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

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Single molecule manipulation: Atomic Force Microscopy

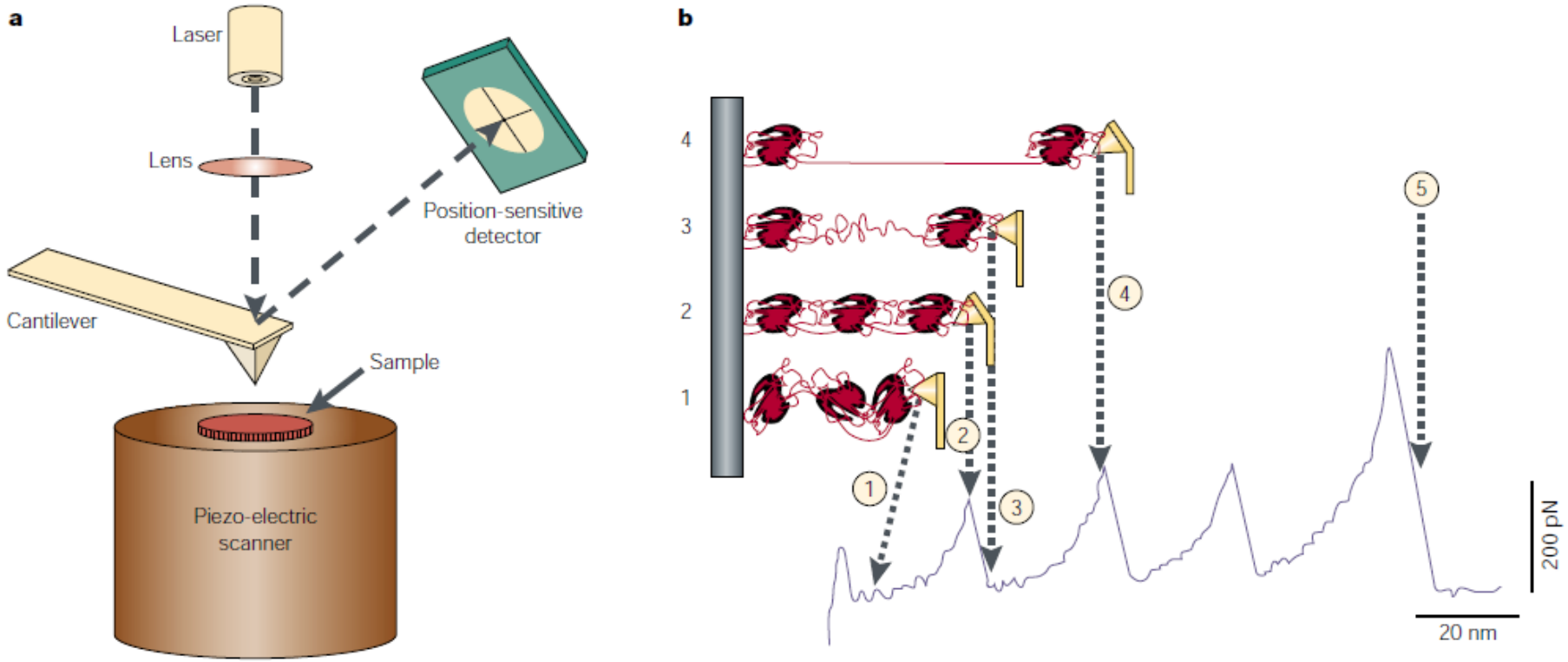
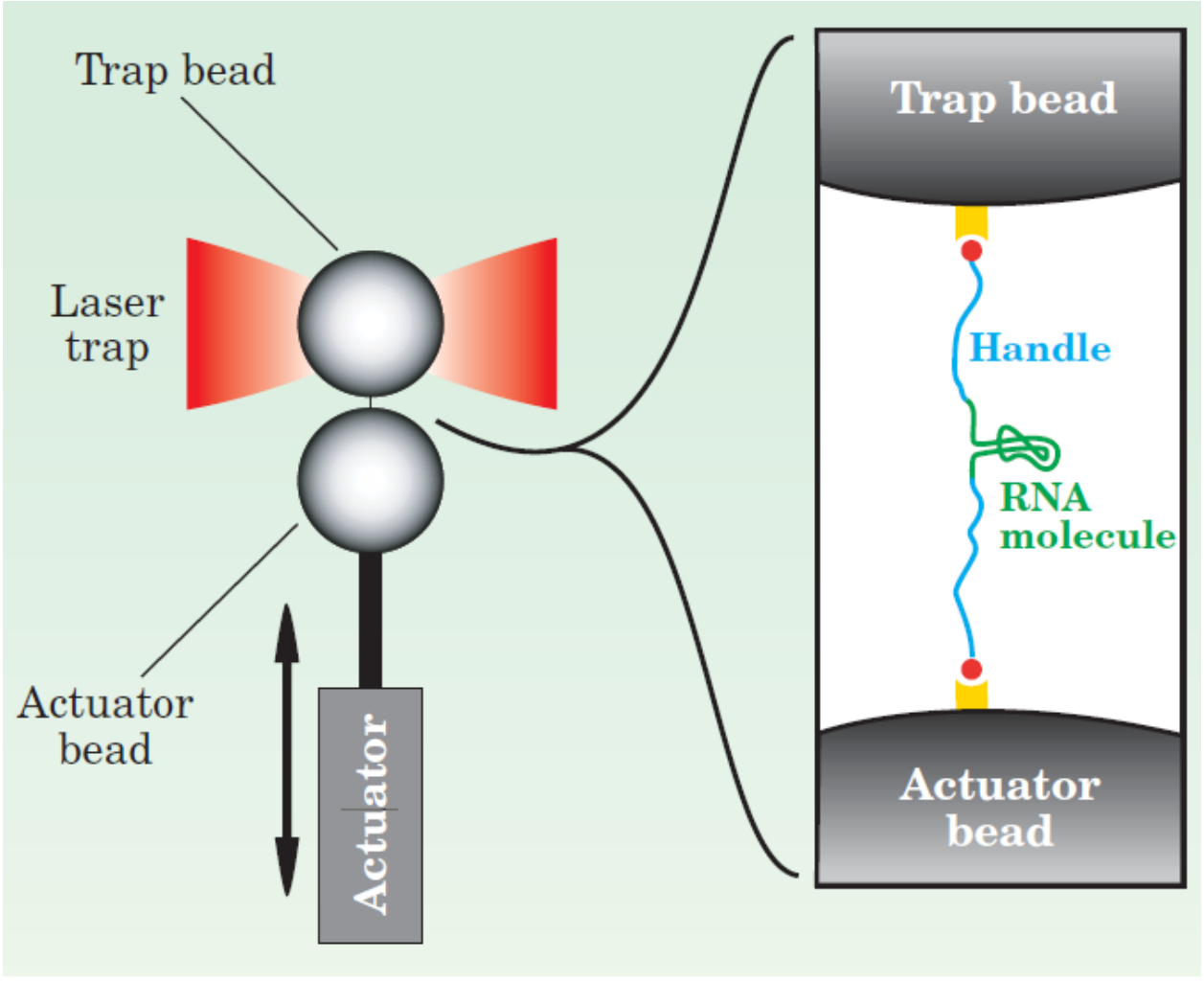


Figure 1 | **Applications of the scanning force microscope (SFM). a** | The principal SFM components. Laser light is focused onto the back of a cantilever that ends with a nanometre-scale tip. The reflection and corresponding position of the tip is detected by a position-sensitive photodiode. A piezo-electric 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[6,64]. 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

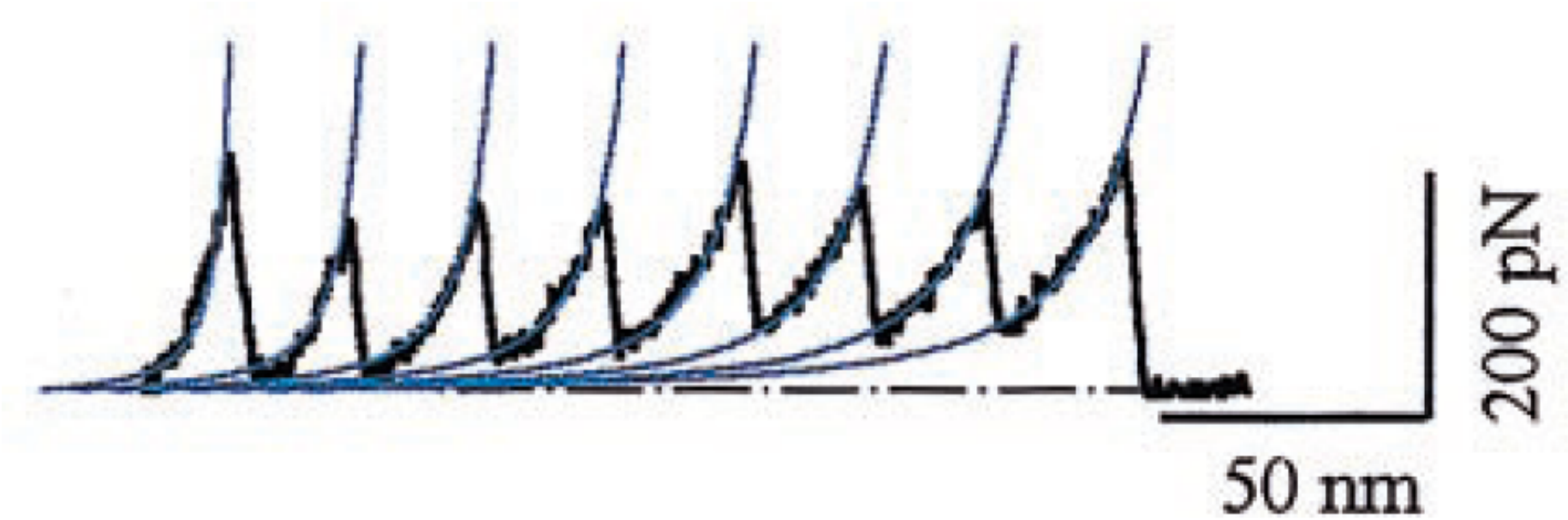


Single molecule manipulation: protocols

- 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

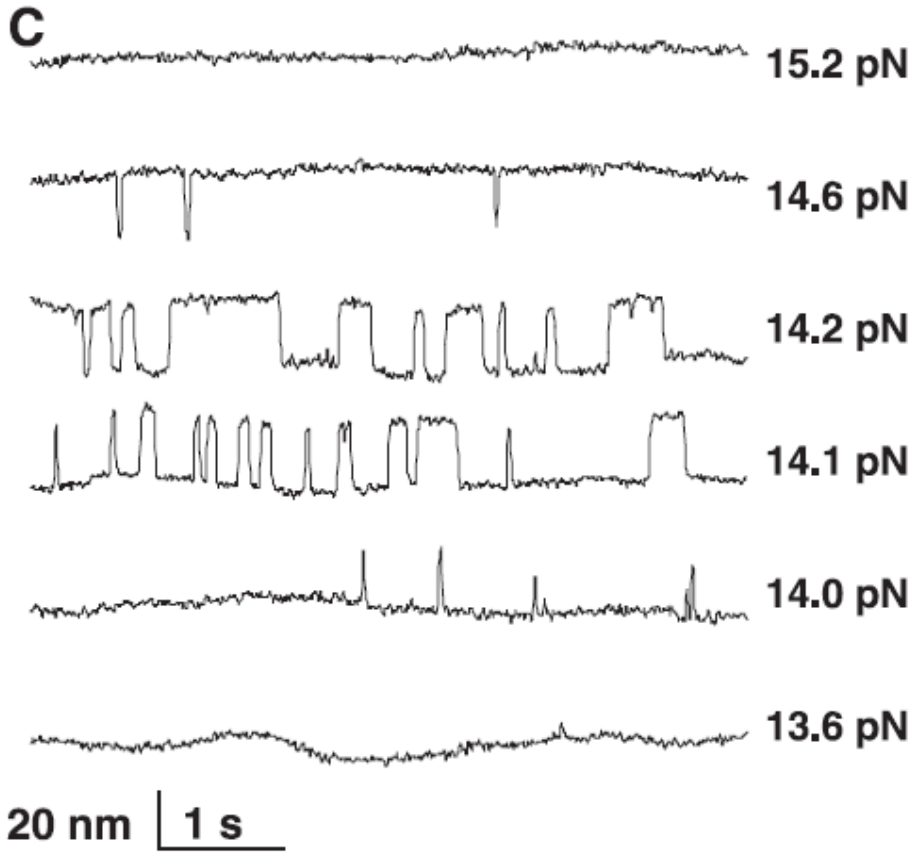
- Constant force:
  - the force on the molecule is kept constant using a feedback apparatus
  - elongation is measured as a function of time

Pulling Poly–Titin (I27): AFM, $v = \text{const}$



Worm Like Chain fits $\Rightarrow$ contour length (and variations)

Pulling an RNA hairpin, $f = \text{const}$



2–state behaviour is clearly observed at $f \simeq f_u$

A recent theoretical review

Physics Reports 486 (2010) 1–74

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Biomolecules under mechanical force

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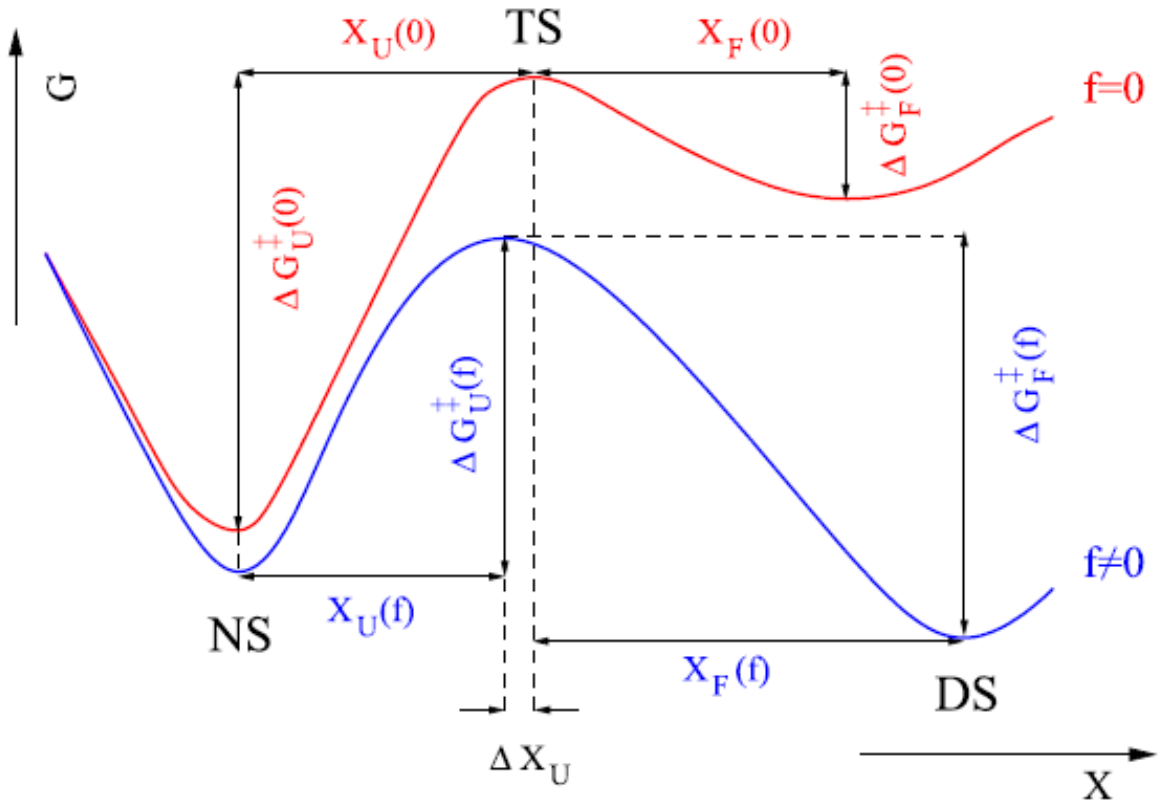
Mechanical unfolding: a simple theory

Elongation is a natural reaction coordinate $\Rightarrow$ Bell's model



Theory: $f = \text{const}$

Assuming TS is not moved by f:

$$\Delta G_u^{\ddagger}(f) = \Delta G_u^{\ddagger}(0) - f x_u$$
$$k_u(f) = k_u(0) \exp\left(\frac{f x_u}{k_B T}\right) \tag{1}$$

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 $f = rt$

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

Probability of unfolding at force f

$$P(f) = \frac{k_u(f)}{r} \exp\left\{\frac{k_B T}{r x_u}\left[k_u(0) - k_u(f)\right]\right\}$$

Most probable unfolding force $f_M = \operatorname{argmax} P(f)$

$$f_M = \frac{k_B T}{x_u} \ln\left[\frac{x_u}{k_u(0) k_B T} r\right]$$

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_α, one or a few beads per aminoacid)
- lattice polymers
- Ising–like (e.g. a binary variable per aminoacid or peptide bond)

Interactions:

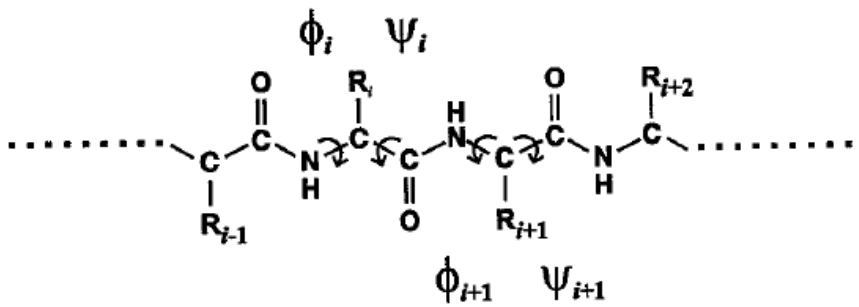
- native (Gō) vs. non–native interactions
- explicit vs. implicit solvent

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 ((ϕ_i, ψ_i) for an aminoacid, (ψ_i, ϕ_{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 Δ 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_α's)

(Wako–Saitô–)Muñoz–Eaton (or ISLAND) model

A microstate (1 = native, 0 = non–native):

000000011111111111000000000011111110111000110

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")

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

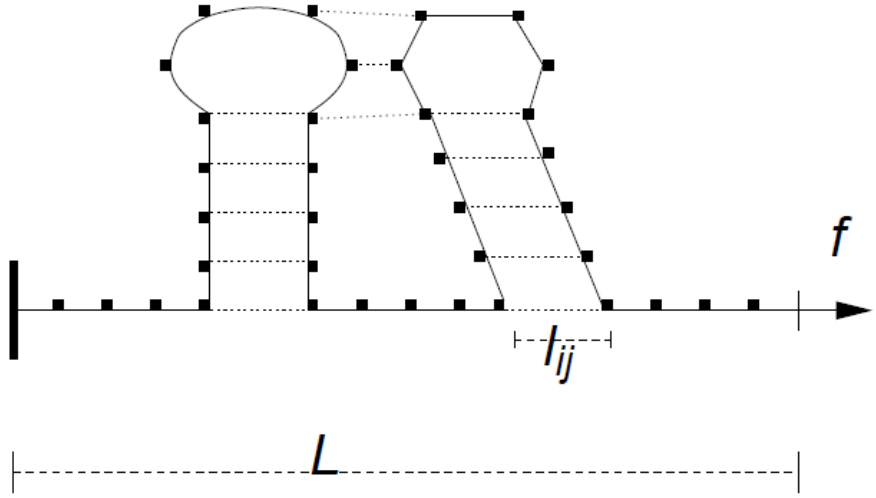
$\epsilon_{ij} \propto$ number of close–by atom pairs

(Wako–Saitô–)Muñoz–Eaton (or ISLAND) model (cont'd)

Several choices for the 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 $\equiv$ sequence of rigid (native) stretches

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

$$H(m, \sigma) = H_0(m) - fL(m, \sigma)$$

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

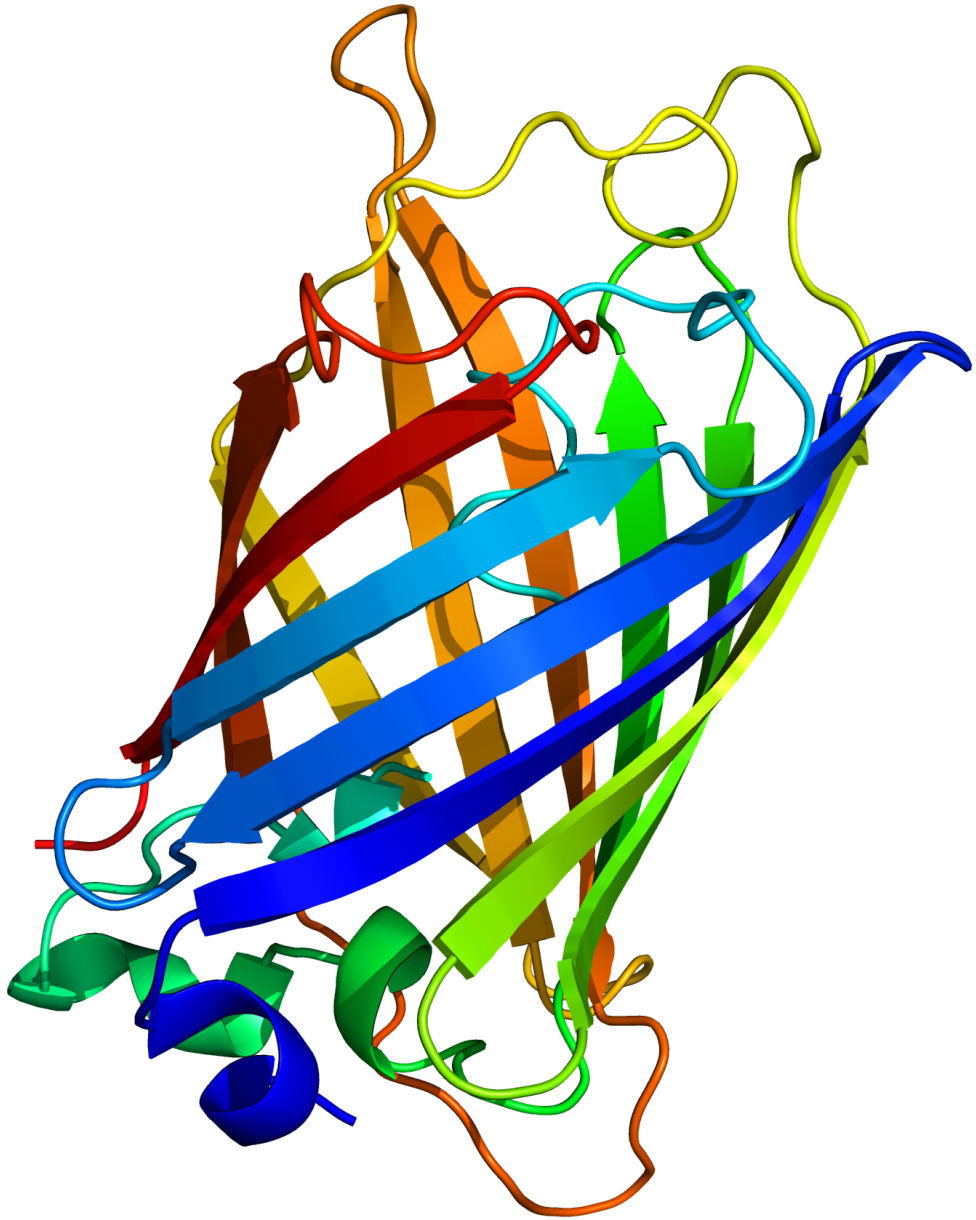
$$L(m, \sigma) = \sum_{0 \le i < j \le N+1} l_{ij} \sigma_{ij} (1 - m_i)(1 - m_j) \prod_{k=i+1}^{j-1} m_k$$

[A. Imparato, A. P. and M. Zamparo, Phys. Rev. Lett. **98**, 148102 (2007)]

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)



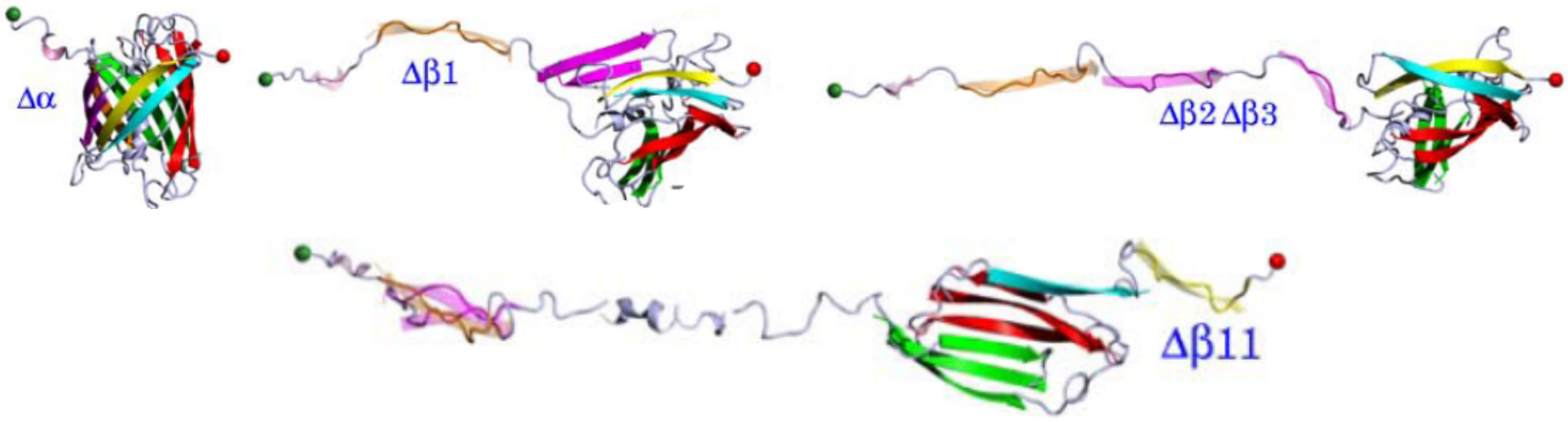
11–strands β–barrel $+$ small helices

Green Fluorescent Protein (GFP)

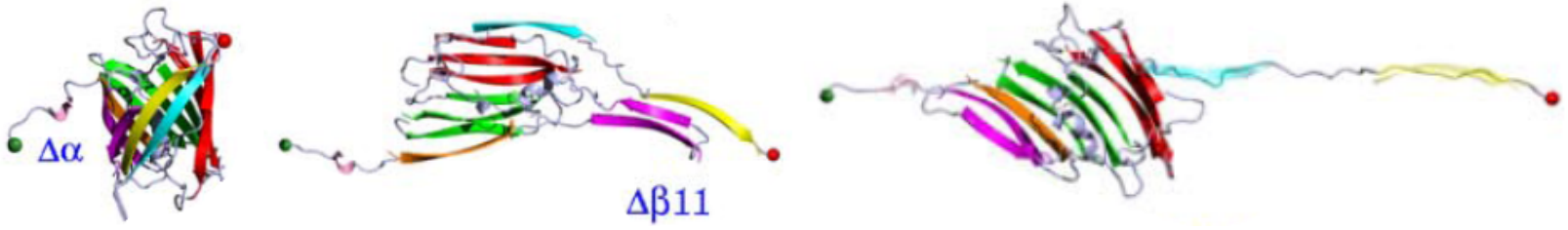
- Large protein: 238 aminoacids
- Bright green fluorescence when exposed to light of a suitable wavelength (395 nm, blue) AND native structure is intact
- Applications in biotechnology
  - localization of proteins in living cells
  - metal ion or pH sensors

Experiments: pulling GFP end–to–end (Reif et al, PNAS '07)

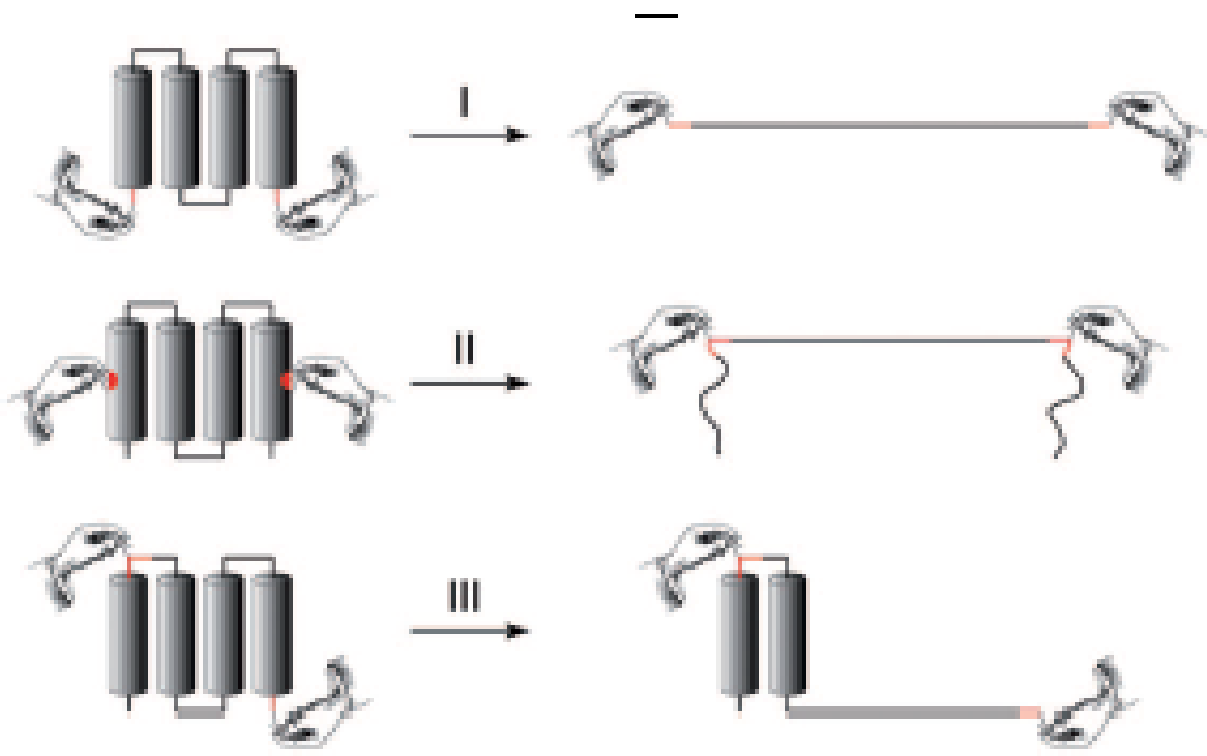
Major unfolding pathway



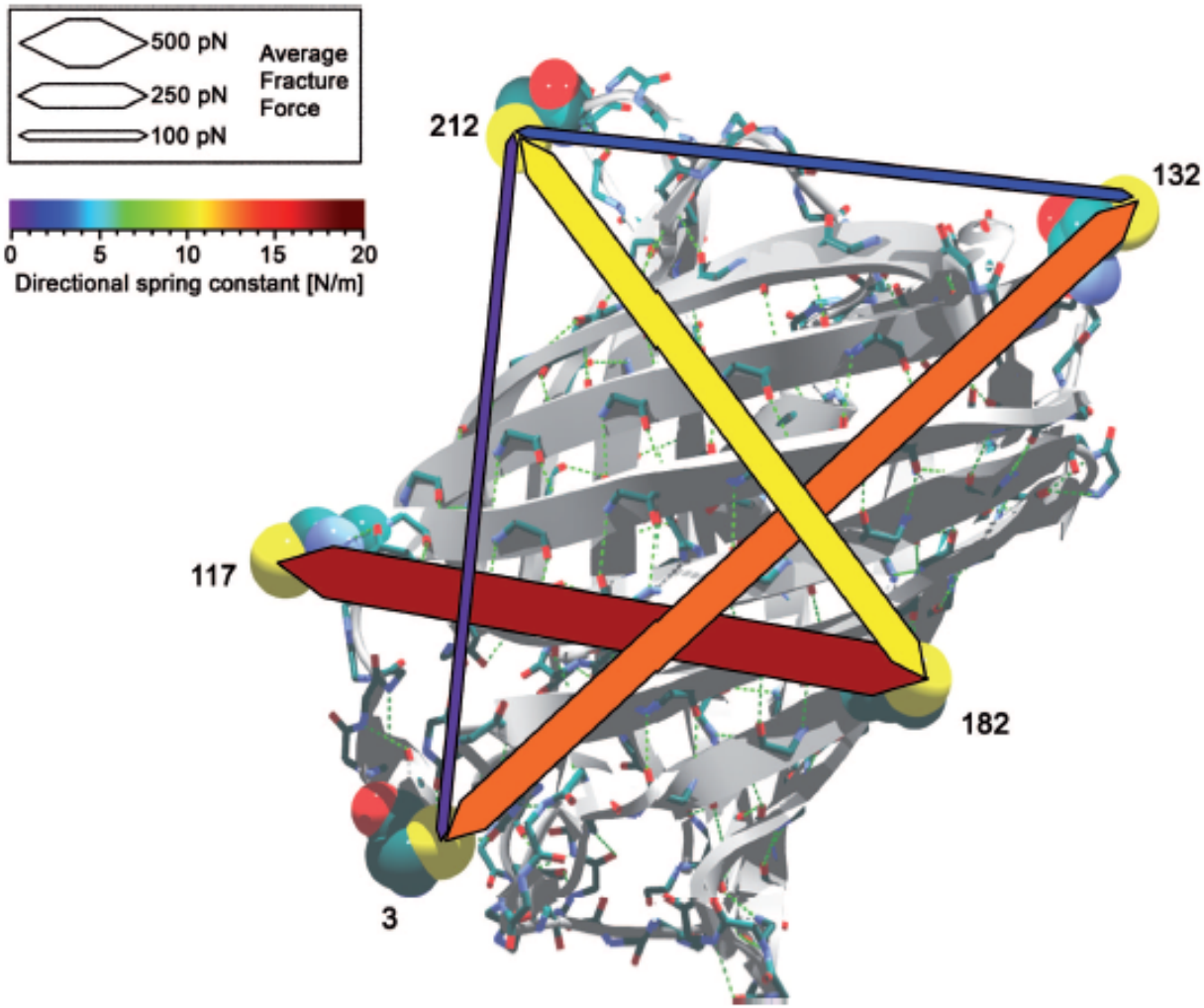
Minor unfolding pathway



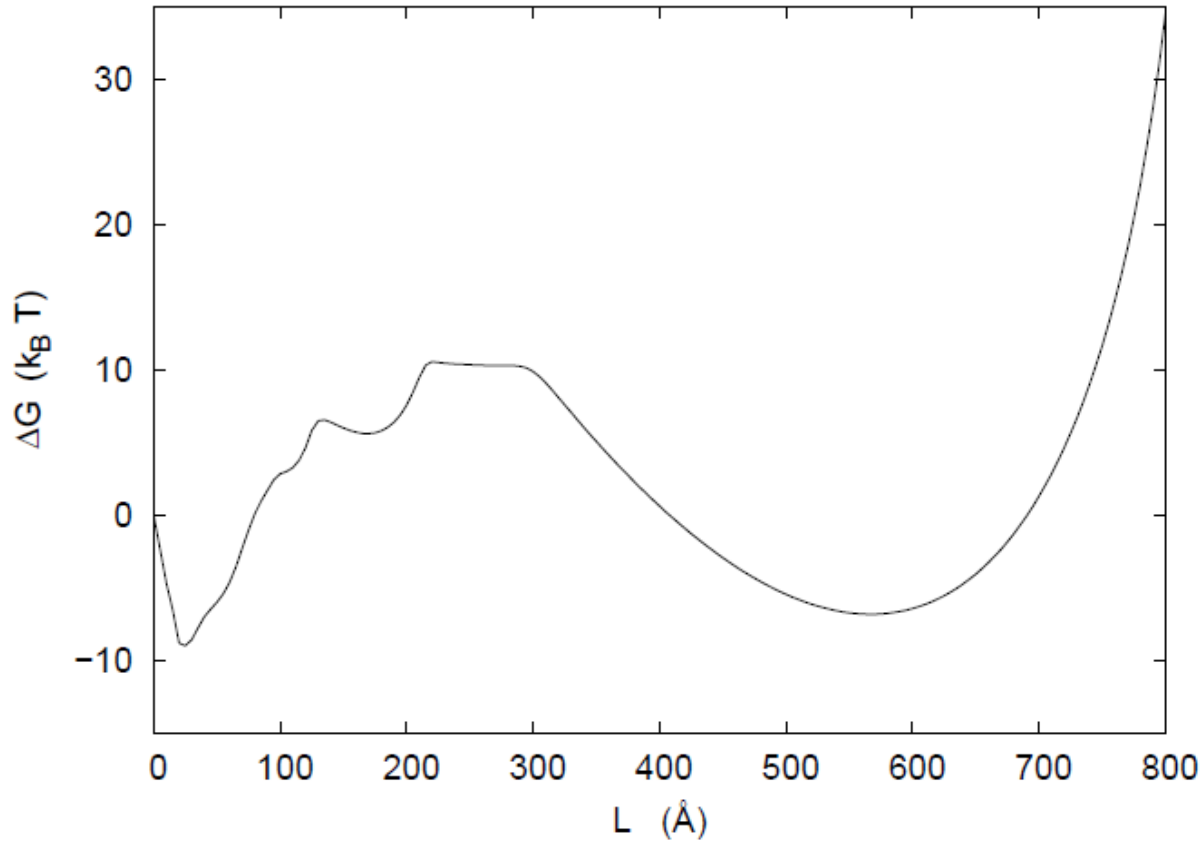
Pulling a protein from different directions



Experiments: pulling GFP from different directions (Reif et al, PNAS '06)



Model: landscape (at equilibrium unfolding f)

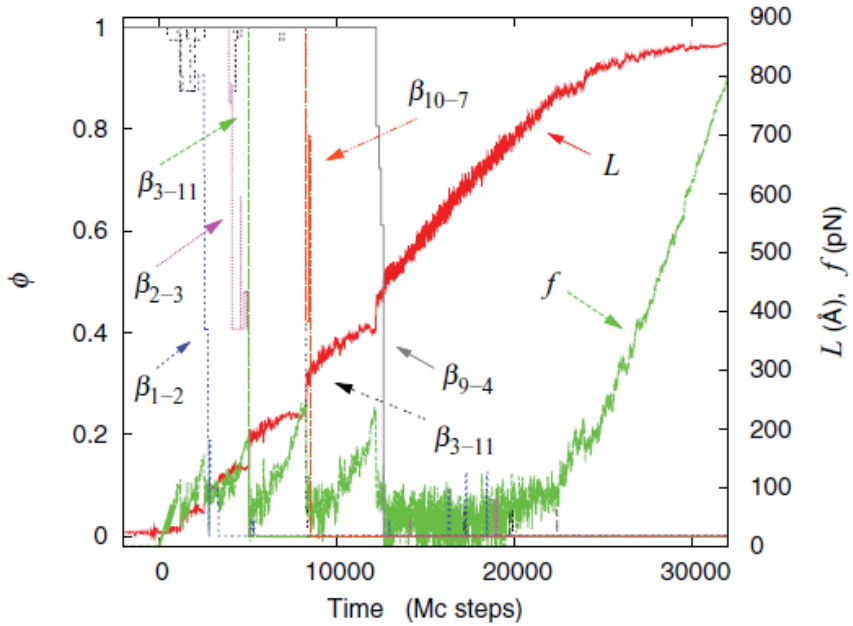


Intermediates: β_1 and β_{11} ($\sim$ 110 Å), $\beta_{10}\beta_{11}$ ($\sim$ 180 Å), $\beta_1\beta_2\beta_3$ ($\sim$ 250 Å)

[A. Imparato, A. P. and M. Zamparo, Phys. Rev. E **84**, 021918 (2011)]

Model: pulling end–to–end

Major unfolding pathway

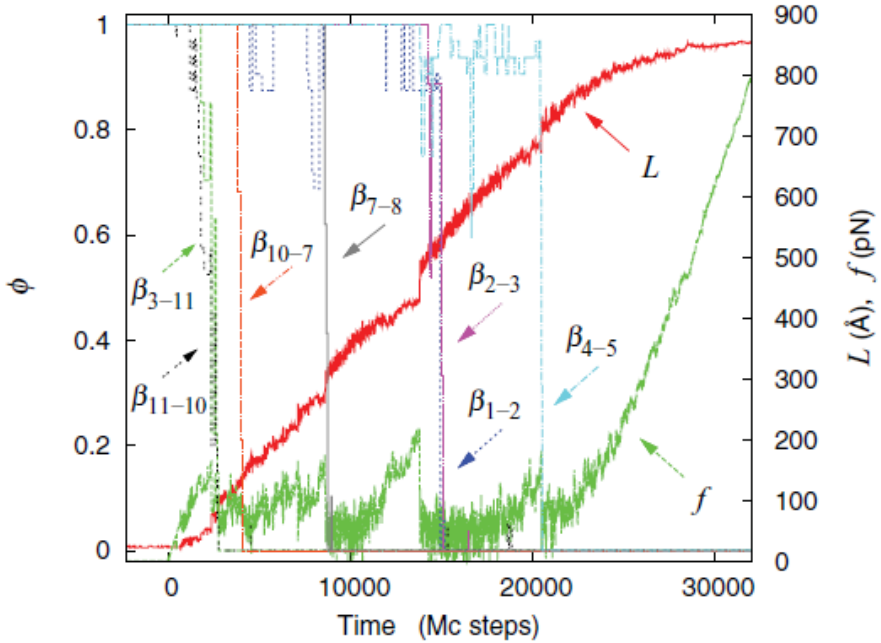


Order of unfolding events

- N–terminal α–helix (small signal)
- β_1
- $\beta_2\beta_3$
- $\beta_{10}\beta_{11}$
- all the rest

Model: pulling end–to–end

Minor unfolding pathway



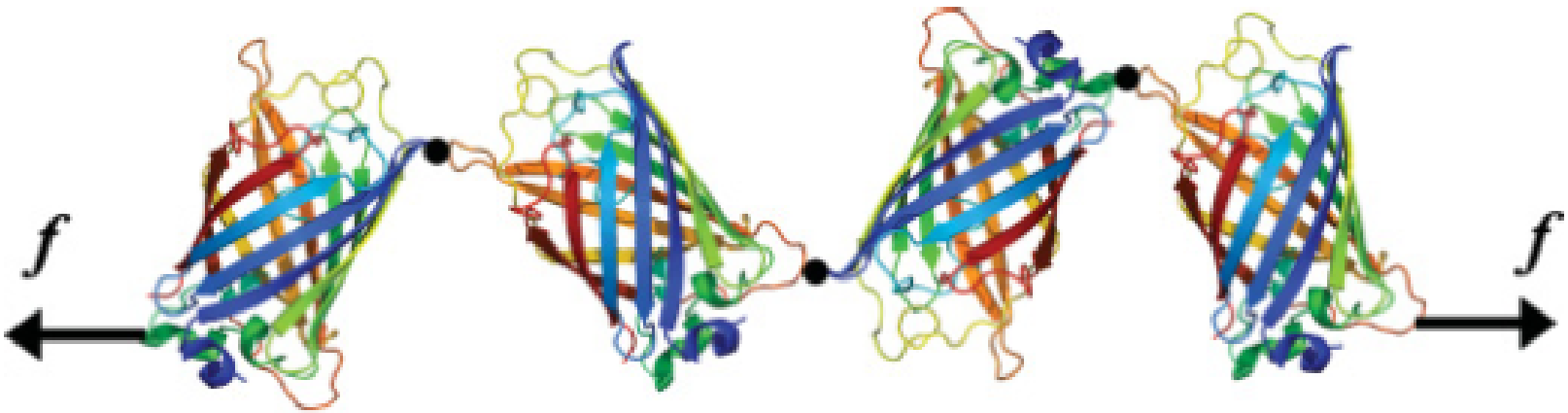
Order of unfolding events

- N–terminal α–helix (small signal)
- β_{11}
- ...

Model: pulling from different directions

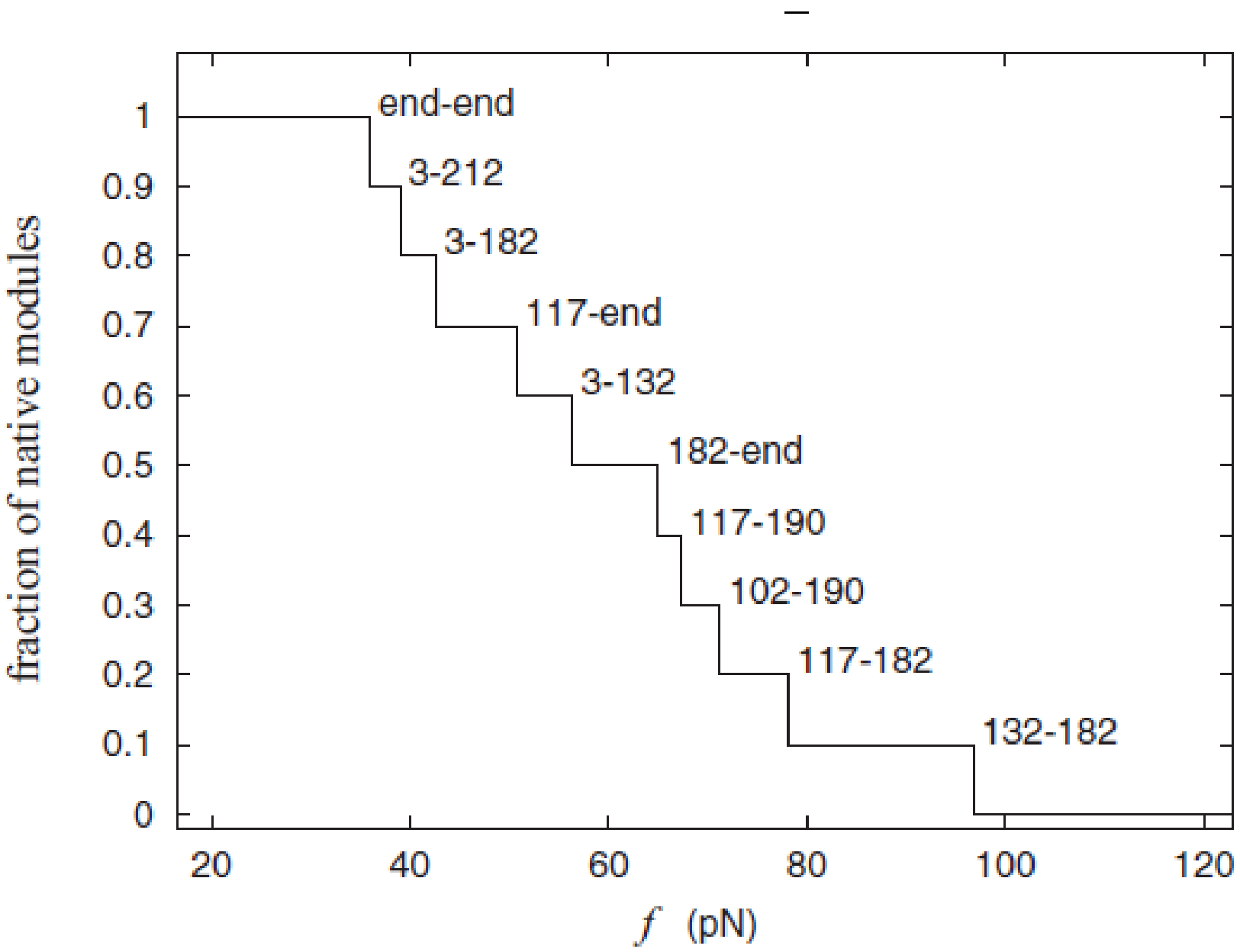
Direction	Unfolding force (pN)		
	$v = 0.3\ \mu$m/s	$v = 2\ \mu$m/s	$v = 3.6\ \mu$m/s
end-end	140 ± 3 (104 ± 40)[a]	177 ± 7	184 ± 13
182-end	196 ± 7	226 ± 6	244 ± 7
3–212	244 ± 12	298 ± 12	317 ± 20 (117 ± 19)[b]
132–212	251 ± 7	266 ± 3	273 ± 6 (127 ± 23)[b]
132-end	306 ± 12	360 ± 20	381 ± 26
182–212	365 ± 2	390 ± 7	409 ± 15 (356 ± 61)[b]
3–132	383 ± 16	471 ± 49 (346 ± 46)[b]	535 ± 80
117–182	467 ± 3	501 ± 11	512 ± 11 (548 ± 57)[b]

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:

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- M. Caraglio, A. Imparato and A. P., Phys. Rev. E **84**, 021918 (2011).

Thanks for your attention