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Supplementary information

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Molecular basis for the regulation of membrane proteins through preferential lipid solvation

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Supplementary Table 1. Number of lipids to visit the first solvation shell of a CLC-ec1 monomer. The first solvation shell is parsed into regions adjacent to the dimerization or non-dimerization interface. Data is taken from an all-atom molecular dynamics simulation of the CLC-ec1 monomer. Numbers shown include repeat visits from the same lipid and the events quantified are used to compute exchange probabilities and mean dwell times shown in Supp. Tables 2 and 3.

	Dimer		**Non-dimer**	
	PO	**DL**	**PO**	**DL**
outer leaflet	1109	617	3076	1439
inner leaflet	1180	617	2735	1260

Supplementary Table 2. Relative lipid exchange probability. The relative exchange probability is defined as the observed probability that DLPC is the replacing lipid subtracting the expected unbiased probability i.e., 0.3. The standard error of the mean is also provided where n=3.

	First Shell		Second Shell	
	Dimer	**ND1/2**	**Dimer**	**ND1/2**
POPC	0.037 ± 0.003	0.01 ± 0.01	0.01 ± 0.01	0.005 ± 0.005
DLPC	0.07 ± 0.02	0.027 ± 0.004	0.034 ± 0.006	0.025 ± 0.002
All	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.012 ± 0.002

Supplementary Table 3. Average lipid residence time. Average lipid residence times (ns) and the standard error of the mean is reported for first and second shell lipids at each interface.

	First Shell		Second Shell	
	Dimer	**Non-dimer**	**Dimer**	**Non-dimer**
POPC	119 ± 1	154 ± 3	59.2 ± 0.6	70.6 ± 0.5
DLPC	127 ± 4	145 ± 6	56.6 ± 0.7	64.3 ± 0.9

Supplementary Table 4. Estimated contributions to the DLPC enrichment factor. The estimated DLPC enrichment factor computed from lipid dynamics data. Note that the subscripts ex and $\bar{t}$ refer to estimates made considering exchange probabilities or mean dwell times only.

	First Shell		Second Shell	
	Dimer	**Non-dimer**	**Dimer**	**Non-dimer**
E_{ex} **(%)**	26 ± 6	12 ± 4	10 ± 5	6 ± 1
$E_{\bar{t}}$ **(%)**	7 ± 5	-6 ± 6	-4 ± 2	-9 ± 2
E **(%)**	35 ± 12	5 ± 7	5 ± 5	-3.8 ± 0.8

Supplementary table 5. Specifications for the molecular system considered in all-atom molecular dynamics simulations of a CLC-ec1 monomer. The molecular composition is provided in addition to the initial box dimensions and the simulation length.

Protein	POPC	DLPC	Na^+	Cl^-	H_20	System size (nm)	Simulation time (μs)
1	446	191	69	72	25,955	15.1 x 15.1 x 7.0	41.3

Supplementary table 6. Multi staged equilibration protocol for all-atom molecular dynamics simulations of the CLC-ec1 monomer. A total of ten equilibration steps were carried out where restraints were gradually reduced.

step[a]	1	2	3	4	5	6	7	8	9	10
Equilibration time (ns)	0.2	0.2	0.2	0.2	0.2	1	4	10	20	20
positional restraints (kcal/mol/Å^2)[b]	60	15	4	-	-	-	-	-	-	-
positional restraints (kcal/mol/Å^2)[c]	60	60	15	4	1	-	-	-	-	-
positional restraints (kcal/mol/Å^2)[d]	60	60	15	4	1	-	-	-	-	-
Distance restraints (kcal/mol/Å^2)[e]	-	-	-	60	60	60	60	60	60	60
RMSD restraint (kcal/mol/Å^2)[f]	-	-	-	45	45	45	45	45	4	1

Distance restraints with ions[e]	
ion 1	**Ion 2**
S107 (OG)	S107 (OG)
S107 (CB)	S107 (CB)
Y445 (OH)	S107 (N)
G149 (N)	G106 (C)
G355 (CA)	G105 (O)
F348 (CE1)	I448 (CG2)
G354 (O)	I448 (CD)
I356 (N)	Y100 (CG)
-	P110 (CD)
-	F348 (CZ)

[a] The protocol is applied using NAMD prior to running simulations on Anton2. The restraints described here are harmonic in nature, where the force constant is listed at each step.
[b] Harmonic position restraints applied to the protein side chain atoms.
[c] Harmonic position restraints applied to protein backbone atoms.
[d] Harmonic position restraints applied to the two bound chlorine ions.
[e] The distance between the two bound chloride ions and select atoms of the protein are maintained with harmonic restraints. The force constant for each is provided as well as the residue and atom type restrained to each ion.
[f] A harmonic restraint is applied to the RMSD, which is calculated over different parts of the protein for each step. Steps 4 and 5 compute the RMSD of all atoms excluding hydrogen. Step 6 focuses on the backbone atoms while step 7 refines the selection to transmembrane backbone atoms. Steps 8, 9, and 10 focus on transmembrane alpha carbons alone.

Supplementary table 7. Harmonic restraints employed at the dimerization interface in all-atom molecular dynamics simulations of the CLC-ec1 monomer. Since we are especially concerned with lipid solvation around the dimerization interface, harmonic restraints are added to better maintain structural integrity here.

Restraint	Res id	atom	Res id	atom	Distance (Å)	Force constant (kcal/mol/Å)
1	E113	Carboxyl-C	E203	Carboxyl-O	3.185	20
2	E202	Carboxyl-C	L406	BB-N	3.463	20
3	E414	Carboxyl-C	L194	BB-N	3.419	20

Supplementary Table 8. Number of molecules simulated for each FEP transformation. Molecules undergoing transformation are specified as POPC_to_DLPC*.

Molecule	0-1 % DLPC	1-10 % DLPC	10-20 % DLPC	20-30 % DLPC
Protein	2	2	2	2
POPC	1,082	984	874	764
POPC_to_DLPC*	10	98	110	110
DLPC*	0	10	108	218
W	14,552	14,552	14,552	14,552
WF	1,616	1,616	1,616	1,616
Na^+	172	172	172	172
Cl^-	185	185	185	185

Supplementary Table 9. Force field parameters for DLPC*.

Bonding Terms		
ATOMS	**LENGTH (NM)**	**FORCE CONSTANT (KJ MOL^{-1})**
NC3-PO4	0.47	1250
PO4 -GL1	0.47	1250
GL1-GL2	0.37	1250
GL1-C1A	0.47	1250
C1A-D2A	0.47	1250
D2A-C3A	0.47	1250
C3A-C4A	0.47	1250
GL2-C1B	0.47	1250
C1B-C2B	0.47	1250
C2B-C3B	0.47	1250
C3B-C4B	0.47	1250
Angle Terms		
ATOMS	**ANGLE (°)**	**FORCE CONSTANT (KJ MOL^{-1} RAD^{-2})**
PO4-GL1-GL2	120	25
PO4-GL1-C1A	180	25
GL1-C1A-D2A	180	25
C1A-D2A-C3A	180	25
D21-C3A-C4A	180	25
GL2-C1B-C2B	180	25
C1B-C2B-C3B	180	25
C2B-C3B-C4B	180	25

Supplementary Table 10. λ values used in FEP simulations. A total of 38 replicas was used for the 0 → 1 % and 93 for the remaining transformation.

Monomers/Dimer (0-1 % DLPC)										
	1's	10's	20's	30's	40's	50's	60's	70's	80's	**90's**
0	0	0.3	0.48	0.625						
1	0.0333									
	3	0.325	0.49	0.65						
2	0.0666									
	7	0.35	0.5	0.675						
3	0.1	0.375	0.5125	0.7						
4	0.1333									
	3	0.4	0.525	0.75						
5	0.1666	0.4166								
	7	7	0.5375	0.8						
6		0.4333								
	0.2	3	0.55	0.9						
7			0.5666							
	0.225	0.45	7	1						
8			0.5833							
	0.25	0.46	3							
9	0.275	0.47	0.6							
Monomers/Dimer (1-10 % DLPC, 10-20 % DLPC, 20-30 % DLPC)										
	1's	**10's**	**20's**	**30's**	**40's**	**50's**	**60's**	**70's**	**80's**	**90's**
0	0	0.11111	0.22	0.32	0.40625	0.465	0.515	0.57143	0.66667	0.9
1	0.01111	0.12222	0.23	0.33	0.4125	0.47	0.52	0.57857	0.68333	0.95
2	0.02222	0.13333	0.24	0.34	0.41875	0.475	0.525	0.58571	0.7	1
3	0.03333	0.14444	0.25	0.35	0.425	0.48	0.53	0.59286	0.72	
4	0.04444	0.15556	0.26	0.35833	0.43125	0.485	0.535	0.6	0.74	
5	0.05556	0.16667	0.27	0.36667	0.4375	0.49	0.54	0.61	0.76	
6	0.06667	0.17778	0.28	0.375	0.44375	0.495	0.545	0.62	0.78	
7	0.07778	0.18889	0.29	0.38333	0.45	0.5	0.55	0.63	0.8	
8	0.08889	0.2	0.3	0.39167	0.455	0.505	0.55714	0.64	0.83333	
9	0.1	0.21	0.31	0.4	0.46	0.51	0.56429	0.65	0.86667	

Supplementary Table 11. Defining the collective variable used in umbrella sampling simulations. The list of amino acids used to define four collective variables for the native and non-native dimers is provided.

Native dimer				Non-native dimer			
Protomer 1		Protomer 2		Protomer 1		Protomer 2	
G_1	G_2	G_1	G_2	G_1	G_2	G_1	G_2
L194	T138	L194	T138	F53	G364	P129	G395
A195	M143	A195	M143	D54	G393	V130	A396
G196	V144	G196	V144	K55	M394	K131	L397
L198	L145	L198	L145	G56	G395	F132	A399
F199	C347	F199	C347	V57	A396	F133	I402
L406	F348	L406	F348	F132	L397	G134	R403
T407	A352	T407	A352	F133	L398	G135	A404
G408	P353	G408	P353	G135	A400	L136	G408
I410	G354	I410	G354	L136		G137	
L411		L411					