SUPPORTING INFORMATION FOR

Lactadherin's Multistate Binding Predicts Stable Membrane-bound Conformations of Factors V and VIII's C domains

*Kevin J. Cheng, Ashley M. De Lio, Riya Jain, Divyani Paul, James H. Morrissey, and Taras V. Pogorelov**

Center for Biophysics and Quantitative Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 61801

Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 61801

School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 61801

Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 61801

National Center for Supercomputer Applications, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 61801

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI, USA, 48109

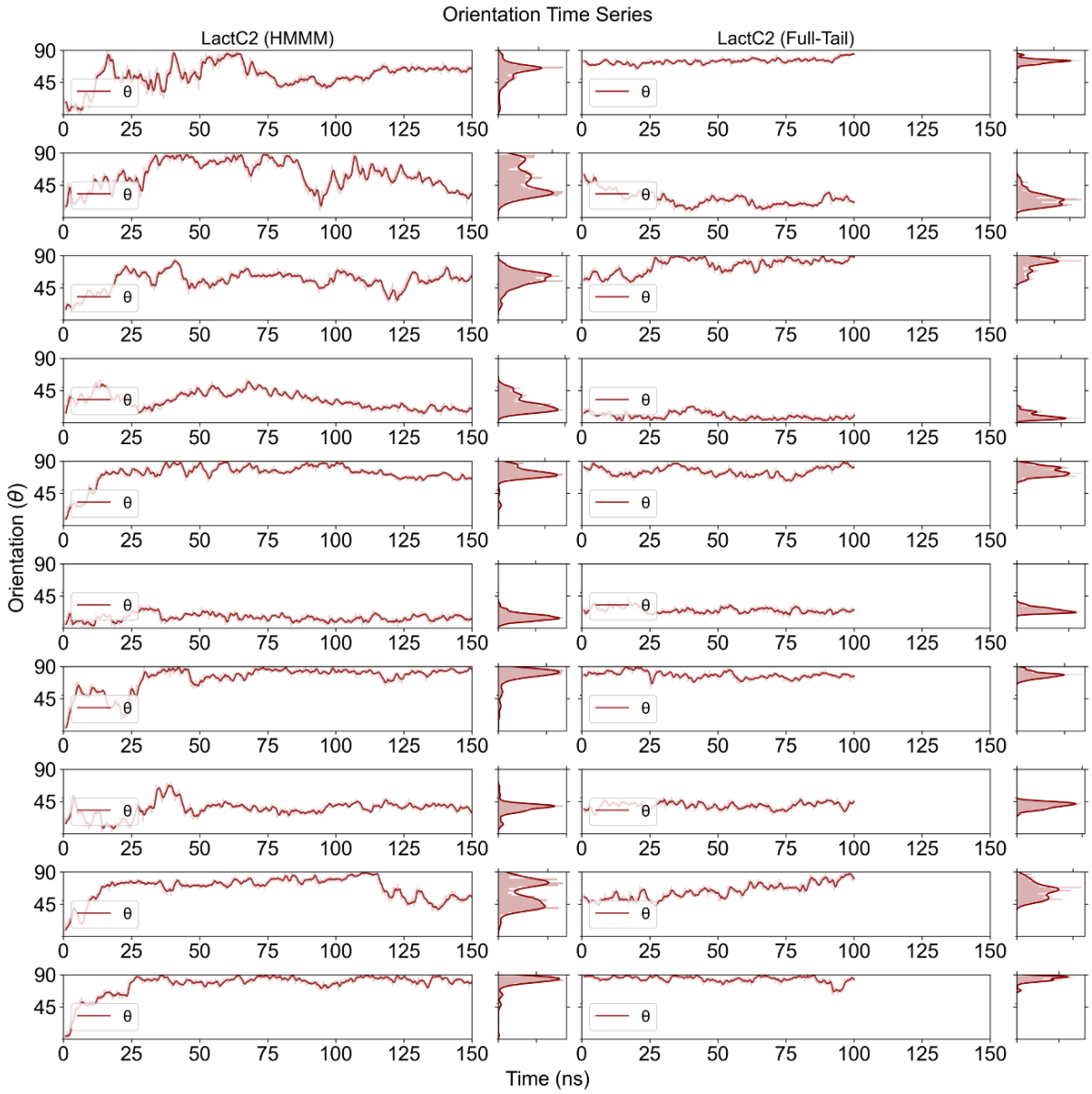


Figure S1. Orientation time series of LactC2 for 10 replicates**.** The left-hand column is the time series from replicates using HMMM, while the right-hand column represents the continuation of simulations with tails regrown in the full-tail representation.

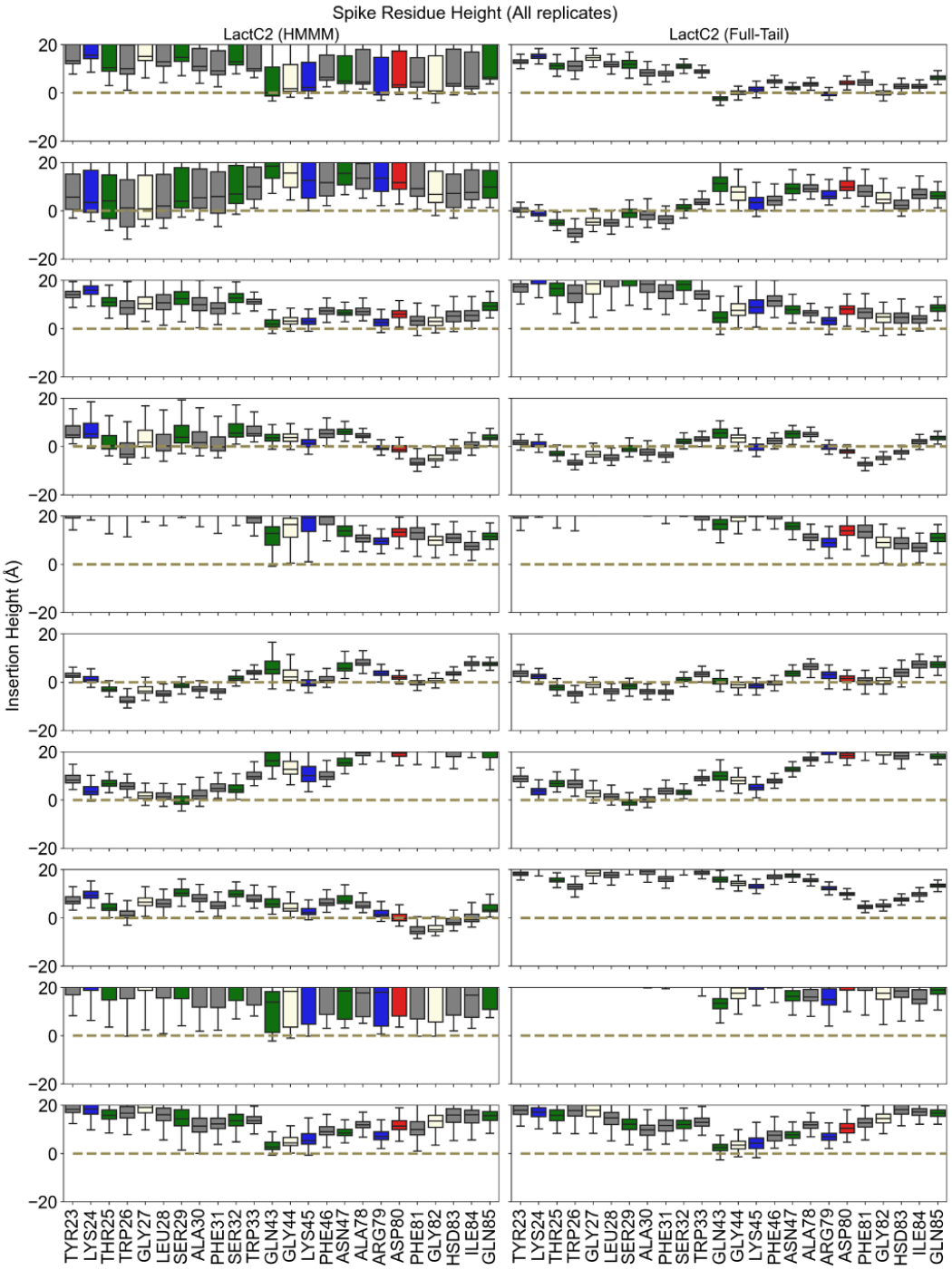
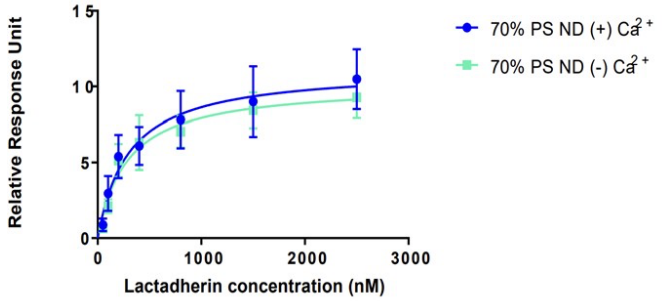


Figure S2. Insertion height box plots for each spike residue for 10 replicates. The left-hand column represents insertion heights from HMMM simulations based on 150 ns of sampling per replicate, while the right-hand column represents insertion heights from simulations with tails regrown in

the full-tail representation (100 ns per replicate). Individual boxplots are colored by residue type: grey-hydrophobic, blue-basic, red-acidic, green-polar, vanilla-other. The dashed line represents the phosphate plane where Lact binds with its spikes for regions greater than z = 0.

(A) Binding study comparison in absence and presence of calcium



(B)

Binding affinity Kd (nM)	
With Calcium	Without Calcium
296 ± 83	283 ± 67

Figure S3. Experimental studies on lactadherin binding to PC/PS nanodiscs. (A) Comparison of binding affinities of lactadherin to nanodiscs containing 70% PS and 30% PC in the presence of (dark blue)) and absence of (light blue) Ca^{2+} at multiple lactadherin concentrations. (B) Binding affinities for both systems.

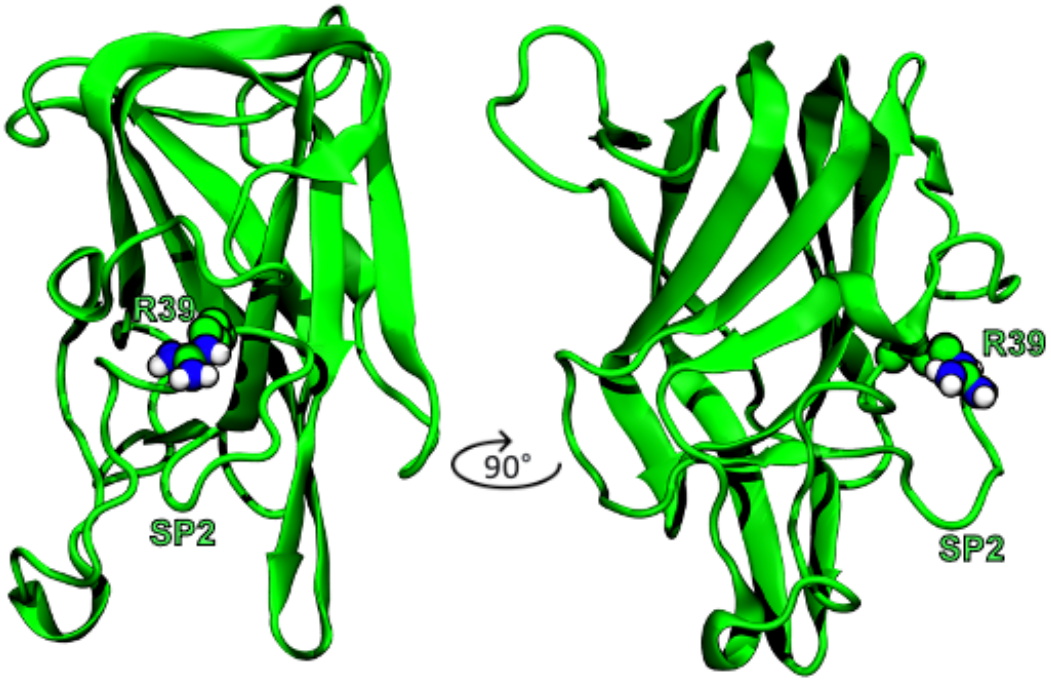


Figure S4. Structure of LactC2 and R39's location. (A) Location of the non-spike R148 on LactC2 with spike 2 (SP2) labeled for positional reference.

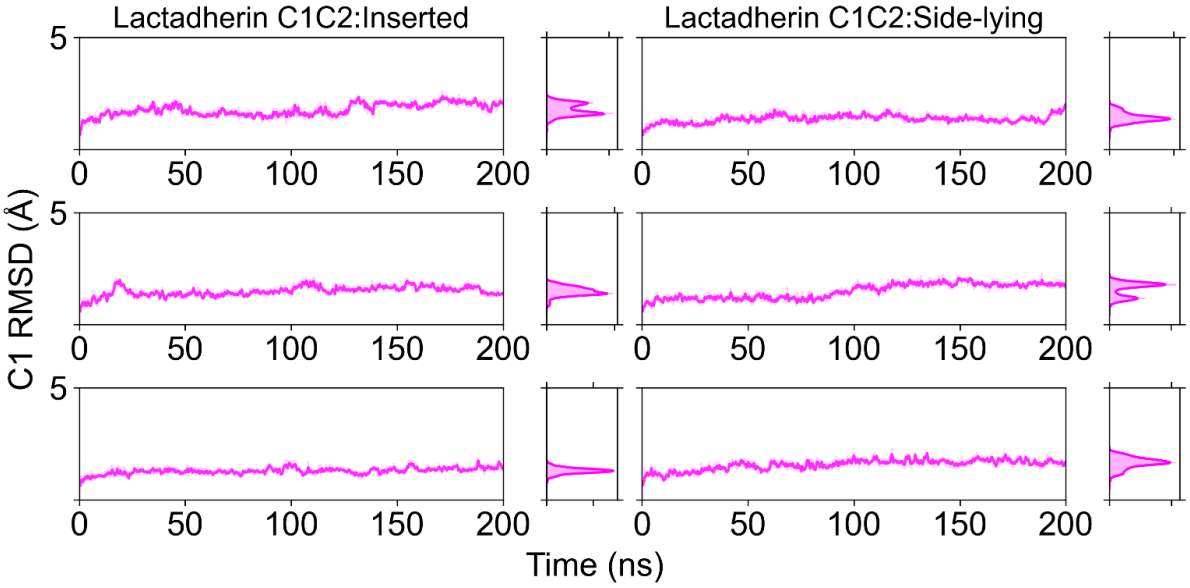


Figure S5. RMSD trace of C1 domain for each replicate with simulations of the LactC1C2 construct in the inserted state (left column) and side-lying state (right column)

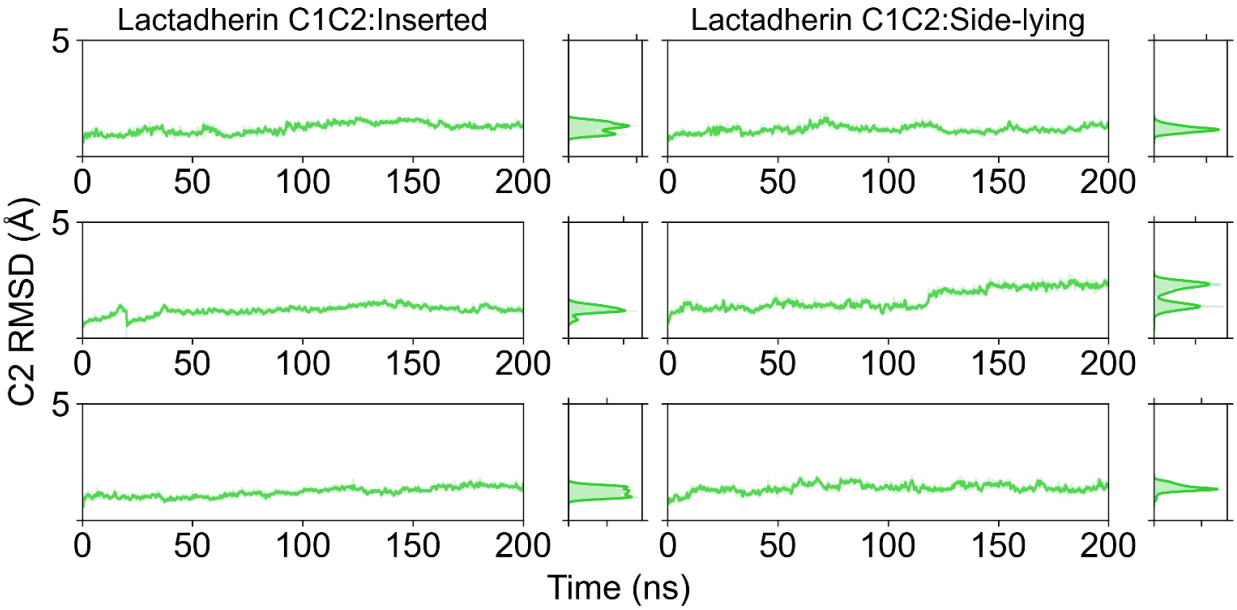


Figure S6. RMSD trace of C2 domain for each replicate with simulations of the LactC1C2 construct in the inserted state (left column) and side-lying state (right column)

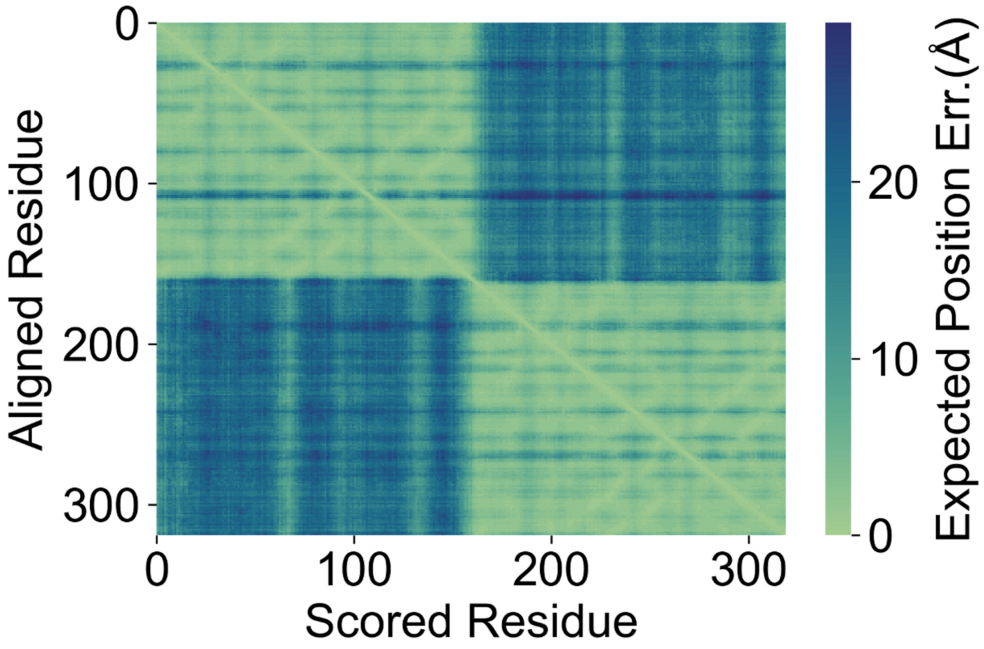


Figure S7. Predicted alignment error output for the LactC1C2 construct generated from AlphaFold structure Q95114.

	Residue (Simulation)	Residue (7KVE)	# POPS Contacts	Percent of Total Contacts
C1 Domain	R29	R1907	6762	11.03%
	S38	S1916	2625	4.28%
	K76	K1954	2370	3.87%
	H77	H1955	3048	4.97%
	Y78	Y1956	5011	8.18%
	R145	R2023	4114	6.71%
C2 Domain	W185	W2063	6735	10.99%
	W186	W2064	5783	9.44%
	R202	R2080	10522	17.17%
	K236	K2114	5015	8.18%
	L238	L2116	5341	8.71%
	S239	S2117	3960	6.46%

Table S1. Coagulation factor V residue contacts with POPS greater than 3% of total contacts. The dashed line separates residues from the C1 and C2 domains. Equivalent residues from PDB: 7KVE are shown. Percent contacts calculated from the total number of contacts recorded in table (i.e., 61286).

	Residue (Simulation)	Residue (7K66)	# POPS Contacts	Percent of Total Contacts
C1	K116	K2136	6762	28.26%
C2 Domain	K163	K2183	2625	10.97%
	S166	S2186	2370	9.90%
	D167	D2187	3048	12.74%
	K187	K2207	5011	20.94%
	R195	R2215	4114	17.19%

Table S2. The residue contacts between fVIIIC1C2 and POPS greater than 8%. The dashed line separates residues from the C1 and C2 domains. Equivalent residues from PDB: 7K66 are shown. Percent contacts calculated from the total number of contacts recorded in table (i.e., 23930)

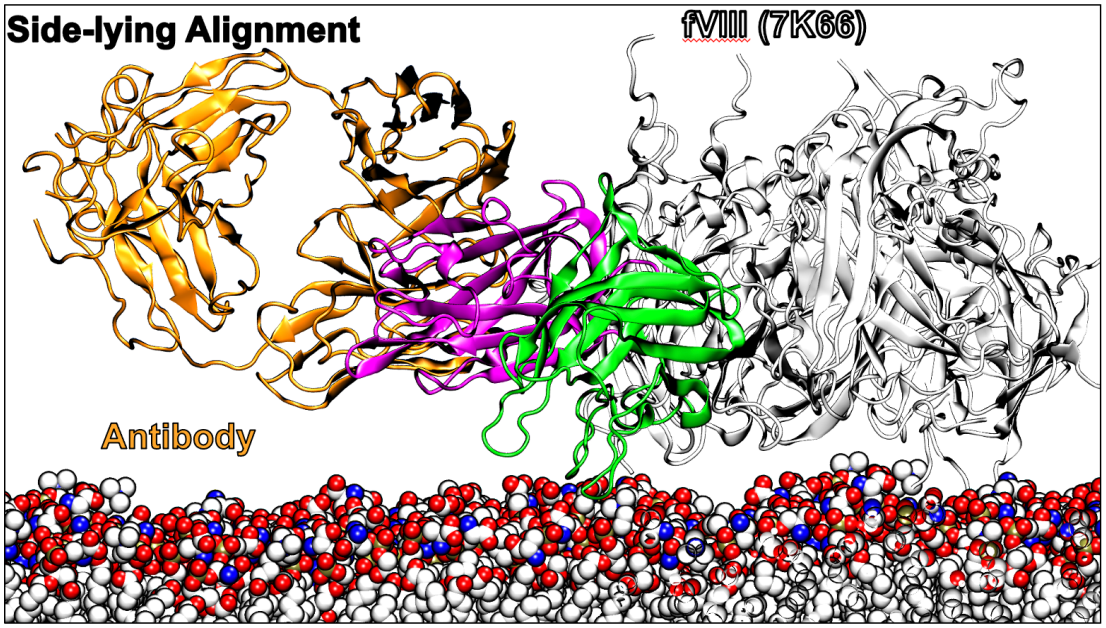


Figure S8: Alignment of a converged structure from simulations of factor VIII C1 (magenta) and C2 (green) domain with 3.3 Å cryo-EM structure 7K66 with antibody bound (orange).

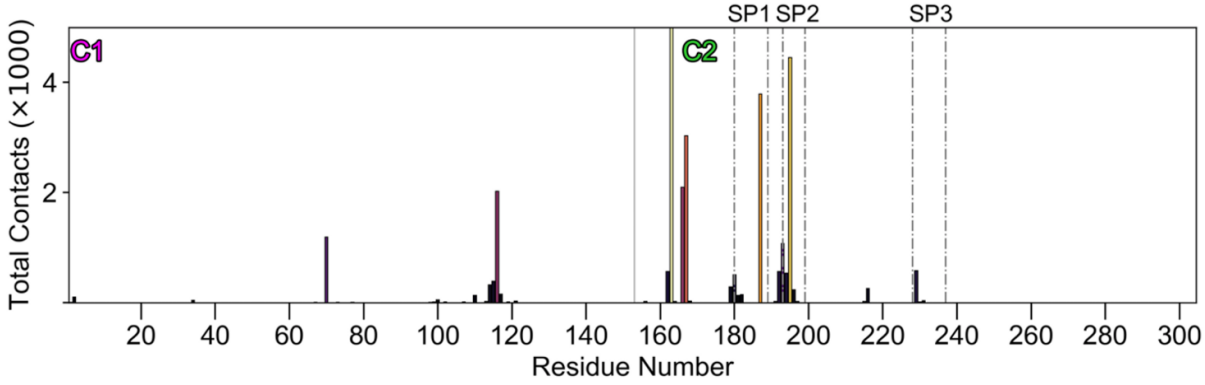


Figure S9. Contact frequency distribution between fVIIIC1C2 residues and phosphatidylserine lipids within 2.5Å. The distribution is divided between the C1 and C2 domain. Each residue is colored by its peak intensity with light colors corresponding to high intensity, and dark colors corresponding to low. Total contacts above maximum are not shown for visual clarity. Spikes 1-3 of fVIII are distinguished with dotted-dashed lines.

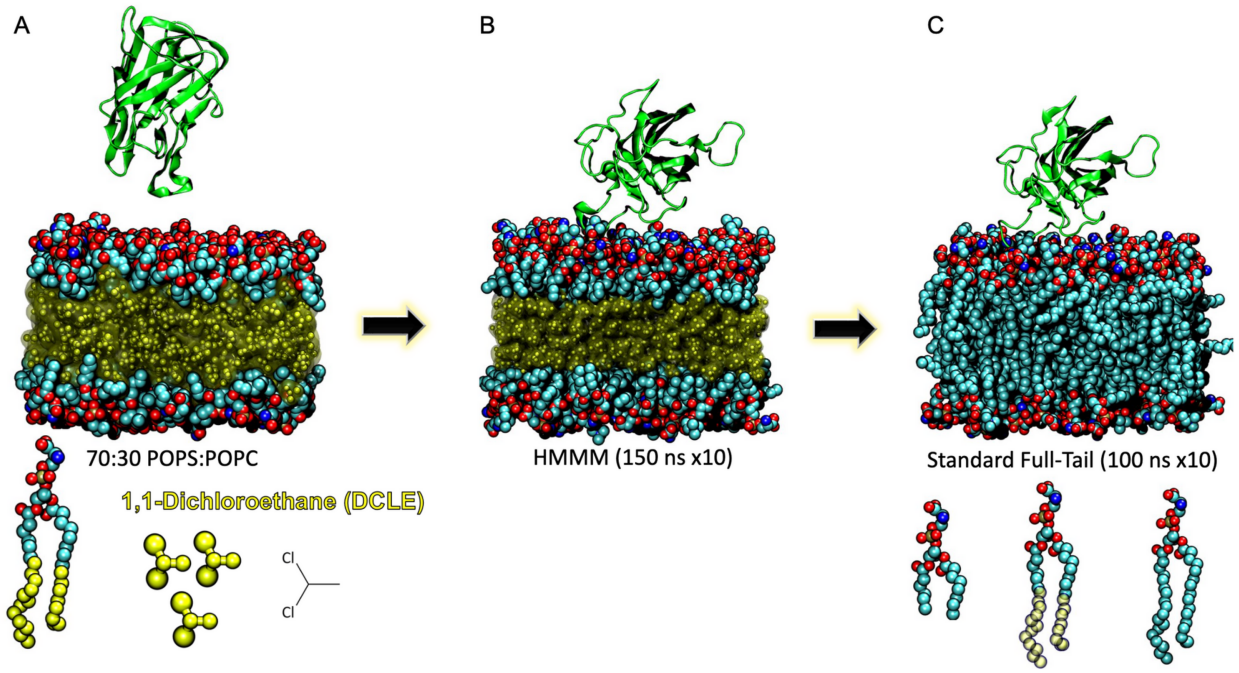


Figure S10. Computational workflow used in this study to efficiently sample and capture the LactC2 membrane-bound state. A. Initial Lact structures start above the membrane using the highly mobile mimetic model. Lipid tails are replaced with DCLE solvent to increase lateral lipid diffusion. B. After 150 ns of simulations, Lact converges to a membrane-bound pose. C. The DCLE solvent is removed and the lipid tails are regrown. 100 ns of simulations are then carried out to sample the conformation with a more realistic membrane representation.

Supplementary Information Experimental Methods.

Materials

POPC and POPS lipids, purchased from Avanti Polar Lipids (Alabaster, AL), were used to prepare the Nanodiscs used in this study. Bio-Beads® SM-2 adsorbent was purchased from Bio-Rad. Bovine Lactadherin was purchased from Hematologic Technologies (Essex Junction, VT), Uniprot accession ID: Q95114. For the Biacore binding studies we purchased the series S Nitrilotriacetic acid Biacore sensor chips from GE Healthcare. The recombinant His-Tagged MSP was expressed in Escherichia coli BL21 DE3 cells (Agilent technologies) and purified in AKTA start system (GE Healthcare Life Science) by Ni NTA(Qiagen) column.

Preparation of Nanodiscs

Quantification of the binding constants for lactadherin to PS containing membranes were done with Nanodiscs. Nanodiscs are soluble monodisperse discoidal lipid/protein particles with controlled size and phospholipid composition where the lipid bilayer is surrounded by a helical protein belt termed as membrane scaffold proteins (MSP) [1]. To prepare the Nanodiscs, a total of 3.9 μmol of phospholipids comprised of 70% POPS and 30% POPC were prepared in chloroform and dried under compressed nitrogen. Nanodiscs comprised of 100 % PC were also prepared as a negative control for the binding studies. The residual chloroform from the lipids was removed overnight under high vacuum. The lipids were dissolved in a 100 mM sodium deoxycholate, 20mM Tris, and 100mM NaCl buffer solution and incubated with MSP for 2 hrs at 4°C. The detergent was removed post incubation via Bio-Beads at 4°C for 4 hours in a rotator. This allowed the self-assembly of the Nanodiscs. The Nanodiscs were further purified by size exclusion

chromatography (SEC) in Akta Pure with the Superdex 200 10/300 GL column (GE healthcare Life Sciences).

Surface Plasmon Resonance Analysis of Lactadherin binding to Nanoscale Bilayers

The binding affinities for lactadherin to the Nanodiscs in the presence and absence of 5 mM Ca2+ were quantified by surface plasmon resonance (SPR) on a GE Biacore T-200 instrument. His-Tagged Nanodiscs were loaded on the Ni-NTA surface sensor chip in a solution of 20 mM HEPES and 100 mM NaCl. The flow rate of lactadherin was set to 30 μL/min and the binding was monitored in real time as the concentration of lactadherin was increased from 25 nM to 2.5 μM (Fig. S12). A representative result for 800 nM lactadherin can be found in Fig. 5B. The surface equilibration time for the binding studies was 110 seconds. Three biological replicate studies were performed with different Nanodisc samples.

As reported previously [2], the binding isotherms were plotted from maximal steady-state response units versus the protein concentration flowed over the chip surface, from which the equilibrium binding constant, Kd, values were derived by fitting the single-site ligand binding equation to the data in Graph Pad Prism 8.0 [3]. The single-site ligand binding equation is:

$$Y = \frac{B_{max}*X}{K_d+X},$$

Specific binding of lactadherin was corrected by normalizing it to the 100% PC Nanodiscs. The non-linear curve of the binding data was analyzed by fitting it to the monomolecular growth model equation:

$$y = m_1 - m_2^{m_3 x},$$

as shown previously [3]. We used this model to calculate the $k_{observed}$ for various LactC2 concentration. The k_{on} was derived from the slope of the $k_{observed}$ versus concentration plot in Figure S12.

Supplementary Information References

1. Bayburt, T.H., Y. V. Grinkova, and S.G. Sligar. 2002. Self-Assembly of Discoidal Phospholipid Bilayer Nanoparticles with Membrane Scaffold Proteins. Nano Lett. 2: 853–856.

2. Del Vecchio, K., and R. V. Stahelin. 2018. Investigation of the phosphatidylserine binding properties of the lipid biosensor, Lactadherin C2 (LactC2), in different membrane environments. J. Bioenerg. Biomembr. 50: 1–10.

3. Single-Site Ligand Binding Equation Fitting was performed use GraphPad Prism version 8.0.0 for Windows.