Free-Energy Relationships for the Interactions of Tryptophan with Phosphocholines

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Supplementary Information

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1. Equations Used for Curve Fitting

1.1 Equilibrium Constants

$$K_{\mathrm{m}} = \frac{[L_{\mathrm{m}}]}{[L]^{n}} \quad \text{(S1)} \qquad K_{1\mathrm{wl}} = \frac{[WL]}{[W][L]} \quad \text{(S2)} \qquad K_{2\mathrm{wl}} = \frac{[W_2L]}{[WL][W]} \quad \text{(S3)} \qquad K_{1} = \frac{[LT]}{[L][T]} \quad \text{(S4)} \qquad K_{2} = \frac{[LT_2]}{[LT][T]} \quad \text{(S5)}$$

$$K_{1\mathrm{w}} = \frac{[WLT]}{[W][LT]} \quad \text{(S6)} \qquad K_{1'} = \frac{[WLT]}{[WL][T]} \quad \text{(S7)}$$

where:
$[L_{\mathrm{m}}]$ is the concentration of lipid in micellar form (*NOT the concentration of micelles*)
$[L]$ is the concentration of free lipid
$[WL]$ is the concentration of 1:1 water:lipid complex
$[W]$ is the concentration of free water
$[W_2L]$ is the concentration of 2:1 water:lipid complex
$[LT]$ is the concentration of 1:1 lipid:tryptophan complex
$[T]$ is the concentration of free tryptophan
$[LT_2]$ is the concentration of 1:2 lipid:tryptophan complex
$[WLT]$ is the concentration of 1:1:1 water:lipid:tryptophan complex
K_{m} is the equilibrium constant for micelle formation
$K_{1\mathrm{wl}}$ is the equilibrium constant for formation of the 1:1 water:lipid complex
$K_{2\mathrm{wl}}$ is the equilibrium constant for formation of the 2:1 water:lipid complex
K_1 is the equilibrium constant for formation of the 1:1 lipid:tryptophan complex
K_2 is the equilibrium constant for formation of the 1:2 lipid:tryptophan complex
$K_{1\mathrm{w}}$ is the equilibrium constant for formation of the 1:1:1 water:lipid:tryptophan complex from the 1:1 lipid:tryptophan complex and water
$K_{1'}$ is the equilibrium constant for formation of the 1:1:1 water:lipid:tryptophan complex from the 1:1 water:lipid complex and tryptophan

1.2 Equations for Water/Lipid (DMPC) Titrations

1.2.1 Binding Isotherms

$$\delta_{\mathrm{w}} = \frac{[WL](\delta_{1\mathrm{wl}} - \delta_{\mathrm{fw}})}{[W]_{\mathrm{t}}} + \frac{2[W_2L](\delta_{2\mathrm{wl}} - \delta_{\mathrm{fw}})}{[W]_{\mathrm{t}}} + \delta_{\mathrm{fw}} \quad \text{(S8)}$$

$$\delta_{\mathrm{l}} = \frac{[WL](\delta_{1\mathrm{wl}'} - \delta_{\mathrm{fl}})}{[L]_{\mathrm{t}}} + \frac{[W_2L](\delta_{2\mathrm{wl}'} - \delta_{\mathrm{fl}})}{[L]_{\mathrm{t}}} + \frac{[L_{\mathrm{m}}](\delta_{\mathrm{lm}} - \delta_{\mathrm{fl}})}{[L]_{\mathrm{t}}} + \delta_{\mathrm{fl}} \quad \text{(S9)}$$

where:
$[L]_{\mathrm{t}}$ is the total concentration of lipid
$[W]_{\mathrm{t}}$ is the concentration of water
δ_{w} is the calculated water chemical shift
δ_{fw} is the chemical shift of free water
$\delta_{1\mathrm{wl}}$ is the water chemical shift in the 1:1 water:lipid complex
$\delta_{2\mathrm{wl}}$ is the water chemical shift in the 2:1 water:lipid complex
δ_{l} is the calculated lipid chemical shift
δ_{fl} is the chemical shift of free DMPC
$\delta_{1\mathrm{wl}'}$ is the DMPC chemical shift in the 1:1 water:lipid complex
$\delta_{2\mathrm{wl}'}$ is the DMPC chemical shift in the 2:1 water:lipid complex
δ_{lm} is the DMPC chemical shift in micellar aggregates

1.2.2 Mass Balances

$$[L]_t = [L] + [WL] + [W_2L] + [L_m] \quad \text{(S10)} \quad \{\text{used to calculate } [L_m]\}$$

$$[W]_t = [W] + [WL] + 2[W_2L] \quad \text{(S11)} \quad \{\text{used to calculate } [W]\}$$

1.2.3 Speciation

[*L*]

$$[L] = \frac{-b_{L'} - \sqrt{b_{L'}^2 - 4K_m^2([L]_t - [WL] - [W_2L])^2}}{2K_m^2([L]_t - [WL] - [W_2L])} \quad \text{(S12)}$$

where:

$$-b_{L'} = 1 + 2K_m\{[L]_t - [WL] - [W_2L]\} \quad \text{(S13)}$$

[*WL*]

$$[WL] = \frac{-b_{WL'} - \sqrt{b_{WL'}^2 - 4y^2K_{1wl}^2([W]_t - 2[W_2L])([L]_t - [W_2L])}}{2yK_{1wl}} \quad \text{(S14)}$$

where:

$$-b_{WL'} = 1 + yK_{1wl}\{[L]_t - 3[W_2L] + [W]_t\} \quad \text{(S15)}$$

$$y = 1 + K_m^2[L]^2 - 2K_m[L] \quad \text{(S16)}$$

[W_2L]

$$[WL] = \frac{-b_{W_2L'} - \sqrt{b_{W_2L'}^2 - 16K_{2wl}^2([W]_t - [W])([W]_t - [WL])}}{8K_{2wl}} \quad \text{(S17)}$$

where:

$$-b_{W_2L'} = 1 + 2K_{2wl}\{2[W]_t - [W] - [WL]\} \quad \text{(S18)}$$

1.3 Equations for Tryptophan/Lipid (DMPC) Titrations

1.3.1 Tryptophan Binding Isotherm

$$\delta = \frac{K_1[L](\delta_1 - \delta_f) + K_{1'}[WL](\delta_{1'} - \delta_f) + 2K_2[LT](\delta_2 - \delta_f)}{1 + K_1[L] + K_{1'}[WL] + 2K_2[LT]} + \delta_f \quad \text{(S19)}$$

where:
δ is the calculated chemical shift
δ_f is the chemical shift of free tryptophan
δ_1 is the tryptophan chemical shift in the 1:1 lipid:tryptophan complex
δ_2 is the tryptophan chemical shift in the 1:2 lipid:tryptophan complex
$\delta_{1'}$ is the tryptophan chemical shift in the 1:1:1 water:lipid:tryptophan complex

1.3.2 Water Binding Isotherm

$$\delta_w = \frac{[WLT](\delta_{1w} - \delta_{fw})}{[W]_t} + \frac{[WL](\delta_{1wl} - \delta_{fw})}{[W]_t} + \frac{2[W_2L](\delta_{2wl} - \delta_{fw})}{[W]_t} + \delta_{fw} \quad \text{(S20)}$$

where:

δ_{1w} is the water chemical shift in the 1:1:1 water:lipid:tryptophan complex

1.3.3 Mass Balances

$$[L]_t = [L] + [WL] + [W_2L] + [L_m] + [WLT] + [LT] + [LT_2] \quad \text{(S21)} \quad \{\text{used to calculate } [L_m]\}$$

$$[W]_t = [W] + [WL] + 2[W_2L] + [WLT] \quad \text{(S22)} \quad \{\text{used to calculate } [W]\}$$

$$[T]_t = [T] + [WLT] + [LT] + 2[LT_2] \quad \text{(S23)} \quad \{\text{used to calculate } [T]\}$$

where:
$[T]_t$ is the total tryptophan concentration

1.3.4 Speciation

[*L*]

$$[L] = \frac{-b_L - \sqrt{b_L^{\,2} - 4K_m^{\,2}([L]_t - [WL] - [WLT] - [W_2L] - [LT] - [LT_2])^2}}{2K_m^{\,2}([L]_t - [WL] - [WLT] - [W_2L] - [LT] - [LT_2])} \quad \text{(S24)}$$

where:
$$-b_L = 1 + 2K_m\{[L]_t - [WL] - [WLT] - [W_2L] - [LT] - [LT_2]\} \quad \text{(S25)}$$

[*LT*]

$$[LT] = \frac{-b_{LT} - \sqrt{b_{LT}^{\,2} - 4K_1^{\,2}([T]_t - 2[LT_2] - [WLT])([L]_t - [WL] - [WLT] - [LT_2] - [W_2L] - x)}}{2K_1} \quad \text{(S26)}$$

where:
$$-b_{LT} = 1 + K_1\{[T]_t - 3[LT_2] + [L]_t - [WL] - 2[WLT] - [W_2L] - x\} \quad \text{(S27)}$$

$$x = [L]/(1 - K_m[L])^2 - [L] \quad \text{(S28)}$$

[*LT*$_2$]

$$[LT_2] = \frac{-b_{LT_2} - \sqrt{b_{LT_2}^{\,2} - 16K_2^{\,2}([T]_t - [WLT] - [T])([T]_t - [LT] - [WLT])}}{8K_2} \quad \text{(S29)}$$

where:
$$-b_{LT_2} = 1 + 4K_2\{[T]_t - [WLT] - 0.5[T] - 0.5[LT]\} \quad \text{(S30)}$$

[*WLT*]

$$[LT_2] = \frac{-b_{WLT} - \sqrt{b_{WLT}^{\,2} - 4K_{1w}^{\,2}([T]_t - 2[LT_2] - [T])([W]_t - [WL] - 2[W_2L])}}{2K_{1w}} \quad \text{(S31)}$$

where:
$$-b_{WLT} = 1 + K_{1w}\{[T]_t - 2[LT_2] - [T] + [W]_t - [WL] - 2[W_2L]\} \quad \text{(S32)}$$

[*WL*]

$$[WL] = \frac{-b_{WL} - \sqrt{b_{WL}^{\,2} - 4K_{1wl}^{\,2}([W]_t - 2[W_2L] - [WLT])([L]_t - 2[W_2L] - x - [WLT] - [LT] - [LT_2])}}{2K_{1wl}} \quad \text{(S33)}$$

where:
$$-b_{WL} = 1 + K_{1wl}\{[W]_t - 3[W_2L] - 2[WLT] + [L]_t - x - [LT] - [LT_2]\} \quad \text{(S34)}$$

$$x = [L]/(1-K_m[L])^2 - [L] \quad \text{(S28)}$$

[W_2L]

$$[W_2L] = \frac{-b_{W_2L} - \sqrt{b_{W_2L}{}^2 - 16K_{2wl}{}^2([W]_t - [W] - [WLT])([W]_t - [WL] - [WLT])}}{8K_{2wl}} \quad \text{(S35)}$$

where:

$$-b_{W_2L} = 1 + 4K_{2wl}\{[W]_t - 0.5[W] - [WLT] - 0.5[WL]\} \quad \text{(S36)}$$

2. Competitive Binding Data

Table S1 Association constants and limiting complexation-induced chemical shift changes for the competitive association of 5-substituted tryptophan derivatives (**1a-h**) with DMPC (**2**)[a]

R	K_1 / M^{-1}	$\Delta\delta_1$ / ppm	$K_{1'}$ / M^{-1}	$\Delta\delta_{1'}$ / ppm	K_2 / M^{-1}	$\Delta\delta_2$ / ppm	K_{1w} / M^{-1}
OMe (**1a**)	73 ± 7	5.37	86 ± 8	5.67	232 ± 23	-0.11	35 ± 3
Me (**1b**)	66 ± 7	5.27	71 ± 7	5.25	123 ± 12	0.07	32 ± 3
H (**1c**)	60 ± 6	4.23	76 ± 8	4.80	48 ± 5	0.76	38 ± 4
F (**1d**)	99 ± 10	5.37	107 ± 11	5.02	64 ± 6	0.49	32 ± 4
Cl (**1e**)	188 ± 19	4.61	175 ± 17	4.58	85 ± 8	2.26	28 ± 3
Br (**1f**)	182 ± 18	4.53	162 ± 16	4.55	90 ± 9	0.96	27 ± 3
I (**1g**)	196 ± 20	4.83	204 ± 20	4.54	84 ± 8	1.36	31 ± 3
NO_2 (**1h**)	306 ± 43	4.66	248 ± 40	5.18	475 ± 47	3.66	25 ± 2

[a] Experiments were conducted in $CDCl_3$ at 20 °C. Association constants and limiting complexation-induced chemical shift changes ($\Delta\delta_1 = \delta_1 - \delta_f$; $\Delta\delta_2 = \delta_2 - \delta_f$; $\Delta\delta_{1'} = \delta_{1'} - \delta_f$) were calculated using non-linear least squares fitting of eqs S19-S36 to the experimental data assuming a competitive binding model (*i.e.* $K_{1w} \neq K_{1wl}$ and $K_1 \neq K_{1'}$ was permitted). Each value is the mean of duplicate experiments. Standard errors in $\Delta\delta$ are ± 10%. Errors in K are ± 10% or the value calculated by curve fitting if the latter was greater than the former.

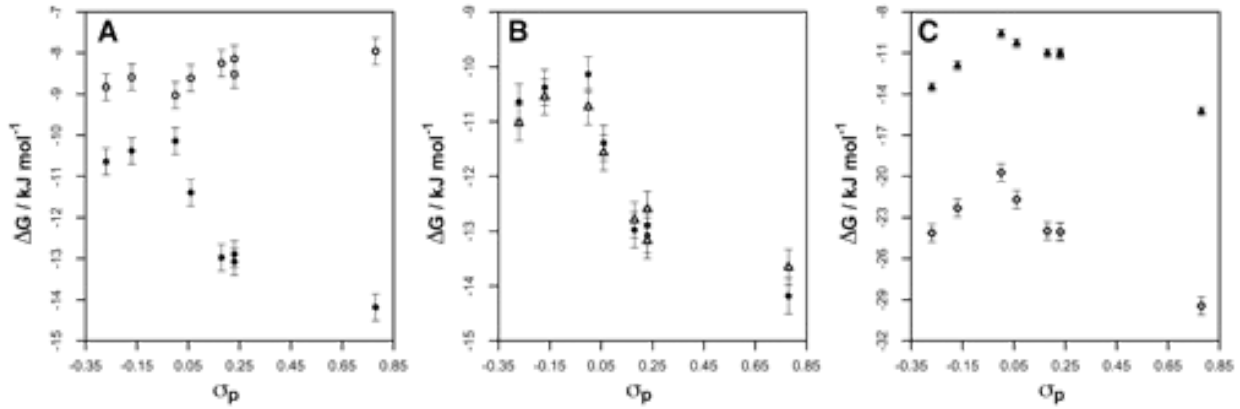

Figure S1 Free energy trends for the association of 5-substituted tryptophan derivatives (**1a-h**) with DMPC (**2**) in $CDCl_3$ at 20 °C as a function of the *p*-Hammett parameter (σ_P) of the 5-substituent. (A) ΔG_1 (●) and ΔG_{1w} (○). (B) ΔG_1 (●) and $\Delta G_{1'}$ (△). (C) ΔG_2 (▲) and $\Delta G_1+\Delta G_2$ (◇). The points are calculated from the data presented in Table S1.

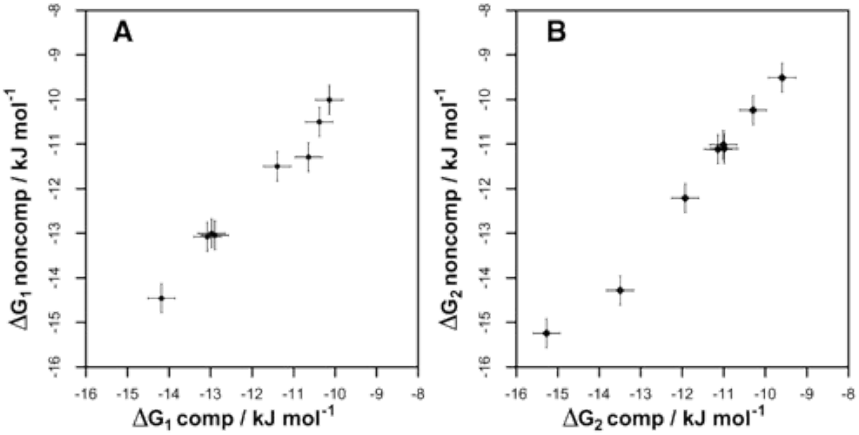

Figure S2 Correlations between values for ΔG_1 (A) and ΔG_2 (B) obtained from competitive and non-competitive binding models to fit the experimental data.

3. Chemical Shift Changes for the Indole NH

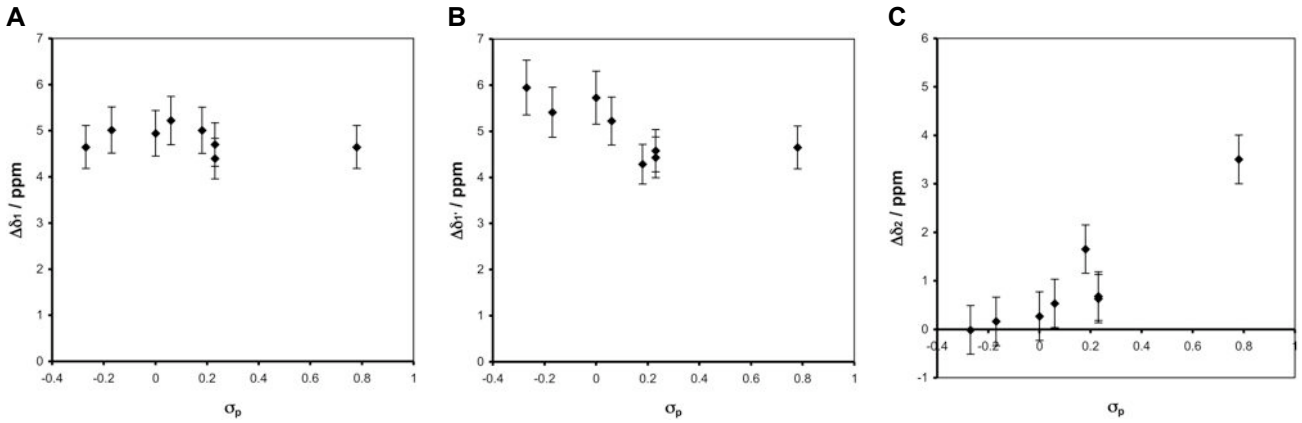

Figure S3 Complexation-induced chemical shift changes for the indole-NH using a non-competitive binding model ($K_{1w} = K_{1wl}$ and $K_1 = K_{1'}$ enforced). (A) $\Delta\delta_1$. (B) $\Delta\delta_{1'}$. (C) $\Delta\delta_2$. Errors in $\Delta\delta_1$ and $\Delta\delta_1$ are ± 10%. Errors in $\Delta\delta_2$ are estimated at ± 0.5.

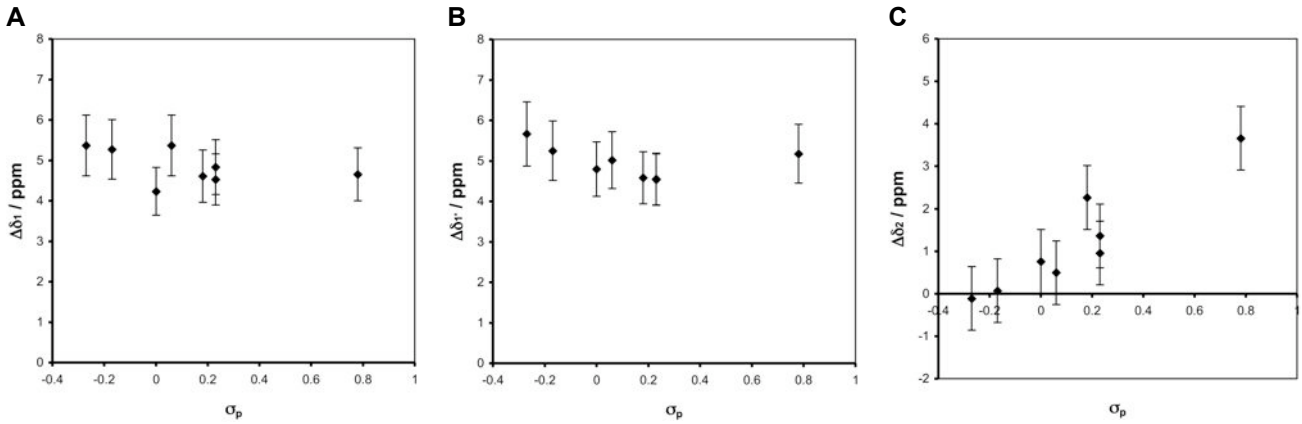

Figure S4 Complexation-induced chemical shift changes for the indole-NH using a competitive binding model ($K_{1w} \neq K_{1wl}$ and $K_1 \neq K_{1'}$ permitted). (A) $\Delta\delta_1$. (B) $\Delta\delta_{1'}$. (C) $\Delta\delta_2$. Errors in $\Delta\delta_1$ and $\Delta\delta_1$ are ± 10%. Errors in $\Delta\delta_2$ are estimated at ± 0.5.

4. Correlations with Resonance and Inductive Components of Hammett Parameter

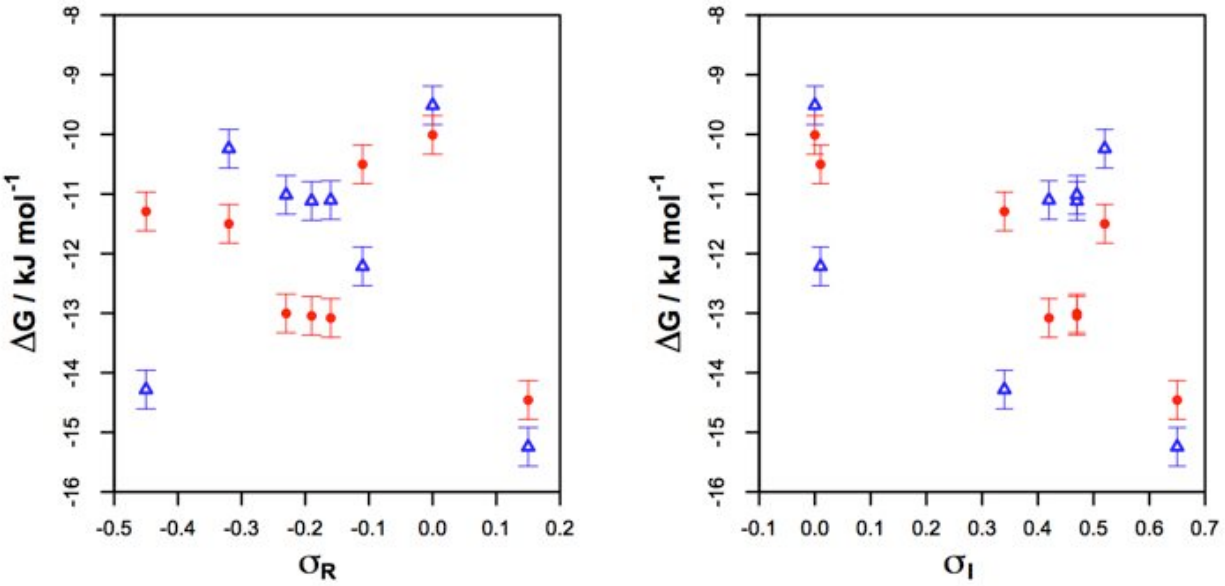

Figure S5 Free energy trends for the association of 5-substituted *L*-tryptophan derivatives (**1a-h**) with DMPC (**2**) in $CDCl_3$ at 20 °C as a function of the resonance (σ_R) and inductive (σ_I) components of the *p*-Hammett substituent parameter (σ_P) of the 5-substituent. The points are calculated from the data presented in Table 1 of the main paper, which assume non-competitive binding of **1a-h** and water to **2**, with ΔG_1 represented as red points (●) and ΔG_2 as open blue triangles (△).

5. Dynamics Trajectories

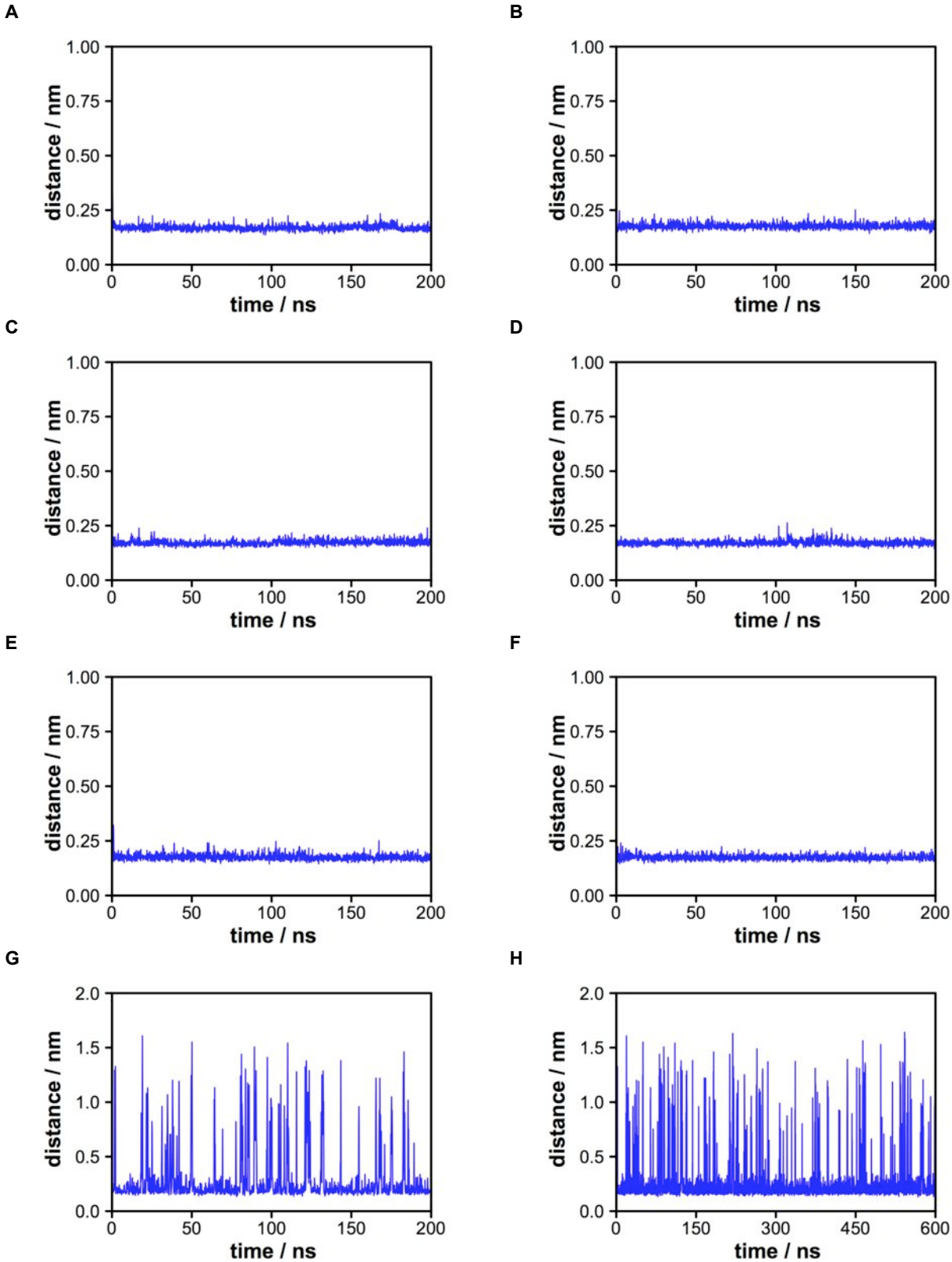

Figure S6 Molecular dynamics trajectories for simulation of tryptophan **1c** and DMPC **2** in $CHCl_3$. (A)-(F) correspond to six different starting configurations for *NpT* simulations at 300 K; (G) shows the first 200 ns of a 600 ns *NVT* simulation at 600 K; (H) shows the full trajectory corresponding to (G).

6. Torsional Distributions

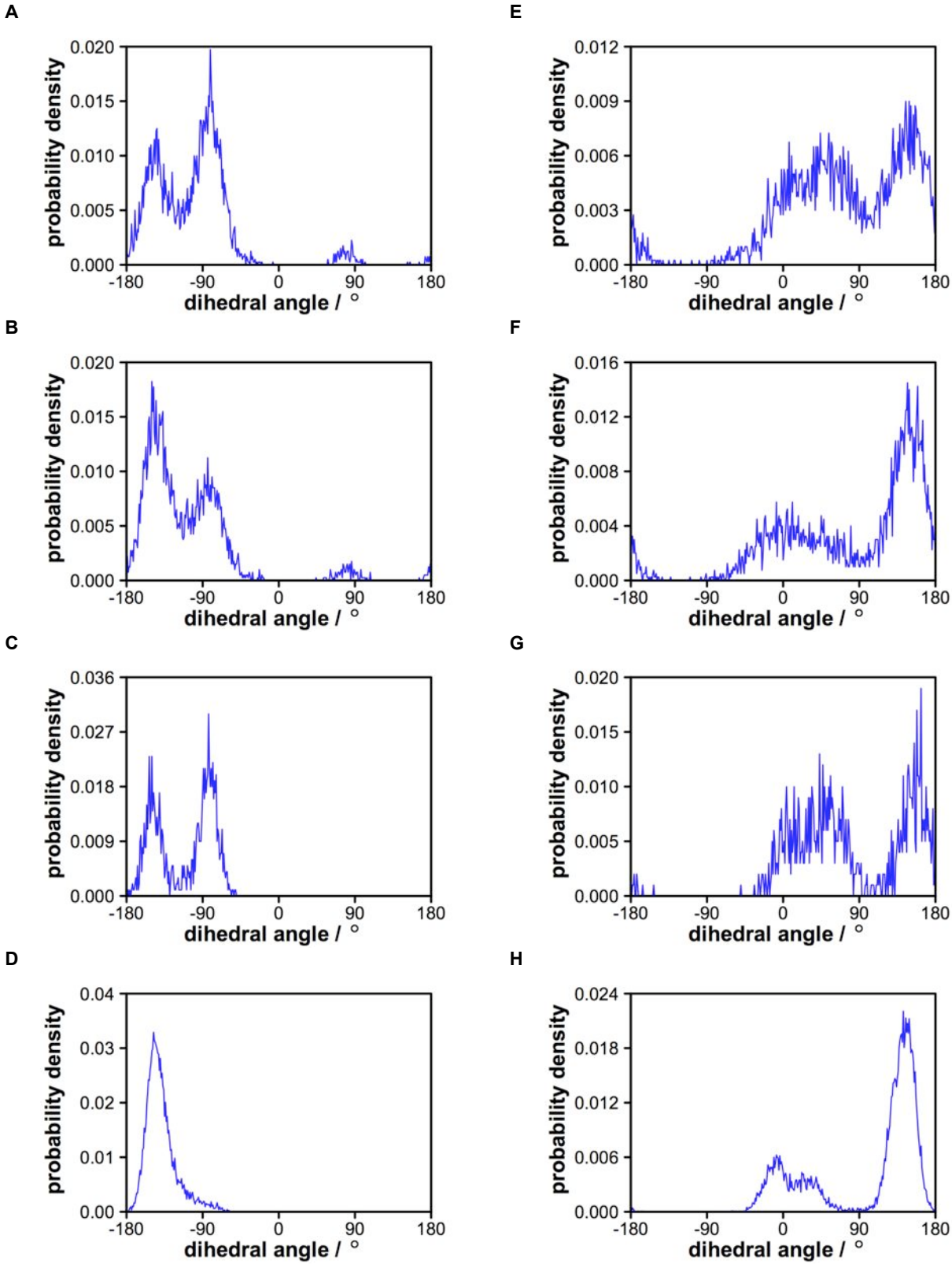

Figure S7 Torsional distributions for selected dihedrals of tryptophan **1c**. (A) Phi (ϕ) from an *NVT* simulation of **1c** in $CHCl_3$ at 600 K; (B) ϕ from an *NVT* simulation of **1c** and DMPC **2** in $CHCl_3$ at 600 K; (C) ϕ from an *NpT* simulation of **1c** in $CHCl_3$ at 300 K; (D) ϕ from an *NpT* simulation of **1c** and DMPC **2** in $CHCl_3$ at 300 K; (E)-(H) are the corresponding distributions for Psi (ψ).

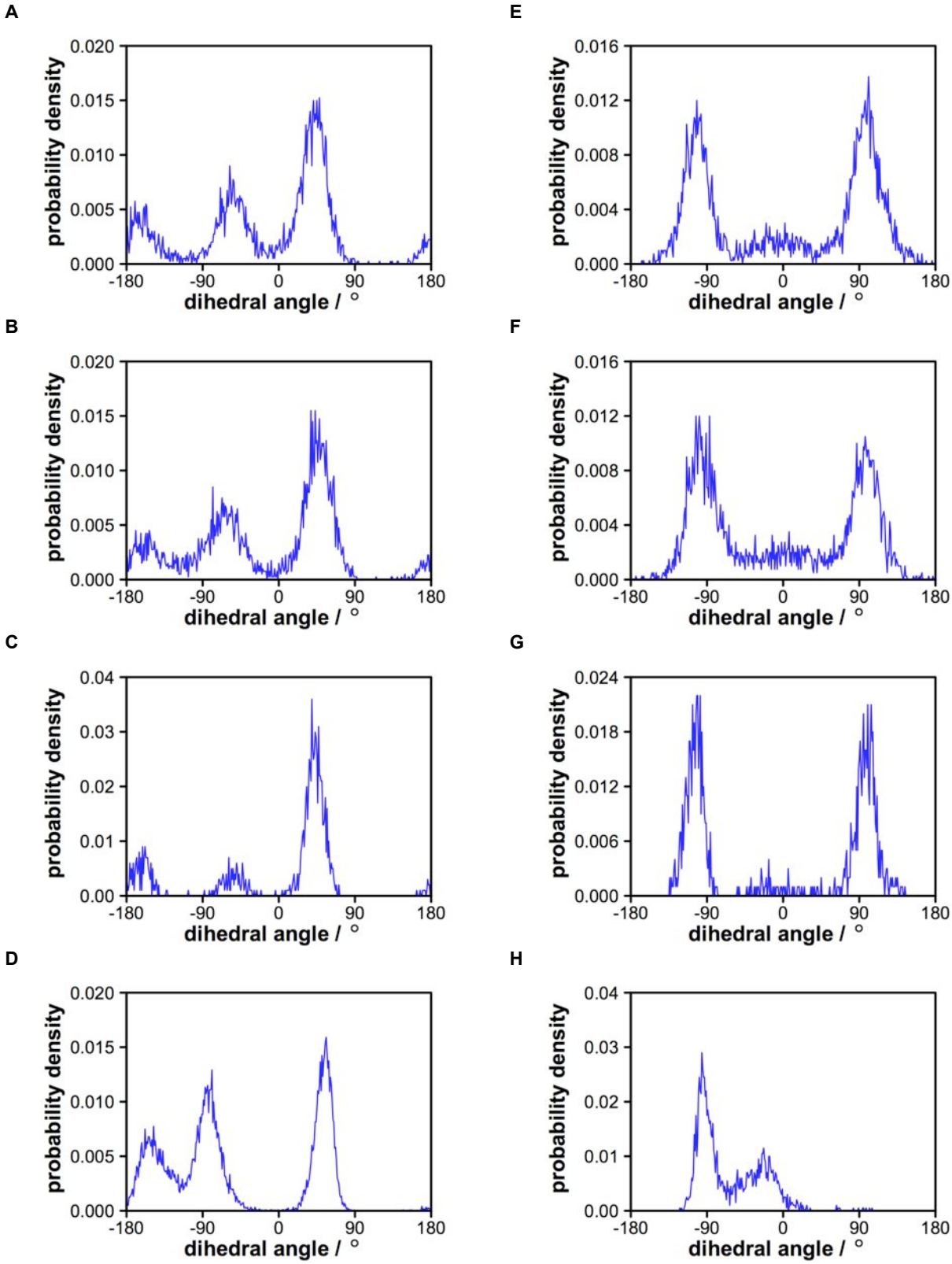

Figure S8 Torsional distributions for selected dihedrals of tryptophan **1c**. (A) Chi_1 (χ_1) from an *NVT* simulation of **1c** in $CHCl_3$ at 600 K; (B) χ_1 from an *NVT* simulation of **1c** and DMPC **2** in $CHCl_3$ at 600 K; (C) χ_1 from an *NpT* simulation of **1c** in $CHCl_3$ at 300 K; (D) χ_1 from an *NpT* simulation of **1c** and DMPC **2** in $CHCl_3$ at 300 K; (E)-(H) are the corresponding distributions for Chi_2 (χ_2).

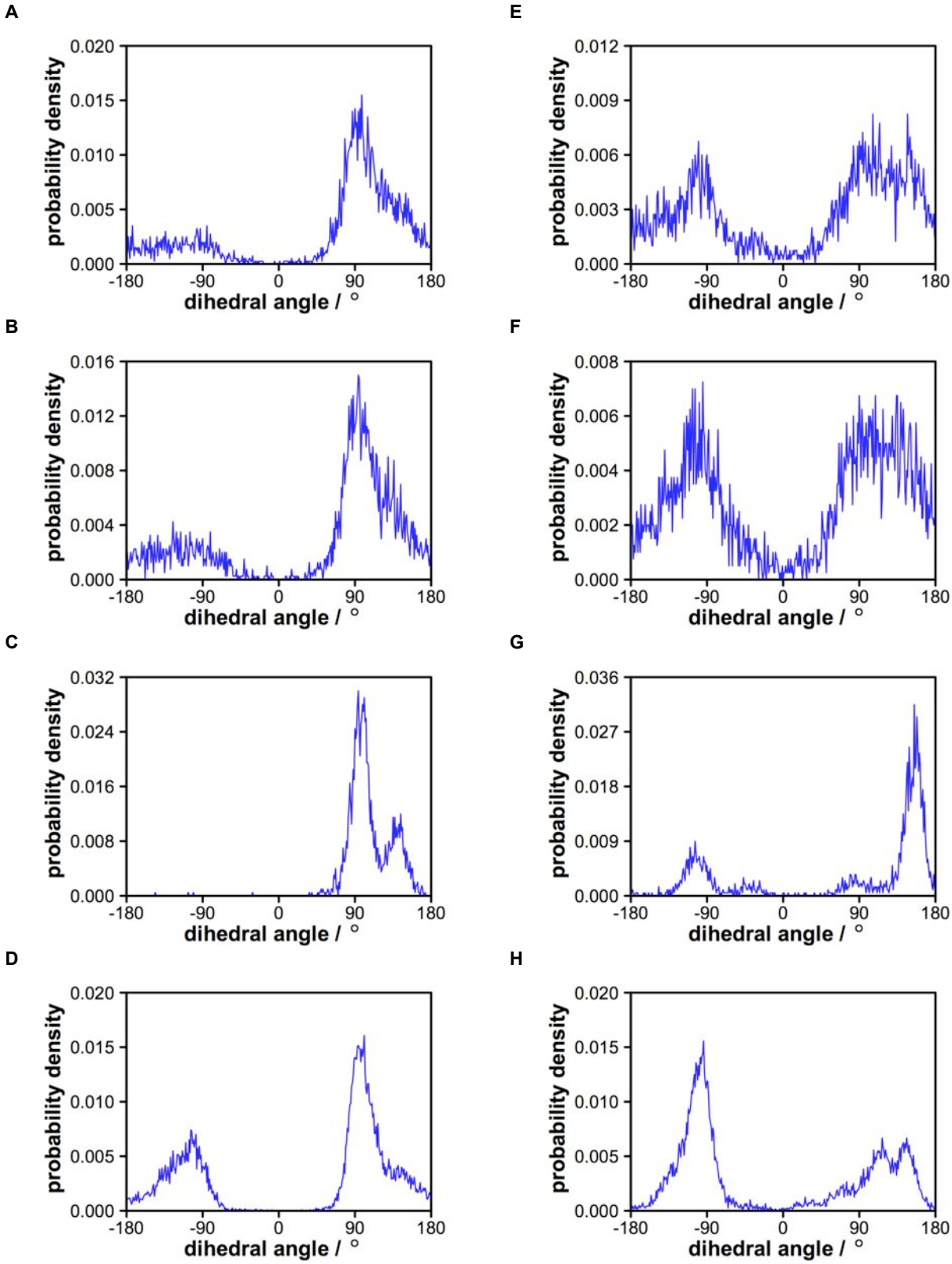

Figure S9 Torsional distributions for selected dihedrals of DMPC **2**. (A) α_2 from an *NVT* simulation of **2** in $CHCl_3$ at 600 K; (B) α_2 from an *NVT* simulation of **1c** and DMPC **2** in $CHCl_3$ at 600 K; (C) α_2 from an *NpT* simulation of **2** in $CHCl_3$ at 300 K; (D) α_2 from an *NpT* simulation of **1c** and DMPC **2** in $CHCl_3$ at 300 K; (E)-(H) are the corresponding distributions for α_3. {α_2 and α_3 are defined as in Marsh, D, *Protein Sci.*, **2003**, *12*, 2109; α_2 = atoms 12-11-8-7, α_3 = atoms 11-8-7-6, Fig. 1}.

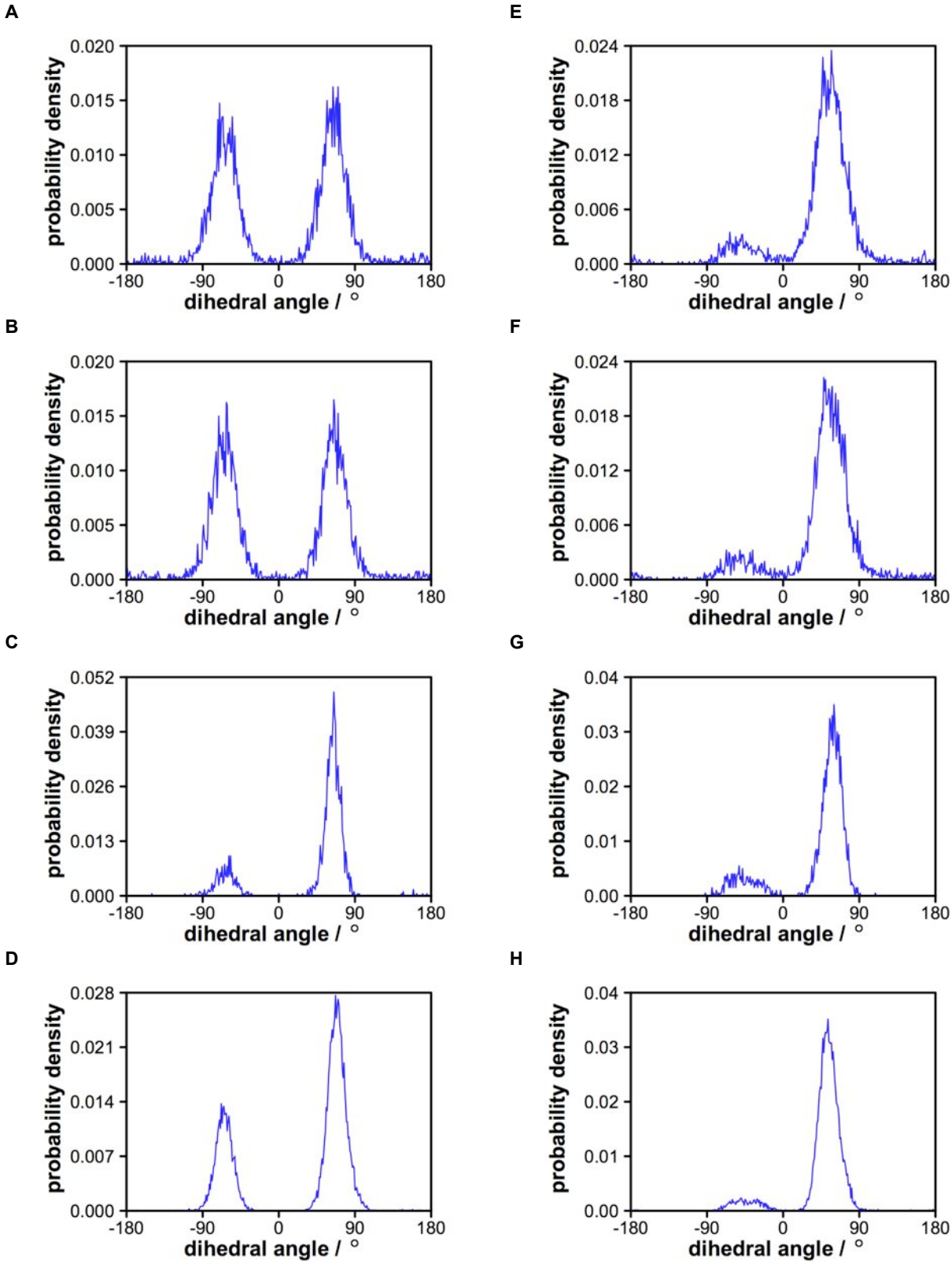

Figure S10 Torsional distributions for selected dihedrals of DMPC **2**. (A) α_5 from an *NVT* simulation of **2** in $CHCl_3$ at 600 K; (B) α_5 from an *NVT* simulation of **1c** and DMPC **2** in $CHCl_3$ at 600 K; (C) α_5 from an *NpT* simulation of **2** in $CHCl_3$ at 300 K; (D) α_5 from an *NpT* simulation of **1c** and DMPC **2** in $CHCl_3$ at 300 K; (E)-(H) are the corresponding distributions for θ_4. {α_5 and θ_4 are defined as in Marsh, D, *Protein Sci.*, **2003**, *12*, 2109; α_5 = atoms 7-6-5-4, θ_4 = atoms 18-17-13-14, Fig. 1}.

7. Radial Distribution Functions

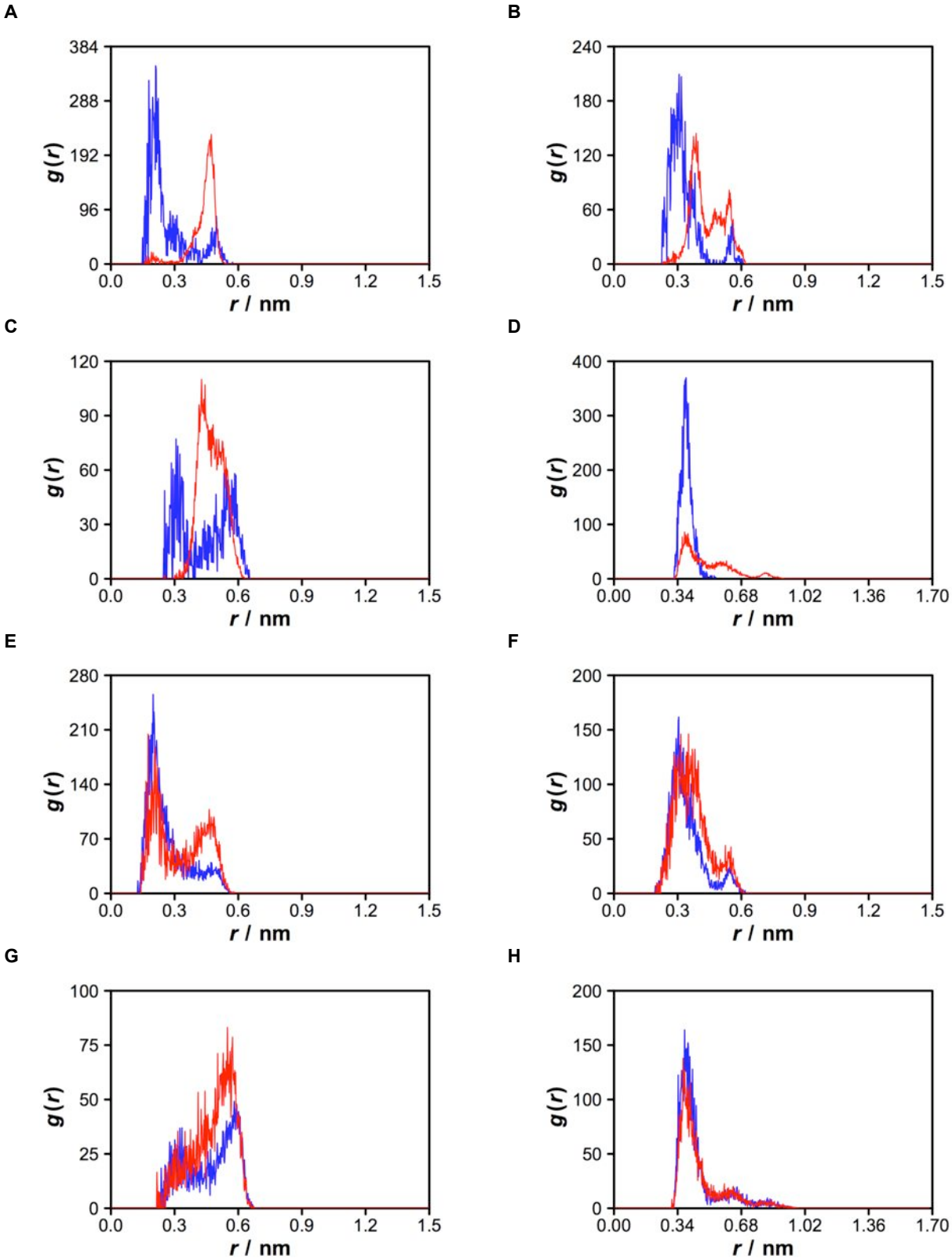

Figure S11 Radial distribution functions for groups of atoms from simulations in $CHCl_3$. (A) 78-52; (B) 54-(59,60,61,62,63); (C) 78-(59,60,61,62,63); (D) 16-(1,2,3,5). (A)-(D) are from *NpT* simulations at 300 K and correspond to Fig. 6 (A)-(D) in the main paper. Data from simulations of **1c** and **2** together are shown in red; data from corresponding simulations of individual molecules of **1c** are shown in blue. (E)-(H) are the corresponding plots for *NVT* simulations at 600 K.

8. Clustered Structures

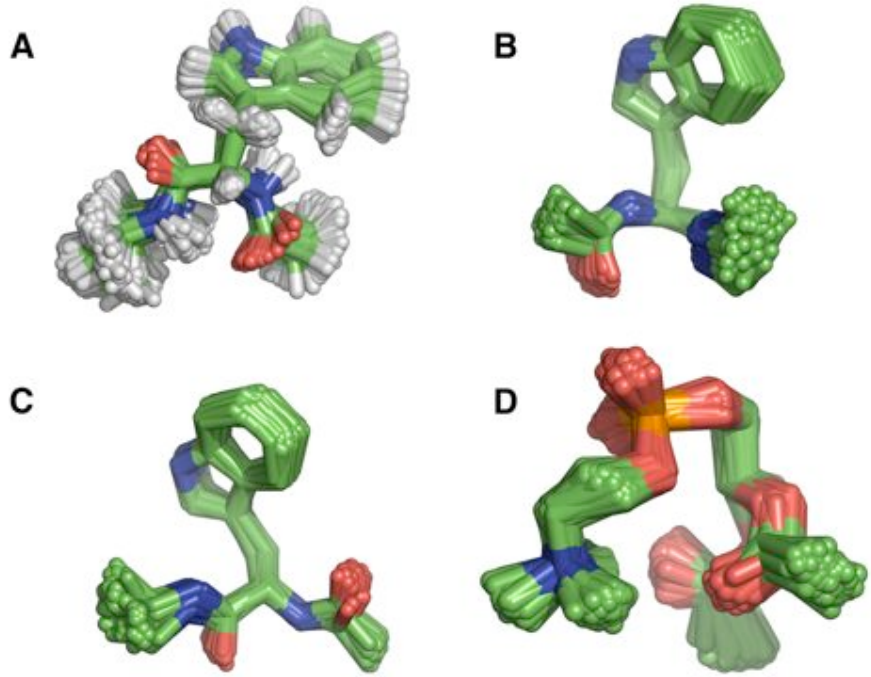

Figure S12 The most abundant conformations of N-acetyl tryptophan N-ethyl amide (**1c**) and DMPC (**2**) from constant-*NpT* simulations of single molecules of each in a chloroform box at 300 K. A, tryptophan derivative **1c** (153 structures); B, **1c** (107); C, **1c** (89 structures); D, DMPC (**2**, 308 structures). Some atoms of the acyl chains have been omitted for clarity.

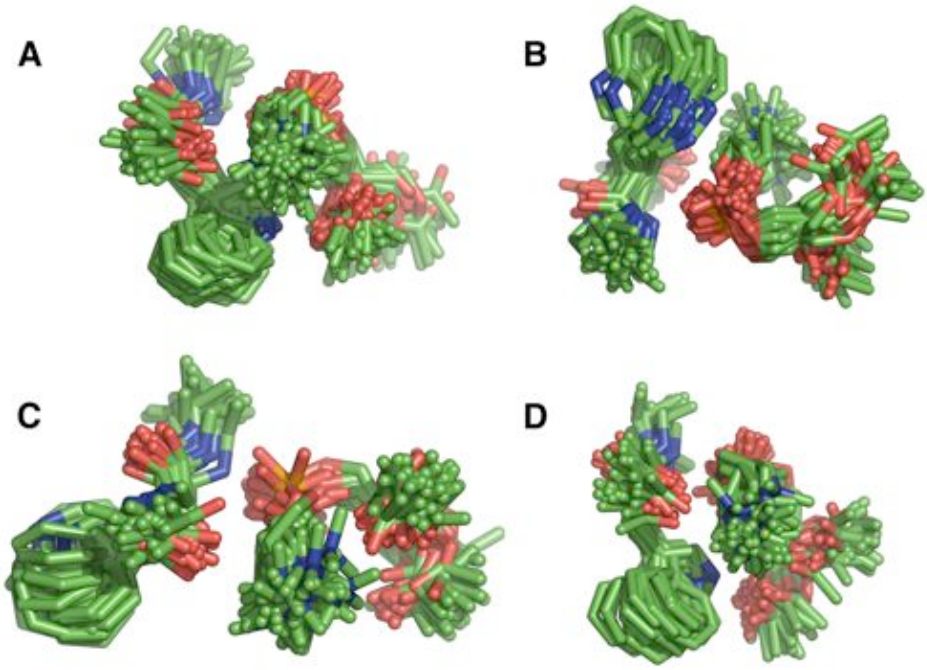

Figure S13 The four most abundant binding interactions from cluster analysis of all of the structures from a 600 ns *NVT* simulaton of tryptophan derivative **1c** and DMPC (**2**) in a chloroform box at 600 K.

9. 1:2 Lipid:Tryptophan Simulation Data

Table S2. Analysis of binding interactions during *NVT* simulations of 2 molecules of tryptophan derivative **1c** and DMPC (**2**) in chloroform at 600 K: tryptophan_1

Entry	Interacting Pair[a]	Cutoff / nm[b]	% time ≤ cut-off	% time as sole interaction[c]
1	20-19	0.35	2 ± 1	-
2	20-16	0.35	2 ± 2	-
3	20-(7,9,10,11)	0.435	16 ± 6	4
4	22-19	0.35	2 ± 2	-
5	22-16	0.35	5 ± 2	-
6	22-(7,9,10,11)	0.495	36 ± 8	4
7	24-19	0.35	5 ± 3	6
8	24-16	0.35	9 ± 4	8
9	24-(7,9,10,11)	0.385	14 ± 3	22
10	4-(Ar)	0.737	34 ± 7	1
11	4-(Py)	0.665	35 ± 8	<1
12	21-(1,2,3,5)	0.613	38 ± 11	10
13	23-(1,2,3,5)	0.539	18 ± 6	11

[a] atom numbers correspond to those in Figure 1. The centre of mass of the atoms grouped in parentheses was used for calculations.

[b] cutoff set to 0.35 nm for hydrogen bonding interactions, or determined from point of zero gradient in the radial distribution function of the corresponding interaction.

[c] the proportion of the time spent at a distance less than the cut-off during which this was the only intermolecular interaction.

Table S3. Analysis of binding interactions during *NVT* simulations of 2 molecules of tryptophan derivative **1c** and DMPC (**2**) in chloroform at 600 K: tryptophan_2

Entry	Interacting Pair[a]	Cutoff / nm[b]	% time ≤ cut-off	% time as sole interaction[c]
1	20-19	0.35	2 ± 2	-
2	20-16	0.35	2 ± 2	-
3	20-(7,9,10,11)	0.413	14 ± 6	2
4	22-19	0.35	2 ± 1	-
5	22-16	0.35	5 ± 3	-
6	22-(7,9,10,11)	0.503	38 ± 11	5
7	24-19	0.35	5 ± 3	4
8	24-16	0.35	9 ± 4	9
9	24-(7,9,10,11)	0.387	14 ± 4	20
10	4-(Ar)	0.765	37 ± 8	1
11	4-(Py)	0.693	39 ± 10	<0.1
12	21-(1,2,3,5)	0.585	39 ± 8	7
13	23-(1,2,3,5)	0.581	19 ± 7	10

[a] atom numbers correspond to those in Figure 1. The centre of mass of the atoms grouped in parentheses was used for calculations.

[b] cutoff set to 0.35 nm for hydrogen bonding interactions, or determined from point of zero gradient in the radial distribution function of the corresponding interaction.

[c] the proportion of the time spent at a distance less than the cut-off during which this was the only intermolecular interaction.

10. 1:2 Lipid:Tryptophan Simulation - Clustering

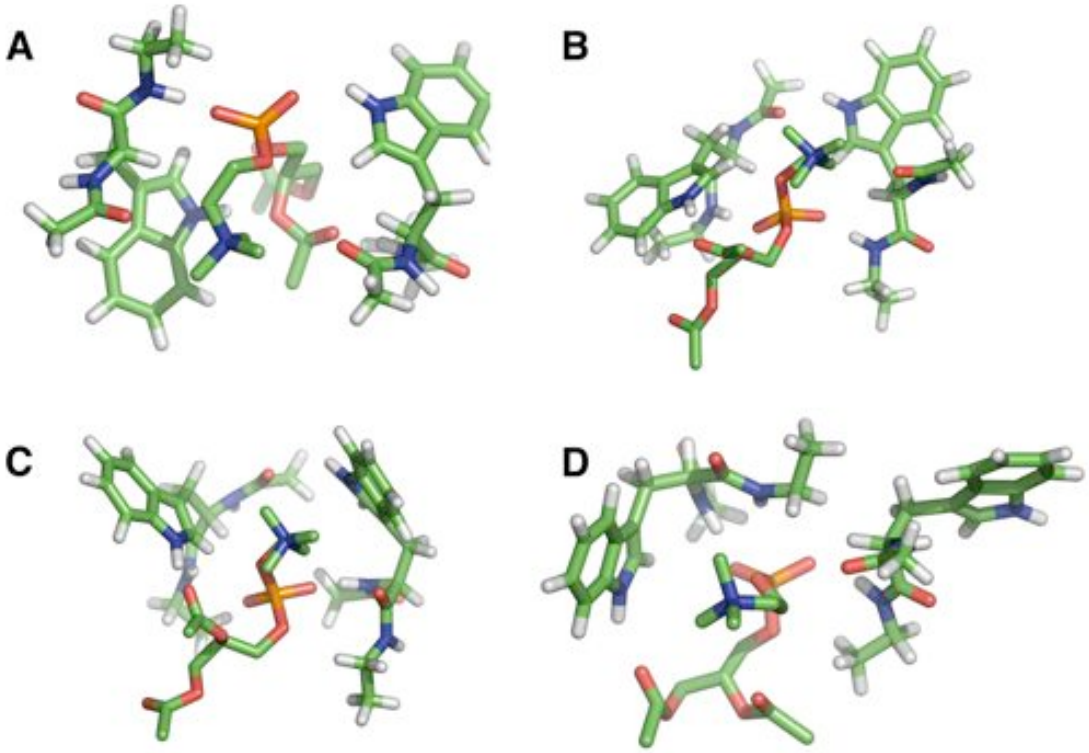

Figure S14 The four most abundant binding interactions from cluster analysis of all of the structures from a 600 ns *NVT* simulaton of 2 molecules of tryptophan derivative **1c** and DMPC (**2**) in a chloroform box at 600 K. The median structure is shown in each case. The numbers of structures per cluster are 9, 8, 7 and 6 for a), b), c) and d) respectively.