si after the ion bombardment. The observable peak around 99.5 eV (3d) correlates well with the energy for the Si 2p state of non-irradiated Si$^7,8$. This peak shifts marginally to 99.2 eV after the irradiation process. At a binding energy of 103.2 eV we found an additional peak that originates from the respective Si 2p peak of SiO$_2$$^5,6$. According to the review article by Naguib et al.$^4$, an amorphization of TiO$_2$, Si, and SiO$_2$ is expected for ion fluences higher than $10^{13}$ ions/cm$^2$, which was the case in this study. Although we found a slight decrease of the oxygen content for irradiated TiO$_2$, a stoichiometric change can be excluded for both Si and TiO$_2$ by these XPS results.


Fig. 3s: XPS spectra for TiO$_2$ and Si with a native SiO$_2$ layer on top. The Shirley underground was subtracted from the original data and a Gaussian fit was applied to the lines (dashed lines). (a) Oxygen and (b) titanium lines for flat non-irradiated and irradiated TiO$_2$. (c) Oxygen and (d) silicon lines for flat non-irradiated and irradiated Si.

**AFM measurements of HPF adsorption on flat Si**

We observe that a dense film of fibrinogen is adsorbed in partly globular conformation (compare to Figure 1 of the manuscript) on top of flat, non-irradiated Si, as shown in Figure 4s(a) as well as on flat, irradiated Si (Figure 4s(b)). Both pictures reveal some networks of the adsorbed HPF, which indicates that it was not possible for the $\alpha$ chains to interact. This interaction probably originates from some proteins that adsorbed in a stretched conformation, which is in well agreement with the hydrophilic
character of the SiO$_2$ layer on top of Si\. Apparently, there is no difference in the adsorption behavior of HPF between crystalline and amorphous Si.

Fig. 4s: AFM images of (a) flat, non-irradiated and (b) flat, irradiated Si. A dense film of HPF proteins was observed on both substrates. The bright spots are most probably agglomerations of HPF molecules. The protein layer underneath the agglomerations consists of HPF proteins in a globular conformation with little networks of some molecules.

---