Investigation of the Effect of Bilayer Composition on PKCα-C2 Domain Docking Using Molecular Dynamics Simulations

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Supporting Information

Lipid Bilayer Characteristics The area per lipid and bilayer thickness were calculated for each system to validate the lipid force fields, as well as to determine if PKCα-C2 docking affected the overall bilayer structure (Table S1). The pure POPC results agree with the previous experimental [1,2] and MD simulation [3–6] data. The presence of POPS in POPC/POPS/PIP2/PKCα-C2 system reduced the area per lipid to 61.1 $Å^2$, and increased the bilayer thickness to 40.3 Å. POPS tended to decrease the area per lipid, as shown previously by Jurkiewicz *et al.* [6] On the other hand, PIP2 molecules increased the bilayer thickness due to their long acyl chains. Adding 10 mol% of POG to POPC/POPS/PIP2 system decreased the area per lipid to 58.7 $Å^2$ and increased the bilayer thickness to 41.6 Å. This is in accordance with previous studies that showed that diacylglycerol decreases the bilayer area and increases the acyl chain order and the bilayer thickness. [5,7] For each bilayer, PKCα-C2 had little to no effect on the lipid area or bilayer thickness.

Table S1. Lipid bilayer characteristics. Area per lipid and bilayer thickness for different systems. Error bars represent the standard error of the mean. All values were extracted and averaged over the last 150 ns of the simulation.

Systems	**Dock**	**Latch**	**Area per lipid ($Å^2$)**	**Bilayer Thickness (Å)**
PKCα-C2/POPC	No	No	63.6±0.1	38.7±0.4
	Perpendicular	No	60.7±0.1	42.2±0.4
PKCα-C2/POPC/POPS/PIP2	Parallel	No	61.2±0.1	40.9±0.2
	No	No	61.0±0.1	41.1±0.2
	Parallel	Yes	59.0±0.1	40.8±0.3
PKCα-C2/POPC/POPS/PIP2/POG	Parallel	No	59.1±0.1	39.4±0.3
	No	No	58.7±0.1	41.4±0.2

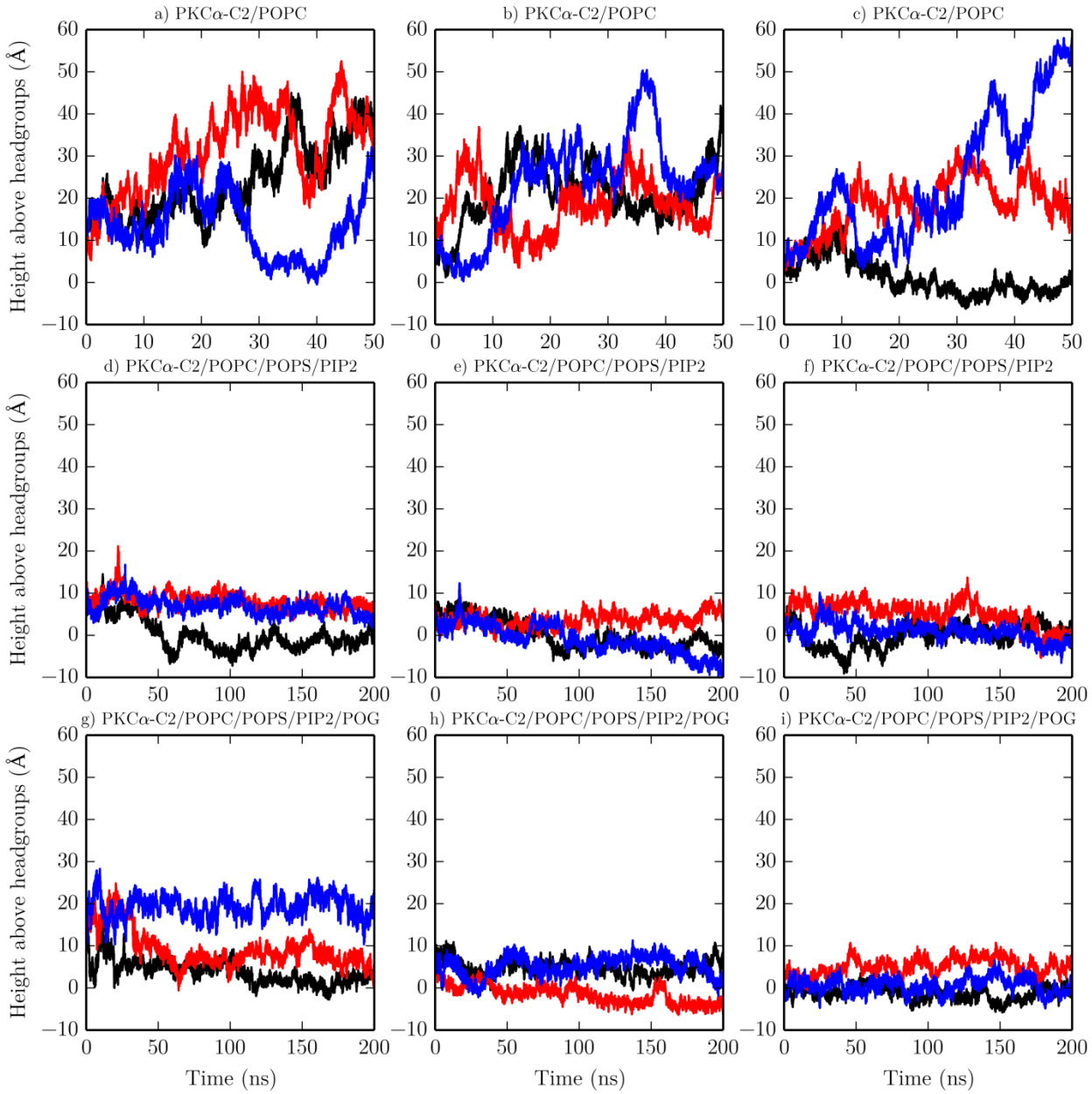

Figure S1. Height above headgroups versus time for different systems. Each system has three initial tilt angles: 0^{o} (a, d, and g), 30^{o} (b, e, and h) and 60^{o} (c, f, and i). The three colors represent different simulation sets.

Table S2. Height above the headgroup region for non-lysine residues. Height above the headgroup region for the following residues: I184, G190, L219, and R252. Error bars represent the standard error of the mean. All values were extracted and averaged over the last 150 ns of the simulation.

Systems	Dock	Latch	Z_{M186}(Å)	Z_{G190}(Å)	Z_{L219}(Å)	Z_{R252}(Å)
Without POG	Perpendicular	No	10.6±0.3	1.2±0.2	10.7±0.2	4.7±0.2
	Parallel	No	11.0±0.3	7.6±0.4	15.4±0.3	0.6±0.2
	No	No	17.4±0.3	9.0±0.3	12.4±0.4	16.2±0.5
With POG	Parallel	Yes	15.3±0.2	10.0±0.3	16.7±0.4	4.5±0.4
	Parallel	No	16.3±0.3	9.0±0.2	15.5±0.2	6.0±0.3
	No	No	17.2±0.3	12.3±0.3	16.3±0.3	14.2±0.4

Table S3. Height above the headgroup region for the lysine residues. Height above the headgroup region for the following residues: K197, K199, K209, and K211. Error bars represent the standard error of the mean. All values were extracted and averaged over the last 150 ns of the simulation.

Systems	Dock	Latch	Z_{K197}(Å)	Z_{K199}(Å)	Z_{K209}(Å)	Z_{K211}(Å)
Without POG	Perpendicular	No	18.2±0.2	21.1±0.2	21.1±0.3	20.9±0.3
	Parallel	No	11.5±0.3	14.3±0.3	11.5±0.5	12.2±0.3
	No	No	19.7±0.3	23.8±0.3	20.8±0.5	16.5±0.3
With POG	Parallel	Yes	10.8±0.3	15.3±0.5	7.7±0.5	6.7±0.2
	Parallel	No	13.3±0.3	18.4±0.4	12.1±0.4	8.3±0.4
	No	No	19.4±0.4	21.6±0.4	19.4±0.6	17.1±0.6

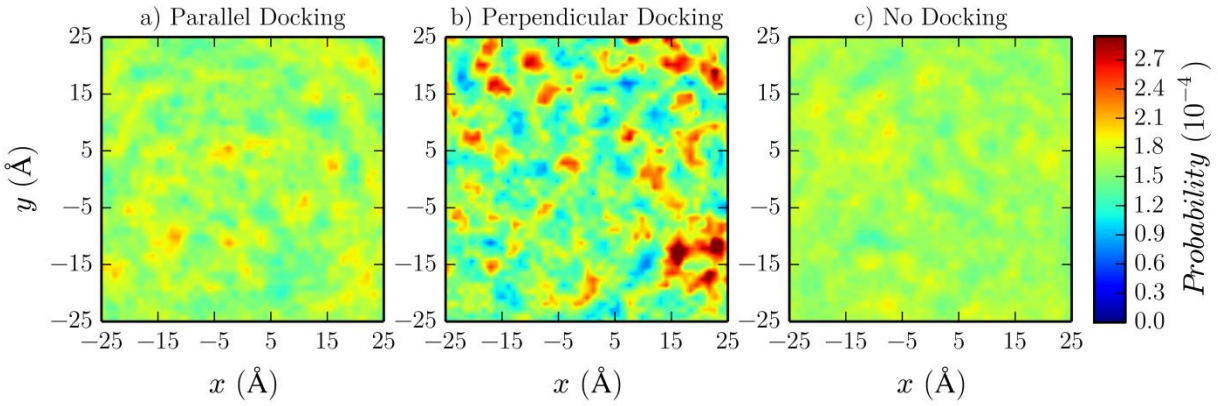

Figure S2. Lipid phosphorus atoms density maps in the PKCα-C2/POPC/POPS/PIP2 system (lower leaflet) for different docking states: a) perpendicular, b) parallel, and c) no docking. The map represents the phosphorus atoms position density - in POPC, POPS and PIP2 - with respect to the center of mass of CBL3.

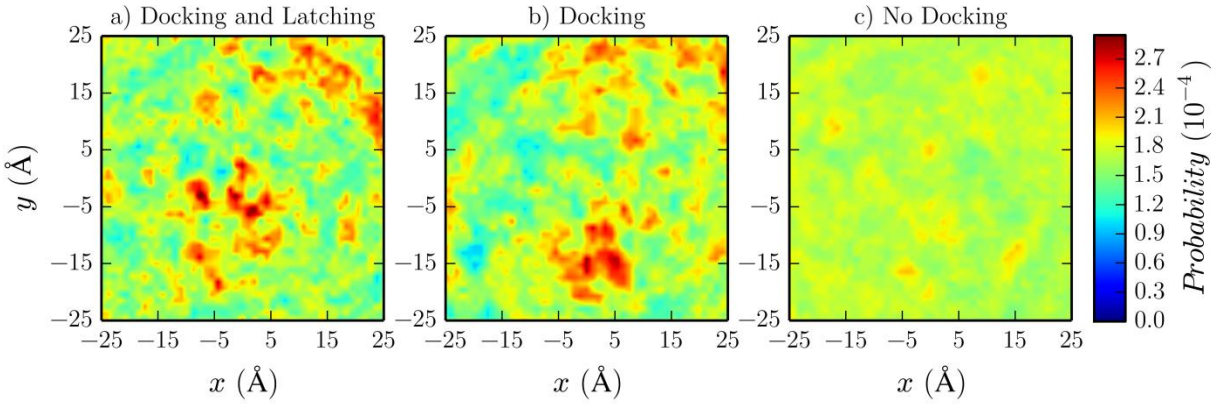

Figure S3. Lipid phosphorus atoms density maps with respect in the PKCα-C2/POPC/POPS/PIP2/POG systems (lower leaflet) for different docking cases: a) docking, b) docking and latching, and c) no docking. The map represents the phosphorus atoms position density - in POPC, POPS and PIP2 - with respect to the center of mass of CBL3.

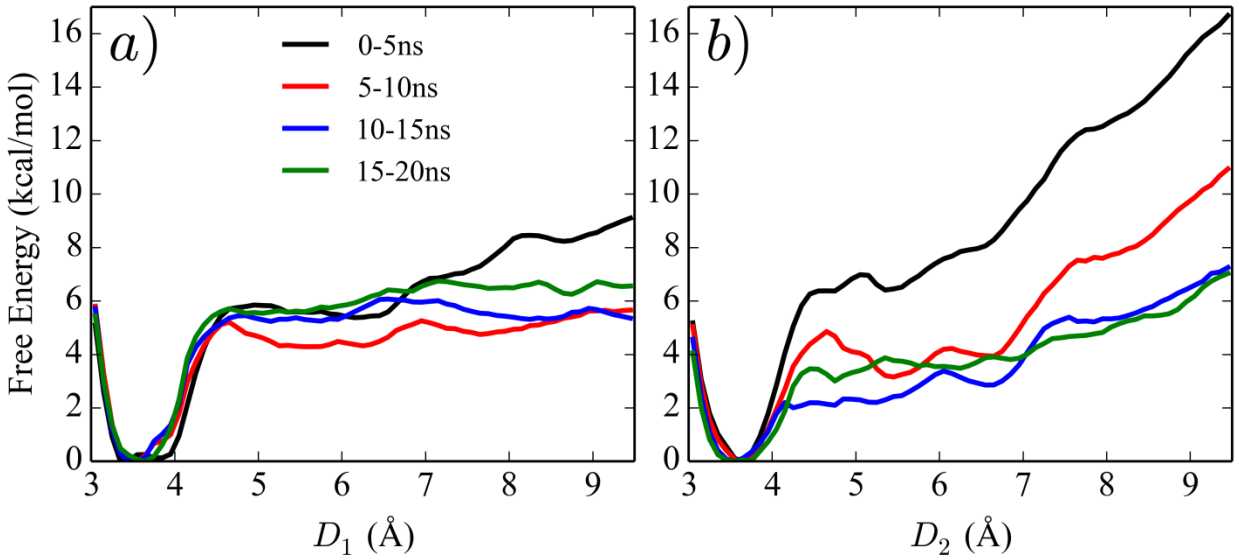

Figure S4. One-dimensional PMF calculations for the system lacking POG for D1 (a) and D2 (b) distances. The degree of similarity between 10-15ns and 15-20ns, suggest there is good convergence in the PMF results.

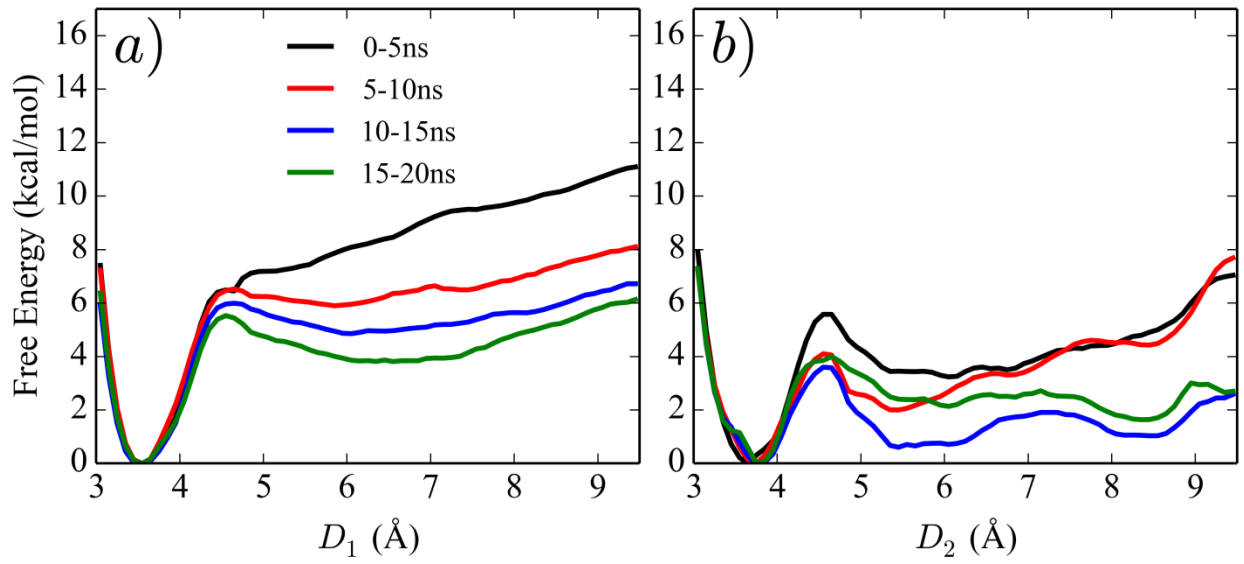

Figure S5. One-dimensional PMF calculations for the system containing POG for D1 (a) and D2 (b) distances. The degree of similarity between 10-15ns and 15-20ns, suggest there is good convergence in the PMF results.

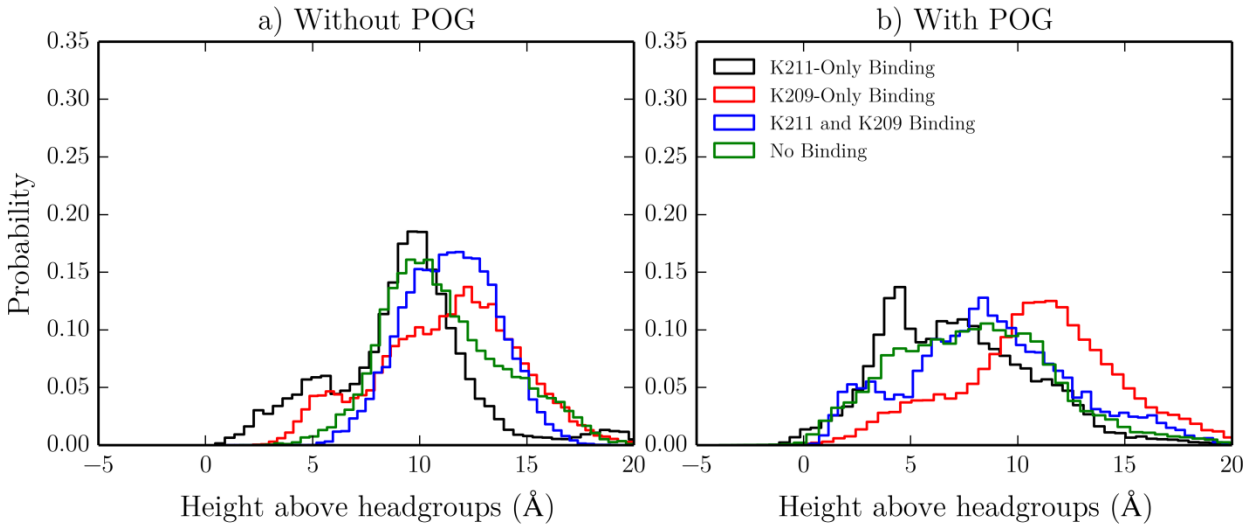

Figure S6. Height above the headgroups for residue N189. The height above the headgroups was calculated from the PMF trajectories for the following four regions: K211-only binding (black), K209-only binding (red), K211 and K209 binding (blue), and no binding (green).

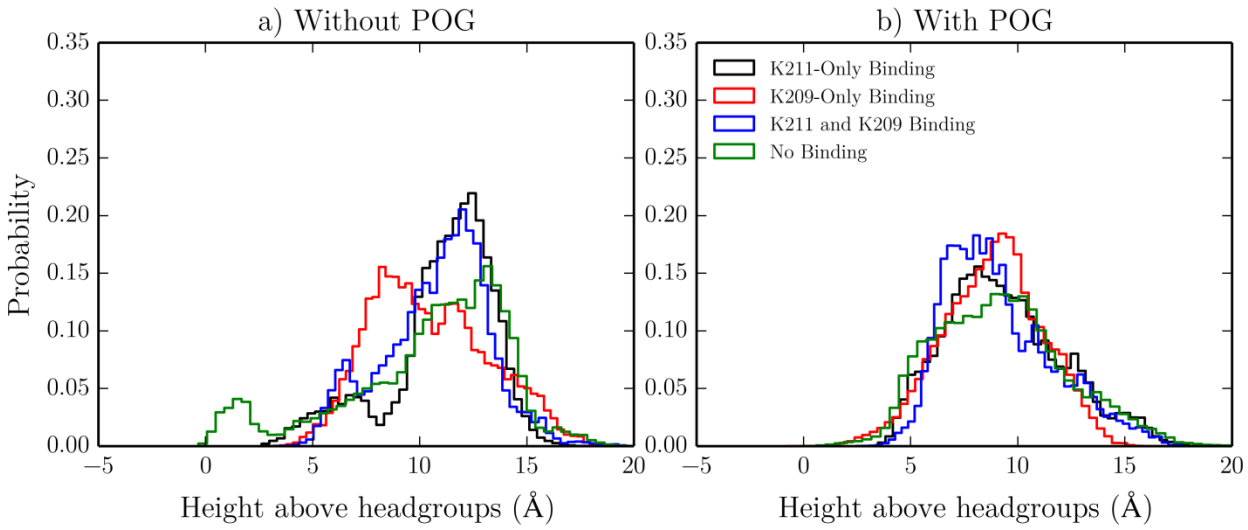

Figure S7. Height above the headgroups for residue N206. The height above the headgroups was calculated from the PMF trajectories for the following four regions: K211-only binding (black), K209-only binding (red), K211 and K209 binding (blue), and no binding (green).

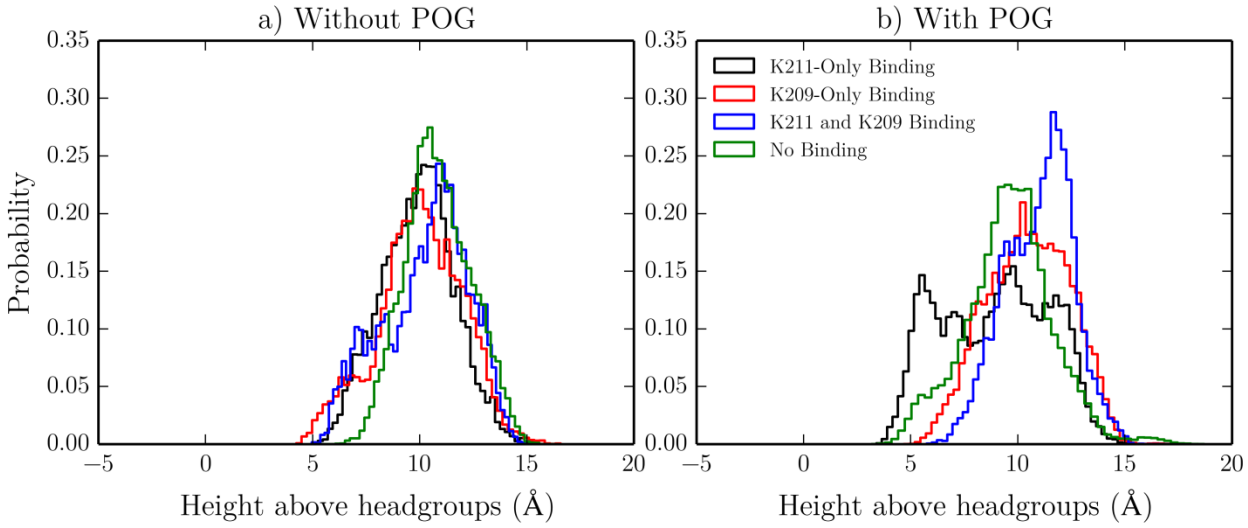

Figure S8. Height above the headgroups for residue T214. The height above the headgroups was calculated from the PMF trajectories for the following four regions: K211-only binding (black), K209-only binding (red), K211 and K209 binding (blue), and no binding (green).

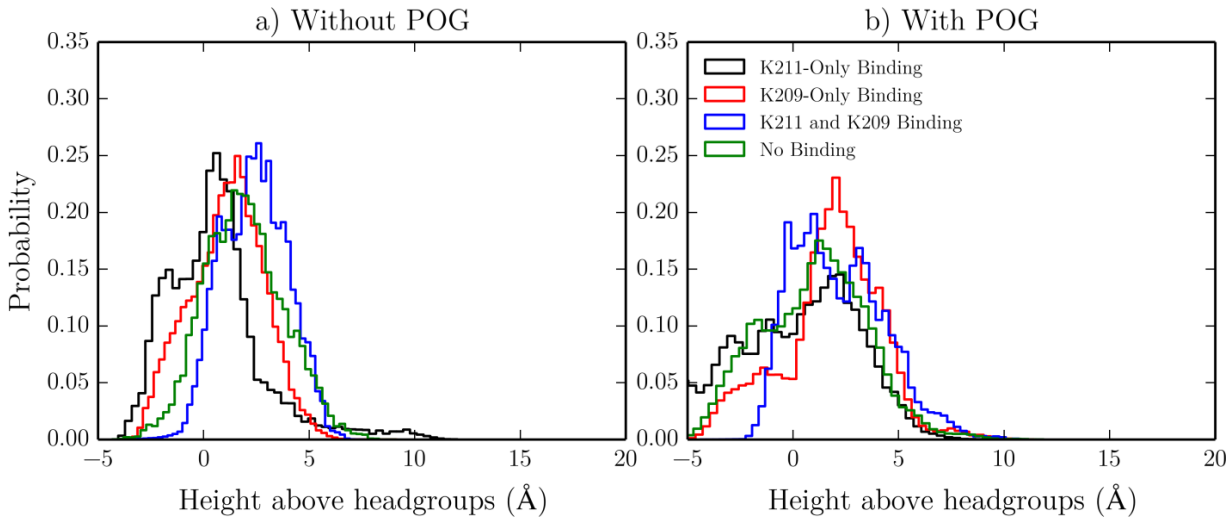

Figure S9. Height above the headgroups for residue R249. The height above the headgroups was calculated from the PMF trajectories for the following four regions: K211-only binding (black), K209-only binding (red), K211 and K209 binding (blue), and no binding (green).

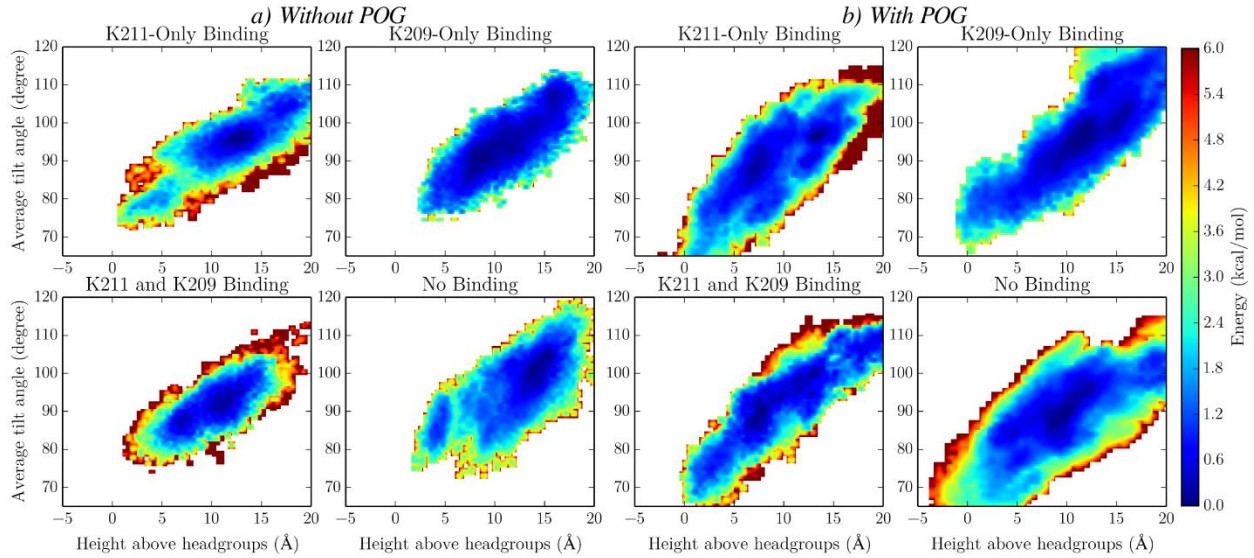

Figure S10. Domain tilt angle versus height above the headgroups for residue N189 in the absence (a) and presence (b) of POG. The two-dimensional free energy profiles for residues N189, N206, and R249 were computed based on their joint probability distributions for the following binding state: K211-only binding, K209-only binding, K211 and K209 binding, and no binding.

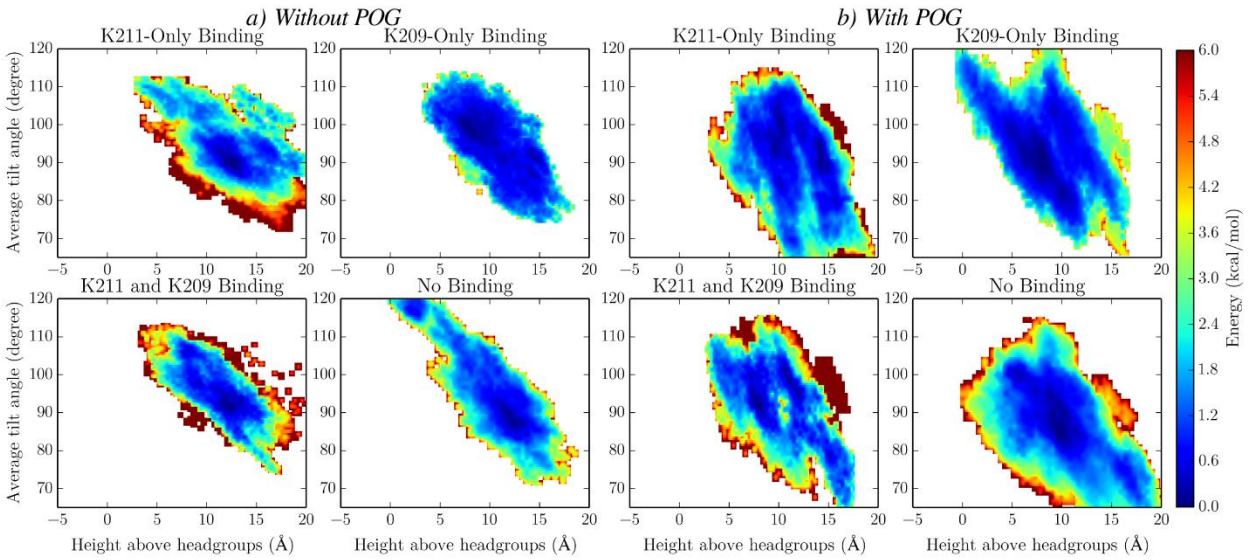

Figure S11. Domain tilt angle versus height above the headgroups for residue N206 in the absence (a) and presence (b) of POG. The two-dimensional free energy profiles for residues N189, N206, and R249 were computed based on their joint probability distributions for the following binding state: K211-only binding, K209-only binding, K211 and K209 binding, and no binding.

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