Supporting Information

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SI Methods

Molecular Dynamics Simulations. Simulation setup. Two PDB structures of the E4K4 peptide (CH3CO-EEEEKKKKKKKKKKKKKK-NHCH3), one starting as a complete α-helix and the other as a random coil, were generated with the molecular modeling program SYBYL (Tripos). SYBYL output to AMBER PDB was used to facilitate use with the AMBER2003 force field. The PDB files were converted to GROMACS (1, 2) coordinate and topology files by using the GROMACS utility pdb2gmx (version 3.3.1). Hydrogen atoms in the PDB structure were ignored, new protons were added back by pdb2gmx. The AMBER2003 force field (3) ported for use with GROMACS was used. The structure was solvated in a cuboid box of dimensions 4.231 nm × 5.463 nm × 7.224 nm with 5547 TIP3P water molecules. The genion utility in GROMACS was used to add Na+ and Cl− atoms to a final ionic strength of 150 mM at neutral pH. Glutamic acid (E) and lysine (K) side chains were charged corresponding to neutral pH of the solution. For replica exchange, 48 replicas were created with exponentially distributed temperatures from 274 K to 465 K with final exchange rates for both configurations ranging from 12–24%. Solvated replicas were subject to a preliminary energy minimization step by using the method of steepest descent with 1.4-nm cutoff for neighborlist, and 9-Å cutoff for Coulombic and van der Waals interactions, until a maximum value of less than 10,000 kJ mol−1 was achieved. Long-range electrostatics are treated throughout with the particle-mesh Ewald (PME) algorithm (4). The system was equilibrated at 274 K through 10 ps of molecular dynamics, by using 2-5 time steps with protein-atom coordinates frozen and water-bond lengths constrained. Initial velocities were assigned randomly from a Maxwell-Boltzmann distribution. For both equilibration and the complete MD simulation, a grid-based neighbor search to 9 Å was conducted every 5 steps. Linear and angular motion of the protein and solvent groups about the center-of-mass were removed at every step. A cutoff of 9 Å was used for both Coulombic and van der Waals interactions. The LINCS algorithm with default GROMACS parameters was used to constrain all bond lengths. Periodic boundary conditions were used. REMD simulations for both random and α-helical conformations were continued until the fraction helix at 274 K did not show appreciable change in fraction helix with further simulation time (Fig. S1). Fraction helix was computed from the backbone (ϕ, ψ) by using the Lifson-Roig model, where ϕ = −60 ± 30 and ψ = −47 ± 30° for ‘n’ consecutive angles results in a helical segment of length ‘n’-2. The (EiKj)2 peptide has an acetyl group at the N terminus and a methyl-amide group at the C terminus and, therefore, can have a maximum helical length of 14. For the random initial configuration each replica was simulated for 80 ns for a total REMD simulation time of 3.8 μs. For the α-helical conformation, each replica was simulated for 42 ns for a total REMD simulation time of 2 μs. As shown in Fig. S1 and Fig. 1A, both simulations converge to the same fraction helix at all temperatures simulated. To study the dynamics of side-chain interactions in real time, three folded replicas were randomly selected from the random initial conformations at 274 K and simulated for 50 ns each. With increasing simulation temperature, fraying of the helix ends becomes more prominent and could disrupt the dynamic pattern of side-chain interactions seen at lower temperatures. Because naturally occurring ER/K α-helices are not seen at the ends of proteins but bridge gaps between protein subdomains, we propose that fraying of ends is an artifact of simulating short peptides due to computational limitations. Fraction helix at 274 K is only 6% higher than fraction helix at the temperature used for the SAXS and single-molecule analyses (295 K). Data from the last 25 ns of simulation were used to analyze distances between side-chain atoms. Distance histograms obtained for each of the three simulations were found to be virtually identical, indicating that we sampled sufficient times to capture different modes of interaction.

Simulation hardware. All simulations were performed on the Bio-X2 cluster at Stanford University.

Data Analysis. Analysis of side-chain interactions was performed on explicit atom trajectories output from GROMACS by using custom Matlab (The Mathworks) computer programs. Maximum likelihood estimator (MLE) fit to the sum of three Gaussians was also performed by using custom Matlab code. The goodness of fit was assessed by generating multiple random datasets based on MLE parameters and estimating their likelihood relative to the experimental dataset. We find that the likelihood estimates generated from MLE parameters are equally distributed about the experimental likelihood, verifying goodness of fit. Water density distribution (Fig. S4) around the helix backbone was computed by using data from the last 1 ns of the simulation. TIP3P water coordinates were output explicitly in GROMACS trajectory files. The axis of the α-helix was defined by a least-squares-fit to backbone Cα coordinates. Coordinate transformation was performed to align the axis of the α-helix with the z axis. Distances of water oxygen atoms, between the minimum and maximum z-coordinate of the peptide, were computed by using the custom Matlab program. Water atoms were binned into 0.5-Å wide annuli around the helix axis and normalized to the volume of the annuli to get the water density distribution.

Theoretical Model for Bending of The ER/K α-Helix. To understand the contribution of side-chain interactions to bending stiffness of the ER/K α-helix, we constructed a simplified theoretical model wherein each side-chain interaction is considered to be a harmonic spring at distance d = 6 Å from the axis of the α-helix (Fig. S6A). The side-chains interact through a combination of hydrogen bonding (3 Å) and intermediate (5 Å) modes (Fig. 2C). However, these two modes are not independent of each other, because they derive from the same distribution. Bending of the α-helix changes the side-chain interaction distances (Fig. 3B). The hydrogen-bonding interactions centered at 3 Å can accommodate only very small extensions (0.3 Å). But, the presence of a second interaction centered at 5 Å with a larger reach of 0.9 Å, that follows the hydrogen-bonding interaction, prevents the α-helix from falling apart in the presence of lateral bending forces, such as those that occur during the swing of the myosin VI or myosin X lever arm. Also, the harmonic spring constant for the 3-Å interaction is 10-fold larger than that for the 5-Å interaction. For side-chain interaction distances corresponding to the 5-Å potential well each E(i) interacts simultaneously with K(i − 4) and K(i + 3). However, for the 3-Å potential well each E(i) interacts with only one of K(i − 4) and K(i + 3). Thus, the 5-Å potential well, in addition to its longer reach, provides a continuous sheet of interactions without breaks along the α-helical backbone. Therefore, we model the side-chain interactions as a continuous narrow tube with stiffness corresponding...
to the 5-Å potential well. For mechanoenzymes such as myosin VI or X, the ER/K α-helix will experience a lateral bending force at one end (for example the cargo binding domain of myosin VI), which is then transmitted to the other end held fixed by stiff protein structural elements (for example the proximal tail of myosin VI). Therefore, we model the ER/K α-helix as a cantilever beam (Fig. S6A) (5). The force and deflection of a cantilever beam are related by Eq. 3 (Fig. S6). The ER/K α-helix has continuous series of interacting residues along multiple faces of the α-helical backbone (Fig. 1 C and D). Therefore, each lateral cross section of the ER/K α-helix is stabilized by either three or four side-chain pairs. A pitch of approximately N = 3.6 residues for the α-helix results in the location of the side-chain pairs, in successive cross sections from its N to C terminus, staggered relative to each other (Fig. S6B). Therefore, we model the side-chain pairs at each cross section as N springs of stiffness k_{3,4} = 440 pN/nm (corresponding to the 5-Å peak), uniformly distributed at d_α = 6 Å from the α-helix axis. The stiffness of these springs provides for the Young’s modulus (E) and their distribution provides for the second moment of area (I) (Fig. S6, Eqs. 1 and 2). Cantilever beam theory (Fig. S6, Eq. 3) was then used to estimate a force per unit deflection (stiffness) of 0.46 pN/nm for the ER/K α-helix, limited to small deflections (~1 nm for a 10-nm α-helix).

Simplifications and Uncertainties. The theoretical model assumes that the α-helix can be treated as a cantilever beam, with the side-chain interactions represented as harmonic springs that resist the deflection of the α-helix. To construct this model, we interpret the two prominent Gaussian peaks seen in the histogram of minimum distances as harmonic potential wells, in which side-chain interactions prefer to reside 82% of the time in equilibrium MD simulations. This interpretation is based on the assumption that the attractive forces between oppositely-charged atoms in the side chains would resist change in distance between them during bending of the α-helix. The 3-Å potential well represents a hydrogen-bonding interaction between E and R/K side chains, but the source of the 5-Å potential well is not completely clear. Importantly, we note that there is no evidence that the 5-Å potential well will resist bending. However, this is a reasonable assumption given that for conformations corresponding to the 5-Å potential well, several charged H atoms in the R/K side chain are in close proximity to the electronegative O, atom of E contributing to attractive forces between side chains. Our model imposes the simplification of treating the bending of the ER/K α-helix as a cantilever beam based on neutral axis bending theory (5). Despite these caveats, our model successfully accounts for the observed stiffness of the ER/K α-helix based on physically reasonable side-chain interactions.

Charge Interaction Map of ER/K α-Helices. ER/K sequences are arranged such that R/K at i are aligned vertically with E at i − 3 or i + 4. This implies also that E at i is aligned vertically with R/K at i − 4 or i + 3. To construct this map (see Fig. 1C and Fig. S4) the sequence is written from right to left and bottom to top starting with the first residue of the ER/K motif at the bottom right hand corner. For most ER/K sequences this alignment is easily done by switching lines when the charge changes sign (E↔R/K). On changing lines, the subsequent R/K or E residue is placed above its interacting partner. In regions where multiple interactions are possible, the map is aligned to maximize the number of displayed interactions. Vertical lines through this map show charged residues that can interact with each other along the α-helix backbone.

SAXS Experiments. Protein expression and purification. For IQ-PT-CAM the IQ-PT human myosin VI cDNA (814–918) was cloned into a pGEX 6P1 vector (GE Healthcare, Piscataway NJ) with human calmodulin (CAM) cloned into a pET28a vector (EMD Chemicals, San Diego, CA). Myosin VI medial tail (MT) [human myosin VI cDNA 916–981] was cloned into a modified pET28a vector (EMD Chemicals, San Diego, CA). This specific MT construct was designed with additional sequences to enable dye/ligand conjugation at each end. The N terminus has the additional sequence EEVEKKC providing a highly reactive cysisteine. The C terminus has the additional sequence KK-DSLEFIASKLA-KKKW with the 11 central residues for labeling with serine phosphoryl transferase (sfp) at the first serine (5) (6). The modified vector contains a His_6 tag, maltose binding protein and a TEV protease cleavage site 5’ to the cloning site. IQ-PT and CAM vectors were coexpressed in Escherichia coli Rosetta (DE3) cells (EMD Chemicals, San Diego CA). pET28a provides kanamycin resistance, whereas pGEX 6P1 provides ampicillin resistance. Hence, cells were grown in triple selection media (including chloramphenicol for Rosetta cells). The MT vector was expressed independently in E. coli Rosetta (DE3) cells. Cells were lysed by sonication in the presence of 1–1.5 mg/ml lysozyme. Lysates were clarified by centrifugation at 100,000 × g for 30 min. IQ-PT-CAM lysates were bound to a GST column (GE Healthcare, Piscataway NJ), followed by elution with reduced glutathione. Eluted proteins were dialyzed in the presence of Precision protease to cleave the GST from the IQ-PT-CAM domain. Dialysis products were further purified by GST column chromatography to remove GST. After the GST column chromatography, the relative amounts of IQ-PT and CAM were assessed by Coomassie stained SDS/PAGE gels. Purified human CAM was added back to obtain approximate equimolar amounts of IQ-PT and CAM, followed by dialysis of the sample into scattering buffer containing 20 mM phosphate, 100 mM NaCl, 1 mM DTT, 1 mM EGTA, and 1 mM EDTA. Samples were further purified on a superdex 200 gel filtration column (GE Healthcare) to obtain a single species of the protein complex containing equimolar amounts of IQ-PT and CAM. The presence of a single species of protein complex was verified independently by using analytical size exclusion column (Superdex 75), multangle laser light scattering (MALLS), and dynamic light scattering (DLS) measurements, from which we estimated sample dispersity at <1%. MT lysates were bound to Ni-NTA resin (Qiagen). The eluted proteins were dialyzed in the presence of TEV protease (1–100 by weight) overnight to cleave the His6 and MBP portions from the MT domains. MT domains were further purified by running the cleavage reaction over a Ni-NTA column to remove His6 containing fragments, followed by a superdex 200 column (GE Healthcare) by using either Circular Dichroism (CD) buffer (10 mM phosphate, pH 7.4/25 mM NaCl) or scattering buffer (see above).

SAXS measurements. SAXS measurements were carried out at the XOR/BESSRC undulator beam line 12-ID of the Advanced Photon Source (Argonne, IL) employing a sample-detector distance of 2 m and CCD detector read out (MAR USA). The data were collected by using a custom-made sample cell (7) at an x-ray energy of 12 keV. Details of the beam line were as described previously (7–9). Samples were clarified by centrifugation at 10,000 × g for 10 min before measurement and five 0.5 s exposures were obtained. Data were image corrected, normalized by incident flux and circularly averaged. The five profiles for each condition were averaged to improve signal quality. Buffer profiles were collected by using identical procedures and subtracted for background correction. The data showed no signs of radiation damage, based on comparison of consecutive scattering profiles from the same sample (data not shown).

SAXS data analysis. Scattering intensities as a function of the momentum transfer were obtained at different protein concentrations. The SAXS profiles for the IQ-PT-CAM complex were superimposable after scaling by forward scattering intensity,

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suggesting that there was no detectable aggregation or interparticle interference effects (Fig. S8E). The MT construct has a highly reactive cysteine, attached for use in future experiments, which could react to form a dimer under very high concentrations. Force results by using lower protein concentration were used (Fig. S8A). Radii of gyration ($R_g$) were determined from Guinier analysis of the low angle scattering data (10) (Fig. S8 B and F). $R_g$ obtained from Guinier analysis agree within experimental errors with the values from the real space distribution function $P(r)$ computed by using the regularized transform method implemented in the program GNOM (11). The $R_g$ for the IQ-PT-CAM complex and the MT are $\sim$2.3 and 3.2 nm, respectively. Our modeled structures (Fig. 4B) have corresponding $R_g$ values of 2.2 and 3.1 nm, respectively.

**SAXS Structure Reconstructions.** The programs DAMMIN (12) and GASBOR (13) were used to construct 3-D bead models that fit the scattering data (Fig. S8 C, D, G, and H). Both programs employ a simulated annealing procedure and a compactness criterion. Ten independent DAMMIN and GASBOR runs were performed for each scattering profile, by using default parameters, the “slow” mode for DAMMIN, no symmetry assumptions (P1) structure for the full recorded scattering profiles. The models resulting from independent runs were superimposed and compared by using the program SUPCOMB (14), based on the normalized spatial discrepancy (NSD) criterion. Models with NSD values $<1$ were considered similar. For all data presented in the main text, the 10 independent repeat runs yielded models with pairwise NSD values $<1$, indicating that the algorithms converged reproducibly to similar structures. The 10 independent structures for each scattering profile were subsequently averaged, and “filtered” consensus models were computed by using the program DAMAVER with default settings (15). Consensus models constructed with DAMMIN and GASBOR gave similar results. For visualization, the reconstructed bead models were converted to electron density maps with the program Situs (16).

**CD.** CD Spectra were acquired by using an Aviv 62DS instrument (Aviv Biomedical) with a 1-mm path length cell in CD buffer. Spectra were taken at 0°C with data collected every 1 nm with a 10 second averaging time, and are the average of 2 repeat scans. Protein concentrations ranged from 1–17 µM. Melt data were collected every 1°C with a 30 s averaging time and a 2 min equilibration. In all cases the reverse melt showed at least 90% reversibility. CD data were converted from mean residue ellipticity (MRE) to fraction helix by using equations outlined in Rohil and Baldwin (17, 18).

**MALLS.** In solution molecular mass of the IQ-PT-CAM complex was determined by using a size exclusion chromatography system coupled to a multiple angle light scattering detector. Protein concentrations were determined with an Optilab REX refractive index detector, and scattering was detected with a Dawn 18 angle MALLS light scattering instrument (Wyatt Technology Corporation). MALLS molecular mass of the IQ-PT-CAM complex was found to be 27.8 ± 0.3 kDa at a maximum concentration of 100 µM with $<5%$ polydispersity. The expected molecular mass of the IQ-PT-CAM complex is 29 kDa.

**DLS.** DLS measurements were made by using a DynaPro instrument (Protein Solutions) running Dynamics version 6 software. IQ-PT-CAM samples at 1–10 mg ml$^{-1}$ in scattering buffer were assayed at 25°C with an acquisition interval of 10 s. Samples were spun at 15,000 $\times$ g for 10 min immediately before analysis. Results were derived by using a regularization fit and with PBS buffer settings. DLS measured a hydrodynamic radius ($R_h$) of 2.3 ± 0.2 nm for the IQ-PT-CAM complex.

**Single-Molecule Optical-Trap Assays.** Protein expression and purification. Two different porcine myosin VI sequences, beginMT [1–918] and endMT [1–981], followed by (GS)$_n$, eYFP, and an N-terminal FLAG tag (DYKDDDDK), were cloned into pBieX-1 (Novagen) and expressed by transfection of plasmid DNA into Sf-9 cells (19).

**Optical trapping.** Optical trapping was performed by using the custom dual-beam optical trap (20–22). All trapping was done without feedback. Dumbbells of actin were formed by using a streptavidin-biotin link between streptavidin coated beads and 100% biotinylated F-actin. Phallolidin was added to a final concentration of 10 µM to stabilize actin filaments. The actin dumbbell was pulled taut with trap stiffness of $\sim$0.005 pN/µm. Motors were attached to nitrocellulose coverslips with monoclonal anti-GFP antibody (Chemicon MAB3580). Motor dilutions were chosen such that $<10%$ of tested platforms showed myosin VI binding to actin, to minimize binding of more than one myosin head to the F-actin filament. Binding events were determined from data traces, by eye, by using both decrease in bead position variance and decrease in bead-to-bead correlation.

Fig. S1. Convergence of fraction helix in REMD simulation from α-helical and random initial conformations. Change in fraction helix at 274 K was computed by using the Lifson-Roig model as a function of time during REMD simulation. Data are block averaged over 0.2-ps intervals. Blue symbols, starting with fully α-helical conformation; red symbols, starting with random initial conformation. The convergence of simulations is seen by near zero slope toward the end of each simulation. Additionally, both simulations converge to the same final value.
**Fig. S2.** The ER/K motif in the medial tail of myosin VI is α-helical in solution. MRE measured by using circular dichroism at 273 K (0°C) for the medial tail of myosin VI shows characteristic minima at 208 and 222 nm. The inset shows the MRE at 222 nm as a function of temperature (thermal melt).
Fig. S3. K residues at $i$ preferentially interact with E at $i - 3$ and $i + 4$ positions, whereas E residues at $i$ preferentially interact with K at $i - 4$ and $i + 3$ positions. Histograms of distance distributions between N/H$_9$/O$_{12}$ atom of residue $i$ at positions K5-E12 with O$_{12}$/N$_9$ atom at $i - 4$, $i - 3$, $i + 3$, and $i + 4$ positions. The x-axes are distance in Å. Data were acquired over 25 ns of simulation time. Columns left to right are $i - 3$, $i + 4$, $i - 4$, and $i + 3$ distances, respectively, whereas rows correspond to residues K5-E12 bottom to top. Note the clear 3-Å peaks with at $i - 3$ and $i + 4$ for K residues, whereas E residues have 3-Å peaks with $i - 4$ and $i + 3$ residues. Importantly, residue K8 ($i$) does not interact with E4 ($i - 4$), and residue E9 ($i$) does not interact with K13 ($i + 4$), as evidenced by the absence of 3-Å peaks in the corresponding distributions. Also, K8 ($i$) does not have a partner at $i - 3$ (K5), whereas E9 ($i$) does not have a partner at $i + 3$ (E12).
Fig S4. Charge interaction map for naturally occurring ER/K motifs. Charge interactions for proteins shown in Table S1 are displayed using this interaction map (for human) and are shown in Fig. 1. Based on MD simulations of the E/AK/ER/K motif, sequence residues E (i) and K (i) interact with EK located at i - 1 or i + 3, and residue EK (i) interacts with RK located at i - 4 or i - 3. Sequences are read right to left, bottom to top. Residue numbers relative to the first residue of the ER/K motif are listed beside the sequences. Vertical lines represent the interacting residues. Sequences with multiple residues that share interactions along the c-helix backbone (see yellow boxed groups of residues in column).
Fig. S5. The 5-Å preferred distance between N$_i$ of K and the centers of mass of O$_i$ of E in the (E$_i$K$_i$)$_2$ α-helix is accompanied by multiple interactions between charged H-atoms in the lysine side chain and the O$_i$ atom of glutamic acid. (A) Histogram of minimum distances between K7-N$_i$ (i) and the centers of mass of O$_i$ for E3 (i − 4), E4 (i − 3), E10 (i + 3), and E11 (i + 4). Red and blue boxes indicate the two peaks at 3 Å and 5 Å, respectively. (B) The smallest distance between three H$_i$ atoms of K7 and two O$_i$ atoms of E3, E4, E10, and E11 for the portion of the histogram in (A) indicated by the red box. H$_i$ and O$_i$ are at hydrogen-bonding distance for N$_i$–O$_i$ distances corresponding to the 3-Å peak in (A). (C) The smallest distance between three H$_i$ atoms (Left) or two H$_i$ atoms (Right) of K7 and the two O$_i$ atoms of E3, E4, E10, and E11 for the portion of the histogram in (A) indicated by the blue box. For convenience, 4 Å is used as the demarcation between the red and blue boxes in (A). (B) and (C) list the partial positive charges of the H$_i$ and H$_i$ atoms resulting from direct and indirect-bonding interactions with the electronegative N$_i$. Note that several H-atoms in the K7 side chain, each with significant partial positive charges are within close proximity to the highly electronegative O$_i$ atom.
Fig. S6. Model of ER/K α-helix as a cantilever beam. (A) (Upper) The α-helical backbone is shown in ribbon representation (yellow) with the location of the interacting atoms of the E and R/K side chains shown as a gray tube surrounding the ER/K α-helix. L is the length of the α-helix, whereas F is the lateral bending force applied at one end, resulting in δy deflection at that end. \( d_o \) is the average distance of side-chain \( N_e \) and \( O_k \) atoms, of K and E respectively, from the axis of the α-helix. (Lower) Side-chain pairs are modeled as harmonic springs located at fixed distance \( d_o = 6 \text{ Å} \) from the α-helix axis, each of which has a spring constant \( k_{5\text{Å}} = 440 \text{ pN/nm} \), estimated from distribution of side-chain pair distances (Fig. 2B). Although the schematic representation only shows residues above and below the neutral axis for clarity, the model is based on side-chain pairs distributed continuously around the α-helix. (B) (Left) The cross section of the ER/K α-helix is approximated as an annulus of width \( q \) with area \( A = 2\pi d_o \, q \) and second moment of area \( I = \pi d_o^3 \, q \) (Eq. 1). (Right) A view down the axis of a 29-aa long \((E4K4)_n\) α-helix (approximately eight turns) represents the staggered placement of residues. (C) The derivation of equivalent Young’s modulus (E) (Eq. 2) of our model as a function of harmonic spring stiffness of side-chain interactions is shown. (D) The force-deflection relationship is shown by using cantilever beam theory (Eq. 3) (5), with E and I approximated for the ER/K α-helix as described in A–C. Substitution of E and I yields a bending stiffness of 0.46 pN/nm for small deflections (<1 nm) for a 10-nm long ER/K α-helix.

1) Spring Stiffness = \( k_{5\text{Å}} = \frac{\text{dForce}}{\text{dExtension}} \)
   For each spring:
2) Young’s modulus = \( (\frac{\text{dForce}}{\text{Area}} \times \frac{\text{Length}}{\text{Area}}) \)
   \( = \frac{\text{dForce}}{\text{dExtension}} \times \frac{\text{Length}}{\text{Area}} \)
   \( = k_{5\text{Å}} \times (rN)/A \)
   For entire cross section:
3) Young’s modulus (E) = \( N \times k_{5\text{Å}} \times (rN)/A \)
   \[ E = \frac{k_{5\text{Å}} rN^2}{2\pi d_o q} \]  
   \[ N \] - number of residues per turn

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2) Young’s modulus = \( (\frac{\text{dForce}}{\text{Area}} \times \frac{\text{Length}}{\text{Area}}) \)
   \( = \frac{\text{dForce}}{\text{dExtension}} \times \frac{\text{Length}}{\text{Area}} \)
   \( = k_{5\text{Å}} \times (rN)/A \)
   For entire cross section:
3) Young’s modulus (E) = \( N \times k_{5\text{Å}} \times (rN)/A \)
   \[ E = \frac{k_{5\text{Å}} rN^2}{2\pi d_o q} \]  
   \[ N \] - number of residues per turn

Young's modulus of cross section:
\[ E = \frac{3EI}{L^3} \delta y \]  
Stiffness = \( \frac{F}{\delta y} \) \[ \frac{3d_o^3 k_{5\text{Å}} rN^2}{2L} \]  

Parameters:
\[ d_o = 0.6 \text{ nm} \]  
\[ r = 0.15 \text{ nm} \]  
\[ k_{5\text{Å}} = 440 \text{ pN/nm} \]  
\[ N = 3.6 \]  
\[ L = 10 \text{ nm} \]
Fig. S7. Hydrophobic residues in the ER/K motif could reduce the local stability of the ER/K α-helix. Thermal melts are shown for different segments of the MT ER/K α-helix in myosin VI. Three different segments of the myosin VI medial tail domain containing different numbers of hydrophobic residues (A, L, I, and M) were folded into fully α-helical conformation and simulated in explicit solvent at 400 K to study their relative stabilities. Sequence (E2K2)4 is simulated as a negative control as it is known to have low α-helical content. The (E2K2)4 helix unravels completely within 20 ns, whereas the three segments of the myosin VI medial tail remain stable over the same time period. However, segments containing hydrophobic residues show transient reductions in fraction helix, which is a result of local unraveling and refolding of helix turns in the hydrophobic regions (data not shown).
Fig. S8. SAXS results for myosin VI MT (A–D) and IQ-PT-CAM (E–H). (A and E) Scaled scattering profiles are shown of $I$ (scattering intensity normalized by forward scattering intensity) versus $s$ ($2 \sin \theta/\lambda$, where $\theta$ and $\lambda$ are the scattering angle and x-ray wavelength, respectively) at indicated concentrations. (B and F) The linear Guinier fit to the scattering data are shown for the IQ-PT-CAM construct for different protein concentrations. The color scheme used is identical to (A and E). The fit residuals (Lower) do not show any significant deviation from zero, nor any consistent curvature. Fits to all three datasets gave consistent $R_g$ values. SAXS bead reconstructions were obtained by using DAMMIN (C and G) and GASBOR (D and H) reconstruction algorithms.

ER/K Helix Myosin VI

Myosin VI IQ-PT-CAM

A

E

B

F

C

G

D

H

0.72 mg/ml
1.44 mg/ml
2.88 mg/ml

1.06 mg/ml
2.13 mg/ml
4.25 mg/ml
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| Saccharomyces cerevisiae      | Mannosyltransferase                              | EEKKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQEEEKKQCEE