Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Electrostatically-driven interaction of ferritin with supported lipid bilayers

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Supporting Informations

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1. Experimental details

1.1. Sensors coating and oxidation

Poly(hydroxymethyl)siloxane (PHMS) thin films were spin coated on gold electrodes of QCM-D sensors (Q-Sense AB, Sweden) and on SPR Au sensor chips (Biacore AB, Sweden). Prior to the spin coating step the gold coated sensors were cleaned by 10 minutes of treatment at 75°C with $H_2O/NH_3/H_2O_2$ mixture (5/1/1 volume ratio), followed by 10 minutes of treatment by UV-ozone at atmospheric pressure and multiple rinsing with Millipore water. Plasma treatments were performed in radiofrequency plasma equipment (Plasma Therm Batchtop PE/RIE m/95a) at the following conditions: gas flow of 20 sccm O_2 (99.9% purity); base pressure of 600 mTorr; power 100 W; 1 min of plasma time. Repeated oxidation treatments were performed by a three-step procedure consisting of (i) 1 minute of oxygen plasma oxidation, (ii) thermal treatment in air during one hour at 200 °C (1.01×10^5 Pa), (iii) a second oxygen plasma oxidation of 1 minute, with water rinsing and nitrogen blow drying in between each two steps. Immediately before running QCM-D and SPR experiments the oxidized PHMS samples were additionally treated by 30 minutes UV-ozone exposure as usually done for PVD-coated SiO_2 sensor crystals, in order to remove accumulated surface contaminants and to cancel aging effects.

1.2. Vesicle preparation

Zwitterionic 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (POPC), anionic 1-hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycero-3-phospho-L-serine (POPS) and cationic 1,2-dioleoyl-sn-glycero-3-ethylphopshocholine (POEPC) lipids were purchased from Avanti Polar Lipids Inc. (AL, US). All other chemicals were purchased from Sigma-Aldrich (US). Water was deionized (resistivity > 18 $M\Omega \cdot cm^{-1}$) and purified using a MilliQ unit (MilliQ plus, Millipore, France). Phosphate Buffer Saline (PBS) solution was prepared from tablets (0.01 M phosphate buffer containing 0.003 M KCl and 0.14 M NaCl, pH 7.4). For the preparation of negatively charged bilayers, 5 mM $MgCl_2$ was added to the PBS buffer. The buffers were filtered and degassed.

Lyophilized lipids were dissolved in chloroform (stock solutions were stored at -20°C), and mixed in desired proportions. The solvent was evaporated under a flow of nitrogen, while rotating the round-bottomed flask, to form a film on the wall of the flask. The lipid film was emulsified in buffer at room temperature, vortexed, and extruded 11 times through a 100 nm polycarbonate membrane, followed by 11 times through a 30 nm membrane (Avanti Polar Lipids Inc., AL, US). Vesicles prepared in this way typically measure 80-100 nm, as determined by dynamic light scattering. The vesicle solutions were stored refrigerated under N_2 and used within two weeks. Charged vesicles were prepared from POPC/POPS 3:1, POPC/POEPC 3:1 or POEPC.

1.3. Protein solutions

Horse spleen ferritin (Sigma) solutions of 50 μg/ml and 100 μg/ml of bulk concentration were used. Either positively or negatively charged ferritin molecules were prepared by diluting the original ferritin solution either in acetate buffer (0.01 M, containing 0.15 M NaCl, pH 3.5) or phosphate buffer (0.01 M, containing 0.003 M KCl and 0.14 M NaCl, pH 7.4), respectively. Fluorescein labelled bovine serum albumin (Sigma) lyophilized powder was dissolved in phosphate buffer (pH=7.4) to the final concentration of 100 mg/mL and kept under dark.

1.4. Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)

QCM-D measurements were carried out in flow mode (50 μL/min) on a Q-sense E4 unit (Q-Sense AB, Gothenburg, Sweden). Prior to each measurement series, the crystals (AT-cut quartz crystals with a fundamental resonance frequency of 5 MHz) were cleaned by immersion in 10 mM sodium dodeceyl sulfate (SDS, > 1 h), followed by rinsing with water, drying with nitrogen, and UV-Ozone treatment (30 min). Frequency shifts were normalized by division by the overtone number.

1.5. Surface Plasmon Resonance (SPR)

Measurements were performed with a BIAcore2000 system from Biacore AB (Uppsala, Sweden). A flow rate of 10 μL/min was used, and a stable baseline was attained before adsorption. The procedure followed that of a QCM-D experiment with the same buffers and chemicals.

2. QCM-D results for the formation of SLBs of different composition

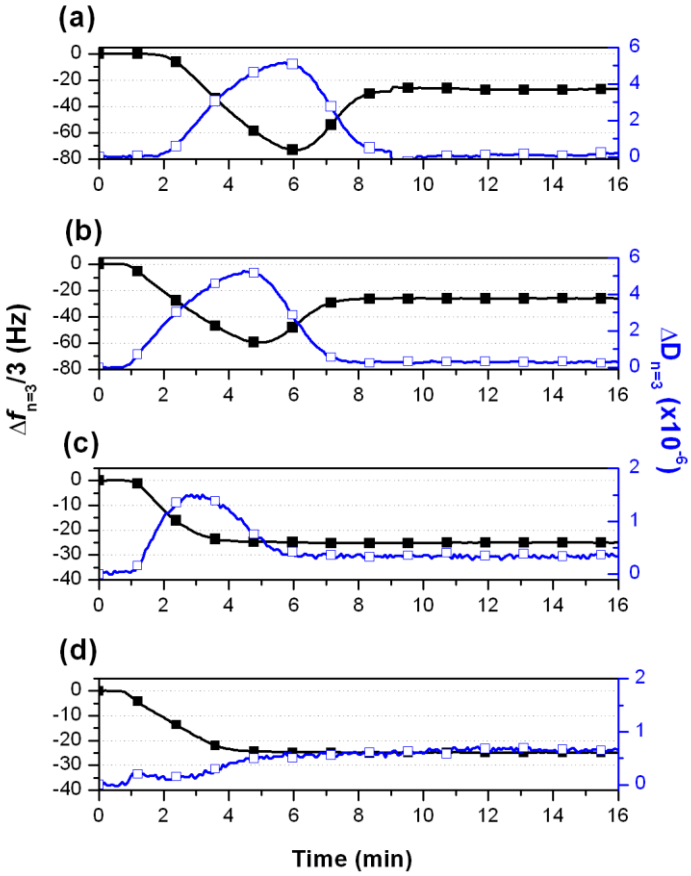

Figure S1- QCM-D curves of frequency (solid symbols) and dissipation (open symbols) shifts for SLBs formation (a: POPC/POPS 3:1; b: POPC; c: POPC/POEPC 3:1; d: POEPC).

In order to prepare supported lipid bilayer nanoplatforms at different electrostatic charge the well characterized SLBs made from zwitterionic POPC vesicles, positively charged POEPC vesicles and the mixture POPC/POPS 75:25 (negatively charged) and POPC/POEPC 75:25 (positively charged were employed [A. Kunze, S. Svedhem and B. Kasemo, Langmuir 2009, 25,5146–5158].

As the negatively charged POPC/POPS (3/1) vesicles (Fig. S1a) or zwitterionic POPC vesicles (Fig. S1b) are entering the chamber, they initially adsorb intact to the surface, resulting in a decrease in Δf (increase in coupled mass) as well as an increase in ΔD (viscoelastic character of the vesicles). As a critical surface coverage is reached, the vesicles start to rupture. At this point both the energy losses (ΔD) and the coupled mass (Δf) have reached their extreme values and start to decrease. At the completion of the bilayer formation, the QCM-D responses, Δf~-26 Hz and $\Delta D \sim 0.2 \times 10^{-6}$, indicate the formation of a coherent and homogeneous bilayer for both cases [C.A. Keller and B. Kasemo, Biophys. J. 1998, 75, 1397–1402].

As to the formation kinetics of the bilayer from positively charged POPC/POEPC (3/1) (Fig. S1c) and POEPC (Fig. S1d) vesicles, the spontaneous vesicle rupture occurs immediately after interaction with the surface, i.e., there is no initial phase of adsorption of intact vesicles. In this representative result, ΔD stabilizes at ~0.5×10^{-6}, which is higher than normally observed for POPC bilayers. Δf decreases monotonically until it reaches the final values respectively of ~ -25 Hz for POPC/POEPC (3/1) and ~ -24 Hz, about 5% lower than for the zwitterionic and negatively charged bilayers.

Both frequency and dissipation shift values at the equilibrium are consistent with the studies reported in literature for supported lipid bilayers having the same composition on SiO_2 [A. Kunze, S. Svedhem and B. Kasemo, Langmuir 2009, 25,5146–5158; M. Edvardsson, S. Svedhem, G. Wang, R. Richter, M. Rodahl and B. Kasemo, Analytical Chemistry, 2009, **81**, 349-361].

3. QCM-D curves for ferritin interaction with SLBs at pH=3.5.

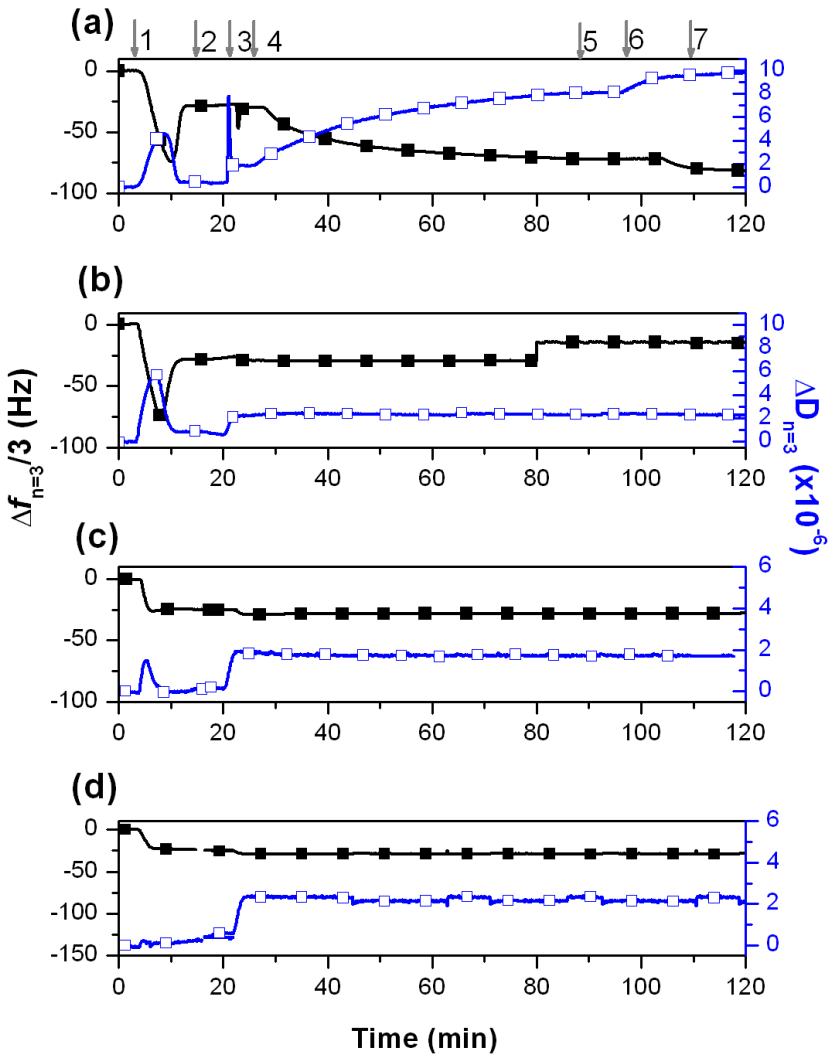

Figure S2. QCM-D curves of frequency (solid symbols) and dissipation (open symbols) shifts for SLB formation (a: PS 25%; b: PC 100%; c: EPC 25%; d: EPC 100%) followed by the adsorption of ferritin at pH=3.5 (0.01 M acetate buffer, containing 0.15 M NaCl). Arrows indicate time points corresponding to: (1) vesicles addition, (2) pH=7.4 rinsing, (3) exchange to buffer pH= 3.5 pH, (4) HSF 50 μg/ml addition, (5) rinsing, (6) HSF 100 μg/ml addition, (7) rinsing.

QCM-D data in Figure S2 show that, except the curve shifts due to the exchange from phosphate buffer (pH=7.4) to acetate buffer (pH=3.5), upon the addition of positively charged Fer molecules (arrows "4" and "6" in the figure) to zwitterionic or positively charged SLBs there are no detectable changes of frequency and dissipation.

On the contrary, a significant protein mass uptake is found on the negatively charged SLB.

The saturation values for HSF adsorption on POPC/POPS SLBs are Δf=-36±4 Hz and ΔD=5.3±0.6 x10^{-6}, respectively, for 50 μg/ml HSF concentration, and Δf=-45±5 Hz and ΔD=6.7±0.8 x10^{-6}, for 100 μg/mL HSF concentration. The $\Delta D/\Delta f$ ratio points as well to a significant contribution of dissipative forces at the protein-lipid bilayer interface.

4. *D-f* plots for the interaction of ferritin with the different SLBs.

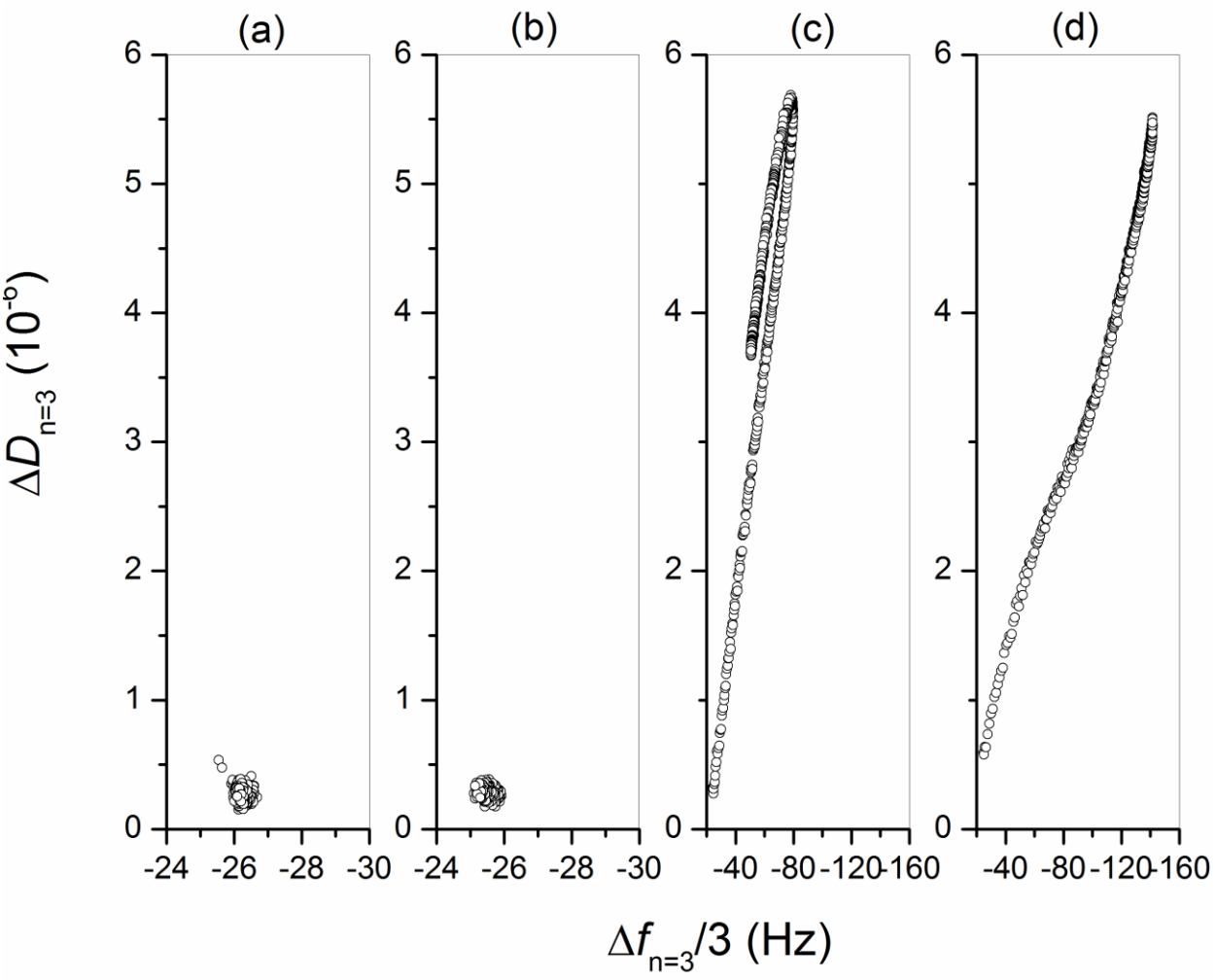

Figure S3. *D-f* plots for the third overtone (n=3) corresponding to the interaction, at pH= 7.4, of 50 uM HSF with: (a) PS 25%; (b) PC 100%; (c) EPC 25%; (d) EPC 100%).