

SUPPLEMENTARY MATERIAL

Simulation Systems and Structures

These interactive displays are available in the web version: <https://hits-mbm-dev.github.io/paper-talin-loop/>

Scripts

Data analysis code is available at https://hits-mbm-dev.github.io/paper-talin-loop/_analysis.html and in the repository at <https://github.com/hits-mbm-dev/paper-talin-loop>.

Input files and raw data

Input files and raw data are available on [heiData](#) here:
<https://doi.org/10.11588/data/BQTQUN>

Supplementary Plots and Tables

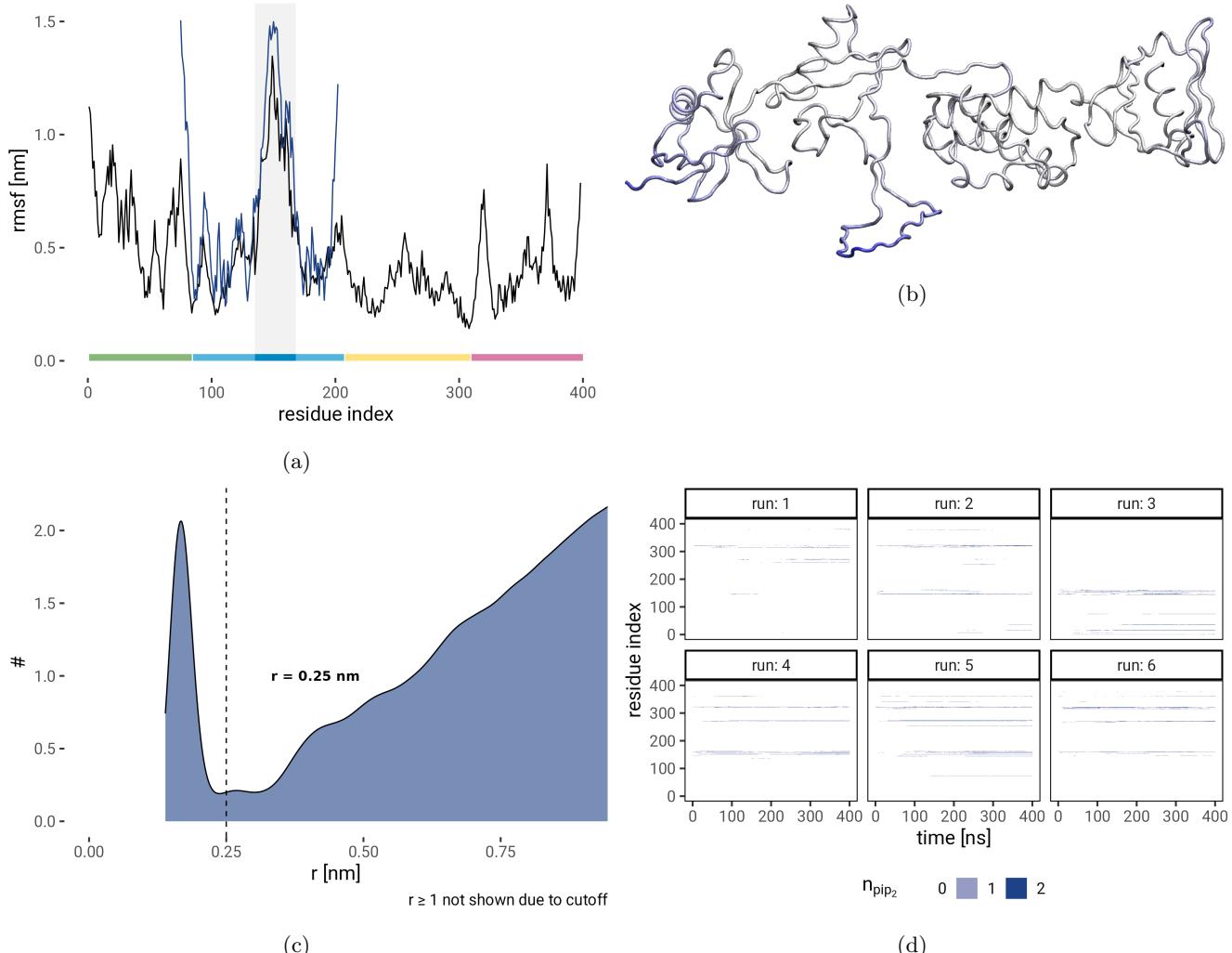


Figure 7: **a)** Root Mean Squared Fluctuation (RMSF) per residue in an equilibrium simulation of the full-length FERM domain (black). The additional blue line shows the RMSF of the NMR ensemble of F1 for comparison (2KC2 (13)). The region of the loop in F1 is highlighted by a grey shade. Color bars as in Figure 6b. **b)** The relative magnitude of the RMSF is shown by coloring the backbone in a render of the FERM structure. **c)** Density plot of distances between PIP₂ and the protein residues to decide on a cutoff for defining interactions. A distance of 0.25 nm was chosen. **d)** A heatmap of the number of PIP₂ molecules bound per residue over time for the 6 individual simulations with the whole FERM domain over the membrane.

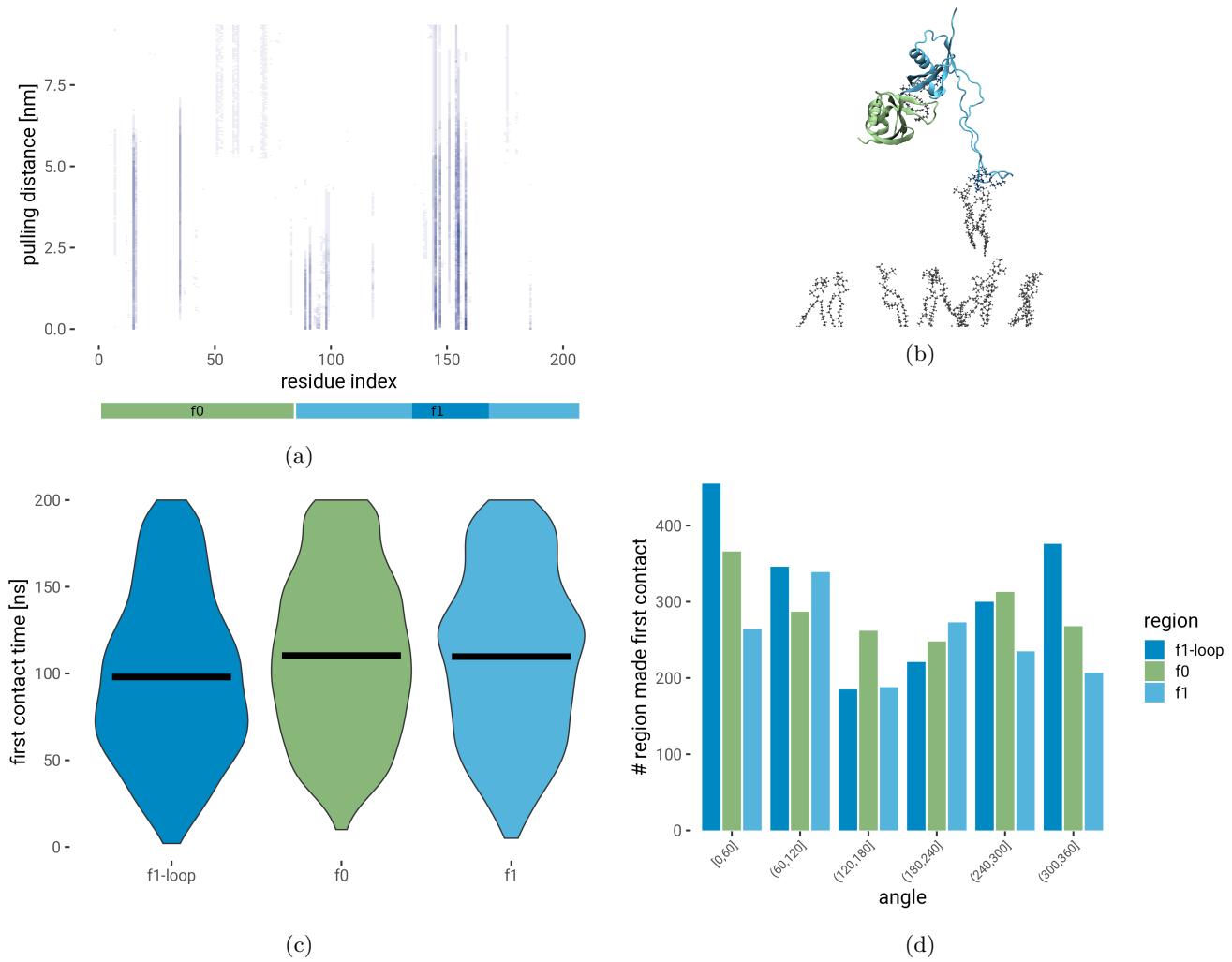


Figure 8: **a)** A closer look at the residues involved in the interaction during pulling reveals the instrumental role of both the F1 loop as well as the F0 subdomain in keeping the connection to the membrane. **b)** Run 4 of the vertical pulling of F0F1. Interactions between the protein and PIP₂ were so strong that a total of 3 molecules of PIP₂ (gray) were pulled out of the membrane (1 by F0 (green) and 2 by the F1 loop (blue)). **c)** Time to first contact of a residue with PIP₂ in the rotational sampling of F0F1. Residues belonging to the F1 loop on average make contact earlier. **d)** Number of times a residue belonging to a region made the first contact in the rotational sampling of F0F1.

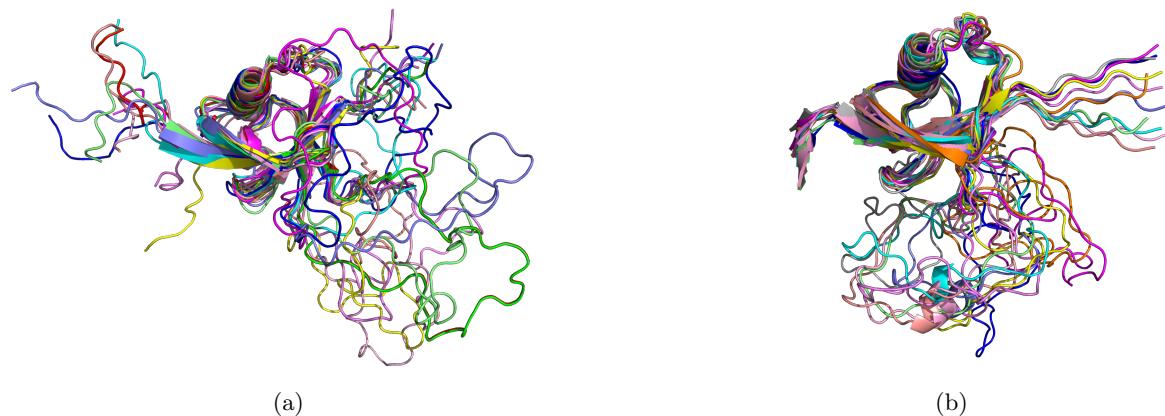


Figure 9: **a)** NMR ensemble of F1, pdb ID 2KC2 ([13](#)) **b)** Ensemble of the F1 domain in equilibrium simulations of the completed FERM domain. The other domains (F0,F2,F3) are not shown, but their steric influence can be seen, as the flexible loop can now no longer occupy the space of the F2 domain as in the NMR structure in **a**.

Table 1: Top residues interacting with F0F1

residue	mean_n_pip
M 1	0.118
K 15	0.114
R 30	0.104
R 35	0.155
K 98	0.101
R 118	0.127
R 144	0.179
K 147	0.155
R 151	0.209
K 154	0.144
K 155	0.152
K 158	0.154
K 160	0.139
K 162	0.107
R 193	0.120

Table 2: Top residues interacting with FERM

residue	mean_n_pip
M 1	0.144
K 15	0.154
T 16	0.134
R 35	0.202
R 74	0.195
R 144	0.555
K 145	0.199
K 147	0.426
R 151	0.295
K 154	0.149
K 155	0.391
K 158	0.506
K 160	0.679
K 162	0.174
K 254	0.157
K 270	0.242
K 272	0.412
R 275	0.416
K 314	0.281
K 316	0.310
K 320	0.742
N 321	0.298
K 322	0.507
K 341	0.248
K 362	0.447

Molecular dynamics Parameters

Production runs

```

integrator          = md
dt                 = 0.002
nsteps             = 100000000
cutoff-scheme      = Verlet
nstlist            = 20
rlist              = 1.2
coulombtype       = pme
rcoulomb           = 1.2
vdwtype            = Cut-off
vdw-modifier      = Force-switch
rvdw_switch        = 1.0
rvdw               = 1.2
tcoupl             = Nose-Hoover
tc_grps            = SYSTEM
tau_t              = 1.0
ref_t              = 303.15
pcoupl             = Parrinello-Rahman
pcoupltype         = semiisotropic
tau_p              = 5.0
compressibility    = 4.5e-5 4.5e-5
ref_p              = 1.0   1.0
constraints         = h-bonds
constraint_algorithm = LINCS
continuation        = yes
nstcomm            = 100
comm_mode          = linear
comm_grps          = SYSTEM
refcoord_scaling   = com

```