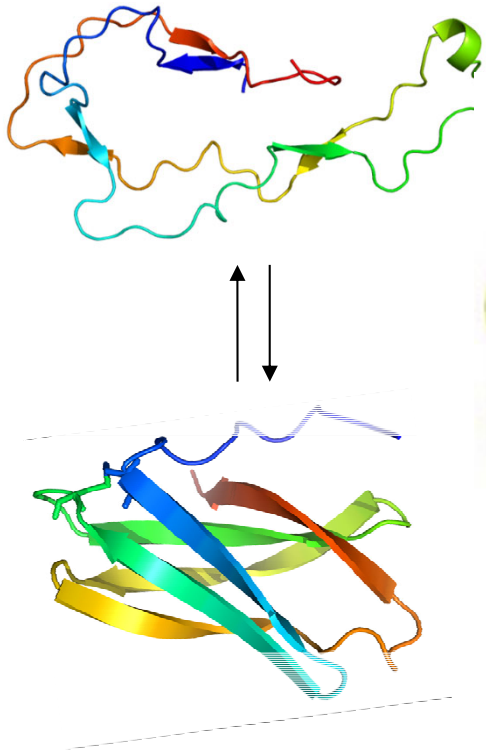
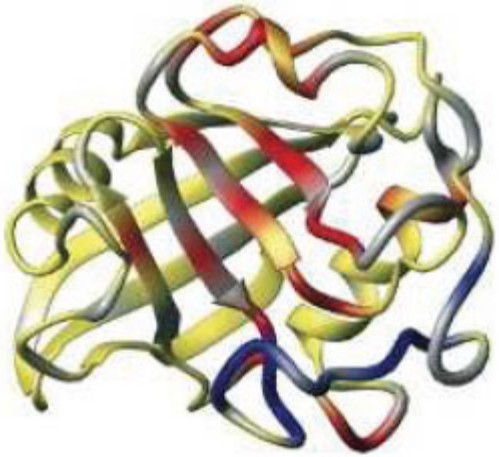
Structrual dynamics in proteins

Protein folding

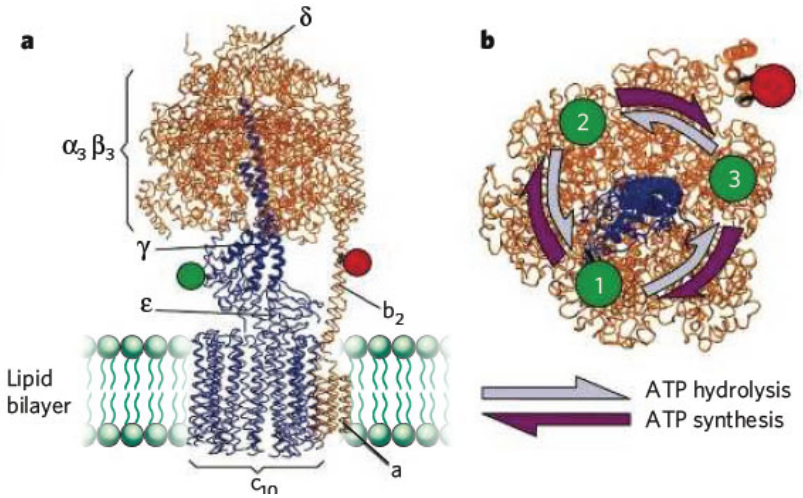


Protein equilibrium fluctuations



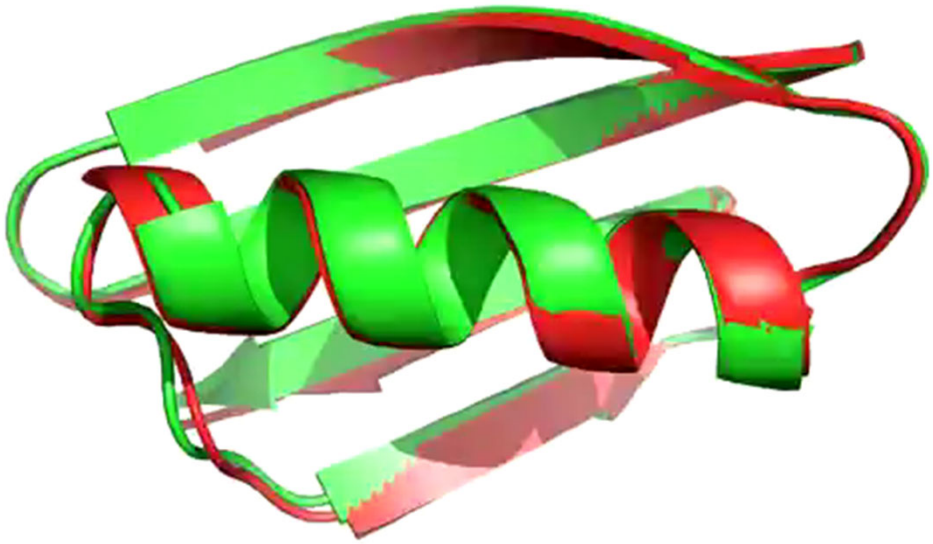
Protein function

Enzymes
Sensors
Channels



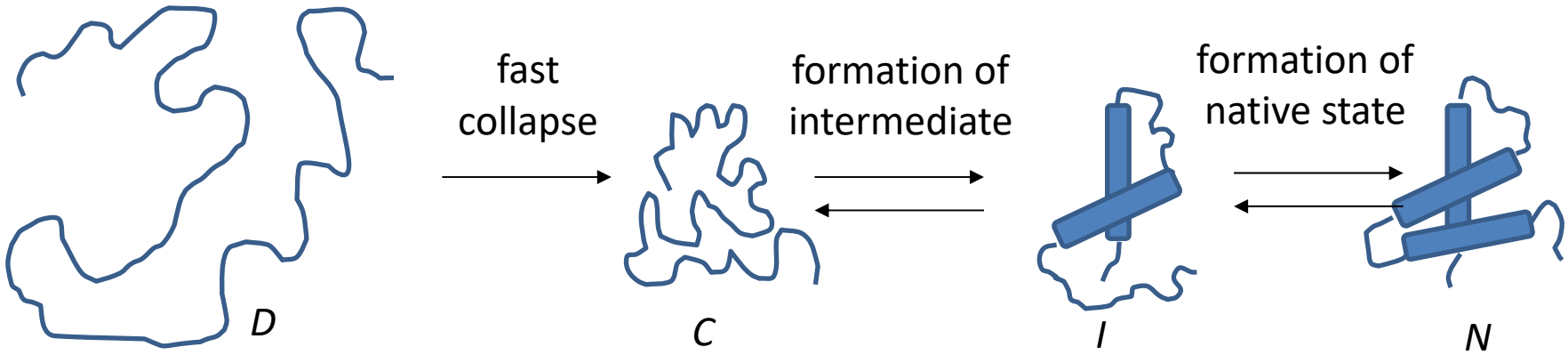
Dynamic nature of proteins

- **X-ray structure:**
  - most abundand state
  - static picture
- **In reality:**
  - proteins are very dynamic
  - dynamics over many timescales
- **Visualization**:
  - Molecular dynamics simulation



Protein G, OPLS-AA
AS Christensen

A protein folding reaction



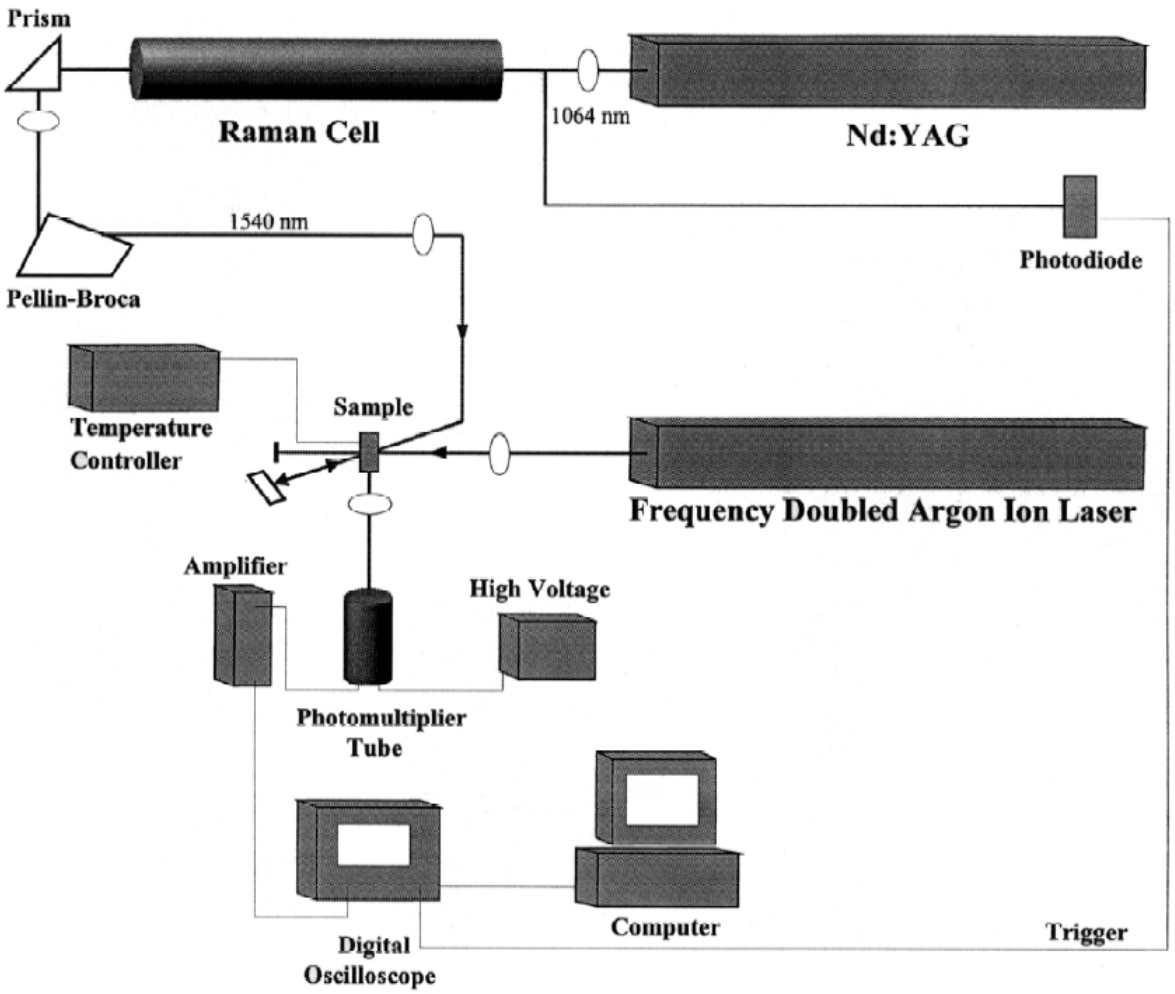
under denaturing conditions

native conditions

small proteins: two state folding

-> cont. notepad

Measuring relaxation kinetics: T-jump



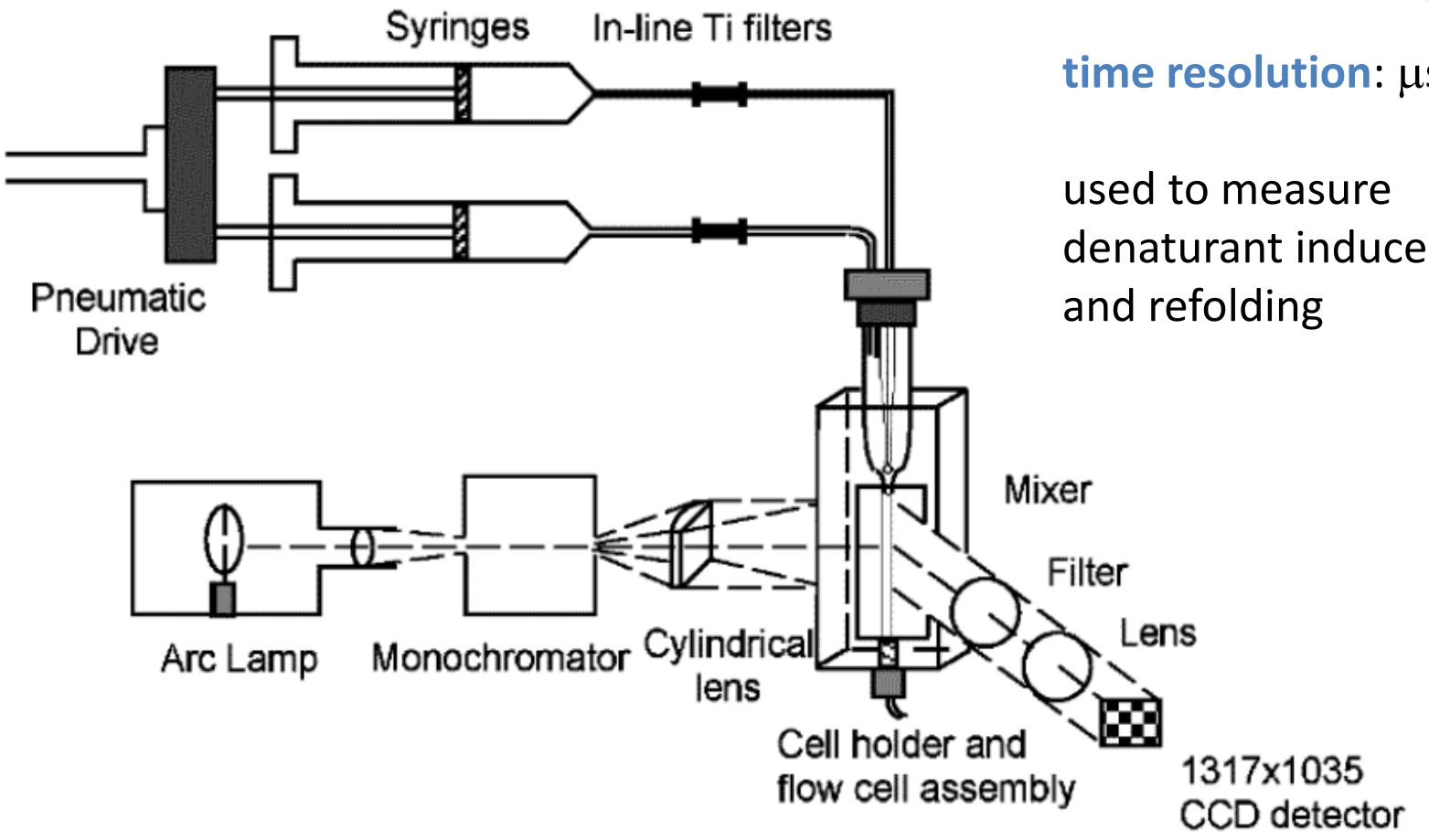
prerequisite: $\Delta H^0 \neq 0$

time resolution: ns-ms

thermal unfolding of secondary structure elements

refolding of cold-denatured proteins

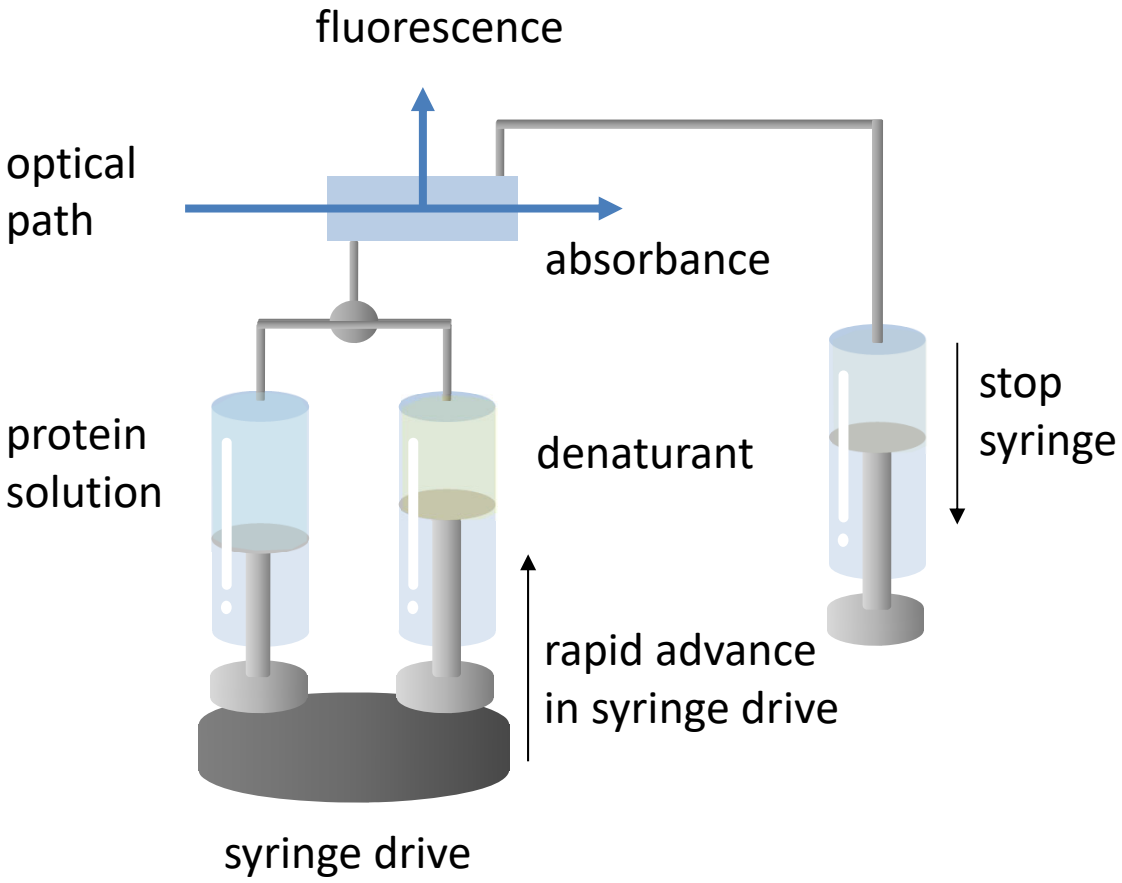
Measuring relaxation kinetics: Continuous flow



time resolution: μs-ms

used to measure denaturant induced un- and refolding

Measuring relaxation kinetics: Stopped flow



Stopped flow experiment:

time resolution: 1 ms – 1000 s

Relaxation kinetics are recorded using:

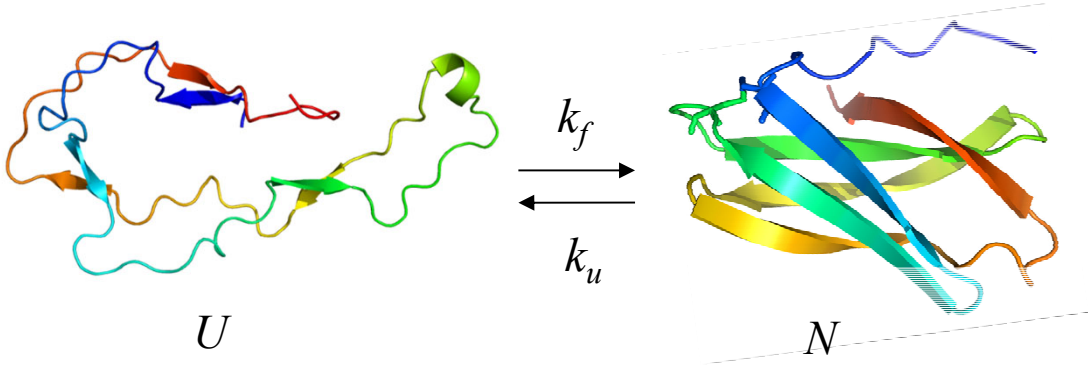
- absorbance
- fluorescence
- FRET
- CD
- anisotropy

Protein folding/unfolding

Protein-protein interactions

protein ligand interactions

Case study: Folding kinetics of a small protein



apparent rate constant:
$\lambda = k_f + k_u$

refolding branch

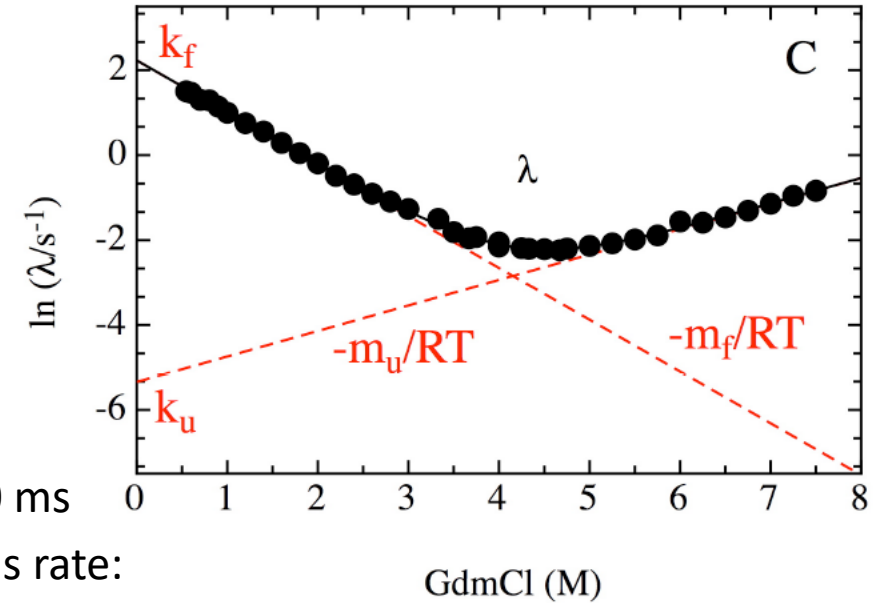
rapid dilution of unfolded protein into refolding buffer

$k_f(H2O) = 9.3\ s^{-1}$

$m_f = 3.0\ (kJ/mol)/M$

relaxation time: 100 ms

vgl. protein synthesis rate: 20 amino acids/s



unfolding branch

rapid dilution of folded protein into unfolding buffer

$k_u(H2O) = 4.8 \cdot 10^{-3}\ s^{-1}$

$m_u = -1.5\ (kJ/mol)/M$

relaxation time: 208 s

$\alpha = 0.67$

Bachmann & Kiefhaber, Protein Folding Handbook, Wiley 2005

Temperature dependence of protein folding

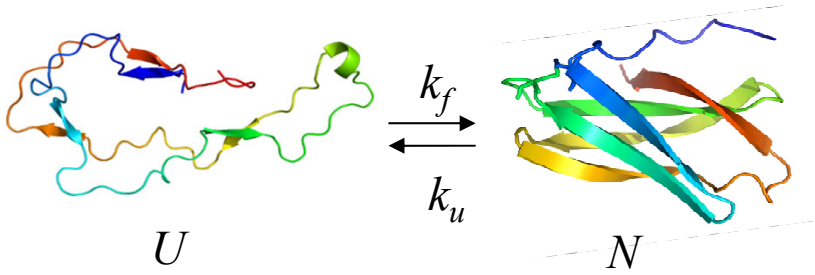
The rate constants are highly dependent on temperature:

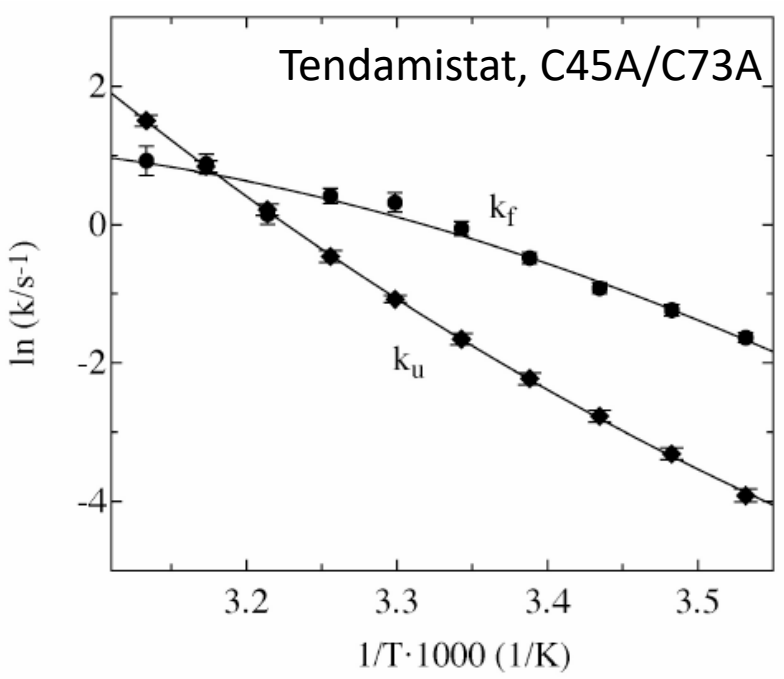
$$k = k_0 e^{-\Delta G^{0\ddagger}/RT}$$

with $\Delta G^0 = \Delta H^0 - T\Delta S^0$

$$k = k_0 e^{-\Delta G^{0\ddagger}/RT} = k_0 e^{-\Delta S^{0\ddagger}/R} \cdot e^{-\Delta H^{0\ddagger}/RT}$$

→ determination of $\Delta H^{0\ddagger}$ and $\Delta S^{0\ddagger}$.





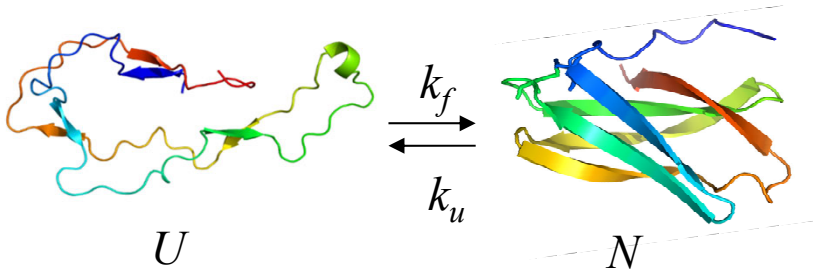
Temperature dependence of protein folding

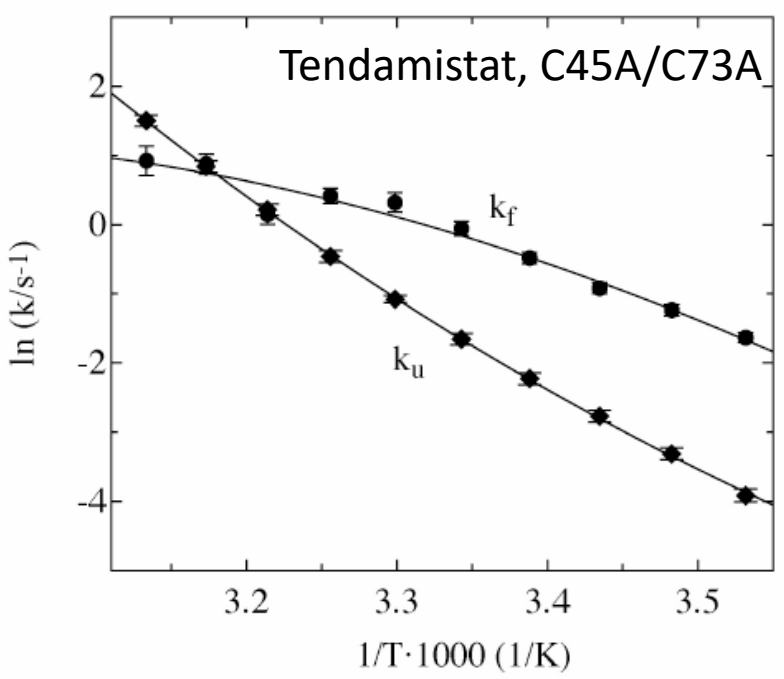
Curvature in k_f and k_u:

temperature dependence must include contributions from $\Delta C_p^{0\ddagger}$

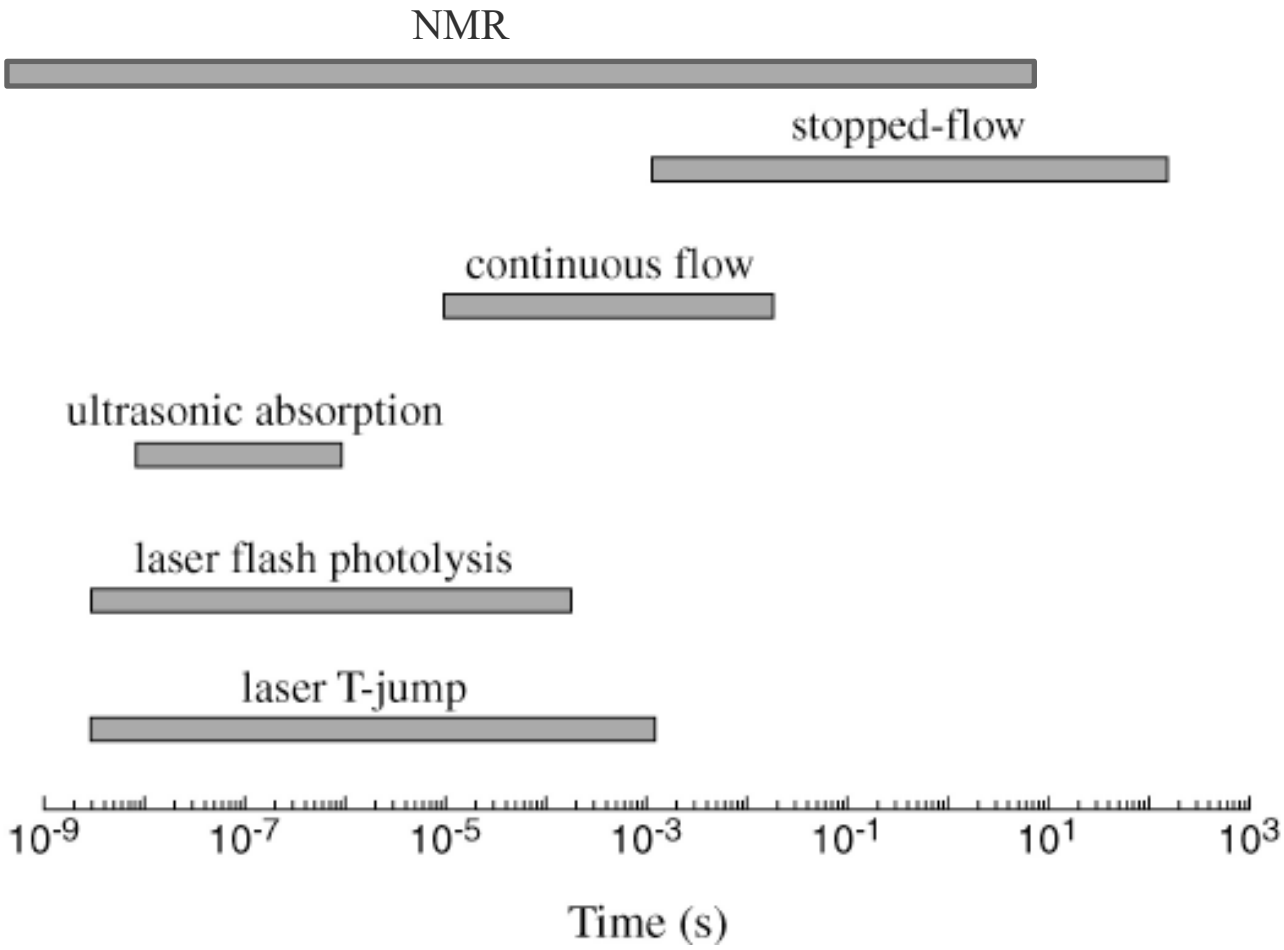
thus, the rate equation must be modified:

$$\ln k = \ln k_0 - \frac{1}{RT}[\Delta H^{0\ddagger}(T_0) - T\Delta S^{0\ddagger}(T_0) + \Delta C_p^{0\ddagger}\left(T - T_0 - T\ln\frac{T}{T_0}\right)]$$

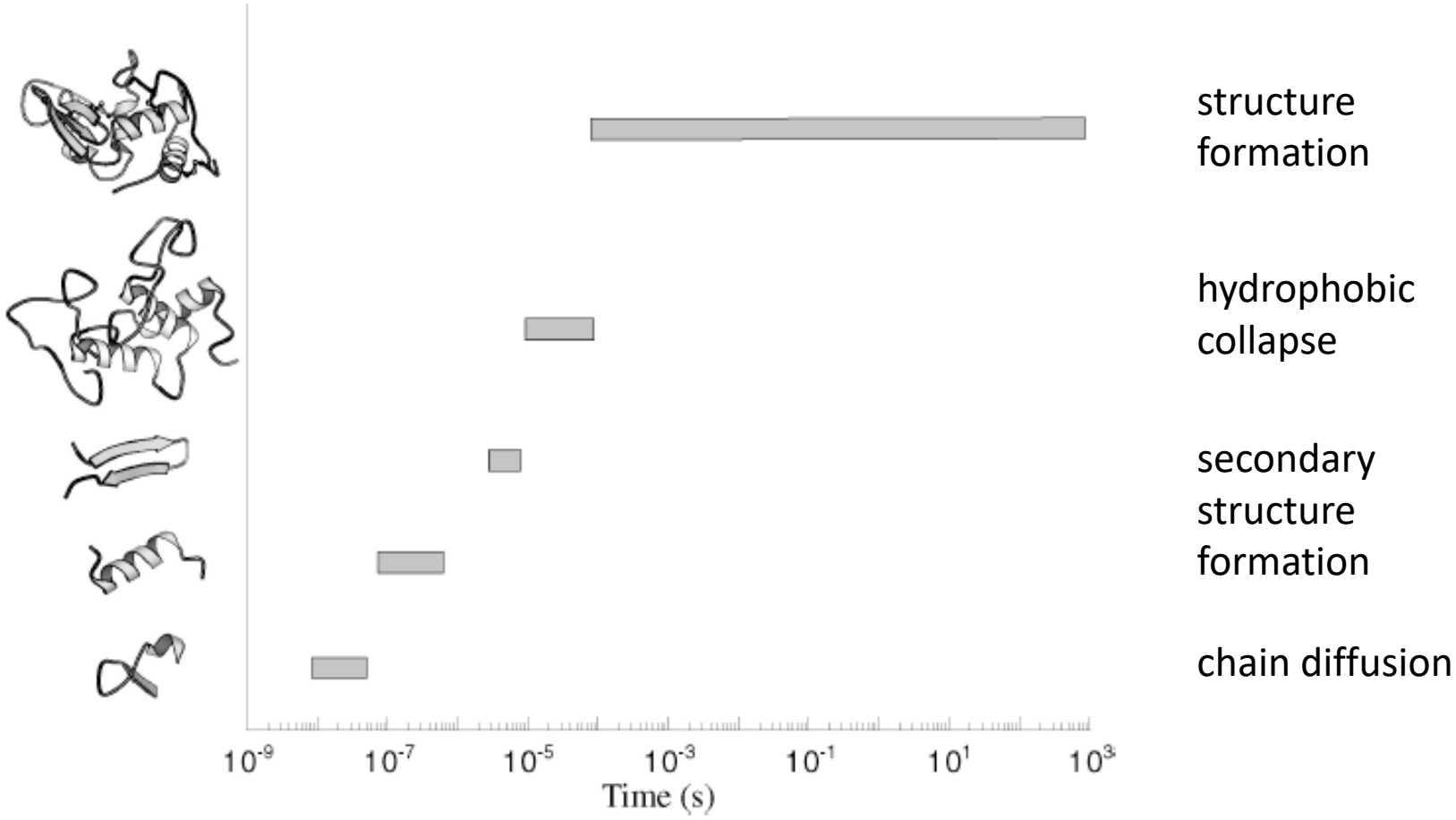




Overview of the time-resolution of different methods

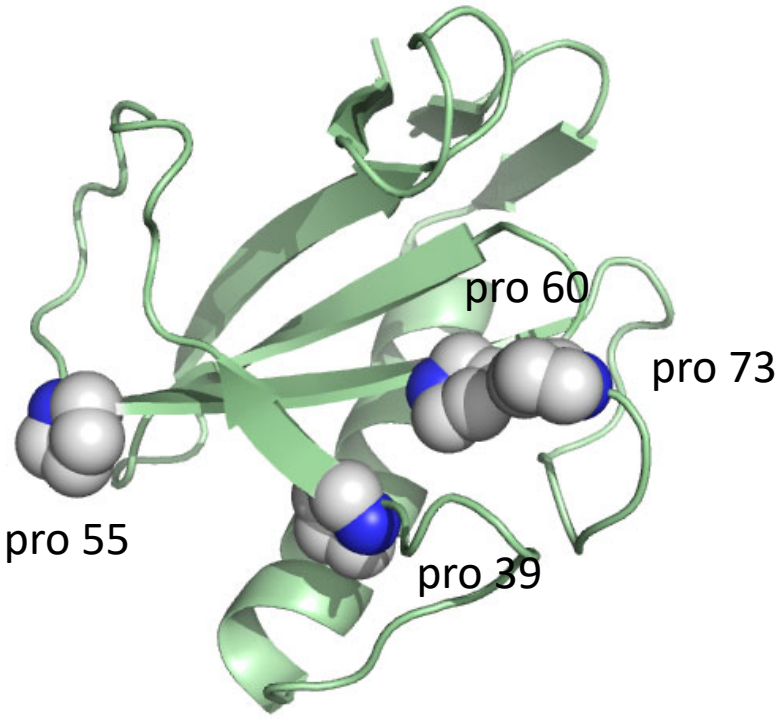


Time scale of protein folding reactions



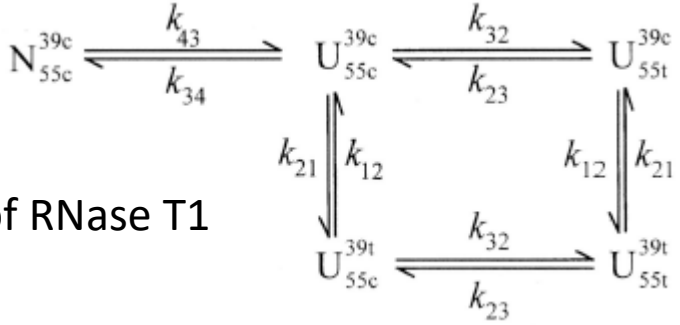
Slow reactions in protein dynamics

Proline isomerization



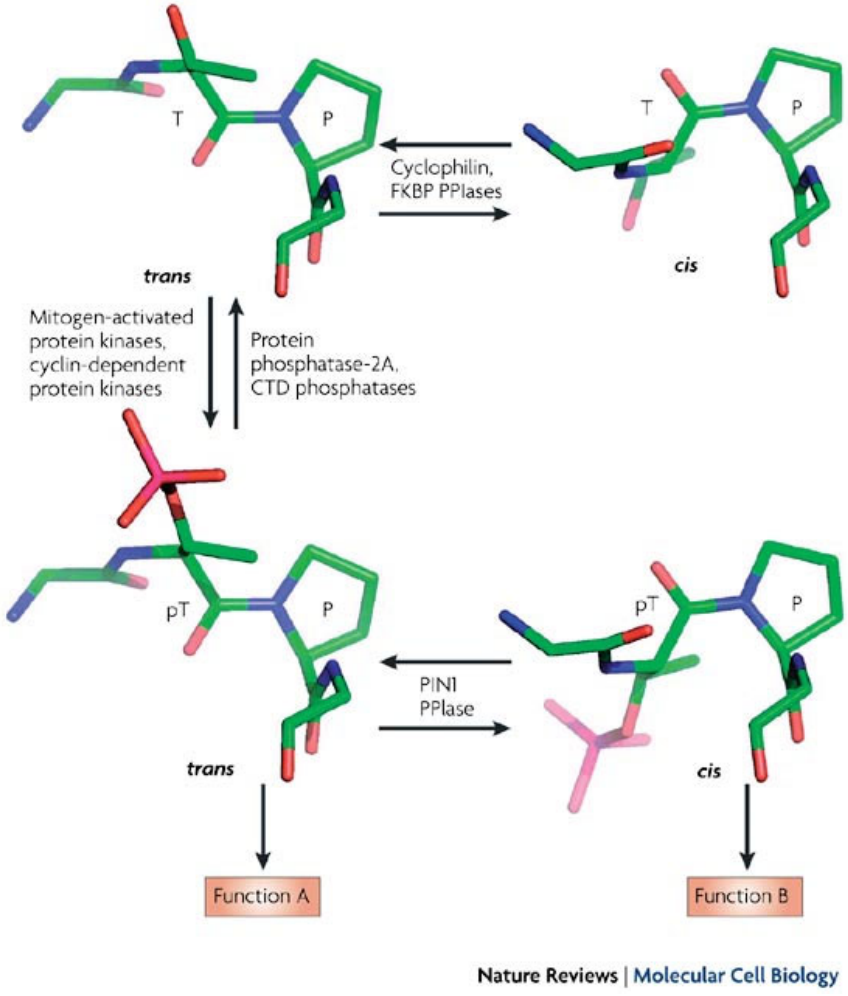
RNase T1

- A high fraction of Xaa-Pro residues are found in *cis*-conformation
- cis-trans isomerization of Xaa-Pro is slow: $t = k_{ct} + k_{tc} \sim 60\ s$
- activation energy is high: $E_A \sim 84\ kJ/mol$
- this results in slow folding reactions
- the number of slow-folding molecules increases with the number of prolines present



folding mechanism of RNase T1

Proline cis-trans isomerases



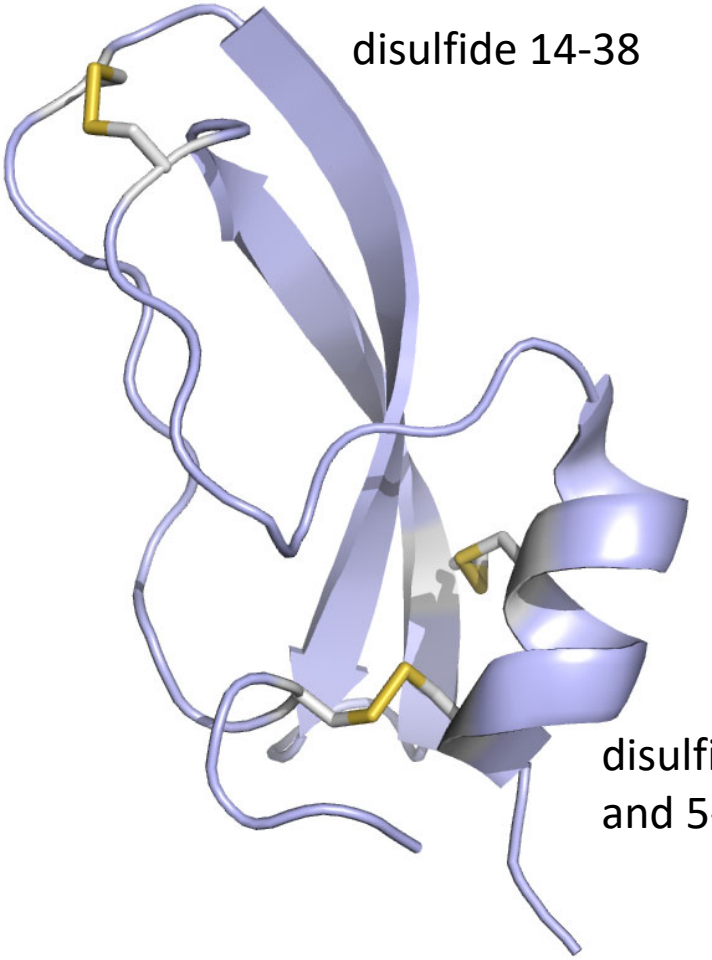
Folding acceleration

Signaling function

PIN1: Phospho-switch

Kun Ping Lu & Xiao Zhen Zhou
Nat Rev Mol Cell Biol 2007

Slow reactions in protein dynamics: Disulfide bonds



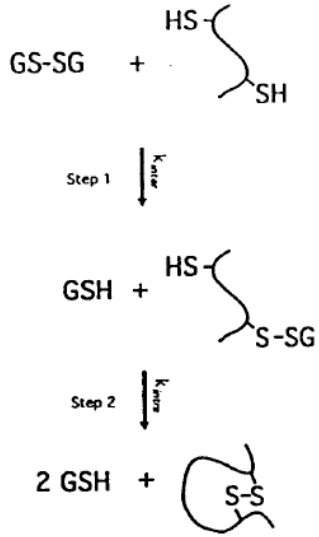
Bovine pancreatic trypsin inhibitor BPTI

Scrambling of disulfide bonds and re-oxidation can produce 15 isomers

$$\frac{1}{5}\cdot\frac{1}{3}\cdot\frac{1}{1}=\frac{1}{15}$$

with all disulfides reduced, BPTI is in a semi-folded state

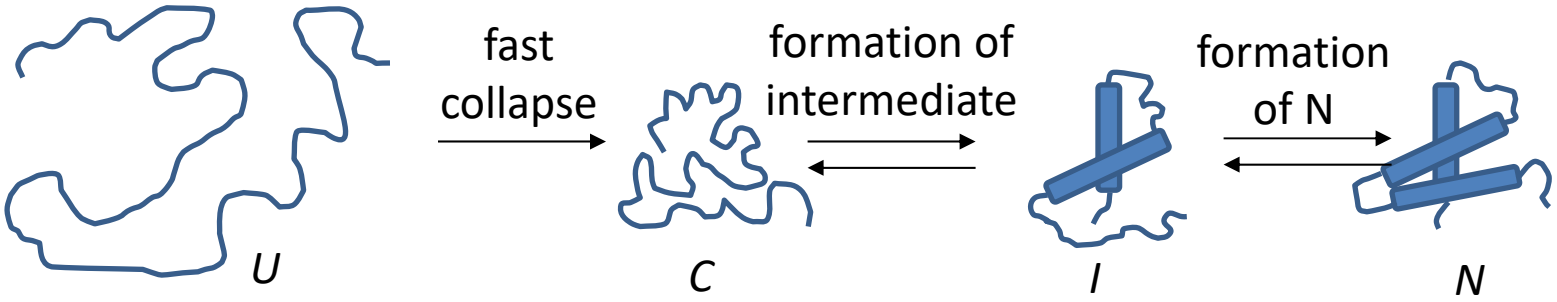
Refolding requires a redox-system, e.g. glutathione



→ coupling of oxidation and folding

Protein folding mechanisms

Folding mechanism: (for larger/more complex proteins)



$$S_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} S_2 \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} S_3 \rightleftharpoons \cdots \underset{k_{N\,N\text{-}1}}{\overset{k_{N\text{-}1\,N}}{\rightleftharpoons}} S_N$$

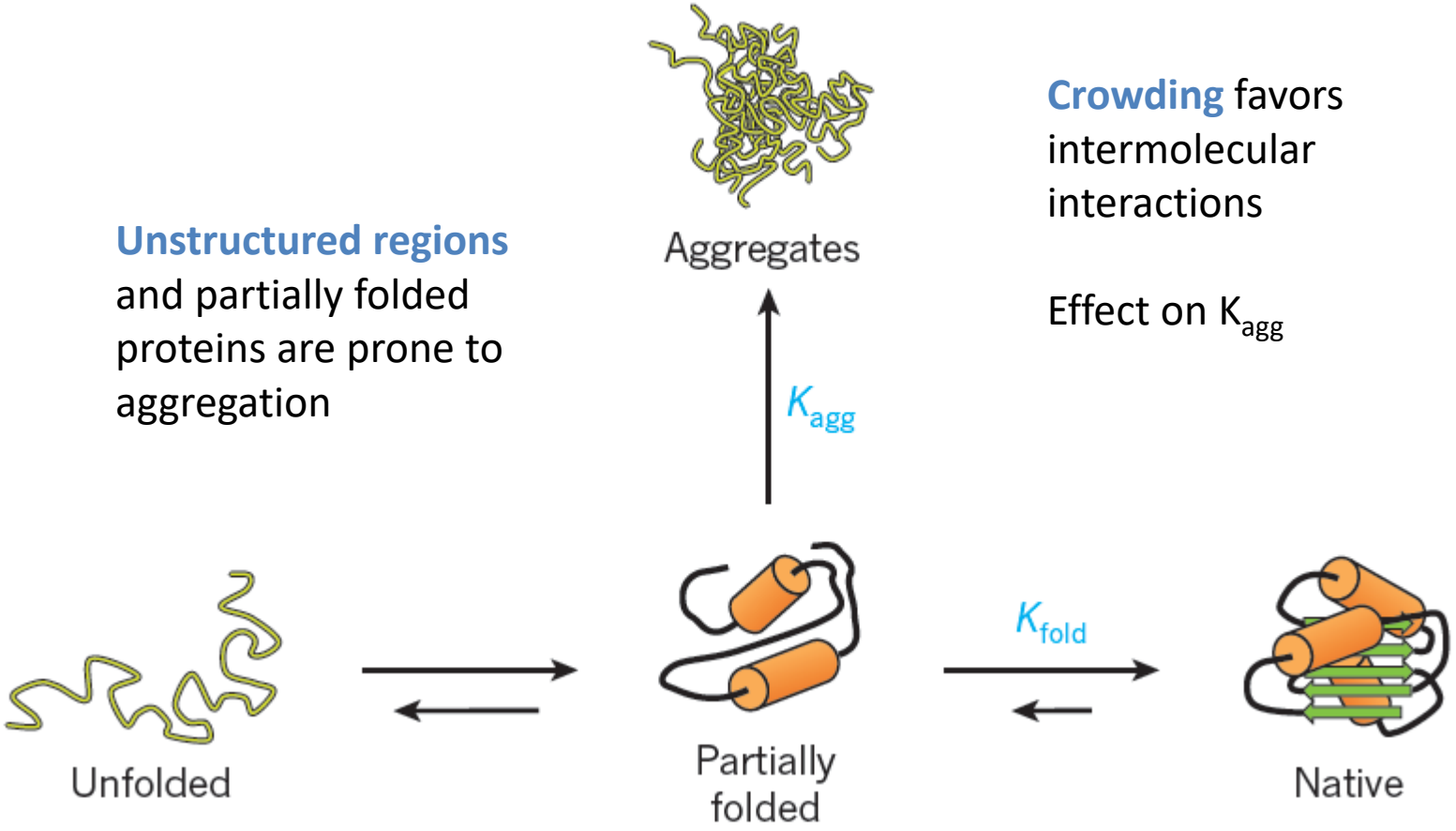
kinetics of formation of S_N

$$x(t) = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + \ldots + A_{N-1} e^{-k_{N-1} t}$$

for N kinetic species, N-1 exponential phases can be observed

Protein aggregation as a side reaction of folding

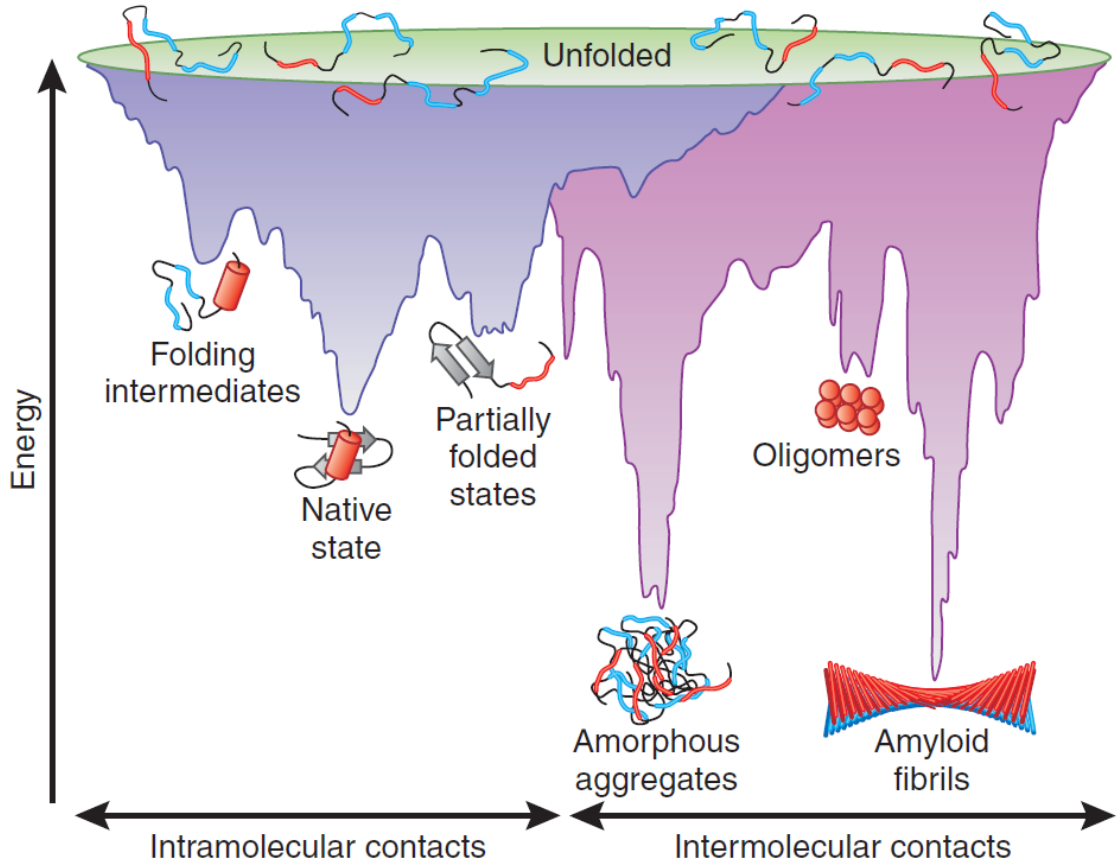
Unstructured regions and partially folded proteins are prone to aggregation



Crowding favors intermolecular interactions

Effect on K_{agg}

Free energy and aggregation



Hartl & Hartl, NSMB 2009

Non-productive folding intermediates - aggregation

Protein misfolding and aggregation are associated with some (neurological) diseases

- *Bovine spongiform encephalopathy (mad cow disease)*
- *Creutzfeld-Jacob disease (human) / Scrapie (sheep)*
- *Alzheimer, Parkinson* *(ca 20% of persons >75 years)*
- *Diabetes II* *(2009: 57 Mio in the USA)*

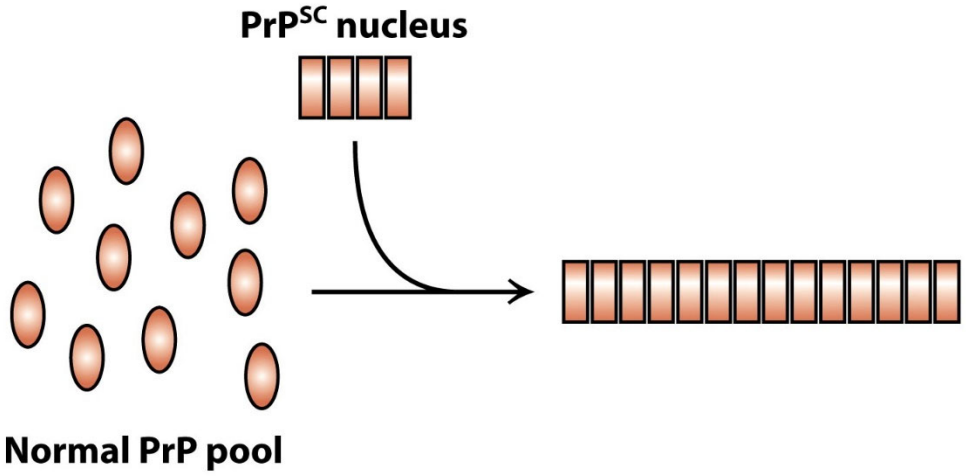
These diseases result in the deposition of protein aggregates called ***amyloid fibrils*** which in a later stage form ***plaques***.

A normally soluble protein is converted into insoluble fibrils rich in b-sheets.

The correctly folded protein is only marginally more stable than the incorrect form.

However, as the incorrect form aggregates, it pulls more correct forms into the incorrect form.

Prion-hypothesis



Prion hypothesis: Disease purely transmitted by the misfolded proteins (prions, amyloids)**xx**

A nucleus of proteins in the abnormal conformation grows by the addition to proteins in the normal form

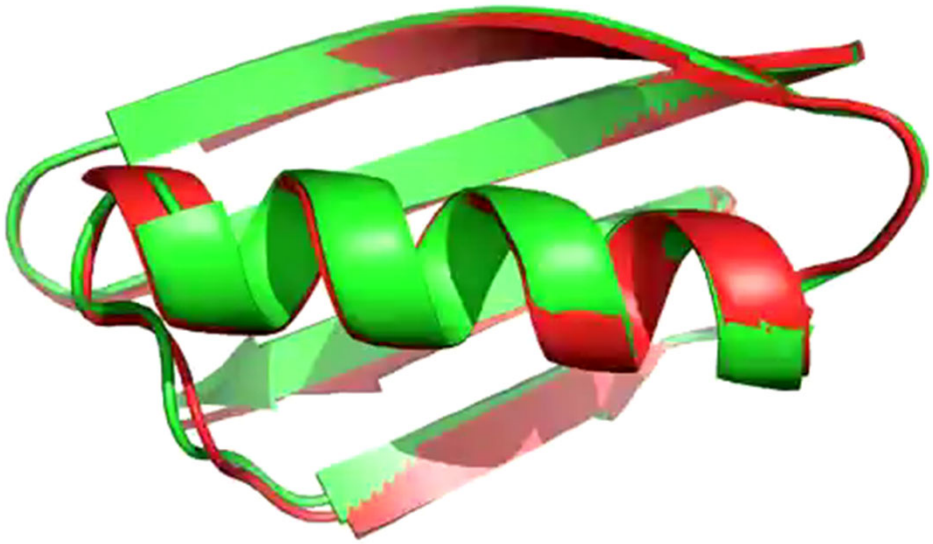
Stanley Prusiner (Nobel Prize in medicine 1997)

1. *Transmissible agent: aggregated form of a specific protein*
2. *Protein aggregates are resistant to degrading agents*
3. *Protein is largely derived from cellular protein called PrP, normally present in brain*

Dynamic nature of proteins

- **X-ray structure:**
  - most abundand state
  - static picture

- **In reality:**
  - proteins are very dynamic
  - dynamics over many timescal



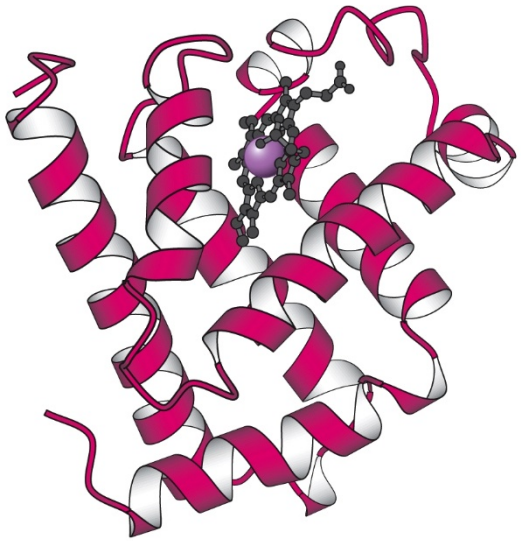
Protein G, OPLS-AA
AS Christensen

→ **functions of dynamic modes**

energy landscape of protein fluctuations

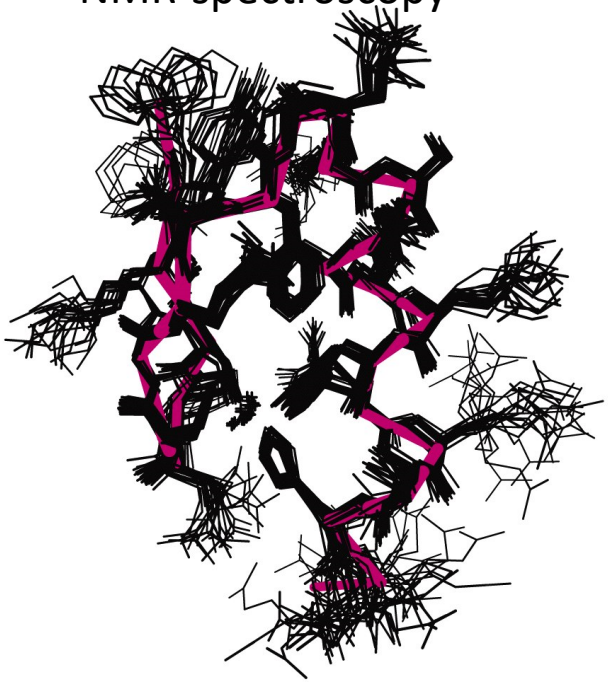
Protein dynamics

X-ray crystallography



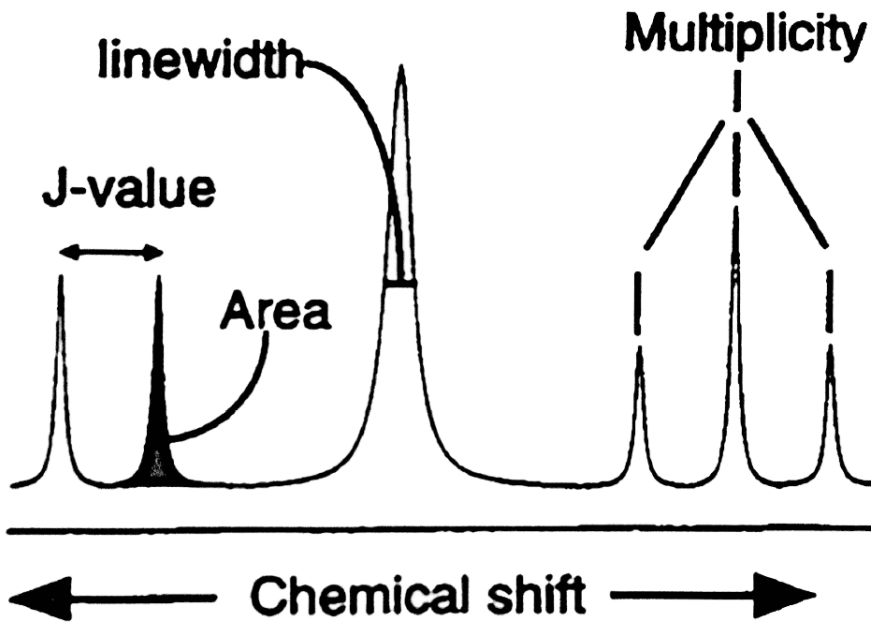
Crystallization required
averaged picture, no dynamics
no size limit

NMR-spectroscopy



Size limit (smaller proteins)
calculated ensemble of structures
protein solubility (μM)
Dynamics can be observed

The 5 parameters of a 1D ^{1}H NMR spectrum



- **chemical shift**
  expressed in ppm of the larmor frequency, shielding of the magnetic field due to the **chemical environment**

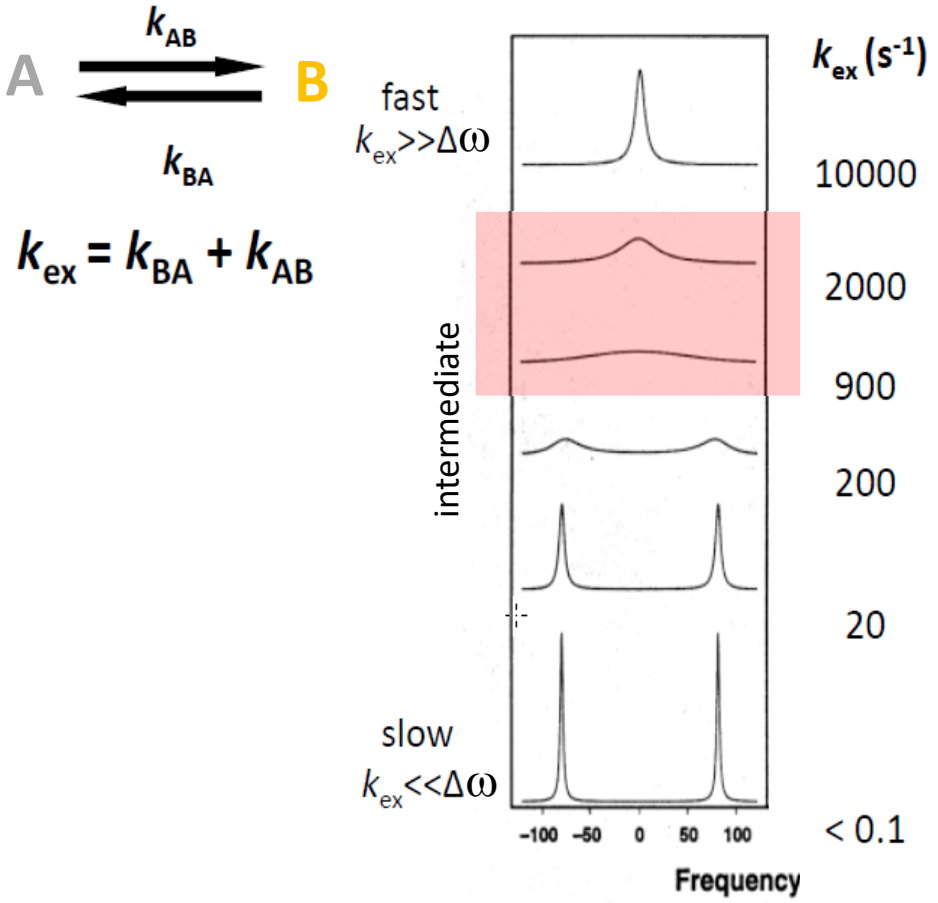
- **Integral**
  proportional to the **number of nuclei**

- **multiplicity & coupling constant**
  interactions between spins in the same molecule

- **linewidth**
  proportional to 1/T2, and thus dependent on **molecular motion**

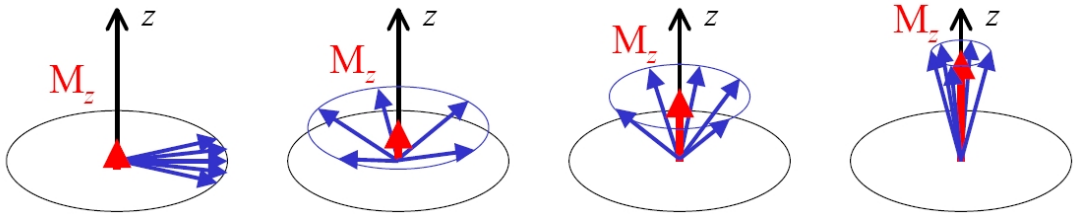
NMR peak shape - dynamics

- Equilibrium experiment
- chemical shift differences between two states → different ω (in Hz)
- ω (and thus also Δω) depends on magnetic field strength $\hbar\omega = \hbar\gamma_H B_0$
- if $k_{ex} >> \Delta\omega$, then only one average peak is measured, **fast exchange**
- if $k_{ex} << \Delta\omega$, then both peaks are detected, **slow exchange**
- in most cases, **intermediate exchange** is observed, here the line-width of the peak yields information about the exchange rate



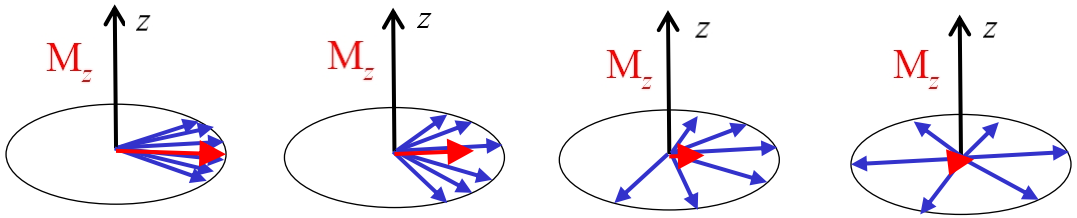
If we are in the intermediate exchange regime: what is the exact value of k_{ex}?

T1 and T2 relaxation



T1 (longintudinal, spin-lattice) relaxation

energy transfer to neighboring spins



loss of phase coherence

T2 (transverse, spin-spin) relaxation

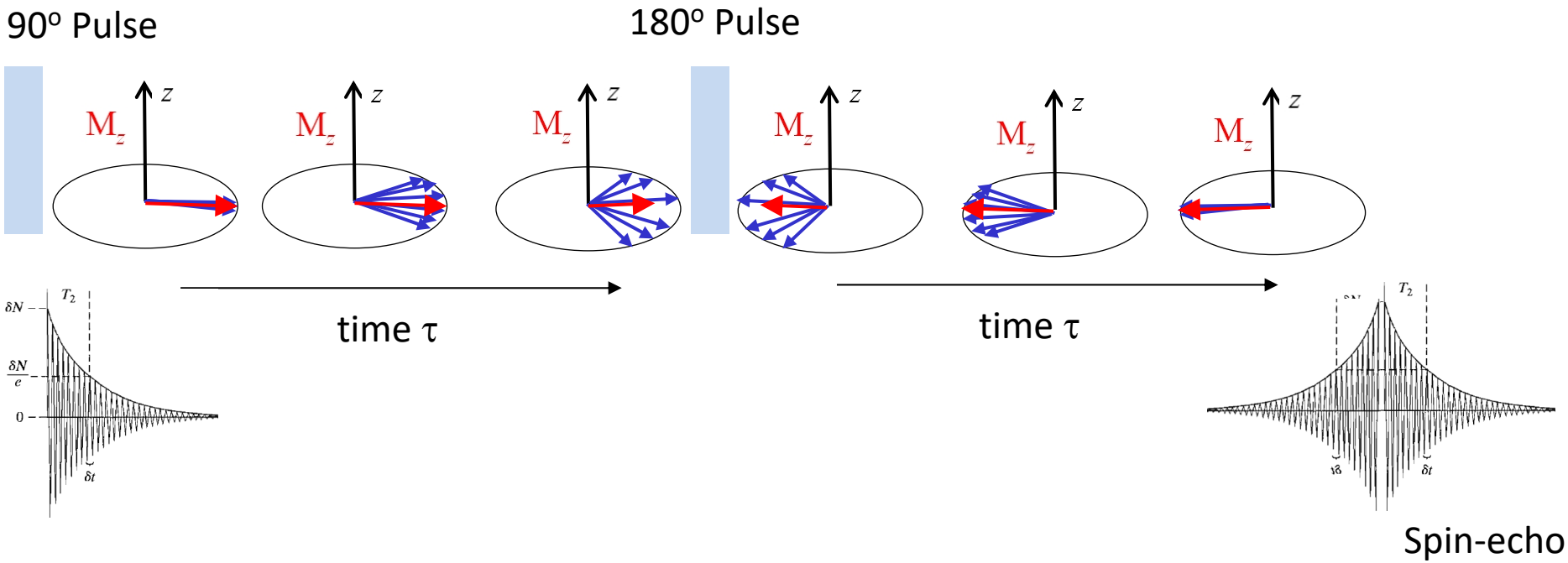
same or shorter as T1

loss of phase coherence due to neighboring dipolar fields

in **<u>solution</u>**, these dipolar fields average out -> **long T2**

in **<u>solid</u>**, dipolar field are almost static -> **short T2**

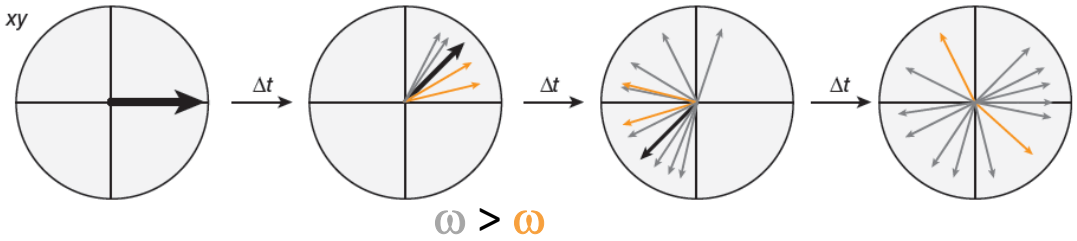
Spin-Echo experiments: Refocussing the spins



Spin-echo

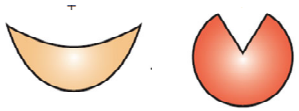
Using a 180° pulse after a waiting time τ, and measuring after the same time τ the spins were refocused, and the signal is back, independent of T2

k_{ex} can be determined by measuring transversal relaxation rates



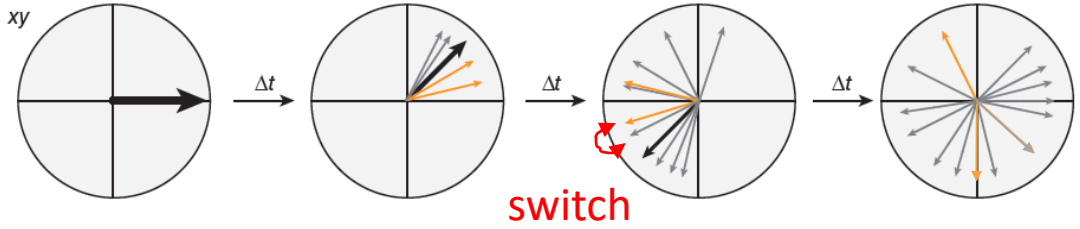
two chemical environments

$\omega > \omega$



A enzyme open

B enzyme closed



exchange

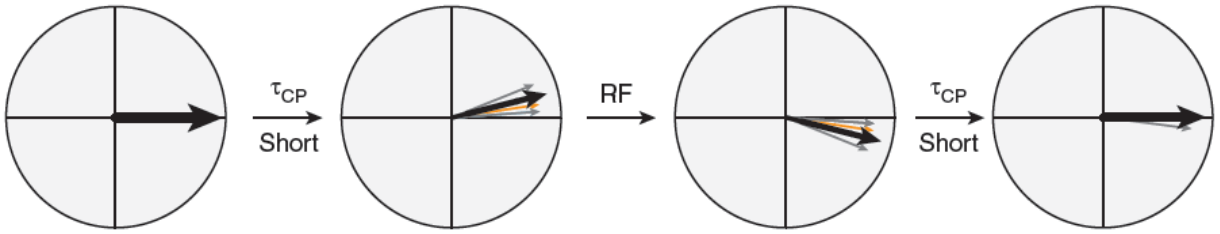
enzyme open

enzyme closed

What happens now in a spin-echo experiment?

Spin-echo experiment with exchange

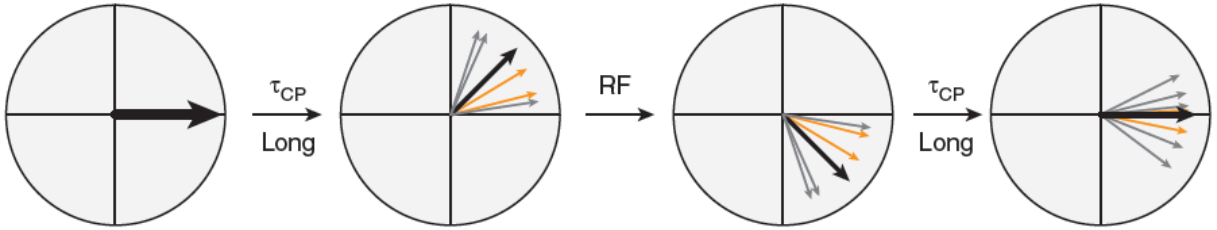
Fast exchange



if τ is shorter than $1/k_{ex}$, then the spins can still be efficiently refocused

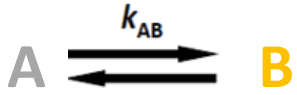
Signal intensity is recovered

Slow exchange



if τ is longer than $1/k_{ex}$, then the spins cannot be efficiently refocused

Signal intensity is lost



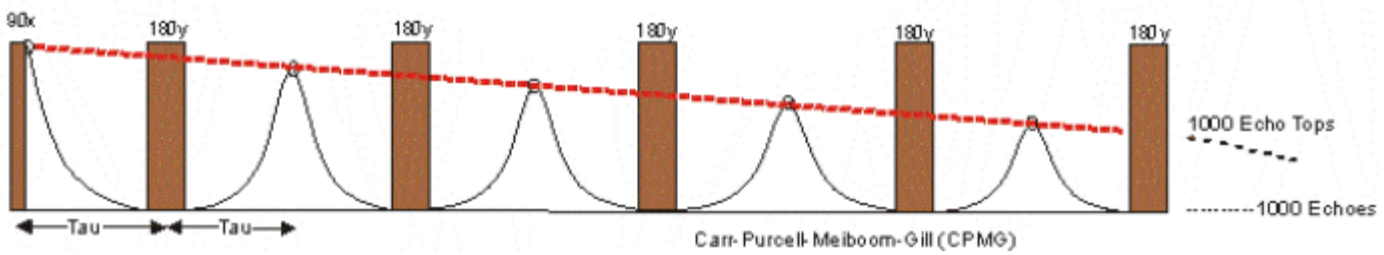
enzyme open

enzyme closed

CPMG experiment: Measuring $R_{2,eff}$ yields information on exchange kinetics

The Carr-Purcell-Meiboom-Gill sequence (*CPMG*): Sequence of n spin-echos

measure transverse T_2 relaxation times, or
relaxation rates $R_2 = 1/T_2$ of any nucleus



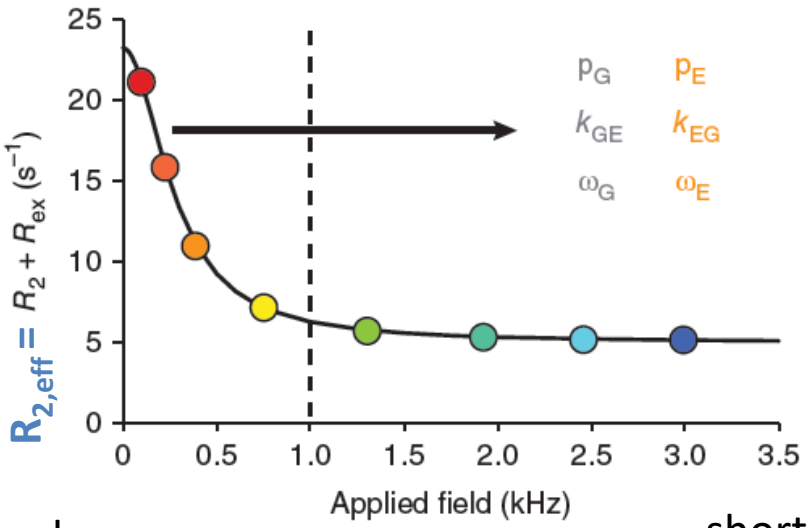
decay with $R_{2,eff}$

$R_{2,eff} = R_2 + R_{ex}$

when chemical exchange occurs and τ is longer than $1/k_{ex}$, intensity is lost with every spin-echo

this relaxation process, characterized by $R_{2,eff}$ depends on τ and contains all information about the chemical exchange process

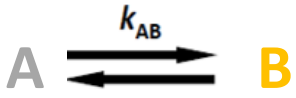
CPMG experiment: Measuring $R_{2,eff}$ yields information on exchange kinetics



long τ

$\nu_{CPMG} = 1/4\tau$

short τ



enzyme open

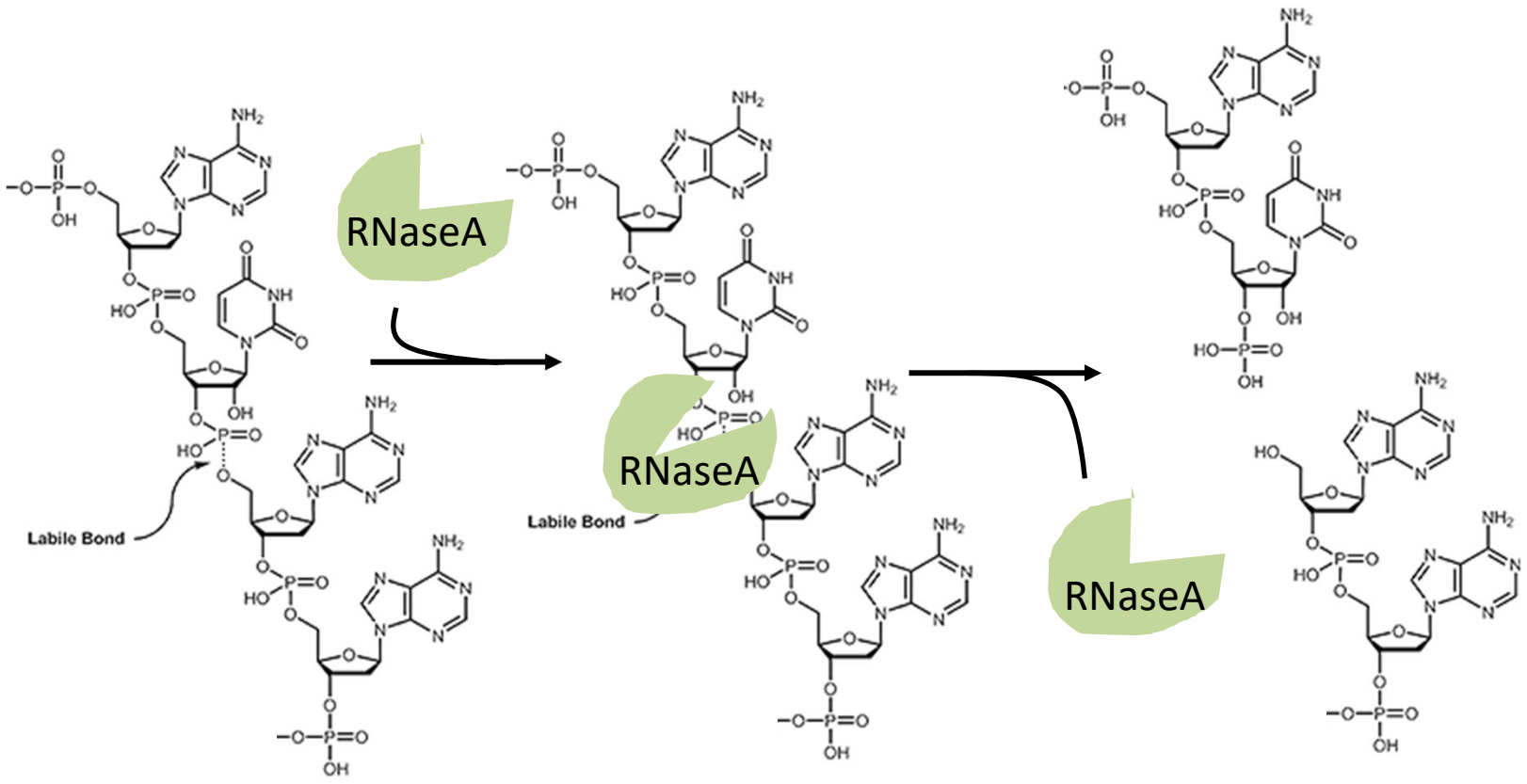
enzyme closed

at small CPMG frequencies (= long τ), the $R_{2,eff}$ is high, and signal is rapidly lost

at high CPMG frequencies (= short τ), the $R_{2,eff}$ is low, and signal decay is slow

Analyzing this decay curve yields the following parameters (on a per residue basis):	k_{AB}, k_{BA} population of A, population of B chemical shift of A, chemical shift of B

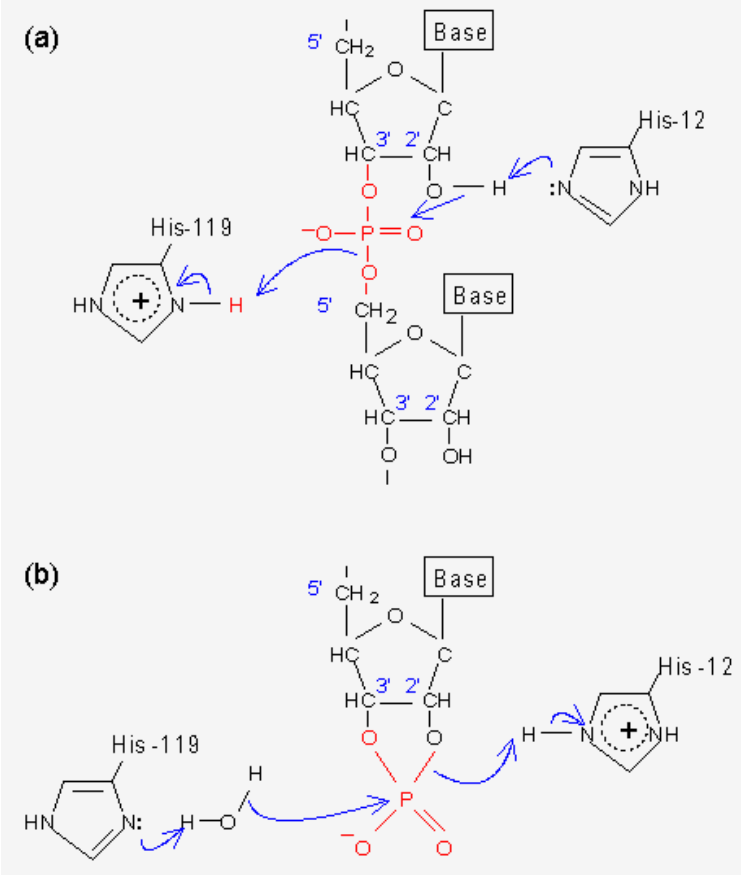
Example: Ribonuclease A



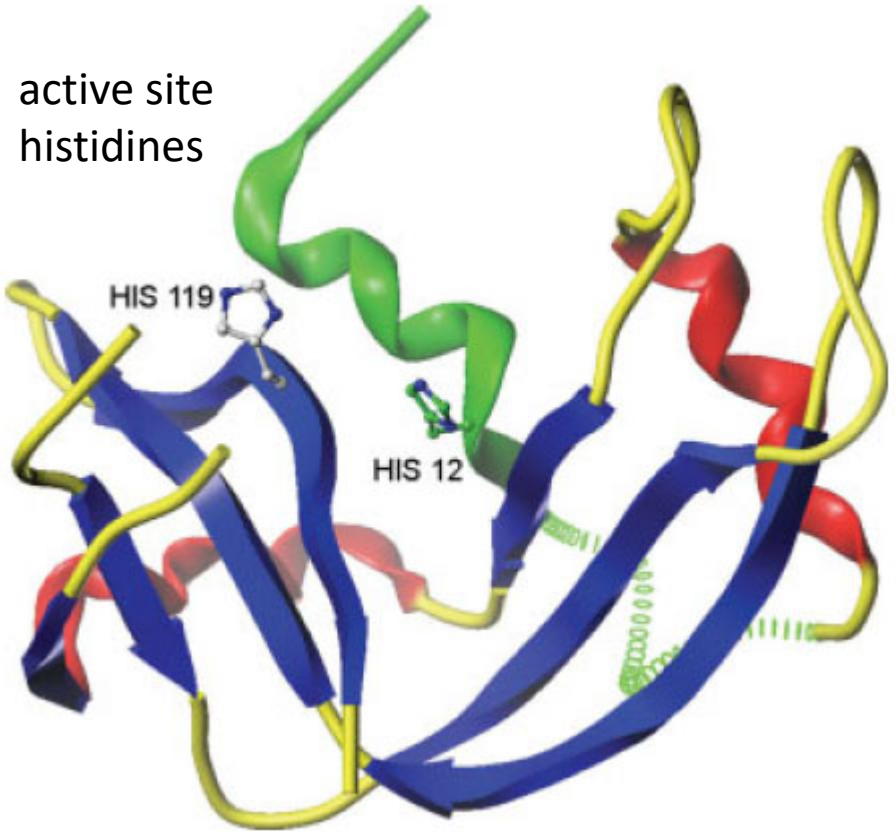
RNA RNaseA:RNA RNA_{cut}

Ribonuclease A – catalytic mechanism

hydrolysis of RNA in a two-step process:



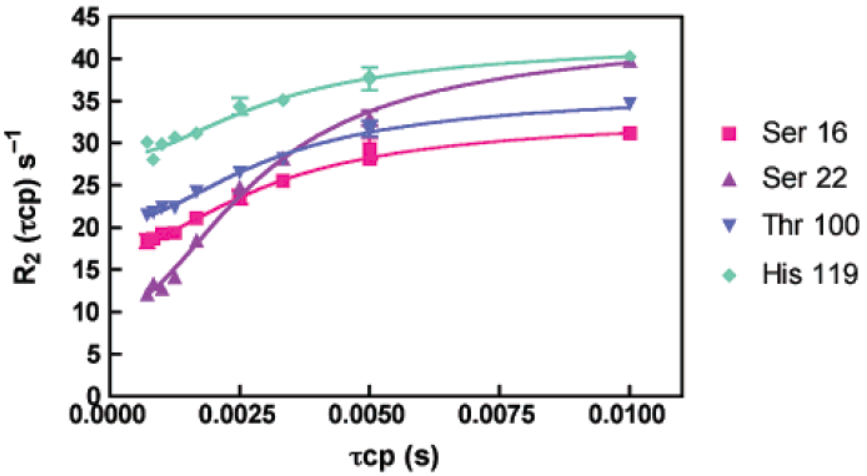
active site histidines



PDB entry 1DY5

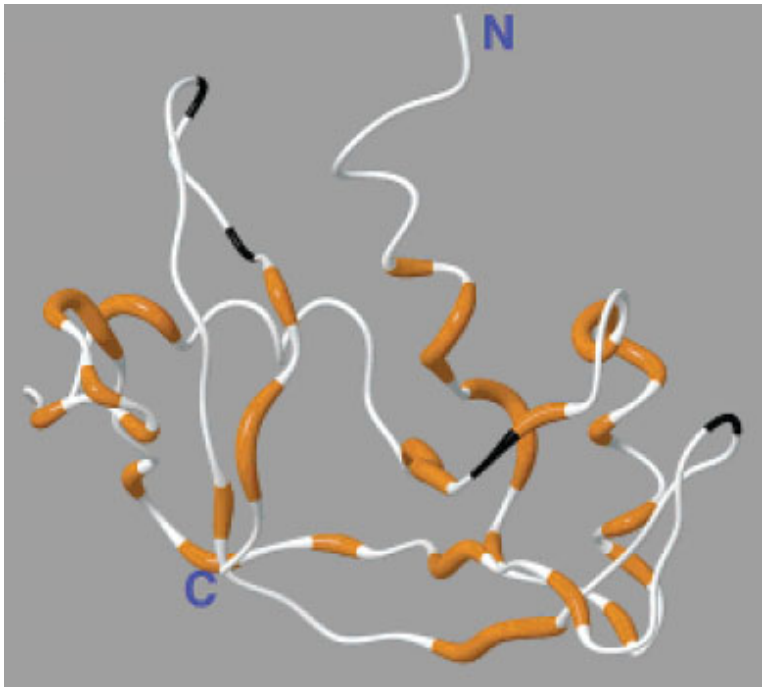
CPMG relaxation measurements

^{15}N relaxation dispersion data for RNase A at 283 K and 600 MHz.



apo RNase A
*k*ex = 1080 ± 80 s^{-1} (283K)

substrate bound RNAse A
*k*ex = 1316 ± 160 s^{-1} (283K)



Flexible residues can be mapped on protein surface

→ dynamics match enzyme kinetics

Beach et al. JACS 2005

Induced fit vs. Conformational Selection

Protein dynamics and substrate interaction

Proteins can adopt bound conformation in absence of ligand

ligand stabilizes this conformation

Protein dynamics limit enzymatic reaction speed