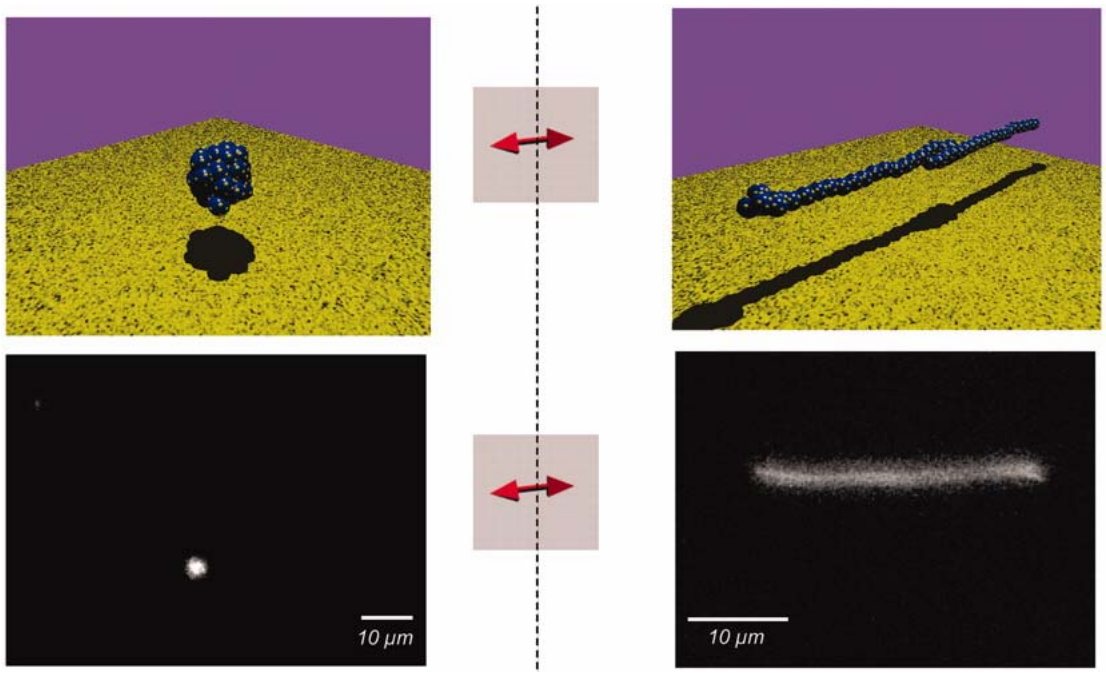
Hydrodynamic effects in protein dynamics



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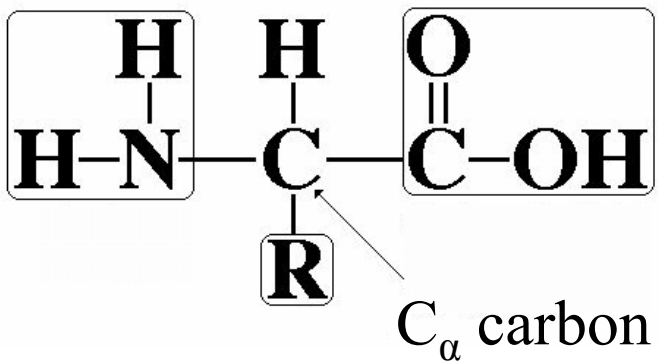
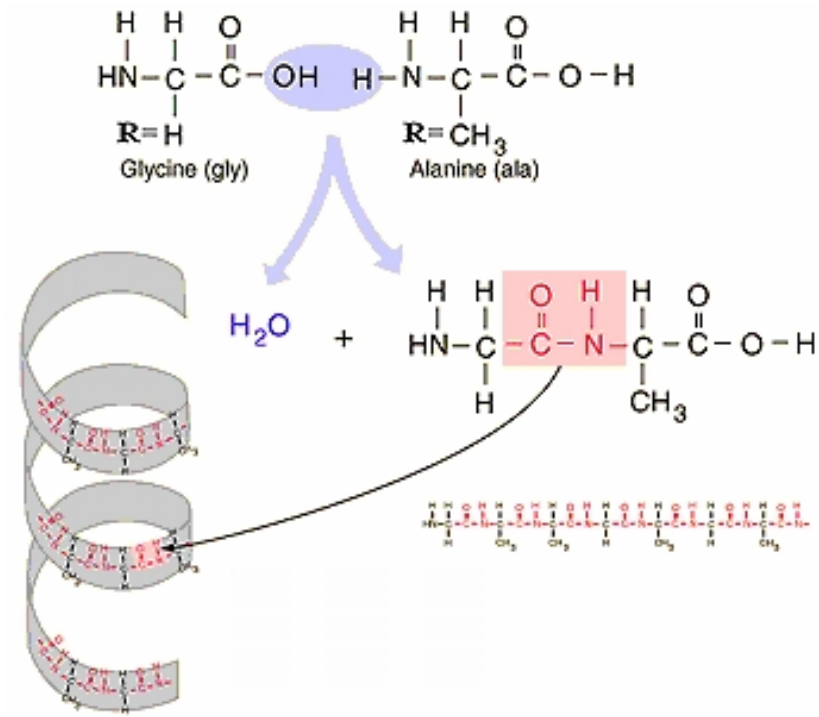
Outline:

- Proteins, their structure and folding properties
- Coarse grained models
- Hydrodynamic interactions
- Stretching of proteins by mechanical forces
- Stretching by a fluid flow
- Influence of hydrodynamic interactions on protein stretching
- Conclusions

Proteins

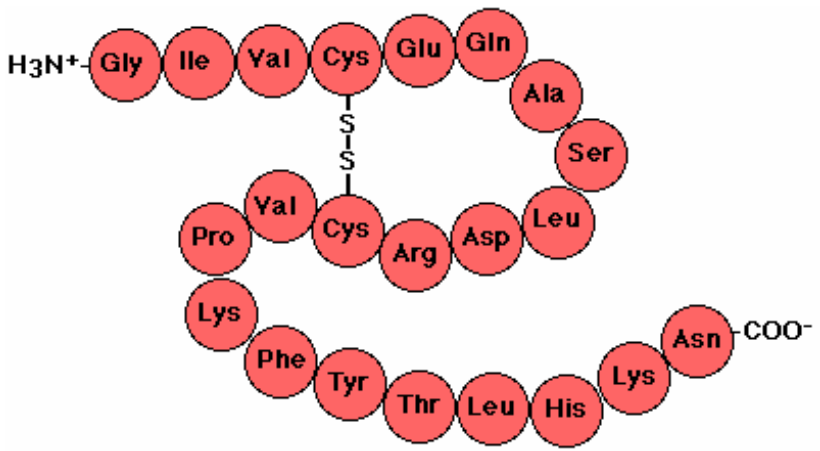
- large (10^3-10^7 Da) biopolimers, made of amino acids joined by peptide bonds





Primary structure

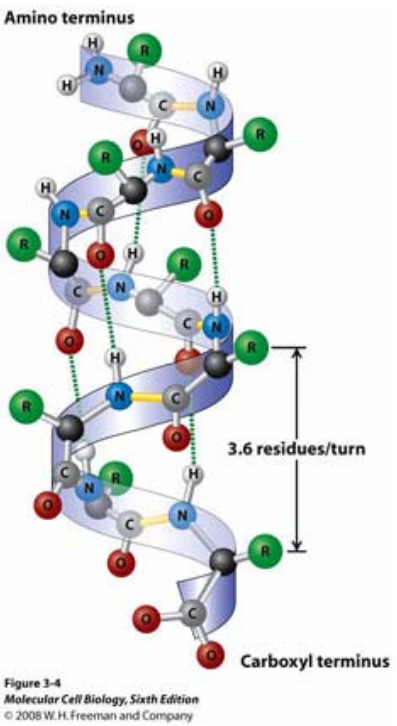
- amino acid sequence



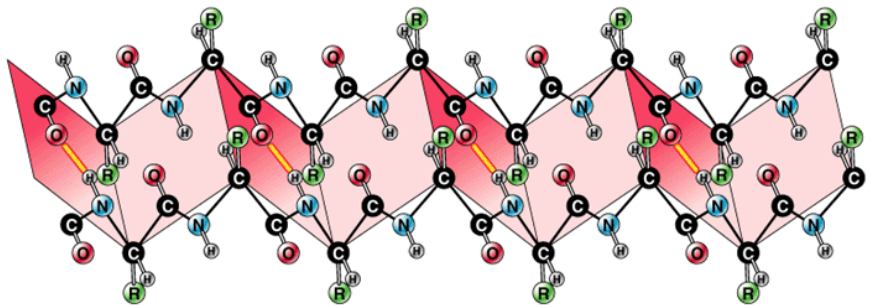
Secondary structure

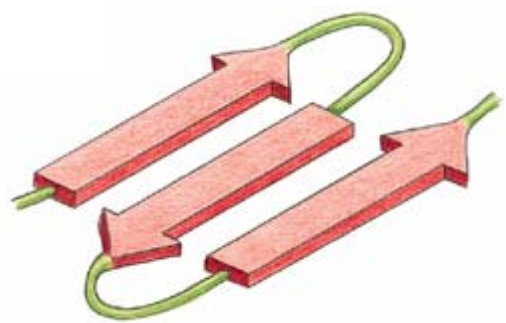
local structures stabilized by hydrogen bonds

- α helices



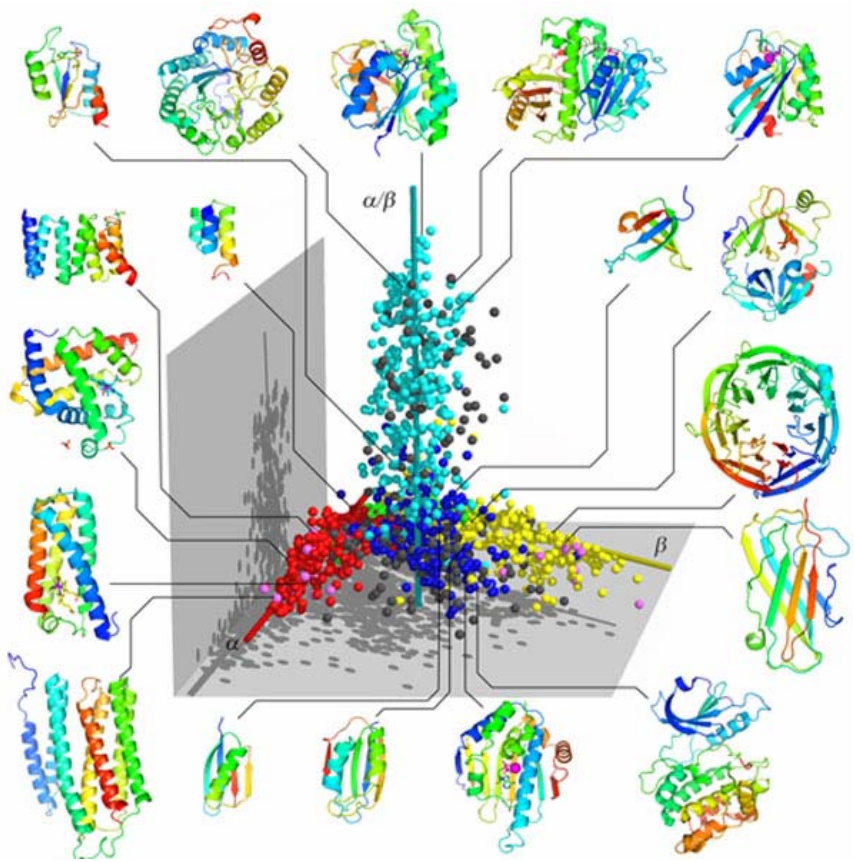
- β structures





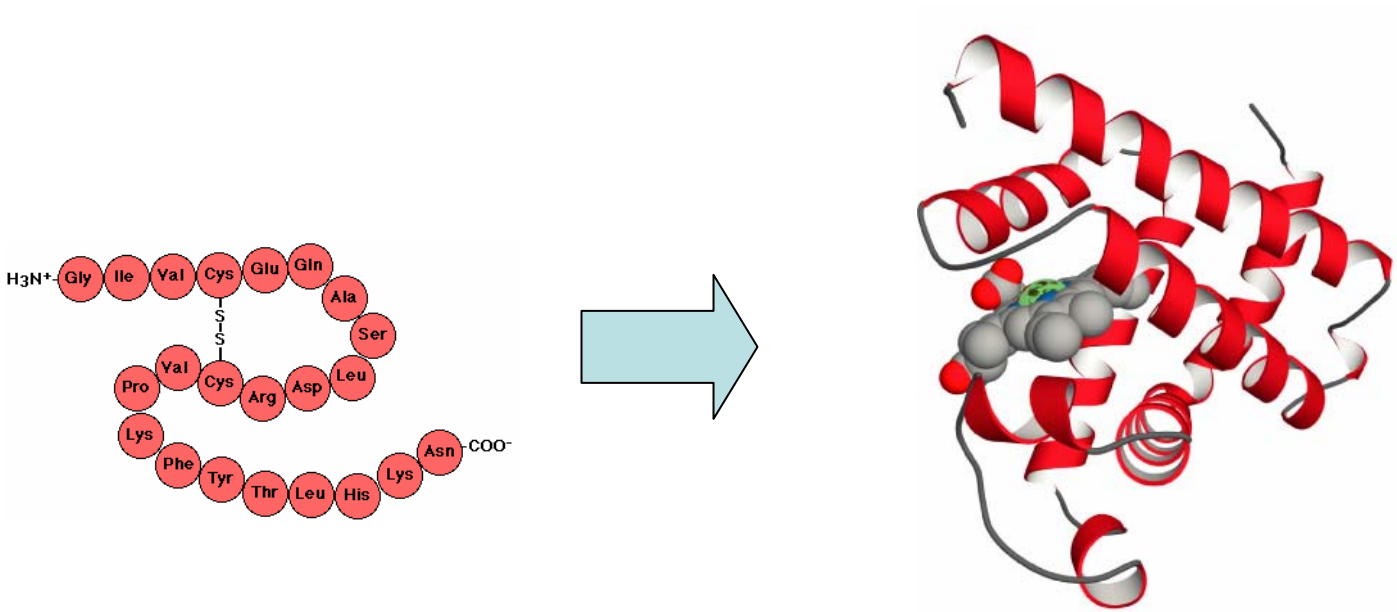
Tertiary structure

Full, three-dimensional structure of the whole protein chain



Protein folding

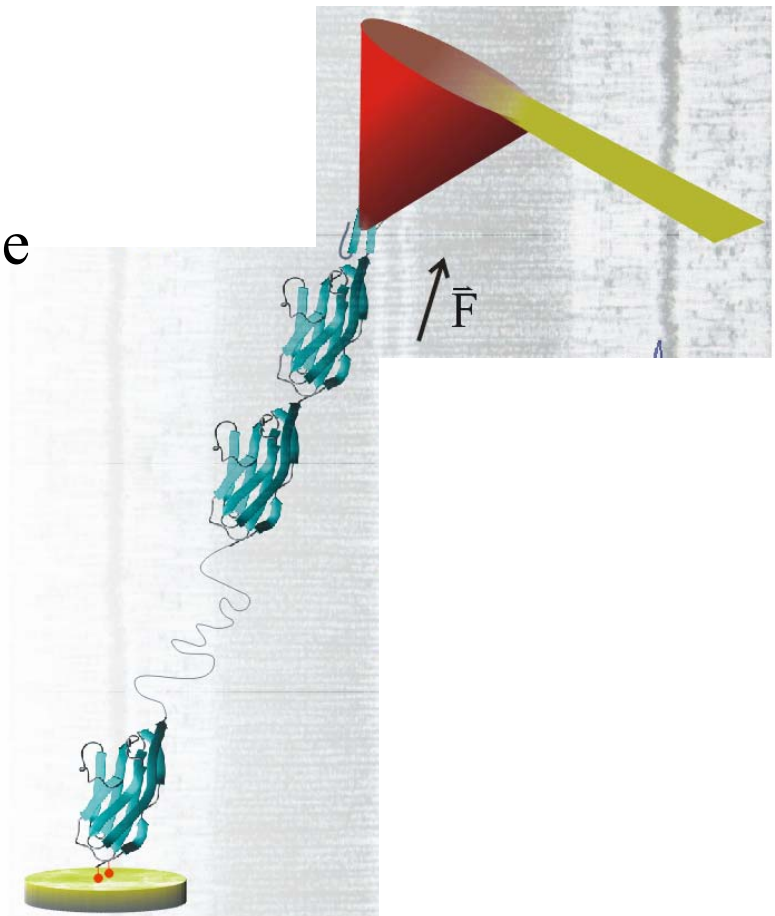
Under physiological conditions proteins fold spontaneously into its characteristic tertiary structure (native state).



primary structure determines the tertiary structure

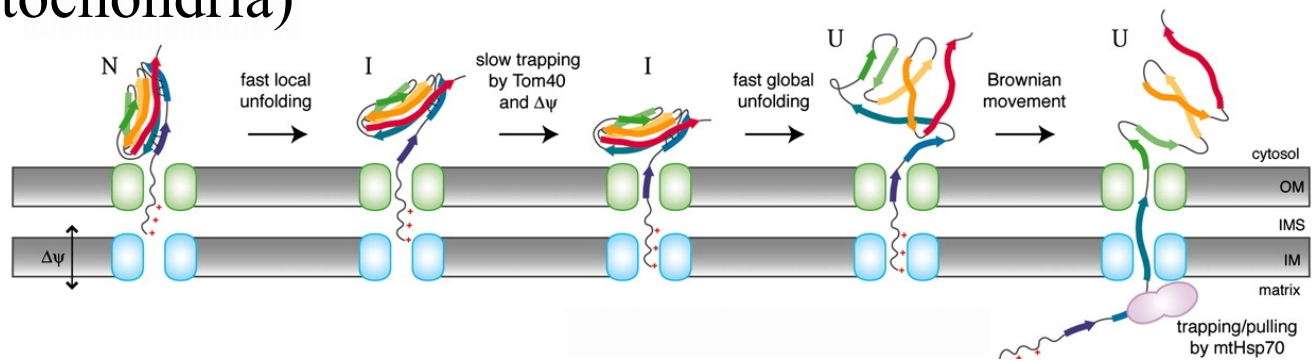
Mechanical stretching of proteins

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.

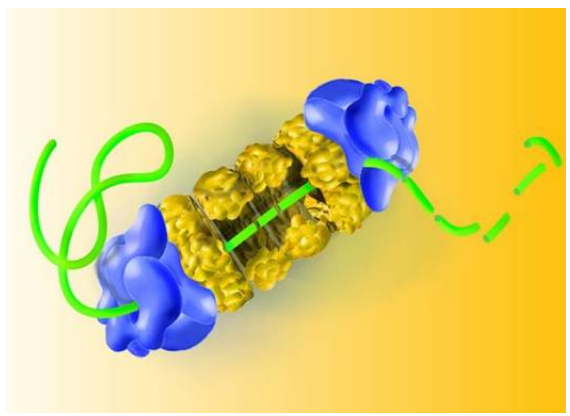


Protein unfolding in the cell

- protein translocation across the membranes (e.g. during import into mitochondria)

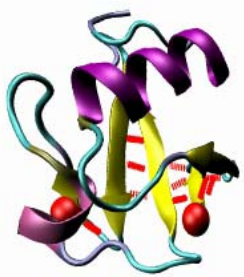


- degradation process in proteasome

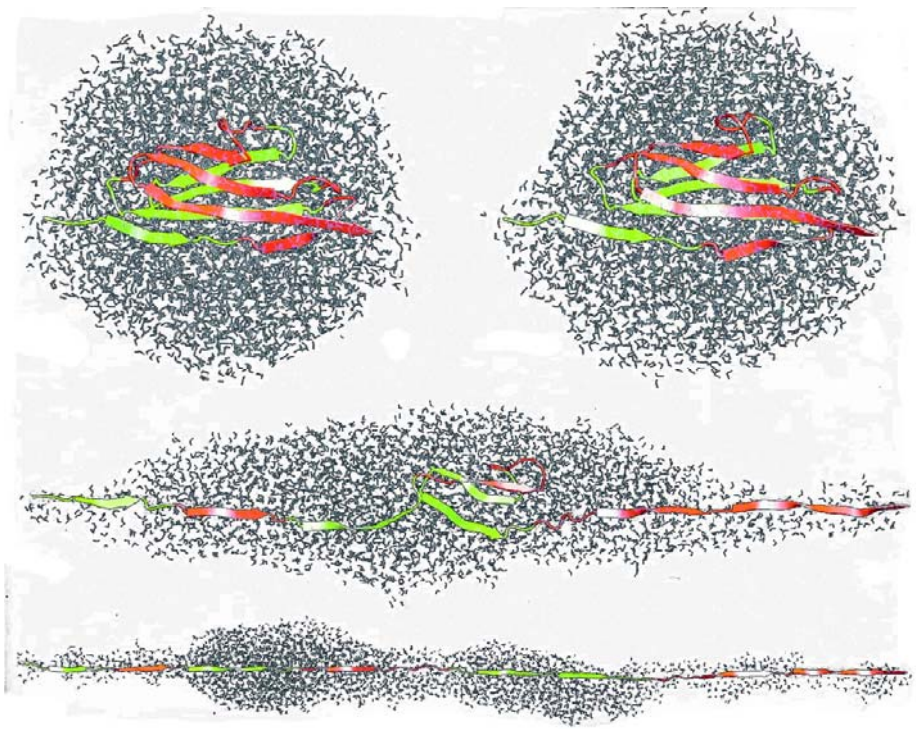


Importance of numerical models:

- 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



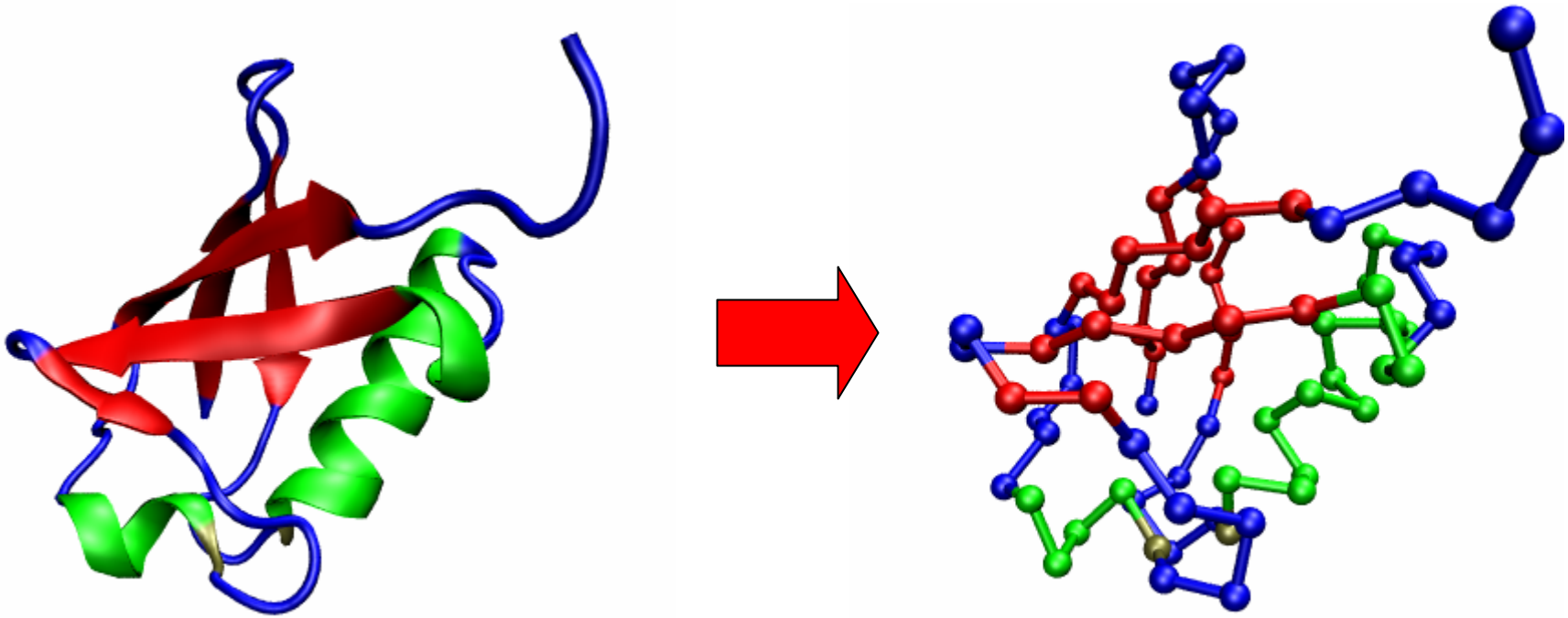
All-atom models



- most exact and relablie, 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

Lu et al., Biophys J. **75**, 662 (1998)

Coarse-grained model of a protein



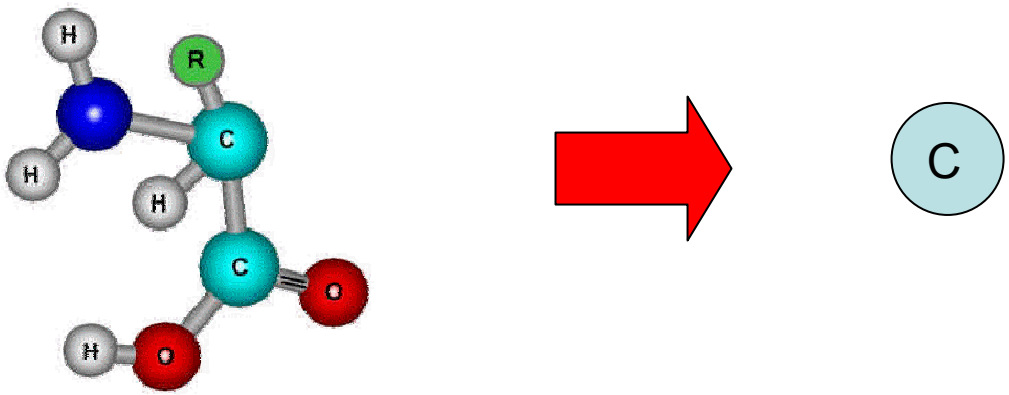
- reduction of the number of degrees of freedom
- effective interactions

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

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

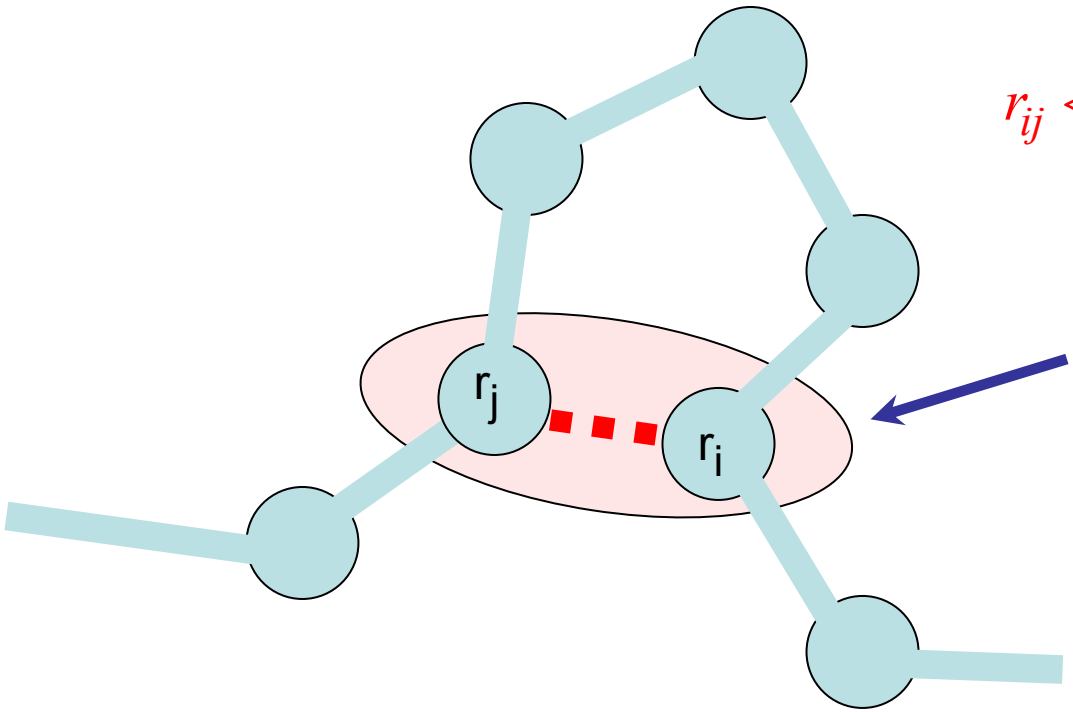
Details of the model

(implementation: Cieplak, Hoang, Robbins)



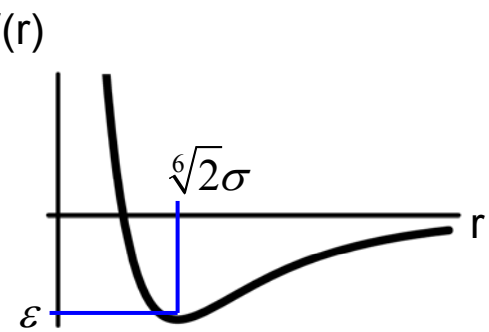
Each amino acid residue replaced by one "bead", located at the C_{α} position

Potential: native contacts



$r_{ij} < r_0$ in the native state

native contact

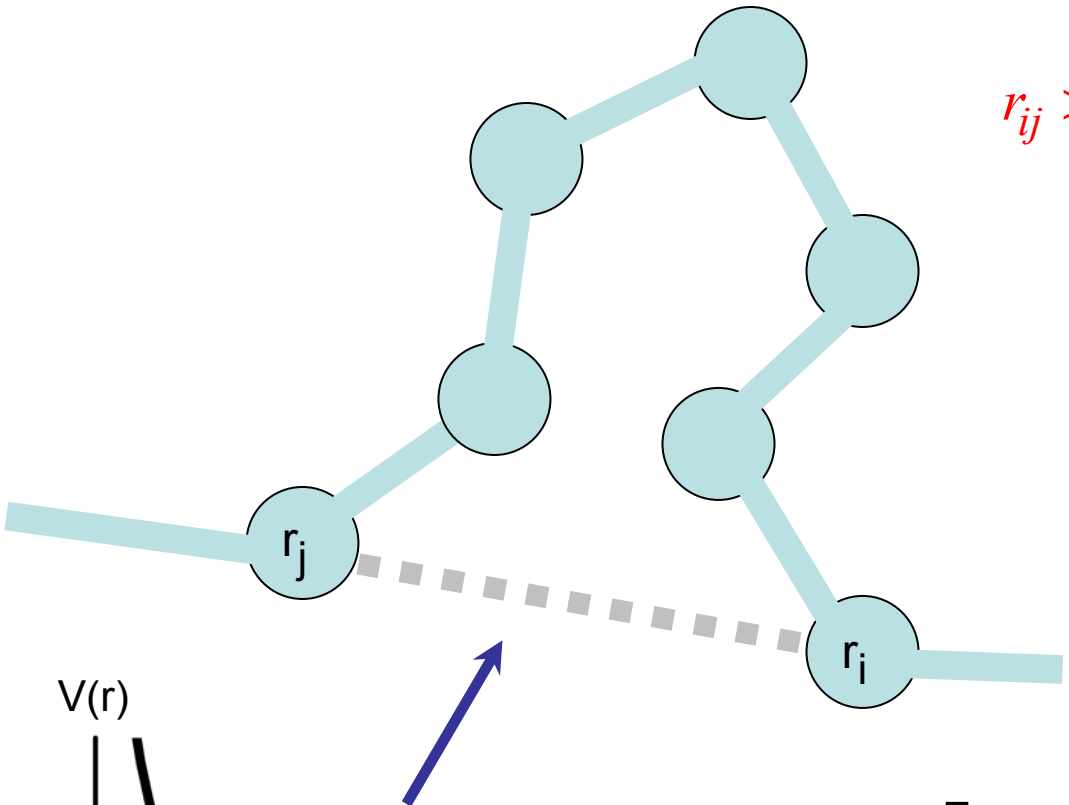


Lennard-Jones potential

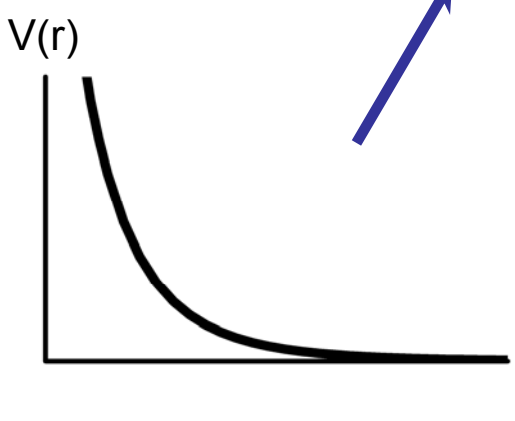
by constraction, the native configuration is the energy minimum

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

Potential: non-native interactions



$r_{ij} > r_0$ in the native state

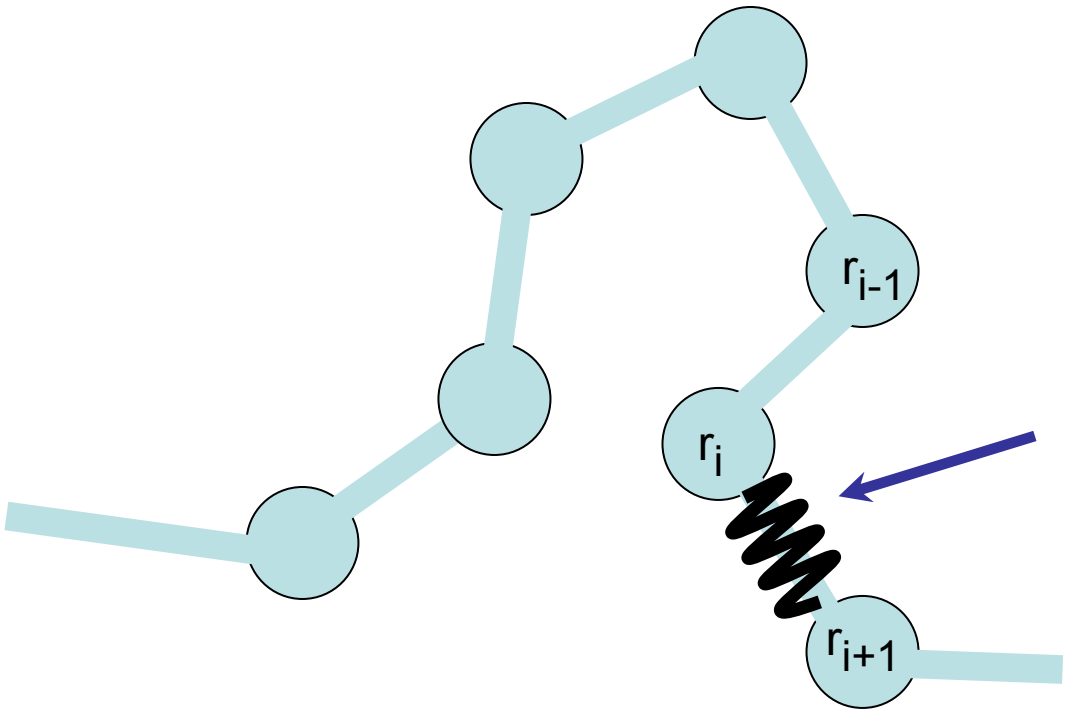


$$V_{ij}(r) = 4\varepsilon\left[\left(\frac{\sigma}{r_{ij}}\right)^{12}\right]$$

pure repulsion

σ = 4Å

Potential: backbone



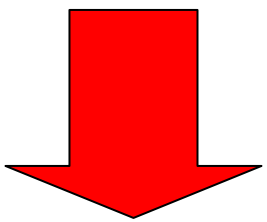
$$V(r) = \frac{1}{2} k (r_{i,i+1} - a)^2$$

$a = 3.8$ Å

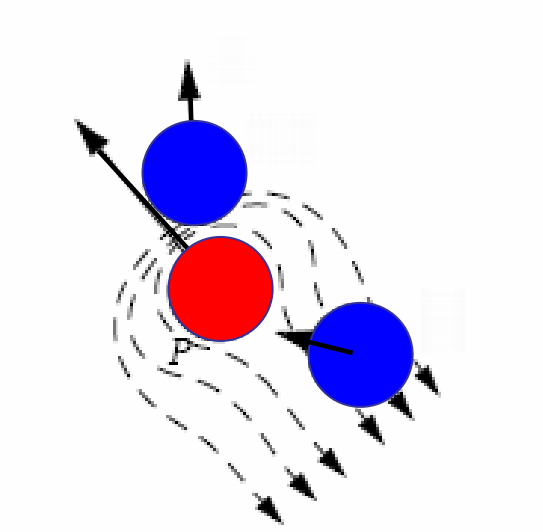
$k = 100\ \varepsilon/\text{Å}^2$

Hydrodynamic interactions

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



hydrodynamic interactions



Hydrodynamic problem

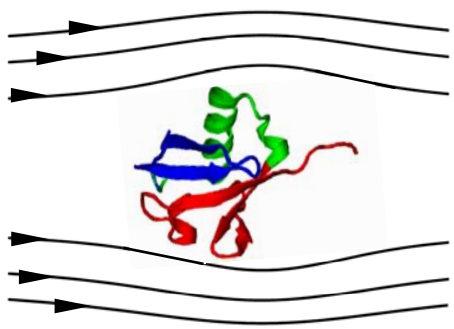
Flow field (**u**) described by Stokes equations:

$$\begin{cases} \eta \nabla^2 \mathbf{u} = \nabla p \\ \nabla \cdot \mathbf{u} = 0 \end{cases}$$

which govern fluid flows in the low Reynolds number regime

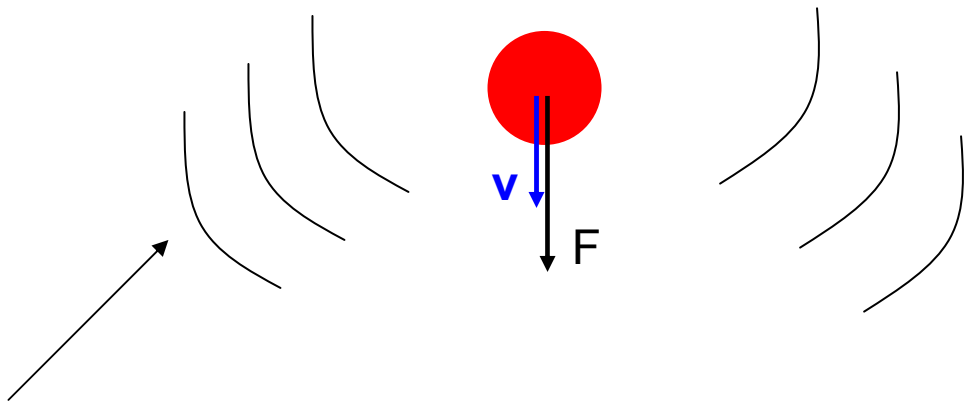
$$\mathrm{Re} = \frac{a \rho v}{\eta}$$ ← characteristic flow velocity

For proteins: $\mathrm{Re} \approx 10^{-6} - 10^{-4} \ll 1$



Single particle

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



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

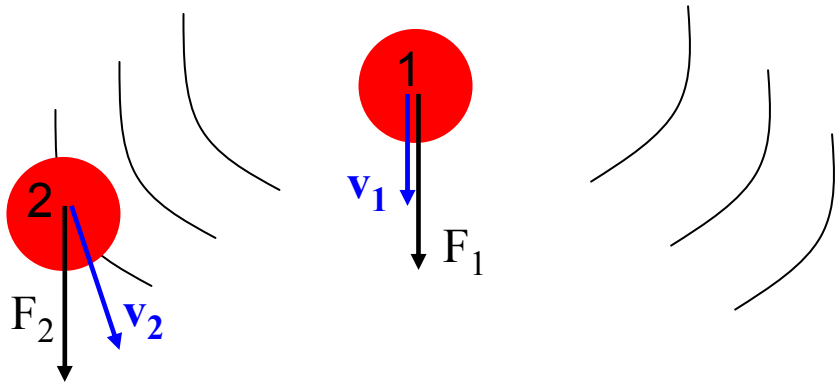
flow disturbance caused by the sphere :

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

long-ranged (1/r)!

Stokes (1863)

Two particles



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

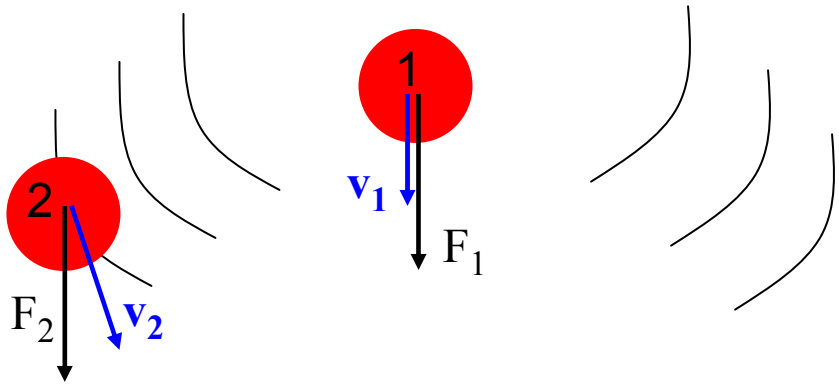
$$\mathbf{v}_2(\mathbf{r}_{12}) = \frac{\mathbf{F}_2}{6\pi\eta a} + \frac{3a}{4r_{12}}(1+\hat{\mathbf{r}}_{12}\hat{\mathbf{r}}_{12})\cdot\frac{\mathbf{F}_1}{6\pi\eta a} + \ldots.$$

higher order terms

single-particle term

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

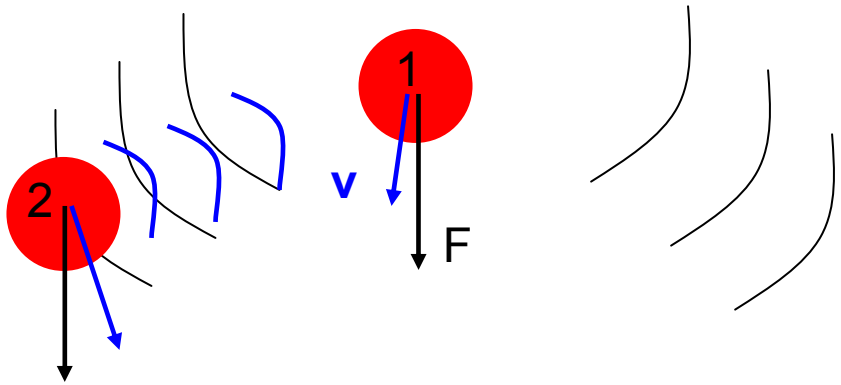
the Oseen tensor



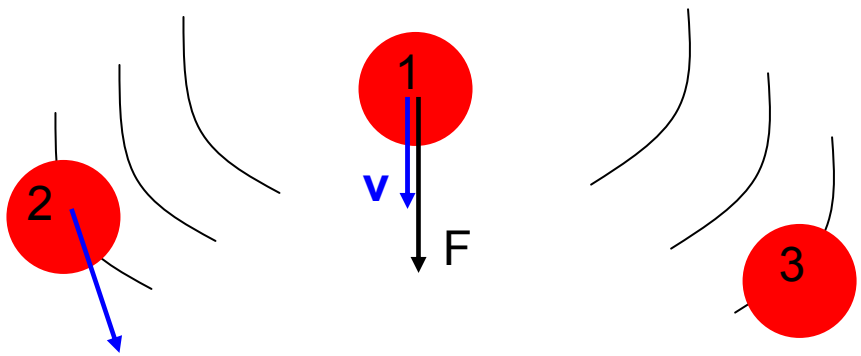
$$\mathbf{v}_2(\mathbf{r}_{12}) = \frac{\mathbf{F}_2}{6\pi\eta a} + \frac{3a}{4r_{12}}(\mathbf{1} + \hat{\mathbf{r}}_{12}\hat{\mathbf{r}}_{12}) \cdot \frac{\mathbf{F}_1}{6\pi\eta a} =$$

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

Higher order terms



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



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

N particles

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

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:

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

multipole methods (Cichocki, Felderhof, Ekiel-Jeżewska, Wajnryb)

Overdamped motion

velocity relaxation time:

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

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

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

$$a \approx 5\text{Å}$$

characteristic diffusion time:

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

$$\tau \gg \tau_v$$

Brownian dynamics

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

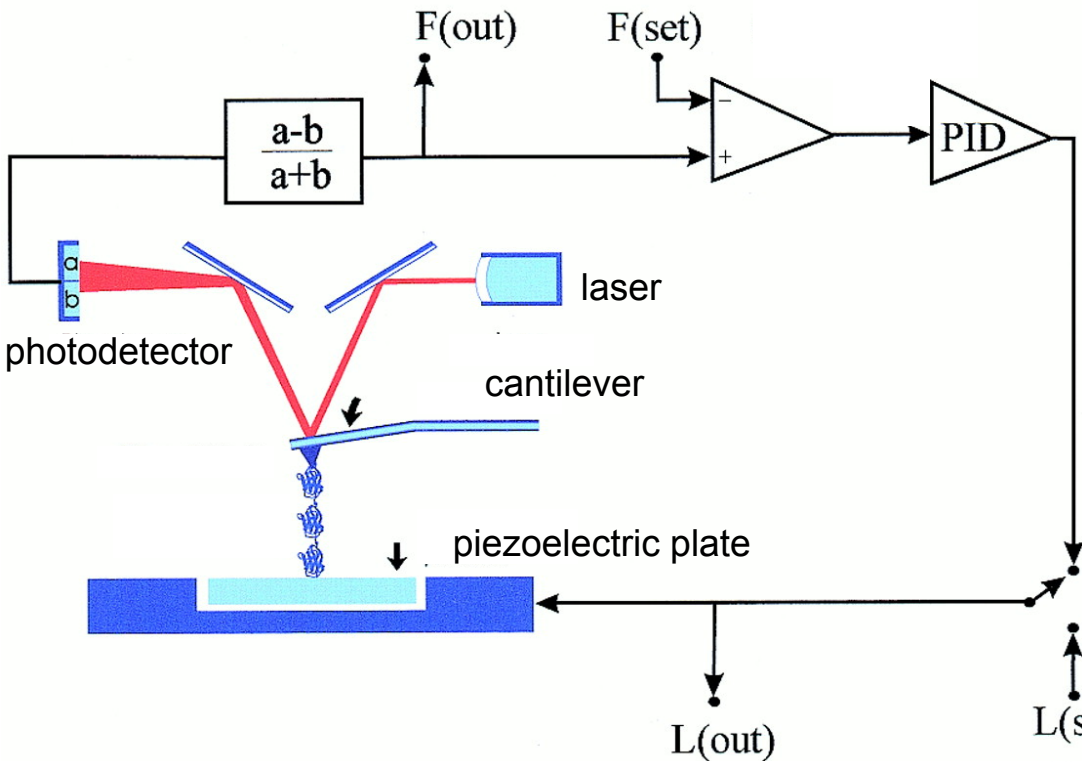
fluctuation-dissipation theorem

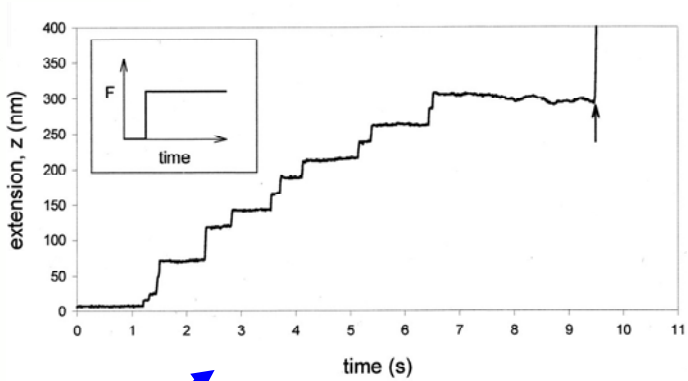
$\mathbf{\Gamma}_i(\Delta t)$ - Gaussian noise with a variance of:

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

(Ermak, McCammon, 1978)

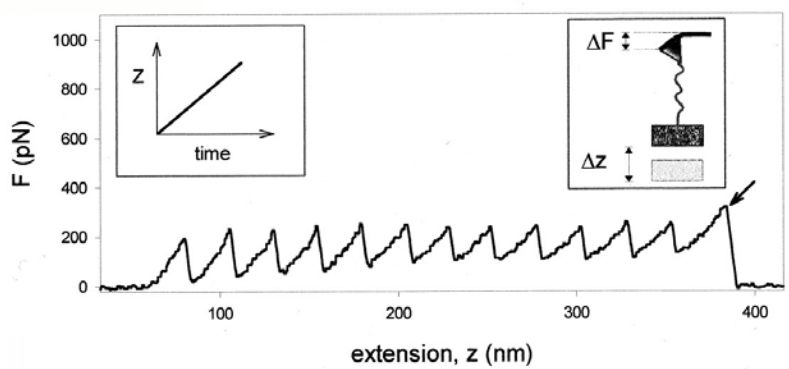
Protein stretching (AFM)





force clamp (constant force)

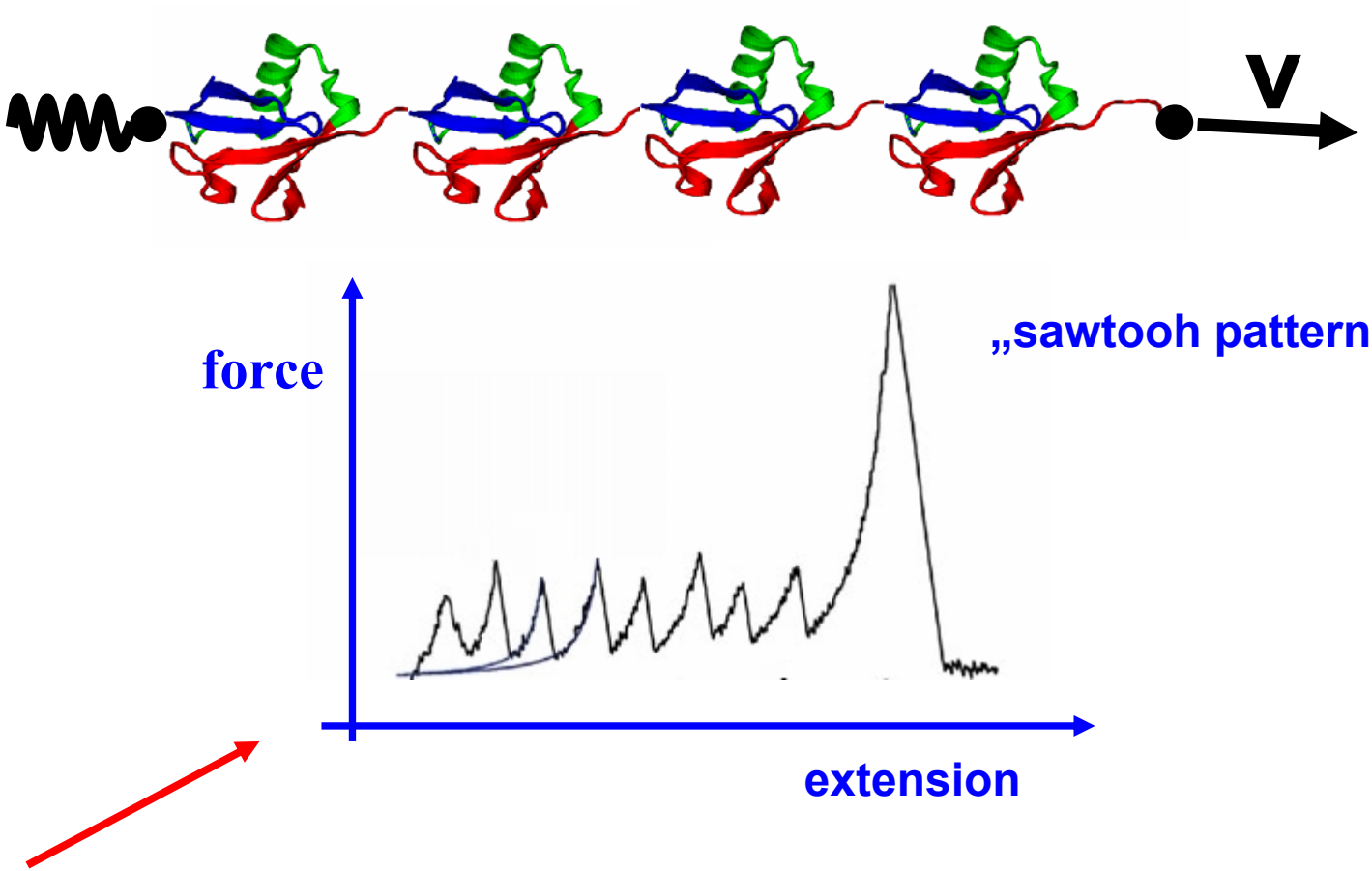
length clamp (constant velocity)



force clamp:

Oberhauser et al. PNAS **98**, 468 (2001)

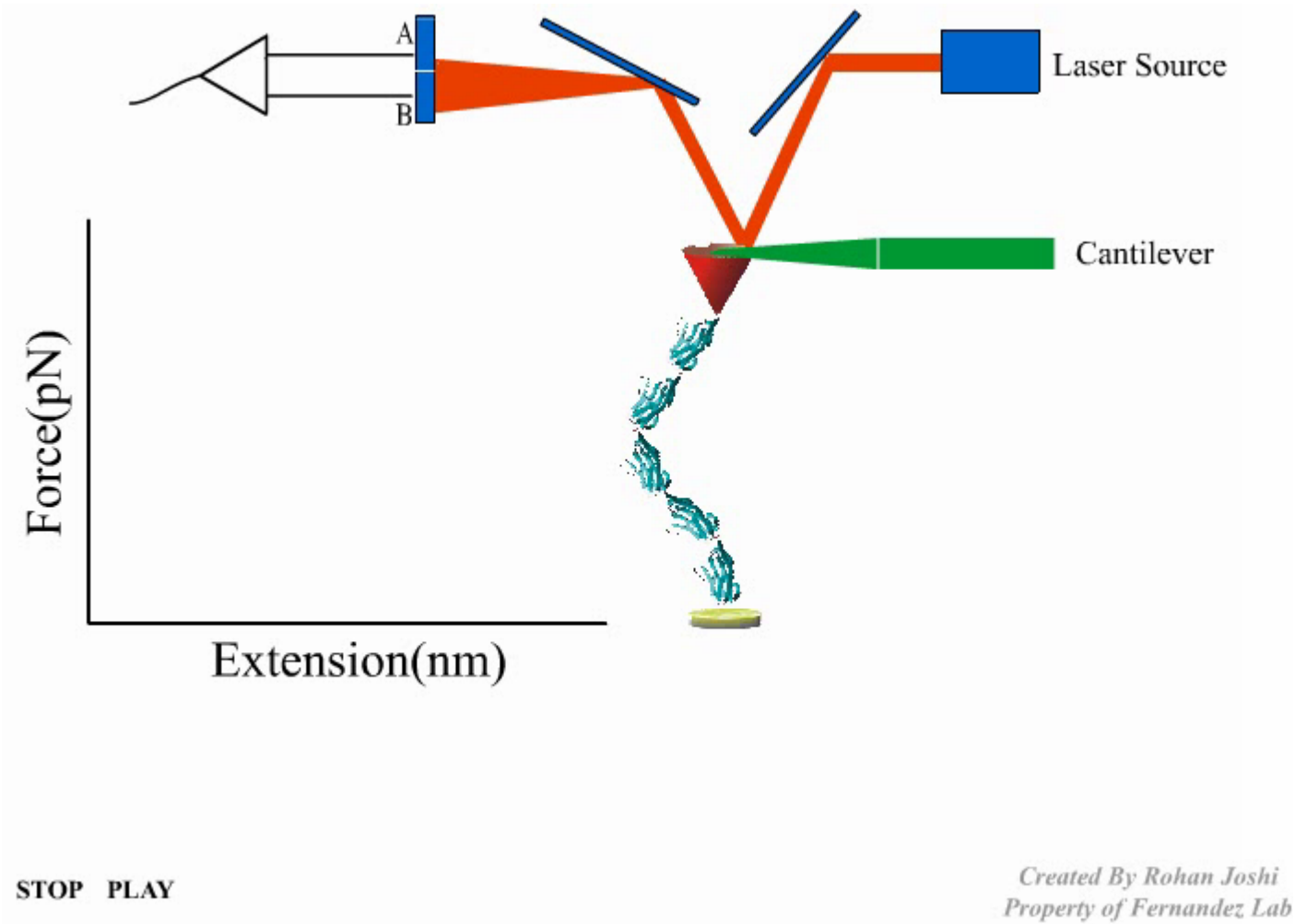
Length clamp



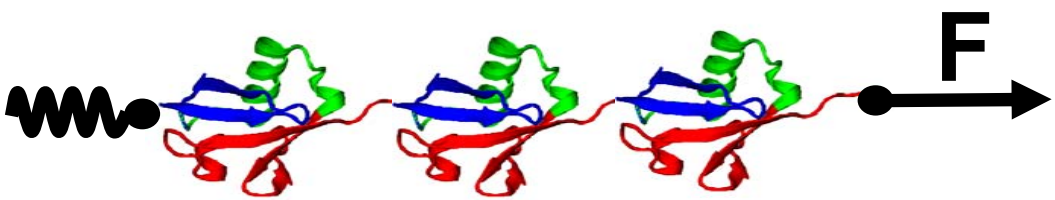
ubiquitin chain:

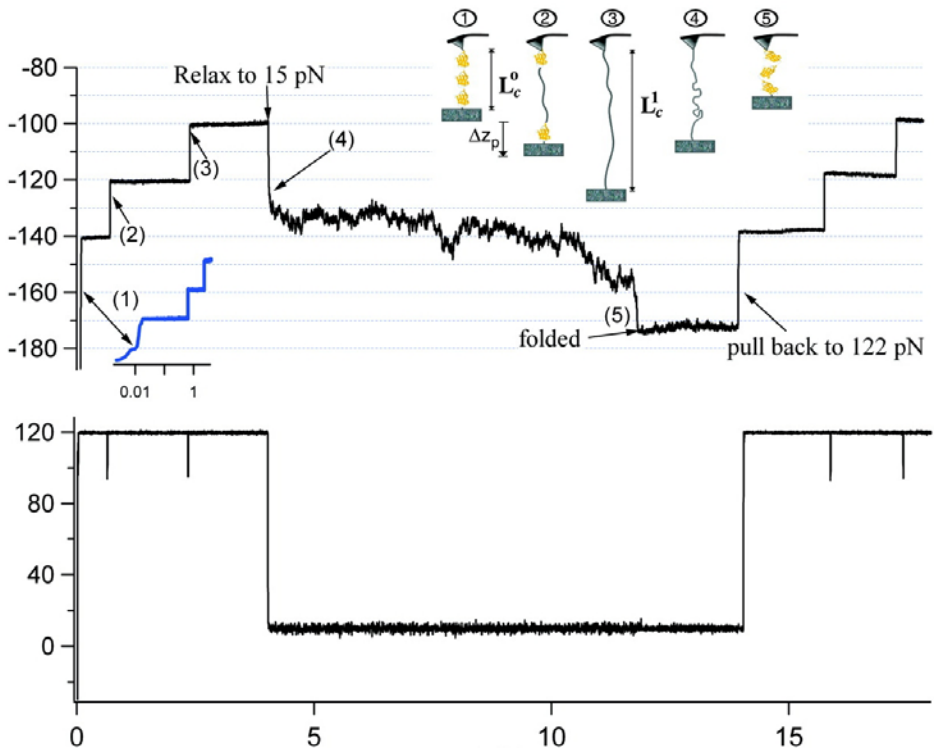
Carrion-Vazquez et al, Nature Struct. Biol. **10**, 738 (2003)

Length clamp



Force clamp



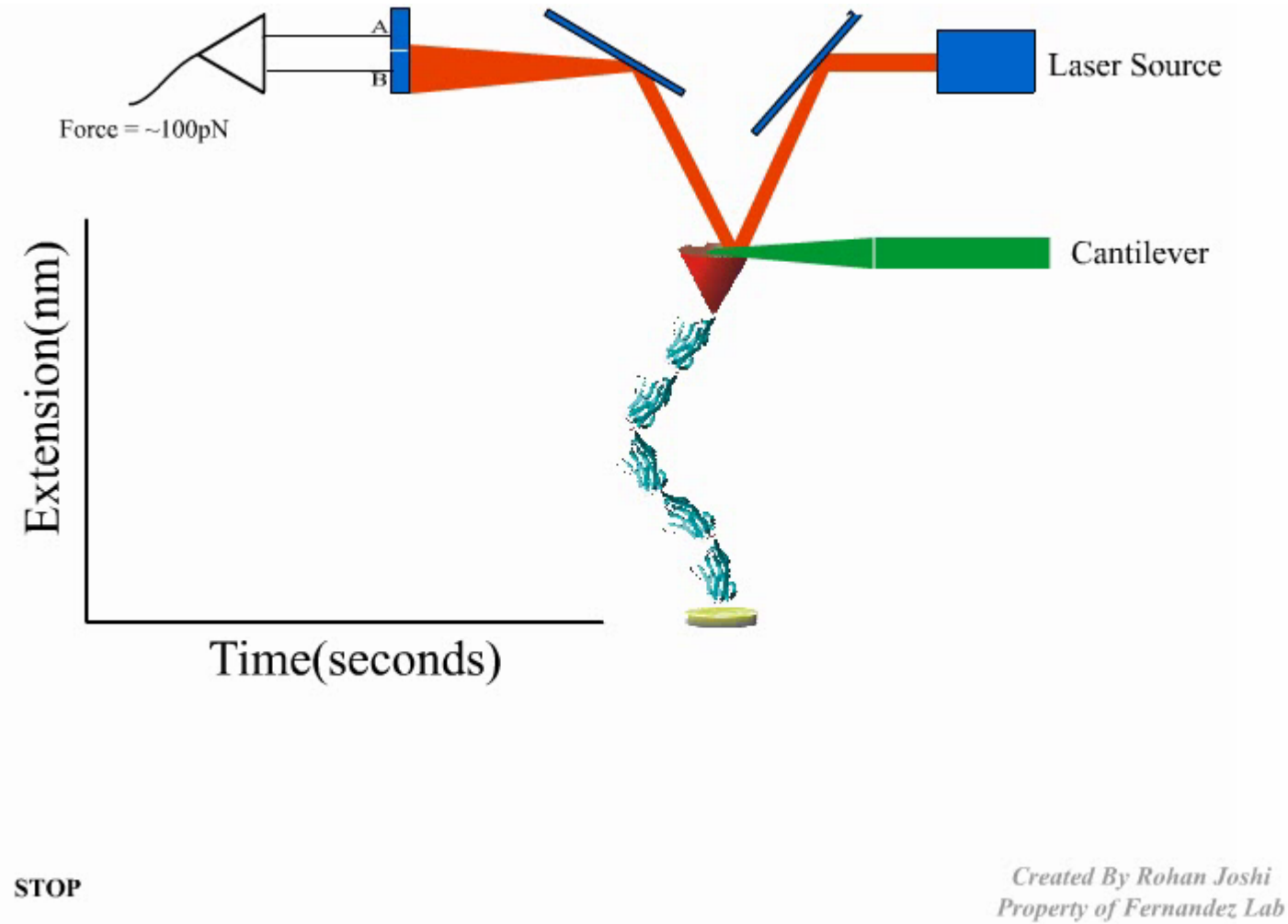


ubiquitin chain:

Fernandez and Li,

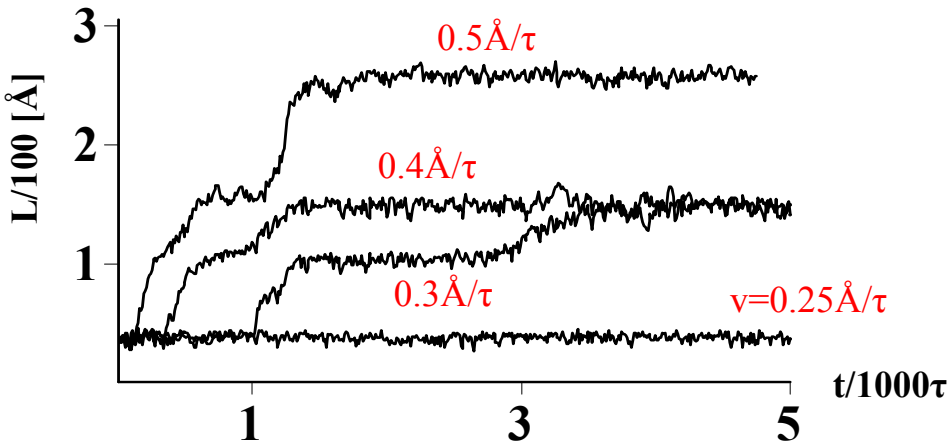
Science **303**, 1674 (2004)

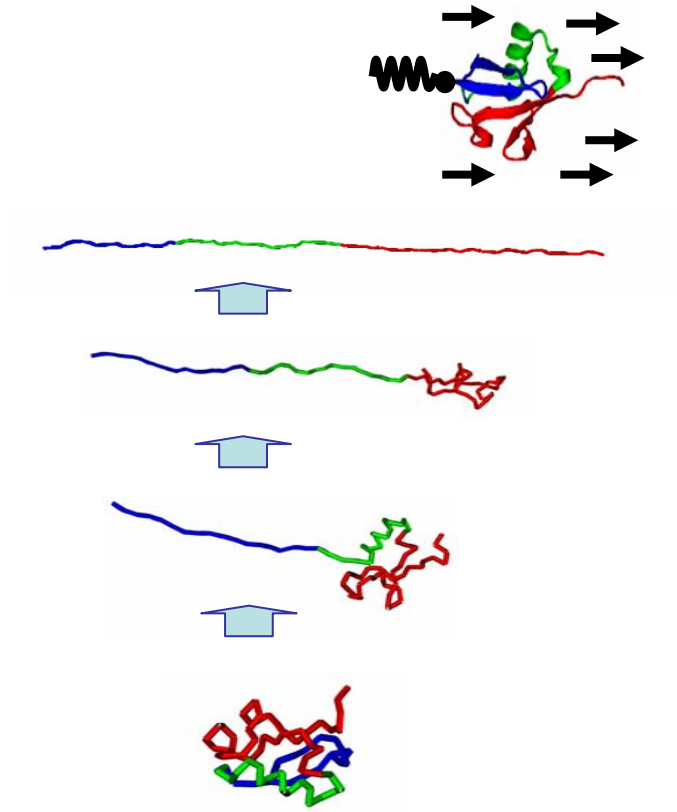
Force clamp


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

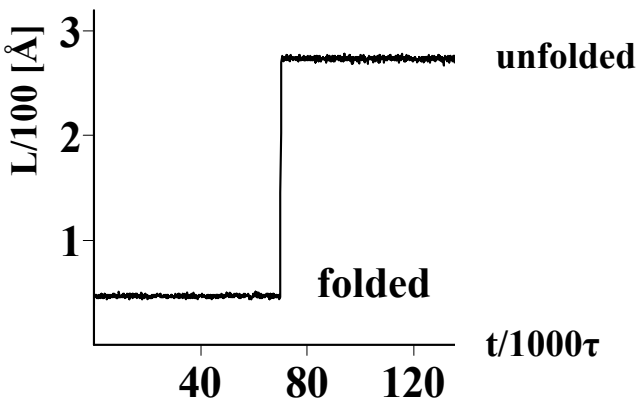
Stretching by a fluid flow

ubiquitin in a uniform flow (v=const.)





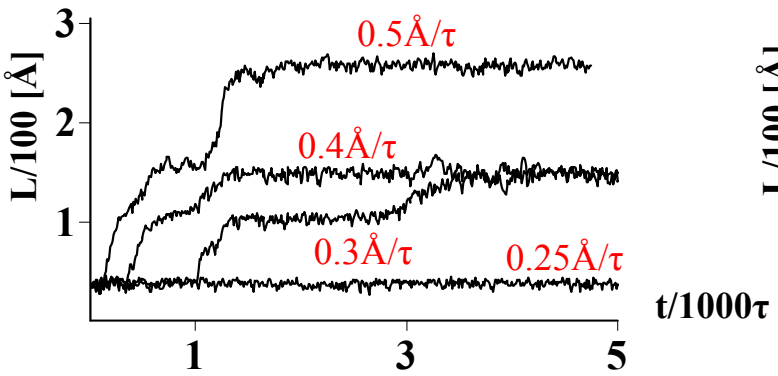
ubiquitin in a force clamp (F=const.)



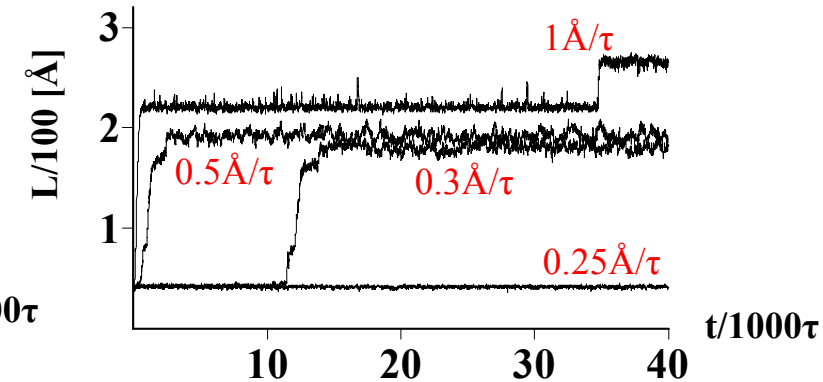
In contrast to force clamp, ubiquitin unfolding in a flow is a multi-step process, involving several intermediate states.

Asymmetry between the ends

N terminus fixed



C terminus fixed



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

- different unfolding times

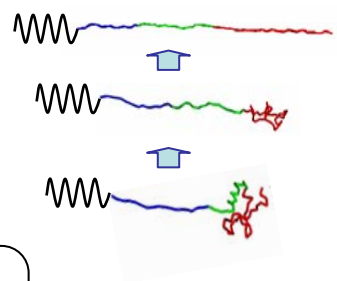
- different set of intermediates

- 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

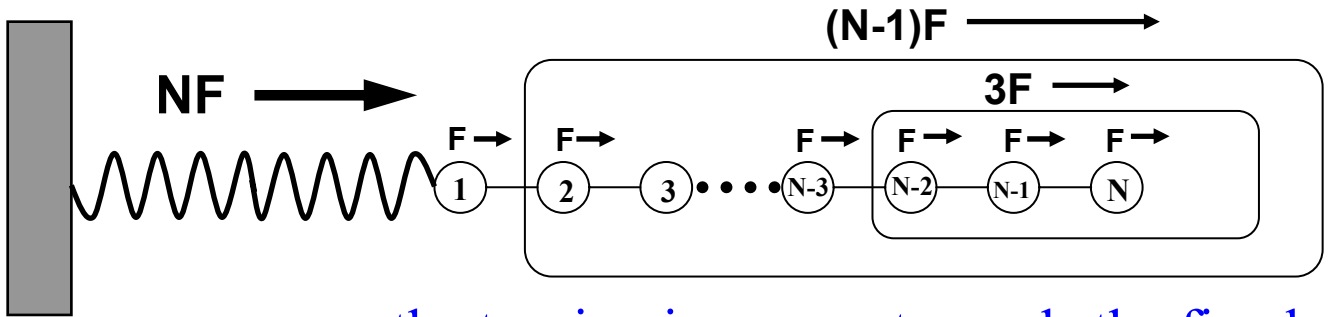
for the protein in a flow, the tension is nonuniform along the chain and depends on the tethering point.



$F = -\gamma v$

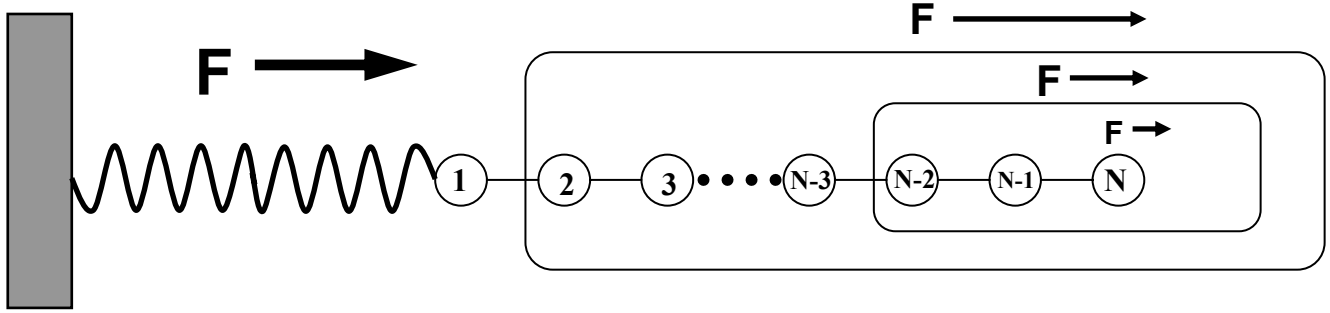
example: linear chain

flow



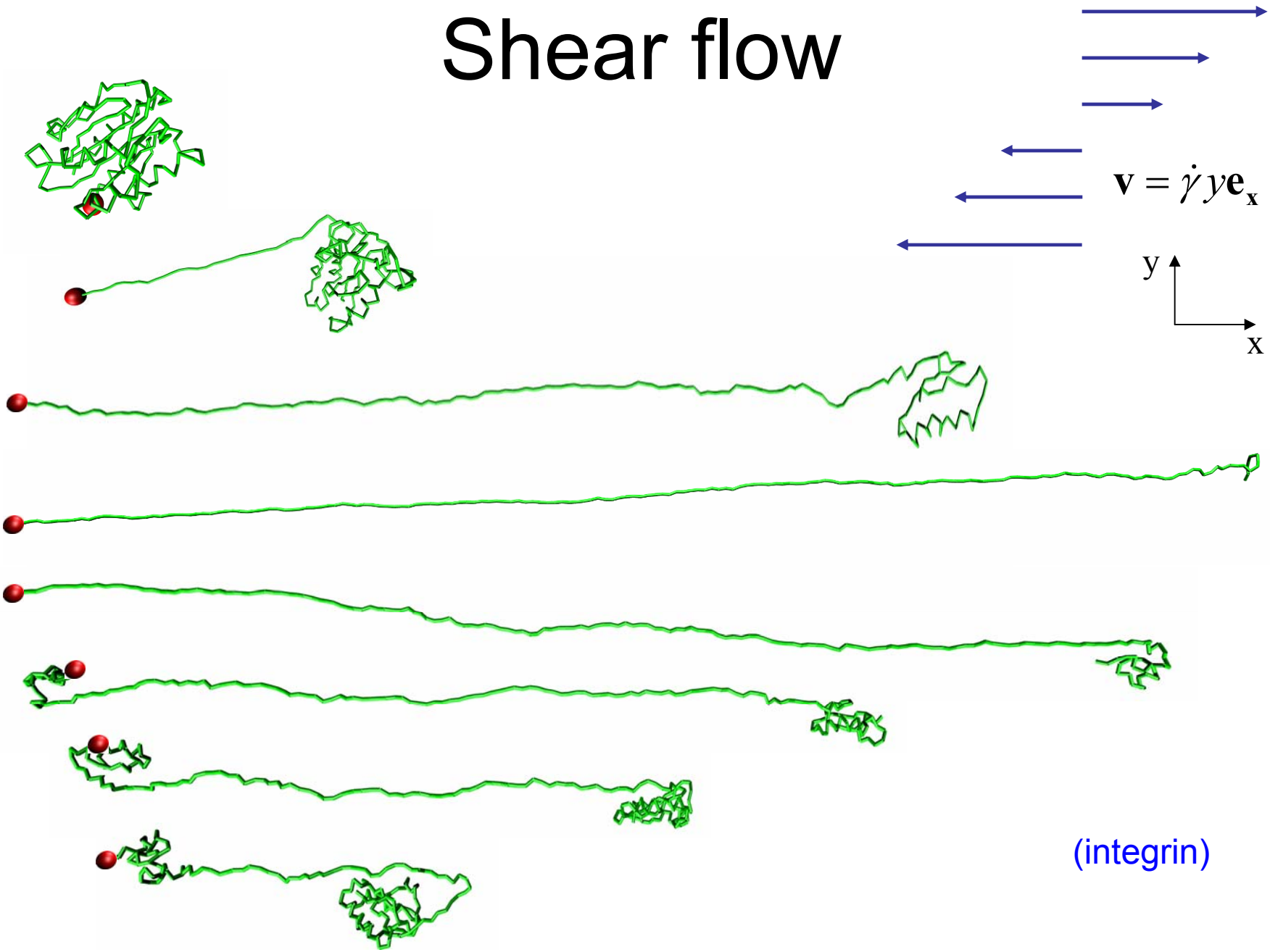
the tension increases towards the fixed end

force-clamp

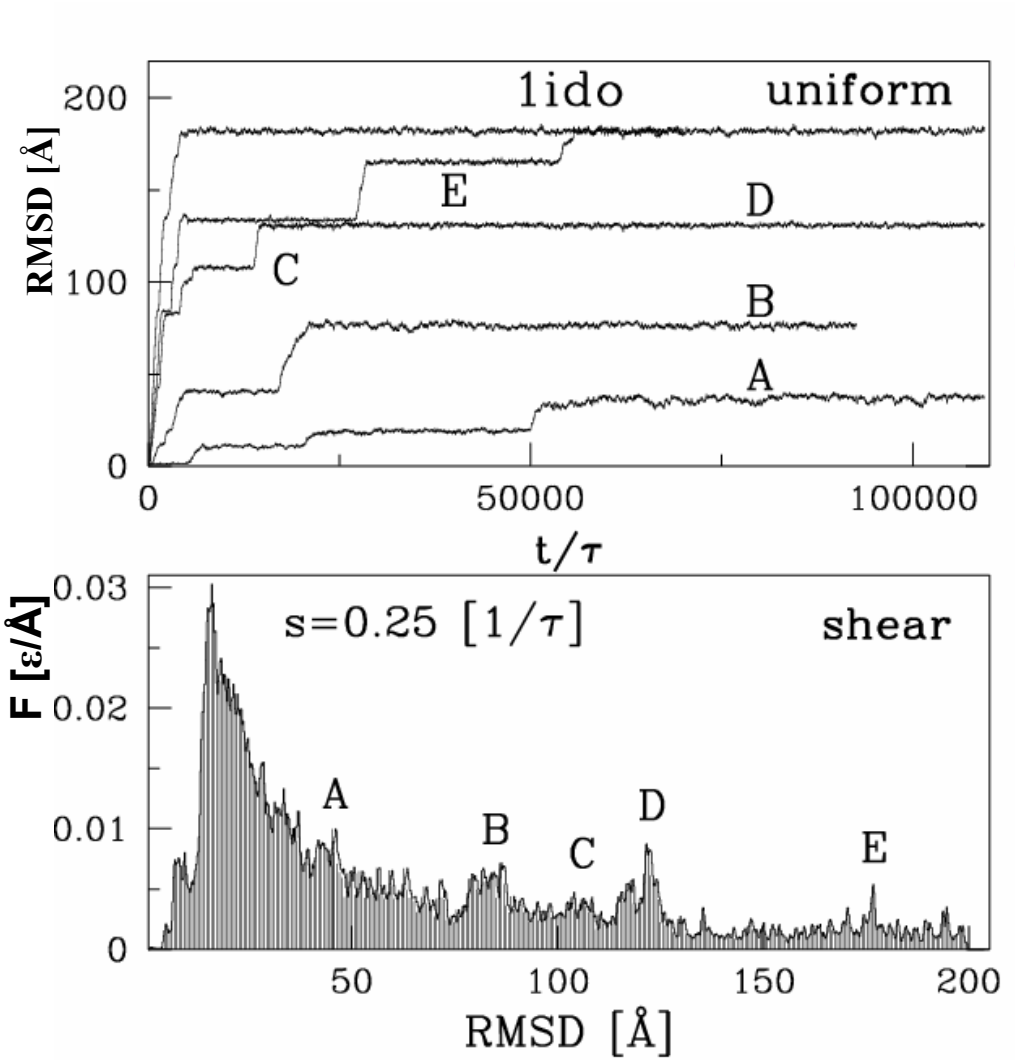


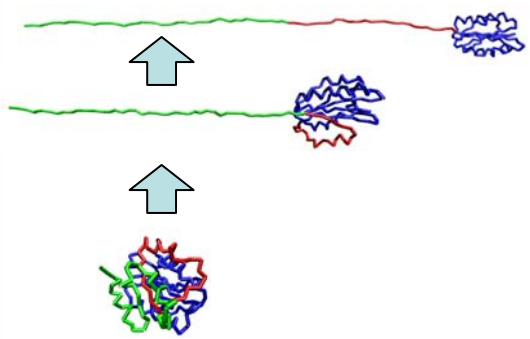
the tension is constant along the chain

Shear flow



Shear flow (2)



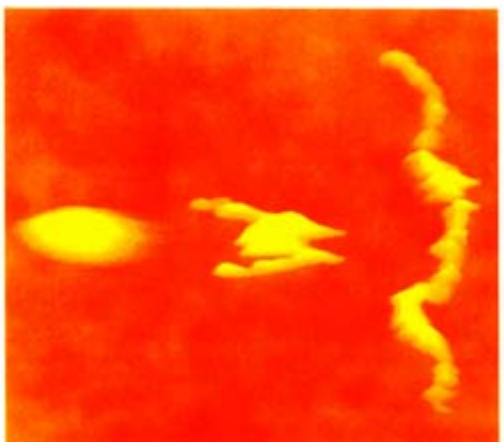


(integrin)

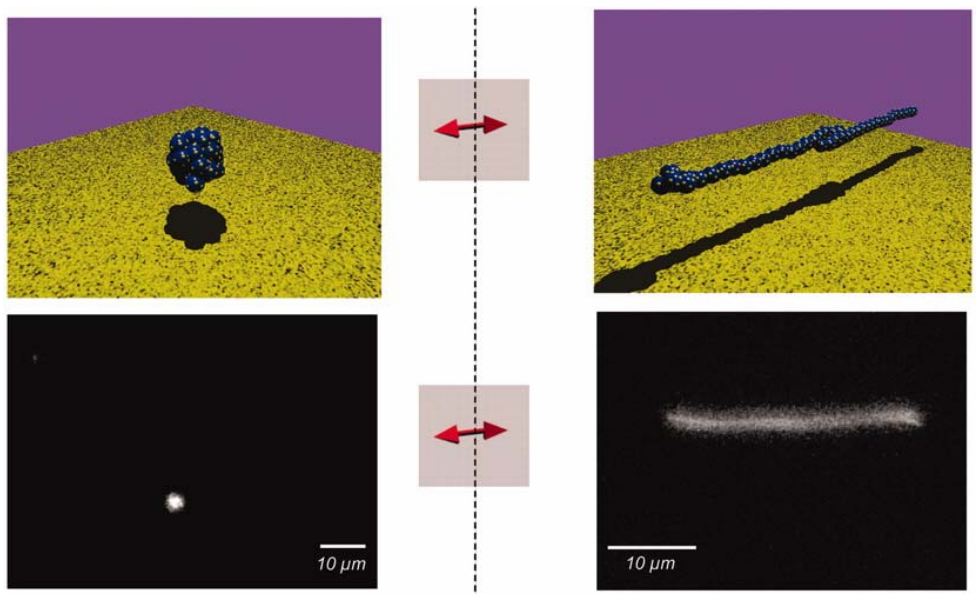
Experiments

1) 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 even strong shear flows of $\dot{\gamma} = 10^5 s^{-1}$ are unable to destabilize this protein

2) stretching of von Willebrand factor – large, multidomain protein playing a major role in blood coagulation:



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



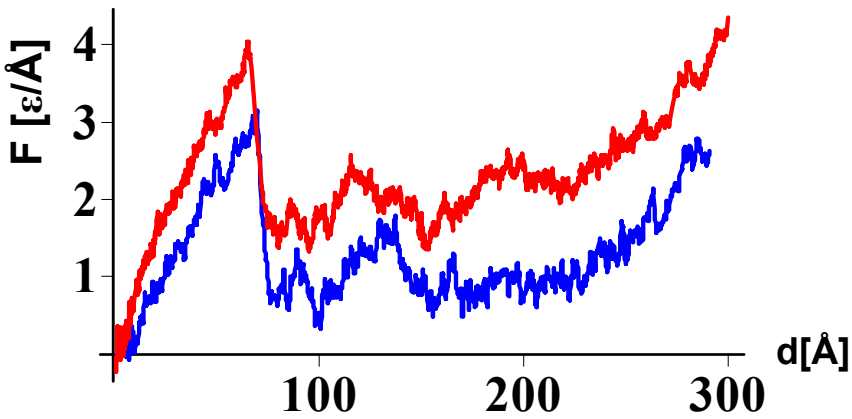
Schneider et al., *PNAS*, **104**, 7899, (2007)

Influence of hydrodynamic interactions on protein unfolding

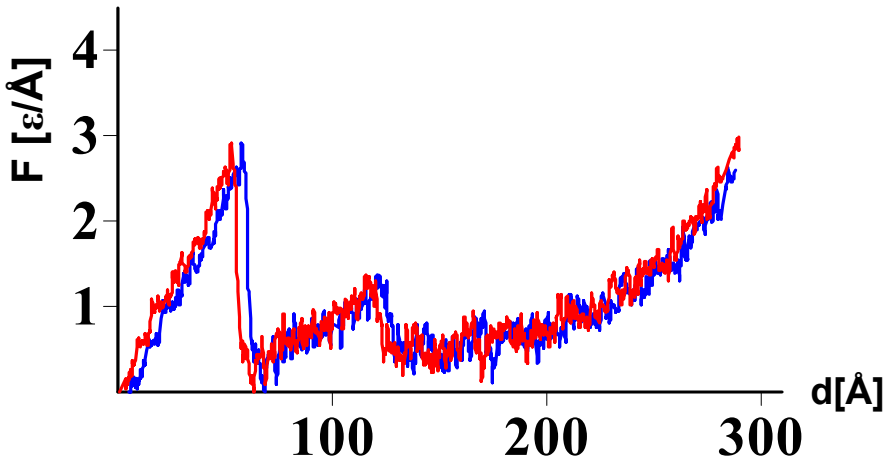
- constant velocity unfolding
- constant force (force-clamp)
- stretching by a fluid flow

Constant velocity stretching

high stretching speeds (v=0.5Å/τ)



low stretching speeds (v=0.05Å/τ)

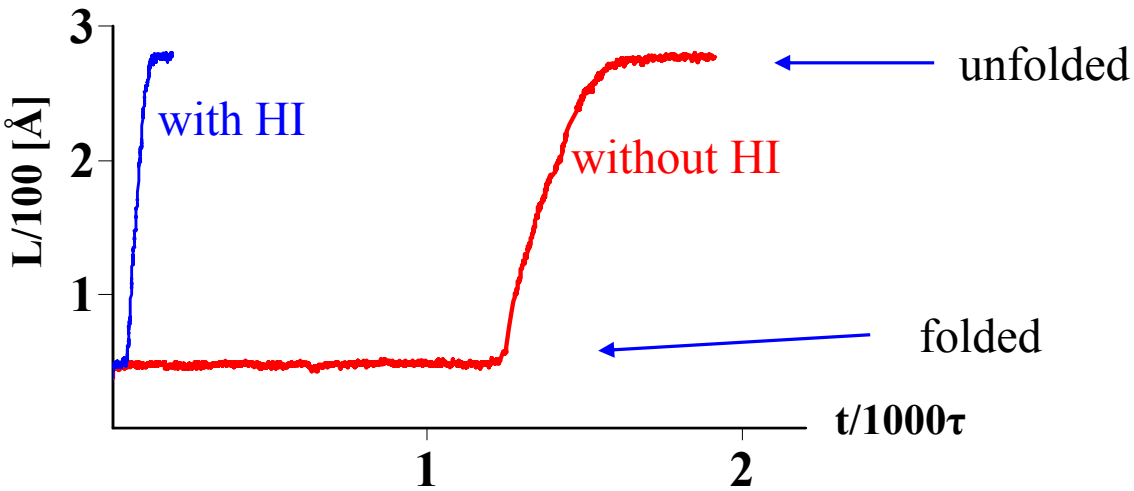


with hydrodynamic interactions

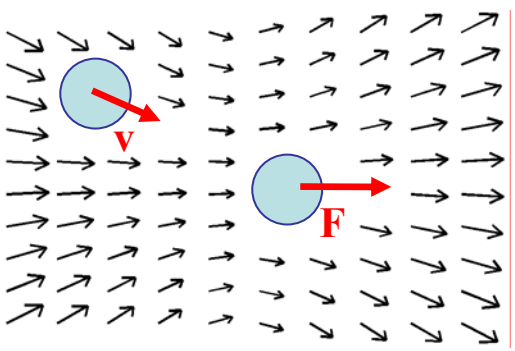
without hydrodynamic interactions

Hydrodynamic interactions reduce the peak force at high stretching speeds

Constant force stretching

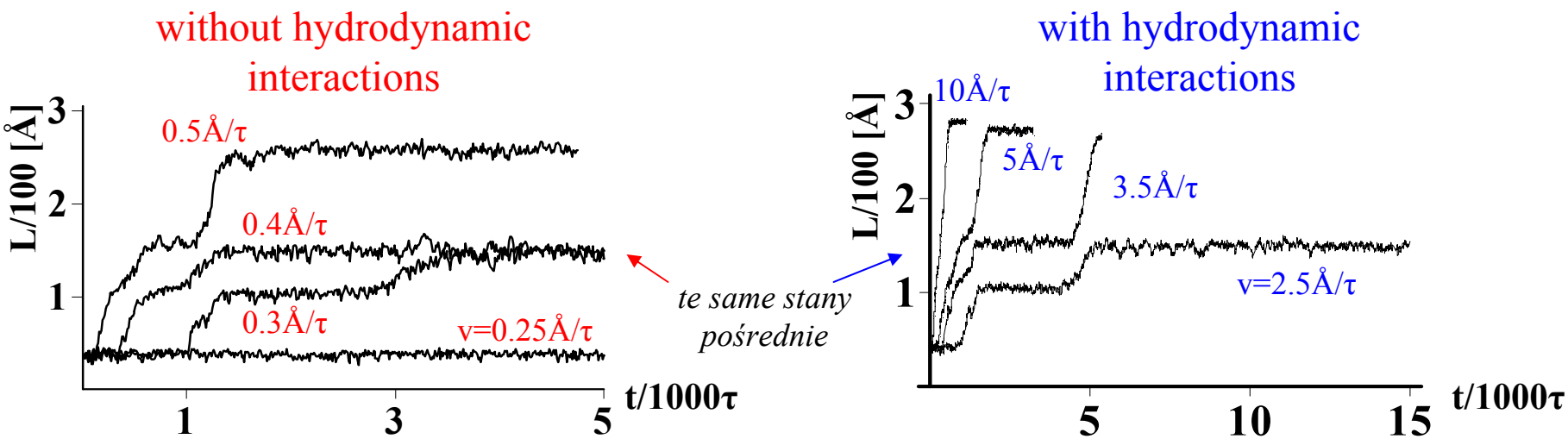


Hydrodynamic interactions considerably facilitate force clamp unfolding due to the dragging effect

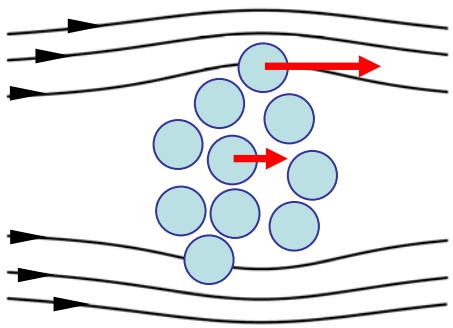


the moving particle creates a flow pattern which affects other particles by pulling them in the direction of its motion

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.
- HI also reduce peak forces in unfolding at a constant speed, although this effect weakens with the diminishing stretching speed.