Supporting Information

Interplay between Folding Mechanism and Binding Modes in Folding Coupled to Binding Processes

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MATERIALS AND METHODS

Coarse Grained Model

For all the simulations described in this work we used the Karanicolas and Brooks[1] C-alpha coarse grained protein folding model. The potential energy function consists of harmonic terms for bonds and angles, a statistical potential for the pseudo-dihedrals, and non-bonded interactions for residue pairs

$$E = V_{nb}(r_{ij}) + V_{bond}(r) + V_{angle}(\theta) + V_{torsion} \qquad (1)$$

Favorable non-bonded interactions are used for the residue pairs that are in contact in the native conformation of the complex having at least one side-chain-side-chain heavy atom pair at distances shorter than 4.5 Å. For these pairs of residues the following functional form is used

$$V_{nb}(r_{ij}) = \epsilon_{ij}\left[13\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - 18\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{10} + 4\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right] \qquad (2)$$

where σ_{ij} is the distance at which the interaction energy is at its minimum (i.e. the separation between alpha carbons in the reference structure) and ϵ_{ij} depends on the type of interaction and residue type. For residue pairs not in contact in the reference structure, a repulsive interaction potential is used in which the repulsive distance is determined by the sum of the radii of residues *i* and *j*. The repulsive radius of a given residue is set to the distance to the closest residue that is not assigned a native contact. For the downhill model the repulsive distance was fixed at a value of 6 Å, resulting in smaller beads but at the same time avoiding the beads to cross over during simulations. Additionally, the force constants for the angular and dihedral contributions were scaled down. A Debye–Hückel[2] form, was used to represent electrostatic interactions:

$$V_{ele}(r_{ij}) = \frac{1}{4\Pi D r_{ij}}\left[q_i * q_j \, exp\left(-\frac{r_{ij}}{\xi}\right)\right] \qquad (3)$$

where q_i and q_j are the net amino-acid charges at pH = 7, ξ is the screening length (10 Å) and *D* is the dielectric constant (80, for water).

Structural parameters defining the PSBD folding interactions were obtained using the contact map derived from the 1w3d structure (45 amino acids). Non-bonded interaction energies were tuned to reproduce the folding temperature. PSBD folding simulations were done at temperatures ranging from 300 to 400K every 10K (totaling 11 simulations). The PSBD downhill folding model was obtained by reducing the effective size of the beads representing the C^{α} atoms of the amino acid residues (i.e. enforcing the same repulsive contribution for all residues) and scaling down the dihedral and angle terms by 50% and 93%, respectively, from their original value.

The parameters to describe binding to E1 were derived from the intermolecular contacts observed in the 1w85 X-ray structure. The modeller software package was used to add missing atoms and residues in the complex, including the missing N-terminal residues of PSBD. To avoid significant deviations from the experimental structure the number of optimization steps in the modeller run was kept to a minimum. The resulting coordinates were used to obtain intra E1 and inter E1-PSBD interactions for the complex. We excluded any new inter-molecular contacts emerging from the added N-terminal segment of PSBD to keep the binding energies/contacts consistent with the 1w85 X-ray crystal structure.
Therefore, the final hybrid potential for folding coupled to binding simulations included the PSBD intra-molecular interactions from 1w3d and the inter-molecular E1-PSBD interactions from the modeller refined 1w85 structure. Folding coupled to binding simulations were performed starting from the Cα coordinates for the hybrid E1-PSBD complex modeller-refined structure, in which the C_{alpha} RMSD for PSBD is 0.38 Å relative to the 1w3d NMR structure used for folding simulations.

Fraction of Native Contacts calculation:

$$Q = \frac{1}{N} \sum_{i,j \epsilon \, contacts} (1 + e^{\beta(r_{ij} - \gamma r_{ij}^{0})})^{-1} \qquad (4)$$

The sum runs over all the native contact pair. r_{ij} and r_{ij}^{0} are the distances between the i and j residues in the instantaneous and pdb reference/pdb conformation. The β and γ have a value of 50 $nm^{-1}$and 1.0 respectively.

Dissociation Constant (K_D):
The dissociation constant for the complex was calculated as described in ref. 2:

$$K_D = \frac{p_u^{\,2}}{p_b} * [\text{Protein}] \qquad (5)$$

where the populations of the unbound state, p_u is obtained from the potential of mean force.

$$p_u = \int_0^{Q^*} e^{-\beta E(Q)} \, dQ \Big/ \int_0^{1} e^{-\beta E(Q)} \, dQ \qquad (6)$$

and p_b= 1-p_u and Q* ~0.025 is the value of the dividing line.

Replica exchange simulations

Replica exchange molecular dynamics simulations[3] (REMD) on the E1-PSBD complex were carried out using 48 parallel replicas running at the following temperatures (K) with exchange attempts every 5 ps, totaling 96 microseconds of simulation: 270.00, 272.18, 274.39, 276.62, 278.88, 281.15, 283.45, 285.79, 288.14, 290.53, 292.92, 295.35, 297.81, 300.30, 302.81, 305.34, 307.91, 310.52, 313.14, 315.79, 318.48, 321.18, 323.92, 326.70, 329.49, 332.32, 335.19, 338.08, 341.00, 343.96, 346.95, 349.97, 353.03, 356.11, 359.24, 362.41, 365.60, 368.83, 372.10, 375.40, 378.74, 382.11, 385.52, 388.97, 392.45, 395.98, 399.57, 403.18.

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Fig. SI 1: Additional examples of folding coupled to binding trajectories for the downhill folding scenario.