Supporting Information for:
“Dependence of Internal Friction on Folding Mechanism”

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1. Supplementary Methods

1.1. Molecular simulation methods. Molecular dynamics was performed in GROMACS[1] with a leap-frog integrator, and the velocity rescaling thermostat[2] with 0.1 ps coupling time is used for all cases involving water-mass scaling. Langevin dynamics is used in REMD with a friction coefficient 1 ps$^{-1}$. Pressure is controlled with the Parrinello-Rahman method[3]. LJ pair interactions were cut off at 1.4 nm and electrostatic energies were calculated by particle-mesh Ewald[4] with a grid spacing of 0.12 nm and a real-space cut-off of 0.9 nm. The force field in all cases is Amber ff03ws—a derivative of Amber ff03[5] with a backbone modification to match the population of the helical states[6, 7] and a scaling of protein and water interaction to capture the dimensions of the unfolded structures[8].

The sequence of 16-residue GB1 is cut from 41 to 56 residues of the full length GB1 protein (PDB: 1PGB[9]); the hairpin native structure is taken from the same PDB structure. 70 ns REMD trajectories of GB1 are generated with a temperature range from 300 to 495 K, starting from the folded state. The native structure of Trp cage is taken from PDB: 1L2Y[10]. 100 ns REMD simulations of Trp cage are generated with a temperature range from 300 to 495 K. All simulations except REMD are performed at 350 K.

The biased all-atom model is constructed by modifying the Lennard-Jones parameters of the original forcefield Amber ff03ws[8]. Atom pairs separated by less than 6 Å in the native configuration were stabilized by multiplying the $\epsilon$ of their LJ potential by 1.15, while the remaining Lennard-Jones interactions were modified by using a small $\epsilon = 10^{-5}$ kJ mol$^{-1}$ and defining $\sigma$ such that the new and old potentials coincide at an energy of 2.5 kJ mol$^{-1}$. In doing so, the attractive part of the non-native nonbonded interactions was removed, while the effective radius of the atom was preserved. We keep all the other terms of the original forcefield.

The duration for all simulations are shown in Table S5.

1.2. Relaxation time from transition path sampling. Relaxation times in Trp cage and hairpin-biased GB1 are calculated by sampling transition paths using the method proposed by Hummer[11], and summarized briefly below. The method uses a reaction coordinate $q(x)$ which is a function of the Cartesian coordinates $x$ of the molecules. An ensemble of structures on a dividing surface $q_0$ near the top of the apparent barrier in the REMD-derived free energy surface $F(q)$ are generated by umbrella sampling. Configurations $x$ for which $|q(x) - q_0| < 0.001$ are randomly selected from this ensemble. “Forward” and “reverse” trajectory pairs were generated by reversing the sign of the initial Maxwell-Boltzmann velocities, and each trajectory was terminated once it reached a region of $q$ defined as a stable state (boundaries used to determine when a stable state was reached, for TPS purposes, are included in Table S1). Successful transition paths were those where the forward and reverse parts terminated in different end states.
By considering the time spent by the system in each stable state, and on transition paths, the rate coefficients for two-state kinetics can be expressed as

\[ k' = \frac{2}{k_1^{-1} + k_2^{-1}} = \frac{p(\text{TP})}{\langle t_{\text{TP}} \rangle} \]

where \( k_1 \) and \( k_2 \) are the rate coefficients for the forward and backward transitions between the two states, \( p(\text{TP}) \) is the fraction of time spent on transition paths, \( \langle t_{\text{TP}} \rangle \) is the average duration of a transition path, and we define \( k' \) with units of rate. Here \( p(\text{TP}) \) can be calculated by

\[ p(\text{TP}) = \frac{p(\text{TP}|q)p_{\text{eq}}(q)}{p(q|\text{TP})} \]

where \( p_{\text{eq}}(q) \) is the equilibrium distribution of \( q \) and \( p(\text{TP}|q) \) is the probability density to be on a trajectory on the transition path given a particular \( q \). \( p(\text{TP}|q) \) can be estimated by shooting forward and backward from phase points \( x \) on the surface. To collect transition paths efficiently, \( q_0 \) needs to be chosen to be close to the isoenergetic surface in which the probabilities of going forward and backward are equal. Each transition path collected in this way must be unbiased by the time spent at the initial value of the coordinate, using the relative weight

\[ w = \left( \sum_{\text{crossing}} |v_i|^{-1} \right)^{-1} \]

where \( v_i \) is the velocity of the \( i^{th} \) crossing event at the dividing surface on the given transition path. The time for each path to spend in \((q_0, q_0 + \delta q)\) in the limit of \( \delta q \rightarrow 0 \) is proportional to \( 1/w \) and can be used to calculate the probability density

\[ p(q_0|\text{TP}) = \frac{\langle \theta_{\text{TP}} \rangle |_{q_0, p_{\text{eq}}}}{\langle t_{\text{TP}} \rangle \langle \theta_{\text{TP}} w \rangle |_{q_0, p_{\text{eq}}}} \]

where \( \theta_{\text{TP}} \) takes a value of one for trajectory pairs forming a transition path and zero otherwise. The average of \( \theta_{\text{TP}} \) is thus equal to \( p(\text{TP}|q_0) \). At last, \( k' \) can be simplified to

\[ k' = p_{\text{eq}}(q_0) \langle \theta_{\text{TP}} w \rangle |_{q_0, p_{\text{eq}}}. \]

For comparison to the MSM, we report the relaxation time \( \tau \), defined by \( \tau^{-1} = k_1 + k_2 \) instead of \( k' \). The relation between them for a two-state model is:

\[ \tau = \frac{2P_1(1 - P_1)}{k'} \]

where \( P_1 \) is the equilibrium probability of the first state.

To calculate the global folding relaxation time of Trp cage from the rate coefficients of the two barriers, we assumed the folding kinetics of Trp cage can be modeled using...
three-states:

\[
\text{(S7)} \quad S_1 \xrightleftharpoons[k_2]{k_1} S_2 \xrightleftharpoons[k_4]{k_3} S_3.
\]

We built a rate matrix from the rate coefficients and calculated the relaxation time from the first eigenvalue of the rate matrix as

\[
\text{(S8)} \quad \tau = \frac{1}{2} \left[ k^* - \sqrt{(k^*)^2 - 4(k_1 k_3 + k_1 k_4 + k_2 k_4)} \right]
\]

where \( k^* = k_1 + k_2 + k_3 + k_4 \).

500 pairs of forward and backward shooting were performed for each barrier of Trp cage and hairpin-biased GB1 by using the fraction of native contacts \( Q \) as the reaction coordinate. The errors of the relaxation time are estimated by using box averages of every 100 pairs of shooting events. When calculating the average transition path time, individual path length is reweighted by using the time spent by that path near the dividing surface, that is the normalized \( w \) of Equation S3, so that a correct transition path ensemble is reproduced[11]. The statistics of the transition path trajectories are shown in Table S1.

1.3. Fraction of native contacts/dihedrals. Fraction of native contacts \( Q \), were defined from the \( N_{ij} \) distances between atom pairs in contact in the native state

\[
\text{(S9)} \quad Q(x) = \frac{1}{N_{ij}} \sum_{(i,j) \in \text{native}} \frac{1}{1 + e^{\beta(d_{ij}(x) - \gamma d_{ij}^0)}},
\]

where \( d_{ij}(x) \) is the distance between atoms \( i \) and \( j \) in configuration \( x \), \( d_{ij}^0 \) is the corresponding distance in the native state, the factor \( \gamma = 1.5 \) allows for fluctuations of distance within the native state, and \( \beta = 50 \text{ nm}^{-1} \). The native contacts were defined with the same 0.6 nm cut-off between all pairs of atoms as was used for building the all-atom native-biased model, except that the contacts within the same and nearest residues were excluded from \( Q \) (since these are almost always in “contact”). Similarly, a fraction of native dihedral angles \( Q_{\text{dih}} \) was defined for all \( N_k \) dihedral angles \( \phi, \psi \).

\[
\text{(S10)} \quad Q_{\text{dih}}(x) = \frac{1}{N_k} \sum_{(k) \in \text{native}} \frac{1}{1 + e^{\beta(D_k(x) - \gamma)}} ,
\]

where \( D_k = (\theta_k - \theta_k^0) - 2\pi \text{ round}[(\theta_k - \theta_k^0)/2\pi] \), and \( \theta_k \) are the \( \phi, \psi \) angles, \( \theta_k^0 \) their corresponding native values and “round” function returns the nearest integer and \( \gamma = \pi/3, \beta = 11.46 \) in units of radians and inverse radians respectively.

1.4. Markov state model. To complement the analysis of the simulations we use a Markov state model (MSM)[12]. This analysis is particularly important in the case of the helix-biased model, since the dynamics of this system does not conform to a two-state picture. First, the simulation data at each value of the viscosity were discretized into microscopic states using a hydrogen bond or a torsion angle description (detailed below).
Assignment of the transitions was performed using transition based assignment[13]. In the case of the torsion angle discretization, typically hundreds to thousands of states are populated during the simulation. To simplify the analysis and increase the statistics of observed transitions, at each value of the viscosity, a consensus set of states corresponding to the most populated ones reaching a 99% of the total population were considered. The transition count matrix \( N(\Delta t) \), was then constructed by counting the number of transitions between every pair of states, after a lag time \( \Delta t \). Then the left transition probability matrix \( T(\Delta t) \), was computed using the maximum likelihood estimator[14]

\[
T_{ji}(\Delta t) = \frac{N_{ji}(\Delta t)}{\sum_k N_{ki}(\Delta t)}.
\]

The relaxation times were calculated from the eigenvalues \( \lambda_i \) of \( T \) as \( \tau_i = -\Delta t / \ln(\lambda_i) \). The errors of the relaxation times were derived from a bootstrap analysis. The lag times for the MSM were chosen to be the shortest ones, in each case, where the slowest relaxation time of the model had approximately converted (Figure S1. The lag times used in the current work is shown in Table S6.

In the torsion angle case, the \( \alpha \) and extended well states are narrowly defined for the Ramachandran angles \( \phi \) and \( \psi \) (see Table S7). In the case of the hairpin biased model, the native state has a residue (Lys10) in the left handed-helix configuration, and in previous work one of us has found that the torsional transition to that state is correlated with folding events[15]. Hence, in this case we use three possible states for each residue (alpha, extended and left handed) instead just two. This is not so important in the case of the helix forming peptide, and hence only two states are considered. In any case, the torsional discretization results in an intractable number of possible microstates \( 2^{14} \) or \( 3^{14} \) in case the L state is explicitly accounted for). For the helix forming system, we simplify the analysis, considering helical stretches of at least three continuous amino acid residues (corresponding to a helix “nucleus”), which reduces the state space to a maximum of 739 microstates. In Table S8 we show the number of states that correspond to the consensus set of observed states for each model and discretization. In practice, at each value of the viscosity only a subset of these states are used, as we consider only the largest ergodic transition matrix.

The other discretization method we use here is based on the hydrogen bonds appearing in the native states for the helix and the hairpin. For each of these hydrogen bonds (9 for the helix and 6 for the hairpin), we define formed and broken states based on the distance between the oxygen and hydrogen atoms \( d_{H-O} \), relative to the distance in the native state \( d_{H-O,eq} \). The H-bonded state corresponds to \( d_{H-O} < 1.1d_{H-O,eq} \) while the broken H-bond state is reached when \( d_{H-O} > 3.6d_{H-O,eq} \). Intermediate values of \( d_{H-O} \) are assigned using transition based assignment[13].

1.5. **Displacement of local residues.** To determine in folding different topologies what is affected by the solvent viscosity, we developed a metric closely related to the
solvent viscosity, that focuses on the motion of the protein residues involved in the folding process.

We first defined the transition-related part of the peptide by looking at the difference in the average contact probabilities corresponding to the helix and hairpin formation respectively (Figure S5). We find that in Trp cage the first barrier (helix) corresponds to the formation of secondary structure from residues 2 to 7, whereas the second barrier (hairpin) involves the collapse of the entire peptide and therefore all residues.

Using these transition residues, we structure-aligned consecutive frames in the transition path trajectories. Let the coordinates of these atoms at time $t$ and $t + \Delta t$ be represented by the set of vectors $\{x_1, x_2, \ldots\} \in X(t)$ and $X(t + \Delta t)$ respectively.

We define the minimum displacement between a vector $x$ and a set of vectors $\{v_1, v_2, \ldots\} \in V$ as

$$m(x, V) = \min_{v_i \in V} \|x - v_i\|,$$

thus $m(X_i(t), X(t + \Delta t))$ is the distance that a particular atom was displaced from the original coordinates of $X(t)$ after correcting for the bulk translational and rotational motion of the protein. The metric for atomic displacement is then

$$M_{\text{atom}}(Q) = \frac{1}{\Delta t |X|} \sum_{i \in S} m(X_i(t), X(t + \Delta t))$$

In Figure S7 we plot $M_{\text{atom}}(Q)$ as a function of solvent viscosity.

1.6. Scaling of water mass. We show the values of the scaling factor that we used for the masses of atoms in the water molecules in Table S9. For low viscosity simulations, we scaled down the integration time-step by a factor of $\sqrt{m/m_0}$, whereas for high viscosity simulations, we kept the standard 2 fs time step limited by the dynamics, the same as our previous work[16].

1.7. Bimolecular simulations. We run two typical biomolecular systems—blocked Trp-Trp and Lys-Asp in Amber ff03ws forcefield with TIP4P/2005 water model. The parameters for MD simulation are the same as mentioned in Molecular simulation methods section. We show the association and dissociation rate coefficients in Figure S8.

1.8. Torsion transitions in alanine dipeptide. We run alanine dipeptide using Amber ff03ws force field and TIP4P/2005 water model with different water mass-scaling. The parameters for MD simulations are the same as mentioned in Molecular simulation methods section. One long trajectory of 500 ns is analyzed for each water mass-scaling. We show the viscosity dependence of the rate coefficients of transitions between polyproline II (ppII) and $\alpha$ states and between ppII and $\beta$ states in Figure S10. We find similar evidence for internal friction in both cases, however, the ppII to $\alpha$ dynamics, being an order of magnitude slower, are more relevant to the slowest relaxation of the system.
Figure S1. Convergence of the slowest relaxation times with the lag time used to build the Markov state model, for the various MSMs considered. All data shown are in the case of solvent viscosity $\eta = \eta_0$. A: helix-biased GB1 using torsion angles; B: helix-biased GB1 using H-bond; C: hairpin-biased GB1 using torsion angles; D: hairpin-biased GB1 using H-bond; E: unbiased GB1 using torsion angles. The increase in relaxation times at long times is due to the lag time approaching the relaxation times.
Figure S2. Viscosity dependence of the transition-path time of hairpin-biased GB1 (a), barrier A of Trp cage (b) and barrier B of Trp cage (c). Histograms are weighted transition path durations and red line shows the average transition path duration.
Figure S3. Time series of the secondary structure sequences of hairpin-biased GB1 (a), helix-biased GB1 (b) and non-biased GB1 (c) in different solvent viscosities using DSSP[17].
Figure S4. Viscosity dependence of the relaxation times of GB1 from MSM. Top: hairpin-biased model. The inset shows the all other relaxation times except the slowest. Bottom: helix-biased model. Error bars are derived from a bootstrap analysis. For both figures, filled symbol and solid lines correspond to MSM using hydrogen bond, whereas empty symbols and dash lines correspond to MSM using torsion angles. All lines are from the power-law fits.
Figure S5. Difference of the average contact probability over barrier A (top) and B (bottom) of Trp cage. The average contact probability is calculated from the transition path configurations on one side of a barrier along $Q$. The difference of the average contact probability is then taken between the right and left side of the barrier. Positive values on the contact map show the contacts forming from left to the right side of the barrier, whereas negative values show the contacts disappearing when crossing the barriers.
Figure S6. Free energy of Trp cage in Amber ff03 with TIP3P water in a previous work[16] (a), Amber ff03ws with TIP4P/2005 water (b), and Amber ff03ws with TIP4P/2005 water and native bias in the present work (c) projected onto fraction of native contacts Q and radius of gyration. Free energy shown in colorbar is in units of kcal/mol.
Figure S7. Changes in atom displacement (Equation S13) for the two Trp cage transition paths averaged over a small $Q$ window for different solvent viscosity.
Figure S8. Association (red) and dissociation (blue) rates between two blocked peptides. Left: Trp-Trp; Right: Lys-Asp. For both figures, solid lines are from the power-law fit and dash lines are from the linear fit. The power-law fit exponents of Trp-Trp are 0.94 ± 0.09 for association and 1.13 ± 0.13 for dissociation; and the power-law fit exponents of Lys-Asp are 0.95 ± 0.07 for association and 0.76 ± 0.06 for dissociation.
**Figure S9.** Viscosity dependence of the relaxation times of unfolded GB1 from the torsion angle MSM. Top: hairpin-biased model. Bottom: non-biased model. For both figures, error bars are derived from a bootstrap analysis. All lines are from the power-law fits.
**Figure S10.** Left: Free energy (in units of kcal/mol) of alanine dipeptide projected onto torsion angles. Right: Viscosity dependence of the rate coefficients of transitions between polyproline II (ppII) and α (red), and between ppII and β basins (blue). Lines show the power-law fit with the exponent of $0.73 \pm 0.02$ for ppII-α transitions and $0.52 \pm 0.06$ for ppII-β transitions.
3. Supplementary Tables

Table S1. Statistics of sampled transition path trajectories. For each system, the value of $Q_0$ used for selecting starting structures and the boundary values of $Q$ used for terminating transition paths ($Q_u$ for unfolding and $Q_f$ for folding) are given. $P_+$ is the average probability of folding (versus unfolding) over all shooting moves and $P_{\text{finished}}$ is the fraction of pairs of shooting moves where both members of the pair terminate in a stable state (versus reaching the maximum allowed time before reaching a stable state).

| $\eta/\eta_0$ | $P_+$ | $P(\text{TP}|Q_0)$ | $t_{\text{max}}$ (ns) | $P_{\text{finished}}$ |
|--------------|-------|---------------------|-----------------------|----------------------|
| GB1 hairpin-biased, $Q_0 = 0.64, Q_u = 0.35, Q_f = 0.85$ |
| 0.1          | 0.52  | 0.47                | 5                     | 1.000                |
| 0.5          | 0.48  | 0.48                | 10                    | 0.996                |
| 1.0          | 0.47  | 0.45                | 10                    | 0.992                |
| 1.4          | 0.49  | 0.47                | 20                    | 1.000                |
| 2.0          | 0.49  | 0.44                | 20                    | 0.993                |
| 2.5          | 0.47  | 0.42                | 20                    | 0.993                |
| 3.0          | 0.46  | 0.43                | 30                    | 0.995                |
| Trp cage Barrier A, $Q_0 = 0.35, Q_u = 0.25, Q_f = 0.44$ |
| 0.1          | 0.60  | 0.25                | 5                     | 1.000                |
| 0.5          | 0.62  | 0.27                | 10                    | 1.000                |
| 1.0          | 0.60  | 0.23                | 10                    | 1.000                |
| 1.4          | 0.63  | 0.25                | 20                    | 1.000                |
| 2.0          | 0.61  | 0.28                | 20                    | 1.000                |
| 2.5          | 0.63  | 0.31                | 20                    | 1.000                |
| 3.0          | 0.61  | 0.30                | 30                    | 1.000                |
| Trp cage Barrier B, $Q_0 = 0.74, Q_u = 0.52, Q_f = 0.87$ |
| 0.1          | 0.32  | 0.36                | 5                     | 1.000                |
| 0.5          | 0.36  | 0.32                | 10                    | 1.000                |
| 1.0          | 0.36  | 0.30                | 10                    | 0.994                |
| 1.4          | 0.38  | 0.33                | 20                    | 0.999                |
| 2.0          | 0.41  | 0.28                | 20                    | 1.000                |
| 2.5          | 0.37  | 0.38                | 20                    | 0.998                |
| 3.0          | 0.36  | 0.35                | 30                    | 1.000                |
Table S2. Helix propensity of helix-biased GB1

| $\eta/\eta_0$ | helix propensity |
|--------------|-----------------|
| 0.1          | 0.25            |
| 0.5          | 0.23            |
| 1.0          | 0.22            |
| 1.4          | 0.25            |
| 2.0          | 0.21            |
| 2.5          | 0.27            |
| 3.0          | 0.20            |

Table S3. Population of states

| GB1 hairpin-biased |          |
|--------------------|----------|
| REMD: folded $Q > 0.64$ | 0.29     |
| unfolded $Q < 0.64$  | 0.71     |
| MSM: folded         | 0.14     |
| unfolded             | 0.86     |
| Trp cage             |          |
| REMD: folded $Q > 0.74$ | 0.11     |
| intermediate $Q \in (0.35, 0.74)$ | 0.56     |
| unfolded $Q < 0.35$  | 0.33     |
Table S4. Coefficient $\beta$ of power-law fits and normalized intercept $\tau_0/\tau(\eta_0)$ of linear fits for different models of GB1. Numbers in brackets are the errors in the last reported digit.

| Model                        | $\beta$  | $\tau_0/\tau(\eta_0)$ |
|------------------------------|----------|------------------------|
| hairpin, hydrogen bond MSM   | $\lambda_1$ | 1.1 (5) | 0.1 (1) |
|                              | $\lambda_2$ | 0.80 (4) | 0.21 (3) |
|                              | $\lambda_3$ | 0.73 (6) | 0.26 (6) |
| hairpin, torsion angle MSM   | $\lambda_1$ | 0.70 (2) | 0.3 (1) |
|                              | $\lambda_2$ | 0.9 (5) | 0.1 (2) |
|                              | $\lambda_3$ | 1.1 (3) | $-0.1$ (1) |
| helix, hydrogen bond MSM     | $\lambda_1$ | 0.72 (9) | 0.26 (5) |
|                              | $\lambda_2$ | 0.49 (6) | 0.48 (5) |
|                              | $\lambda_3$ | 0.52 (6) | 0.46 (4) |
| helix, torsion angle MSM     | $\lambda_1$ | 0.7 (1) | 0.27 (8) |
|                              | $\lambda_2$ | 0.67 (6) | 0.29 (4) |
|                              | $\lambda_3$ | 0.62 (5) | 0.34 (4) |
| hairpin unfolded, torsion angle MSM | $\lambda_1$ | 0.7 (2) | 0.3 (1) |
|                              | $\lambda_2$ | 0.9 (1) | 0.19 (5) |
|                              | $\lambda_3$ | 0.69 (6) | 0.30 (4) |
| non-biased unfolded, torsion angle MSM | $\lambda_1$ | 0.64 (7) | 0.33 (4) |
|                              | $\lambda_2$ | 0.46 (6) | 0.52 (4) |
|                              | $\lambda_3$ | 0.41 (8) | 0.58 (6) |
Table S5. Length of MD trajectories of GB1 and Trp cage.

| $\eta/\eta_0$ | time length (ns) | $\eta/\eta_0$ | time length (ns) |
|---------------|------------------|---------------|------------------|
| GB1           |                  | helix-biased long simulations |                  |
|               |                  | 0.1           | 200              |
|               |                  | 0.5           | 1000             |
|               |                  | 1.0           | 2000             |
|               |                  | 1.4           | 2000             |
|               |                  | 2.0           | 2000             |
|               |                  | 2.5           | 2000             |
|               |                  | 3.0           | 2000             |
| total         | 11450            | total         | 13000            |
| short simulations |                | transition-path barrier A |                  |
| 0.1           | 2(ns) x150       | 0.1           | 0.1(ns) x500     |
| 0.5           | 5 x150           | 0.5           | 0.3 x500         |
| 1.0           | 10 x150          | 1.0           | 0.5 x500         |
| 1.4           | 12 x150          | 1.4           | 0.8 x500         |
| 2.0           | 16 x150          | 2.0           | 0.9 x500         |
| 2.5           | 18 x150          | 2.5           | 1.2 x500         |
| 3.0           | 22 x150          | 3.0           | 1.1 x500         |
| total         | 12750            | total         | 2450             |
| long simulations |                | transition-path barrier B |                  |
| 0.1           | 200              | 0.1           | 0.4(ns) x500     |
| 0.5           | 1000             | 0.5           | 0.9 x500         |
| 1.0           | 2000             | 1.0           | 1.2 x500         |
| 1.4           | 2000             | 1.4           | 1.4 x500         |
| 2.0           | 2000             | 2.0           | 3.2 x500         |
| 2.5           | 2000             | 2.5           | 4.2 x500         |
| 3.0           | 2000             | 3.0           | 3.9 x500         |
| total         | 13000            | total         | 7600             |
| Trp cage      |                  | Trp cage total | 13250            |
| REMD          | 1.0 100 x32      |               |                  |
| transition-path barrier A | 0.1 0.1(ns) x500 |               |                  |
| transition-path barrier B | 0.1 0.4(ns) x500 |               |                  |

S21
Table S6. Lag time of GB1 MSMs in units of saving frequency (dt)

| Model            | MSM type | Lag time (dt) |
|------------------|----------|---------------|
| helix-biased     | Torsion  | 1             |
| helix-biased     | H-bond   | 10            |
| hairpin-biased   | Torsion  | 3             |
| hairpin-biased   | H-bond   | 1             |
| unbiased         | Torsion  | 1             |

saving frequency (ps)

| Model            | $\eta/\eta_0 = 0.01$ | 0.25 | 1   | 2   | 4   | 6.25 | 9   |
|------------------|-----------------------|------|-----|-----|-----|------|-----|
| helix-biased     | 2(ps)                 | 10   | 20  | 20  | 20  | 20   | 20  |
| hairpin-biased   | 2                     | 10   | 10  | 20  | 20  | 20   | 20  |
| unbiased         | 1                     | 5    | 10  | 10  | 10  | 10   | 10  |

Table S7. Limits for transition based assignment regions for the $\phi$ and $\psi$ Ramachandran dihedral angles.

| State            | $\phi$       | $\psi$       |
|------------------|--------------|--------------|
| Alpha            | $[-100, -40]$| $[-60, 0]$   |
| Extended         | $[-180, -40]$| $[120, 180]$ |
| Left handed      | $[50, 100]$  | $[-40, 70]$  |

Table S8. Number of microstates for each of the simulation models corresponding to the torsion angle based and H-bond based discretizations. In the case of the helix-biased and the unbiased models, in the torsional discretization, helical stretches were required to contain a minimum of 3 continuous helical residues.

| Model            | Torsions | H-bonds |
|------------------|----------|---------|
| Helix-biased     | 38       | 165     |
| Hairpin-biased   | 263      | 29      |
| Unbiased         | 143      |         |

References

[1] Hess, B.; Kutzner, C.; Van Der Spoel, D.; Lindahl, E. J. Chem. Theory Comput. 2008, 4, 435–447.
[2] Bussi, G.; Donadio, D.; Parrinello, M. J. Chem. Phys. 2007, 126, 014101.
[3] Parrinello, M.; Rahman, A. J. App. Phys. 1981, 52, 7182–7190.
[4] Darden, T.; York, D.; Pedersen, L. J. Chem. Phys. 1993, 98, 10089–10092.
Table S9. The scaling of water mass, the relative viscosity and the time-step used in the simulations.

| $m/m_0$ | $\eta/\eta_0$ | $\delta t$ [fs] |
|---------|----------------|-----------------|
| 0.01    | 0.1            | 0.2             |
| 0.25    | 0.5            | 1               |
| 1       | 1              | 2               |
| 2       | 1.4            | 2               |
| 4       | 2              | 2               |
| 6.25    | 2.5            | 2               |
| 9       | 3              | 2               |

[5] Duan, Y.; Wu, C.; Chowdhury, S.; Lee, M. C.; Xiong, G.; Zhang, W.; Yang, R.; Cieplak, P.; Luo, R.; Lee, T.; Caldwell, J.; Wang, J.; Kollman, P. A. J. Comp. Chem. 2003, 24, 1999–2012.

[6] Best, R. B.; Hummer, G. J. Phys. Chem. B 2009, 113, 9004–9015.

[7] Best, R. B.; Mittal, J. J. Phys. Chem. B 2010, 114, 14916–14923.

[8] Best, R. B.; Zheng, W.; Mittal, J. J. Chem. Theory Comput. 2014, 10, 5113–5124.

[9] Gallagher, T.; Alexander, P.; Bryan, P.; Gilliland, G. L. Biochemistry 1994, 33, 4721–4729.

[10] Neidigh, J. W.; Fesinmeyer, R. M.; Andersen, N. H. Nat. Struct. Mol. Biol. 2002, 9, 425–430.

[11] Hummer, G. J. Chem. Phys. 2004, 120, 516–523.

[12] Chodera, J. D.; Noé, F. Curr. Opin. Struct. Biol. 2014, 25, 135 – 144.

[13] Buchete, N.-V.; Hummer, G. J. Phys. Chem. B 2008, 112, 6057–6069.

[14] Prinz, J.-H.; Wu, H.; Sarich, M.; Keller, B.; Senne, M.; Held, M.; Chodera, J. D.; Schutte, C.; Noe, F. J. Chem. Phys. 2011, 134, 174105.

[15] Best, R. B.; Mittal, J. Proc. Natl. Acad. Sci. USA 2011, 108, 11087–11092.

[16] De Sancho, D.; Best, R. B. Nat. Commun. 2014, 5, 4307.

[17] Kabsch, W.; Sander, C. Biopolymers 1983, 22, 2577–2637.