Supporting Information for

Oncogenic mutations in the DNA-binding domain of FOXO1 that disrupt folding: quantitative insights from experiments and molecular simulations

Dylan Novack*[1], Lei Qian*[2], Gwyneth Acker[2], Vincent A. Voelz[1], Richard H. G. Baxter[2]*

[1]Department of Chemistry, Temple University, Philadelphia, Pennsylvania 19122, USA.

[2]Department of Medical Genetics & Molecular Biochemistry, Lewis Katz School of Medicine at Temple University, 3440 North Broad Street, Philadelphia, PA, 19140, USA.

*These authors contributed equally

Supplemental Tables

Table S1.

Mean first passage times (MFPTs) between macrostates.

| MFPT (µs)	to U	to I	to N
from U	-	9.4	20.7
from I	6.4	-	6.9
from N	15.5	5.9	-

Table S2.
Hydrophobic transfer (HT) model parameters for each amino acid. Normalized maximal accessible surface areas A_0 are taken from Tien et al.[53] Consensus hydrophobicities are taken from Eisenberg et al.[81]

Residue	A_0 (nm^2)	Consensus hydrophobicities (kcal/mol)
Glycine	0.97	0.48
Alanine	1.21	0.62

|		
Serine	1.43	-0.18
Arginine	2.65	-2.53
Proline	1.54	0.12
Threonine	1.63	-0.05
Methionine	2.03	0.64
Valine	1.65	1.08
Leucine	1.91	1.06

Supplemental Figures
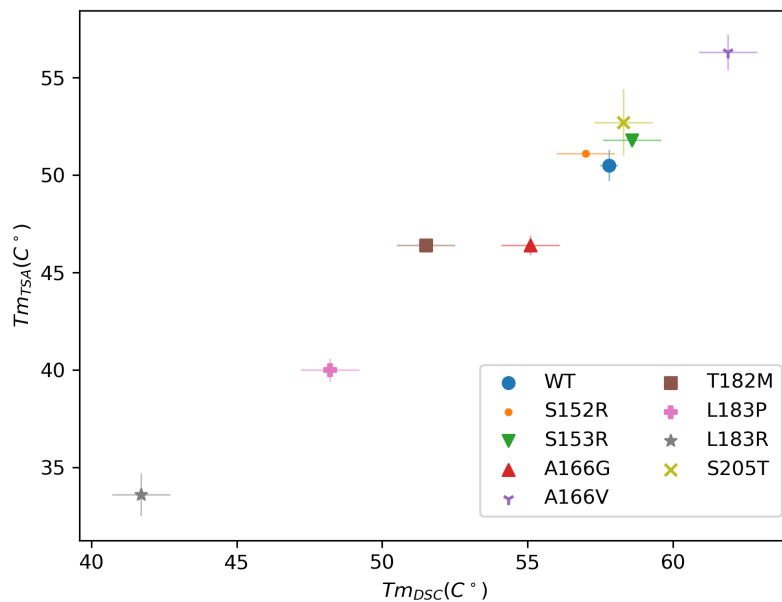
Figure S1.

Comparison of melting temperatures T_m measured in thermal shift assays (TSA) versus those measured using differential scanning calorimetry DSC. Uncertainties in DSC T_m measurements for mutants are estimated to be ± 1 C.
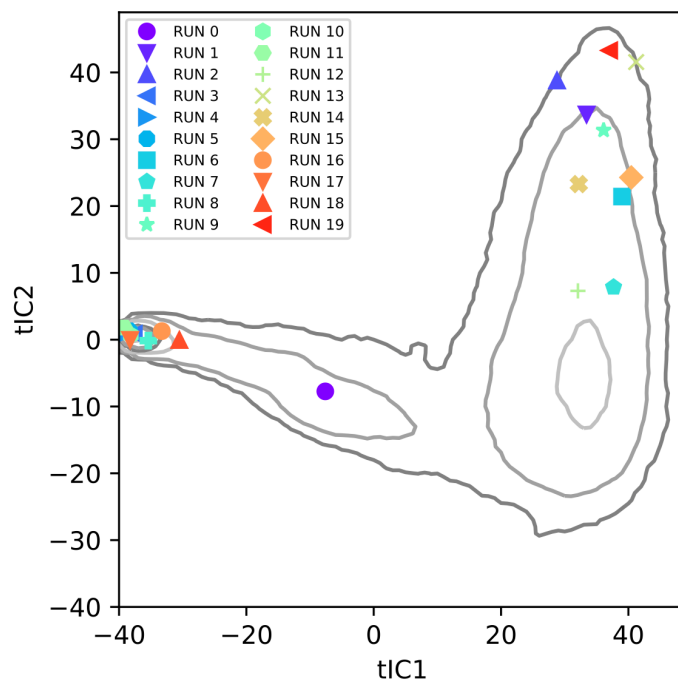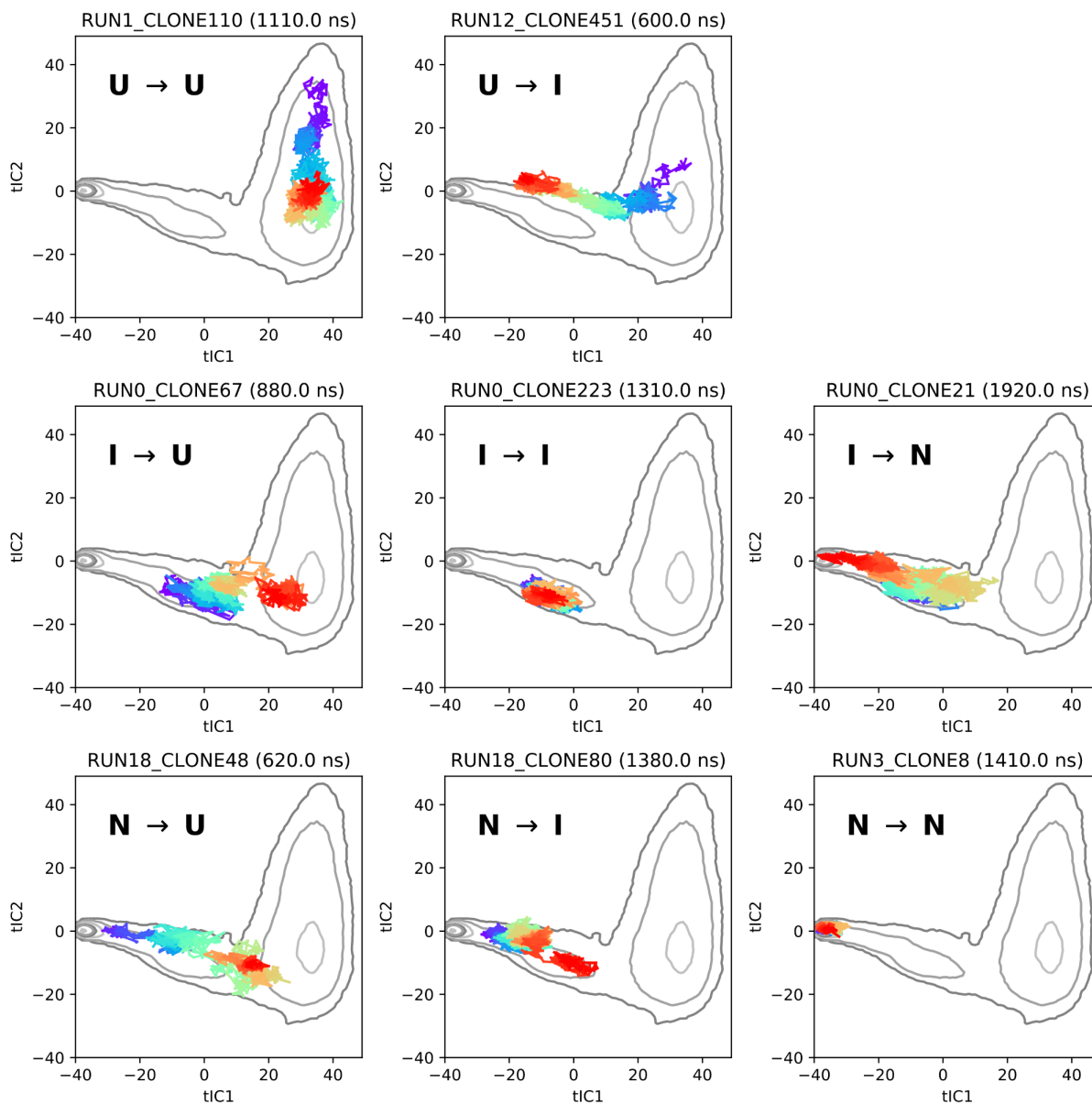
Figure S2.

The locations of the twenty conformations used to initiate trajectories, projected onto the (tIC1, tIC2) free energy landscape.
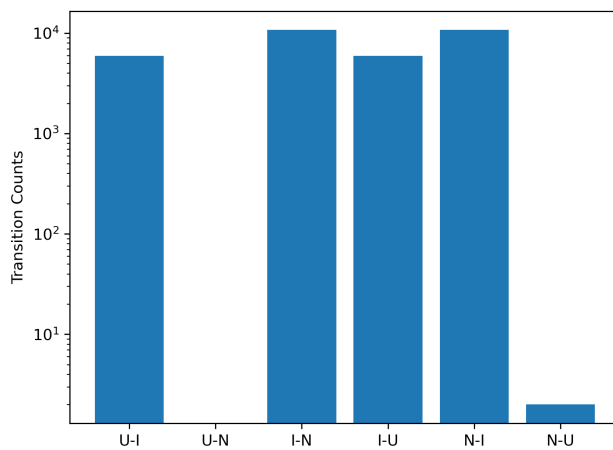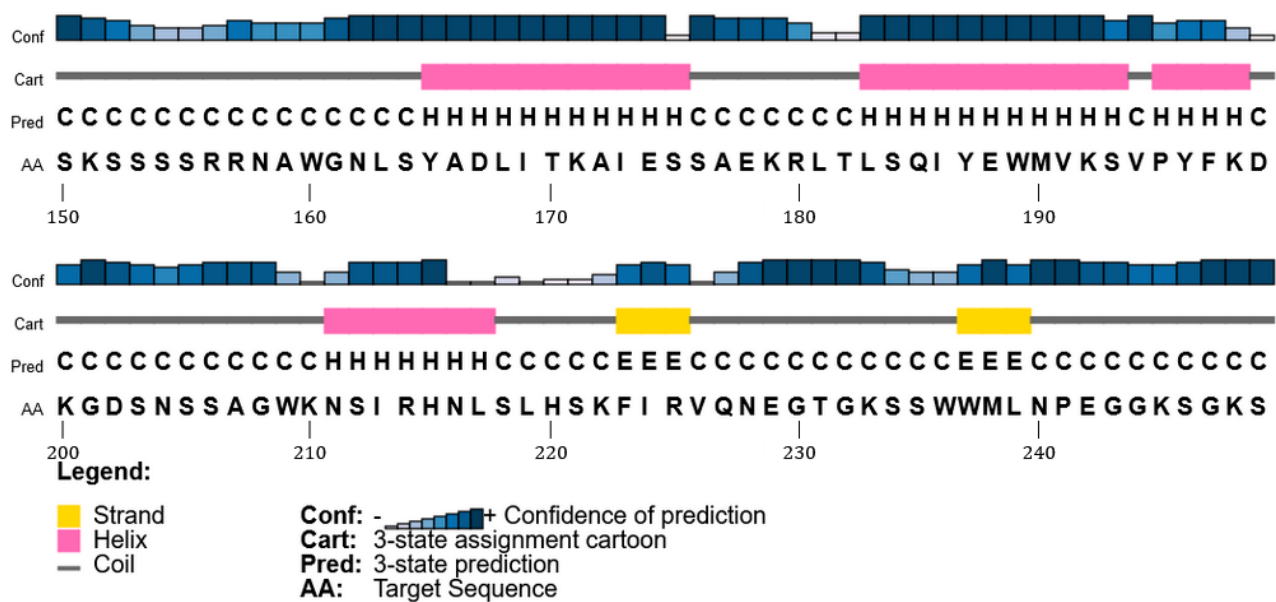

Figure S3.

Transition counts observed between macrostates U, I and N (at lag time 5.0 ns) in the simulation trajectory data.
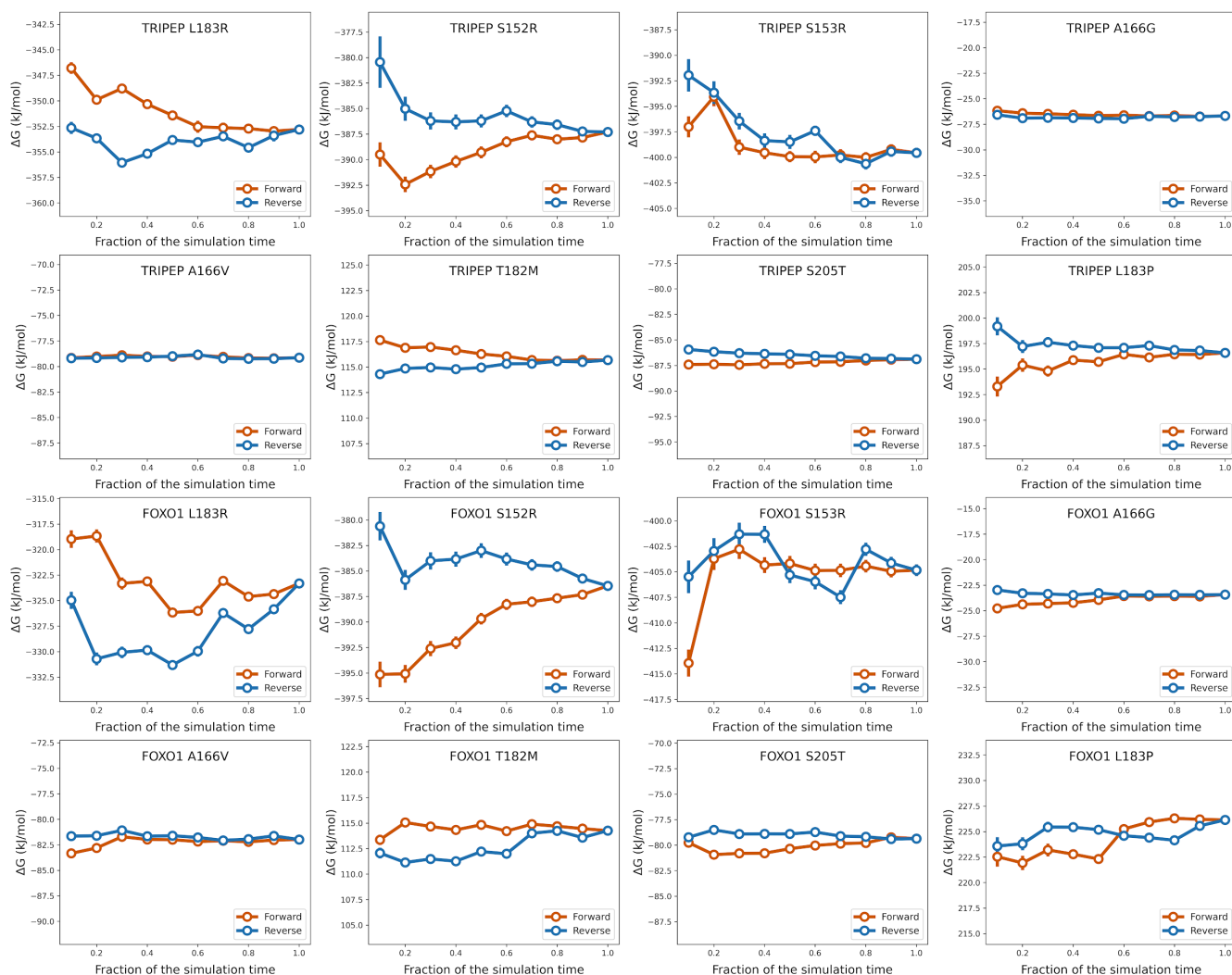
Figure S4.

Examples of trajectories involved in observed macrostate transitions. Traces are plotted on the TICA free energy landscape, with the color denoting the direction of time (violet to red denotes the start to finish). Animated visualizations of each of these trajectories are included in Supplemental Movies M1–M8.
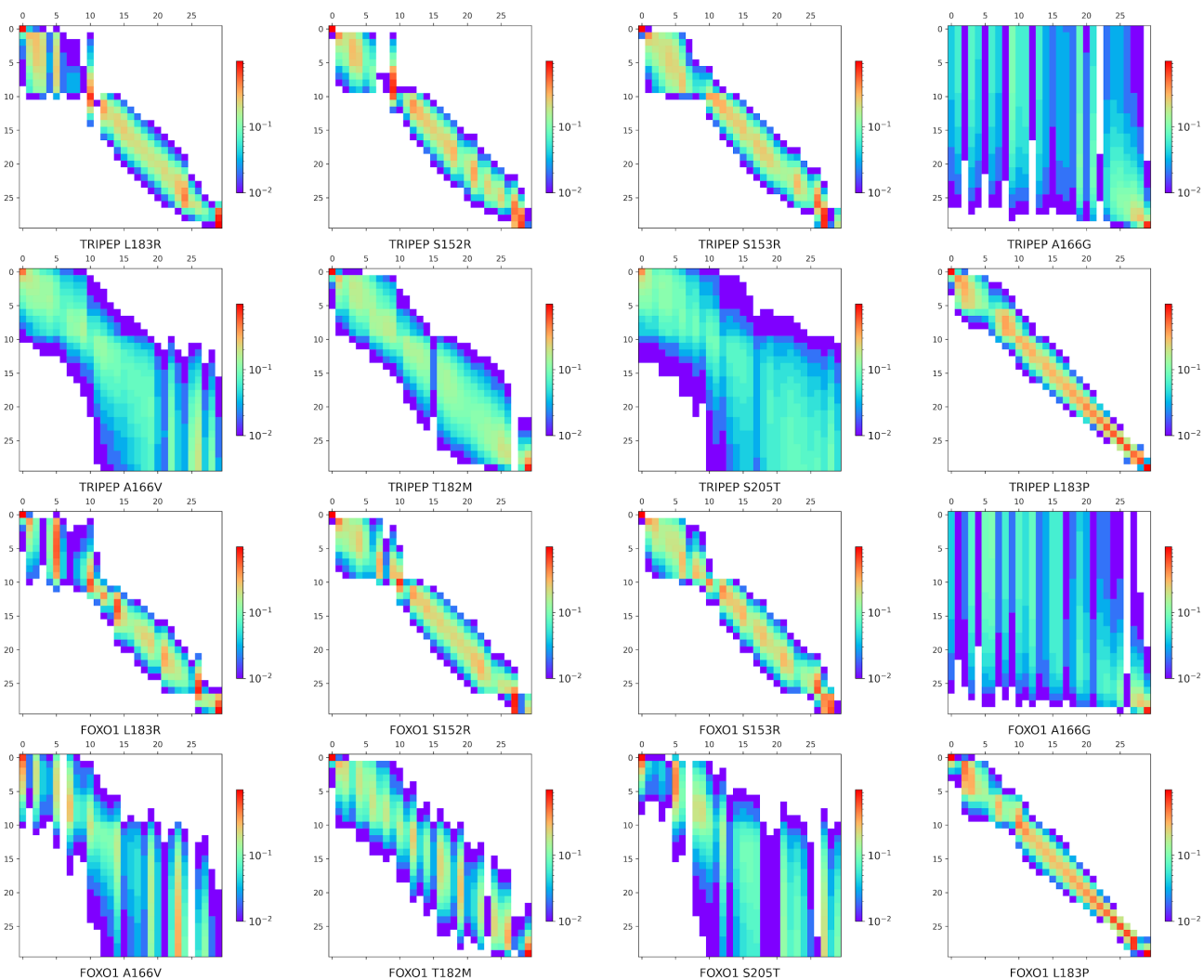
Figure S5.

PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) predictions of FOXO1 secondary structure.

Figure S6.

Time-reversed convergence plots for ΔG estimates were used to identify and discard non-equilibrated regions of the trajectories. In this analysis, two calculations are compared in which increasing amounts of data from a 8-ns trajectory are given to the MBAR free energy estimator; one from a time-forward trajectory and the other from a time-reversed trajectory. When all the data is included, the two calculations yield the same estimate; disagreement of the estimates when only part of the data is included may indicate convergence problems. The results show that most of the FEP simulations are sufficiently converged by the last 4 ns, with the possible exception of folded-state S152R. Therefore, we report FEP estimates computed from only the last 4 ns of each 8-ns trajectory.
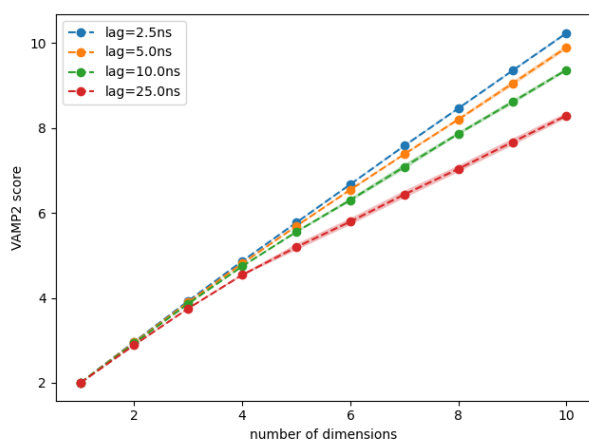
Figure S7.

The overlap matrix, whose elements O_{ij} quantify the overlap between the distributions of $\Delta U_{ij} = U(\lambda_j) - U(\lambda_i)$ for alchemical intermediate indices i and j (see Klimovich et al. 2015), is shown for each FEP calculation.
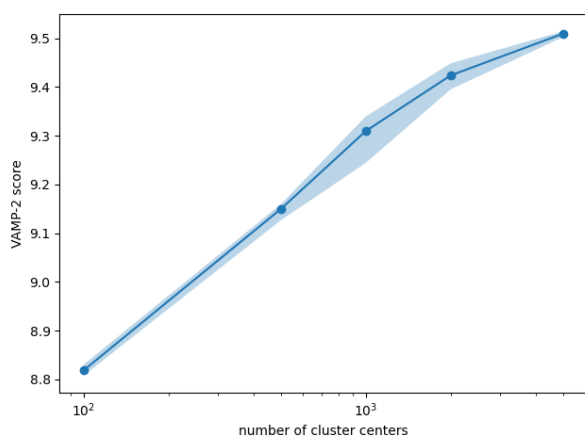
A

B

Figure S8.

(A) To choose an appropriate TICA lag time and number of TICA dimensions, VAMP2 scores were computed for different microstate MSMs constructed using various numbers of TICA dimensions and TICA lag times: $\tau_{\mathrm{TICA}} = 2.5$ ns (blue), 5.0 ns (orange), 10.0 ns (green), and 25.0 ns (red). All models used alpha-carbon distance features, and 1000 k-means cluster centers. Based on these results, we chose to construct MSMs of FOXO1 using a TICA lag time of $\tau_{\mathrm{TICA}} = 2.5$ ns, and four TICA dimensions. (B) To determine an appropriate number of cluster centers, VAMP2 scores were computed for microstate MSMs constructed using different numbers of cluster centers. All calculations were performed using an MSM lag time of $\tau_{\mathrm{MSM}} = 5.0$ ns and TICA lag time of $\tau_{\mathrm{TICA}} = 2.5$ ns.

A


Commute Distance v. Total Kinetic Variance

B
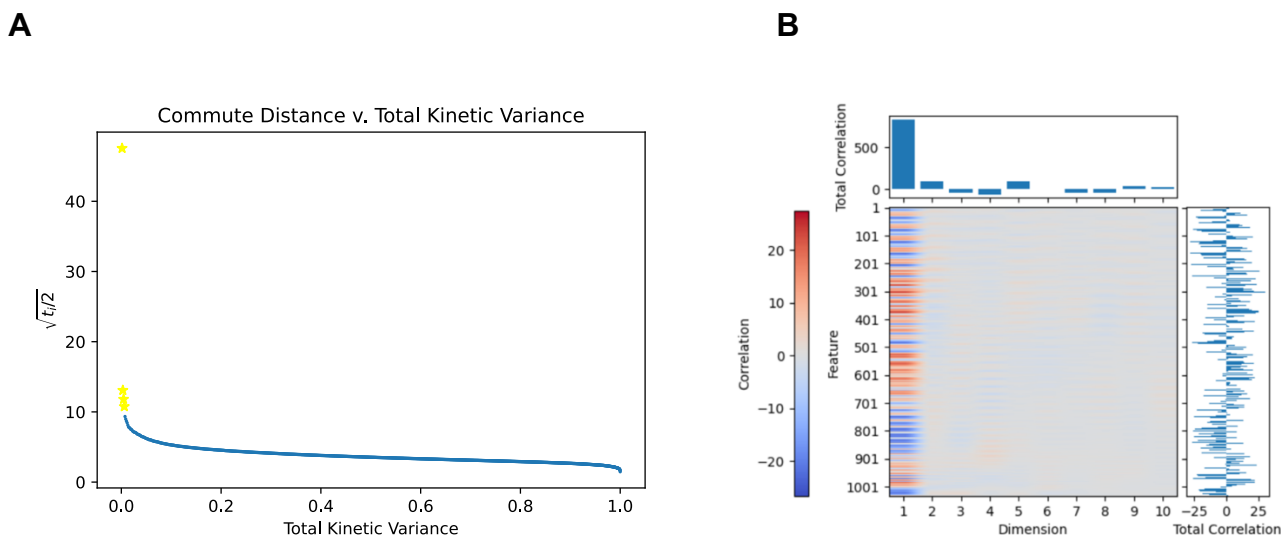
Figure S9.

To demonstrate that four TICA components are sufficient to capture the slowest motions, we consider the "commute map" scaling of the TICA components. While TICA returns eigenvectors of the time-lagged correlation matrix that have unit variance by definition, scaling each eigenvector by its corresponding eigenvalue results in a "kinetic map" (Noé, F. and Clementi, C. 2015), a space in which the Euclidean distance between any two points approximates a diffusion-based commute time. A measure of the commute time ascribed to each tIC_i is provided by $(t_i/2)^{1/2}$ where t_i is the ith implied timescale (Noé et al. 2016). The fractional kinetic variance captured by each tIC_i can be expressed as $t_i/(\Sigma_i\, t_i)$, and the total kinetic variance captured by the first n tICs is given by $(\Sigma_{i=1\,to\,n}\, t_i)/(\Sigma_i\, t_i)$. (A) A plot of the commute time vs. total kinetic variance shows that first four tICs (yellow stars) capture the slowest motions. The total kinetic variance, however, comprises contributions from a large number of higher-dimensional tICs, likely arising from the heterogeneous and high-dimensional dynamics in unfolded states. (B) The correlation of each distance feature j with each tIC_i. The total correlation with the input feature data is by far the largest for the first tIC, which corresponds primarily to the folding of helix α3.

References

Noe, F. and Clementi, C. 2015. Kinetic distance and kinetic maps from molecular dynamics simulation. J. Chem. Theory. Comput. http://doi.org/10.1021/acs.jctc.5b00553

Noé, Frank, Ralf Banisch, and Cecilia Clementi. "Commute Maps: Separating Slowly Mixing Molecular Configurations for Kinetic Modeling." Journal of Chemical Theory and Computation 12, no. 11 (November 8, 2016): 5620–30. https://doi.org/10.1021/acs.jctc.6b00762.
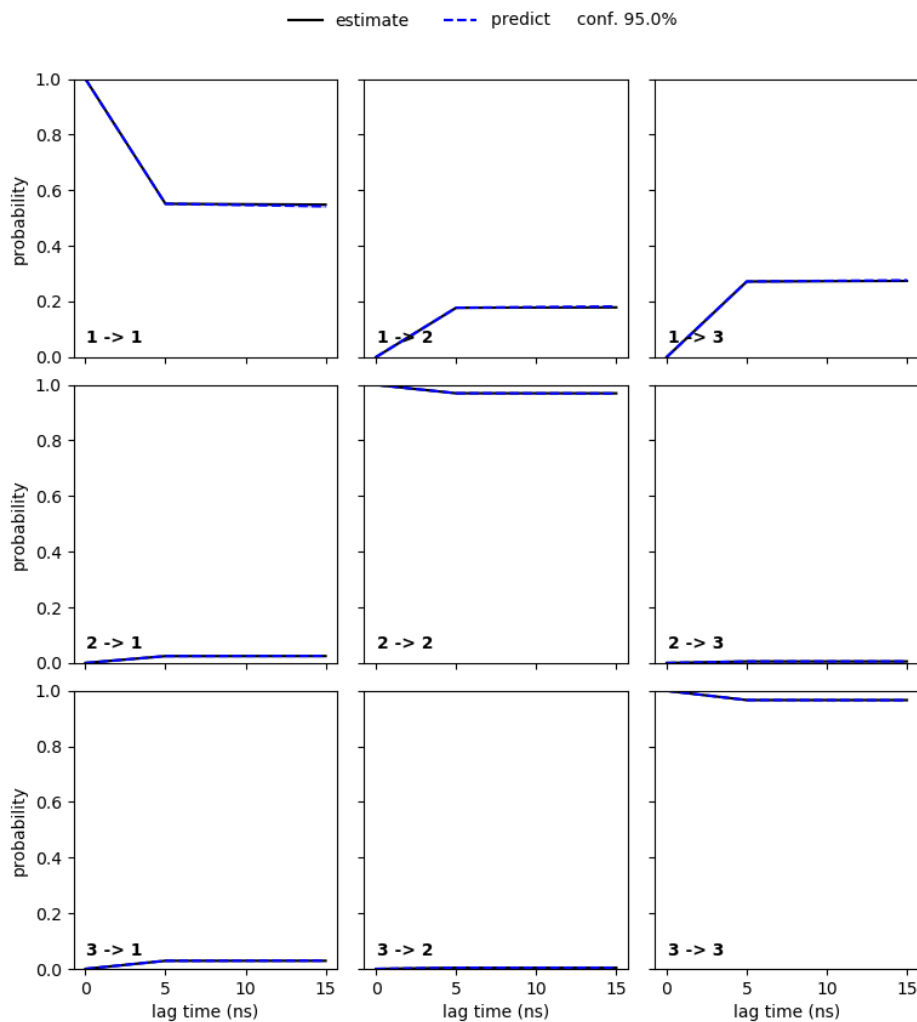
Figure S10.

The Chapman-Kolmogorov test is used to check whether the macrostate MSM is sufficiently Markovian. In this test, empirical estimates of the 3×3 macrostate transition probability matrix $\mathbf{T}^{(n\tau)}$ constructed at lag times $n\tau$, $n = 1, 2, 3\ldots$ (solid lines) are compared to predictions $[\mathbf{T}^{(\tau)}]^n$ (dashed lines). Each row of panels labeled "$i \to j$" shows the time evolution of populations for macrostate j where the initial population is placed entirely in macrostate i. Here, $i{=}1$ is macrostate I, $i{=}2$ is macrostate N, and $i{=}3$ is macrostate U. The results show that the two models (empirical vs. prediction) are virtually indistinguishable, indicating the dynamics is Markovian.

Supplemental Movies

Movie M1. Visualization of a U→U trajectory (1110 ns, RUN 1, CLONE 10).

Movie M2. Visualization of a U→I trajectory (600 ns, RUN 12, CLONE 451).

Movie M3. Visualization of a I→U trajectory (880 ns, RUN 0, CLONE 67).

Movie M4. Visualization of a I→I trajectory (1310 ns, RUN 0, CLONE 223).

Movie M5. Visualization of a I→N trajectory (1920 ns, RUN 0, CLONE 21).

Movie M6. Visualization of a N→U trajectory (620 ns, RUN 18, CLONE 48).

Movie M7. Visualization of a N→I trajectory (1380 ns, RUN 18, CLONE 80).

Movie M8. Visualization of a N→N trajectory (1410 ns, RUN 3, CLONE 8).