Supporting Material

Extension of a Three-Helix Bundle Domain of Myosin VI and Key Role of Calmodulins

Yanxin Liu, Jen Hsin, HyeongJun Kim, Paul R. Selvin, Klaus Schulten

1 Movies

- **Movie S1** shows the trajectory of the extension process of the PT domain (simulation PIC-sr-1 in Table 1) and the subsequent relaxation simulation (PIC-re-1 in Table 1). The simulated system includes the IQ motif (cyan) with a bound apo-CaM (yellow), and the proximal tail domain (blue).

- **Movie S2** shows the trajectory of the extension process of the PT domain with an additional 2 nm extension (simulation PIC-sr-1 followed by PIC-sr-2 in Table 1) followed by a 100 ns relaxation simulation (PIC-re-2, see Table 1). The simulation PIC-re-2 did not show any noticeable difference from simulation PIC-re-1 in terms of α-helical content and stability of the extended conformation.

- **Movie S3** shows the trajectory of the extension process of the PT domain in the absence of IQ motif and bound apo-CaM (simulation P-sr in Table 1) followed by a relaxation simulation (P-re in Table 1). The secondary structure of the PT domain is seen to disrupt quickly and is found not to recover in the 100 ns relaxation simulation.

2 Figures

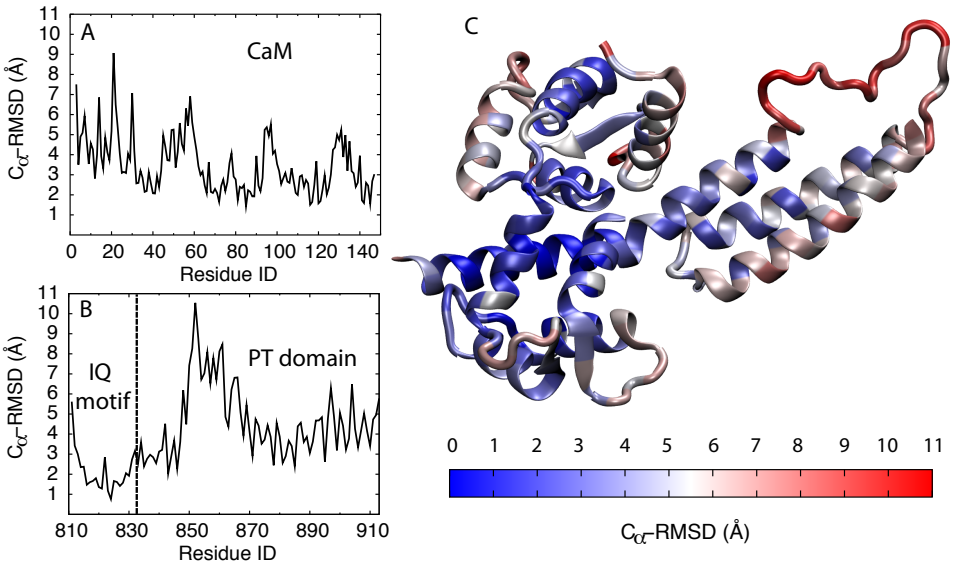

Figure S1: C_α-RMSD of individual residues in the simulated system. (A) C_α-RMSD of individual residues in IQ motif bound apo-CaM. (B) C_α-RMSD of individual residues in IQ motif and PT domain. (C) Simulated system colored by C_α-RMSD. The residues with largest RMSD are colored red, while the ones with smallest RMSD are colored blue. The disordered loop (residues 850-863) between helix I and helix II of the PT domain is seen to be the most flexible part of the system.

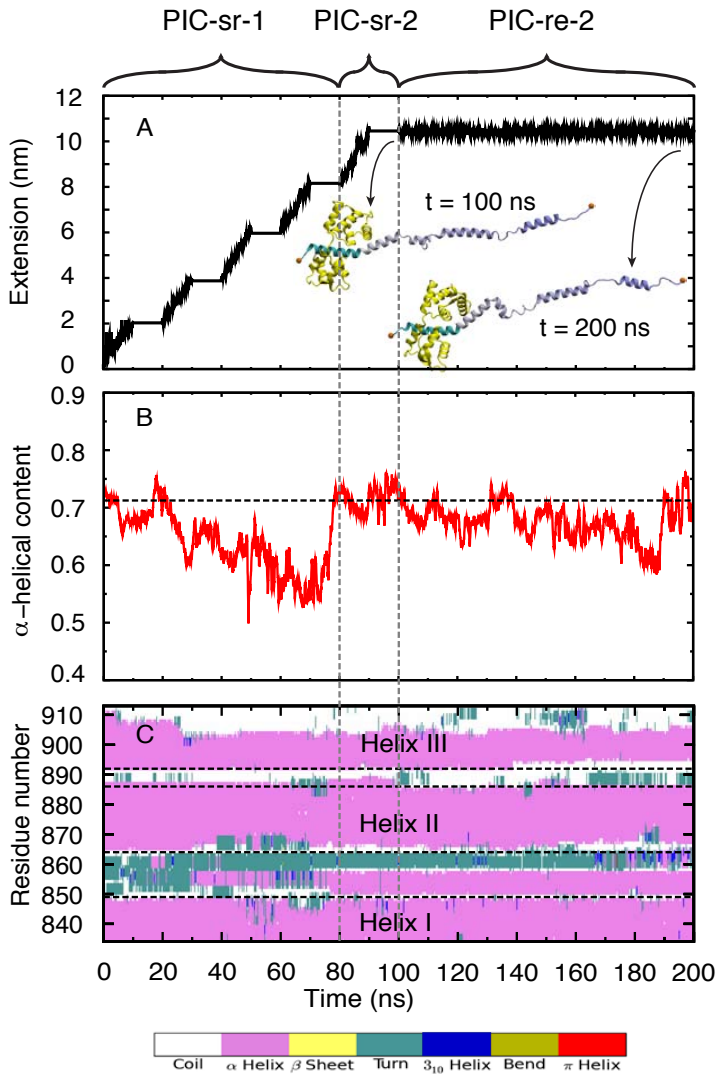

Figure S2: Characterization of PT domain stretching process that further extended the system by 2 nm with one more cycle of SMD/relaxation simulation (PIC-sr-2 in Table 1), in addition to the cycles applied in simulation PIC-sr-1, results of which are shown in Fig. 4. The first 80 ns data is from simulation PIC-sr-1 shown again here for clarity. (A) Extension of the system as a function of simulation time. The conformation at t=100 ns and the final conformation at t=200 are also shown. (B) The α-helical content is not affected by further extension and fluctuated around 0.71 during the relaxation simulation. (C) Time evolution of PT domain secondary structure. α-helices are seen to remain mostly intact; a small portion of helix III looses its α-helical character while a short segment of helix is formed between helices I and II. The analysis for this figure used the Timeline plugin of VMD (33).

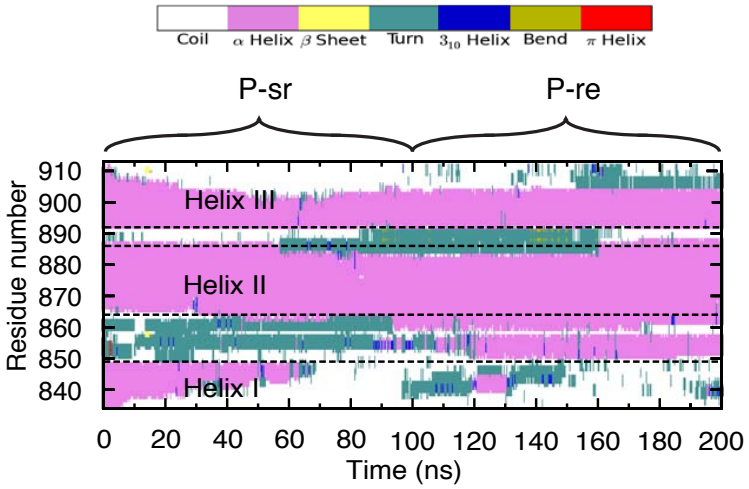

Figure S3: Time evolution of PT domain secondary structure in the absence of IQ motif and CaM. The entire helix I and a part of helix III loose their α-helical character that is not recovered in the 100-ns relaxation simulation. The analysis for this figure used the Timeline plugin of VMD (33).

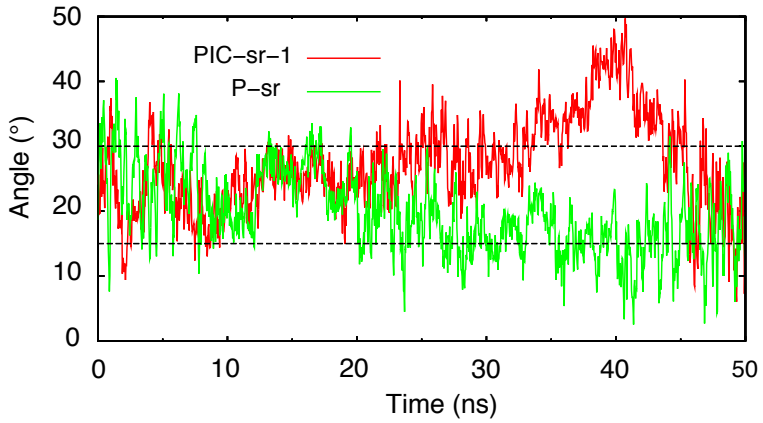

Figure S4: Angles between helix I and x-axis (the latter being the direction of the applied force in the SMD simulations) with and without IQ motif and CaM. Only the first 50 ns of data are shown. In simulation PIC-sr-1 (red trace), helix I is found to dissociate from helices II and III within 50 ns, while in simulation P-sr (green trace), helix I is found to unravel, loosing most of its secondary structure within 50 ns. PT domain with the IQ motif and bound CaM maintains the angle between helix I and x-axis at a value of 30° when helix I dissociation occurred between 25 ns and 45 ns. Without CaM, the same angle diminishes quickly to 15°.