Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information

Repelling and Ordering: The Influence of Poly(ethylene glycol) on protein adsorption

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SI. MATERIALS & METHODS

SIA. Lipid Monolayer Preparation

1 mmol DMPE and DMPE-PEG2000 (both Avanti Polar Lipids, purity >99%) were dissolved in chloroform (VWR Chemicals, purity >99%) and are mixed by volume to get solutions with 0, 1, 5 and 10 mol% DMPE-PEG2000 content. The lipids are then spread on an aqueous phosphate buffered saline subphase (PBS, Sigma Aldrich pH = 7.4) in a translating trough and compressed to an initial surface pressure of 20 mN/m. The average area per DMPE-PEG2000 molecule in the monolayers containing 1, 5 and 10 mol% DMPE-PEG200 is ~60, 12 and 6 nm², respectively. This has been calculated by tracking the amount of molecules present at the surface and the area they occupy. To study the protein adsorption behavior either bovine serum albumin (BSA, Sigma Aldrich, purity ≥ 96%) or fibrinogen (Fbg, Sigma Aldrich, clottable protein >90%) dissolved in PBS buffer solution were injected into the subphase to reach a final concentration of 0.1 mg/mL. During the measurements the surface pressure was monitored at all times using a DeltaPi tensiometer (Kibron, Finland).

SIB. Area-Pressure Isotherms & Brewster Angle Microscopy (BAM)

A commercial setup (Accurion, EP3 BAM) is used for the acquisition of the area-pressure isotherms and BAM images. After the lipids are spread on the liquid surface the barriers are closed at a speed of 30 cm²/min. The air/monolayer interface is illuminated with a polarized 658 nm laser beam with an incident angle of ~53° with respect to the surface normal, which corresponds to the Brewster angle of the pure air/PBS interface. The reflected light is collected with a 10x objective and detected with a CCD camera. All measurements are taken at a room temperature of 22±1°C, in a Nima Langmuir trough (maximum area 700 cm^2) with a volume of ~400 ml. The monolayers are prepared as for the SFG measurements.

SIC. Vibrational sum-frequency generation (SFG) spectroscopy

Experimental setup

In our SFG setup a tunable broadband femtosecond infrared pulse is overlapped in space and time with a narrowband visible pulse. Visible pulses at 800 nm (~40 fs duration) wavelength are generated by a regenerative Ti:Sapphire amplifier (Spitfire Ace, Spectra-Physics, USA) with a repetition rate of 1 kHz. The beam is then split and one part is used to pump an optical parametric amplifier system (TOPAS-C, Spectra-Physics, USA) to generate broadband tunable infrared pulses. The other part is spectrally narrowed by an etalon (SLS Optics Ltd.) to a bandwidth of ~15 cm^{-1}. Before the sample the visible and infrared pulses possess pulse energies of ~20 μJ and ~3 μJ respectively. The polarization of both beams is controlled by polarizers and half-wave plates. Finally, the two beams are focused onto the air/PBS interface and the reflected SFG signal is dispersed with a spectrograph (Acton SpectraPro 300i, Princeton Instruments, USA) onto an electron-multiplied charge coupled device camera (Newton EMCCD 971P-BV, Andor Technology Ltd, UK). The experimental stage is flushed with nitrogen to suppress IR-absorption from ambient water during all measurements. The lipid monolayers are prepared in a Langmuir trough that is constantly moving during the measurements to prevent laser-heating-induced displacement of the phospholipids[S1], and has a volume of ~34 ml. All measurements are taken at a room temperature of 22±1°C.

Data treatment

All spectra were taken in ssp-polarization combination (s-SFG, s-VIS and p-IR), are background subtracted and normalized with a non-resonant reference signal from a gold film deposited on a silicon wafer used to account for the IR spectral shape. ssp-polarization combination was chosen as the interpretation of the spectra is more straightforward than in the ppp case and because the signal to noise ratio in sps configuration is so low that spectra with detectable signal in the amide I region could only be recorded for Fbg at the mixed monolayer containing 5 mol% DMPE-PEG2000. ppp SFG spectra in the amide I region of Fbg at mixed

DMPE:DMPE-PEG2000 monolayers with DMPE-PEG2000 content varying from 0 to 5 mol% are plotted in Figure S6.

SII. SUPPORTING MEASUREMENTS

SIIA. Pressure/area isotherms & BAM

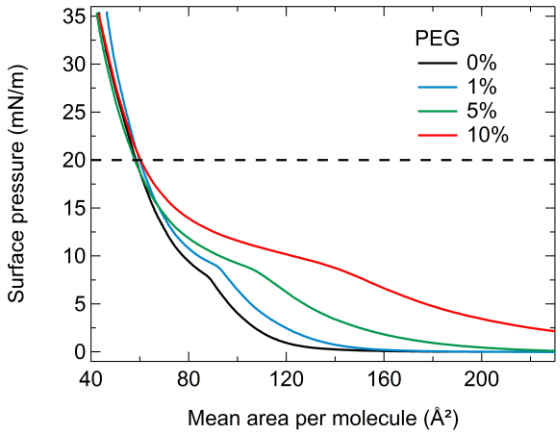

Fig. S1. Monolayer compression (π-A) isotherms measured at 22±1°C of the mixed DMPE:DMPE-PEG2000 monolayers with DMPE-PEG2000 content varying from 0 to 10 mol%.

Figure S1 shows the monolayer compression isotherms of the different DMPE:DMPE-PEG2000 mixtures. The changes in the isotherms with increasing PEG content is comparable to reported results for other mixed monolayer systems with PEGylated phospholipids.[S2–4] As can be seen from the figure, at high surface pressures > 20 mN/m all mixed monolayers behave almost identical as the pure DMPE monolayer, which indicates that the PEG chains are completely submerged into the buffered subphase. At a surface pressure of 20 mN/m, all monolayers are in the liquid-condensed phase. Studies using a mixed monolayer using a very similar phospholipid, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE),[S2,S3] found that by changing the PEG concentration in the monolayers from 1 to 10 mol% the PEG chains are expected to change from mushroom to brush conformation, so we can expect the same is happening in our study.

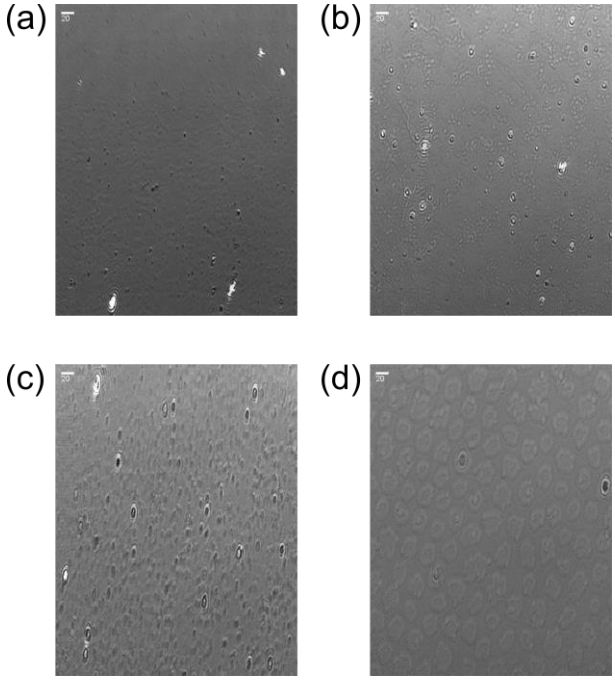

Fig. S2. BAM images of different mixed DMPE:DMPE-PEG2000 monolayers with increasing PEG concentration: (a) 0, (b) 1, (c) 5 and (d) 10 mol%. All images are taken at a surface pressure of 20 mN/m. The scalebar size is 20 μm and the sensitivity of the detecting camera was automatically adjusted: For this reason, the intensities in between the different images are not comparable.

In Figure S2 BAM images of the different mixed phospholipid monolayers with DMPE-PEG2000 concentrations from 0 to 10 mol% are shown. The images are taken at a surface pressure of 20 mN/m, as in the SFG measurements. The BAM images show that as the DMPE-PEG2000 content in the monolayer increases the monolayer's roughness increases in agreement with what reported in literature on similar systems.[S5]

SIIB. SFG

Figure S3 shows the SFG spectra in the amide I region before (open symbols) and after (filled symbols) injection of BSA into the subphase of the DMPE monolayer. This is a rescaled Figure derived from the data also shown in Figure 3. As can be seen a new peak centered at 1660 cm^{-1} appears after injection of BSA, which indicates the presence of the protein at the surface.

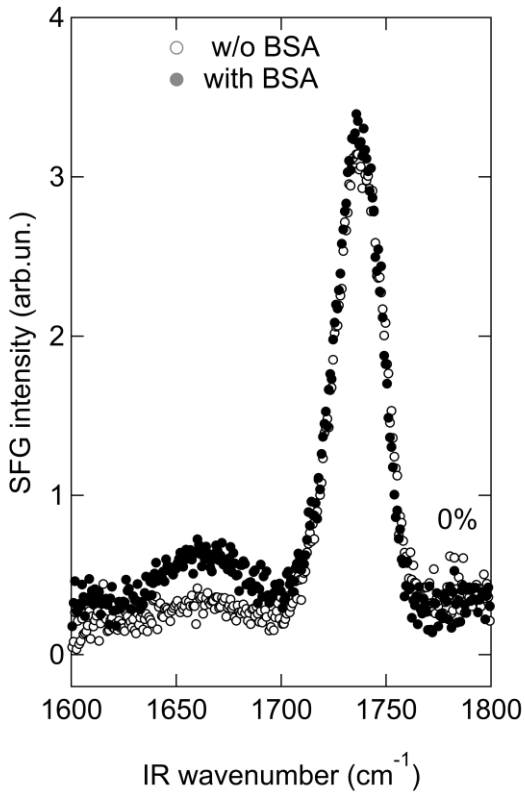

Fig. S3. SFG spectra in the amide I region before (open symbols) and after (filled symbols) injection of BSA at the DMPE monolayer. Data replotted from Figure 3a.

In our raw spectra a broad (~1580-1800 cm^{-1}) and very weak SFG signal is always present, which is generated by the water bending mode. The spectrum is shown in Figure S4. In order to get rid of this background signal, the SFG spectrum of the plain air/PBS interface was subtracted from the all spectra (with and without proteins). However, this contribution is still visible in some measured spectra, since our background subtraction method possibly underestimates the contribution of more ordered water at the mixed air/monolayer interface compared to the plain air/PBS interface.

Furthermore, all shown spectra are normalized on the IR spectral shape after subtraction of the background signal. The weak peak in the amide I region appearing in some of the measured spectra could also be an artifact from that normalization, which can overcompensate the water absorption, since the residual environmental humidity can slightly change from measurement to measurement.

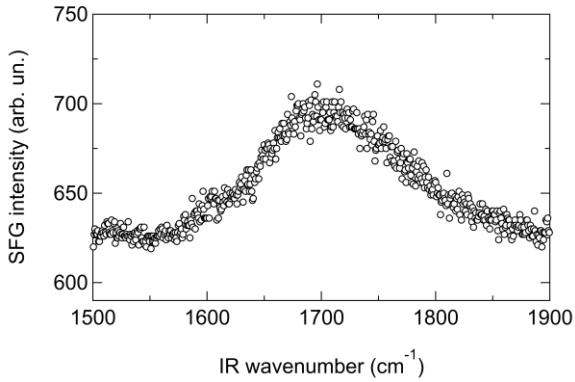

Fig. S4. SFG spectrum of the air/PBS interface in the amide I range. The measurement was done in ssp polarization combination with an integration time of 10 minutes.

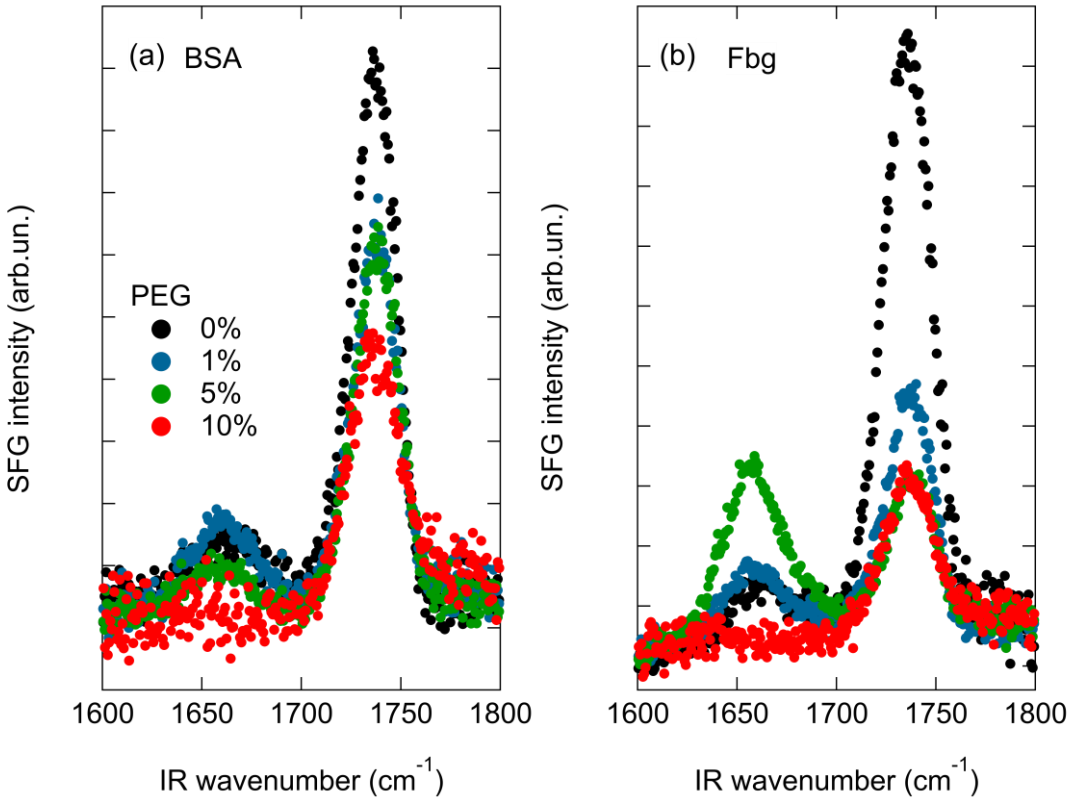

Fig. S5. SFG spectra in the amide I region of (a) BSA and (b) Fbg at mixed DMPE:DMPE-PEG2000 monolayers with DMPE-PEG2000 content varying from 0 to 10 mol%. Data plotted from Figure 3.

The SFG spectra in Figure S5 for the monolayers in presence of proteins show that the shape of the protein signal in the amide I region, does not change changing the DMPE-PEG2000 content in the mixed monolayers. Therefore we conclude that the protein secondary structure is not dramatically changed with increasing DMPE-PEG2000 content.

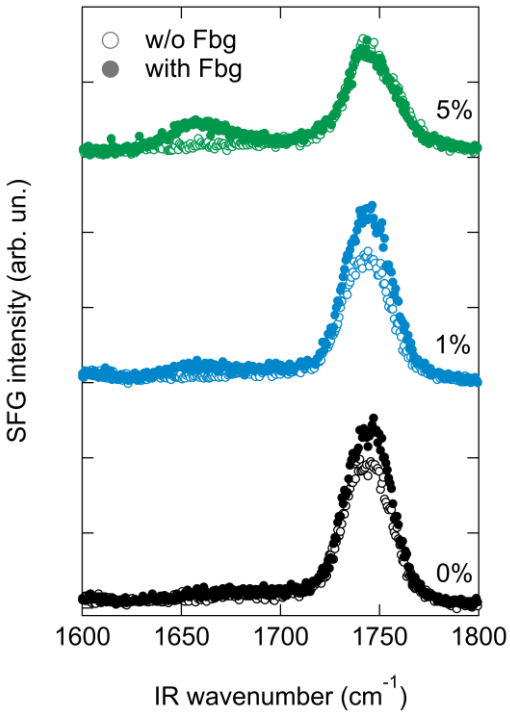

Fig. S6. SFG spectra in ppp polarization combination in the amide I region of Fbg at mixed DMPE:DMPE-PEG2000 monolayers with DMPE-PEG2000 content varying from 0 to 5 mol%.

Figure S6 depicts SFG spectra in ppp polarization combination for different mixed DMPE:DMPE-PEG2000 monolayers in presence of Fbg at the surface. As in the case of ssp polarization combination, the intensity of the protein amide I signal increases with increasing DMPE-PEG2000 concentration in the monolayer. In ppp polarization combination several $\chi^{(2)}$ tensor elements are probed simultaneously (see Table S1) and can interfere with each other making the interpretation of measurement in this polarization combination less straightforward.

Table S1. $\chi^{(2)}$ tensor elements contributing to the SFG spectra for different polarization combinations for an azimuthally isotropic surface.

Polarization combination (SFG, VIS, IR)	**Probed $\chi^{(2)}$ elements (x≡y ∥ surface, z ⊥ surface)**
ssp	$\chi^{(2)}_{xxz}$
ppp	$\chi^{(2)}_{zzz}, \chi^{(2)}_{zxx}, \chi^{(2)}_{xzx}, \chi^{(2)}_{xxz}$

SIII. Calculation of vibrational SFG spectra

The spectral vibrational SFG calculations have been performed based on the formalism described in Ref. [S6] Briefly, the atom coordinates were obtained from the PDB files, from which the one-exciton Hamiltonians were constructed. Then, after diagonalization the eigenvalues and eigenvectors of the systems were obtained, from which their spectroscopic responses were calculated. The couplings in the Hamiltonians were determined (I) for nearest neighbors using a parametrized map of an *ab initio* calculation of a glycine dipeptide (Ac-Gly-$NHCH_3$) with the 6-31G+(d) basis set and B3LYP-functional, that gives the coupling as a function of the dihedral angle, and (II) for non-nearest neighbors using the transition dipole coupling (TDC) model that gives the through-space coupling in a Coulomb-like fashion. The rationale behind this is that the neighbor coupling is dominated by through-bond effects, while non-nearest neighbors couple mainly through space. The local amide I mode frequencies of the amide groups are redshifted according to the same empirical hydrogen-bond shift model used in Ref. [S7], which is a more detailed version of the model described in Ref. [S8] as described in Ref [S6], however with 20% smaller hydrogen bond length – redshift ratios.

The vibrational SFG spectrum is generally very sensitive for the orientation of the protein. In order to reproducibly define the molecular axis of the proteins, we aligned them according to their moments of inertia, using the *orient* script in VMD (see Figures S7 b and c).[S9] We found that the experimental spectra of Fbg could be reproduced well with $\Theta = 5.9°$, with Φ and Ψ averaged from 0 to 2π due to the azimuthal isotropy (Θ, Φ and Ψ being the Euler angles as usually defined, see Fig. S7 a) and also Ref. [S6]). The Θ-angle of the lipid C=O-mode was set to 64° in accordance with IRRAS measurements on similar phospholipids.[S10] The fitted width of the Lorentzians was 5.1 cm^{-1} for the protein normal modes and 6.3 cm^{-1} for the lipid C=O groups, while the gas phase frequency of the amide I local modes was found to be 1678 cm^{-1}. For BSA the azimuthal isotropy seems less pronounced, so we fitted the data

without averaging Φ. The experimental BSA spectra could be reproduced well with a rather broad orientation distribution centered around (Θ,Φ) = (~100°, ~210.0°). The broad distribution around these values, probably a result of the more-globular structure of BSA compared to Fbg, is reflected in the much larger width of the normal modes of the protein (15.7 cm^{-1}, as compared to 5.1 cm^{-1} for Fbg); all other parameters remained the same as for Fbg.

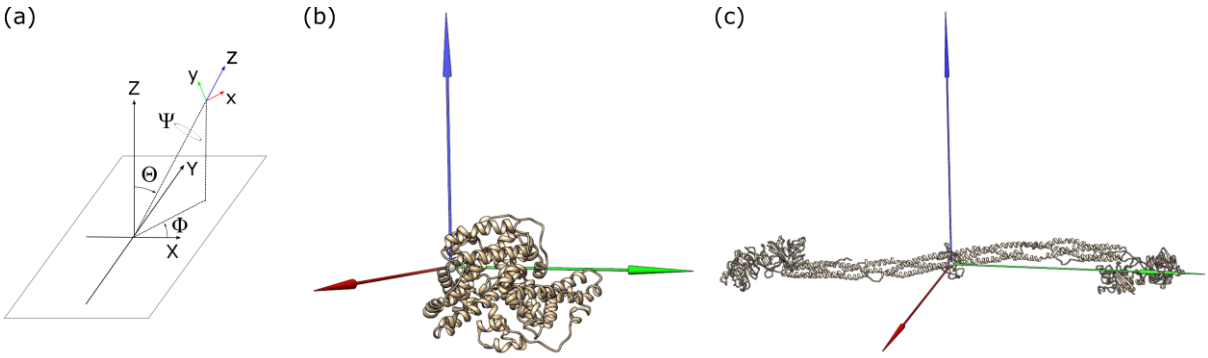

Fig. S7. (a) Definition of the molecular axis and the Euler angles (Θ, Φ, Ψ), orientation of (b) BSA (Θ,Φ) = (96.7°, 213.0°) and (c) Fbg in the molecular frame (Θ = 5.9°, Φ is averaged from 0 to 2π). Red, green and blue represent the x, y and z-axes respectively. The z-axis is parallel to the surface normal in the lab frame.

References

S1 E. H. G. Backus, D. Bonn, S. Cantin, S. Roke and M. Bonn, Laser-Heating-Induced Displacement of Surfactants on the Water Surface, *J. Phys. Chem. B*, 2012, **116**, 2703–2712.

S2 T. L. Kuhl, D. E. Leckband, D. D. Lasic and J. N. Israelachvili, Modulation of interaction forces between bilayers exposing short-chained ethylene oxide headgroups, *Biophys. J.*, 1994, **66**, 1479–1488.

S3 J. Majewski, T. L. Kuhl, M. C. Gerstenberg, J. N. Israelachvili and G. S. Smith, Structure of Phospholipid Monolayers Containing Poly(ethylene glycol) Lipids at the Air−Water Interface, *J. Phys. Chem. B*, 1997, **101**, 3122–3129.

S4 C. Ohe, Y. Goto, M. Noi, M. Arai, H. Kamijo and K. Itoh, Sum Frequency Generation Spectroscopic Studies on Phase Transitions of Phospholipid Monolayers Containing Poly(ethylene oxide) Lipids at the Air−Water Interface, *J. Phys. Chem. B*, 2007, **111**, 1693–1700.

S5 M. N. Shahid and V. Tsoukanova, Effect of Saline on Transitions in Poly(ethylene glycol)-Grafted Succinyl-Phosphoethanolamine Monolayers Bearing C 16 Aliphatic Chains, *J. Phys. Chem. B*, 2011, **115**, 3303–3314.

S6 S. J. Roeters, C. N. Van Dijk, A. Torres-Knoop, E. H. G. Backus, R. K. Campen, M. Bonn and S. Woutersen, Determining in situ protein conformation and orientation from the amide-I sum-frequency generation spectrum: Theory and experiment, *J. Phys. Chem. A*, 2013, **117**, 6311–6322.

S7 K. Meister, S. J. Roeters, A. Paananen, S. Woutersen, J. Versluis, G. R. Szilvay and H. J. Bakker, Observation of pH-Induced Protein Reorientation at the Water Surface, *J. Phys. Chem. Lett.*, 2017, **8**, 1772–1776.

S8 P. Hamm, M. Lim, W. F. DeGrado and R. M. Hochstrasser, The two-dimensional IR nonlinear spectroscopy of a cyclic penta-peptide in relation to its three-dimensional structure, *Proc. Natl. Acad. Sci.*, 1999, **96**, 2036–2041.

S9 W. Humphrey, A. Dalke and K. Schulten, VMD: Visual molecular dynamics, *J. Mol. Graph.*, 1996, **14**, 33–38.

S10 A. Gericke, C. R. Flach and R. Mendelsohn, Structure and orientation of lung surfactant SP-C and L-alpha-dipalmitoylphosphatidylcholine in aqueous monolayers, *Biophys. J.*, 1997, **73**, 492–499.