Membrane-Induced Structural Rearrangement and Identification of a Novel Membrane Anchor in Talin F2F3

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Video S1. **Membrane Binding and Insertion of Talin at Atomic Resolution.** The movie depicts the three-stage binding mechanism of talin as a dynamic process. The first stage is the initial attraction to the membrane surface via the basic MOP residues (0–13 s, blue). The movie then zooms in on the underside of the F2 subdomain where the release and insertion of the Phe-rich hydrophobic membrane anchor (red) into the membrane core is demonstrated (15–28 s). The movie then zooms back out and shows the large-scale, membrane-induced interdomain conformational change the brings the F3 into contact with the membrane surface (30–38 s) via the FAP residues (gray). Here, talin is represented by the green cartoon, DBPS molecules are in brown, DCLE molecules are yellow spheres, and water/ions are omitted for clarity.

Table S1: Tumbling angle for talin F2F3 subdomain measured over the first 10 ns of each of the five trajectories reported. This was calculated by measuring the angle between the vector connecting the two center of masses of the F2 and F3 subdomains and the membrane normal. Presented is the maximum and minimum angles talin makes with the membrane normal as well as the tumbling angle, which is the difference between maximum and minimum angles. The average tumbling angle is also included ± standard deviation across the five runs.

Simulation	Max Angle (°)	Min Angle (°)	Tumbling Angle (°)
1	120.89	102.24	18.65
2	135.60	102.45	33.15
3	132.92	113.52	19.40
4	120.95	102.93	18.02
5	132.86	114.07	18.79
Avg.	–	–	21.60±6.47

Table S2: Average (±SD) number of phospholipids in contact with talin F2F3 in its membrane-bound state. The contacts were counted every 0.05 ns over the last 10 ns of each trajectory and averaged across all five membrane-binding simulations. A contact is defined as either a phosphate (geometric center of PO_4^-), carboxylate (geometric center of COO^-), or ammonium (geometric center of NH_3^+) group of the lipid within 4.5 Å of any side chain ("Side Chain") or backbone ("Backbone") atoms in the protein.

		Side Chain		Backbone		
	Residue	PO_4^-	COO^-	PO_4^-	COO^-	NH_3^+
F2/MOP	H255	0.42±0.24	0.08±0.04	0.19±0.24	0.00±0.00	0.09±0.10
	K256	0.44±0.10	0.18±0.05	0.05±0.01	0.00±0.00	0.01±0.01
	K258	0.73±0.19	0.14±0.06	0.01±0.01	0.00±0.00	0.00±0.00
	K274	0.41±0.33	1.09±0.92	0.14±0.08	0.12±0.05	0.20±0.05
	R276	1.60±0.04	0.36±0.25	0.69±0.09	0.04±0.03	0.03±0.02
	K280	0.67±0.23	0.63±0.54	0.39±0.29	0.04±0.04	0.00±0.00
	K287	0.53±0.14	0.53±0.18	0.08±0.07	0.09±0.03	0.11±0.09
F3/FAP	K325	0.37±0.27	0.06±0.02	0.14±0.10	0.01±0.01	0.02±0.01
	N326	0.55±0.02	0.06±0.02	0.14±0.10	0.01±0.01	0.03±0.03
	K327	0.08±0.01	0.32±0.12	0.02±0.01	0.01±0.01	0.02±0.01

Table S3: Average internal RMSD values (±SD) during the DOPS (full) membrane simulation of talin F2F3 are shown for the whole protein (residues 206-408), F2 subdomain backbone (residues 206-306), the F3 subdomain backbone (residues 312-408), and the MOP/Membrane Anchor moiety (residues 250 to 290). In each calculation, the reference structure was given by the first frame of the trajectory and the protein backbone was superimposed on the reference each frame. The data presented are average RMSD ± standard deviation. The average is calculated every 0.1 ns for the last 90 ns of the trajectory.

Structure	RMSD (Å)
Whole Protein	2.99 ± 0.41
F2 Subdomain	1.63 ± 0.21
F3 Subdomain	2.54 ± 0.39
MOP/Anchor	1.00 ± 0.24

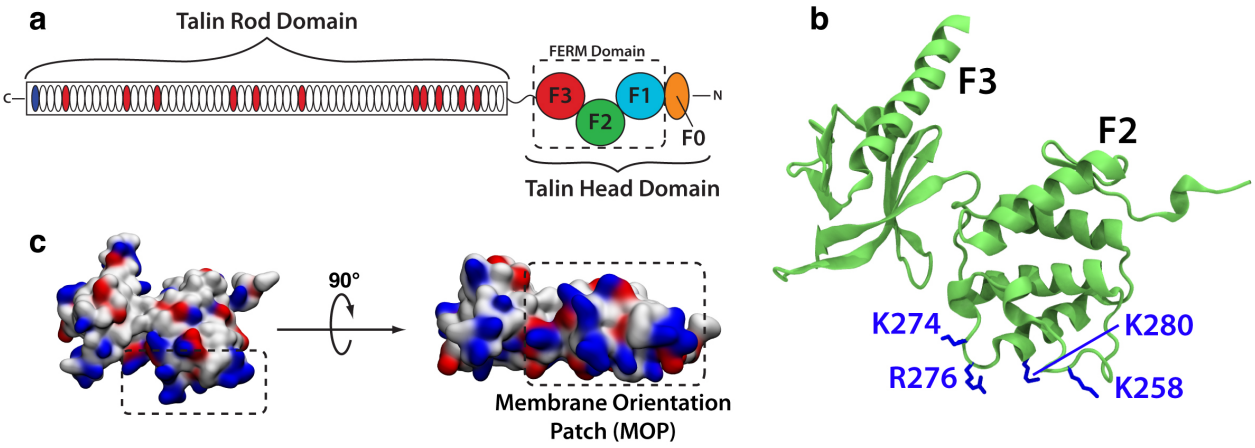

Figure S1: **Talin Structure.** (a) A schematic displaying the overall arrangement of domains in talin including the extended talin rod domain and the talin head domain composed of the F0 (orange), F1 (blue), F2 (green), and F3 (red) subdomains. (b) Crystal structure (PDB 3G9W) of talin showing the F2 and F3 subdomains together with the proposed membrane orientation patch (MOP) residues. The talin backbone is shown in green and MOP residues are shown in blue and labeled. (c) A surface respresentation of talin F2F3 together with basic (blue) and acidic (red) residues shown. The boxed area highlights the proposed MOP and the high number of positive residues that are proposed to directly interact with the membrane.

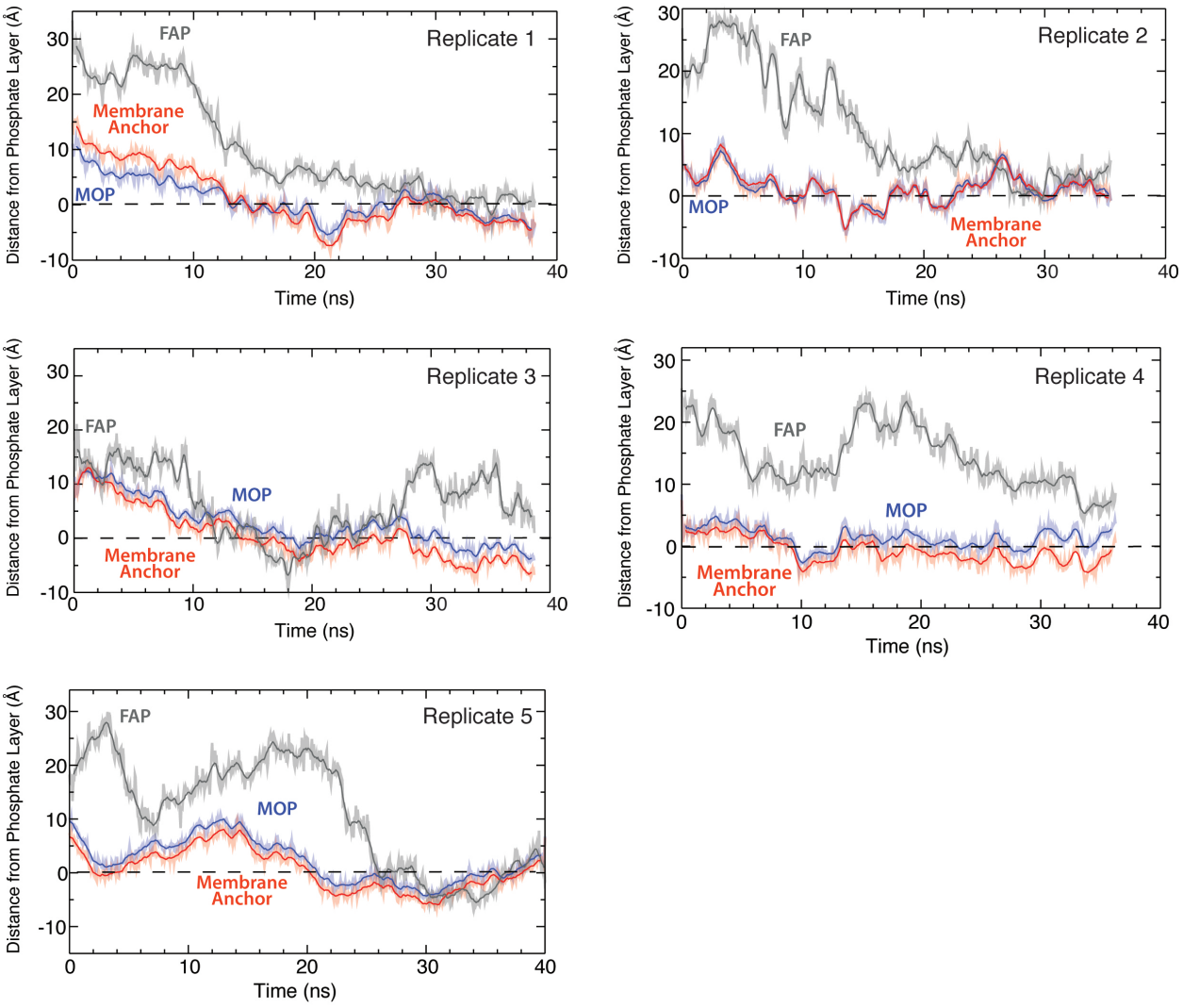

Figure S2: **Reproducible Membrane Binding.** Plots of the five independent membrane-binding simulations demonstrating the reproducible membrane binding and insertion of talin F2F3. The MOP is represented by the blue trace, the Phe-rich membrane anchor by the red trace, and the FAP by the gray curve. The dashed lines represent the phosphate layer of the *cis*-leaflet (similar to Fig. 1).

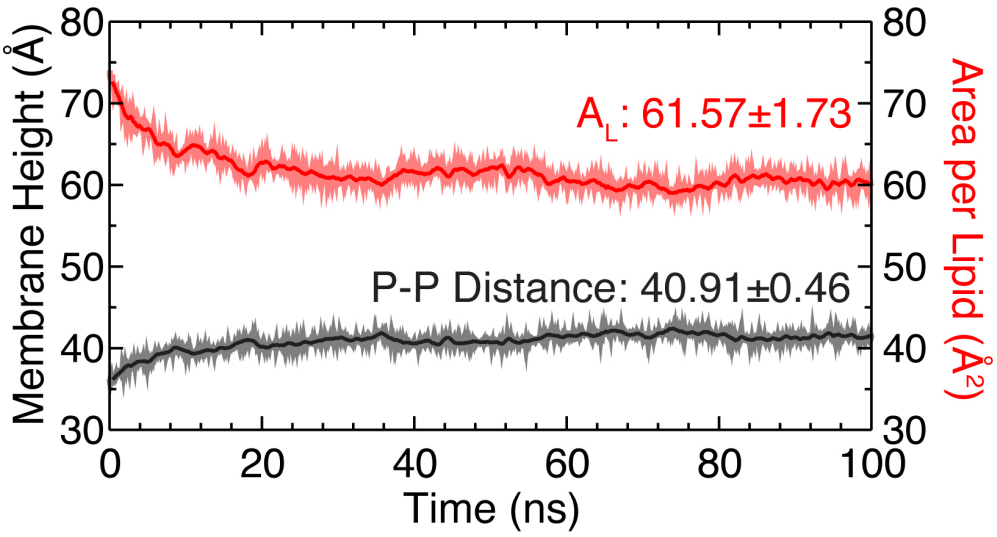

Figure S3: **Structural Properties of Converted Membrane** Shown on the plot is the membrane height (black) and area per lipid (red) of the DOPS membrane constructed from the HMMM system. The membrane height was measured by finding the distance between the center of mass of the phosphate layer of the *cis*- and *trans*-leaflets every 0.05 ns (P-P distance). The area per lipid was also calculated every 0.05 ns and was measured by finding the area of the simulation cell and dividing by the number of lipids in the *cis*-leaflet (96 DOPS, same number as the *trans*-leaflet). The number presented in the plots are the mean ± SD over the last 30 ns of the trajectory.

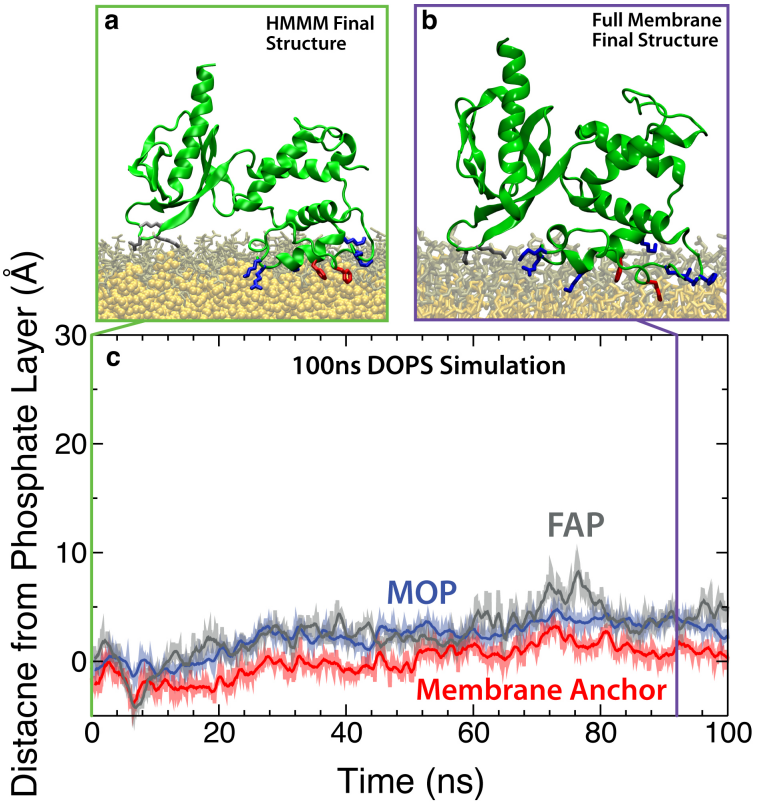

Figure S4: **Talin F2F3 is Stable in DOPS Membrane.** (a; green frame) Snapshot of the membrane bound form of talin F2F3 obtained from the HMMM simulations. Here, talin is shown in green, with MOP (blue), membrane anchor (red), and FAP residues (gray) highlighted. Here, DBPS molecules are shown in brown and DCLE molecules in yellow, similar to Fig. 1. (b; black frame) Snapshot from t=98 ns in the DOPS simulations. Here, the coloring scheme is retained from (a). For easy comparison, all carbons below C4 on the fatty acyl chains are colored to match the DCLE in (a) and the headgroup is colored the same as (a). (c) Plot of the 100 ns simulation of membrane-bound talin in a conventional DOPS membrane. The data are from a production run following the equilibration procedure described in the Methods section. Here, the height of each membrane-binding moiety above the phosphate layer is plotted (MOP in blue, Phe-rich membrane anchor in red, and FAP in gray similar to Fig. 1). This plot shows that the height above the phosphate layer of each moiety is conserved from the HMMM model to a full membrane model, demonstrating the stability of the membrane-bound conformation of talin F2F3, observed using the HMMM model, in a conventional membrane such as DOPS.