

TiO₂ nanoparticle interactions with supported lipid membranes

– an example of removal of membrane patches

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Supporting Information.

MATERIALS AND METHODS

Combined QCM-D and reflectometry measurements. Combined QCM-D and reflectometry measurements were performed in flow mode using a prototype instrument.¹ Gold-coated QCM-D sensor crystals (Q-Sense AB, Sweden) were modified to meet the requirement for sensitive reflectometry measurements by deposition of a 100 nm layer of Ti followed by a 110 nm layer of SiO₂ onto the sensor surface. Prior to the experiment, the crystals were cleaned as for ordinary QCM-D measurements, and the experiments were performed similarly. The detection of mass adsorbed to the sensor surface in optical reflectometry is based on changes in the reflectivity of a solid surface when material adsorbs to

it. The measured optical signal, ΔR , is the relative change of the intensity ratio of the two polarizations of the reflected light, which is different from ellipsometry in that the phase shift of the reflected light is not taken into account. By optical modeling, ΔR can be related to the thickness d and the refractive index n of the material adsorbed to the sensor surface relative to the surrounding buffer (n_0) via:²

$$\Delta R = d (n - n_0) A$$

where A is a sensitivity factor dependent on the optical setup.

RESULTS AND DISCUSSION

Combined QCM-D and reflectometry results confirms bilayer removal. By combining QCM-D and optical reflectometry measurements in a novel experimental setup,¹ the QCM-D data and optical data are obtained simultaneous on the same surface. As opposed to QCM-D, which measures acoustically coupled mass (here adsorbed lipid and NP mass, as well as solvent associated with it), reflectometry measures changes in refractive index near the surface due to adsorption of material on the sensor surface. The optical result is given as ΔR .

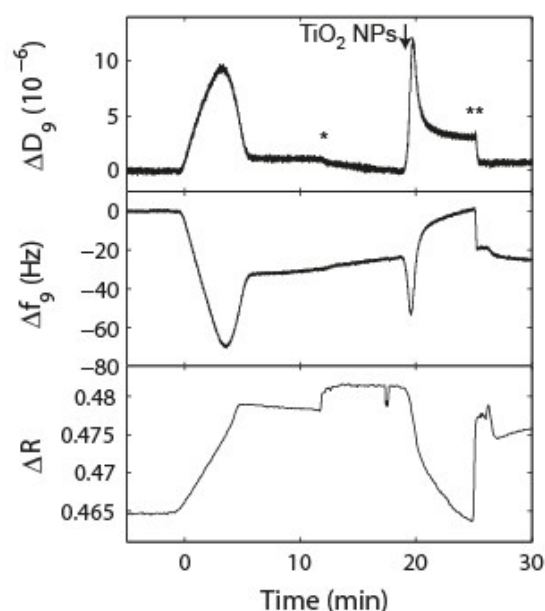


Figure S1 QCM-D frequency and dissipation shifts (Δf and ΔD , respectively) and reflectometry data (ΔR) for the interaction between TiO_2 NPs and a POPG/POPC (1:1) membrane. These complementary results were collected simultaneously in a combined QCM-D and reflectometry setup. The lipid membrane formation (in TRIS-NaCl- CaCl_2) is followed by a buffer change to TRIS (indicated by *), the addition of TiO_2 NPs in TRIS, and a buffer change to TRIS-NaCl- CaCl_2 (indicated by **).

As shown in Figure S1, the scenario of Ca^{2+} mediated membrane damage induced in PG-containing membranes upon exposure to TiO_2 NPs was confirmed in experiments with optical detection of the mass at the sensor during the QCM-D experiment. The same experimental set up has been used before to characterize different supported lipid membrane systems, while performing reflectometry and QCM-D was performed simultaneously on the same surface. This is a particularly powerful combination when it comes to differentiate between different mechanisms for formation of the supported lipid membranes. QCM curves show similar behavior as described above. For membrane formation, the signal of reflectometry first increases and then levels off, which is consistent with previous reports.³ After introducing TiO_2 NPs, reflectometry curve initially shows sharp drop in signal and stabilizes at almost the same value of baseline prior to membrane formation. This indicates a big loss in the optical mass (or dry mass) of lipid membrane due to TiO_2 addition. However, the signal almost goes back after rinsing with Tris-NaCl- CaCl_2 buffer, namely, the membrane returns to the substrate again but with some lipid patches removed during the interaction with TiO_2 NPs. Thus, the results from the combined QCM-D and optical setup further supports our assumption.

Two control experiments supporting the Ca^{2+} -ions mediated mechanism. Control experiments by QCM-D, where EDTA was added instead of TiO_2 NPs, are shown in Figure S2. In A it is shown that the re-exposure of the membrane to more Ca^{2+} ions is required for TiO_2 particles to bind when added again to the lipid membrane, and in B, it is shown that EDTA, a high-affinity Ca^{2+} chelator, has a similar effect

on the membrane as the TiO₂ NPs. In yet another series of experiments, Mg²⁺ ions were found to have a similar effect as Ca²⁺ (not shown).

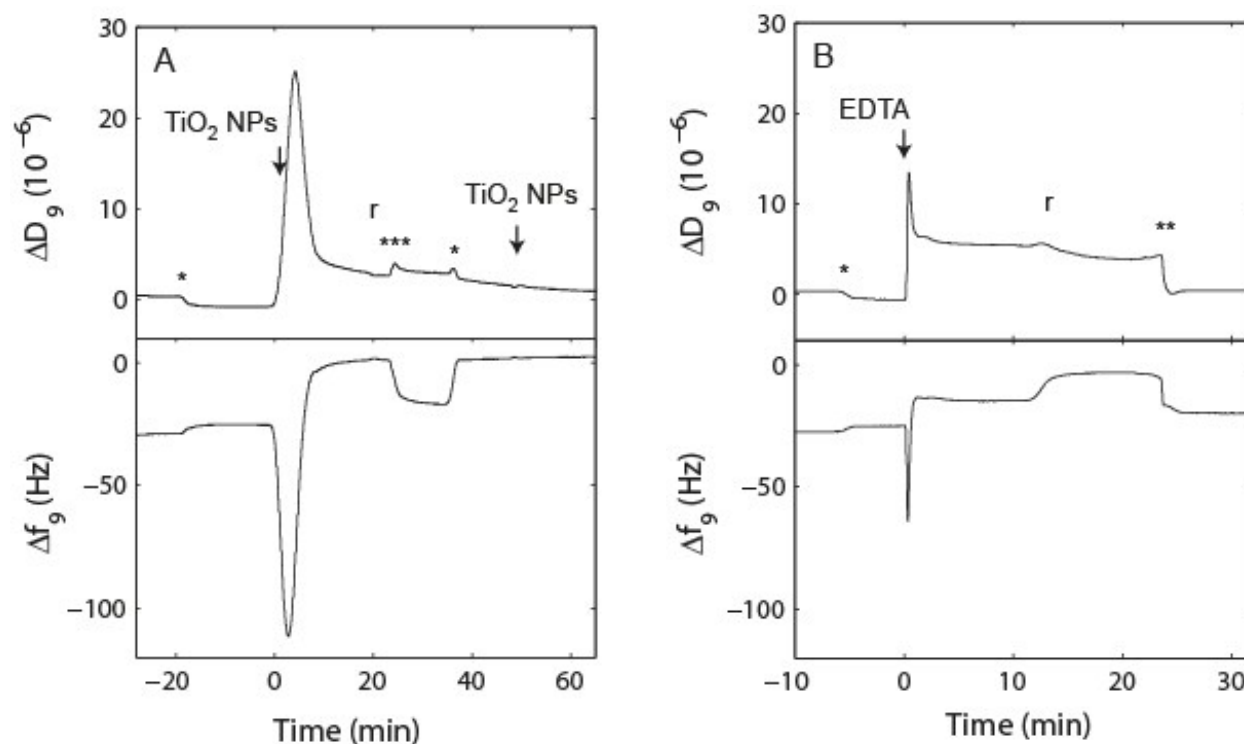


Figure S2 QCM-D control experiments (frequency and dissipation shifts; Δf and ΔD , respectively) with the POPC/POPG 1:1 membrane, to which (A) TiO₂ NPs were added twice to the membrane while rinsing only with a non-Ca²⁺ containing buffer (TRIS-NaCl) in between, or (B) EDTA was added instead of TiO₂ NPs. In both cases, the lipid membrane formation (in TRIS-NaCl-CaCl₂) is followed by a buffer change to TRIS (*) before the addition of TiO₂ NPs or EDTA in TRIS.

DLVO results

In the DLVO theory the total interaction energy is calculated as the sum of electrostatic and van der Waals energies. The electrostatic interactions are described by a screened Coulomb potential whereas the van der Waals interaction is usually obtained by using the Hamaker theory.⁴

We used a sphere-plate interaction model to describe the interaction between a TiO₂ particle and a silica surface with a lipid bilayer. The size of the sphere was set to the average hydrodynamic size of the

TiO₂ NPs (57 nm, see Table 1). Zeta potential values for TiO₂ NPs (measured, see Table 1) and the supported lipid membrane (estimated by the corresponding value obtained for liposomes in bulk, see Table 1) were used to obtain the interaction energies between TiO₂ NPs and the lipid membrane, as suggested by Hogg *et al.*⁵ The van der Waals energies were calculated by using an expression which takes into account the retardation effects.⁶ The retardation effects were found to be necessary when considering attractive interactions between two bodies at distances larger than few nanometers. The value of 1×10^{-20} J was used for TiO₂-water-silica system.

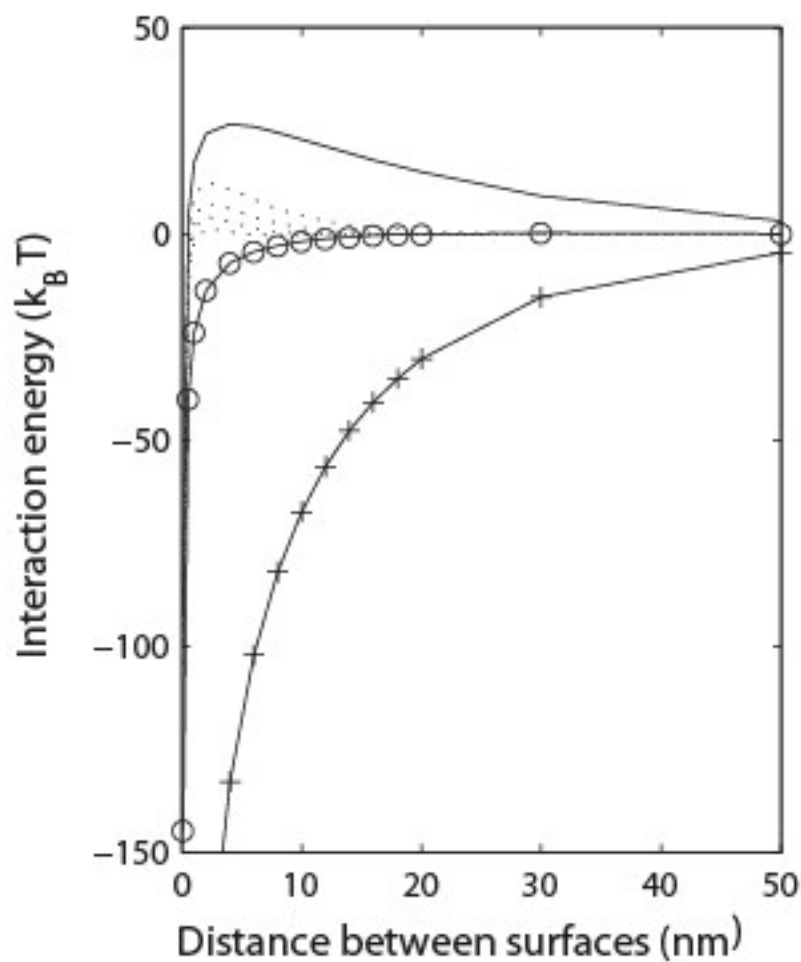


Figure S3 Total interaction energy between a planar lipid membrane and TiO₂ NPs obtained by DLVO theory using the experimental conditions given in Table 1. Solid lines represent surface zeta potentials

of POPC (o), POPC-POEPC (1:1) (+), and POPC-POPG (1:1) (no symbol) membranes in 10 mM TRIS, and dotted lines represent POPC-POPG (1:1) membranes in 10 mM TRIS + r mM CaCl_2 ($r = 1, 2, 4,$ and 8), where higher CaCl_2 concentrations leads to lower interaction energies.

The interaction energy profiles show that the interaction between the positively charged bilayers and negatively charged TiO_2 NPs is attractive. As expected, and in accordance with our experimental results, the attractive interaction increases as the positive charge on the membrane increases, e.g, the POPC/POEPC 1:1 membrane has highest positive charge and shows the strongest attractive interaction, which is well consistent with our experimental results (Figure 1A). On the other hand the strongly negatively charged membrane POPC/POPG 1:1 shows strongest repulsion. More weakly negatively charged bilayer (zeta potential -13 mV) formed by POPC/POPG 1:1 in the presence of CaCl_2 shows slightly repulsive interaction energies. In contrast, our experimental results show that TiO_2 NPs adsorb on these bilayers. We note that the zeta potential is measured for liposomes in solution and it may not correctly represent the zeta potential of bilayers, due to geometric reasons as well as due to potential asymmetries in the distribution of lipids between the two membrane leaflets. For example, it is likely that the highly negatively charged SiO_2 surface attracts Ca^{2+} ions which results in high concentrations of Ca^{2+} ions at the interface between the substrate and the lipid bilayer. The increased Ca^{2+} concentration in the membrane will induce strong screening of the negatively charged groups on lipids and this will result in a zeta potential of the supported membrane which is much lower than for the liposome in bulk solution. DLVO calculations show that a zeta potential of -5 mV on the membrane will lead to an energy barrier of $0.3 k_B T$, and at a -2 mV potential, the attraction is purely attractive (Supporting Information, Figure S3).

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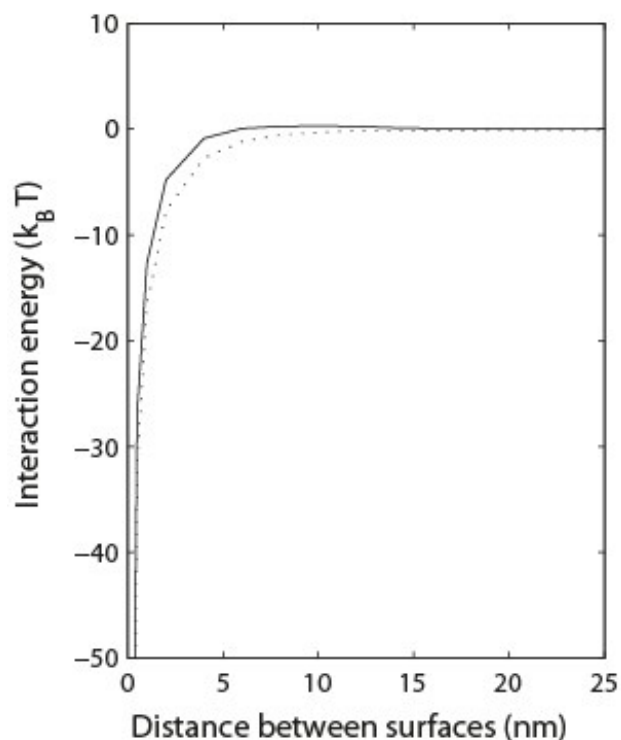


Figure S4 Total interaction energy between a planar lipid membrane and TiO₂ NPs in 10 mM TRIS buffer obtained by applying DLVO theory. The interaction energies are calculated by considering -27 mV zeta potential on the particle surface, while assuming -5 mV and -2 mV on the membrane (solid and dotted lines, respectively).

References:

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