Supporting information for: Structure determination of Hen Egg-White Lysozyme Aggregates adsorbed to Lipid/Water and Air/Water Interfaces

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The supporting information (12 pages in total) contains 3 tables and 10 figures.

AFM Measurements

Table S1: Information about aggregates determined from AFM images.

		Height and Type of Aggregates	
Incubation time	Oligomers	Oligomers	Fibrils
0.5 h		~2.5 nm	
24 h	0.8 - 1.5 nm	~2.5 nm	1.5 - 3 nm
130 h			1.5 - 4 nm

Surface Pressure Data

The surface tension was monitored continuously during VSFG measurements with a commercial KSV NIMA instrument (Biolin Scientific, Sweden) using a platinum rod. Surface pressure (π) is defined as $\pi = \gamma_0 - \gamma$, where γ_0 is the surface tension of air/D_2O and γ is either the surface tension of a DOPG monolayer on the surface, or the surface tension of air/D_2O with some adsorbates.

Figure S1 A and B show surface pressure data for air/D_2O and DOPG/D_2O interfaces after injection of lysozyme sample's aliquots heated for various times. These data were only used for qualitative analysis and to monitor when the whole system reached equilibrium. A quantitative analysis was not possible due to a significant inaccuracy in the equilibrium surface pressure. An example of a lack of reproducibility is given for 130 h aliquot at air/D_2O surface (with an area between two measurements shaded in light red) is shown in S1 A.

An increase in the surface pressure demonstrates that lysozyme and/or its aggregates were adsorbed to the interface. The shape of the initial increase and the speed depended on the depth at which the aliquot was injected, which was varied within 1 - 5 mm under the visual surface. Only for air/D_2O interface when 0.5 h heated aliquot was injected, a monotonous decrease in the surface pressure was registered, which most likely appeared due to evaporation of the sample or a drift of the surface tensiometer reading. For other

aliquots surface pressure increased, however, no clear trend could be identified. Surprisingly, at DOPG/D_2O (initial surface pressure of DOPG/D_2O was ~30 mN/m) approximately the same equilibrium surface pressure value $\pi_{eq} \simeq 35$ mN/m was reached for all aliquots and even with varying pD. It was shown in a previous study that lysozyme can penetrate phosphatidylglycerol lipid monolayer when surface pressure of the lipid film is up to 20 mN/m. In our case, almost constant π_{eq} suggests that the lipid monolayer is saturated with lysozyme and its aggregates in all cases.

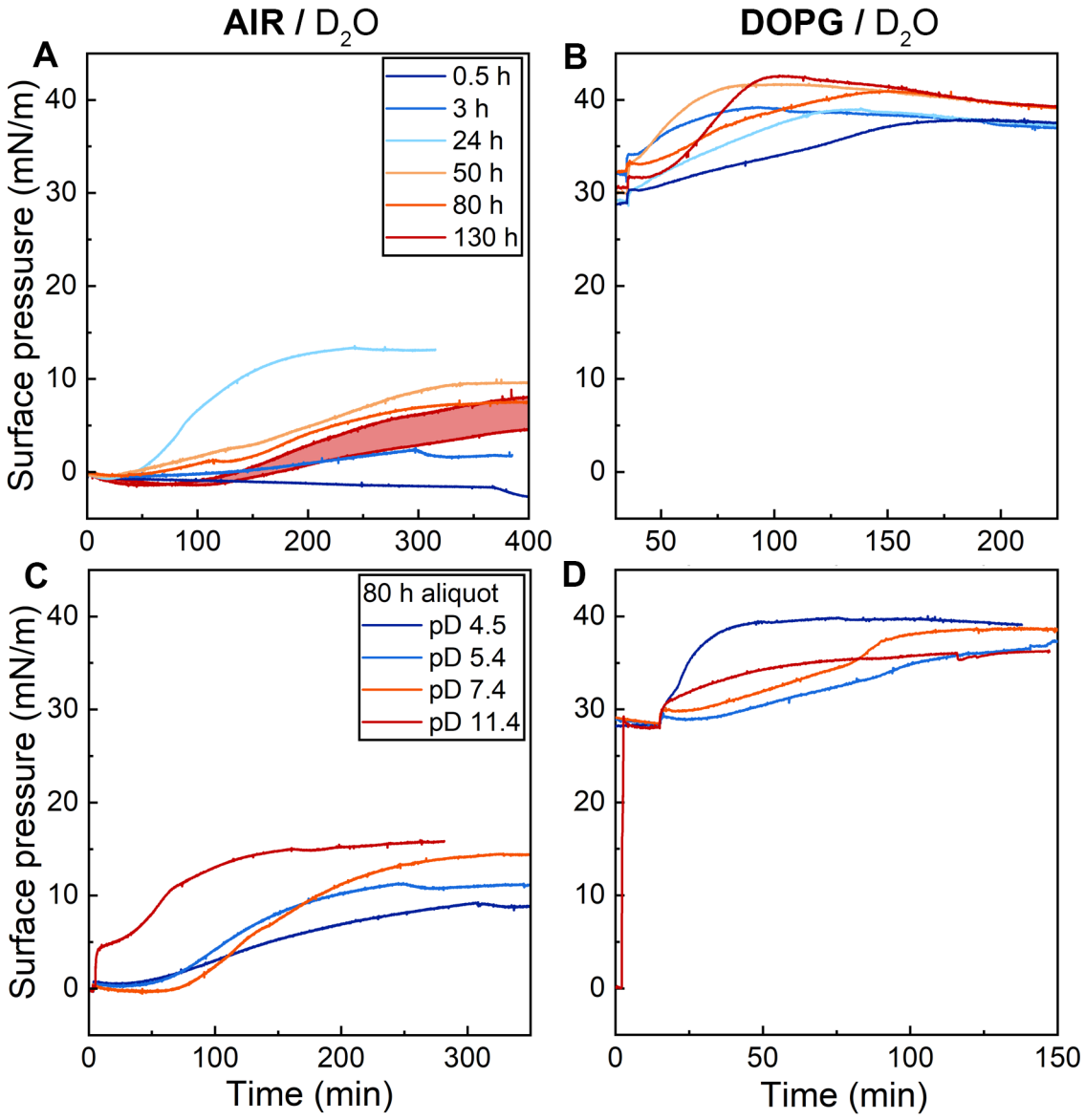

Figure S1: Surface pressure of air/D_2O (A) and DOPG/D_2O (B) interfaces during the adsorption of lysozyme aliquots, heated for different times (see legend). Surface pressure at different pD values during the adsorption of 80 h lysozyme aliquot to the air/D_2O (C) and DOPG/D_2O (D) interfaces.

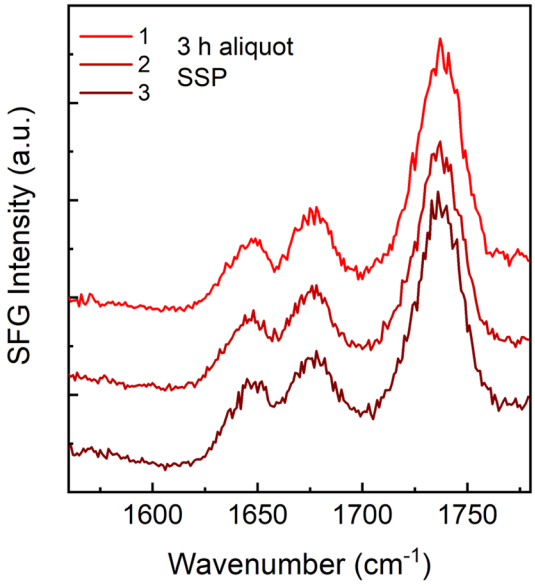

Figure S2: VSFG spectra in SSP polarization of 3 hours heated aliquot adsorbed to the DOPG/D_2O interface. Each spectrum corresponds to a different experiment (numbered as 1, 2 or 3 in the legend).

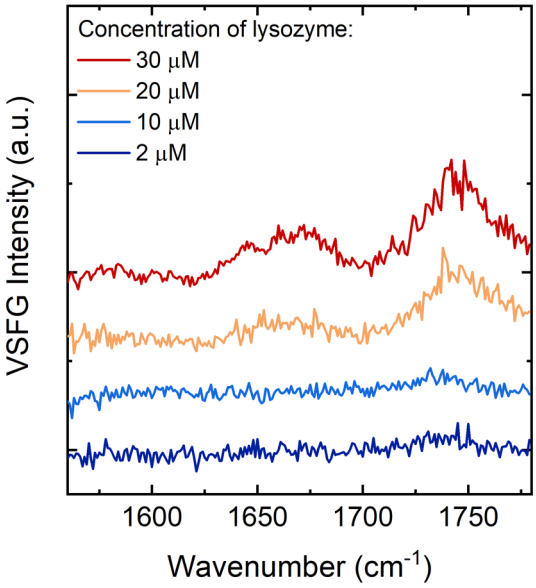

Figure S3: VSFG spectra in SSP polarization combination of 0.5 h aliquot adsorbed to air/D_2O interface at various bulk concentrations (see legend).

VSFG spectra comparison

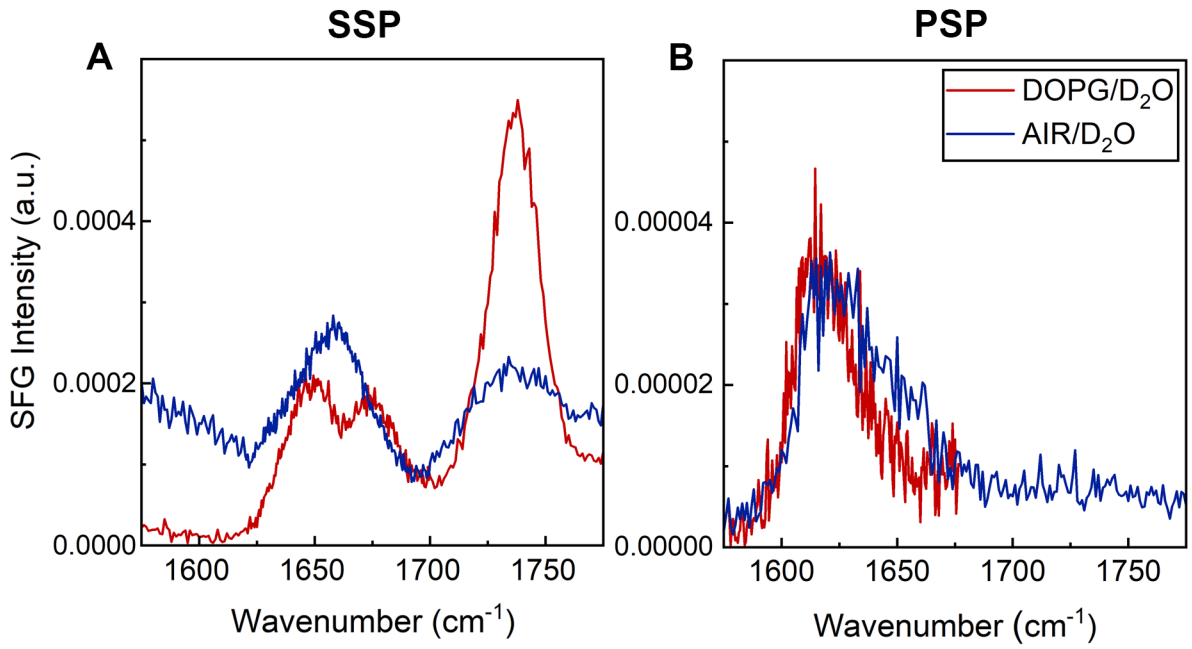

Figure S4: VSFG spectra of 80 h aliquot adsorbed to air/D_2O and DOPG/D_2O interfaces in SSP and PSP polarization combinations.

VSFG spectra at H_2O

A solvent has an affect on the spectral shape and band positions in VSFG spectrum as can be seen from Figure S5. VSFG spectra of 3 h aliquot adsorbed at DOPG/D_2O shows two pronounced peaks, whereas at DOPG/H_2O those peaks are less separated and also shifted by $\sim$5 cm^{-1} to higher frequencies. The bending mode of H_2O contributes to the spectrum at $\sim$1655 cm^{-1}, thus we chose D_2O as a solvent in our study.

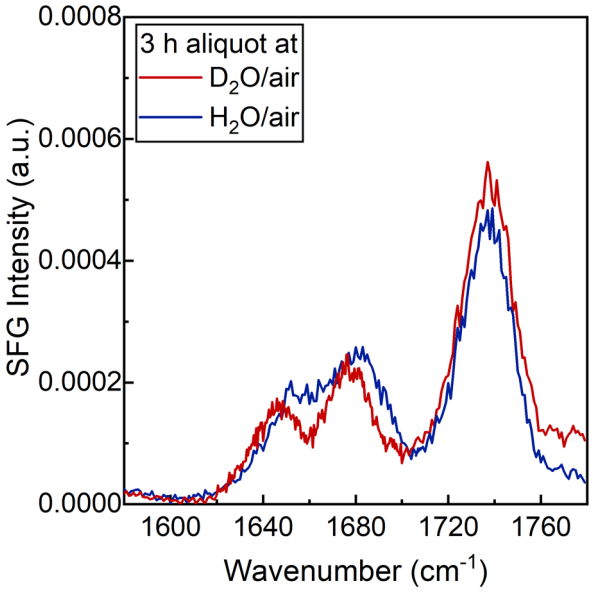

Figure S5: VSFG spectra in SSP polarization combination of 3 h aliquot adsorbed to air/D_2O and air/H_2O interfaces.

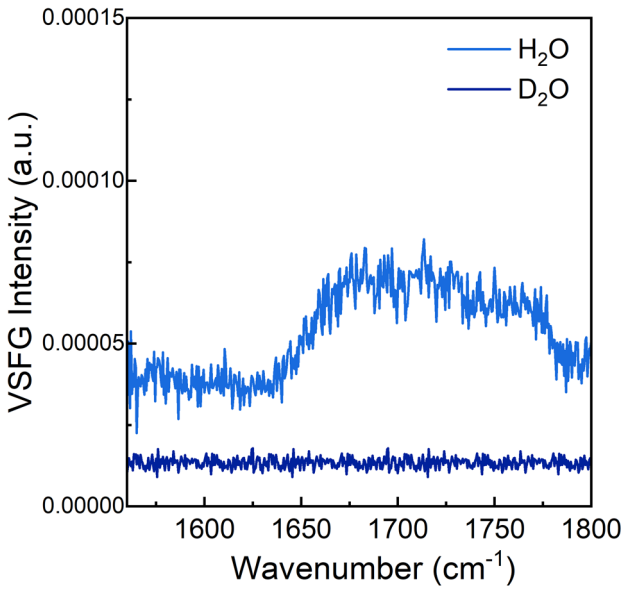

Figure S6: VSFG spectra of air/H_2O and air/D_2O interfaces in SSP polarization combination.

Fitting VSFG spectra

To extract the number of peaks contributing to the spectrum and the exact position of those peaks, the VSFG spectrum was fitted according to the formula:

$$I_{\mathrm{VSFG}} \propto \left|\chi^{(2)}_{\mathrm{NR}} + \chi^{(2)}_{\mathrm{R}}\right|^2 = \left|A_{\mathrm{NR}} e^{i\varphi_{\mathrm{NR}}} + \sum_q \frac{A_q}{\omega_{\mathrm{IR}} - \omega_q + i\Gamma_q}\right|^2 \tag{1}$$

where $\chi^{(2)}_{\mathrm{NR}}$ is a non-resonant second order non-linear susceptibility and $\chi^{(2)}_{\mathrm{R}}$ is the effective resonant second order non-linear susceptibility. A_{NR} and φ_{NR} are the non-resonant amplitude and phase. A_q, ω_q and Γ_q are the amplitude, the center frequency and the width of the q-th resonance, respectively.

Table S2: Parameters obtained from fitting VSFG spectra of lysozyme 80 h aliquot adsorbed to air/D_2O.

$\chi^{(2)}_{NR}$	φ_{NR}	A_{NR}	
	2.83	0.4	
$\chi^{(2)}_{R}$	ω_q, cm^{-1}	A_q	Γ_q, cm^{-1}
Peak 1	1646	2.15	15
Peak 2	1675	-2.51	17
Peak 3	1720	1.52	14
Peak 4	1616	-0.59	14

Table S3: Parameters obtained from fitting VSFG spectra of lysozyme 80 h aliquot adsorbed to DOPG/D_2O.

$\chi^{(2)}_{NR}$	φ_{NR}	A_{NR}	
	2.02	0.17	
$\chi^{(2)}_{R}$	ω_q, cm^{-1}	A_q	Γ_q, cm^{-1}
Peak 1	1650	2.8	11
Peak 2	1675	2.4	11
Peak 3	1734	6.83	13
Peak 4	1618	-2.10	15

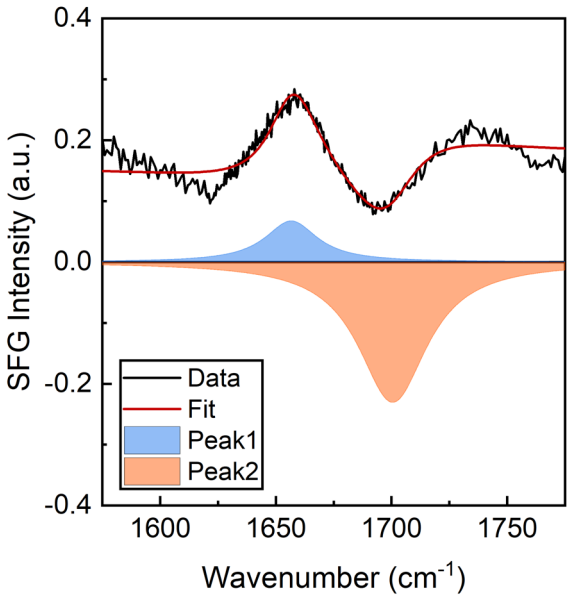

Figure S7: VSFG spectrum of 80 h aliquot adsorbed to air/D_2O interface (SSP polarization combination) and fitting of the data with only one peak in the Amide I region.

Packing of DOPG monolayer

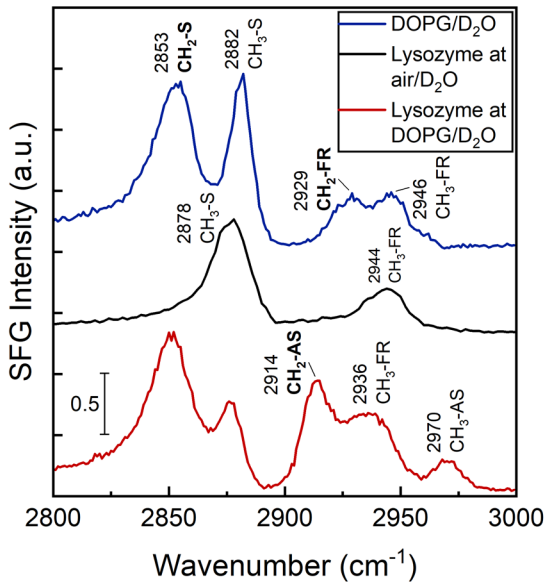

Figure S8: VSFG spectra of DOPG/D_2O interfaces, and 20 h lysozyme's aliquot adsorbed to air/D_2O and DOPG/D_2O, in C-H vibrational region.

Packing of the lipid monolayer can be evaluated from the strength of CH_2 and CH_3 symmetric vibrational modes, the intensity ratio of those two modes are often used to estimate

molecular ordering. It is well known that when the lipid monolayer is ideally packed, only the signal from CH_3 vibrational modes will be visible in the VSFG spectrum. When monolayer starts to lose its ordering, the intensity of CH_3 peaks decreases, and vibrational bands of CH_2 start to appear. In our case, we see intense CH_2 vibrational bands in the VSFG spectra of DOPG/D_2O interface, which suggests that monolayer is loosely packed. DOPG is unsaturated lipid with two cis-double bonds in its hydrocarbon chains (see Figure 1B in the manuscript for the molecular structure). Unsaturated lipids tend to form loosely packed monolayers due to defects in their hydrocarbon chains that are caused by cis-double bonds. Previous reflection absorption infrared spectroscopy (RAIRS)[S1] and SFG[S2,S3] studies have revealed that monolayers constructed from lipids with unsaturated linkages show higher disorder and fluidity comparing with saturated lipids. After adsorption of 20 h aliquot, the intensity of CH_3-S decreases even further, showing that monolayer got even less ordered.

In Figure S9 we show DOPG isotherm from which it is evident that 30 mN/m surface pressure of DOPG monolayer corresponds to liquid condensed phase. Our measured isotherm is consistent with isotherms presented in the literature.[S4,S5]

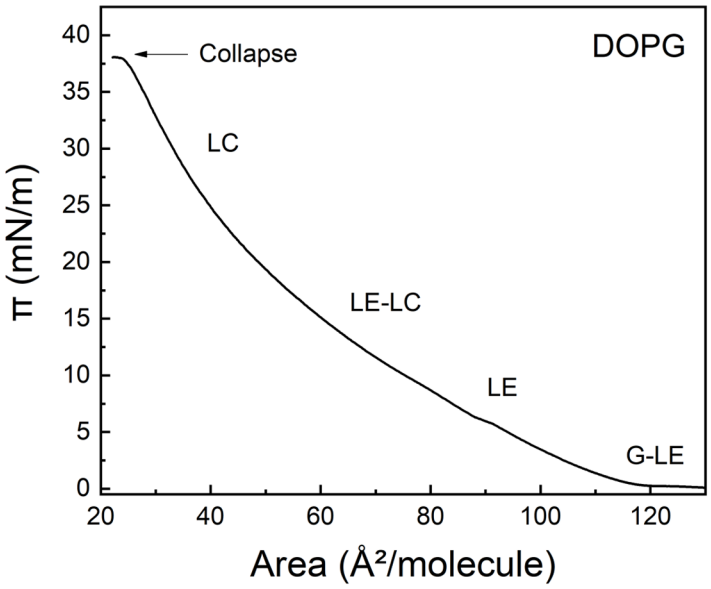

Figure S9: Surface pressure (π) - mean molecular area (Å) isotherm of DOPG. G-LE stands for gas phase and liquid-expanded phase coexistence, LE: liquid-expanded phase, LC: liquid-condensed phase, LE-LC: coexistence of LE and LC phases. The phase transitions are shown only as a guideline.

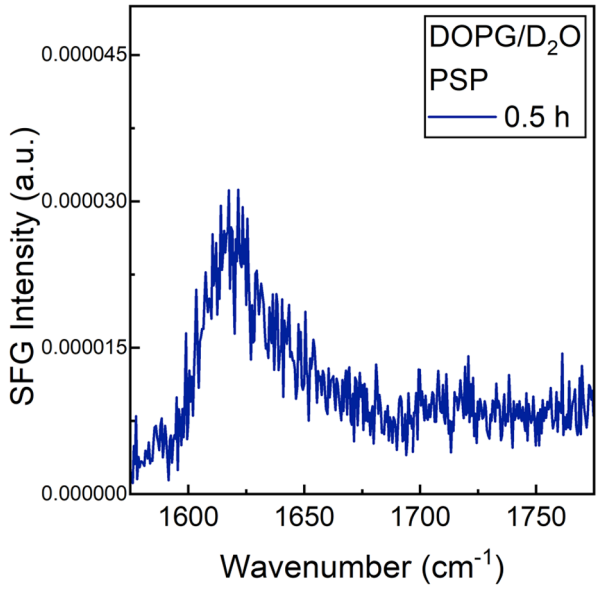

Figure S10: VSFG spectrum of 0.5 h aliquot adsorbed to DOPG/D2O interface in PSP polarization combination in the 1560 - 1760 cm^{-1} region. No signal above 1700 cm^{-1} shows that there is no polarization leakage.

References

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