Kinetic network models of tryptophan mutations in β-hairpins reveal the importance of non-native interactions

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Overview

- 9.4 ms of explicit-solvent simulation on Folding@home
- Multiple sequences compared by constructing Markov state models (MSMs) using tICA to achieve a unified set of metastable states.
- Quantitative agreement with experimental kinetics and structural NMR observables
- Multi-modal kinetics are due to specific non-native kinetic traps
- Unfolded-state stabilization and transition state movement as Trp mutations are introduced
- Kinetic frustration and glass-like behavior characterized
- β-capped sequences trpzip4 and trpzip5 are able to overcome frustration and remain strongly two-state
- Results may explain under-representation of Trp in natural proteins.

Name	Sequence
GB1	GEWTYDDATKTFTVTE
trpzip4	GEWTWDDATKTWTWTE
trpzip5	GEWTYDDATKTFTWTE
trpzip6	GEWTWDDATKTWTVTE

	τ_{obs} (μs)	τ_f (μs)	τ_u (μs)	T (K)
GB1[1]	3.5	6	6	297
trpzip4[2]	14.0	14.9	234.0	297

Table 1: Experimental folding rates for GB1 and trpzip hairpins.

Methods

Molecular Simulation

- All simulations performed in GROMACS 4.5, using AMBER ff99sb-ildn + 1507 TIP3P water molecules, 6 Na^+ and 3 Cl^- ions in a (36.4 Å)^3 box
- Average trajectory length of 400 ns, and maximum trajectory length of >2 μs
- MSMBuilder2[3] was used for all MSM construction and analysis. Hybrid k-medoids clustering into 2000 microstates was performed using four tICA components based on backbone+C_β interatomic distances.[4] 150-macrostate MSMs were calculated similarly, using the combined data for all sequences, and lumping by the BACE algorithm.[5]

Analysis

- Committor values (p_{fold}) and pathway fluxes were computed using Transition Path Theory (TPT).[6]
- Kinetic frustration scores by Savol and Chennubhotla. [7]
- Glass activities were computed using the s-ensemble method, as by Weber et al.[8]

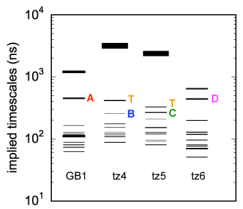

Figure 1: Spectrum of ten slowest implied timescales for 150-macrostate MSM models (10 ns lag time). Line widths are proportional to equilibrium fluxes $||\phi_n||^2$ through each eigenmode ϕ_n. Labeled are particular eigenmodes dominated by trap macrostates T, A, B, C, D.

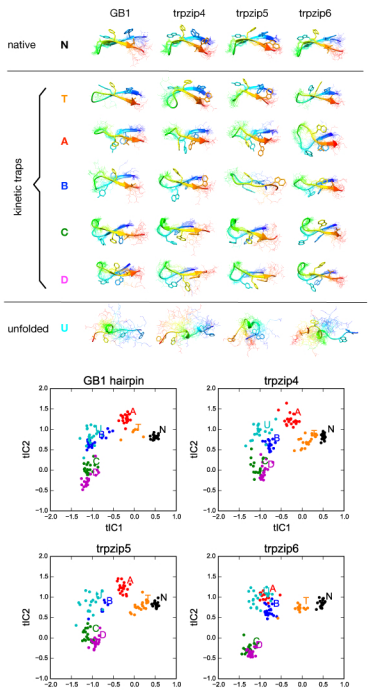

Figure 2: Conformations from selected macrostates projected onto the first two time-lagged independent components (tICs).

number of states	2000		150	
lagtime τ	10 ns	40 ns	10 ns	40 ns
GB1	2.0	3.5	1.2	2.6
trpzip4	4.7	10.3	3.2	8.1
trpzip5	2.6	6.3	2.4	6.0
trpzip6	1.7	5.9	0.64	1.7

Table 2: Predicted folding relaxation rates τ_{obs} (μs) at 300K.

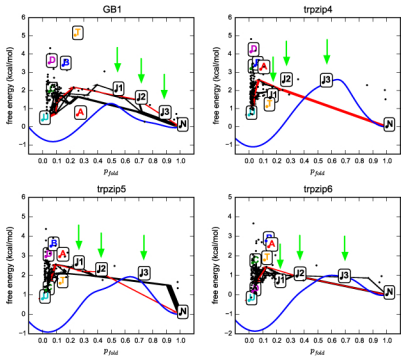

Figure 3: Macrostate free energies versus p_{fold} (committor) values for the U→N reaction, for all hairpin sequences, calculated by Transition Path Theory (TPT). Key macrostates are labeled. Lines denote the top ten pathways of reactive flux from U→N, with the top path in red.

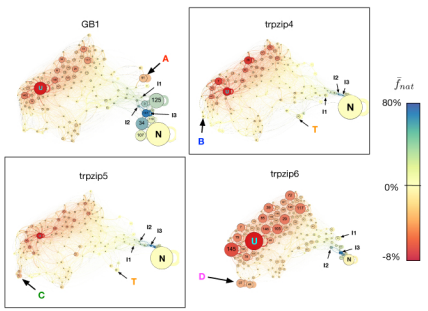

Figure 4: Network representations of 150-macrostate MSMs for GB1 hairpin, trpzip4, trpzip5 and trpzip6, each built using the same metastable definitions, with key macrostates labeled. Nodes are sized in proportion to macrostate equilibrium populations, and colored according to computed kinetic frustration scores, $\bar{f}_{nat}(i)$. Graph layout was performed using Gephi.

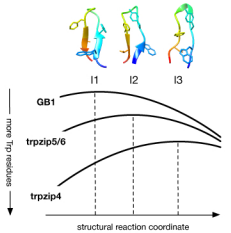

Figure 5: A schematic cartoon illustrating movement of transition state intermediates I1, I2 and I3 along a structural reaction coordinate as tryptophans are introduced.

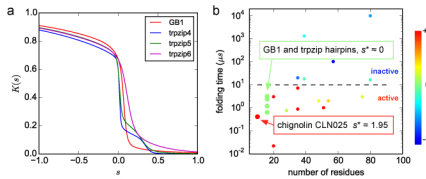

Figure 6: (a) Mean activities $K(s)$ versus s computed from 150-macrostate MSMs. Activities are the mean number of non-self-transitions for an ensemble of modified transition matrices $\mathbf{T}(s) = \mathbf{U}e^{-s} + \mathbf{D}$, where $\mathbf{U}$ and $\mathbf{D}$ are the off-diagonal and diagonal elements of an unbiased MSM transition matrix, respectively. (b) Coexistence values s^* near zero suggests dynamics intermediate between active and glassy phases, in accordance with findings from Weber et al. Superposition of results on the plot of 16 MSMs studied by Weber et al., with dashed line denoting their proposed 10 μs boundary between active and inactive dynamics. These results suggest that these hairpins are slightly more inactive than mini-proteins of similar size.

References

[1] Victor Muñoz, Peggy A Thompson, James Hofrichter, and William A Eaton, *Nature*, **1997**, *390*(6656), 196–199.

[2] Deguo Du, Yongjin Zhu, Cheng-Yen Huang, and Feng Gai, *Proceedings of the National Academy of Sciences*, **2004**, *101*(45), 15915–15920.

[3] Kyle A Beauchamp, Gregory R Bowman, Thomas J Lane, Lutz Maibaum, Imran S Haque, and Vijay S Pande, *Journal of Chemical Theory and Computation*, **2011**, *7*(10), 3412–3419.

[4] Christian R Schwantes and Vijay S Pande, *Journal of Chemical Theory and Computation*, **2013**, *9*(4), 2000–2009.

[5] Gregory R Bowman, *The Journal of Chemical Physics*, **2012**, *137*(13), 134111.

[6] Frank Noé, Christof Schütte, Eric Vanden-Eijnden, Lothar Reich, and Thomas R Weikl, *Proceedings of the National Academy of Sciences of the United States of America*, **2009**, *106*(45), 19011–19016.

[7] Andrej J Savol and Chakra S Chennubhotla, *Journal of Chemical Theory and Computation*, **2014**, *10*(8), 2964–2974.

[8] Jeffrey K Weber, Robert L Jack, and Vijay S Pande, *Journal of the American Chemical Society*, **2013**, *135*(15), 5501–5504.

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