Supporting Information

Cations Stiffen Actin Filaments by Adhering a Key Structural Element to Adjacent Subunits

Glen M. Hocky,1,† Joseph L. Baker,2,† Michael J. Bradley,3,4 Anton V. Sinitskiy,1,5 Enrique M. De La Cruz,3* and Gregory A. Voth1*

1Department of Chemistry, James Franck Institute, Institute for Biophysical Dynamics, and Computation Institute, The University of Chicago
2Department of Chemistry, The College of New Jersey
3Molecular Biophysics and Biochemistry, Yale University
4Current address: Syros Pharmaceuticals, Cambridge, MA
5Current address: Department of Chemistry, Stanford University

†Authors contributed equally
*Corresponding authors:

enrique.delacruz@yale.edu (203) 432-5424
gavoth@uchicago.edu (773) 702-9092

Molecular dynamics simulations

Actin 13-mers were constructed following the procedure described previously.1 Subunit conformations came from either the models of Oda (PDB ID 2ZWH)2 or Namba (PDB ID 3MFP).3 Water molecules, ADP and magnesium ion were positioned inside the binding pocket in the manner found in the Oda PDB structure.2 A periodic filament of 13 subunit was constructed by duplicating each configuration, and translating it in the Z-direction by 27.6 Å and then rotating by -166.7 degrees using VMD.4 The system was solvated by water molecules with at least 12 Å of water padding in the x and y directions. The autoionize tool in VMD was used to replace some waters with potassium and chloride ions such that the system was neutralized and had a concentration of 0.180 KCl.4

Molecular dynamics simulations were performed with NAMD5 using the CHARMM 27 force field with CMAP correction,6 using the TIP3P model for water molecules.7 Bonds to hydrogen atoms were constrained using the SHAKE algorithm,8 permitting the use of a 2 fs integration timestep. Temperature was maintained using the Langevin thermostat5 with a coefficient of 5 ps⁻¹ and pressure maintained by a Langevin piston9 with a period of 2 ps and a time constant of 1 ps.

The system was energy minimized in four stages of 1000 steps of steepest descent, with 10 kcal/mol/Å² constraints on specific atoms in the system. In the four stages, the following parts of the system were constrained: (1) protein, nucleotide, nucleotide Mg²⁺ and nucleotide waters (within 5 Å of Mg²⁺), (2) protein backbone, nucleotide, nucleotide Mg²⁺, and nucleotide waters,
(3) nucleotide, nucleotide Mg\(^{2+}\), and nucleotide waters, (4) nucleotide Mg\(^{2+}\) and nucleotide waters. The same restraints as in step (2) were then reapplied. The system was heated from 0 K to 310 K at a rate of 5 K/ps in the NVT ensemble using a 2 fs timestep and then NVT simulation was further continued for 50 ps. The system was then simulated at 1 atm in the NPT ensemble, relaxing the constraints after 200 ps by a factor of two, and repeating this process 5 more times. The system was then simulated with no constraints for 400 ps.

**Construction of subunits containing stiffness and polymerization ions**

**Construction of the Namba-cation starting subunit configuration**

The Namba model with stiffness and polymerization cations was constructed starting from the “low resolution” prediction obtained in Ref. 10 using the webFEATURE algorithm\(^{11}\). This algorithm assigns energetic scores to certain structural features of binding pockets in order to score potential areas of the protein as candidate ion binding sites. As described in in Ref. 10, the resulting actin/ion model can be thought of as “low resolution” since the input actin model has a 6.6 Å resolution and the webFEATURE algorithm looks in an area 7 Å out from a test position.\(^{11}\)

In order to obtain Mg\(^{2+}\) ions that are hexa-coordinated to oxygen atoms in the stiffness and polymerization ion sites, we further refined this starting model using a multi-stage minimization protocol with restraints, which is described below. The refinement was carried out using an actin 5-mer, as the 5-mer environment guarantees that the central actin monomer has all appropriate contacts as would be found in a complete filament. Furthermore, the use of a 5-mer allowed for repositioning of the D-loop of each monomer further into the actin target binding cleft of the n+2 monomer (except for the two monomers closest to the barbed end of the filament) during the refinement process. The actin 5-mer was built following the same procedure as followed for an actin 13-mer, except that only five subunits were used.

In the starting 5-mer model, before MD refinement, the stiffness cation is in contact with E167’ and surrounded by several other potential coordinating side chains (Table S1). Analysis of structures in the PDB\(^{12}\) containing bound magnesium ions show that magnesium ions are generally coordinated by four side-chain oxygen atoms and two water oxygen atoms in an octahedral geometry at a distance of approximately 2-3 Å from the ion center.\(^{13}\) Therefore, each of the two low affinity cations were coordinated by four amino acid oxygen atoms and two water oxygen atoms in our model. In order to select amino acids that would contribute oxygen atoms to complete the coordination, we relied on the proximity of the ions to amino acids in the binding sites and sequence conservation data. In the stiffness site, K50, E57, Q49 and E167’ were positioned such that oxygen atoms from each of these amino acids could be coordinated to the magnesium ion using the restraints listed in Table S2. In the polymerization site, the amino acids D286’, D288’, E205’ and T202 were chosen to coordinate to the magnesium ion using the restraints listed in Table S2. D-loop restraints to position the D-loop further into the n+2 TBC are also listed in in Table S2. In the first stage of minimization amino acid residues were positioned to coordinate the ions, and in a second stage the additional two water molecules were positioned to coordinate the ions and complete the hexacoordination. The interactive molecular dynamics feature of VMD/NAMD (4, 5) was then used to remove side chain clashes. The central subunit in the pentamer was then used with the protocol in the previous section to construct and equilibrate our Namba-cation 13-monomer periodic filament model. The resulting model exhibited magnesium ions that did not unbind even after extensive periods of simulation time.
However, the initial prescribed coordination was not fully maintained at either site, exhibiting some polymorphism, as discussed in the main text.

**Table S1.** Distances of oxygen atoms from Mg$^{2+}$ ion when the ion is placed centrally in the stiffness site described in Kang, *et al.*

| Residue | Namba distance (Å) | Oda distance (Å) |
|---------|--------------------|------------------|
| Gly36   | 3.57               | 2.66             |
| Gln49   | 3.20               | 9.98             |
| Lys50   | 2.82               | 4.39             |
| Asp51   | 3.66               | 2.48             |
| Glu57   | 5.77               | 6.77             |
| Glu167' | 6.47               | 12.1             |

**Table S2.** Restraints for construction of the ion binding pockets, labels are atom names in CHARMM.

| Group 1 | Group 2 | Distance (Å) | Force Constant (kcal/mol/Å$^2$) |
|---------|---------|--------------|---------------------------------|
| D-loop to TBC restraints |
| K61(n)  | D292(n+2) | 1.8 | 50.0 |
| I64(n)  | Y169(n+2) | 1.8 | 50.0 |
| I64(n)  | L171(n+2) | 1.8 | 50.0 |
| M44(n)  | L142(n+2) | 1.8 | 50.0 |
| M44(n)  | Y143(n+2) | 1.8 | 50.0 |
| V43(n)  | L346(n+2) | 1.8 | 50.0 |
| V43(n)  | F352(n+2) | 1.8 | 50.0 |
| V43(n)  | M355(n+2) | 1.8 | 50.0 |
| V43(n)  | F375(n+2) | 1.8 | 50.0 |
| M47(n)  | L349(n+2) | 1.8 | 50.0 |
| M47(n)  | F352(n+2) | 1.8 | 50.0 |
| Stiffness ion restraints |
| K50,O(n) | Mg(s)  | 1.6 | 50.0 |
| E57,OE1 | Mg(s)  | 1.6 | 50.0 |
| E57,OE2 | Mg(s)  | 4.0 | 200.0 |
| E167,OE1| Mg(s)  | 1.6 | 50.0 |
| E167,OE2| Mg(s)  | 4.0 | 200.0 |
| Q49,OE1 | Mg(s)  | 1.6 | 50.0 |
| Polymerization ion restraints |
| D286,OD1 | Mg(p) | 1.6 | 50.0 |
| D286,OD2 | Mg(p) | 4.0 | 200.0 |
| D288,OD1 | Mg(p) | 1.6 | 50.0 |
| D288,OD2 | Mg(p) | 4.0 | 200.0 |
| E205,OE1 | Mg(p) | 4.0 | 200.0 |
| E205,OE2 | Mg(p) | 1.6 | 50.0 |
| T202,OG1 | Mg(p) | 1.6 | 50.0 |
Construction of the Oda-cation starting subunit configuration

Once the Namba-cation had been constructed and we had established that adding coordinating ions increased filament stiffness, we wished to test our hypothetical binding site in the Oda model. Rather than starting from scratch, we chose to add stiffness cations to an Oda filament in roughly the same geometry developed for the Namba model to better make our results comparable to each other. An equilibrated subunit from the Oda simulation was taken. A subunit configuration from the Namba-cation configuration after 40 ns of simulation was extracted. We then compute the rotation matrix that aligns Oda and Namba-cation subunit backbones, and apply this matrix to position of the stiffness cation from the Namba-cation simulation. This was then used as a starting point for the ion position. A pentamer of Oda subunits with these additional aligned cations was created, and the same equilibration procedure as above was performed, except that additionally the restraints Table S2 were added to the potential energy. We did not force water molecules to align in the remaining spots, and assumed these would reach their final positions in the equilibration process. When doing this procedure, we found that Y53 would adhere to the stiffness cation, blocking the location of a bound water in the Namba-cation geometry. Hence we applied an additional one-sided harmonic wall potential between the stiffness cations and the OH1 oxygen of Y53 with a minimum at 4Å and a force constant of 50 kcal/mol/Å^2, which caused the tyrosine oxygen to be released from the cation, and allowed that part of the actin backbone to relax to a different configuration.

After equilibration, the central subunit and stiffness cation was taken and used to build a 13-mer. During the equilibration of the 13-mer, these same additional forces were applied on each subunit. We maintained these constraints for 20 ns NPT simulation as well to allow the Oda monomer to relax while maintaining cation coordination. After these 20ns, the constraints were released, and subsequent results are as discussed in the main text.

Coarse-grained definitions

In order to compute structural properties of actin filaments, we map the position of groups of residues in each subunit to the position of their center of mass. We use the definitions previously proposed in Saunders and Voth\(^{14}\) which capture well the collective motion of actin segments in the monomeric and filamentous form, as well as preserving many traditional designations from crystallographic studies (Table S3).

| Bead | Identity | Residues                  |
|------|----------|---------------------------|
| 1    | SD1      | 5-33, 80-147, 334-349     |
| 2    | SD2      | 34-39, 52-69              |
| 3    | SD3      | 148-179, 273-333          |
| 4    | SD4      | 180-219, 252-262          |
| 5    | D-loop   | 40-51                     |
| 6    | Flap     | 236-251                   |
| 7    | H-loop   | 263-272                   |
| 8    | C-terminus | 350-375                |
| 9    | Nucleotide | (ATP or ADP position)     |
| 10   | N-terminus | 1-4                     |
| 11   | S-loop   | 70-79                     |
| 12   | Hinge    | 220-235                   |
Dimensionality reduction techniques
D-loops (residues 40-51) were extracted every nanosecond from fifty nanosecond segments of trajectories for our simulations termed Namba-cation, Namba, Oda, Oda-f and Cofilin. In the first four cases, there were 13 subunits per simulation resulting in 650 D-loop sample geometries, and in the Cofilin case there were 11 subunits per simulation, resulting in 550 D-loop sample geometries. We performed analysis on this total data set of 3150 D-loop configurations.

Steinhardt-Nelson order parameters
To measure the symmetry of the magnesium ion coordination, we computed the Steinhardt-Nelson bond orientational order parameter\textsuperscript{15} for the S and P site Mg\textsuperscript{2+} ions with respect to their neighboring oxygen atoms. We used the MDTraj\textsuperscript{16} library’s “compute neighbors” function to find oxygen atoms within 3 angstroms of the ions, and computed the parameter of order \(m\) using the equations:

\[
q_{lm} = \frac{1}{N_b} \sum_{i=1}^{N_b} Y_{lm}(\theta_i, \phi_i)
\]

\[
Q_l = \sqrt{\frac{4\pi}{2l+1} \sum_{m=-l}^{m=l} (q_{lm})^*(q_{lm})}
\]

where \(N_b\) is the number of neighboring oxygens, \(\theta_i\) and \(\phi_i\) are the two angles in spherical coordinates between the ion and oxygen neighbor \(i\), \(Y_{lm}(\theta_i, \phi_i)\) is the spherical harmonic of degree \(l\) and order \(m\), and the asterisk denotes complex conjugation.

In order to determine which degree to use, we built an ideal octahedral geometry with a central particle at the origin surrounded by four planar neighbors at the edges of a unit square in the xy plane. Two additional neighbors were added above and below the central particle at a distance of \(\sqrt{2}/2\) so that all 6 neighbors were equidistant. In this geometry, we computed the \(Q_2\) to \(Q_8\) and found that \(Q_2, Q_3, Q_5, Q_7\) are all zero. We found for this geometry \(Q_4 = 0.764, Q_6 =0.354, \) and \(Q_8 = 0.718\). \(Q_6\) is generally used to measure icosahedral order\textsuperscript{15} so this smaller value is reasonable. Because \(Q_4\) was the largest value, we took this as our measure for the ideality of the magnesium ion coordination in this work.

dPCA
To perform dihedral principal component analysis (dPCA) we followed the procedure of Ref.\textsuperscript{17}. From the configurations extracted as described above, we computed 66-dimensional vectors, consisting of the cosines and sines of the dihedral angles \(\varphi, \psi,\) and \(\omega\) for each residue. This calculation gave 6 features for each residue in each D-loop, resulting in a matrix \(X\) of dimensions 3150x66. The mean was subtracted from each column. Standard principal component analysis was performed on the matrix \(X\) by singular value decomposition. As usual, the principal components were determined such that each descending principal component captures the maximal amount of variance of the data subject to the constraint that it orthogonal to all of the preceding ones\textsuperscript{17}. In this case, we found that the top three principal component took up 18%, 12% and 8% of the total variance, respectively. Based on the analysis of the distribution of the data points in the three-dimensional subspace spanned by the first three principal components, we concluded that only the top two principle components were needed to see clear separations in
the data points into clusters. As discussed in the main text, the top component separates the data into three states, folded, cofilin-like, and unfolded. The second component further separates folded from the cofilin-like and unfolded states. The third principal component shows that the D-loops from the simulations with bound cations exist in a smaller set of the unfolded space, as in the diffusion-map data.

**Figure S1.** Transformed dihedral information using the top three principal components from dPCA analysis.\(^{17}\)

**Diffusion map**
Diffusion map is a non-linear dimensionality reduction technique that attempts to separate data by samples that are assumed to be far apart temporally due to large separation in a distance metric.\(^{18,19}\) To perform the diffusion map analysis, we first compute the pairwise distance matrix \(D\) between our 3150 D-loop snapshots. Each entry in the matrix is computed by aligning a pair of structures using the Theobald QCP Algorithm\(^{10}\) as implemented in the MDTraj library,\(^{16}\) and then compute the root-mean-squared distance (RMSD) between the backbone atom positions. We also computed the variance in distances, \(\sigma^2 = \text{Var}(D) = 0.72 \text{ Å}^2\). We then construct the diffusion map matrix \(M\) by first computing \(A = \exp\left(-\frac{D^2}{\epsilon \sigma^2}\right)\), and then setting each element \(M_{ij} = A_{ij} / \sum_j A_{ij}\).\(^{19}\) The term \(\epsilon\) sets the range of dynamical correlations probed, and we choose the value \(\epsilon = 8\), which causes a histogram of values in the matrix \(M\) to fall off at short values (Figure S2), in order to restrict the matrix to local correlations. We solve for the top eigenvalues and vectors using the ARPACK implementation of the Implicitly Restarted Arnoldi Method\(^{21}\) from the Scipy python library.\(^{22}\) The first eigenvalue is equal to unity,\(^{19}\) and we observe a small spectral gap after the third eigenvalue (Figure S2). This suggests that two diffusion map vectors may be enough to characterize the data set as has been done in the main text.
Figure S2. Data from diffusion map analysis. (A) Probability distribution of pairwise RMSDs between D-loop samples. The prominent peak at 3.5 Å shows that the sample distribution is not uniform. (B) The distribution of entries in the matrix $A$ discussed above. (C) Top eigenvalues of the matrix $M$. The largest eigenvalue is 1 because $M$ is a stochastic matrix. The difference between eigenvalue 1 and 2 is 0.13 and between 2 and 3 is 0.07.

Further structural properties of filaments

We find that the actin filaments as studied in this work and as discussed some previously tend to change slightly over the course of many nanoseconds of simulation. This relaxation results in a softer filament, at least at times approximately 200 ns. As discussed in the main text and previous works, it is still true that the Namba filament with cations is more rigid than that without cations, especially in the torsional rigidity (Table S4).

Table S4. Overall filament properties. Persistence length and torsional rigidity were computed on 10ns sliding windows covering simulation times 75 ns up to 175 ns. The values reported are the average and standard deviation from the different time windows.

|               | Namba-cation | Namba   | Oda    |
|---------------|--------------|---------|--------|
| $L_p$ (µm)    | 4.22 +/- 0.65| 3.59 +/- 0.92| 4.92 +/- 0.44 |
| Rigidity      | 0.38 +/- 0.07| 0.17 +/- 0.03| 0.29 +/- 0.05 |
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