Supporting Text S1

A Disorder Induced Domino-Like Destabilization Mechanism Governs the Folding and Functional Dynamics of the Repeat Protein IκBα

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The Wako-Saitô-Muñoz-Eaton (WSME) model

The free-energy of a particular microstate (m,n) (i.e., a string of 1's between and including m and n) in the WSME model with electrostatics and solvation free-energy [19,21] is given by

$$\Delta F = \sum \Delta G_{m,n}^{stab} - T \sum_{m}^{n} \Delta S_{conf} \qquad (S.1)$$

The stabilization free-energy of a microstate (first term in the above equation) is represented as a sum of van der Waals interactions (E_{VdW}), electrostatic potential (E_{elec}) and solvation free-energy (ΔG_{solv}) arising from purely native contributions:

$$\Delta G_{m,n}^{stab} = E_{VdW} + E_{elec} + \Delta G_{solv} \qquad (S.2)$$

The second term corresponds to the entropic cost of fixing residues to the native conformation at the temperature T. The interacting partners that contribute to the van der Waals energy are identified by setting a distance cut-off (r_{cut}) to the pair-wise heavy-atom partners (i, j) calculated from the PDB file.

$$E_{VdW} = \sum_{m,n} \xi_{i,j} \rho \qquad (S.3)$$

where $\rho = 1$ if $r_{ij} \leq r_{cut}$ and $\rho = 0$ otherwise. In atomic-level force-fields the interaction energies are distance dependent but since the WSME model pre-assumes an ensemble derived from the native structure a distance dependent energy is not required. The r_{cut} was fixed to 6 Å and nearest neighbors were excluded from the contact calculation as before[19,21].

A Debye-Hückel (DH) treatment is employed for the electrostatic potential term:

$$E_{elec} = \sum_{m,n} K_{Coulomb} \frac{q_i q_j}{\varepsilon_{eff} r_{ij}} \exp\left(-r_{ij} \kappa\right) \qquad (S.4)$$

wherein $K_{Coulomb}$ is the Coulomb constant (1389 kJ.Å/mol), q_i is the charge on the atom i, r_{ij} is the distance between charge centers i and j, and ε_{eff} is the effective dielectric constant and is fixed to 29. $1/\kappa$ is the Debye screening length that depends on ε_{eff}, solvent ionic-strength (I) and temperature (T) as

$$\kappa^2 = \frac{8\pi e^2 I}{\varepsilon_{eff} k_B T} \tag{S.5}$$

where k_B is the Boltzmann's constant, and e is the elementary charge. Charges were assigned to atoms according to pH 7.0 protonation state and I was fixed to the experimental value of 0.05 M[10].

The solvation free-energy is approximated as being proportional to the number of formed contacts ($x_{cont}^{m,n}$) in that microstate with the proportionality constant being ΔC_p^{cont}, which is the temperature-independent heat capacity change upon fixing a native contact. Therefore,

$$\Delta G_{solv} = x_{cont}^{m,n} \Delta C_p^{cont} \left[\left(T - T_{ref} \right) - T \ln \left(T/T_{ref} \right) \right] \tag{S.6}$$

where T_{ref} is the reference temperature which is fixed to 385 K[34]. A uniform conformational entropy cost (ΔS_{conf}) is assigned to residues independent of sequence or native secondary-structure.

The partition function (Z) is calculated employing the transfer-matrix formalism of Wako and Saitô[16,17]:

$$Z(T) = v_l \left[\prod_{i=1}^{N} X_i \right] v_r^{tr} \tag{S.7}$$

where

$$X_i = \begin{pmatrix} 1 & 1 & 1 & \cdots & 1 & 1 \\ z & 0 & 0 & \cdots & & 0 \\ 0 & H_1^{(i)}z & 0 & & & \vdots \\ & & H_2^{(i)}z & & 0 & 0 \\ & 0 & & & H_{N-2}^{(i)}z & H_{N-1}^{(i)}z \end{pmatrix}$$

$$v_l = \left(1,1,1,\cdots,1\right)$$

$$v_r = \left(1,0,0,\cdots,0\right)$$

and

$$H_k^{(i)} = \exp\left(-\beta \sum_{j=1}^{k} \Delta G^{stab}\right) \qquad (k \le N-i) \qquad \text{(S.8)}$$

$$H_k^{(i)} = 0 \qquad\qquad (k > N-i)$$

Here, $\beta = 1/RT$ and $z = \exp\left(\Delta S_{conf}/R\right)$ where R is 8.314 J mol^{-1} K^{-1}. The one-dimensional free-energy profiles are constructed by accumulating the total statistical weight for members of the ensemble with different number of structured residues.

The overall probability of a particular residue to be folded (p_f^i) can be obtained from

$$p_f^i = Z^{-1} v_l \left[\prod_{j=1}^{i-1} X_j\right]\left[\frac{\partial X_i}{\partial \ln z}\right]\left[\prod_{j=i+1}^{N} X_j\right] v_r^{tr} \qquad \text{(S.9)}$$

The final parameters from fit to the excess heat capacity profile are: ΔS_{conf} = -18.1 J mol^{-1} K^{-1}, ε = -70.1 J mol^{-1} while ΔC_p^{cont} is fixed to -0.358 J mol^{-1} K^{-1}[19].

Chemical Denaturation

Chemical denaturation effects are introduced by a phenomenological constant that decreases the stabilization free-energy linearly with denaturant concentration ([D]):

$$\Delta G_{m,n}^{stab}([D]) = E_{VdW} + E_{elec} + \Delta G_{solv} - x_{cont}^{m,n} m^{cont}[D] \qquad \text{(S.10)}$$

Other parameters including entropic cost were fixed to the original values extracted from the characterization of temperature-induced denaturation.