Equilibrium and Kinetics of the Ankyrin Repeat Protein Myotrophin through a Simple Statistical Mechanical Model

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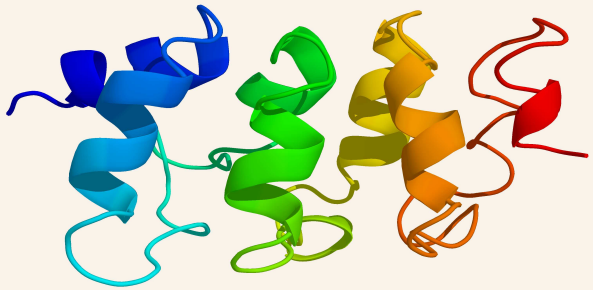
Overview

- Overview of the problem
- WSME model
- Equilibrium methods and results
- Kinetics methods and results
- Conclusions

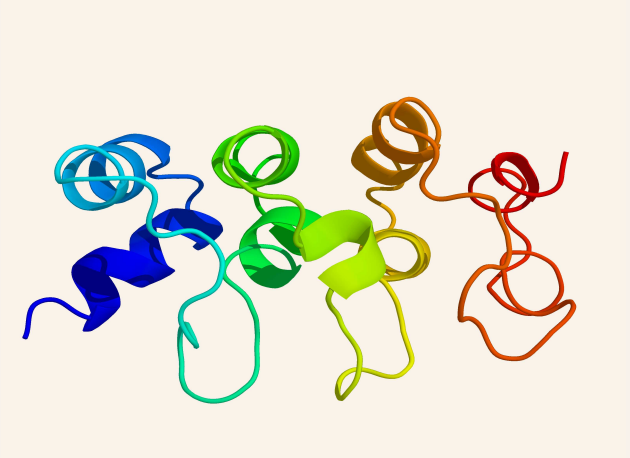
Myotrophin

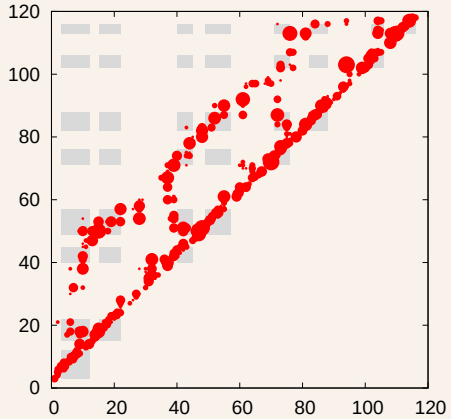


- it's a repeat protein of the ankirin family;
- experimental two-state equilibrium;
- experimental two-state kinetics;
- pathways heterogeneity: N-terminal and C-terminal domain.

Myotrophin

Native contacts map $\Delta_{i,j}$ =number of atomic contacts between residues i and j.





Atomic contact $\Leftrightarrow d(atom(i), atom(j)) < 3.5$Å.

Equilibrium

WSME

The Hamiltonian:

$$H = -\sum_{i=1}^{N-1}\sum_{j=i+1}^{N} \varepsilon_{i,j}\Delta_{i,j}m_{i,j} + \sum_{k=1}^{N}(q_k T + \alpha_k c)m_k, \qquad (1)$$

Dynamic variables:

m_k State of k residue (1 =native,0 =denatured);

$m_{i,j}$ $= \prod_{k=i}^{j} m_k$.

Parameters:

$\Delta_{i,j}$ Number of contacts between residues i and j;

$\varepsilon_{i,j}$ Energy cost of one-contact breaking;

q_k Entropy gain of one-residue unfolding;

α_k Parameter governing the denaturant action;

c Denaturant (urea) molar contentration;

T Temperature.

WSME

Mutations:

- We mimic a mutation modifying the strength of a selected group of native contacts;
- We select a total amount of *mutation strength* constant for all mutation ($\varepsilon_{tot} = 9.21$kJ/mol);
- We decrease of $\Delta\varepsilon_{i,j}$ the energy cost of each contact;
- $\Delta\varepsilon_{i,j}$ vary to satisfy the total perturbation constrain.

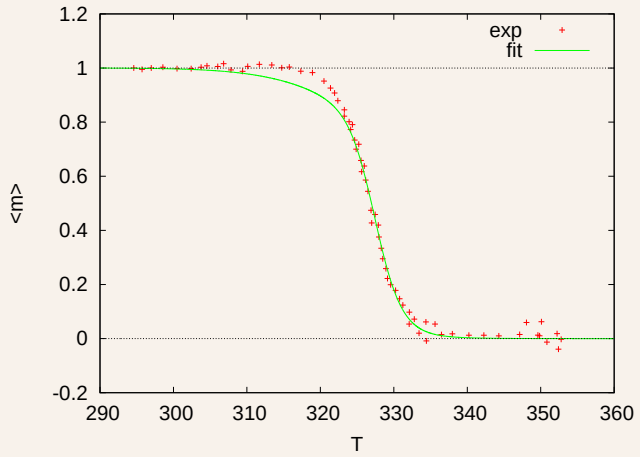
Equilibrium: Methods

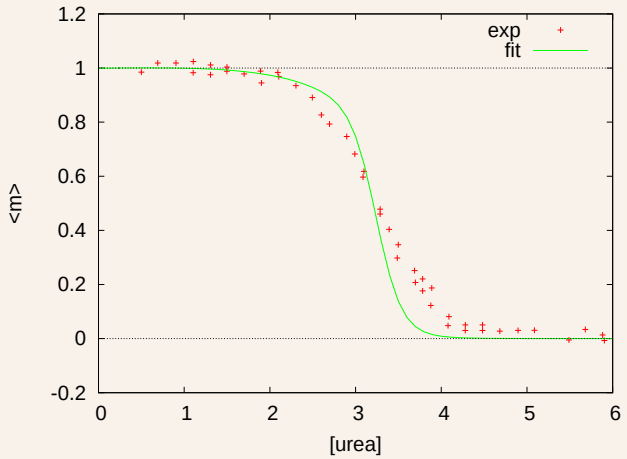
WMSE model:

- Partition function Z can be exactly calculated in equilibrium.
- Thermodynamics quantities can be as well computed:
  - $\rightarrow$ F, E, S, C_p;
  - $\rightarrow$ $< m_k >$;
  - $\rightarrow$ $< s_{i,j} >= \left\langle (1 - m_{i-1}) \left(\Pi_{k=i}^{j} m_k \right) (1 - m_{j+1}) \right\rangle$
- The order parameter $p(z) = \frac{m(z) - u(z)}{n(z) - u(z)}$ (z denaturant concentration, $u(z), n(z)$ baselines for unfolded and native state)

Parameters Fitting

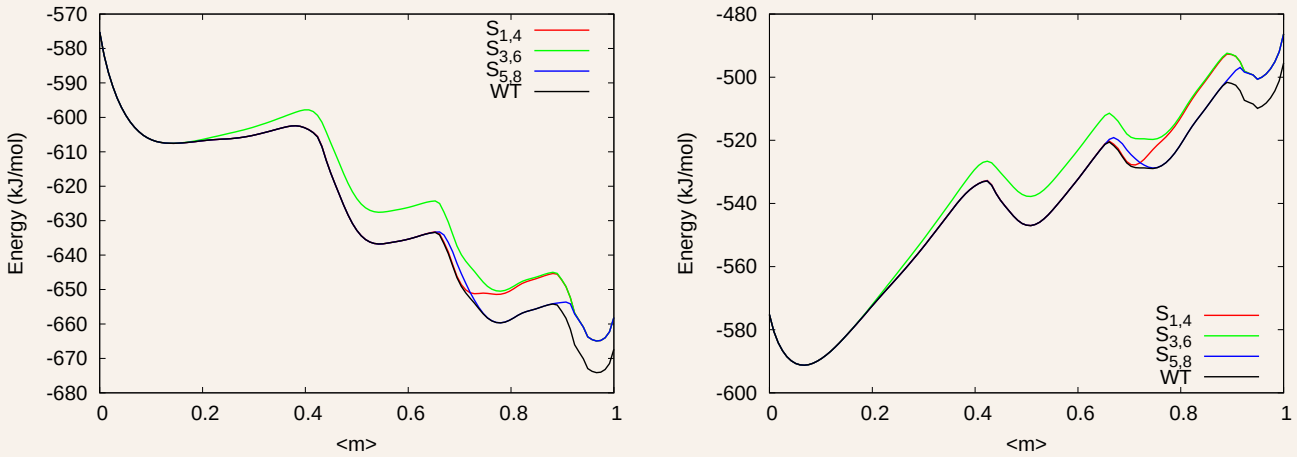
- Parameters are choosen as constants: $\varepsilon_{i,j} = \varepsilon, q_k = q = 2R, \alpha_k = \alpha$.
- The parameters values of ε and α are adjusted to experiments in order to reproduce T_m and c_m.





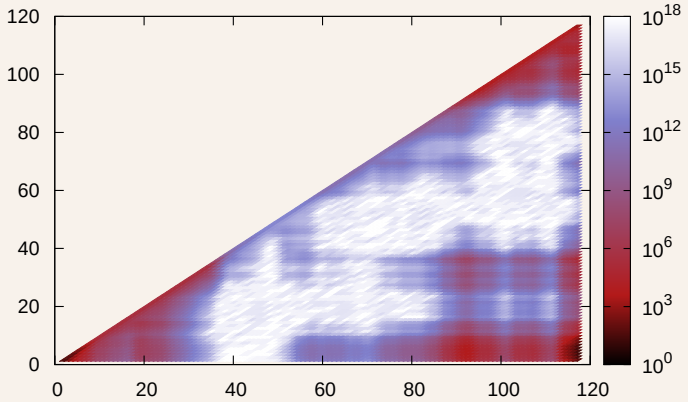
Temperature (left) and denaturant (right) denaturation curves.

Equilibrium



Free-Energy profile in folding (left) and unfolding (right) denaturant conditions. This analysis reveals a four minima landscape.

Landscape



Inverse native string propabilities at strong folding condition ($c = 0$). This is representative of the landscape conformation.

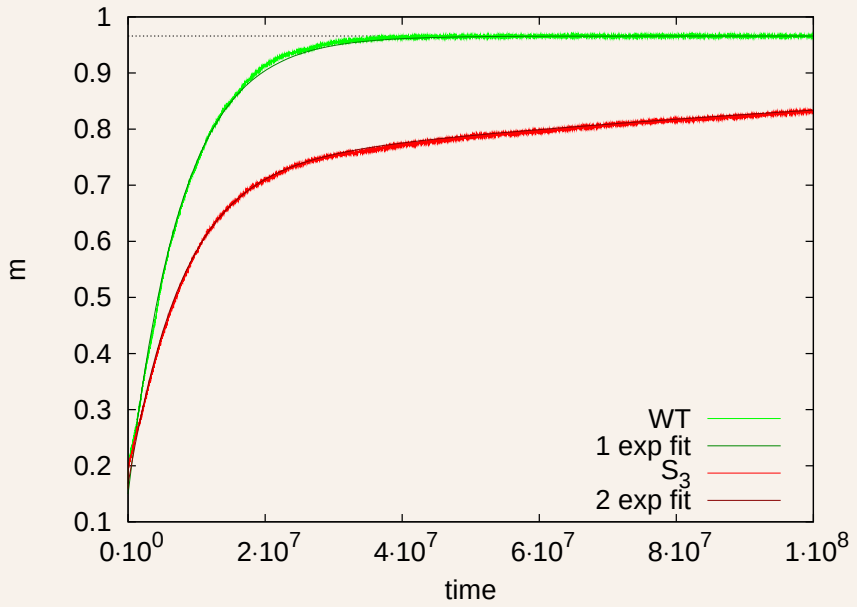
$$< s_{i,j} >^{-1} = \left\langle (1 - m_{i-1}) \left(\prod_{k=i}^{j} m_k \right) (1 - m_{j+1}) \right\rangle^{-1}$$

Kinetics

Kinetics: Methods

- Montecarlo simulation over 2000 proteins;
- Metropolis algorithm, 1-residue flip per step;
- Observables: $m(t)$;
- Helices last [un]folding time t_α.

Kinetics: Results



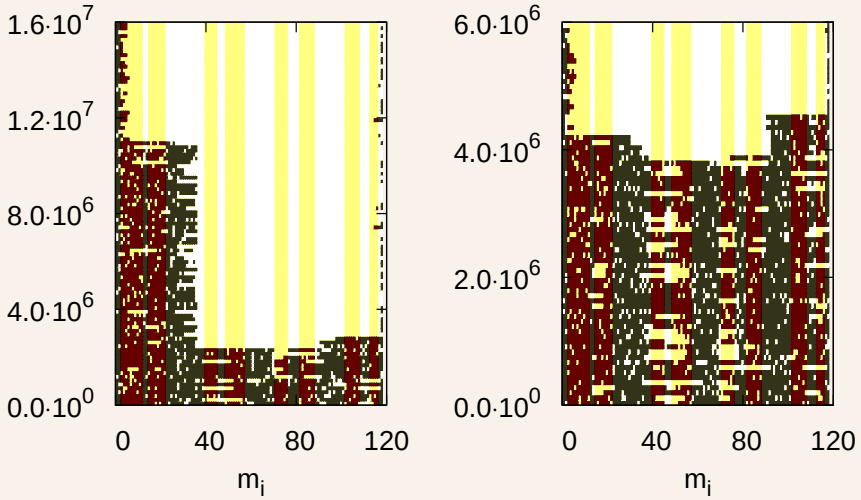
2-state kinetics despite landscape roughness in the WT case:

- $< m > (t)$ fitted with one exponential in the WT case;
- some mutation present a fast folding phase.

Protein relaxation in folding condition as functionof time, starting from a random configuration with infinite temperature probability. Average calculated over 2000 molecules.

Kinetics: Results

Pathway heterogeneity



Example of two single protein folding. It is possible to distinguish differents behaviors that could suggest the pathway heterogeneity.

Kinetics: Results

Definition of pathways:

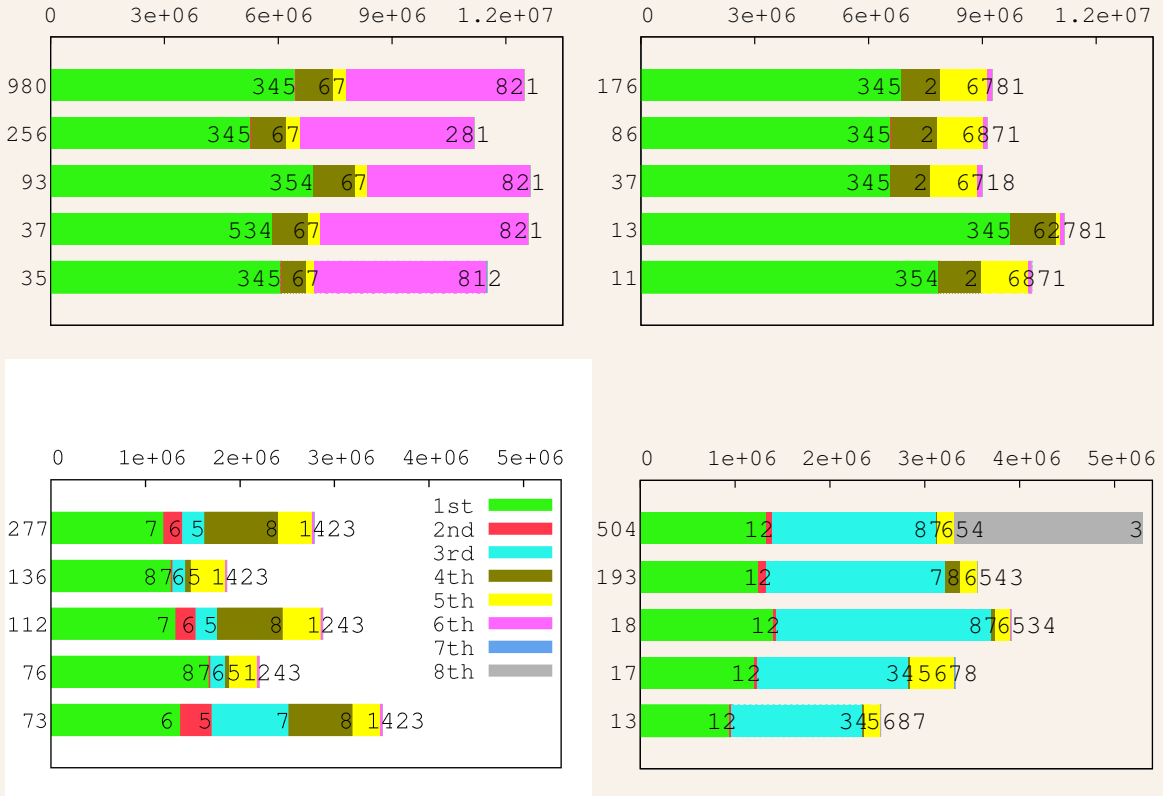
- *a* C-terminal fold first, unfold last;
- *b* N-terminal fold first, unfold last.

	k_1 (10^{-7})	$\lvert c_1 \rvert$	*a* (%)	*b* (%)
folding	1.279(9)	0.814(4)	80.2	19.8
unfolding	4.76(8)	0.965(4)	40.2	59.8

Rates and amplitudes (from 1-exponential fits) and fraction of molecules that select folding pathway *a* and *b*, in the case of folding and unfolding relaxations of the WT protein.

Folding

$< t_\alpha >$ patterns of helices stabilization in folding (top) and unfolding (bottom) processes.



Mutants

	folding				unfolding			
	k_1 (10^{-7})	k_2 (10^{-7})	a_f (%)	b_f (%)	k_1 (10^{-7})	k_2 (10^{-7})	a_u (%)	b_u (%)
WT	1.29	-	80.2	19.8	5.08	-	40.2	59.8
$S_{1,2}$	1.22	-	85.1	14.9	5.20	32.0	85.7	14.3
S_3	0.058	1.26	98.7	1.3	5.00	-	39.6	60.4
$S_{7,8}$	1.26	-	61.7	38.3	6.95	47.8	1.7	98.3

Rate and fraction of molecules that select folding pathway *a* and *b* in the case of folding and unfolding dinamics for different types of perturbation, as compared to the wild type (WT). Missing entries in column k_2 mean that the 1-exponential fit was sufficient, and the 2-exponential one would produce overfitting.

Conclusions

Conclusions

- We have applied the results of WSME model to the not-so-small repeat protein Myotrophin.

Equilibrium:

- WSME model provide a fast algorithm to resolve the equilibrium problem;
- Two-state-like bahavior at equilibrium despite simplicity and non banal energy profile.

Kinetics:

- Two-state-like general bahevior;
- Pathways multiplicity;
- Mutants behavior as pathways switchers;
- Non-symmetry between foldind and unfolding pathways.