

Supporting Information

Charge Controlled Surface Properties of Native and Fluorophore Labeled Bovine Serum Albumin at the Air-Water Interface

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Experimental set-up for vibrational sum-frequency generation

Münster Ultra-fast Spectrometer for Interfacial Chemistry (MUSIC) uses a Spectra-Physics Solstice-Ace Amplifier which is seeded by a fs-oscillator centered at a wavelength of 794 nm and a bandwidth of 22 nm (Spectra Physics Mai Tai SP, 84 MHz, 770 mW) and pumped Q-switched laser (Ascend 60, 1 kHz, >35 W). The Solstice-Ace regenerative amplifier delivers approximately 7 W (1 kHz, at 795 nm) average power, which is divided (50:50) by an internal beam splitter and subsequently guided into an internal and an external compressor. The former (3.2 W, 796 nm and 18 nm bandwidth) pumps the optical parametric amplifier (Light conversion, TOPAS Prime) with the NDFG non-collinear configuration to generate broadband femtosecond IR pulses, which are tunable from 2.7 to 20 μm . The broadband IR has approximately 300 cm^{-1} full-width at half-maximum bandwidth. An air-spaced etalon (SLS Optics LTD, FSR 12.4 nm at 735 nm, R=94.5%) was inserted into the external compressor to generate the VIS narrowband pulse centered at 804.1 nm and 4 cm^{-1} bandwidth. Etalon side bands are removed by beam blocks, which are also placed inside the external compressor.

For the generation of the SFG, the VIS and the IR pulses overlap in time and space at 55° and 60° incident angles, respectively. The mean circularized beam diameters ($1/e^2$) were 530 μm and 260 μm for the VIS and IR beams. The SFG photons were directed into a spectrograph (Kymera-328i-D2-SIL, Andor) with electron multiplied charge-couple device EMCCD (Andor Newton, Du97P-BVF). A short pass filter with cut-off at 763 nm (AHF, F76-789) was used to filter the visible beam. For the liquid-gas interface, all the spectra were recorded using s-polarized SF, s-polarized visible and p-polarized IR (*ssp*) beams.

Influence of the phase change of O-H stretching vibrations on the aromatic C-H band at 3060 cm^{-1}

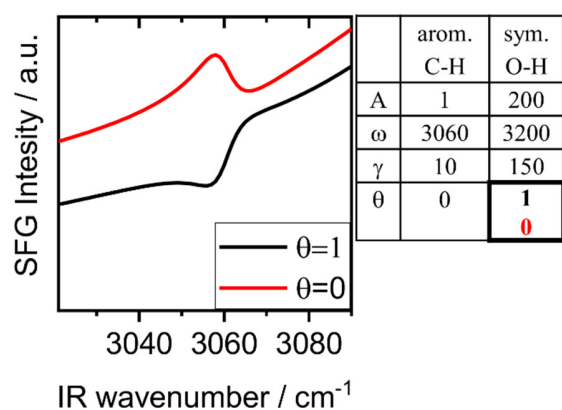


Figure S1. Example of the interference between aromatic C-H stretching band at 3060 cm^{-1} and the broad O-H stretching band centered at 3200 cm^{-1} for two different phases of the O-H band. Note that the phase is directly related to the net orientation of water molecules at the interface. Simulation of the spectra was done according to equation (2) in the main text. $\theta=0$ mimics the situation of BSA molecules at a pH below the IEP (positive net charge and waters' oxygen pointing towards the interface), while $\theta=1$ reflects the situation above the IEP where the waters' hydrogens point towards the interface (negative net charge).

Fluorescein isothiocyanate (FITC) labeled bovine serum albumin (BSA)

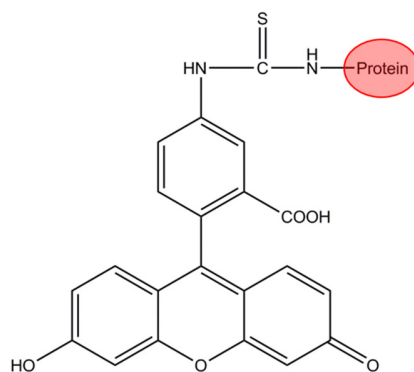


Figure S2. Covalent thiourea bond of FITC at the ϵ -amino group of lysin amino acid residues in BSA.

Dynamic surface tension

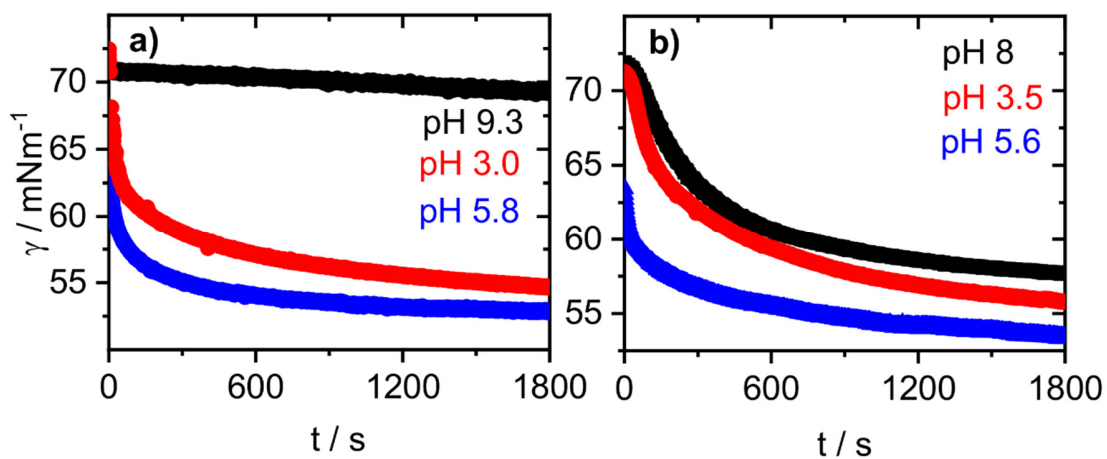


Figure S3. Dynamic surface tension at chosen pH values of a) 50 μM BSA-FITC and b) 50 μM BSA solution.

Foam stability and drainage

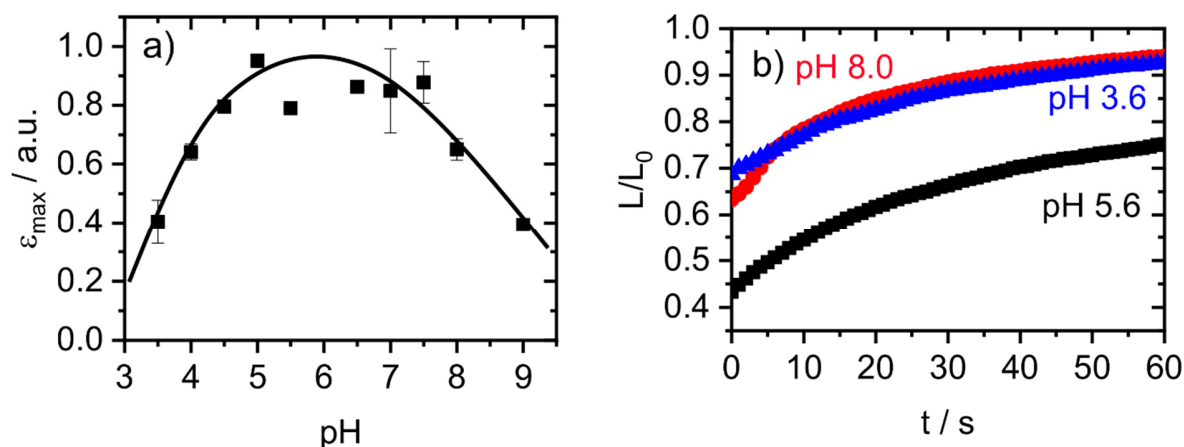


Figure S4. a) Maximum liquid fraction ϵ directly after foaming. b) Drainage curves: liquid level L over the liquid height L_0 before foaming was started. In b) we show a comparison between different pH values higher, smaller and near the bulk IEP (pH 5.3, see main text).

Thin films

BSA foam films were further studied by the thin-film-pressure balance technique. The foam thin-films were produced in a 2 mm hole of a porous glass plate, which is fused to a glass capillary tube under a gas-tight pressure cell with two CaF_2 windows. This chamber is placed under a microscope (Zeiss, Imager.A2.AXIO) coupled to a camera (Nikon D5300). The chamber is equipped with a reservoir filled up with the sample solution to be able to immerse the frit again in case of film rupture, as well as providing constant vapour pressure into the chamber. The pressure is controlled by a syringe pump (Legato210, kdScientific) and measured by a pressure transducer (A-10, 0-50 mbar, WIKA Germany). Further explanation of this technique can be found in the literature (Gochev, G., Retzlaff, I., Exerowa, D., Miller, R., *Coll. Surf. A* **2014**, 460, 272–279).

Films around the IEP (pH 5.4) are stable for about one hour also at significantly higher disjoining pressures without further drainage. Below and above the IEP, the films drained substantially, even at low pressures. Parts of the foam films appeared as thin black films (films are so thin that all the light can be transmitted). At these pH values they ruptured before reaching an equilibrium. Aggregates were found at all pH values and therefore in a broad pH region and not only at the IEP.

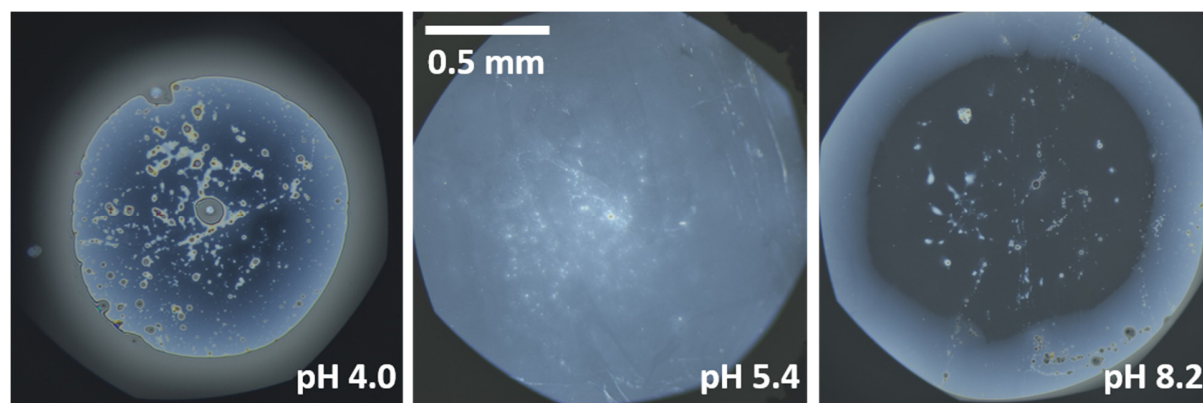


Figure S5. Micrographs of single foam films taken with a thin film pressure balance at different pH values under, close to and above the IEP of BSA ($c=50\mu\text{M}$).