

Intrinsically disordered region of Talin's FERM domain functions as an initial PIP₂ recognition site

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ABSTRACT Focal adhesions (FAs) mediate the interaction of the cytoskeleton with the extracellular matrix (ECM) in a highly dynamic fashion. Talin is a central regulator, adaptor protein and mechano-sensor of focal adhesion complexes. For recruitment and firm attachment at FAs, Talin's N-terminal FERM domain binds to phosphatidylinositol 4,5-bisphosphate (PIP₂)-enriched membranes. A newly published autoinhibitory structure of Talin-1, where the known PIP₂ interaction sites are covered up, lead us to hypothesize that a hitherto less examined loop insertion of the FERM domain acts as an additional and initial site of contact. We evaluated direct interactions of Talin-1 with a PIP₂ membrane by means of atomistic molecular dynamics (MD) simulations. We show that this unstructured, 33-residue-long loop strongly interacts with PIP₂ and can facilitate further membrane contacts, including the canonical PIP₂ interactions, by serving as a flexible membrane anchor. Under force as present at FAs, the extensible FERM loop ensures Talin to maintain membrane contacts when pulled away from the membrane by up to 7 nm. We identify key basic residues of the anchor mediating the highly dynamic Talin-membrane interaction. Our results put forward an intrinsically disordered loop as a key and highly adaptable PIP₂ recognition site of Talin and potentially other PIP₂-binding mechano-proteins.

SIGNIFICANCE FERM domains are modular domains that often harbor PIP₂ binding sites and serve as anchoring points to membranes. Talin's FERM domain features a peculiar long and disordered loop, the function of which has remained fully elusive. We here show by means of atomistic molecular dynamics simulations that the loop serves as a first PIP₂ interaction site and flexible anchor to the membrane. This provides mechanistic insight into the role of intrinsically disordered regions in protein–membrane interactions.

INTRODUCTION

Tip

This is a draft and as such subject to change. The source repository for this manuscript lives at <https://github.com/hits-mbm-dev/paper-talin-loop>. The manuscript and poster are available in multiple formats:

- manuscript web/html: <https://hits-mbm-dev.github.io/paper-talin-loop/>
- manuscript print/pdf: <https://hits-mbm-dev.github.io/paper-talin-loop/index.pdf>
- poster web/html: <https://hits-mbm-dev.github.io/paper-talin-loop/poster.html>
- poster print/pdf: <https://hits-mbm-dev.github.io/paper-talin-loop/poster.pdf>

Cells critically sense the mechanics of their environment at cell adhesion sites for a multitude of biological processes. Contact with the extracellular matrix and surrounding cells regulates growth, differentiation, motility and even apoptosis (1–4). The multiprotein focal adhesion complex is responsible for translating between and integrating biochemical and mechanical signals for both outside-in and inside-out activation (5, 6).

At the center of the focal adhesion complex sits the adaptor protein Talin, which dynamically unfolds and refolds under force (7). A schematic of Talin can be seen in Figure ???. Through interaction with integrin tails (dark green) (8), which in turn interact with collagen fibers via their heads, it links the extracellular matrix to the intracellular cytoskeleton by directly interacting with actin. Talin also features specific interactions with the membrane. Their formation, mechanical stability and role in mechanosensing remain to be fully resolved.

Talin contains an N-terminal FERM domain (F for 4.1 protein, E for ezrin, R for radixin and M for moesin), which is composed of the subdomains F0 to F3 and provides a link to the cytosolic side of the plasma membrane (9). It does so via a conserved binding motif for phosphatidylinositol 4,5-bisphosphate (PIP_2), which is enriched at active focal adhesion sites (10–12). The main PIP_2 binding sites are located in F2 and F3 (highlighted as red spheres in Figure ??).

Two isoforms exist of Talin, Talin-1 and Talin-2, encoded by the *tln1* and *tln2* genes. This work refers to Talin-1 if not otherwise stated. Notably, the Talin-1 FERM domain differs from other FERM proteins through the addition of the F0 subdomain, which is connected to F1 via a charged interface, as well as an insertion in F1, a flexible loop with helical propensity and basic residues (13). Additionally, Talin's FERM domain exists in an extended conformation, as opposed to the cloverleaf-like conformation of other FERM proteins (14). F3 also has a binding site for β -integrin tails (15) and is partly responsible for the enrichment of PIP_2 at the membrane through a binding site for $\text{PIP}K\gamma$ (16). A second integrin binding site is located in the rod domain 11 (R11) (17). Talin interacts with the cytoskeleton through actin binding sites (F2-F3, R4-R8, R13-DH) (18). The review by Klapholz et al. (19) provides an excellent overview of the many interaction sites of Talin and their central role in the focal adhesion complex.

The mechanistic role of the disordered loop in the F1 domain in the many aspects of Talin function remains elusive. Its overall positive charge renders it a prime candidate as a PIP_2 binding site. However, previous studies only identified a minor role of the loop in PIP_2 binding compared to F2-F3 (20, 21). On the other hand, the F1 loop has been shown to contribute to Talin-mediated integrin activation (13).

It was previously shown that F3 can interact with R9, which impedes integrin activation (22). Furthermore, in a recently determined cryo-electron microscopy structure of autoinhibited Talin-1, Dedden et al. (23) showed that the rod domains R9 and R12 shield the established PIP_2 binding surface and the integrin binding site in F3 (see Figure ??, Figure ??). This beckons the question how this autoinhibition can be resolved. Song et al. (12) previously investigated a fragment of Talin consisting of F2-F3 and an inhibiting rod segment and suggested a pull-push mechanism, whereby negatively charged PIP_2 attracts its positively charged binding surface on F2-F3 and simultaneously repels the negatively charged surface of the inhibitory rod segment. However, this still leaves open the question of how Talin can establish a first contact with the membrane and remain within a sufficient proximity for this effect to kick in.

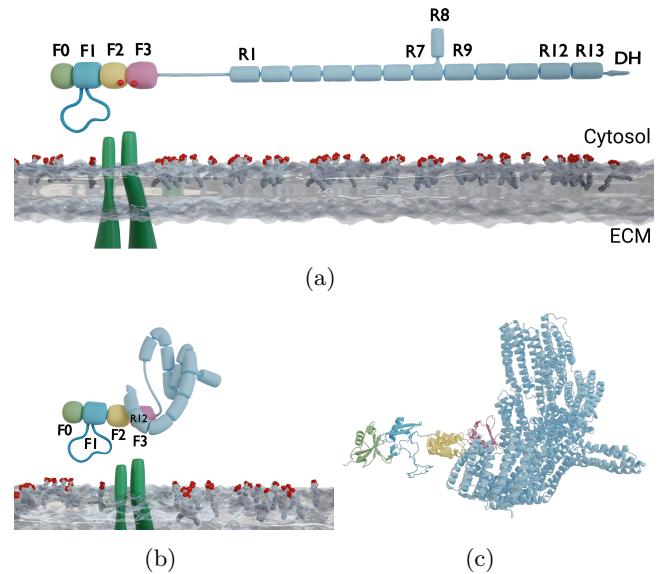


Figure 1: A schematic overview of Talin and our simulation setup. **a)** A schematic rendering of full-length Talin over a POPC membrane enriched with PIP_2 in the upper, intracellular leaflet. The subdomains under scrutiny in this publication, namely F0-F3, which comprise the N-terminal FERM domain (or Talin head), are highlighted in pastel colors (green, cyan, yellow, magenta). The two major PIP_2 binding sites in F2-F3 are marked with red spheres. The Talin rod segments (or Talin tail) are numbered R1 to R13. Note that under physiological conditions, with Talin experiencing force from bound actin, the angle between the FERM domain and the Talin rod would be more akin to 30° as opposed to the linear structure shown here for illustrative purposes. Tails of an integrin α and β heterodimer reaching through the lipid bilayer are represented in green. **b)** A schematic rendering of the autoinhibited structure of Talin as crystallized by Dedden et al. (23) in combination with a cartoon representation in **c**). The completed FERM structure by Elliott et al. (14), with our addition of the modelled F1 loop, is fitted to the autoinhibited structure, as the latter does not include F0F1 due to their flexibility. The complete FERM structure can be explored interactively in the context of our simulation system in the Supplementary Materials. The main PIP_2 binding sites in F2-F3 are occluded by rod domain 12.

We hypothesized that the flexible F1 loop inserted into Talin's FERM domain serves as an additional PIP₂ interaction site. As such it would be readily accessible to PIP₂ even in Talin's autoinhibited conformation and would further mechanically stabilize Talin's interaction with the membrane. To test this hypothesis, we modelled the loop, which, due to its high flexibility, is not included in crystal structures of the FERM domain, such as PDB-ID 3IVF by Elliot et al. (14).

With a complete structure of the Talin FERM domain, we investigated the role of the F1 loop through atomistic molecular dynamics (MD) simulations, which had previously also proven useful to detect the recognition of PIP₂ in membranes by PH domains (24) or the FERM domain of Focal Adhesion Kinase (25).

In F0F1 simulations, we found the loop to have a clear propensity to interact with the PIP₂-containing membrane. It is able to establish a first contact with the membrane even from unfavorable initial orientations due to its large search volume. Furthermore, we show with simulations of the full-length FERM domain that once the loop has established an initial contact, it can anchor the FERM domain to the membrane and allow the known major binding sites in F2-F3 to form.

These results provide mechanistic insight Talin-PIP₂ interactions and highlight the role of secondary intrinsically disordered binding surfaces for membrane recognition.

MATERIALS AND METHODS

Molecular dynamics with GROMACS

MD simulations were performed with GROMACS (26, 27) version 2020.03 (28). A crystal structure of the Talin FERM domain by Elliot et al. (14) with the PDB-ID 3IVF was used as the basis of all simulations.

The deleted or missing residues (134-172) belonging to the F1 domain loop were modeled using MODELLER (29, 30) via the interface to Chimera (31), followed by equilibration with GROMACS. The resulting conformation was compared to an NMR structure of the F1 domain (PDB-ID 2KC2) by Goult et al. (13). The missing residue M1 was also added. The missing residues D125 and E126 as well as I399 and L400 were not modelled, as they are far from the region of interest (the F1-loop). This leaves us with a sequence from residue 1 to 398 with a shift by 2 in numbering compared to the canonical TLN1_MOUSE Talin-1 sequence (uniprot ID P26039) after residue 124. Simulations were performed with the CHARMM36 force field. Topologies, including the membrane, were generated with the CHARMM-GUI web app (32-34) and GROMACS tools. All simulations used the TIP3P water model and were neutralized with 0.15 mol/L of NaCl. A 6-step equi-

libration was performed after gradient descent energy minimization while gradually relieving restraints on protein and membrane atoms. This followed the standard procedure recommended by CHARMM-GUI. The exact Molecular Dynamics Parameter (mdp) files are provided in the data (<https://doi.org/10.11588/data/BQTQUN>) in the assets folder of each simulation system. Production runs used a timestep of 2 fs, a Verlet cut-off scheme for Van-der-Waals interactions and the Particle Mesh Ewald (PME) method for long-range electrostatics. NPT-ensembles were achieved by Nosé-Hoover temperature coupling (35, 36) and Parrinello-Rahman pressure coupling (37). An example .mdp-file can be found in the Supplementary Materials.

The initial equilibrium simulation of the completed FERM domain was run for 75 ns. Subsequently, the root mean squared fluctuation (RMSF) was calculated with GROMACS tools.

The F0F1 FERM sub domains (residues 1 to 197) were simulated to evaluate protein-membrane association using a rotational sampling approach. This entailed placing the protein 1.5 nm away from a 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) membrane, where 12 of the 119 lipids in the upper leaflet were replaced with PIP₂. This results in a physiological concentration of 10% PIP₂. The PIP₂ molecules used in the simulations have a charge of -4, consistent with the deprotonation state of the phosphate groups at physiological pH (38).

60 different starting orientations of the protein were generated, spanning a rotation of 360 degrees. The protein was rotated in such a way that the respective closest residue had the same distance to the membrane for the 0° and the 180° starting positions. 6 replicates of each orientation were run for 200 ns each. However, due to a hardware failure, 2 of these 360 runs are corrupted and thus excluded from the analysis.

From this rotational sampling, we selected representative conformations with loop-membrane interactions as the basis of 6 equilibrium simulations of the complete FERM domain over a POPC membrane with 26 PIP₂ lipids out of a total of 273 lipids in the upper leaflet. Each simulation ran for 400 ns. The initial conformations for perpendicular pulling simulations of the F0F1 subdomains to gauge interaction strength were also chosen from the rotational sampling set. The pulling simulations used an umbrella potential applied to the C-terminus with a harmonic force constant of 500 kJ/mol/nm² and a constant speed of 0.03 m/s (See Supplementary Data in f0f1-vert-pulling/pull_00003.mdp)

Distance information was extracted from trajectories with gromacs tools interfaced via CONAN (39).

Automation, Data Analysis and Availability

Setup scripts written in bash are available for all simulations shown in this work. Computations for data analysis were tracked with the targets R package (40). Plots were generated with ggplot2 (41). Interactive structure representations are embedded using Mol* (42). Schematic visualizations were rendered with blender (43) and VMD (44). Files relevant to this work that are too big to be uploaded to this repository, such as trajectories and blender files, will be uploaded to a separate location. This manuscript was generated with quarto (45–47).

The plotting code and computational graph of targets is available in [this document](#).

RESULTS

The F1 loop can act as a point of first contact

The high flexibility of the F1 loop gave us the confidence to model it from sequence. It retained its flexibility in equilibrium simulations (Figure ??), which in combination with comparisons to NMR structures (13) confirmed this approach. The resulting system that provides the basis for our simulations can be explored interactively in the Supplementary Materials.

When simulating only F0F1 over a POPC membrane containing 10% PIP₂, we noticed that the F1 loop had a clear propensity to establish contact with the membrane. Once the contact had been established, the protein was anchored strongly enough for more contacts to evolve with time, pulling the protein onto the membrane (Figure ??). In order to control for a potential bias towards the loop as a result of the starting position, we performed a rotational sampling of the system, where the starting angle of the loop with respect to the membrane was varied across 60 equally spaced angles. Figure ?? shows that independent of the starting position, the loop is able to find the membrane and bind to it. This is due to the large search space it can cover with its high flexibility (see Figure ??). Binding happens earlier in the simulation when the loop starts oriented favorably towards the membrane (Figure ??, 0 °).

Once a contact has been made, it becomes exceedingly unlikely for F0F1 to dissociate from the membrane (Figure ??). Out of 358 runs¹, 89 runs never made contact with the membrane, but out of the 269 that did, only 10 eventually dissociated. Thus, the simulated timeframe of 200 ns per trajectory allowed for the observation of some reversibility of FERM–PIP₂ binding, albeit only for weakly bound cases and at a small rate of

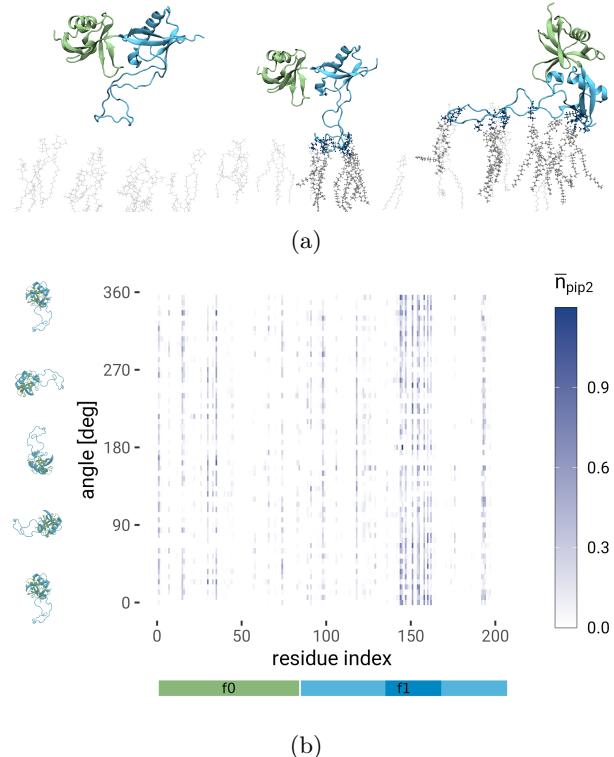


Figure 2: Rotational sampling of F0F1 reveals the lipid binding capabilities of the F1 loop. **a)** Snapshots from a simulation involving F0F1 over a POPC membrane containing 10% PIP₂ in the upper leaflet. POPC is not rendered and PIP₂ is shown as light grey stick models that turn thicker for those molecules that are currently interacting with residues of the protein. The PIP₂-binding residues are then shown as dark blue stick models. **b)** Time-averaged number of PIP₂ molecules bound per residue along the F0F1 sequence (x-axis) as a heatmap summarizing 358 simulations from a rotational sampling, with the starting angle on the y-axis. We sampled 60 different starting positions, rotated equally spaced around the horizontal axis, with 6 replicates each. Each simulation is 200 ns long. 0° corresponds to the loop pointing downwards towards the membrane, as shown in the smaller renders to the left of the heatmap.

¹ 6 replicas each for 60 angles minus 2 runs lost to a storage failure