→ *Electronic Supplementary Information for*

# New Insight of Probe-Location Dependent Polarity and Hydration at Lipid/Water Interfaces: Comparison between Gel- and Fluid-Phase of Lipid Bilayers

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**NMR and Mass Spectroscopic Characterization of 4AP-Cn Probes:** The synthesized 4AP-Cn derivatives were characterized by $^1$H NMR and ESI mass spectrometry. Analytical data for each of the 4AP-Cn derivatives are as follows:

(1) 4AP-C2. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K) 1.2 (t, 3H); 3.7 (q, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 191.094 $(M+H)^+$.

(2) 4AP-C3. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K) 0.9 (t, 3H); 1.6 (m, 2H); 3.6 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 205.0300 $(M+H)^+$.

(3) 4AP-C4. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K): 0.9 (t, 3H); 1.3 (m, 2H); 1.6 (t, 2H); 3.6 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 219.046 $(M+H)^+$.

(4) 4AP-C5. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K): 0.9 (t, 3H); 1.3 (m, 4H); 1.6 (t, 2H); 3.6 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 233.060 $(M+H)^+$.

(5) 4AP-C6. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K): 0.9 (t, 3H); 1.3 (m, 6H); 1.6 (t, 2H); 3.6 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 247.074 $(M+H)^+$.

(6) 4AP-C7. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K): 0.8 (t, 3H); 1.3 (m, 8H); 1.6 (t, 2H); 3.5 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 261.087 $(M+H)^+$.

(7) 4AP-C8. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K): 0.8 (t, 3H); 1.2 (m, 10H); 1.6 (t, 2H); 3.6 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 275.102 $(M+H)^+$.

(8) 4AP-C9. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K): 0.8 (t, 3H); 1.2 (m, 12H); 1.6 (t, 2H); 3.6 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 289.116 $(M+H)^+$.

(9) 4AP-C10. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K): 0.8 (t, 3H); 1.2 (m, 14H); 1.6 (t, 2H); 3.5 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 303.130 $(M+H)^+$.

(10) 4AP-C12. **$^1$H NMR** (500MHz, $CDCl_3$, 298 K): 0.8 (t, 3H); 1.2 (m, 18H); 1.6 (t, 2H); 3.5 (t, 2H); 6.8 (dd, 1H); 7.0 (d, 1H); 7.5 (d, 1H); 4.4 (s, 2H); **ESI-MS** (+): *m/z* 331.159 $(M+H)^+$.

**Supplementary Figures:**

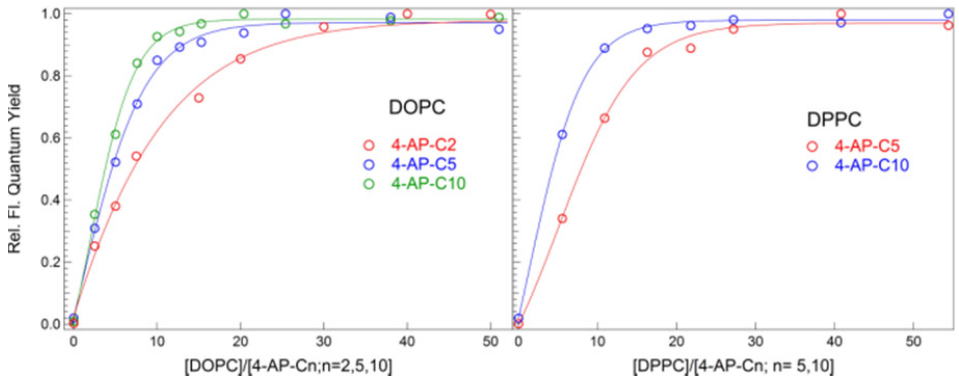


**Figure S1**. Binding kinetics of few 4AP-Cn (n = 2, 5 and 10) probes with DOPC (left) and DPPC (right) vesicles.

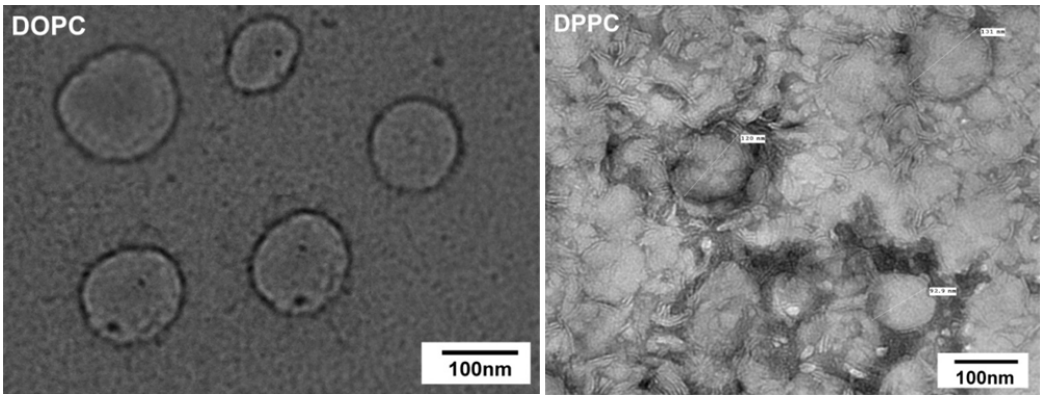


**Figure S2.** TEM images confirm the formation of DOPC and DPPC small unilamellar vesicles (SUVs) of size ~100 nm.

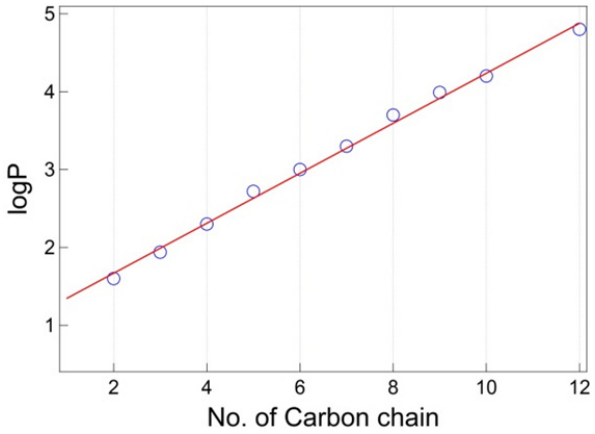


**Figure S3.** Plot of octanol/water partition coefficient (logP) of 4AP-Cn probes with number of carbon atoms in the alkyl-chain obtained from shake-flask method. Line through pints show linear fit to data.

**Table S1.** Experimental and calculated (from software) partition coefficients (logP) of 4AP-Cn probes.

| 4AP-Cn Probes | Octanol/Water Partition Coefficient (logP) | | | | |
|---|---|---|---|---|---|
| | Experiment | Simulation (using various software) | | | |
| | | miLogP | ACD | ALOPS | ClogP |
| 4AP-C2 | 1.67 | 0.93 | 0.81 | 0.15 | 0.72 |
| 4AP-C3 | 1.99 | 1.45 | 1.34 | 0.50 | 1.18 |
| 4AP-C4 | 2.31 | 2.02 | 1.87 | 0.91 | 1.63 |
| 4AP-C5 | 2.63 | 2.52 | 2.55 | 1.50 | 2.09 |
| 4AP-C6 | 2.96 | 3.03 | 2.93 | 2.10 | 2.54 |
| 4AP-C7 | 3.27 | 3.53 | 3.46 | 2.72 | 2.99 |
| 4AP-C8 | 3.59 | 4.04 | 3.99 | 3.41 | 3.45 |
| 4AP-C9 | 3.92 | 4.54 | 4.52 | 3.95 | 3.90 |
| 4AP-C10 | 4.24 | 5.05 | 5.06 | 4.47 | 4.36 |
| 4AP-C12 | 4.88 | 6.06 | 5.60 | 5.44 | 5.27 |

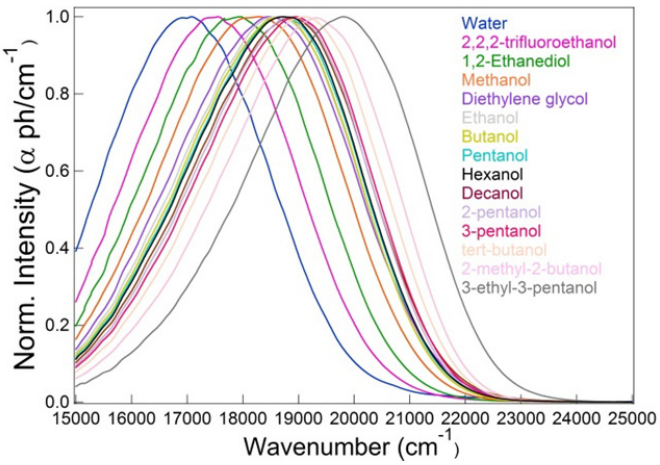


**Figure S4.** Normalized and corrected fluorescence spectra of 4AP-C3 in 15 different solvents from which the $E_T^N$-scale is generated. See the color code for solvents in panel.

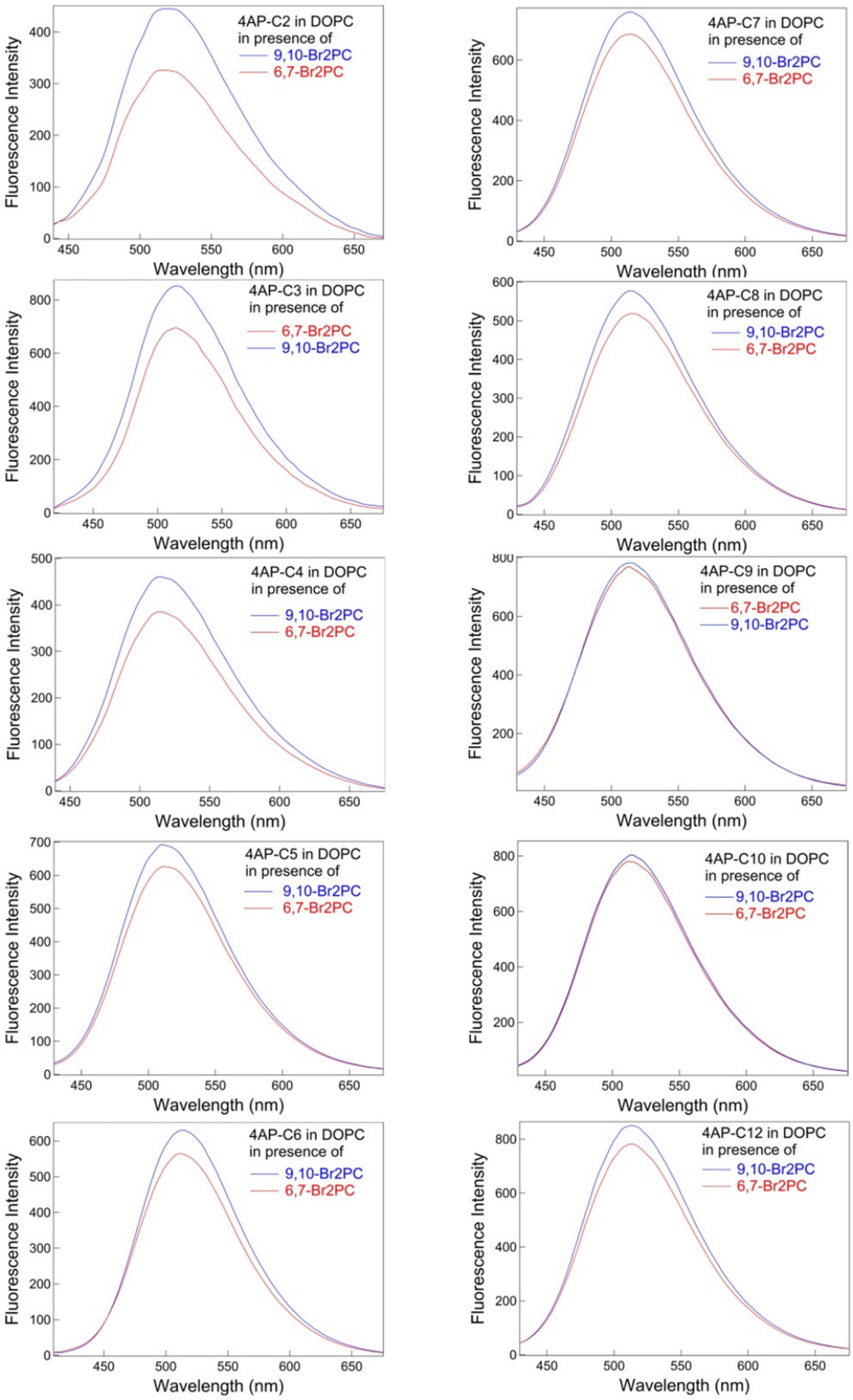


**Figure S5**. Relative fluorescence spectra of 4AP-Cn (n = 2 to 10, 12) in DOPC vesicles in presence of shallow (6,7-$Br_2$PC) and deep (9,10-$Br_2$PC) quenchers. Quenching of fluorescence by the shallow quencher is higher in all cases. See plots for legends.

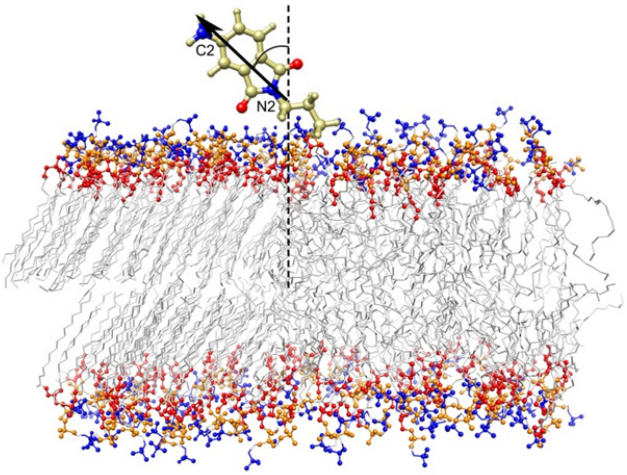


**Figure S7.** Cartoon showing the angle of 4AP-moiety (defined as the angle between vector joining N2 and C2 of 4AP-moiety and the bilayer normal) used for calculation of angle-distributions of probes at the DPPC/water and DOPC/water interfaces from MD trajectories.