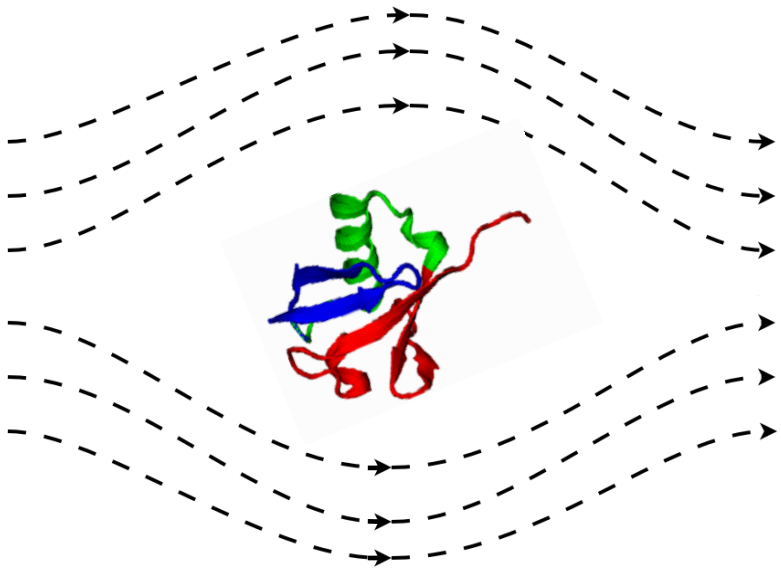
Theoretical models of protein dynamics in hydrodynamic flows



Piotr Szymczak

University of Warsaw

INNOVATIVE ECONOMY
NATIONAL COHESION STRATEGY

FNP
Foundation for Polish Science

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND

Outline:

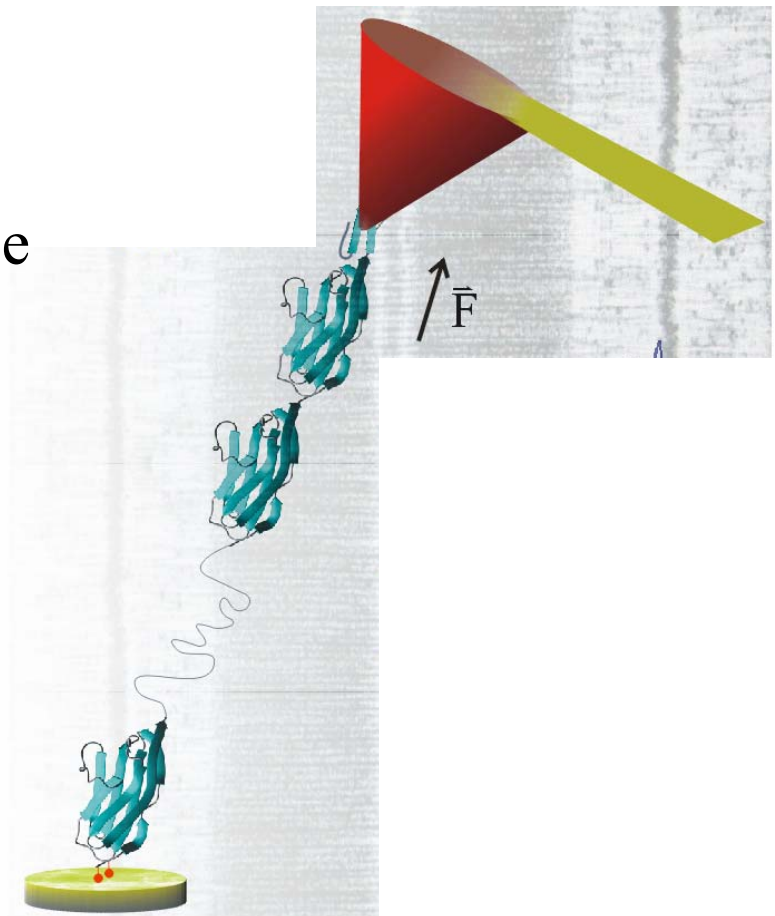
- Stretching of proteins
- Coarse grained protein model
- Stretching of proteins by a fluid flow
- Influence of hydrodynamic interactions on protein stretching

Collaborator:

- Marek Cieplak (Polish Academy of Sciences)

Mechanical stretching of proteins

Stretching of single protein molecules using atomic force microscope or optical tweezers allows us to study the conformational changes under mechanical force, identify the strongest bonds in the structure, etc.



Protein stretching (AFM)

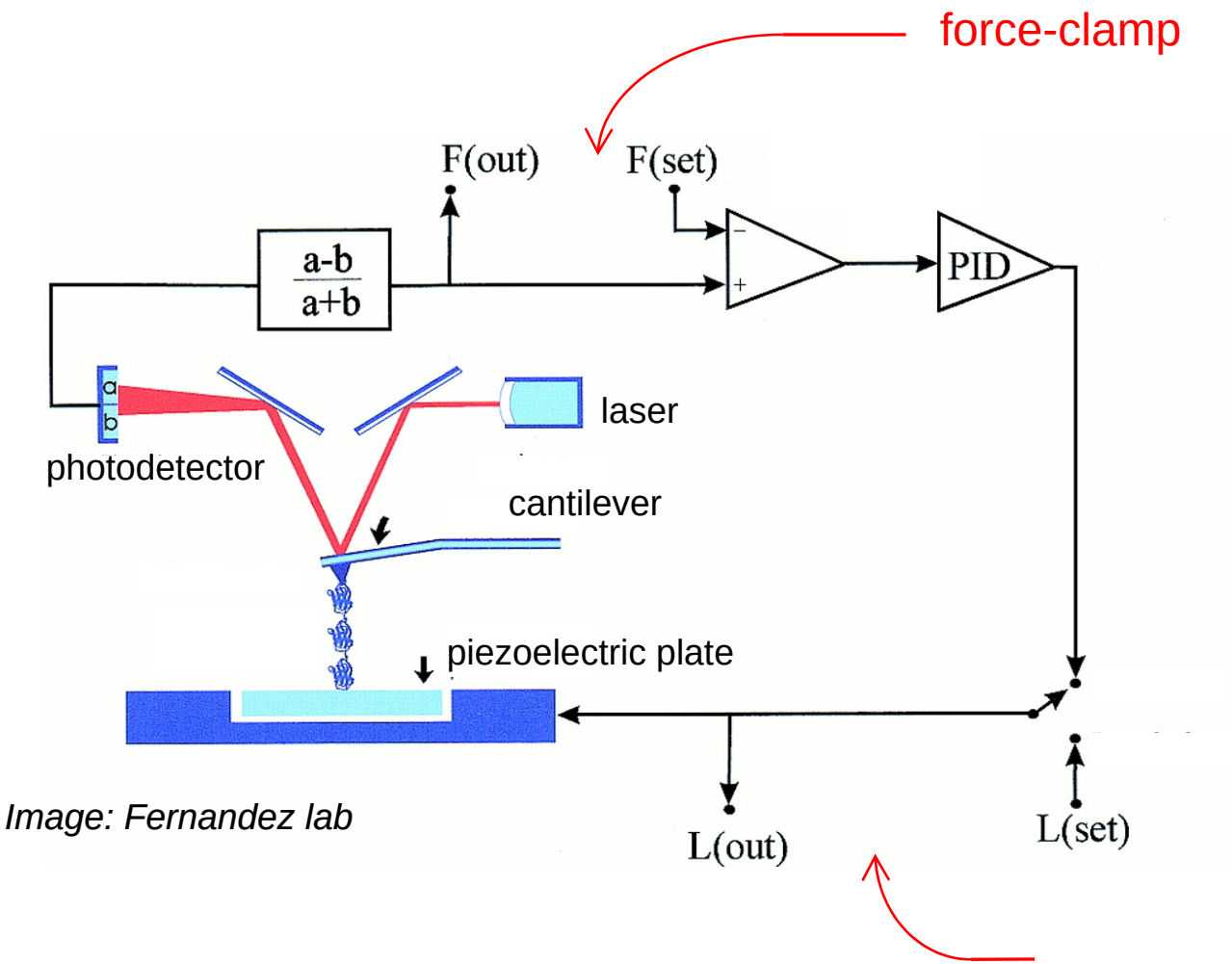
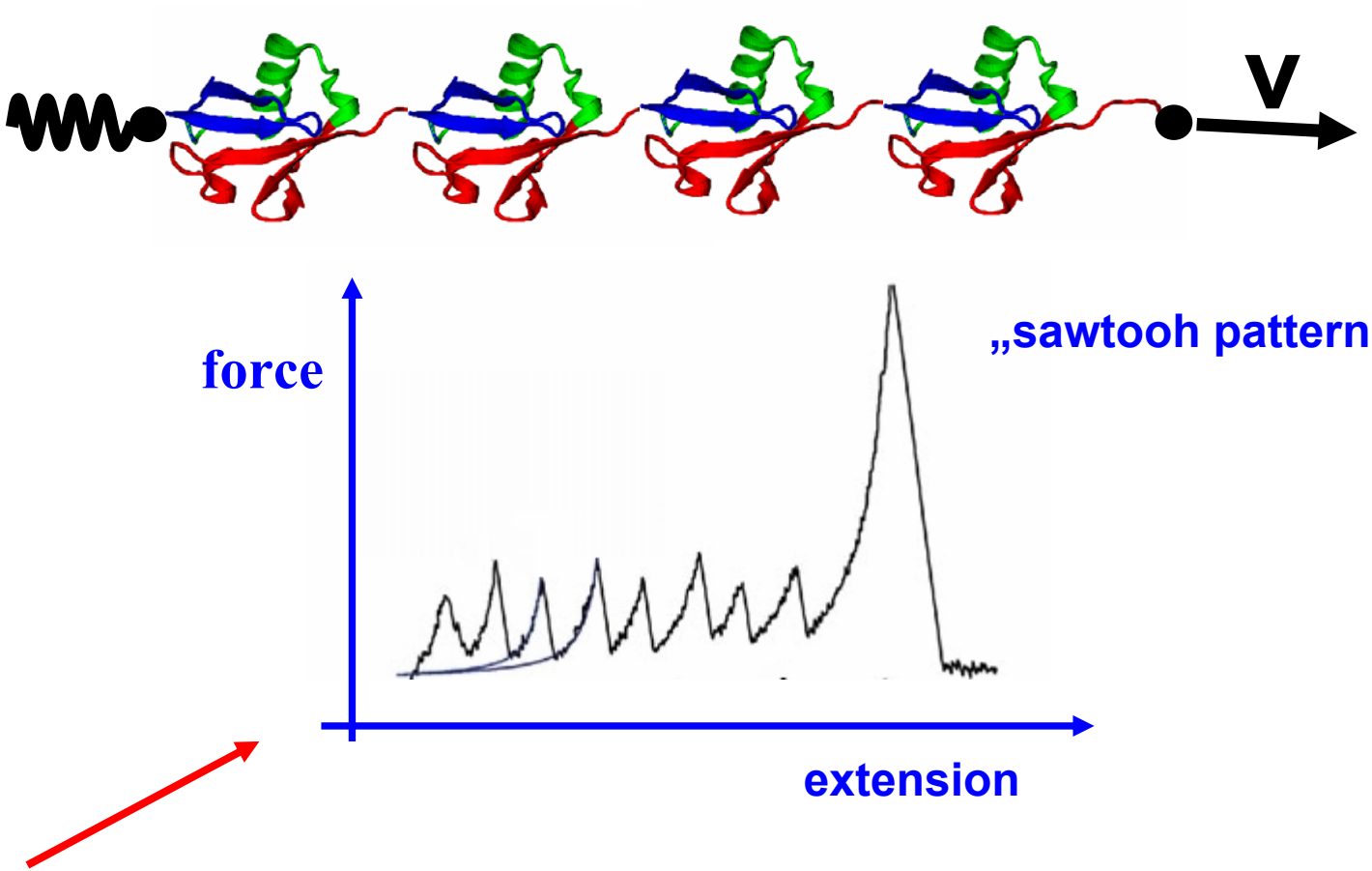


Image: Fernandez lab

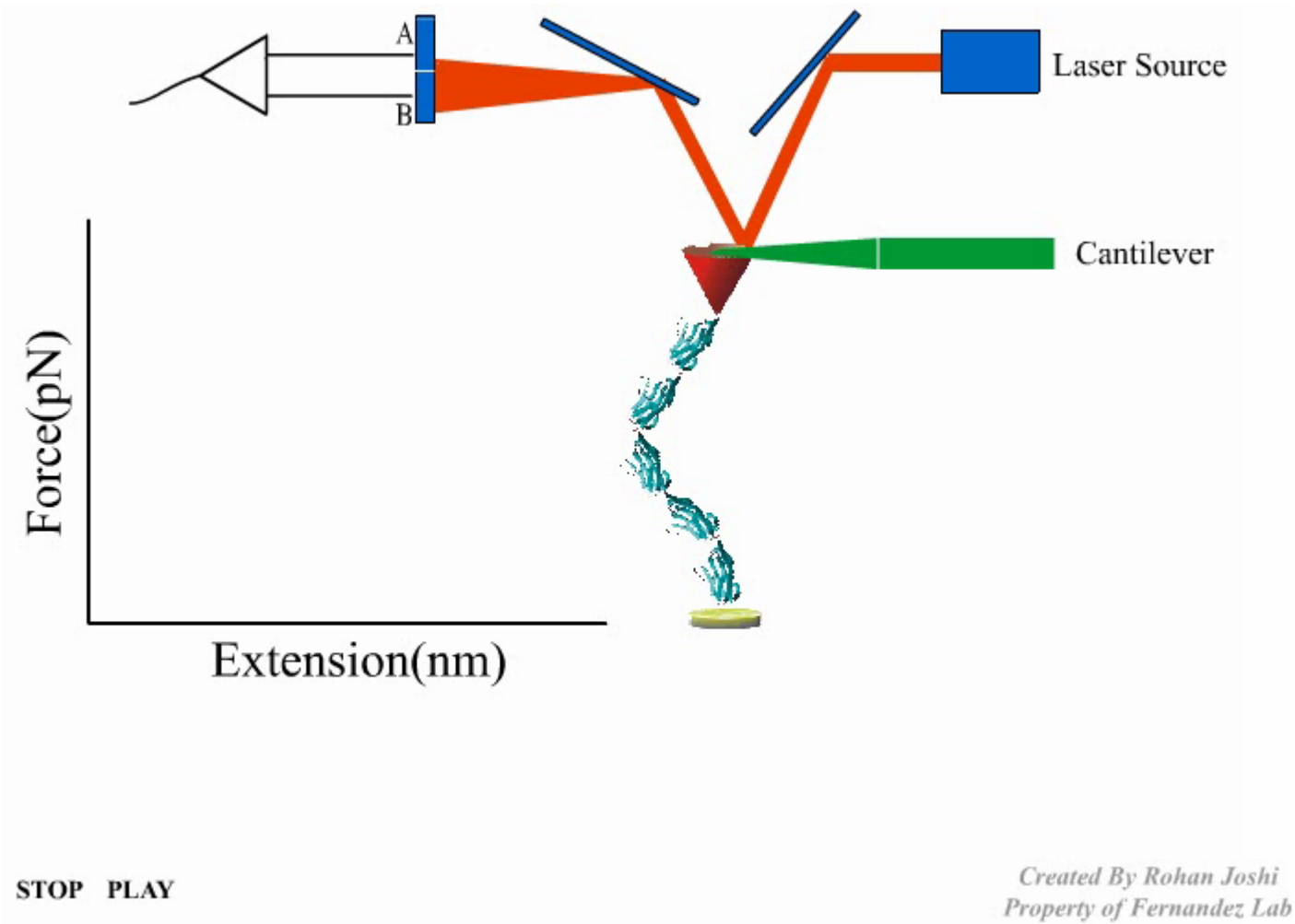
Constant velocity



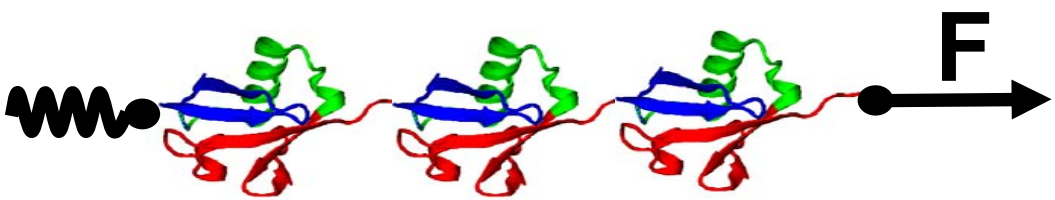
ubiquitin chain:

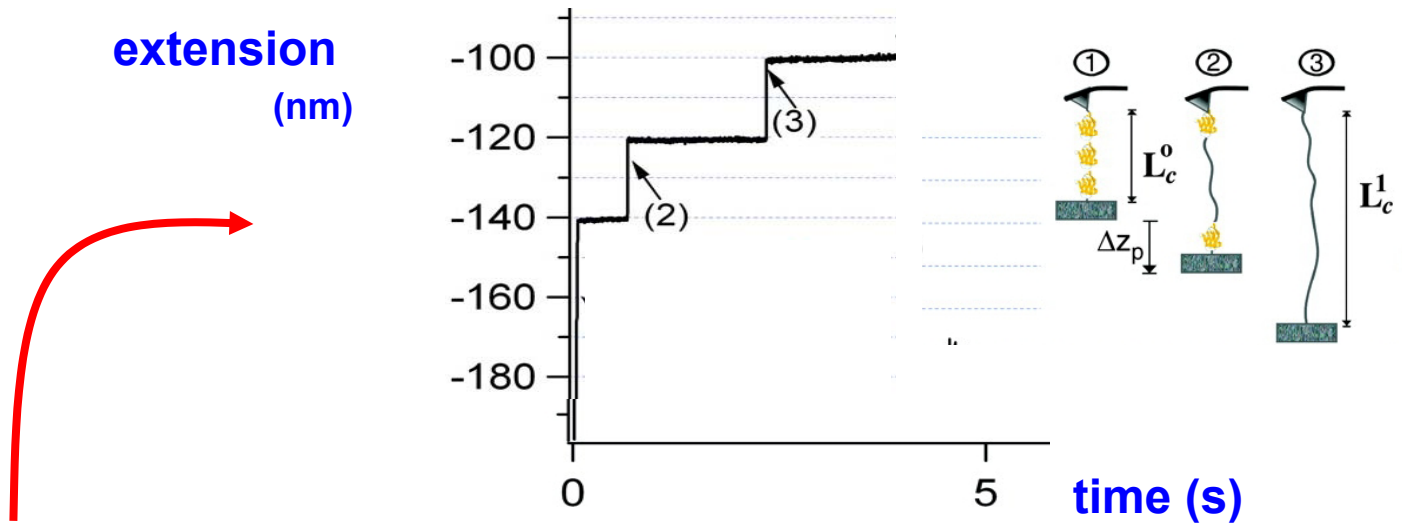
Carrion-Vazquez et al, Nature Struct. Biol. **10**, 738 (2003)

Constant velocity



Force clamp



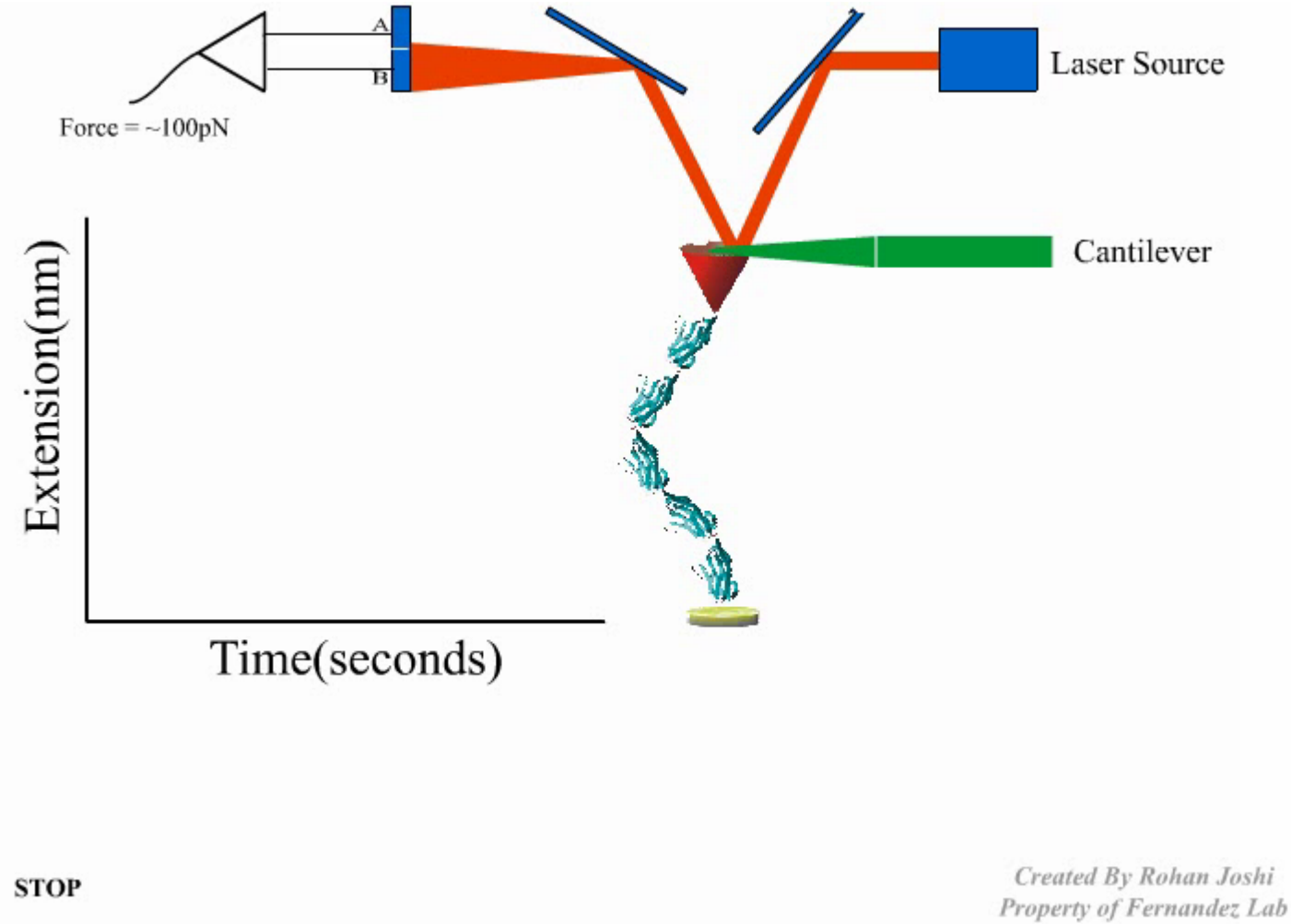


ubiquitin chain:

Fernandez and Li,

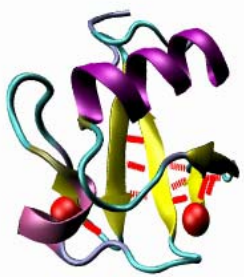
Science **303**, 1674 (2004)

Force clamp

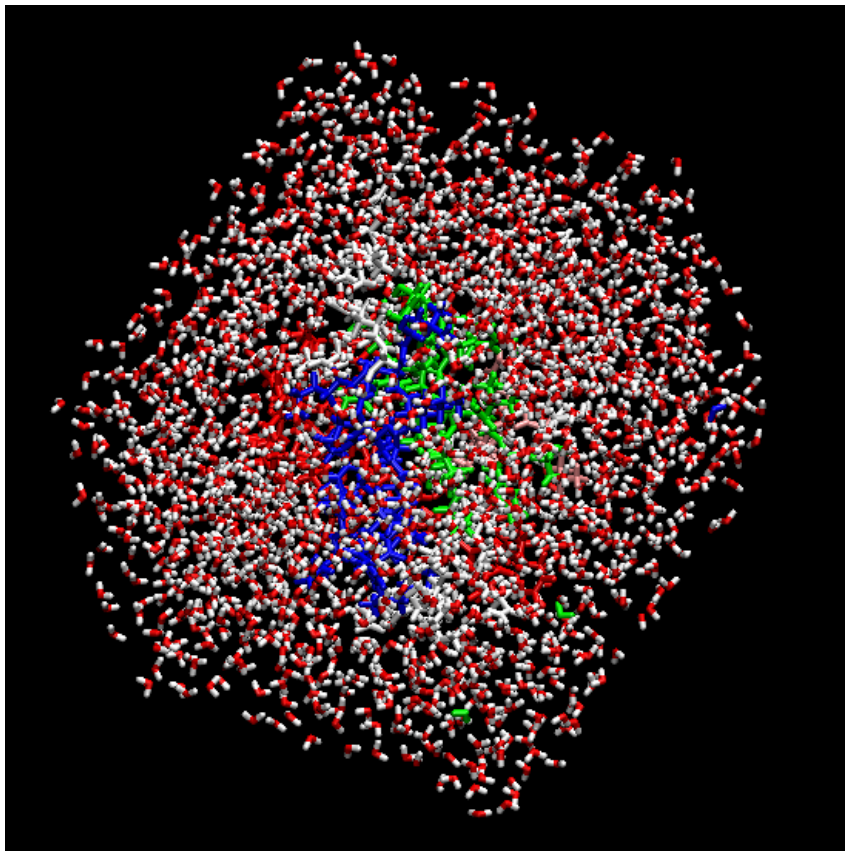

A
B
Force = ~100pN
Laser Source
Cantilever
Extension(nm)
Time(seconds)
STOP
Created By Rohan Joshi
Property of Fernandez Lab

Importance of numerical models:

- experiments give limited information on the unfolding process (end-to-end distance, resistance force, etc.)
- numerical modeling allows to relate the characteristics of individual trajectories to the microscopic events during folding

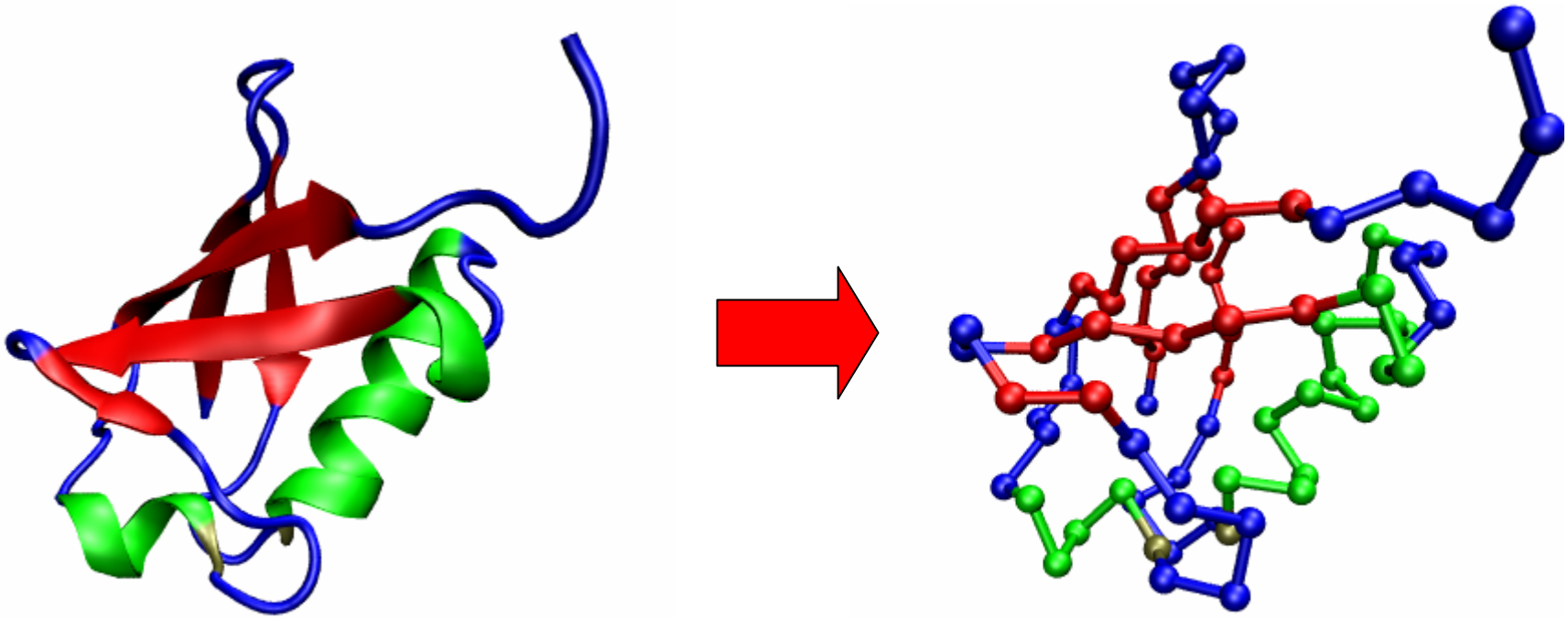


All-atom models



- most exact and reliable, but highly expensive numerically:
- accessible timescales are much shorter than those probed experimentally (5-7 orders of magnitude)
- hard to obtain statistically meaningful results and explore a wide range of forces

Coarse-grained model of a protein

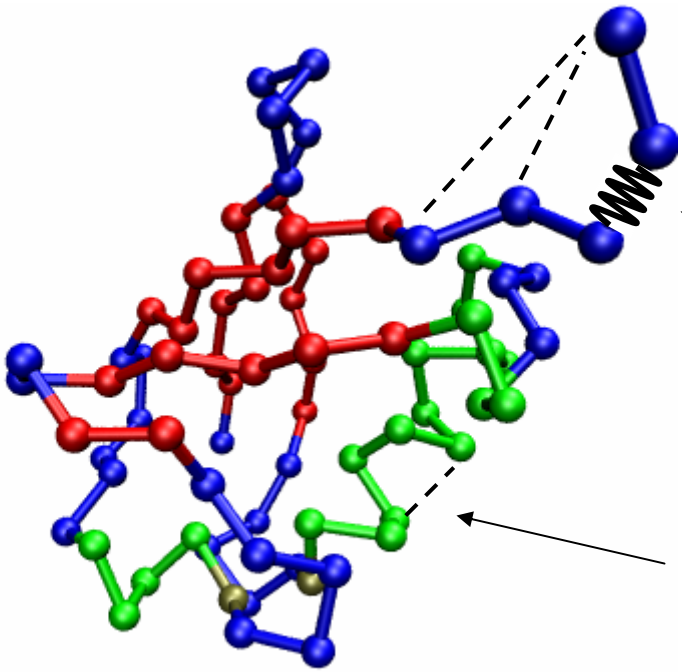


- reduction of the number of degrees of freedom
- effective interactions

Go models – constructed from the explicit structure of protein native state

H. Abe and N. Go, Biopolymers **20**, 1013 (1981)

Coarse-grained model - details

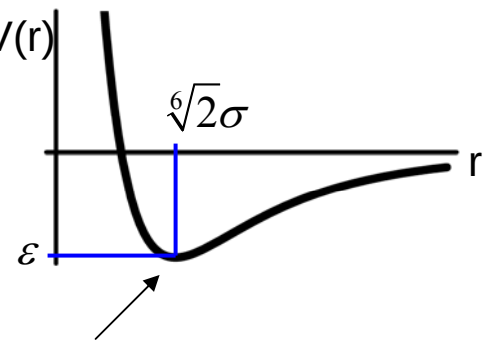


each amino acid residue replaced by one "bead"

stiff harmonic springs representing the peptide bonds

Lennard-Jones forces representing inter-amino acid contacts

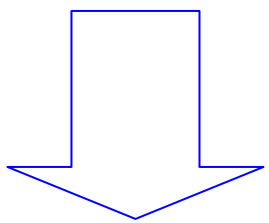
$$V_{ij}(r) = 4\varepsilon\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]$$

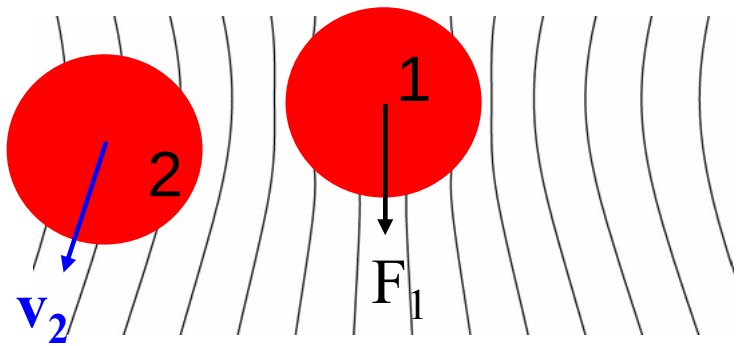


minimum corresponding to the native distance

Hydrodynamic interactions

- proteins are surrounded by a water environment
- each amino acid moves in the flow field created by the other





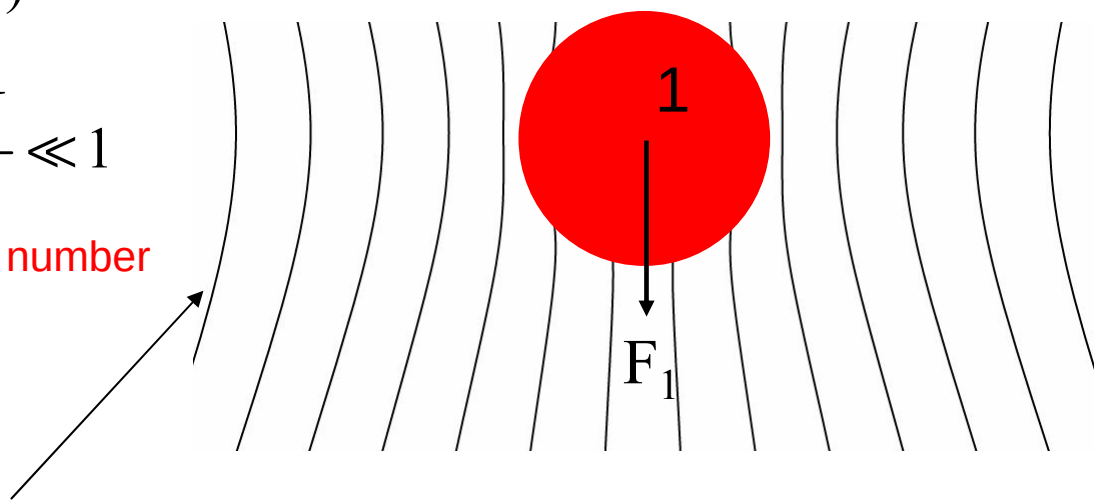
hydrodynamic interactions

Single particle

a single particle moving under the influence of an external force (e.g. gravity)

$$\mathrm{Re} = \frac{\rho a U}{\eta} \ll 1$$

(low Reynolds number regime)



$$v = \frac{F}{\zeta} = \frac{F}{6\pi\eta a}$$

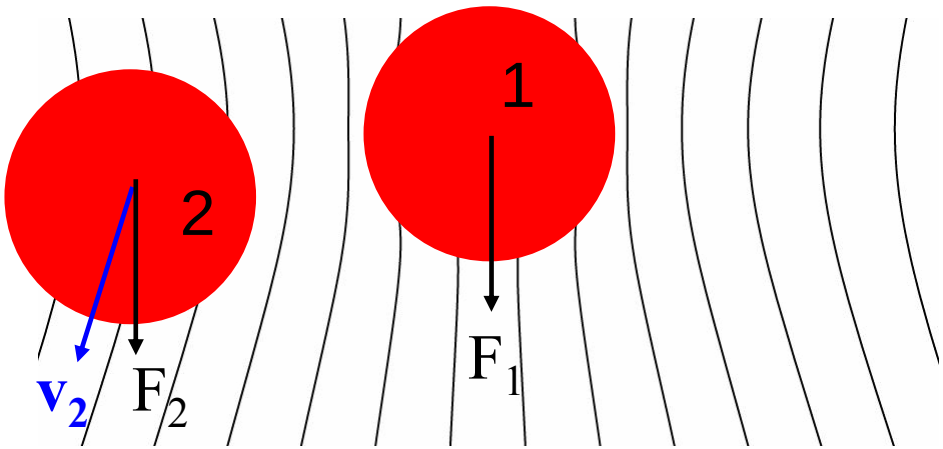
flow disturbance caused by the sphere :

$$\mathbf{u}(\mathbf{r}) = \frac{\mathbf{F}}{6\pi\eta a}\left(\frac{3a}{4r} + \frac{a^3}{4r^3}\right) + \mathbf{r}\frac{\mathbf{F}\cdot\mathbf{r}}{6\pi\eta a}\left(\frac{3a}{4r} - \frac{3a^3}{4r^3}\right)$$

long-ranged (1/r)!

Stokes (1851)

Two particles



Due to the flow from particle 1, the velocity of particle 2 is enhanced with respect to the single particle velocity:

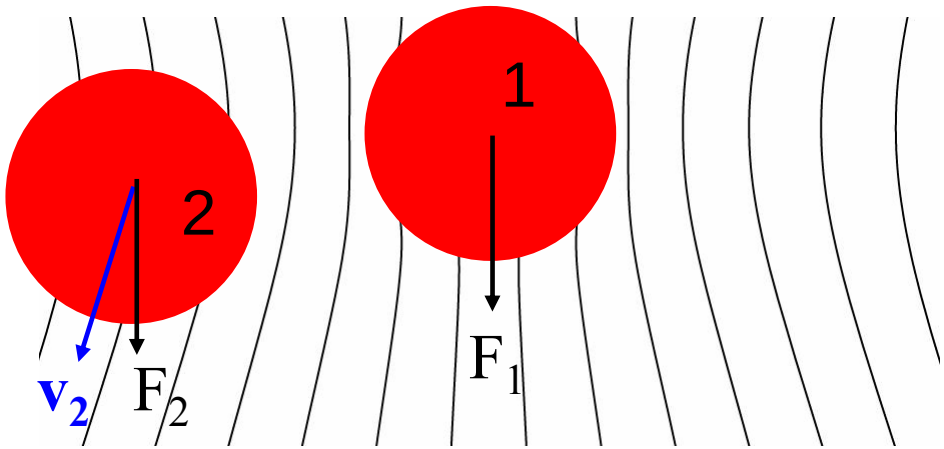
$$\mathbf{v}_2(\mathbf{r}_{12}) = \frac{\mathbf{F}_2}{6\pi\eta a} + \frac{3a}{4r_{12}}(1+\hat{\mathbf{r}}_{12}\hat{\mathbf{r}}_{12})\cdot\frac{\mathbf{F}_1}{6\pi\eta a} + \ldots.$$

higher order terms

single-particle term

hydrodynamic interaction (contribution to velocity of 2 due to the force on 1)

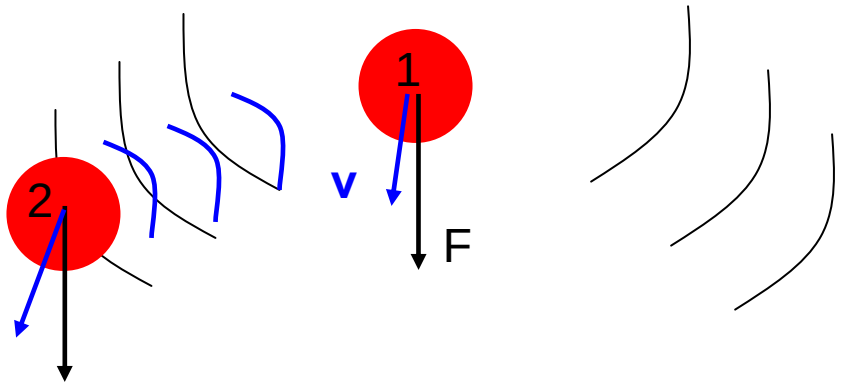
Oseen tensor



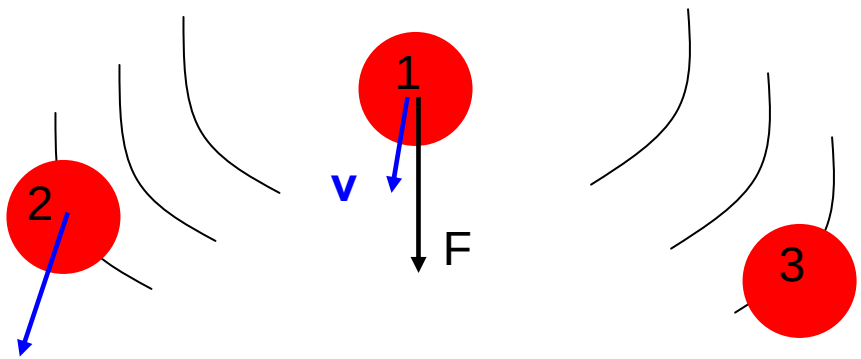
$$\mathbf{v}_2(\mathbf{r}) = \frac{\mathbf{F}_2}{6\pi\eta a} + \frac{3a}{4r_{12}}(1 + \hat{\mathbf{r}}_{12}\hat{\mathbf{r}}_{12}) \cdot \frac{\mathbf{F}_1}{6\pi\eta a} =$$

$$= \frac{\mathbf{F}_2}{6\pi\eta a} + \underbrace{\frac{1}{8\pi\eta r_{12}}(\mathbf{1} + \hat{\mathbf{r}}_{12}\hat{\mathbf{r}}_{12})}_{\text{Oseen tensor, } \mathbf{T}(\mathbf{r}_{12})} \cdot \mathbf{F}_1$$

Higher order terms



Multiple reflections: The flow generated by particle 2 influences 1, which in turn influences 2...



Many-body effects: hydrodynamic interactions between two particles are modified by the presence of the third one

N particles - mobility matrix

$$\mathbf{v}_i = \sum_{j=1}^{N} \boldsymbol{\mu}_{ij} \cdot \mathbf{F}_j$$

mobility matrix

The Oseen approximation:

$$\boldsymbol{\mu}_{ij} = \delta_{ij} \frac{1}{6\pi\eta a}\mathbf{1} + (1-\delta_{ij}) \frac{1}{8\pi\eta r_{ij}} (\mathbf{1} + \hat{\mathbf{r}}_{ij}\hat{\mathbf{r}}_{ij})$$

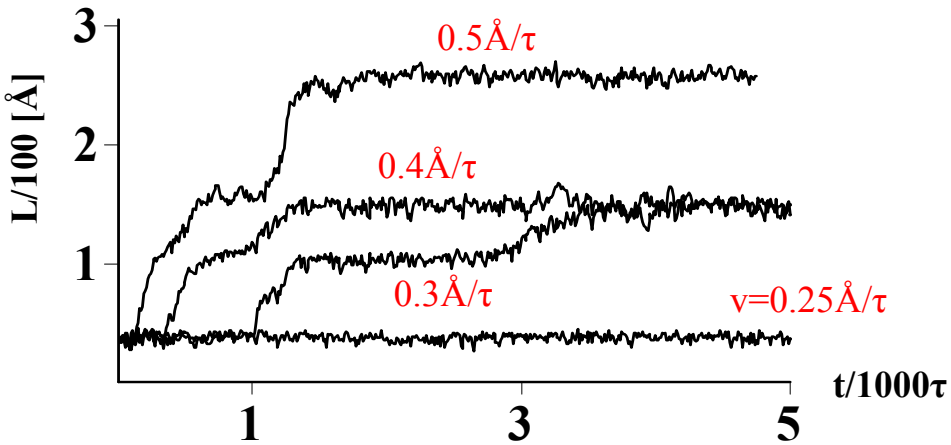
The Rotne-Prager approximation:

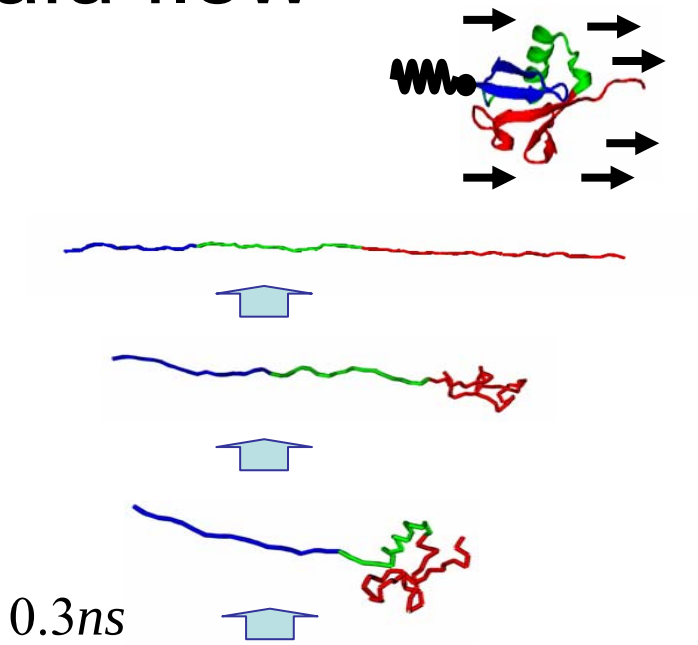
$$\boldsymbol{\mu}_{ij} = \delta_{ij} \frac{1}{6\pi\eta a}\mathbf{1} + (1-\delta_{ij}) \frac{1}{8\pi\eta r_{ij}} \left[(\mathbf{1} + \hat{\mathbf{r}}_{ij}\hat{\mathbf{r}}_{ij}) + \frac{2a_{ij}^2}{r_{ij}^2} \left(\frac{1}{3}\mathbf{1} - \hat{\mathbf{r}}_{ij}\hat{\mathbf{r}}_{ij} \right) \right]$$

The derivation of full hydrodynamic interactions, including many-body terms is possible but much more expensive numerically (Felderhof, Cichocki, Brady)

Brownian dynamics simulation of protein stretching by a fluid flow

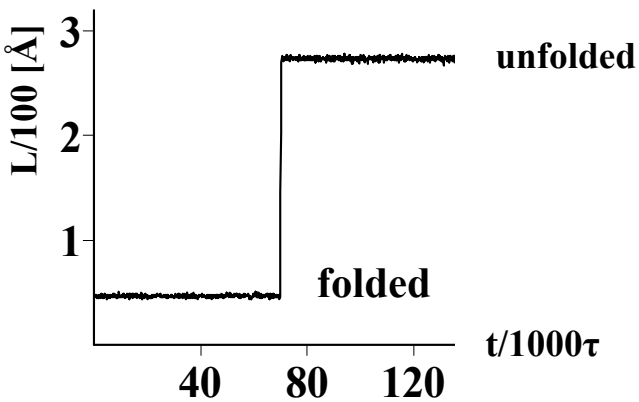
ubiquitin in a uniform flow (v=const.)





$$\tau = \frac{a^2}{D} \approx 0.3ns$$

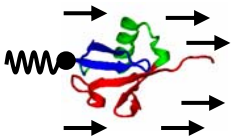
ubiquitin in a force clamp (F=const.)



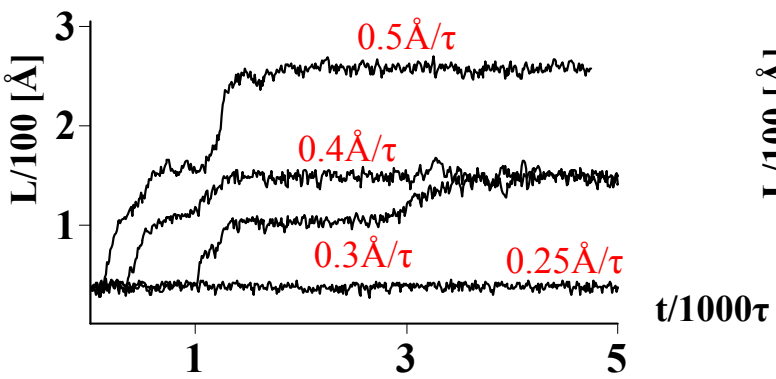
In contrast to force clamp, ubiquitin unfolding in a flow is a multi-step process, involving several intermediate states.

Szymczak, Cieplak, JCP, 125, 164903, 2006

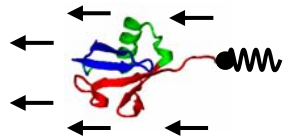
Asymmetry between the ends

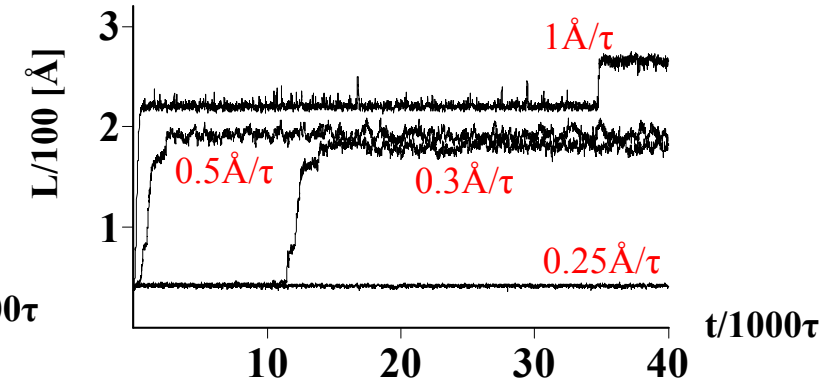


N terminus fixed



C terminus fixed





- **C terminus anchored ≠ N terminus anchored!**

- different unfolding times

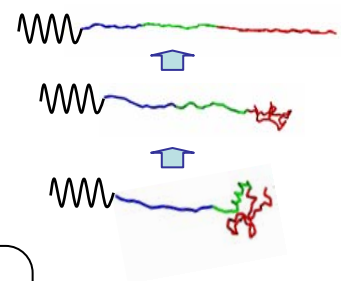
- different set of intermediates

- velocities needed for a full unfolding of ubiquitin (v=0.5Å/τ) correspond to about 15 cm/s.

large velocities (approx 1000 times larger than those used for DNA stretching)!

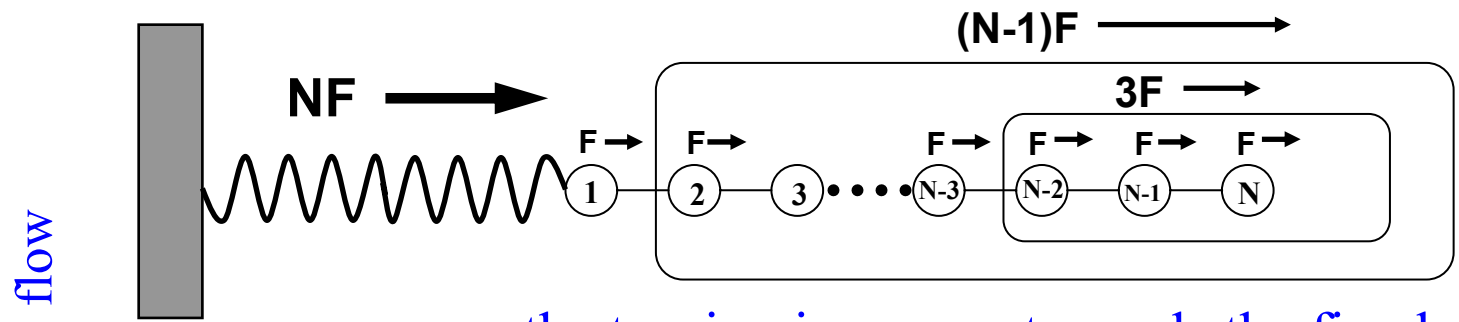
Reasons for N-C asymmetry

for the protein in a flow, the tension is nonuniform along the chain and depends on the tethering point.

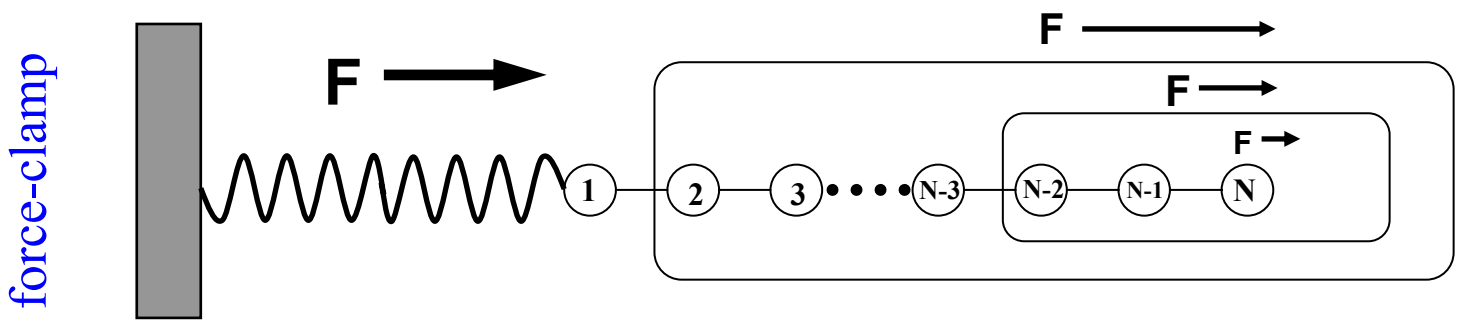


$F = -\gamma v$

example: linear chain

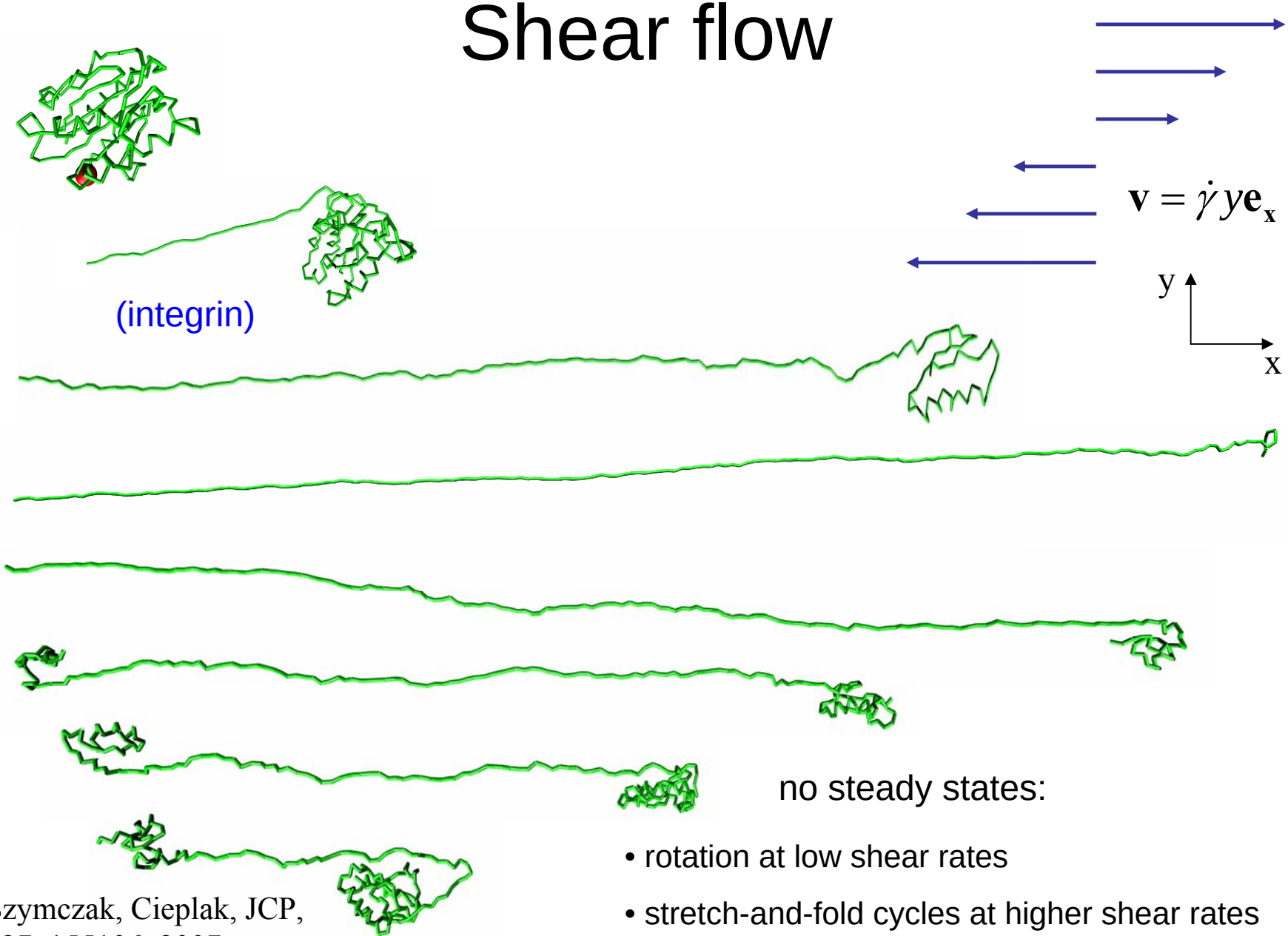


the tension increases towards the fixed end



the tension is constant along the chain

Shear flow



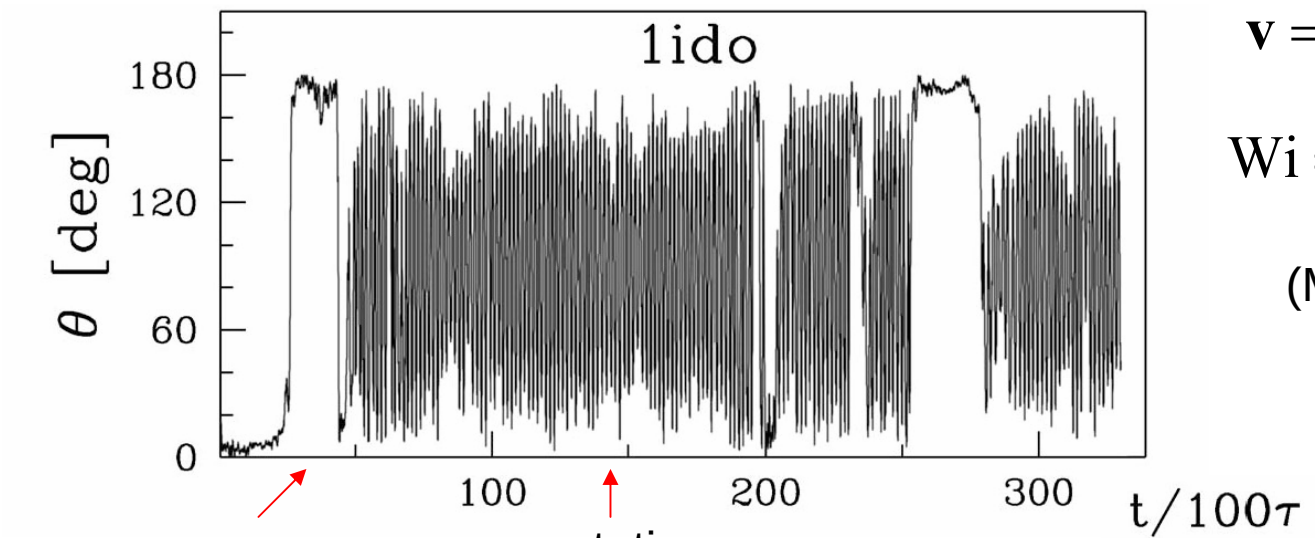
no steady states:

- rotation at low shear rates
- stretch-and-fold cycles at higher shear rates

Szymczak, Cieplak, JCP, 127, 155106, 2007

Tumbling at moderate shear

Time-dependence of the angle between the end-to-end direction of the protein and the direction of the flow



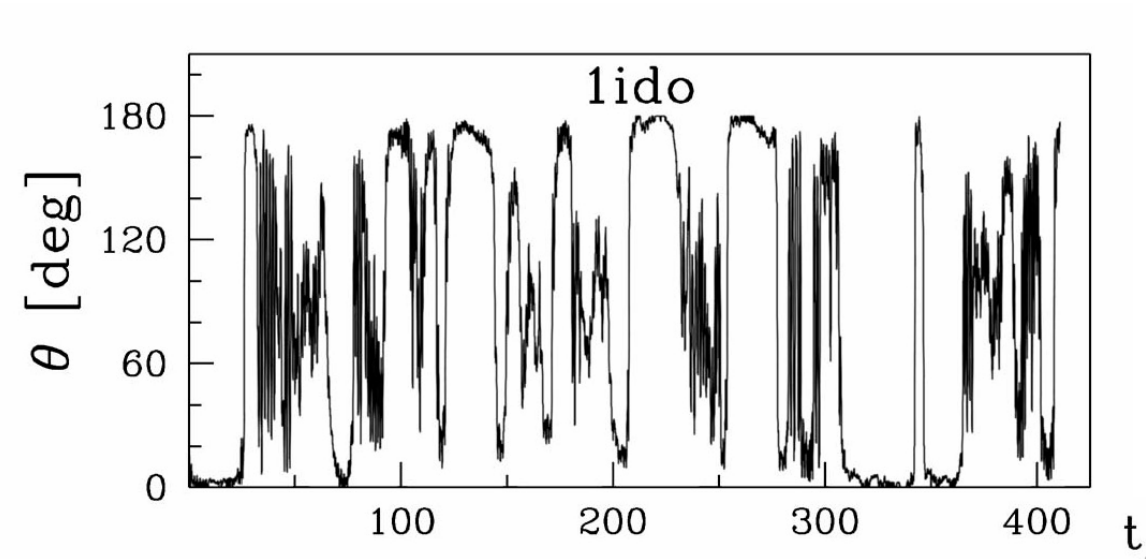
$$\mathbf{v} = \dot{\gamma} y \mathbf{e}_x$$

$$\mathrm{Wi} = \dot{\gamma} t_{fold} = 600$$

(Moderate flow)

Tumbling at stronger shear

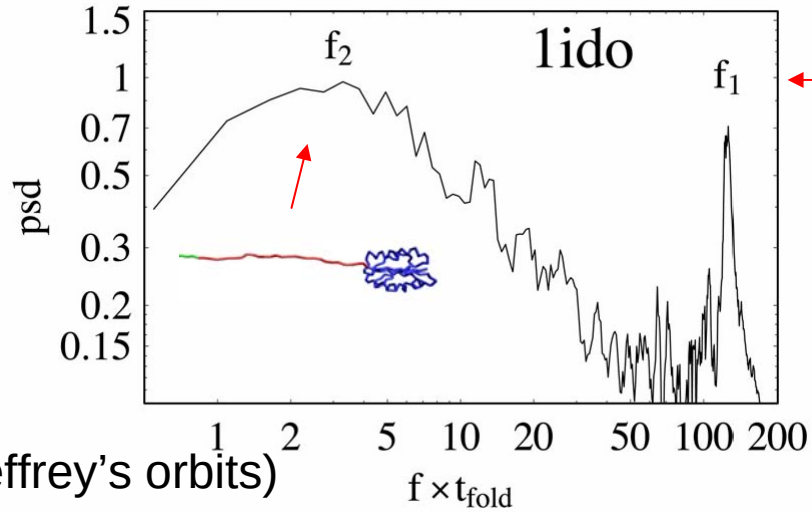
Time-dependence of the angle between the end-to-end direction of the protein and the direction of the flow

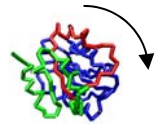


$$\mathrm{Wi} = \dot{\gamma} t_{fold} = 1000$$

(Stronger flow)

Power spectrum: two frequencies

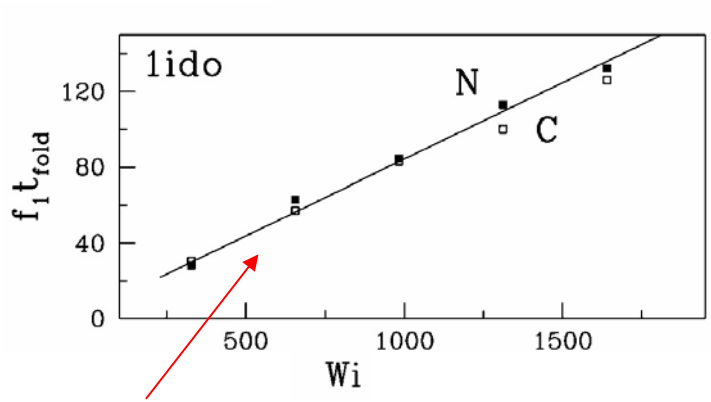




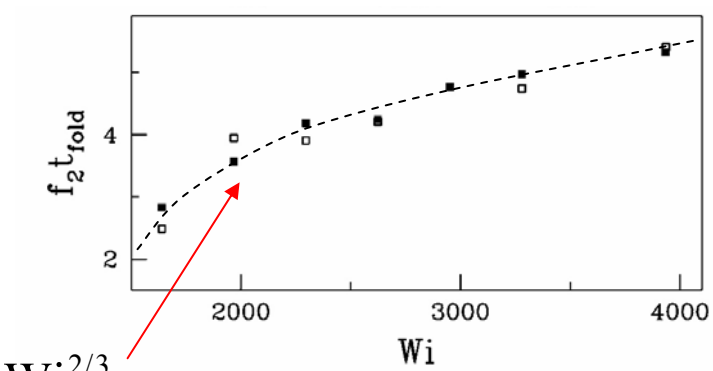
$\left(\mathrm{Wi} = \dot{\gamma} t_{fold} = 1600\right)$

(rotation - Jeffrey's orbits)

(stretch-collapse cycles)



$f_1 t_{fold} = \mathrm{Wi}/4\pi$

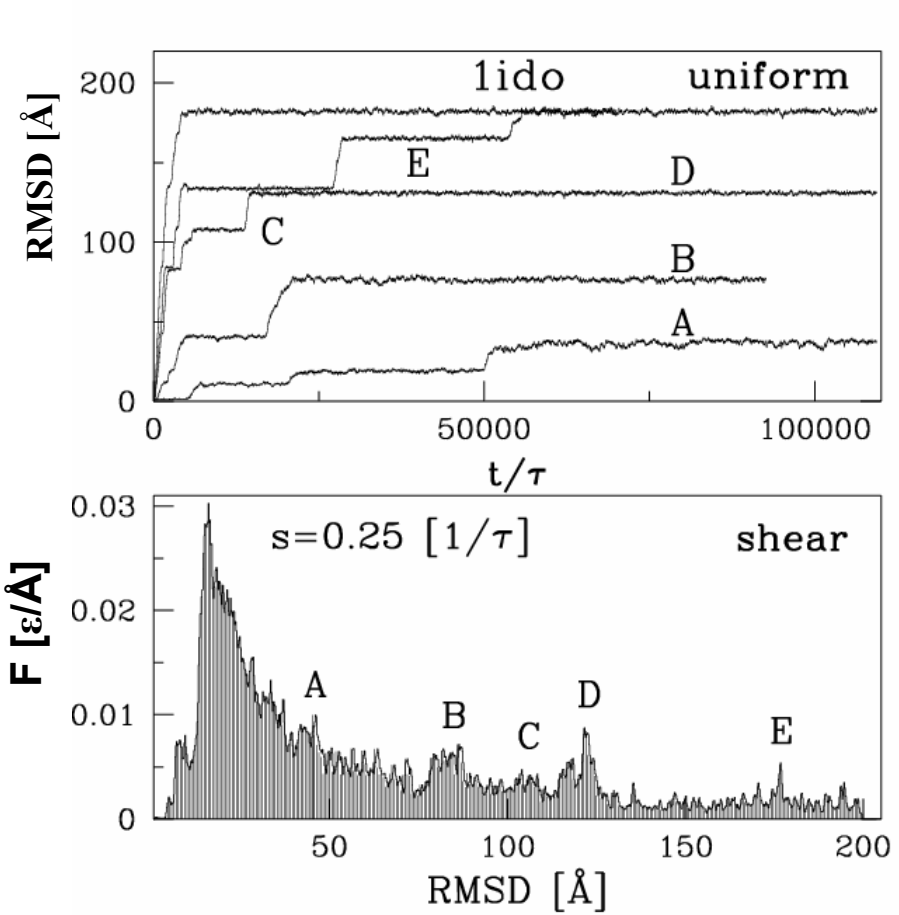


$f_2 t_{fold} \sim \mathrm{Wi}^{2/3}$

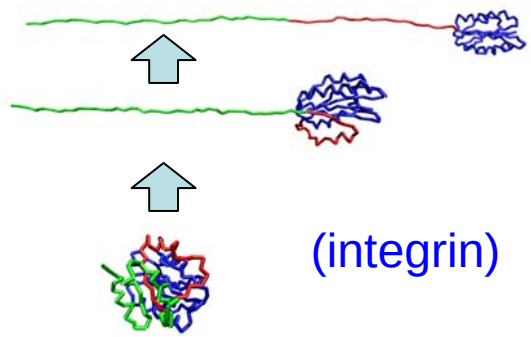
similar behavior in DNA:

Schroeder et al., *PRL*, **95** 018301, 2005

Uniform flow vs shear flow



(RMSD from the native structure)



- fingerprints of the underlying set of intermediate states are observed in RMSD histogram

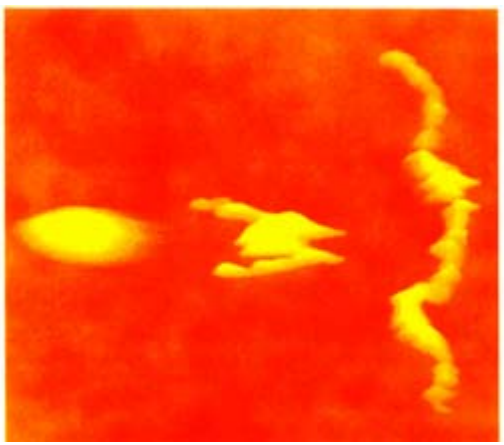
- however, in a shear flow those states are never long lived, as even a small thermal fluctuation moves the protein to the region of smaller (larger) flow and the molecule collapses (stretches)

Experiments

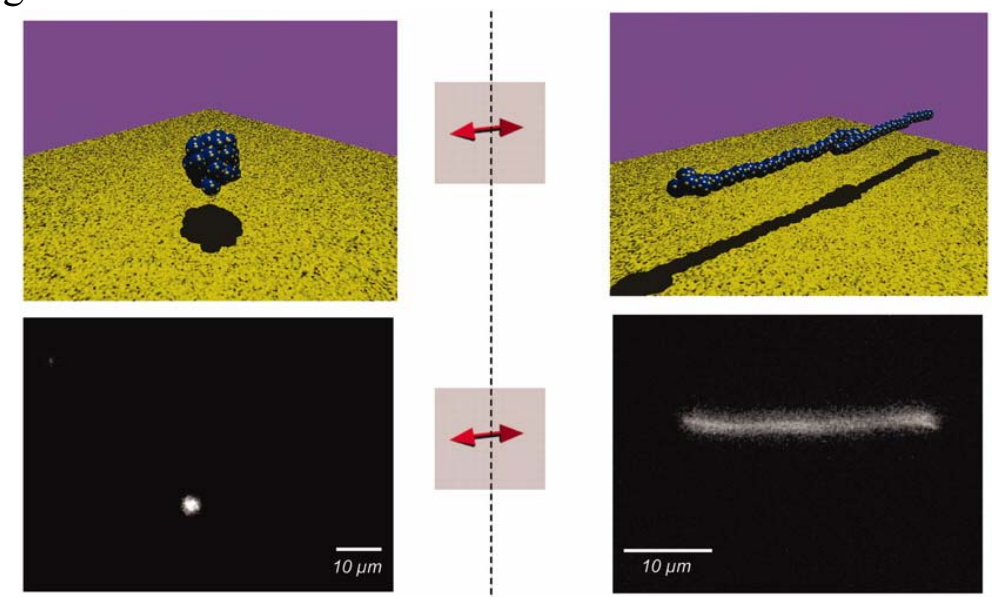
• Jaspe and Hagen „Do protein molecules unfold in a simple shear flow?" *Biophys. J.*, **91**, 3415, 2006: experiments on horse cytochrome c unfolding show that shear flows of $\dot{\gamma}=10^5 s^{-1}$ (Wi=10) are unable to destabilize this protein, the authors estimate the threshold unfolding rate at $Wi=10^3$

• Ashton et al. "Shear-induced unfolding of lysozyme monitored *in situ*", *Biophys. J.* **96** 4231, 2009: lysozyme unfolds at Wi=15

• a number of experiments showing the stretching of von Willebrand factor – large, multidomain protein playing a major role in blood coagulation:



Siedlecki et. al, *Blood*, **88**, 2939 (1996)

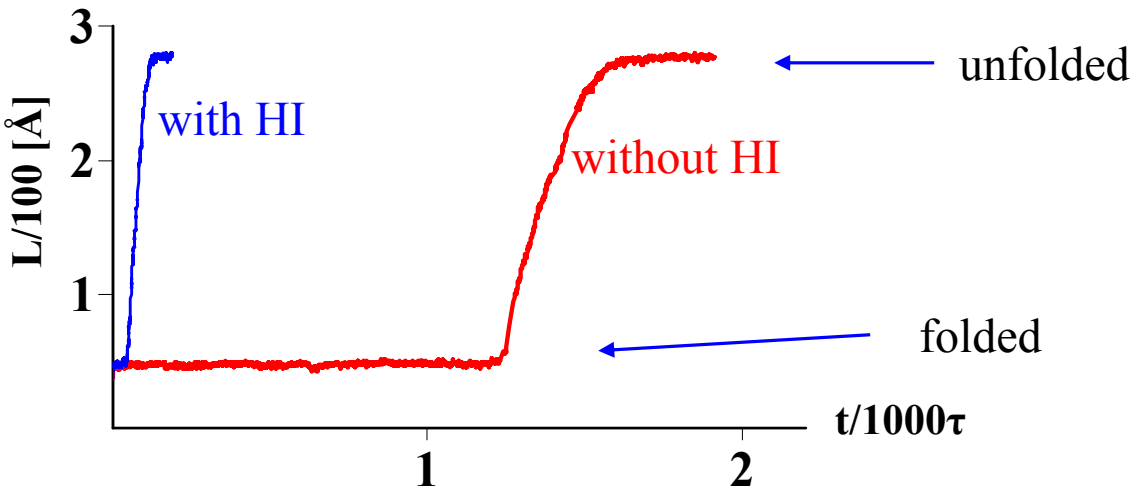


Schneider et al., *PNAS*, **104**, 7899, (2007)

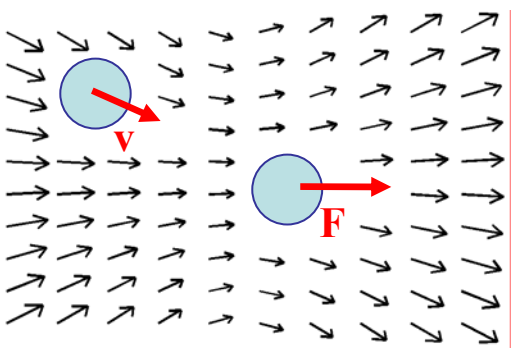
Influence of hydrodynamic interactions on protein unfolding

- constant force (force-clamp)
- stretching by a fluid flow

Constant force stretching

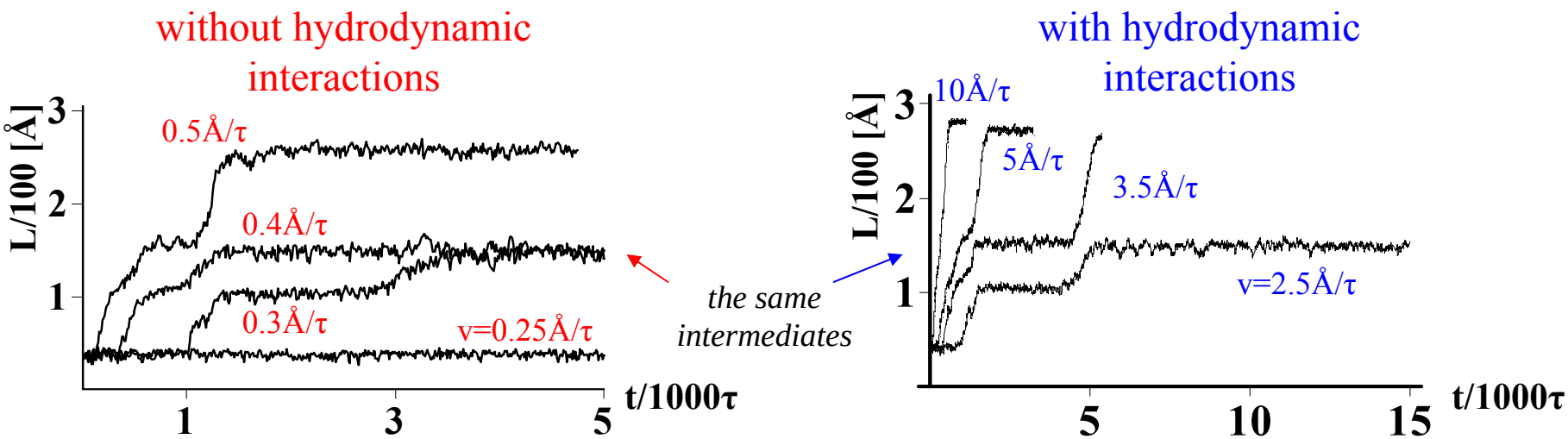


Hydrodynamic interactions considerably facilitate force clamp unfolding due to the dragging effect

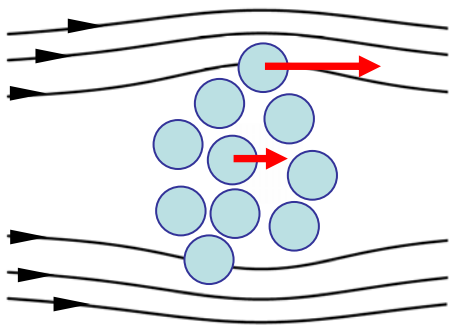


the moving particle creates a flow pattern which affects other particles by pulling them in the direction of its motion

Stretching by a flow



Unfolding of the system with HI requires a much larger flow speed than without HI due to the shielding effect



the particles inside a cluster are shielded from the flow and experience a smaller drag force than those on the surface

Summary

Stretching by a flow:

- Unfolding in a uniform flow usually involves several kinetic transitions between subsequent intermediates and has a richer dynamics than that in the force-clamp
- Due to the non-uniform tension along the protein chain unfolding pathways for the protein in the flow depend on the selection of the point of anchor.
- These features offer potentially wider diagnostic tools to investigate structure of proteins compared to experiments based on the atomic force microscopy.

Influence of hydrodynamic interactions on protein unfolding:

- Hydrodynamic interactions significantly affect the time scales of protein unfolding.
- HI facilitate unfolding at a constant force
- HI inhibit stretching by fluid flows.

Overdamped motion

velocity relaxation time:

$$\tau_v = \frac{m}{\gamma} = 0.05\,ps$$

characteristic diffusion time:

$$\tau = \frac{a^2}{D} = \frac{a^2\gamma}{kT} \approx 0.3ns$$

$$m = 2\times10^{-22}\,g$$

$$\gamma \approx 6\pi\eta a = 3\times10^{-9}\,g/s$$

$$a \approx 5\text{Å}$$

$$\tau \gg \tau_v$$

Brownian dynamics

$$\Delta \mathbf{R}_i = \sum_j \mu_{ij} \cdot \mathbf{F}_j \Delta t + \mathbf{\Gamma}_i(\Delta t)$$

fluctuation-dissipation theorem

$\mathbf{\Gamma}_i(\Delta t)$ - Gaussian noise with a variance of:

$$< \mathbf{\Gamma}_i(\Delta t)\mathbf{\Gamma}_j(\Delta t) >= 2kT\,\mu_{ij}\Delta t\,\mathbf{I}$$

(Ermak, McCammon, 1978)