

Supporting Information to:

β -Lactoglobulin adsorption layers at the water/air surface: 5. Adsorption isotherm and equation of state revisited, impact of pH

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Non-linear dilational surface rheology

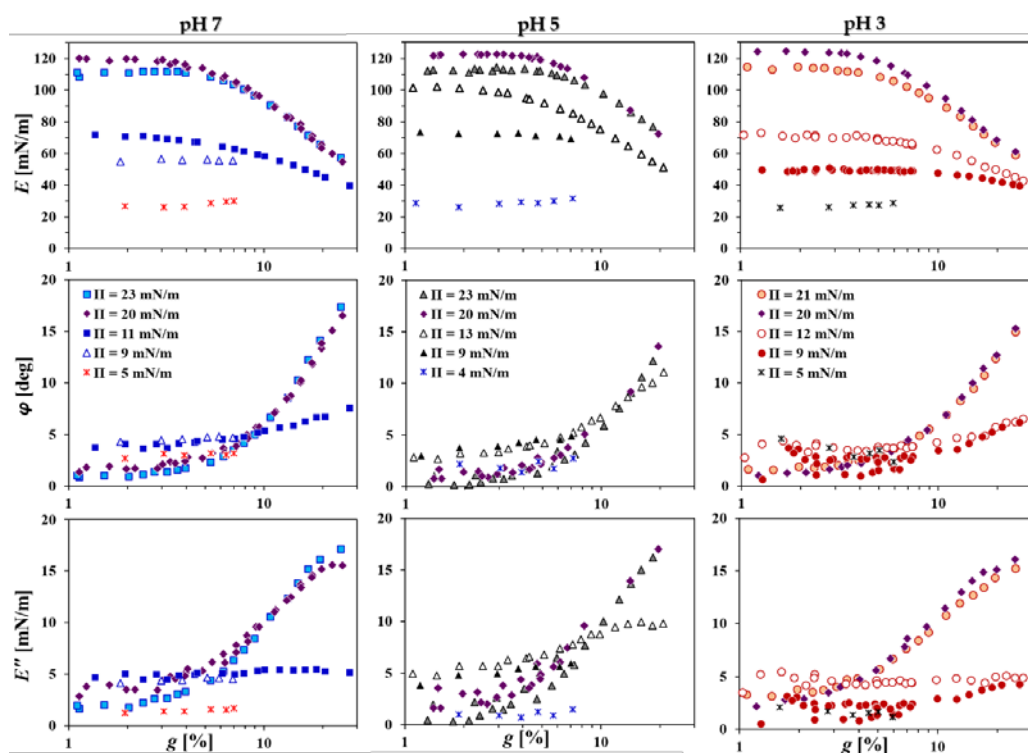
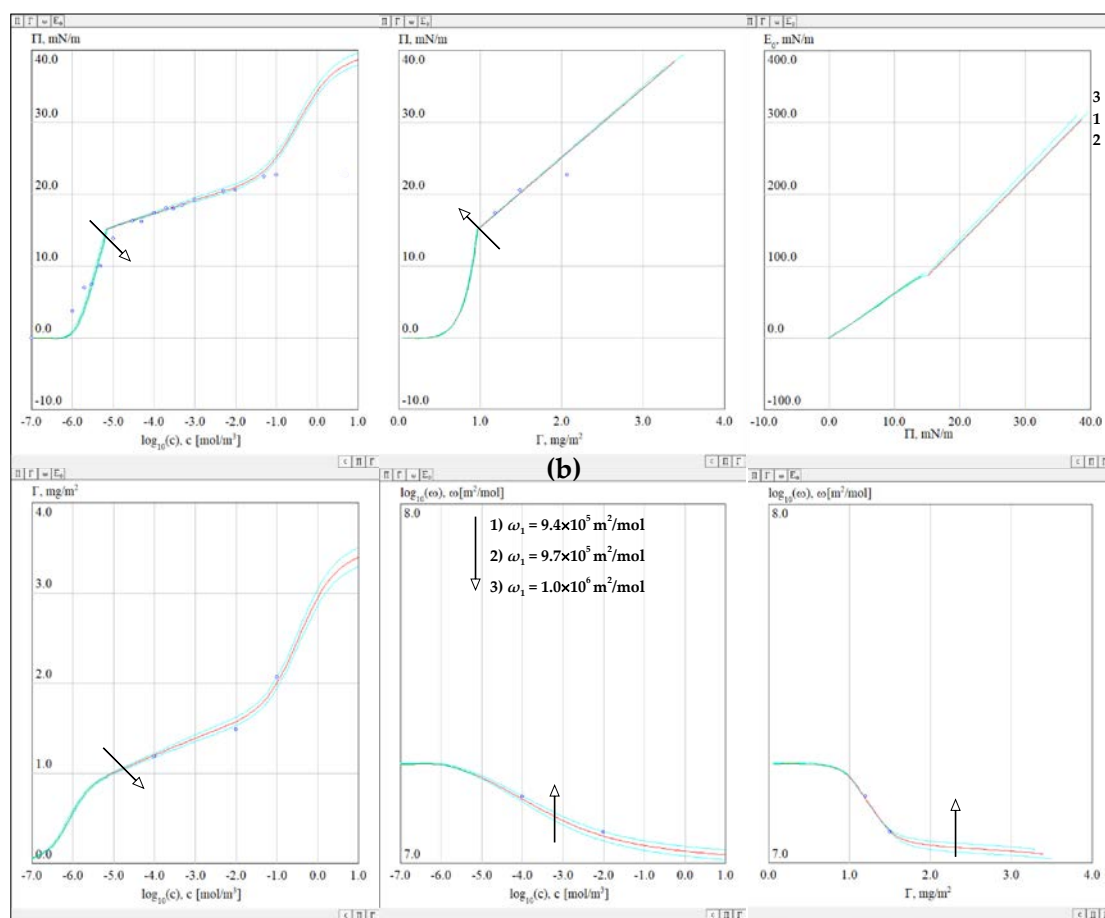
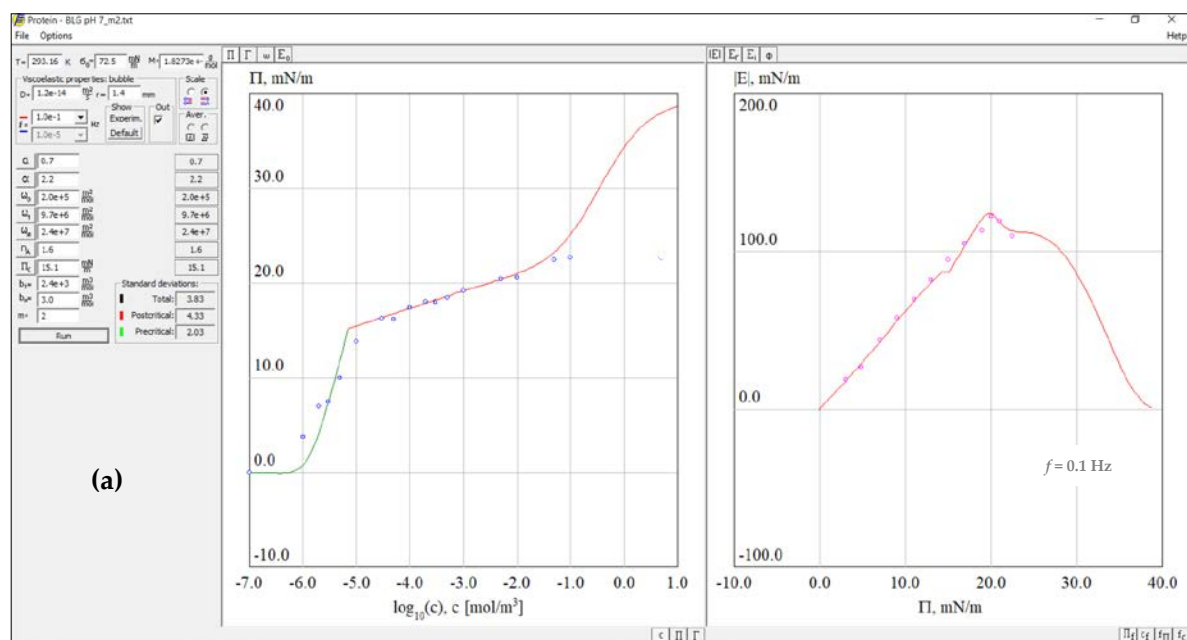


Figure S1. Dilational rheology parameters: E (complex viscoelasticity modulus) and φ (phase angle), and the corresponding E'' (imaginary part of E) as a function of the amplitude g of oscillating area deformation at various surface pressures Π and at constant oscillation frequency of $f = 0.1$ Hz.

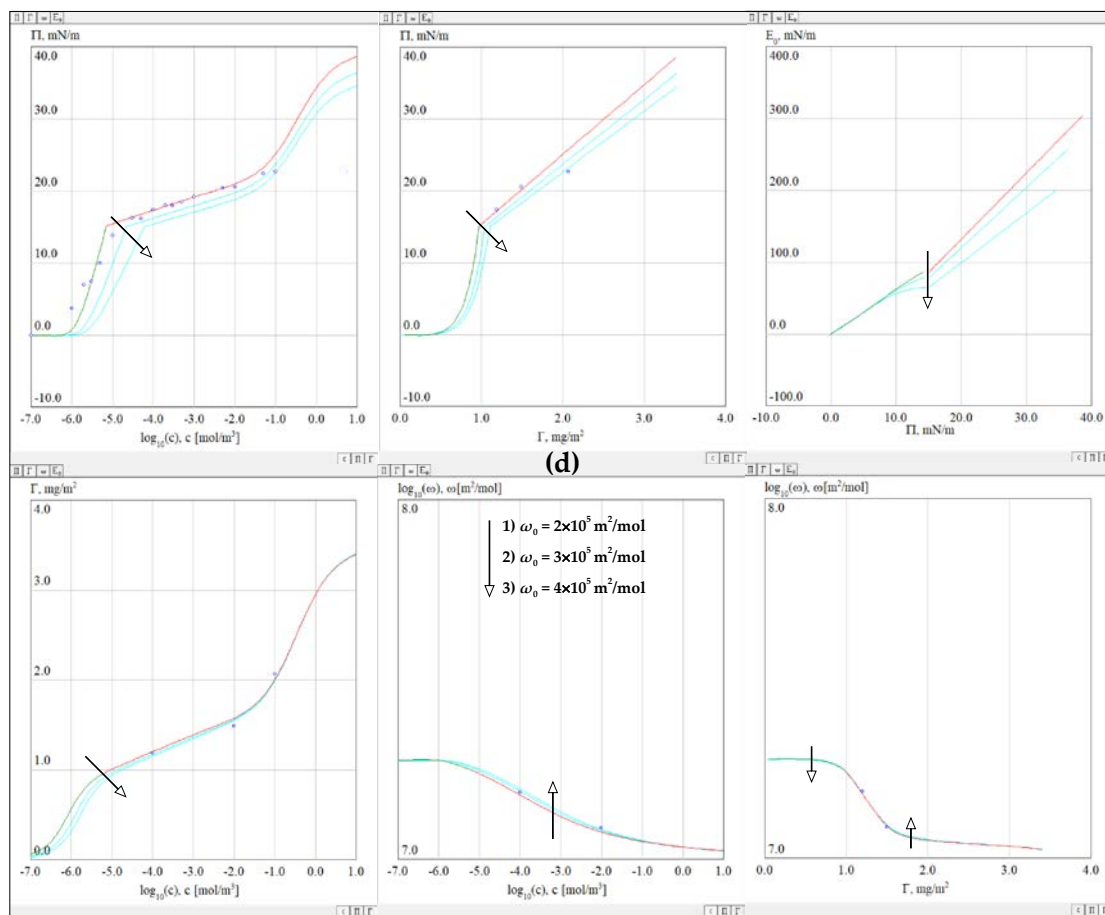
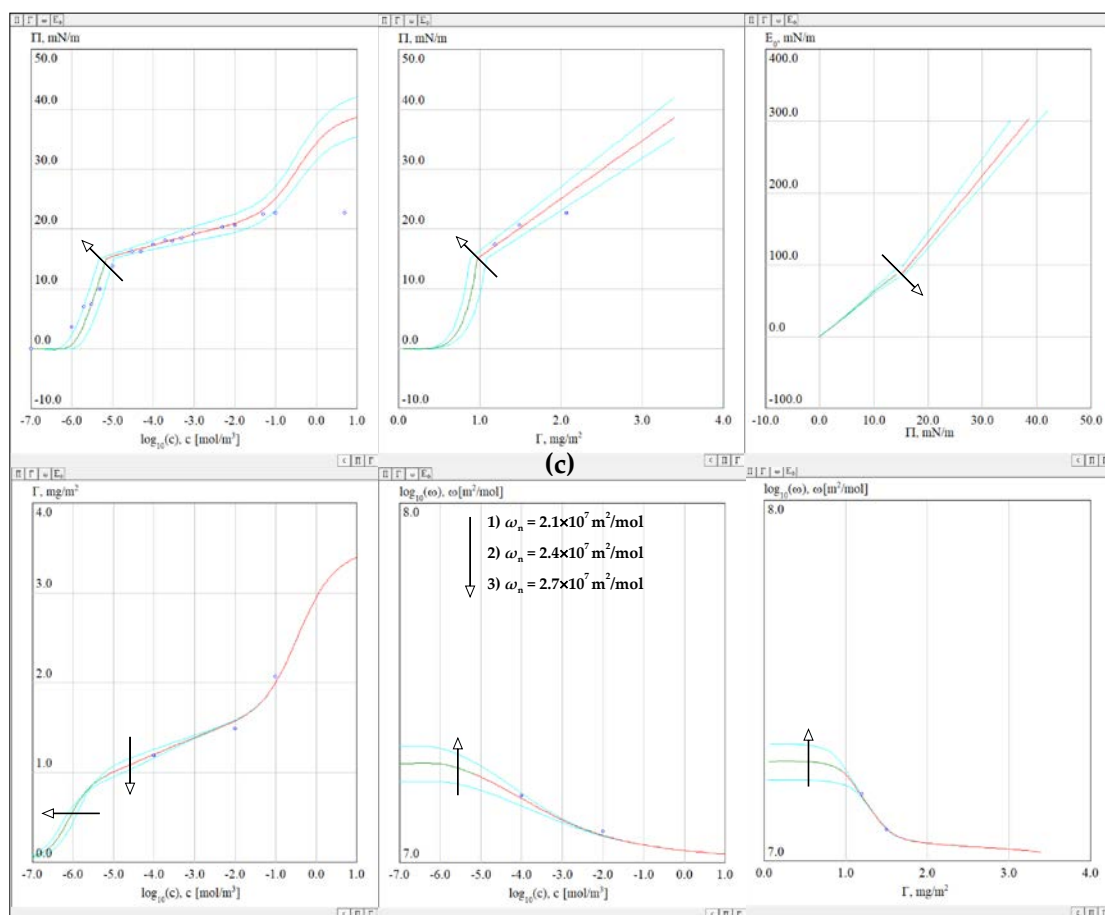
Amplitude sweeps ($g = 1\text{--}30\%$) at constant frequency of $f = 0.1$ Hz were applied always at the end of a given experiment (after ≈ 22.2 hours of adsorption), because such large area deformations were found to cause strong disturbances/destructions of the BLG layers and any surface rheometry measurements after that showed atypically low E -values. In few cases, narrower amplitude sweeps ($g = 1\text{--}7\%$, $f = 0.1$ Hz) were applied in the course of adsorption layer formation.

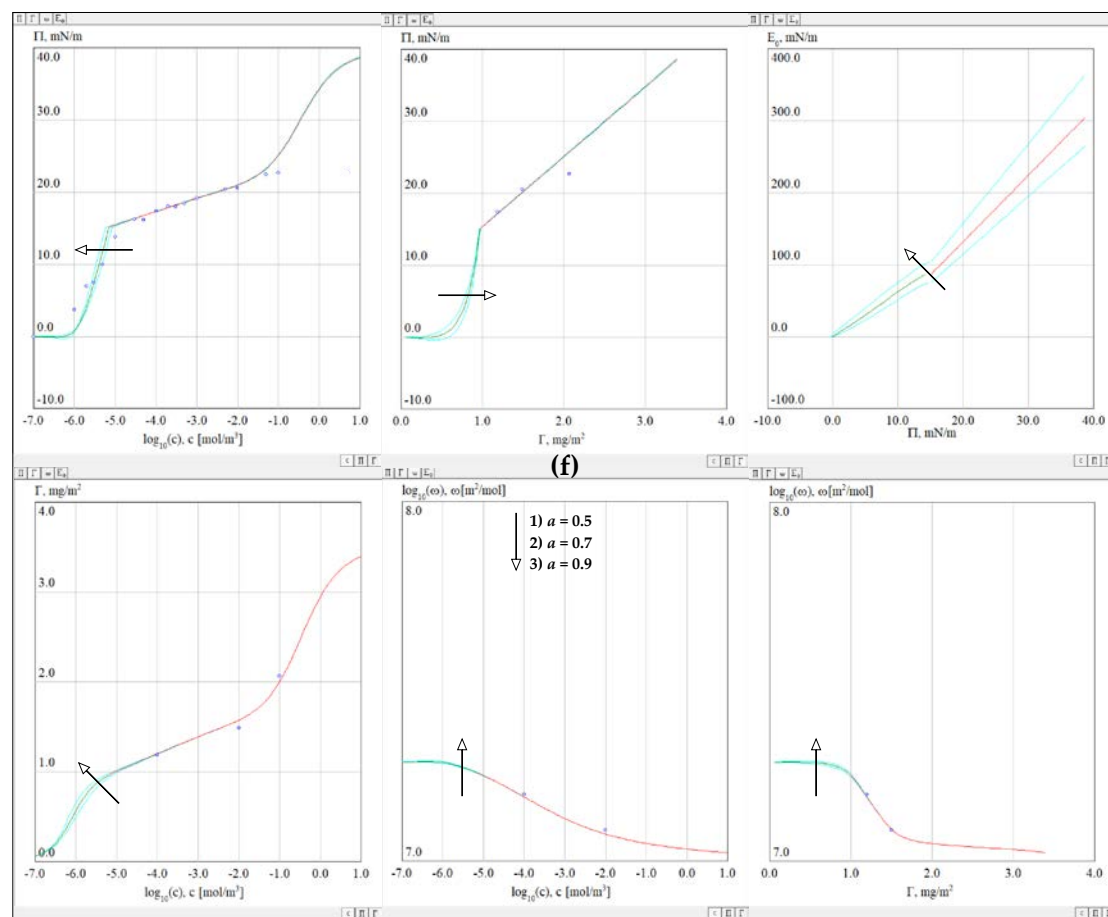
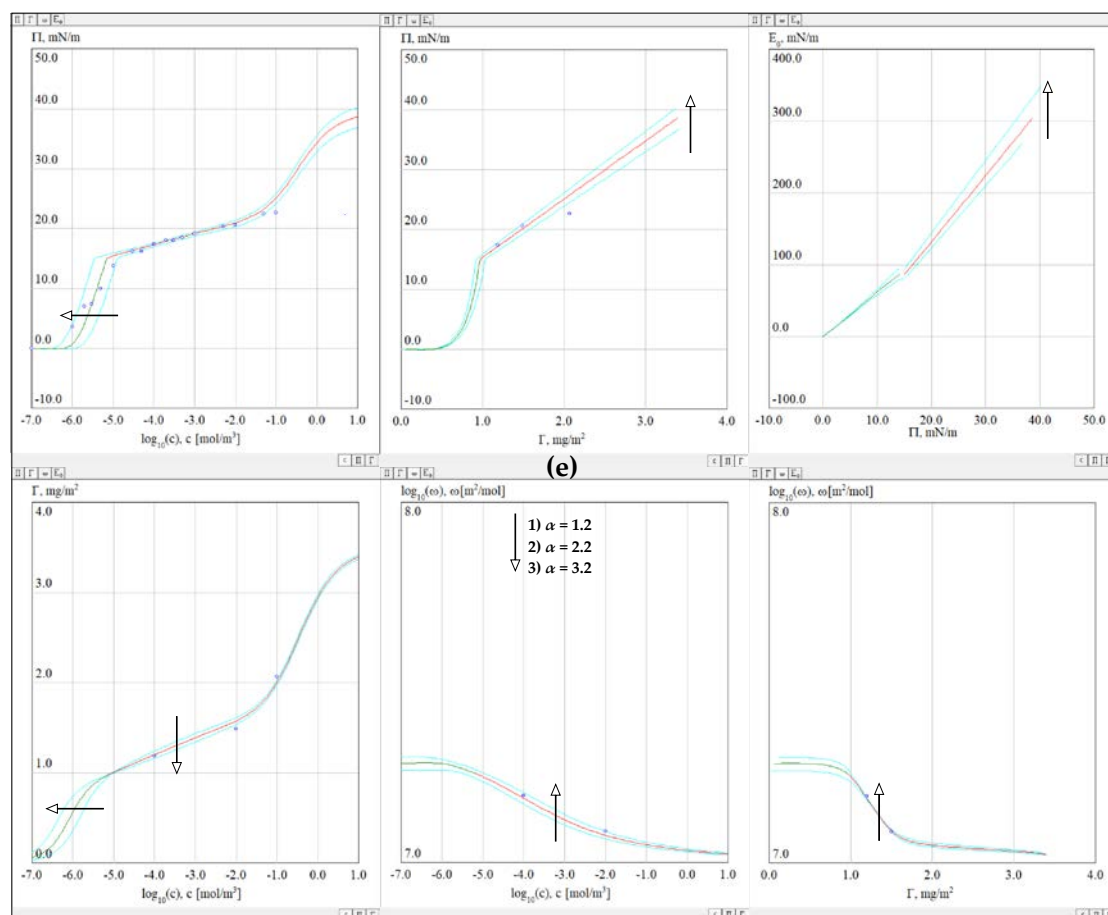
Model simulations

Below in Fig. S2 we show original screenshots of the interactive software tool^a for the fitting process of the theoretical model to the experimental data at pH 7. The effects of variations of the values of some model parameters on the simulation results are illustrated.



^a <http://www.thomascatt.info/Scientific/adso/adso.htm>





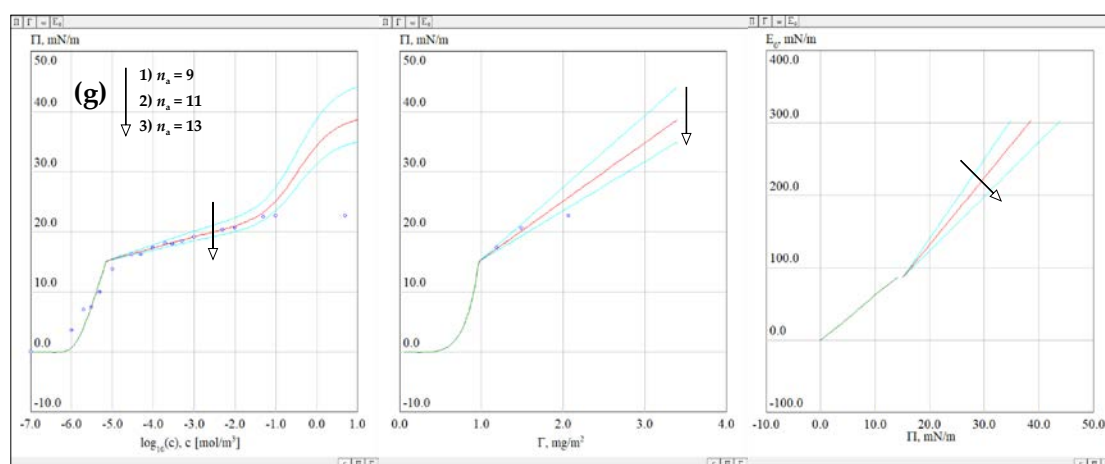


Figure S2. Exemplary screenshots of the interactive software for fitting the theoretical model to experimental data (pH 7). The symbols ($\circ$) are experimental data and the lines are best m2-fits; where the model predictions are presented by green and red lines, the colors indicate the pre-critical (green) and the post-critical (red) ranges divided by the critical parameters Π^* , ω^* , Γ^* and E_0^* .

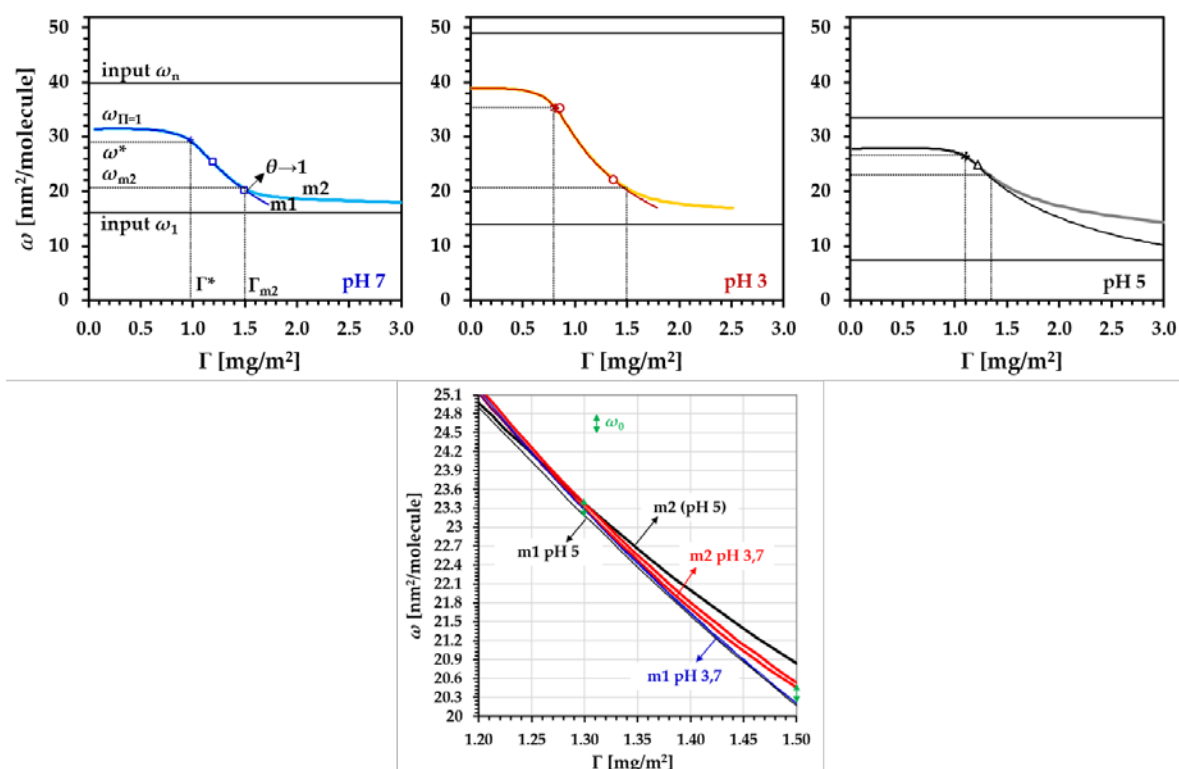


Figure S3. Model simulations of $\omega(\Gamma)$ dependencies by *one-layer* (m1) and *two-layer* (m2) fits. **(top)** The horizontal solid lines are the input (boundary) values for ω_1 and ω_n in the calculation procedure. The horizontal and vertical dotted lines indicate the critical points at coordinates (ω^*, Γ^*) , and the splitting points at coordinates $(\omega_{m2}, \Gamma_{m2})$ at full saturation of the primary monolayer ($\theta \rightarrow 1$). **(bottom)** a zoom-in portion of the $\omega(\Gamma)$ dependencies illustrating the determination of Γ_{m2} ; $\omega_0 \approx 0.33 \text{ nm}^2$ is the area increment used in the calculation procedures for all pH values.

In Figure S2 selected screenshots are presented to demonstrate the strategy for obtaining the optimum parameter values. Note, the number of parameters is very large, however, their values cannot be chosen arbitrarily because of their physical meaning. The sub-groups are screenshots and show the following situations: S2-a) panel of model parameters, the physical meaning of which are explained in the main text. The two graphs show the dependencies of $\Pi(c)$ and $|E|(\Pi)$; the small circles are the experimental points while the continuous lines refer to the calculated dependencies:

S2-b) six different dependencies are shown to show their sensitivity for three different values of the parameter ω_1 ; S2-c) the same six dependencies as in b) are shown for three different values of ω_n ; S2-d) the same six dependencies as in b) are shown here for three different values of ω_0 ; in S2-e) and S2-f) again the six dependencies are shown in order to demonstrate how changes in the parameter values for α and a are obtained; S2-g) the three graphs show how changes in the aggregation number n_a influence the dependencies of $\Pi(c)$; $\Pi(\Gamma)$ and $E_0(\Pi)$.

The $\omega(\Gamma)$ data in Figure S3 are plotted in full length as calculated by the model. For each pH, the curves from m1- and m2-fits run together up to a splitting point at coordinates $(\omega_{m2}, \Gamma_{m2})$. The prolongation of the curves beyond Γ_{m2} (monolayer saturation) correspond to an increase of Γ due to the formation of the secondary layer, however, the parallel decrease of the molar area $\omega > \omega_{m2}$ is physically meaningless.

In Figure S4 are shown the frequency dependencies of the reduced viscoelasticity modulus E/E_0 and of the imaginary part E'' of the modulus as calculated by eqs. (8,9) with a *one-layer* (m1) model with the parameters' values listed in Table 1 (main text) and a diffusion coefficient $D_{\text{intr}} = 1 \times 10^{-10} \text{ m}^2/\text{s}$, which is weakly dependent on pH (3–7) [1–3]. The values for E'' , measured after ≈ 22.2 hours of adsorption, for all BLG concentrations and pHs used in this study are lower than 10 mN/m.

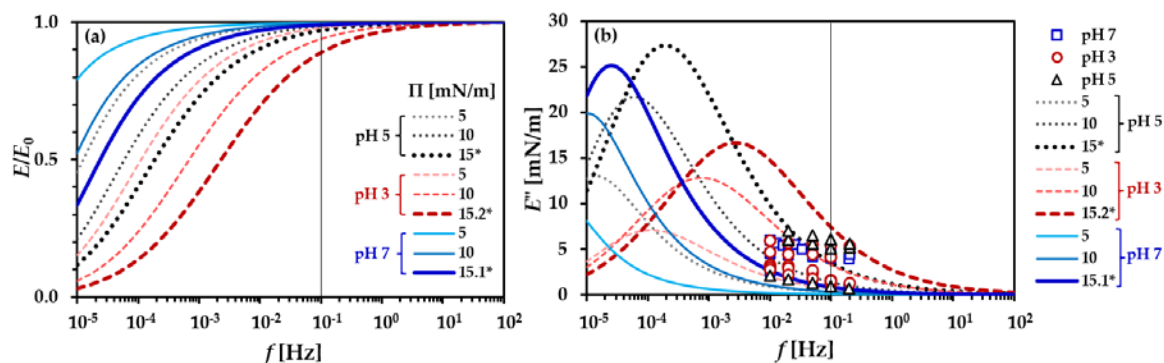


Figure S4. Computed values for the (a) $(E/E_0)(f)$, and (b) $E''(f)$ dependencies for three surface pressures in the pre-critical region including Π^* . Symbols in (b) are experimental data at few surface pressures $\Pi < \Pi^*$. Asterisks denote values at Π^* .

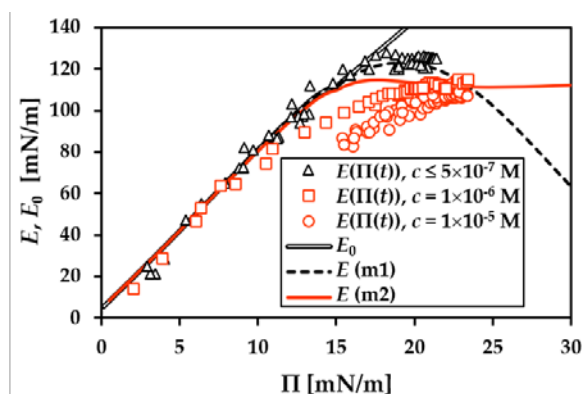


Figure S5. Experimental (symbols) and computed (lines) dependencies of the dilational complex viscoelasticity modulus $E(\Pi)_{fg}$, $f = 0.1 \text{ Hz}$, $g \approx 2.7 \%$; the computed dependency of the limiting elasticity $E_0(\Pi)$ is also given for comparison.

Model for the computation of thermodynamic properties of proteins

Extended model description

The model essentially assumes that the adsorption occurs with the formation of multiple layers. At the same time, the adsorption in the first layer (the one closest to the interface) governs the

behavior of subsequent layers as explained further below. In what follows, the superscript refers to the adsorption layer.

The model assumes a discrete spectrum of n adsorbed states ($1 \leq j \leq n$) for a protein molecule, where the average molar area ω is distributed in the range between the boundary values: ω_1 , which corresponds to state 1 with a minimum area, and ω_n , which corresponds to the n^{th} state with a maximum area. At an intermediate state j the partial molar area is $\omega_j = \omega_1 + (j - 1)\omega_0$, where ω_0 is an area increment taken to be in the order of the area per adsorbed water molecule. The average molar area, ω , is determined via the total coverage of the first layer θ , the partial adsorption in j^{th} state in the first layer $\Gamma_j^{(1)}$ and the total adsorption of molecules in the first layer $\Gamma^{(1)}$:

$$\theta = \omega \Gamma^{(1)} = \sum_{j=1}^n \omega_j \Gamma_j^{(1)}, \quad \Gamma^{(1)} = \sum_{j=1}^n \Gamma_j^{(1)}. \quad (\text{S.1})$$

The adsorption isotherm equation for each j^{th} adsorbed state in the first adsorption layer reads:

$$b_j^{(1)} c = \frac{\omega \Gamma_j^{(1)}}{(1-\theta)^{\omega_j/\omega}} \exp \left[-2a \frac{\omega_j}{\omega} \theta \right], \quad (\text{S.2})$$

where $b_j^{(1)}$ is the adsorption equilibrium parameter for the protein molecules adsorbed in the j^{th} state, and a is a Frumkin-type interaction parameter ($a > 0$ means intermolecular attraction), which accounts for the enthalpic non-ideality. Therefore, the properties of the adsorbed system are seen to be dependent, among others, on n values of the adsorption equilibrium parameter, which makes the problem too complicated to be treated. To simplify the situation, it was proposed in [4,5] to introduce the approximation that the surface activity of protein molecules adsorbed in a particular state j depends on the partial molar area, ω_j , according to a power law with a constant exponent, α :

$$b_j^{(1)} = \left(\omega_j / \omega_1 \right)^\alpha b_1^{(1)}. \quad (\text{S.3})$$

This means that setting $\alpha > 0$ implies that the adsorption of molecules in states with larger molar areas is favored [6,7]. Then, combining eqs. (S.2) and (S.3) one obtains:

$$b_1^{(1)} c = \frac{\omega \Gamma_j^{(1)}}{(\omega_j / \omega_1)^\alpha (1-\theta)^{\omega_j/\omega}} \exp \left[-2a \frac{\omega_j}{\omega} \theta \right]. \quad (\text{S.4})$$

The value b_1 in the left-hand side is the adsorption equilibrium constant for the protein molecules adsorbed in the first layer in the state '1'. Noting that the left-hand side of eq. (S.2) taken at $j = 1$ is equal to the left-hand side of eq. (S.4), one can equate the right-hand side of eq. (S.2) taken at $j = 1$ to the right-hand side of eq. (S.4) to obtain the expression for the adsorption of molecules in the first layer in any j^{th} state via their adsorption in the state '1':

$$\begin{aligned} \Gamma_j^{(1)} &= \Gamma_1^{(1)} \left(\frac{\omega_j}{\omega_1} \right)^\alpha (1-\theta)^{(\omega_j - \omega_1)/\omega} \exp \left(2a\theta \frac{\omega_j - \omega_1}{\omega} \right) \\ &= \Gamma_1^{(1)} \left(\frac{\omega_j}{\omega_1} \right)^\alpha \exp \left\{ \frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta] \right\}. \end{aligned} \quad (\text{S.5})$$

Note that the assumption eq. (S.3) eliminates all $b_j^{(1)}$ except $b_1^{(1)}$ and thus essentially simplifies the mathematical problem. Combining expression (S.5) with the second relation of eq. (S.1) one calculates the total adsorption in the first layer:

$$\Gamma^{(1)} = \sum_{j=1}^n \Gamma_j^{(1)} = \Gamma_1^{(1)} \sum_{j=1}^n \left(\omega_j / \omega_1 \right)^\alpha \exp \left\{ \frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta] \right\}. \quad (\text{S.6})$$

Therefore, the adsorption of molecules in the first layer in the state with minimum molar area ω_1 is:

$$\Gamma_1^{(1)} = \frac{\Gamma^{(1)}}{\sum_{j=1}^n (\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}}. \quad (\text{S.7})$$

Combining eq. (5) and eq. (1) one obtains the adsorption of molecules in any j^{th} state of the first layer:

$$\Gamma_j^{(1)} = \Gamma^{(1)} \frac{(\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}}{\sum_{j=1}^n (\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}}. \quad (\text{S.8})$$

Then, the expression for the surface coverage in the first layer, i.e. the first expression in (S.1) becomes:

$$\theta = \omega \Gamma^{(1)} = \sum_{j=1}^n \omega_j \Gamma_j^{(1)} = \Gamma^{(1)} \frac{\sum_{j=1}^n \omega_j (\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}}{\sum_{j=1}^n (\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}}, \quad (\text{S.9})$$

and hence

$$\omega = \frac{\sum_{j=1}^n \omega_j (\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}}{\sum_{j=1}^n (\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}}. \quad (\text{S.10})$$

This equation interrelates the model variables θ and ω via the model parameters ω_j . In a similar way one can introduce the expression (S.7) into the adsorption isotherm eq. (S.4) taken for $j = 1$ to obtain:

$$\begin{aligned} b_1^{(1)} c &= \frac{\omega \Gamma_1^{(1)}}{(1-\theta)^{\omega_1/\omega}} \exp\left[-2a \frac{\omega_1}{\omega} \theta\right] \\ &= \frac{\omega \Gamma^{(1)} \exp\left(-2a \frac{\omega_1}{\omega} \theta\right)}{(1-\theta)^{\omega_1/\omega} \sum_{j=1}^n (\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}} \\ &= \frac{\theta \exp\left(-2a \frac{\omega_1}{\omega} \theta\right)}{(1-\theta)^{\omega_1/\omega} \sum_{j=1}^n (\omega_j/\omega_1)^\alpha \exp\left\{\frac{\omega_j - \omega_1}{\omega} [\ln(1-\theta) + 2a\theta]\right\}} \end{aligned} \quad (\text{S.11})$$

where the first relation of eq. (S.1), $\theta = \omega \Gamma^{(1)}$ was used. Expression (S.11) provides the second relation between the model variables θ and ω . Hence, the equations (S.10) and (S.11) constitute the set of equation which, if solved for any relevant set of model parameters $b_1^{(1)}$, a , α , ω_1 , ω_n and n , yields the values of protein adsorption in the first layer $\Gamma^{(1)}$ and the values of model variables θ and ω for any protein concentration in the solution c .

The set of equations derived above describe the adsorption for a protein monolayer within a 2-D solution model. We assume that further accumulation of matter onto the monolayer gives rise to the formation of an adjacent protein layer, which in turn becomes the substrate for the adsorption; hence the global interfacial structure tends to become heterogeneous. These secondary layers can be

considered as adsorbed onto the preceding layers with their adsorption governed by a Langmuir type mechanism with the adsorption equilibrium constant, $b^{(2)}$. The total adsorption Γ in L layers then could be calculated as (see [4,8]):

$$\Gamma = \Gamma^{(1)} \sum_{k=1}^L \left(\frac{b^{(2)}c}{1+b^{(2)}c} \right)^{k-1}. \quad (\text{S.12})$$

The surface tension of the adsorbed matter at relatively low protein concentrations is assumed to be governed by the properties of the first adsorbed layer. However, at the higher protein concentrations in the solution, a kink point on the isotherm of surface pressure Π on the protein concentration c is observed, corresponding to the critical bulk (c^*) and surface (Γ^*) protein concentrations, and the surface pressure value (Π^*) which divide the isotherms into a pre-critical region ($c < c^*$) and a post-critical region ($c > c^*$); the superscript ‘*’ refers to the critical values. For relatively low (pre-critical) protein concentrations the equation of state reads:

$$-\frac{\Pi\omega_0}{RT} = \ln(1-\theta) + \theta \left(1 - \frac{\omega_0}{\omega} \right) + a\theta^2. \quad (\text{S.13})$$

The pre-critical region, defined by eq. (S.13), is characterized by a steep increase of Π with increasing c , while in the post-critical region, Π usually increases only slightly. Such behavior of the layer in the post-critical region is attributed to a 2-D condensation (surface aggregation), a compression of the layer [4,5,9,10] and the formation of a multilayer structure [4,5,11,12]. The protein molecules and aggregates are considered as independent kinetic units and it is approximated that the increase of Π is proportional to the increase in Γ with a factor equal to the inverse value of the aggregation number, n_a , [6]:

$$\Pi = \Pi^* \left(1 + \frac{1}{n_a} \frac{\Gamma - \Gamma^*}{\Gamma^*} \right), \quad (\text{S.14})$$

where Γ is calculated using eqs. (S.1)-(S.12). This completes the formulation relevant to the calculation of the thermodynamic properties.

Computations

To obtain the simultaneous solution of eqs. (S.10) and (S.11) it is convenient to introduce the dimensionless variable Ω and dimensionless parameter Ω_0 :

$$\Omega = \frac{\omega}{\omega_1}, \quad \Omega_0 = \frac{\omega_0}{\omega_1}. \quad (\text{S.15})$$

In terms of these quantities, the values of molar areas relevant for the model are:

$$\frac{\omega_j}{\omega_1} = 1 + (j-1)\Omega_0, \quad \frac{\omega_j - \omega_1}{\omega} = (j-1)\frac{\Omega_0}{\Omega}. \quad (\text{S.16})$$

Dividing eq. (S.10) by ω_1 one obtains:

$$\begin{aligned}
\Omega &= \frac{\sum_{j=1}^n [1+(j-1)\Omega_0] [1+(j-1)\Omega_0]^\alpha \exp\left\{(j-1)\frac{\Omega_0}{\Omega} [\ln(1-\theta) + 2a\theta]\right\}}{\sum_{j=1}^n [1+(j-1)\Omega_0]^\alpha \exp\left\{(j-1)\frac{\Omega_0}{\Omega} [\ln(1-\theta) + 2a\theta]\right\}} \\
&= 1 + \Omega_0 \frac{\sum_{j=1}^n (j-1) [1+(j-1)\Omega_0]^\alpha \exp\left\{(j-1)\frac{\Omega_0}{\Omega} [\ln(1-\theta) + 2a\theta]\right\}}{\sum_{j=1}^n [1+(j-1)\Omega_0]^\alpha \exp\left\{(j-1)\frac{\Omega_0}{\Omega} [\ln(1-\theta) + 2a\theta]\right\}} \quad (S.16) \\
&= 1 + \Omega_0 \frac{\sum_{j=2}^n (j-1) [1+(j-1)\Omega_0]^\alpha \exp\left\{(j-1)\frac{\Omega_0}{\Omega} [\ln(1-\theta) + 2a\theta]\right\}}{1 + \sum_{j=2}^n [1+(j-1)\Omega_0]^\alpha \exp\left\{(j-1)\frac{\Omega_0}{\Omega} [\ln(1-\theta) + 2a\theta]\right\}}
\end{aligned}$$

Introducing the auxiliary functions:

$$\xi_j(\theta, \Omega) = [1+(j-1)\Omega_0]^\alpha \exp\left\{(j-1)\frac{\Omega_0}{\Omega} [\ln(1-\theta) + 2a\theta]\right\}, \quad (S.17)$$

$$\begin{aligned}
\Xi(\theta, \Omega) &= 1 + \sum_{j=2}^n [1+(j-1)\Omega_0]^\alpha \exp\left\{(j-1)\frac{\Omega_0}{\Omega} [\ln(1-\theta) + 2a\theta]\right\} \\
&= 1 + \sum_{j=2}^n \xi_j(\theta, \Omega), \quad (S.18)
\end{aligned}$$

one obtains the equation which relates Ω and θ :

$$\Omega = 1 + \Omega_0 \frac{\sum_{j=2}^n (j-1) \xi_j(\theta, \Omega)}{\Xi(\theta, \Omega)}. \quad (S.19)$$

The adsorption isotherm (S.11) in terms of these quantities becomes:

$$b_1^{(1)} c = \frac{\theta}{\Xi} \frac{\exp\left[-2a \frac{\theta}{\Omega}\right]}{(1-\theta)^{1/\Omega}}. \quad (S.20)$$

To obtain the solution of eqs. (S.19) and (S.20) the computation program was developed to implement the Newton-Raphson method as described in Section 9.6 of [13], and thus to yield the values of model variables θ and ω for any protein concentration in the solution c and any relevant set of model parameters $b_1^{(1)}$, a , α , ω_1 , ω_n and n . From thus calculated values all thermodynamical functions are obtained according to the expressions above. The program is designed to provide the fitting of experimental data in the graphical interface.

References

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