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Supplementary Information: Interaction of Glycine, Lysine, Proline and Histidine with Dipalmitoylphosphatidylcholine Lipid Bilayers: a Theoretical and Experimental Study†

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1 FTIR Experiments

Fig. 1 (a), (b) and (c) shows the νC=O band for different DPPC:Gly molar ratios, at three different temperatures (a) 298.2 K gel, (b) 314.2 K transition and (c) 323.2 K crystalline states.

Table 1 reports the experimental frequencies and the frequency shifts of both bonded and free C=O groups ($\nu C{=}O_b$ and $\nu C{=}O_f$, respectively) at Gly:DPPC ratios within 0.0:1 and 4.0:1, and at three different temperatures, corresponding to the gel (298.2 K), the transition temperature (314.2 K) and the liquid crystalline (323.2 K) states.

Table 2 reports the experimental frequencies and the frequency shifts of both symmetric and antisymmetric streching modes of the phosphate groups at three differents temperatures, 298.2 K, 314.2 K and 323.2 K, in water solution.

Finally, Table 3 and Table 4 provide a complete set of FTIR measurements at different Gly:DPPC molar ratios.

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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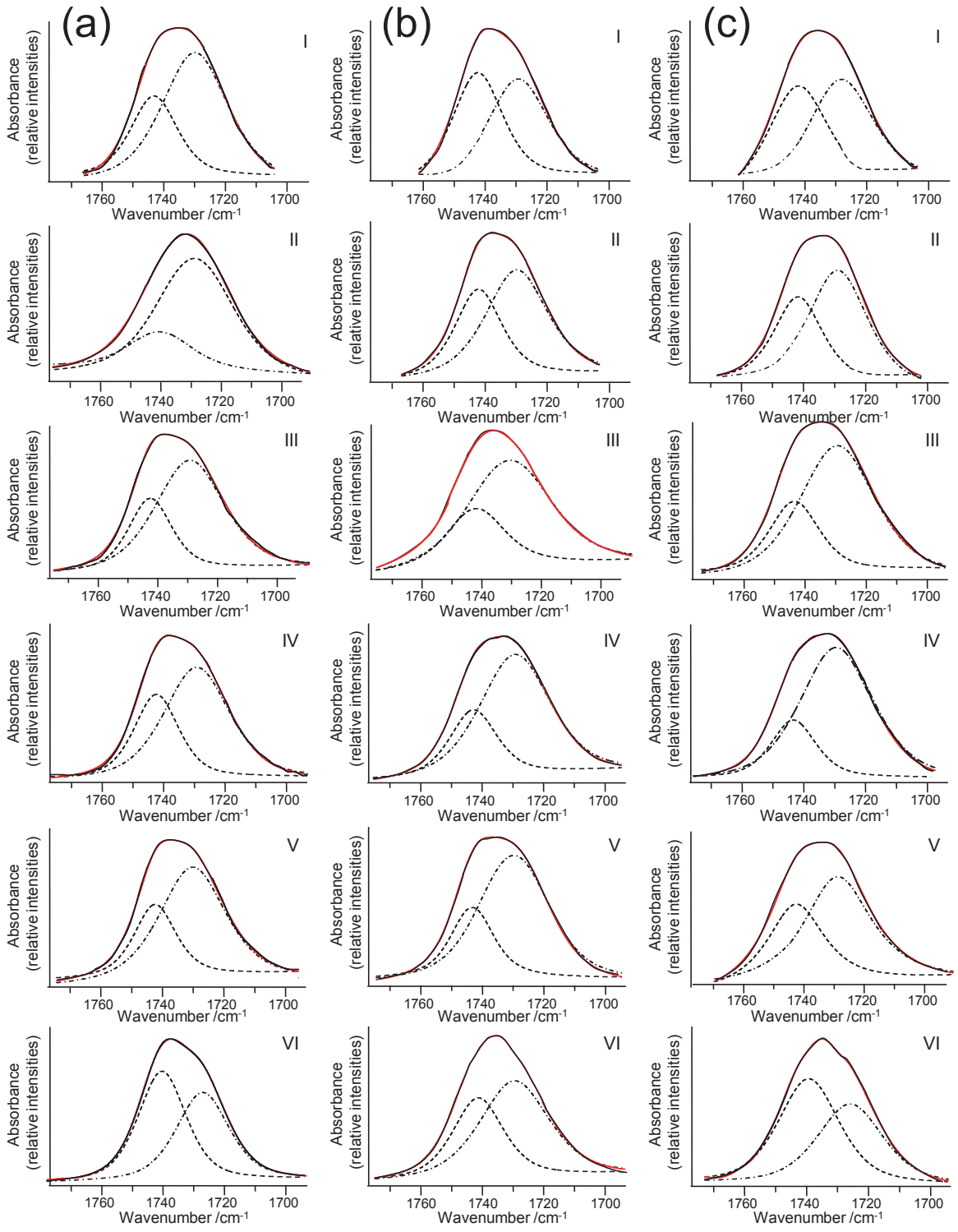

Fig. 1 Deconvolution and curve fitting (Gaussian-Lorentzian functions) of the component bands for the two populations of carbonyl groups (free and H-bonded) at (a) 298.2 K, (b) 314.2 K and (c) 323.2 K. In all scripts, the experimental bands (full black line) and the cumulative peak fit (red lines), as well as the C=O$_{free}$ (dash line) and C=O$_{bonded}$ (dash dot line) populations are shown. Numbers I to VI denote different Gly:DPPC molar ratios: I: 0.0:1; II: 0.4:1; III: 0.9:1; IV: 2.0:1; V: 3.0:1; VI: 4.0:1.

Table 1 Experimental frequencies values (with the corresponding standard deviation) in the free (labeled as subindex *f*) and H-bonded (labeled as subindex *b*) population of C=O groups at 298.2, 314.2 and 323.2 K in D_2O. † In parenthesis and in bracket we listed the corresponding variations in the frequency, with respect of the 0.0:1 Gly/DPPC molar ratio, for the free and H-bonded populations of C=O groups, respectively

Temperature	Molar ratio Gly/DPPC	†$\nu C{=}O_f$	†$\nu C{=}O_b$
298.2 K			
	0.0:1	1742 ± 0.6	1730 ± 0.7
	0.4:1	1743 ± 0.3 (1)	1732 ± 0.5 [2]
	0.9:1	1742 ± 0.1 (0)	1729 ± 0.3 [-1]
	2.0:1	1742 ± 1.0 (0)	1729 ± 0.8 [-1]
	3.0:1	1741 ± 0.7 (-1)	1729 ± 0.4 [-1]
	4.0:1	1741 ± 0.1 (-1)	1728 ± 0.5 [-2]
314.2 K			
	0.0:1	1742 ± 0.5	1729 ± 0.2
	0.4:1	1742 ± 0.5 (0)	1730 ± 0.5 [1]
	0.9:1	1743 ± 0.6 (1)	1731 ± 0.5 [2]
	2.0:1	1742 ± 0.6 (0)	1729 ± 0.8 [0]
	3.0:1	1742 ± 0.2 (0)	1729 ± 0.9 [0]
	4.0:1	1742 ± 0.1 (0)	1730 ± 0.3 [1]
323.2 K			
	0.0:1	1742 ± 0.5	1728 ± 0.7
	0.4:1	1741 ± 0.9 (-1)	1728 ± 0.5 [0]
	0.9:1	1743 ± 0.5 (1)	1730 ± 0.3 [2]
	2.0:1	1742 ± 0.4 (0)	1729 ± 0.3 [1]
	3.0:1	1743 ± 0.2 (1)	1729 ± 0.5 [1]
	4.0:1	1739 ± 0.6 (-3)	1724 ± 0.3 [-4]

Table 2 Experimental frequencies values (with the corresponding standard deviation) in the symmetric and antisymmetric stretching modes of the PO_2^- groups at 298.2, 314.2 and 323.2 K in H_2O. † In parenthesis and in bracket we listed the corresponding variations in the frequency, with respect of the 0.0:1 Gly/DPPC molar ratio, in the antisymmetric and symmetric stretching modes of the PO_2^- groups, respectively

Temperature	Molar ratio Gly/DPPC	$^{\dagger}\nu_{as}PO_2^-$	$^{\dagger}\nu_{s}PO_2^-$
298.2 K			
	0.0:1	1233 ± 0.5	1090 ± 0.5
	0.4:1	1226 ± 1.5 (-7)	1089 ± 0.1[-1]
	0.9:1	1224 ± 0.8 (-9)	1089 ± 0.5 [-1]
	2.0:1	1225 ± 1.0 (-8)	1090 ± 0.9 [0]
	3.0:1	1225 ± 0.9 (-8)	1089 ± 0.7 [-1]
	4.0:1	1224 ± 0.5 (-9)	1089 ± 0.7 [-1]
314.2 K			
	0.0:1	1231 ± 0.5	1089 ± 0.1
	0.4:1	1231 ± 0.3 (0)	1088 ± 0.1 [-1]
	0.9:1	1229 ± 1.0 (-1)	1090 ± 0.5 [1]
	2.0:1	1229 ± 1.0 (-2)	1090 ± 0.5 [1]
	3.0:1	1230 ± 0.9 (-1)	1088 ± 1.0 [-1]
	4.0:1	1230 ± 0.9 (-1)	1088 ± 0.5 [-1]
323.2 K			
	0.0:1	1234 ± 0.3	1088 ± 0.1
	0.4:1	1232 ± 0.5 (-2)	1088 ± 0.1 [0]
	0.9:1	1231 ± 1.0 (-3)	1089 ± 0.3 [1]
	2.0:1	1230 ± 0.8 (-4)	1088 ± 0.2 [0]
	3.0:1	1231 ± 0.8 (-3)	1088 ± 0.2 [0]
	4.0:1	1232 ± 1.0 (-2)	1088 ± 0.5 [0]

Table 3 Frequency values of symmetric, antisymmetric stretching, and bending modes of CH_2 y CH_3 groups in the hydrophobic region of a DPPC bilayer in the presence of Gly. FTIR measurements performed at 298.2 K (gel phase).

Molar ratio Gly/DPPC	ν_{as} [CH_3]	$\Delta\nu_{as}$ [CH_3]	ν_s [CH_3]	$\Delta\nu_s$ [CH_3]	ν_{as} [CH_2]	$\Delta\nu_{as}$ [CH_3]	ν_s [CH_2]	$\Delta\nu_s$ [CH_3]	δ [CH_2]	$\Delta\delta$ [CH_2]
0.0:1	2956	0	2874	0	2919	0	2850	0	1468	0
0.4:1	2955	**-1**	2873	**-1**	2919	**0**	2850	**0**	1468	**0**
0.9:1	2955	**-1**	2872	**-2**	2918	**-1**	2850	**0**	1468	**0**
2.0:1	2955	**-1**	2873	**-1**	2918	**-1**	2850	**0**	1468	**0**
3.0:1	2956	**0**	2871	**-3**	2918	**-1**	2850	**0**	1468	**0**
4.0:1	2956	**0**	2874	**0**	2918	**-1**	2850	**0**	1468	**0**

Table 4 Frequency values of symmetric, antisymmetric stretching, and bending modes of CH_2 y CH_3 groups in the hydrophobic region of a DPPC bilayer in the presence of Gly. FTIR measurements performed at 323.2 K (liquid phase).

Molar ratio Gly/DPPC	ν_{as} [CH_3]	$\Delta\nu_{as}$ [CH_3]	ν_s [CH_3]	$\Delta\nu_s$ [CH_3]	ν_{as} [CH_2]	$\Delta\nu_{as}$ [CH_3]	ν_s [CH_2]	$\Delta\nu_s$ [CH_3]	δ [CH_2]	$\Delta\delta$ [CH_2]
0.0:1	2957	0	2872	0	2923	0	2853	0	1461	0
0.4:1	2956	**-1**	2874	**2**	2923	**0**	2852	**-1**	1456	**-5**
0.9:1	2955	**-2**	2872	**0**	2923	**0**	2853	**0**	1459	**-2**
2.0:1	2955	**-2**	2872	**0**	2923	**0**	2853	**0**	1462	**1**
3.0:1	2955	**-2**	2872	**0**	2923	**0**	2853	**0**	1460	**-1**
4.0:1	2955	**-2**	2872	**0**	2923	**0**	2852	**-1**	1458	**-3**

2 Methods: Molecular Dynamics Simulations

2.1 Criteria to ensure the stability of the Systems

In order to test whether the Umbrella Sampling simulations were long enough to ensure the convergence of the free energy profiles, each 100 ns MD trajectory was split into 5 sub-trajectories of 20 ns. Free energy profiles were computed from each sub-trajectory and used to compute the average profile and the corresponding statistical error. The results are shown in Figs. 2 to 7, and presented in the following order:

- Fig 2: Gly/DPPC system
- Fig 3: $N^{\delta 1}$-H His/DPPC system
- Fig 4: $N^{\varepsilon 2}$-H His/DPPC system
- Fig 5: His^{+}/DPPC system
- Fig 6: Lys^{+}/DPPC system
- Fig 7: Pro/DPPC system

In all cases, it can be clearly appreciated that the free energy profiles obtained form the sub-trajectories fluctuate around the average curve. These data strongly suggest that the simulations have reached a steady state.

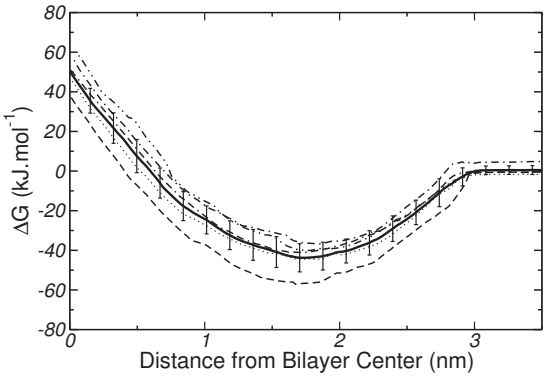

Fig. 2 Free energy profiles for five subtrajectories of Gly, corresponding to: (———) Δt= 0-20ns, (· · · · ·) Δt= 20-40ns, (– – – –) Δt= 40-60ns, (– · – · –) Δt= 60-80ns and (– · · – · · –) Δt= 60-80ns. Thick solid line is the average of the free energy with the corresponding statistical error.

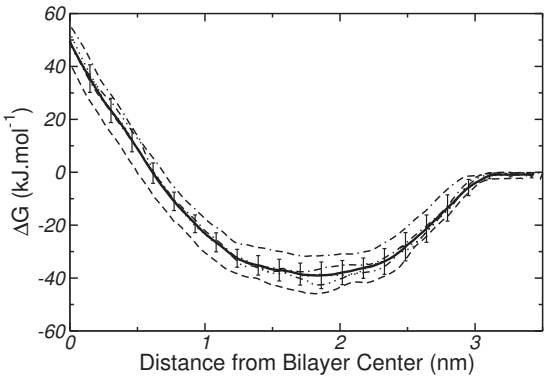

Fig. 3 Free energy profiles for five subtrajectories of $N^{\delta 1}$-H His, corresponding to: (———) Δt= 0-20ns, (· · · · · ·) Δt= 20-40ns, (– – – –) Δt= 40-60ns, (– · – · –) Δt= 60-80ns and (– · · – · · –) Δt= 60-80ns. Thick solid line is the average of the free energy with the corresponding statistical error.

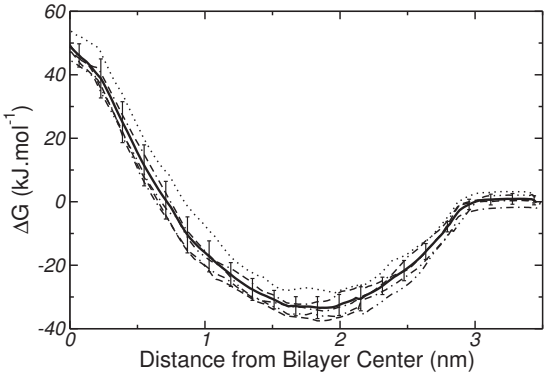

Fig. 4 Free energy profiles for five subtrajectories of $N^{\varepsilon 2}$-H His, corresponding to: (———) Δt= 0-20ns, (· · · · · ·) Δt= 20-40ns, (– – – –) Δt= 40-60ns, (– · – · –) Δt= 60-80ns and (– · · – · · –) Δt= 60-80ns. Thick solid line is the average of the free energy with the corresponding statistical error.

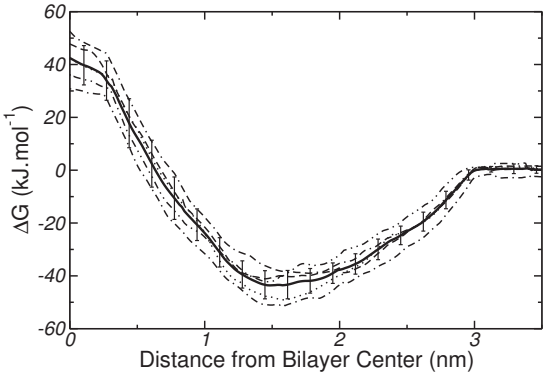

Fig. 5 Free energy profiles for five subtrajectories of His^{+}, corresponding to: (———) Δt= 0-20ns, (· · · · · ·) Δt= 20-40ns, (– – – –) Δt= 40-60ns, (– · – · –) Δt= 60-80ns and (– · · – · · –) Δt= 60-80ns. Thick solid line is the average of the free energy with the corresponding statistical error.

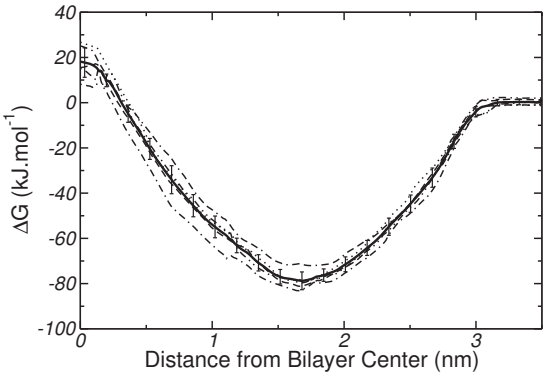

Fig. 6 Free energy profiles for five subtrajectories of Lys^+, corresponding to:: (———) Δt= 0-20ns, (· · · · · ·) Δt= 20-40ns, (– – – –) Δt= 40-60ns, (– · – · –) Δt= 60-80ns and (– · · – · · –) Δt= 60-80ns. Thick solid line is the average of the free energy with the corresponding statistical error.

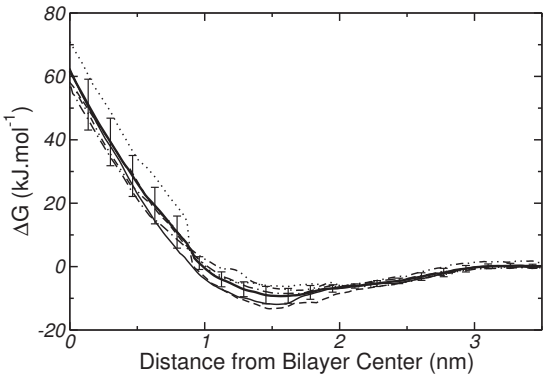

Fig. 7 Free energy profiles for five subtrajectories of Pro, corresponding to: (———) Δt= 0-20ns, (· · · · · ·) Δt= 20-40ns, (– – – –) Δt= 40-60ns, (– · – · –) Δt= 60-80ns and (– · · – · · –) Δt= 60-80ns. Thick solid line is the average of the free energy with the corresponding statistical error.

2.2 Analysis of Hydration/Dehydration

Figure 8 shows the number of coordinating water molecules in the first hydration shield of the carbonyl oxygen atom of Gly. As can be appreciated, Gly losses around 11 water molecules when inserted into the lipid bilayer. This behavior is concomitant to the decrease in the number of H-bonds showed in the upper panel of Figure 13 of the main manuscript.

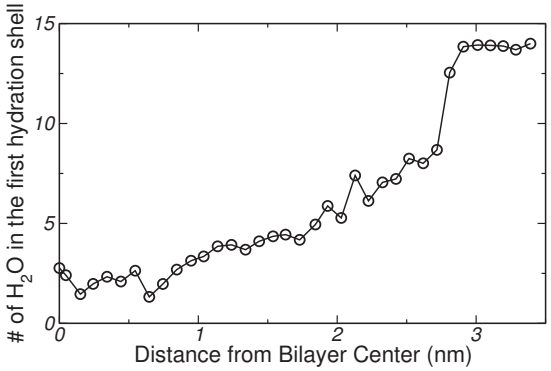

Fig. 8 Number of coordinating water molecules in the first hydration shield of the carbonyl oxygen atom of Gly.