Direction dependent mechanical unfolding and Green Fluorescent Protein as a force sensor

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Single molecule manipulation: Atomic Force Microscopy

Figure 1 | Applications of the scanning force microscope (SFM). a | The principal SFM components. Laser light is focused onto the back of a cantilever that ends with a nanometre-scale tip. The reflection and corresponding position of the tip is detected by a position-sensitive photodiode. A piezo-electric scanner moves the sample in all directions, enabling the tip to scan topography or to extend molecules attached to the surface. b | Diagrams and force curves showing the mechanical unfolding of repeating immunoglobulin-like domains. As the distance between the surface and tip increases (from state 1 to state 2), the molecule extends and generates a restoring force that bends the cantilever. When a domain unfolds (state 3), the free length of the protein increases, relaxing the force on the cantilever. Further extension again results in a restoring force (state 4). The last peak represents the final extension of the unfolded molecule before detachment from the SFM tip (state 5).
Single molecule manipulation: Laser Optical Tweezers
Single molecule manipulation: protocols

- **Constant velocity:**
  - the moving end of the molecule is pulled through an elastic force
  - the center of the corresponding harmonic potential moves at $v = \text{const}$
  - the force on the molecule can be measured as a function of the elongation

- **Constant force:**
  - the force on the molecule is kept constant using a feedback apparatus
  - elongation is measured as a function of time
Pulling Poly–Titin (I27): AFM, $\nu = \text{const}$

Worm Like Chain fits $\Rightarrow$ contour length (and variations)
Pulling an RNA hairpin, $f = \text{const}$

2–state behaviour is clearly observed at $f \sim f_u$
A recent theoretical review

Biomolecules under mechanical force

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Mechanical unfolding: a simple theory

Elongation is a natural reaction coordinate $\Rightarrow$ Bell’s model
Theory: \( f = \text{const} \)

Assuming TS is not moved by \( f \):

\[
\Delta G_u^\dagger (f) = \Delta G_u^\dagger (0) - f x_u \\
k_u(f) = k_u(0) \exp \left( \frac{f x_u}{k_B T} \right)
\]  \( (1) \)

Similarly,

\[
k_f(f) = k_f(0) \exp \left( -\frac{f x_f}{k_B T} \right)
\]
Theory: $f = rt, \ r = \text{const}$

Unfolding rate at time $t$, force $f = rt$

$$k_u(rt) = k_u(f) = k_u(0) \exp \left( \frac{fx_u}{k_BT} \right)$$

Probability of unfolding at force $f$

$$P(f) = \frac{k_u(f)}{r} \exp \left\{ \frac{k_BT}{rx_u} \left[ k_u(0) - k_u(f) \right] \right\}$$

Most probable unfolding force $f_M = \arg \max P(f)$

$$f_M = \frac{k_BT}{x_u} \ln \left[ \frac{x_u}{k_u(0)k_BT} \frac{r}{k_BT} \right]$$
More complex phenomena

- **Intermediates**: metastable states which retain only part of the native structure

- **Pathway diversity**: the unfolding of a protein with many intermediates can proceed through pathways which depend on the details of the pulling protocol

- **Direction dependence**: when the force is not applied end-to-end, but only a portion of the chain is pulled, the unfolding phenomenon depends on the application points of the force
Modeling approaches

Degrees of freedom:
- atomistic (all or heavy atoms)
- coarse–grained ($C_\alpha$, one or a few beads per aminoacid)
- lattice polymers
- Ising–like (e.g. a binary variable per aminoacid or peptide bond)

Interactions:
- native (Gō) vs. non–native interactions
- explicit vs. implicit solvent
Ising–like models

- Galzitskaya and Finkelstein, PNAS 96, 11299 (1999)
- Alm and Baker, PNAS 96, 11305 (1999)
- Muñoz and Eaton, PNAS 96, 11311 (1999)

A binary degree of freedom $m_k$, taking values native/non–native (resp. 1, 0) is associated to each aminoacid or to each peptide bond $\Rightarrow 2^N$ microstates

Can be thought of as an extremely crude discretization of a pair of dihedral angles ($\phi_i, \psi_i$ for an aminoacid, $\psi_i, \phi_{i+1}$ for a peptide bond)
Ising–like models (cont’d)

Many more non–native conformations $\Rightarrow$ excess entropy $q (\sim k_B)$ associated to non–native value (or entropy cost associated to native)

Different (native only) contact interaction energies: contact map $\Delta$ read from the PDB putting some threshold on interatomic distances (typically 0.4–0.5 nm between nonhydrogen atoms, or 0.65–0.7 nm between $C_\alpha$’s)
A microstate (1 = native, 0 = non-native):

\[
000000011111111100000000001111111011100110
\]

**ISLANDS** of 1’s can be identified.

Only aminoacids in the same island can interact: a non-native peptide bond (or aminoacid) breaks the chain into two non-interacting parts.

Effective free energy ("Hamiltonian")

\[
H = - \sum_{i<j} \epsilon_{ij} \Delta_{ij} \prod_{k=i}^{j} m_k - T \sum_i q_i (1 - m_i)
\]

\[\epsilon_{ij} \propto \text{number of close-by atom pairs}\]
Several choices for the \textit{kinetics}:

- Monte Carlo simulations
- diffusion on a 1D free energy profile
Mechanical unfolding: generalizing the island model

To each island we associate an orientational degree of freedom, which in the simplest case is still Ising–like (parallel/antiparallel to the force).

We do not need any more the introduction by hand of an excess entropy for non–native bonds.

The equilibrium thermodynamics is still exactly solvable.

Summing over orientational variables we get back the island model with an excess entropy \( q = k_B \ln 2 \).
Mechanical unfolding: generalizing the island model (cont’d)

PROTEIN ≡ sequence of rigid (native) stretches

For each stretch: native length $l_{ij}$, orientation $\sigma_{ij} = \pm 1$

\[
H(m, \sigma) = H_0(m) - fL(m, \sigma)
\]

\[
H_0(m) = - \sum_{i<j} \epsilon_{ij} \Delta_{ij} \prod_{k=i}^{j} m_k
\]

\[
L(m, \sigma) = \sum_{0 \leq i < j \leq N+1} l_{ij} \sigma_{ij} (1 - m_i)(1 - m_j) \prod_{k=i+1}^{j-1} m_k
\]

Summary of previous results

- **2–state behaviour** in agreement with theory and experiments (PRL ’07, JCP ’07)

- Ubiquitin 3–state behaviour: intermediate has same structure as in all–atom models. Multi–stage refolding as in experiments (PRL ’08)

- Multi(5)–state behaviour in an RNA fragment: pathways consistent with experiments and coarse–grained models (PRL ’09)

- **Pathway diversity** in a fibronectin domain (JCP ’10)
Green Fluorescent Protein (GFP)

11–strands $\beta$–barrel + small helices
Green Fluorescent Protein (GFP)

- **Large** protein: 238 amino acids

- Bright green fluorescence when exposed to light of a suitable wavelength (395 nm, blue) **AND native structure is intact**

- **Applications in biotechnology**
  - localization of proteins in living cells
  - metal ion or pH sensors
Experiments: pulling GFP end–to–end (Reif et al, PNAS ’07)

Major unfolding pathway

Minor unfolding pathway
Pulling a protein from different directions
Experiments: pulling GFP from different directions (Reif et al, PNAS ’06)
Model: landscape (at equilibrium unfolding $f$)

Intermediates: $\beta_1$ and $\beta_{11}$ ($\sim 110 \text{ Å}$), $\beta_{10}\beta_{11}$ ($\sim 180 \text{ Å}$), $\beta_1\beta_2\beta_3$ ($\sim 250 \text{ Å}$)

Model: pulling end–to–end

Major unfolding pathway

Order of unfolding events

- N–terminal $\alpha$–helix (small signal)
- $\beta_1$
- $\beta_2\beta_3$
- $\beta_{10}\beta_{11}$
- all the rest
Model: pulling end-to-end

Minor unfolding pathway

Order of unfolding events

- N-terminal $\alpha$-helix (small signal)
- $\beta_{11}$
- . . .
Model: pulling from different directions

<table>
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<th>Direction</th>
<th>$v = 0.3 , \mu m/s$</th>
<th>$v = 2 , \mu m/s$</th>
<th>$v = 3.6 , \mu m/s$</th>
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<td>177 ± 7</td>
<td>184 ± 13</td>
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<td>(104 ± 40)$^a$</td>
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<tr>
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<td>(117 ± 19)$^b$</td>
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<td>(548 ± 57)$^b$</td>
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GFP as a force sensor

http://pre.aps.org/kaleidoscope/pre/84/2/021918
GFP as a force sensor
Coworkers:

- Marco Zamparo (Padova University)
- Alberto Imparato (Aarhus University, Denmark)
- Michele Caraglio (PoliTO)

Main Refs for our work:

Thanks for your attention