Supplementary Material for:

Characterization of Lipid-Binding Site of Equinatoxin II by NMR and Molecular Dynamics Simulation

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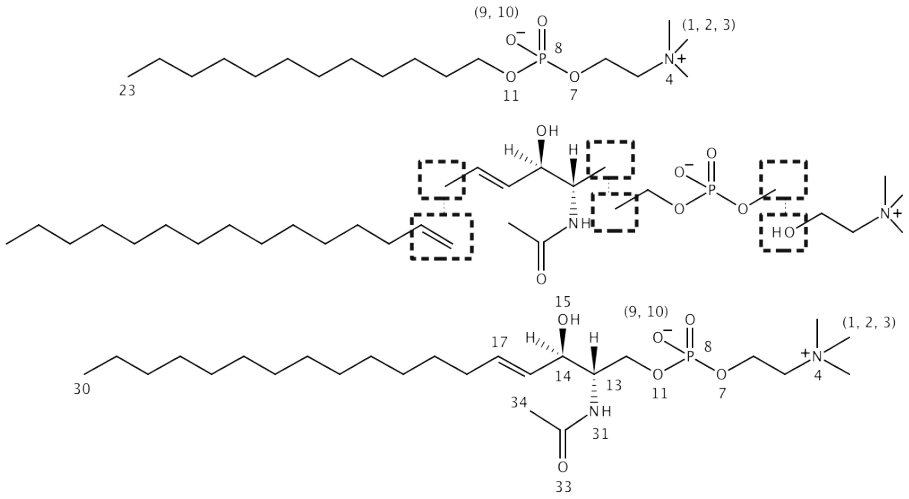

Figure S1: DPC schematic and atom nomenclature (upper). Fragment-based RESP derivation scheme used to for Ac-SM (lower).

Table S1: RESP charges and assigned GAFF/GAFFlipid atom types for DPC.

Name	Type	Charge	Name	Type	Charge
C1-C3	c3	-0.1438	H15 [AIB]	hl	-0.0121
[H1-H3] [AIBIC]	hc	0.1235	C16	a3	0.0351
N4	n4	0.029	H16 [AIB]	hl	-0.0099
C5	c3	-0.036	C17	a3	0.0113
H5 [AIB]	hc	0.1324	H17 [AIB]	hl	-0.0043
C6	c3	0.1414	C18	a3	0.0169
H6 [AIB]	hc	0.0548	H18 [AIB]	hl	-0.0092
O7	os	-0.5766	C19	a3	0.0178
P8	p5	1.2266	H19 [AIB]	hl	-0.0101
O9, O10	o	-0.797	C20	a3	0.0105
O11	os	-0.5059	H20 [AIB]	hl	-0.0073
C12	a3	0.1847	C21	a3	0.0064
H12 [AIB]	hl	0.0059	H21 [AIB]	hl	0.0002
C13	a3	0.0229	C22	a3	0.038
H13 [AIB]	hl	0.0324	H22 [AIB]	hl	-0.0042
C14	a3	-0.0182	C23	a3	-0.0775
H14 [AIB]	hl	-0.0047	H23 [AIBIC]	hl	0.0153
C15	a3	0.0138			

Table S2: RESP charges and assigned GAFF/GAFFlipid atom types for Ac-SM.

Name	Type	Charge	Name	Type	Charge
C1-C3	c3	-0.143	H20 [AIB]	hl	-0.0092
[H1-H3] [AIBIC]	hc	0.1231	C21	a3	0.0255
N4	n4	0.0315	H21 [AIB]	hl	-0.0108
C5	c3	-0.0363	C22	a3	0.0158
H5 [AIB]	hc	0.1329	H22 [AIB]	hl	-0.009
C6	c3	0.1258	C23	a3	0.0158
H6 [AIB]	hc	0.0594	H23 [AIB]	hl	-0.009
O7	os	-0.5005	C24	a3	0.0255
P8	p5	1.2328	H24 [AIB]	hl	-0.0108
O9, O10	o	-0.8009	C25	a3	0.0183
O11	os	-0.5146	H25 [AIB]	hl	-0.0086
C12	c3	0.2501	C26	a3	0.0158
H12 [AIB]	hc	0.0001	H26 [AIB]	hl	-0.009
C13	c3	-0.03	C27	a3	0.0158
H13A	hc	0.135	H27 [AIB]	hl	-0.009
C14	c3	0.1385	C28	a3	0.003
H14A	hc	0.0925	H28 [AIB]	hl	0
O15	oh	-0.6264	C29	a3	0.0442
H15A	ho	0.4203	H29 [AIB]	hl	-0.0055
C16	c2	-0.1899	C30	a3	-0.0801
H16A	hc	0.1871	H30 [AIBIC]	hl	0.0152
C17	c2	-0.1627	N31	n	-0.5588
H17A	hc	0.1244	H31A	hn	0.2854
C18	a3	-0.0446	C32	c	0.6949
H18 [AIB]	hl	0.0394	O33	o	-0.5892
C19	a3	0.0278	C34	a3	-0.3083
H19 [AIB]	hl	0.0012	H34 [AIBIC]	hl	0.0903
C20	a3	0.0178			

Table S3: Additional simulation details

Simulation	Atoms	Waters	Ions	Time (ns)
EqtII only [a]	28137	8465	7 Cl^-	100
Ac-SM_{54} [b]	27633	7681	-	60
EqtII + DPC_{54}	44247 / 60423	12737 / 18129	7 Cl^-	220 / 200
EqtII + Ac-SM_{54}	57681 / 84723	16783 / 25797	7 Cl^-	220 / 200

[a] Chain A of PDB 1IAZ was used as the starting structure and solvated with 11 Å truncated octahedron. Simulations were run as described in text (9 Å cutoff used).

[b] Starting configuration was an interdigitated bilayer of Ac-SM units hydrated with an 8 Å shell of TIP3P water. Simulations were run as described in text (12 Å cutoff used).

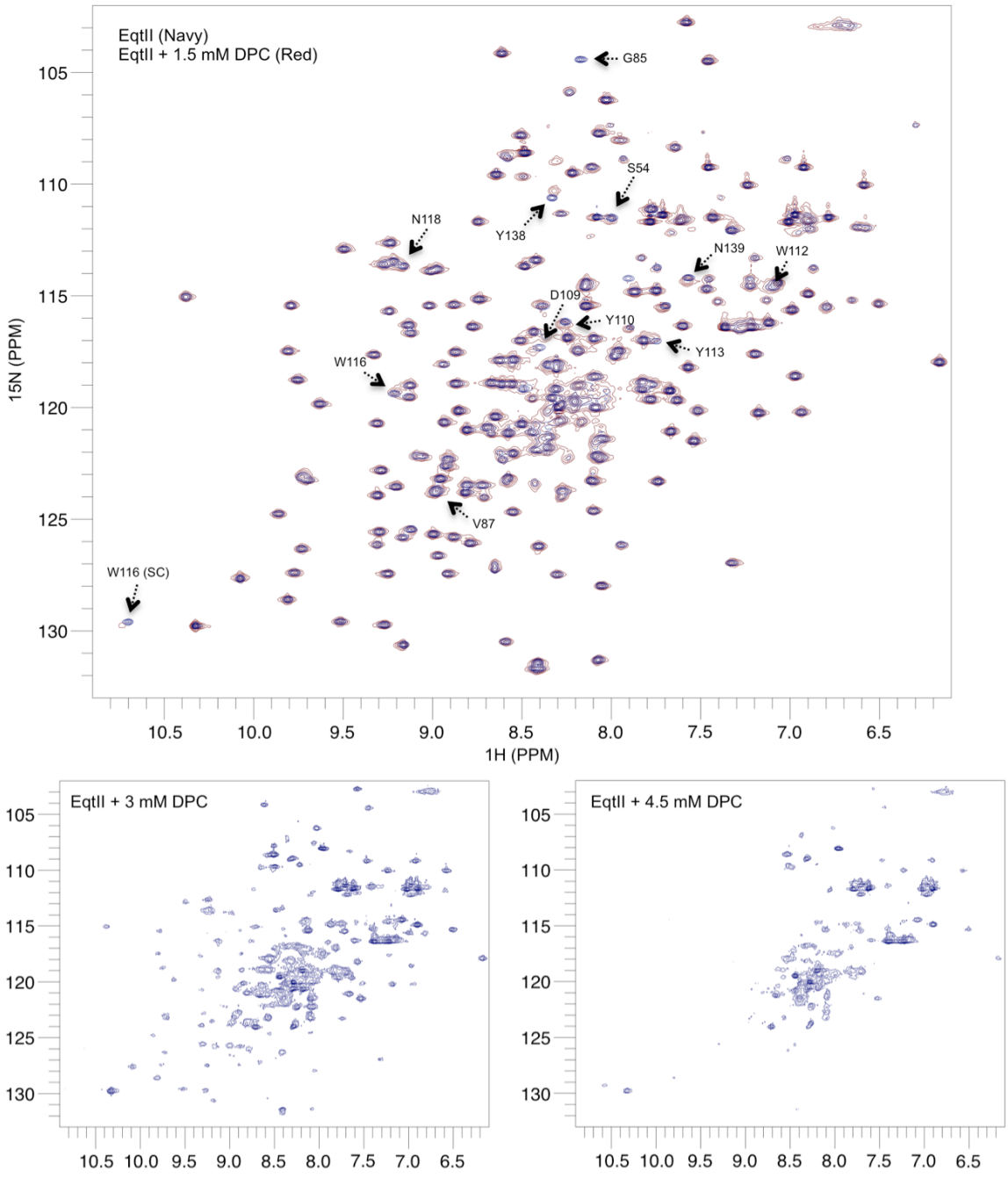

Figure S2: ^{1}H-^{15}N HSQC spectra of 0.3 mM EqtII in buffer (10 mM sodium phosphate, 20 mM NaCl, 0.02% NaN_3, 10% D_2O, pH 5.0) show specific perturbations to loop β3-β4 (S54), strand β6 (G85, A87), loop β7-β8 (D109, Y110, W112, Y113), strand β8 (W116, W116 Nε/Hε, N118) and helix α2 (Y138, N139) regions at the CMC of DPC (*upper*). DPC concentration exceeding the CMC caused considerable reduction in spectral quality (*lower*).

Force field comparisons

Table S4: Force-field comparison of dihedral parameters for selected DPC atoms as measured by relative trans population (%)[a].

Selected Atoms	AMBER99SB (Abel et al.)	Amber99SB[b]	GAFF[b]	GAFFlipid[b]
N4 C5 C6 O7	17.8	30.3	20.6	21.3
C5 C6 O7 P8	83.6	87.6	94.2	94.8
C6 O7 P8 O11	9.0	21.9	58.6	56.7
O7 P8 O11 C12	6.0	25.3	65.1	80.5
P8 O11 C12 C13	12.0	93.4	91.4	90.8
O11 C12 C13 C14	70.0	51.3	49.1	65.2
C12 C13 C14 C15	-	79.6	80.8	74.3
C13 C14 C15 C16	-	81.5	79.7	73.9
C14 C15 C16 C17	-	84.8	81.0	75.6
C15 C16 C17 C18	-	85.5	80.9	73.6
C16 C17 C18 C19	-	86.9	82.3	74.7
C17 C18 C19 C20	-	86.8	81.7	74.0
C18 C19 C20 C21	-	86.1	82.6	74.0
C19 C20 C21 C22	-	83.3	80.8	73.1
C20 C21 C22 C23	-	79.5	76.4	68.3
C C C C	84.3	83.8	80.7	73.5

[a] Dihedral angles were measured for each DPC residue over the final 30 ns (3000 snapshots) of a 40 ns trajectory. Trans dihedral angles were defined as occurring between -180° to -90° and 90° to 180°.

[b] Simulations were prepared by solvating an equilibrated DPC micelle in an 8 Å shell of TIP3P water. Production runs utilized the same conditions as defined in the main text. The system consisted of 17778 total atoms and 4828 water molecules.

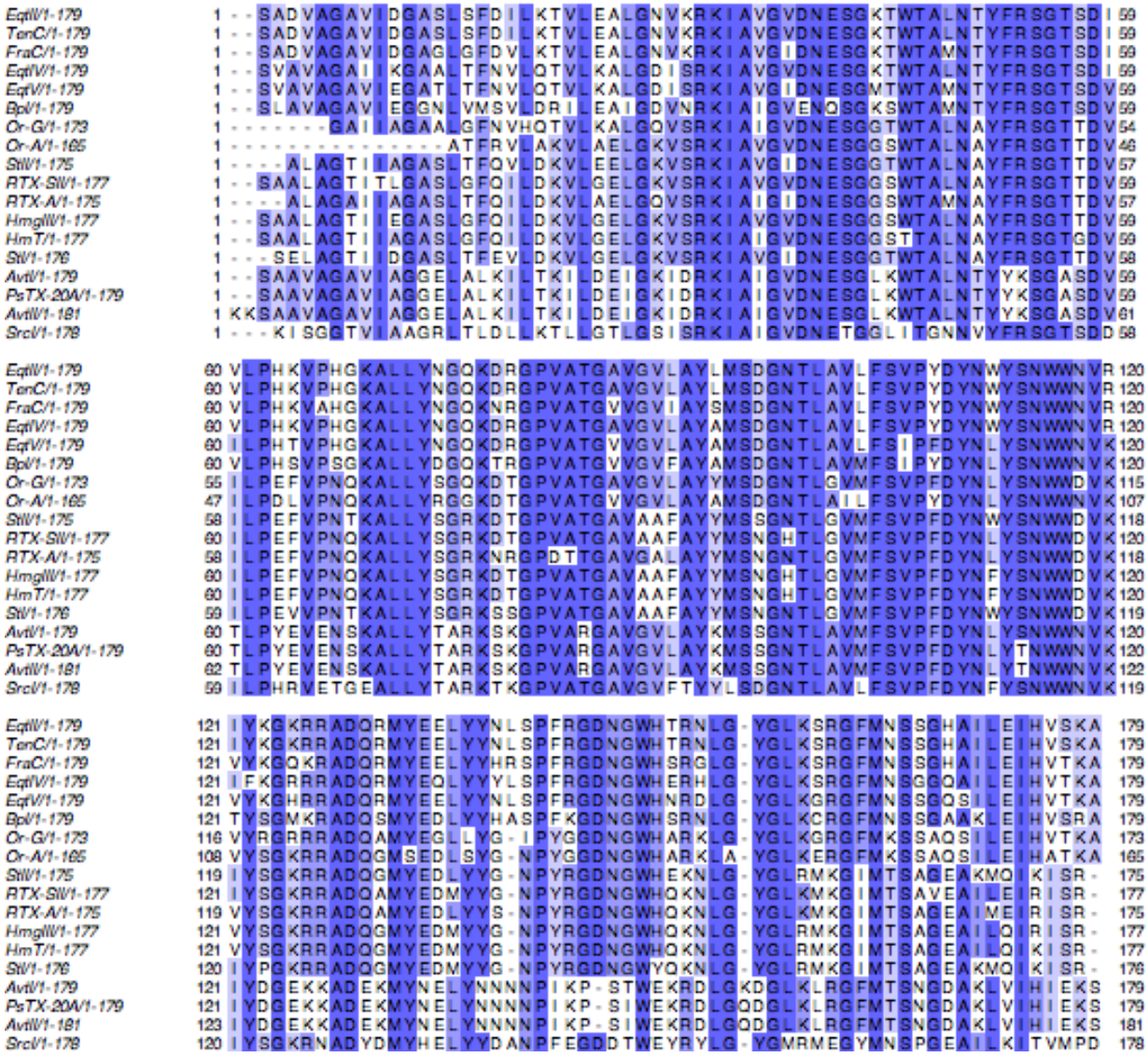

Figure S3: Actinoporin protein sequence alignments. Uniprot accession codes and sequence identities relative to EqtII are detailed in the main text. Alignments were performed using the Clustal Omega webserver. The figure was created using Jalview 2.8.1 with highly conserved residue color-shaded according to conservation.

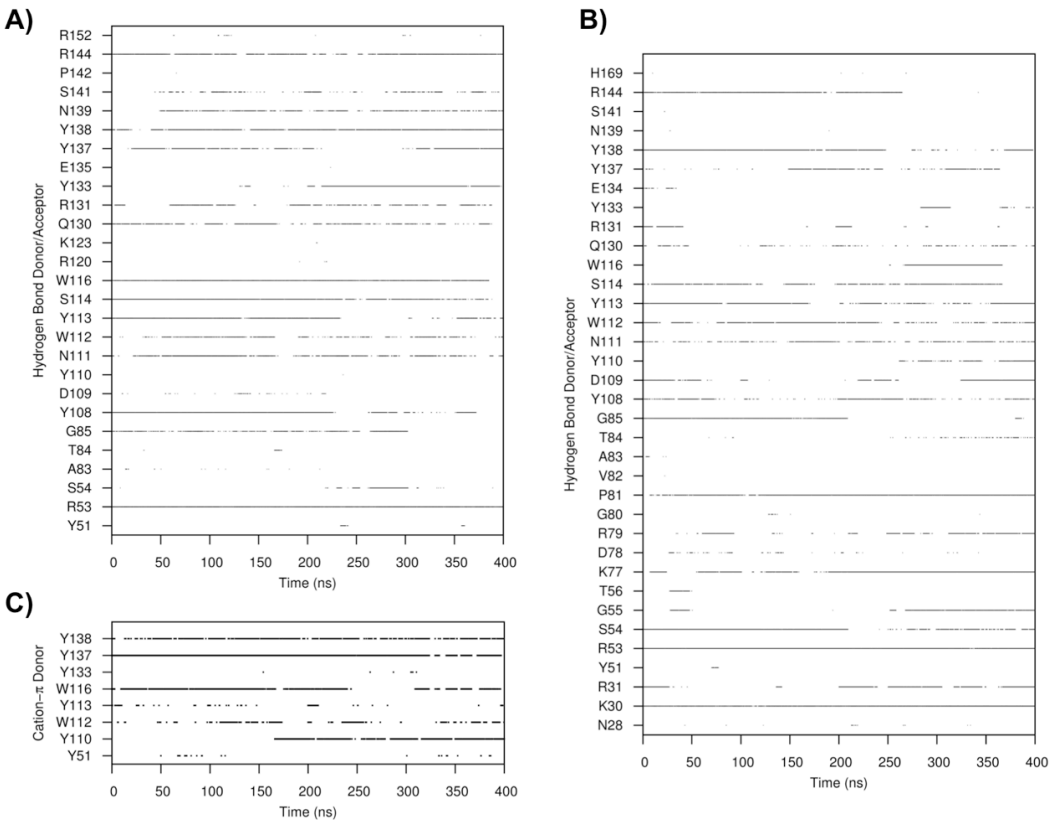

Figure S4: Per-residue time evolution profiles of H-bonds to (A) DPC, and (B) Ac-SM residues; and (C) cation-π interactions to Ac-SM residues. Trajectories were analyzed over 40000 frames.

Size estimates of DPC and Ac-SM

Dynamic light scattering measurements were made using a Malvern Zetasizer Nano ZS in 173° backscattering mode. Triplicate readings for Ac-SM were made at 2, 4, 8, 16, 64, 128 µM in water. DPC was measured at 128 mM. All readings were made at 25 °C and analyses using manufacturer's software. The reported hydrodynamic radius of Ac-SM was averaged over all measurements over 4 µM, where signal quality was adequate.

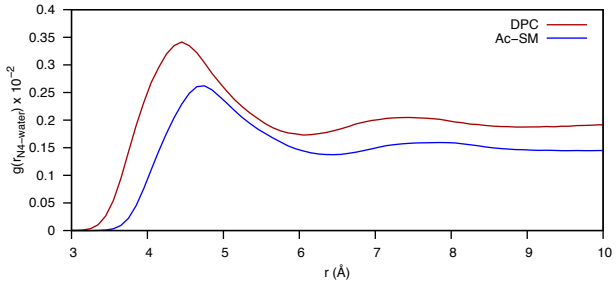

Figure S5: Radial pair distribution of water to the choline nitrogen of DPC and Ac-SM.