

Effect of oil hydrophobicity on adsorption and rheology of β -lactoglobulin at oil-water interfaces

Supporting Information

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Supporting Information Figures 1 and 2 are results referring to adsorption kinetics and elongational rheology. Supporting Information Figure 3 shows interfacial shear rheology results with cyclohexane and toluene. Supporting Information Figure 4 depicts the surprising G'_i and G''_i behavior of octanal. The following Supporting Information Figures 5, 6, and 7 visualized interfacial shear experiments of MCT, air, ethyl acetate, and methyl-*tert*-butylether, respectively. All interfacial shear rheological tests were performed with 1 g/L β -lg injection solution at a deformation of 1 %, $\omega = 1$ rad/s and $T = 20^\circ\text{C}$. Supporting Information Figure 8 presents the effect of COT variations in *n*-alkanes on protein adsorption.

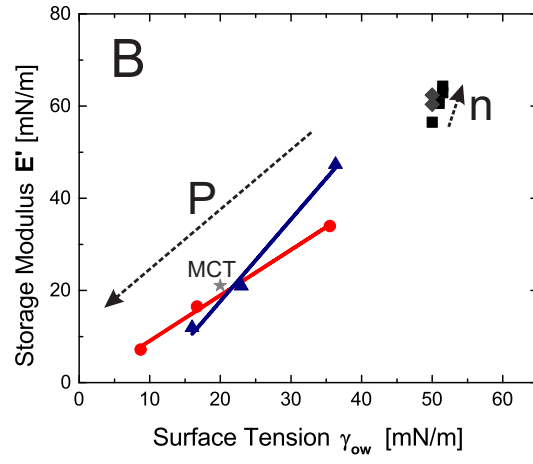


Figure 1: E' as a function of γ_{ow} . The tendency of increased E' with decreased polarity more considerably.

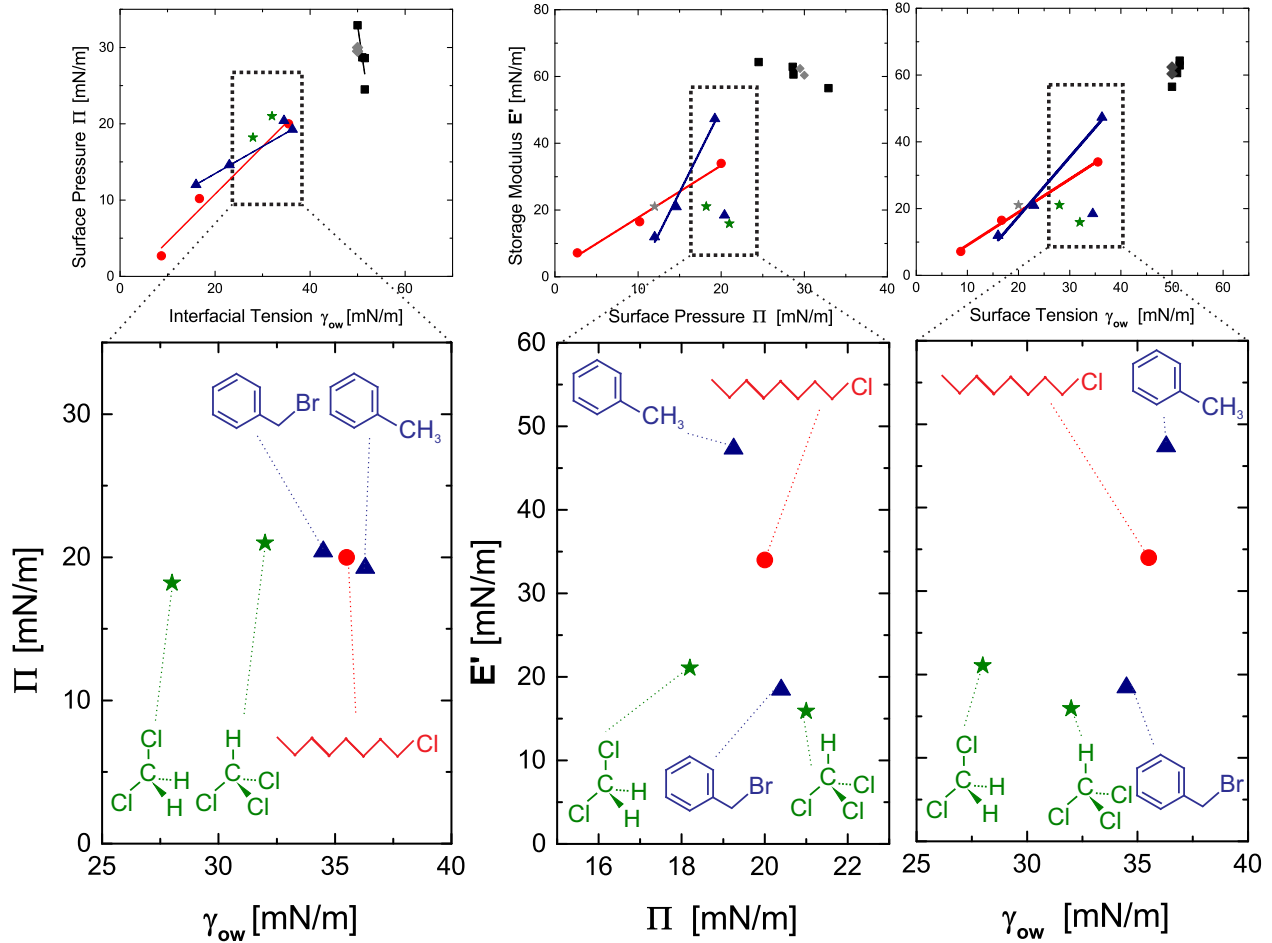


Figure 2: Chloroform(CHCl_3), dichloromethane (CH_2Cl_2) and benzyl bromide ($\text{C}_6\text{H}_5\text{CH}_2\text{Br}$) fit nicely into the γ_{ow}/Π graph and support the claim that Π decrease with increased polarizability. However, regarding their E' , their results are significantly decreased. This supports the fact that the mechanisms controlling surface activity are decoupled from the mode of action governing the viscoelastic properties of the equilibrated protein layer. Benzyl bromide is hydrolyzed in water into benzyl alcohol ($\text{C}_6\text{H}_5\text{CH}_2\text{OH}$) and hydrogen bromide (HBr). Due to this process a behavior as seen for ethyl acetate is expected (see Figure 7).

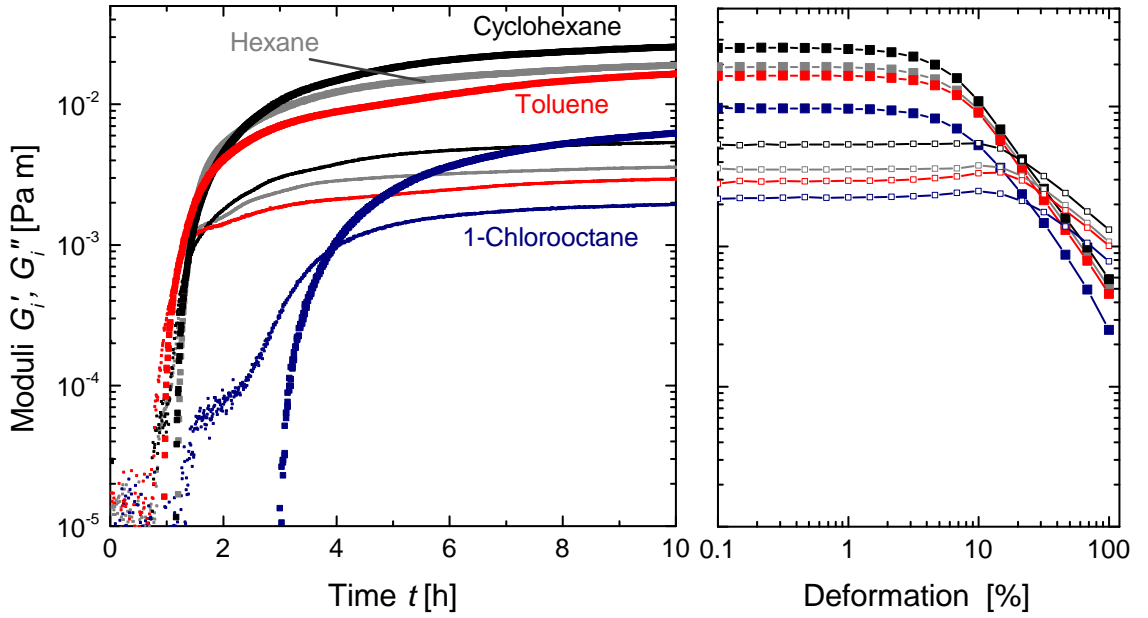


Figure 3: Viscoelastic shear moduli over time during the formation of β -lg layers at toluene (red) compared to n -alkane (gray and black) interfaces. COT of toluene is purely diffusion controlled as for decane and hexane. However, its $G'_{i\infty}$ is indistinguishable from the non polarizable hydrocarbons. Compared to 1-chlorooctane (navy) with a similar γ_{ow} β -lg results in significantly stronger network at toluene interface.

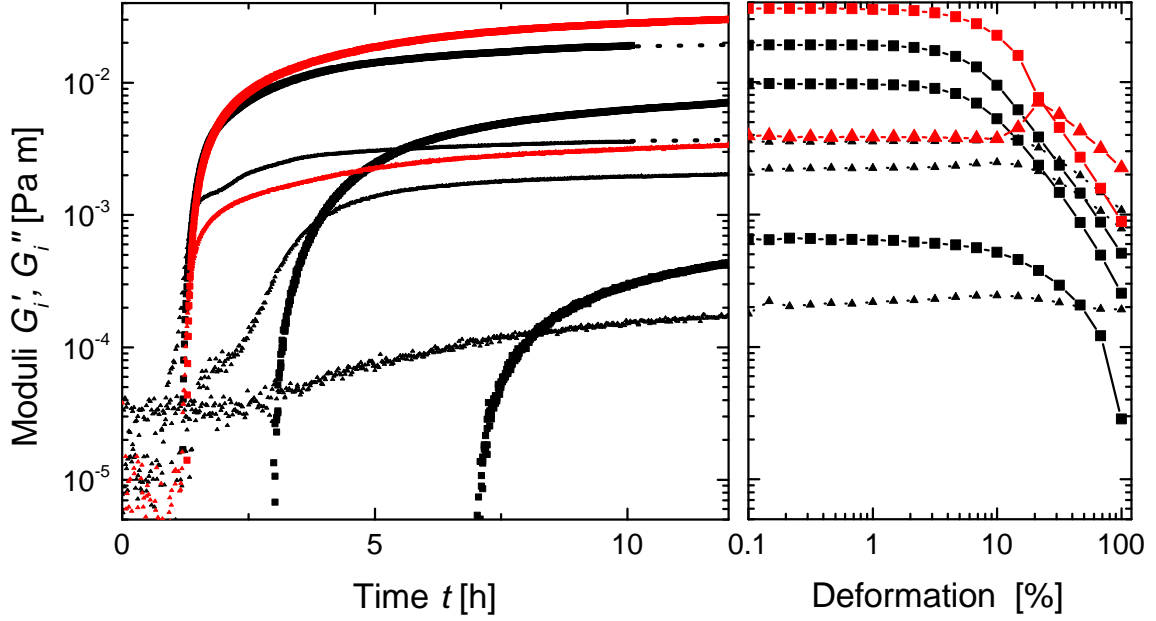


Figure 4: On the left interfacial network formation shown with a time sweep for octanal in comparison to hexane, 1-chlorooctane and 1-octanol. G'_i and G''_i are displayed as squares and triangles. The moduli of hexane after 10 h are extrapolated with dotted lines. On the right, amplitude sweep is shown for the same oils after equilibration of the interfacial layer. Even though the oil polarity behave as $\gamma_{ow}^{hexane} > \gamma_{ow}^{1-chlorooctane} > \gamma_{ow}^{octanal} > \gamma_{ow}^{1-octanol}$, octanal exhibits G'_i and G''_i curves expected for an oil with a lower polarity than n -alkanes. This might be related to interactions between the aldehyde group and the amino groups of proteins: $\text{Protein-NH}_2 + \text{R=O} \longrightarrow \text{Protein-N=R} + \text{H}_2\text{O}$. If octanal binds to the protein it forms a chemical hydrophobic coating and allows the n -octane tail to interact intramolecular, and thereby β -lg unfolding is facilitated and hydrophobic interactions are increased. The strong elastic response is only seen in shear deformation but not in dilatation. This behavior and the chemical hydrophobic coating utmost fascinating and should be further investigated.

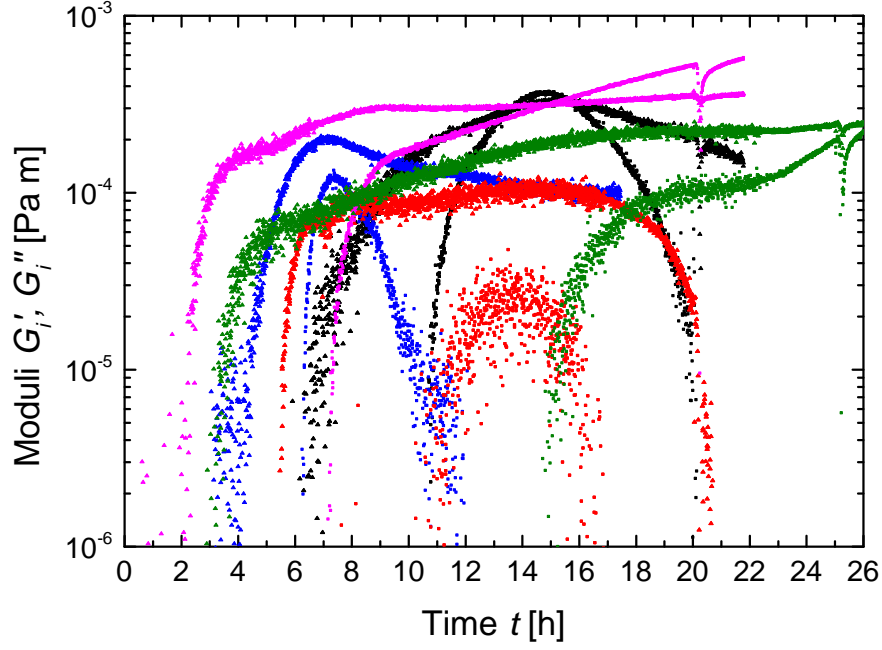


Figure 5: Five results of MCT measurements. It was not possible to unreproducibly measure MCT oil. Impurity are possible reasons. G'_i started at random onset times. In some cases no COT is reached, since G'_i drops again before COT is reached.

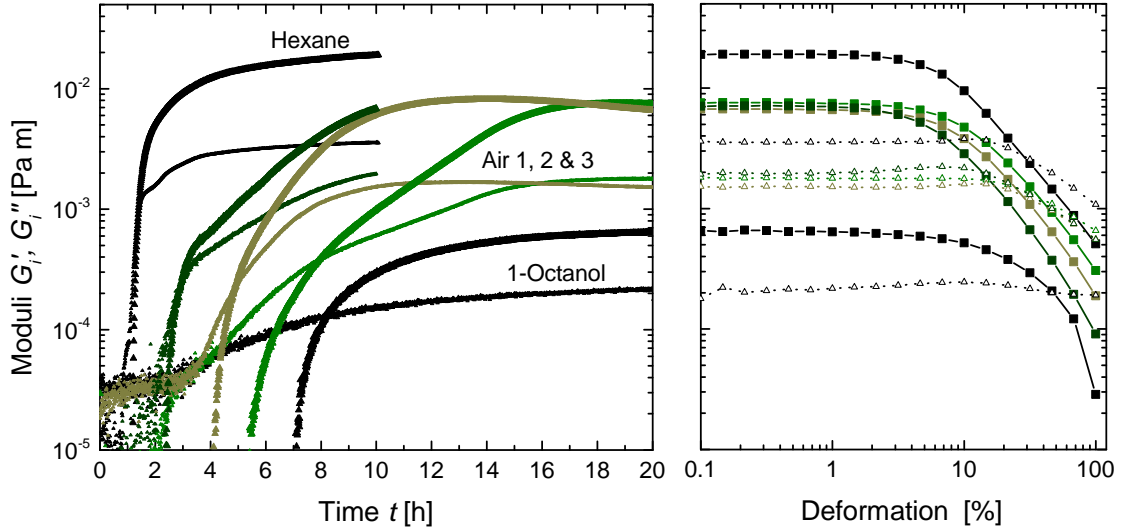


Figure 6: Viscoelastic behavior of β -lg adsorption and network formation at a/w surface in comparison to hexane and 1-octanol. Air results in a constant $G'_{i\infty}$. But contrary to oil interfaces the COT varies considerably. The amplitude sweep on the right show small but negligible variations.

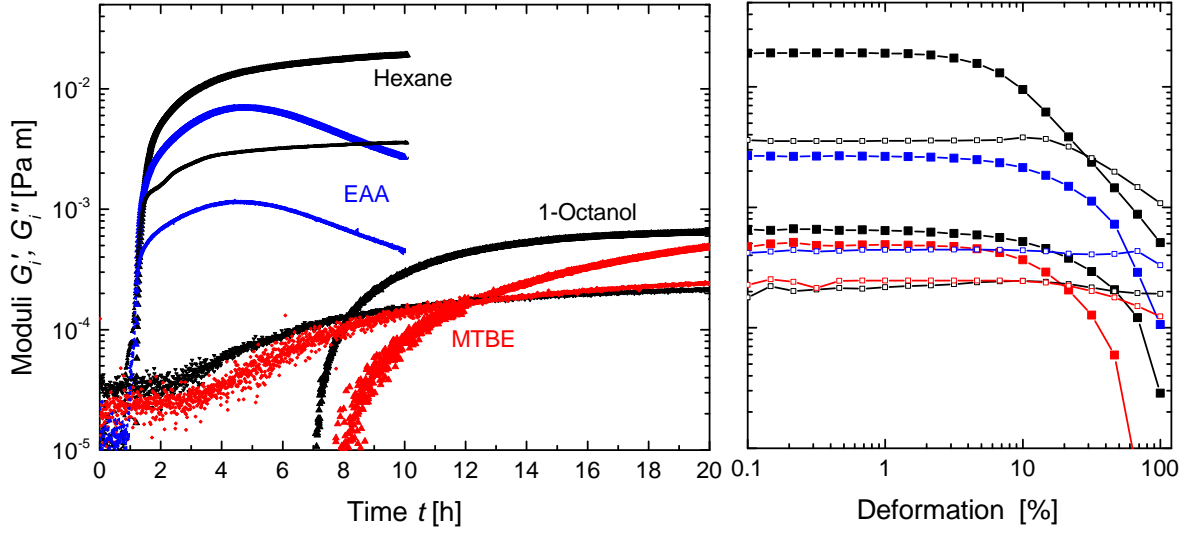


Figure 7: Time (left) and amplitude sweep (right) of ethyl acetate (EAA) and methyl-*tert*-butylether (MTBE). Hexane and 1-octanol are depicted for comparison. At EAA interface an interfacial film is immediately formed as soon as a sufficient amount of β -lg reaches the bulk surface. G'_i increases rapidly, but after 4 h G'_i and G''_i decreases again. This is most likely related to the hydrolysis of EAA to ethanol and acetic acid. These products destabilize the protein network. MTBE has, according to literature, a slightly higher γ_{ow} than 1-octanol. Contrary to expectation, COT is delayed at MTBE interface. The time sweep was ended after 20 h, at this point $G'_{i\infty}$ was not fully reached. $G'_i{}^{MTBE}$ aims at a higher $G'_{i\infty}$ than of 1-octanol.

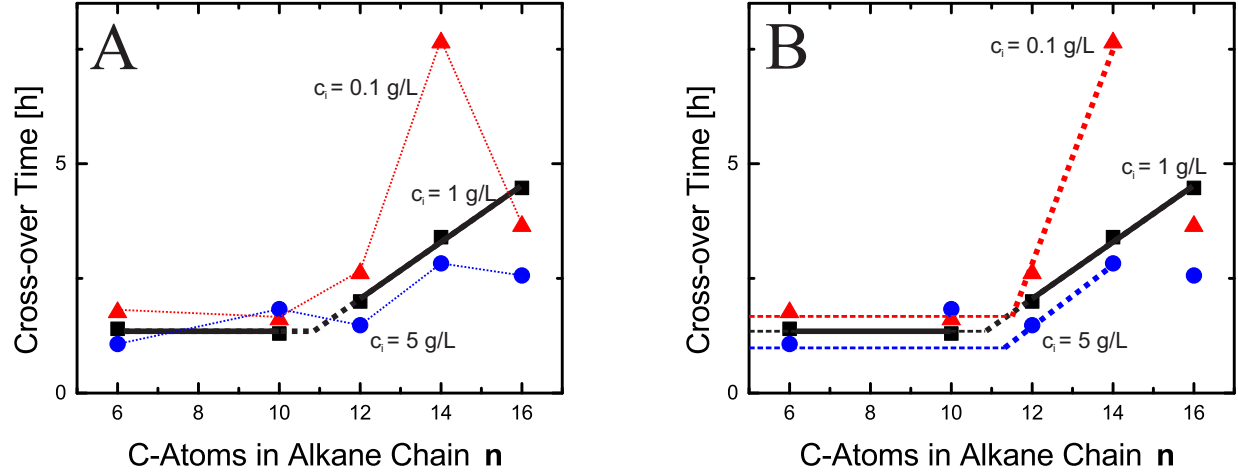


Figure 8: For a better understanding of the mechanisms controlling COT at n -alkane interface, different concentrations were tested. The decane ($n = 10$) results were strongly varying with disturbed G'_i course, presumably due to impurities. In A) the points of 0.1 g/L (red) and 5 g/L (blue) are connected with dotted lines, to guide to the eye. COT increases up to $n = 14$ but decreases again at $n = 16$ for the higher and considerably for the lower concentration. $COT_{n=16}$ fluctuated noticeably, but the typical G'_i behavior indicated no perceptible impurities. This suggests that the mode of action controlling COT is similar for $n = 16$ is similar as for air (Figure 6). In B) the $COT_{n=10}$ and $COT_{n=16}$ were not taken into account. $COT_{n=6}$ is regarded as DT. $COT_{n=12}$ and $COT_{n=14}$ connected and extrapolated (dotted lines) and crossed with the DT (dashed lines). For all concentration the kink is around $n = 11$, supporting the presented mechanism in Figure 7.