Exploring the Rugged Conformational Energy Landscape of Proteins

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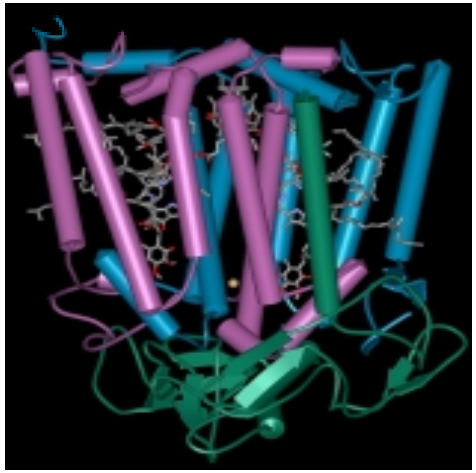
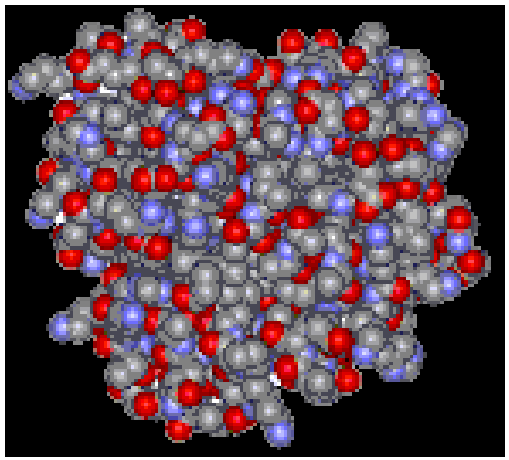
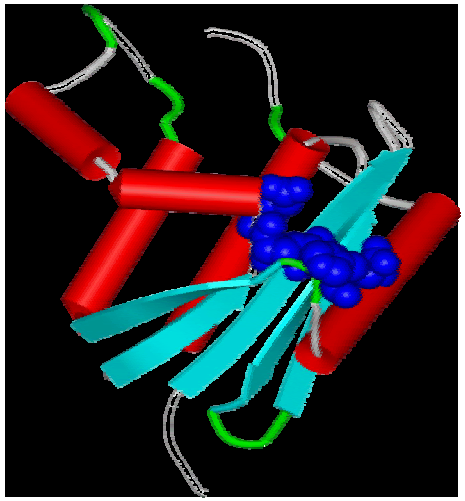
Department of Physics, University of Illinois at Urbana-Champaign







Proteins - Paradigms of Complex Systems

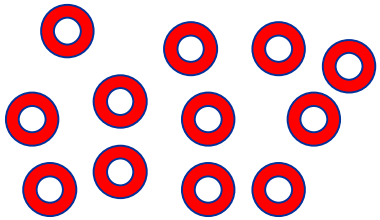
Systems

- Biomolecules
- Glass-forming liquids
- Synthetic polymers
- Spin glasses

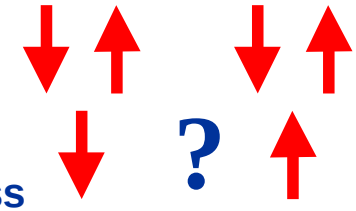
Essential Properties

- Distributed physical parameters
- Wide range of time scales
- Nonlinearity
- Non-Arrhenius temperature dependence

Disorder:

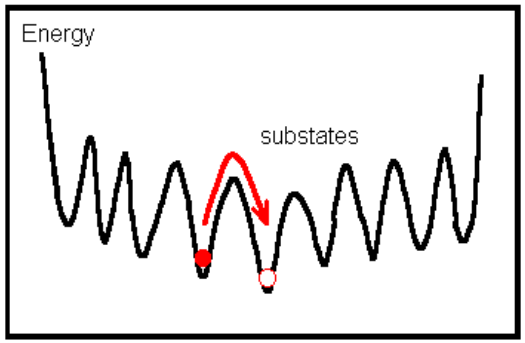


Frustration:



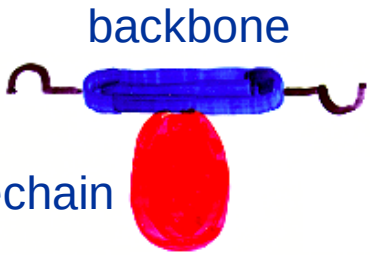
Spin glass

Rugged energy landscape



Proteins

- Building blocks:

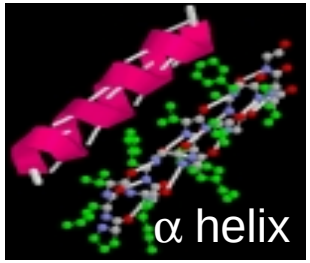


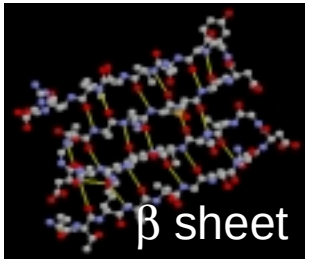
20 different amino acids

- Primary structure:

peptide (covalent) bonds

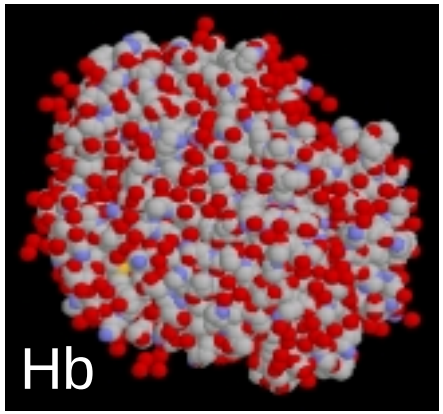
- Secondary structure:





hydrogen bonds

- Tertiary structure:

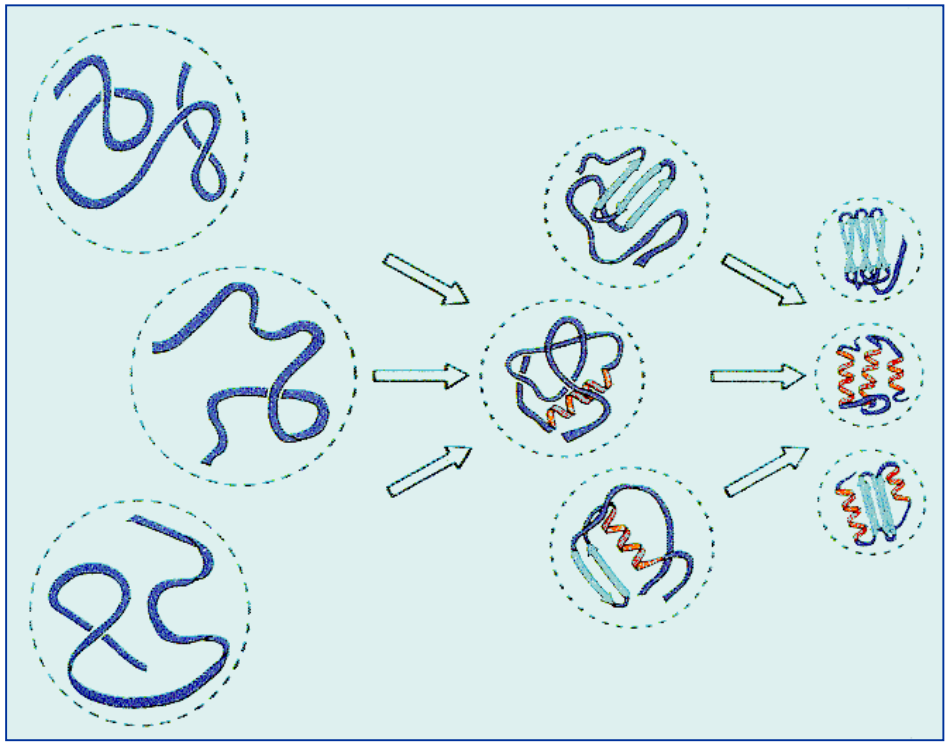


Sequence determines 3D fold

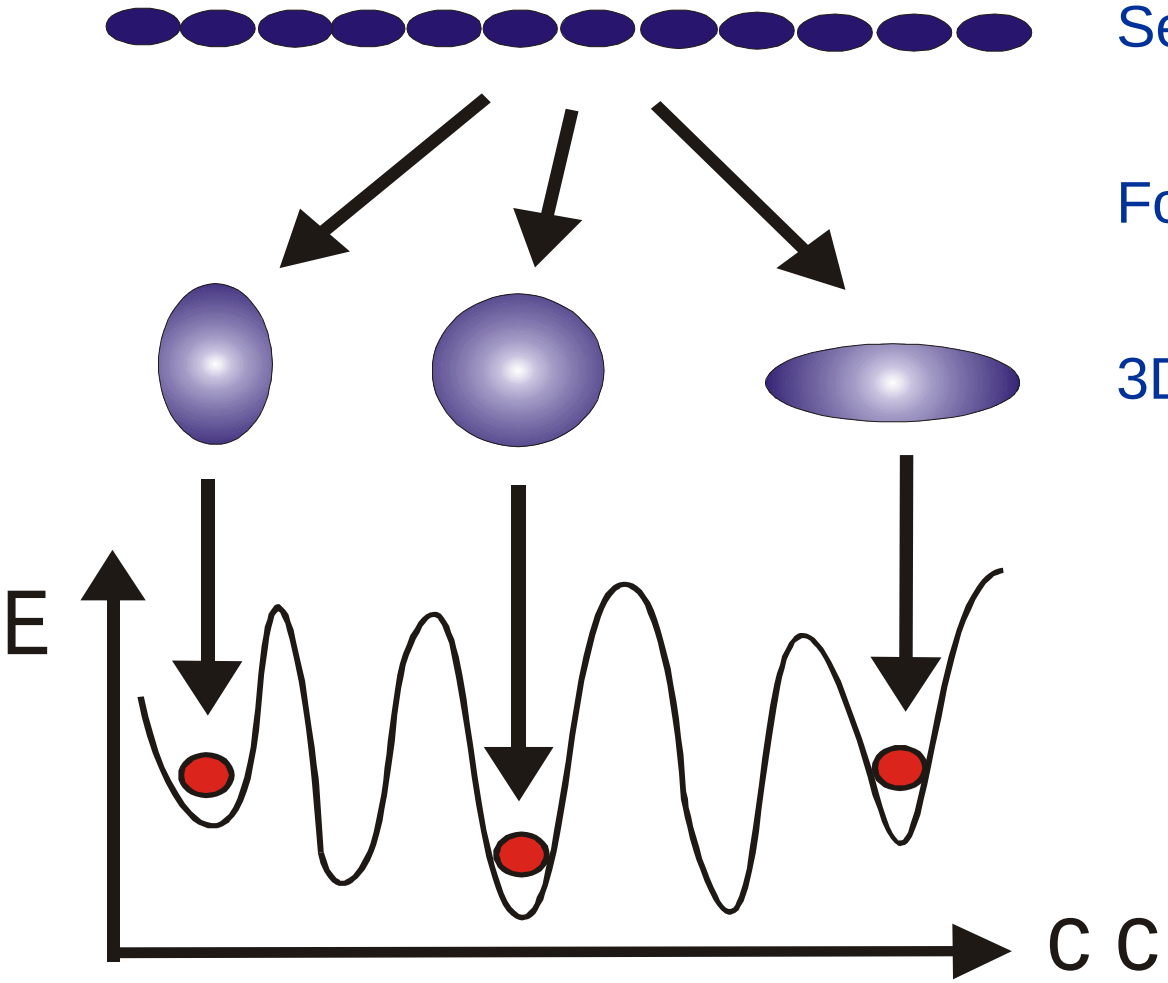
3D interactions:
- hydrophobic
- van der Waals
- H bonds / ionic / S-S

Protein Folding

- Huge number of possible chain conformations (~10^{150} for a protein with 150 amino acids).
- Folding decreases number of available conformations substantially.
- The native fold is not unique, but contains a large number of conformational substates.

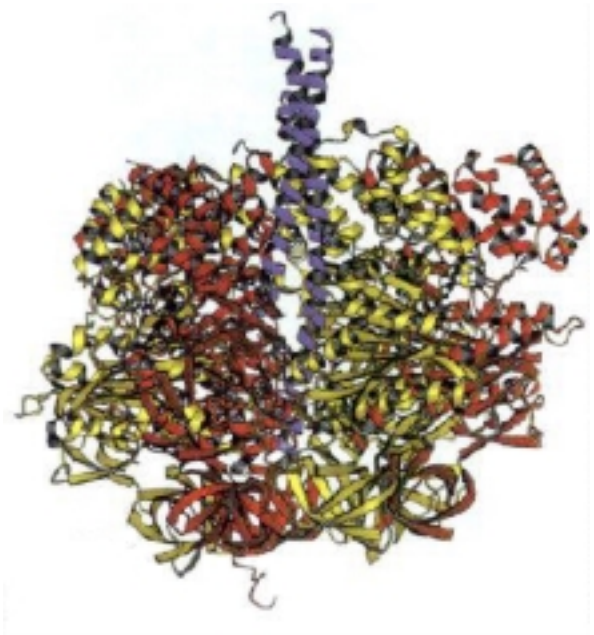


Energy Landscape of Folded Proteins

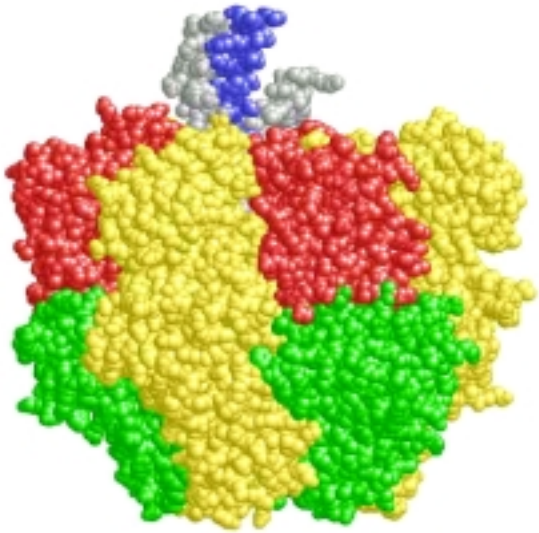


Conformational substates:
same overall structure,
details are different.

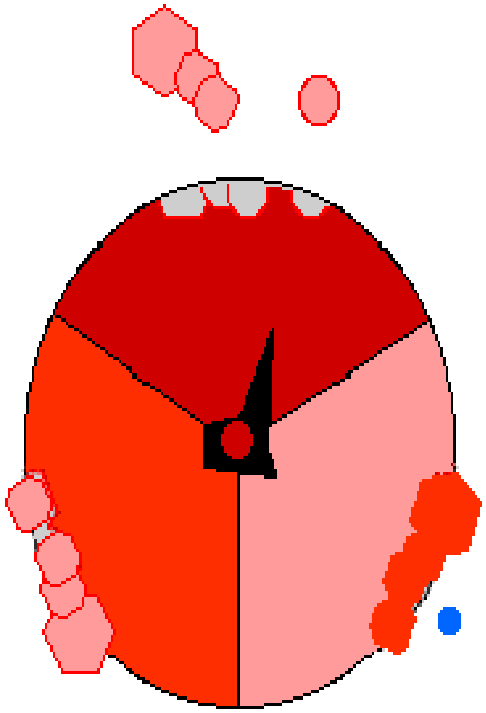
Energy Landscape Governs Structure



Structure



Dynamics



Function

- Theoretical Approaches
- Computer Simulations
- Experiment

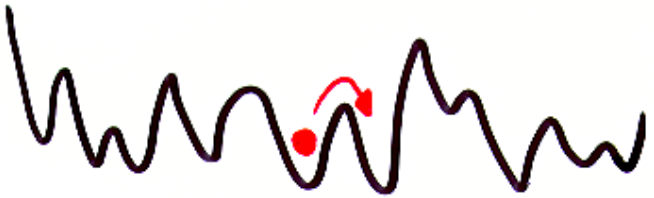
F_1 ATP Synthase

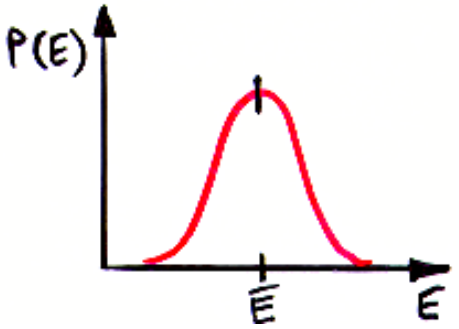
$ADP + P_i \quad ATP + H_2O$

Random Energy Landscape

Thermodynamics:

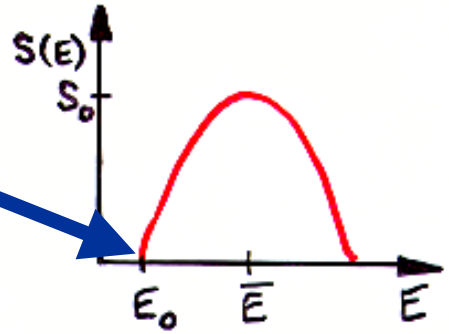
$$P(E) = \frac{1}{\sqrt{2\pi}\Delta E} \exp\left[-\frac{(E-\overline{E})^2}{2\Delta E^2}\right]$$





$$\langle E\rangle = \frac{1}{Z}\int E\,P(E)\exp\left[-\frac{E}{k_B T}\right]dE = \overline{E} - \frac{\Delta E^2}{k_B T}$$

$$S(E) = k_B \ln\left[\Omega_0 \cdot P(E)\right] = S_0 - k_B \frac{(E-\overline{E})^2}{2\Delta E^2}$$



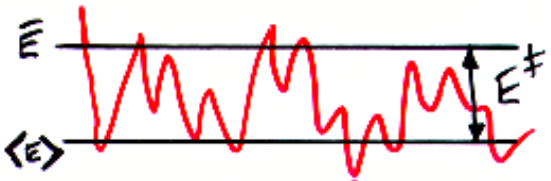
For $\langle E\rangle \rightarrow E_0$: entropy crisis

$$S(E_0) = 0 = S_0 - k_B \frac{(E_0-\overline{E})^2}{2\Delta E^2} \Rightarrow \boxed{T_0 = \frac{\Delta E}{\sqrt{2k_B S_0}}}$$

Kinetics:

$$\kappa = \kappa_0 \exp\left[-\frac{F^{\neq}}{k_B T}\right] = \kappa_0 \exp\left[-\frac{E^{\neq} - TS^{\neq}}{k_B T}\right] = \boxed{\kappa_0 \exp\left[-\left(\frac{\Delta E}{2k_B T}\right)^2\right]}$$

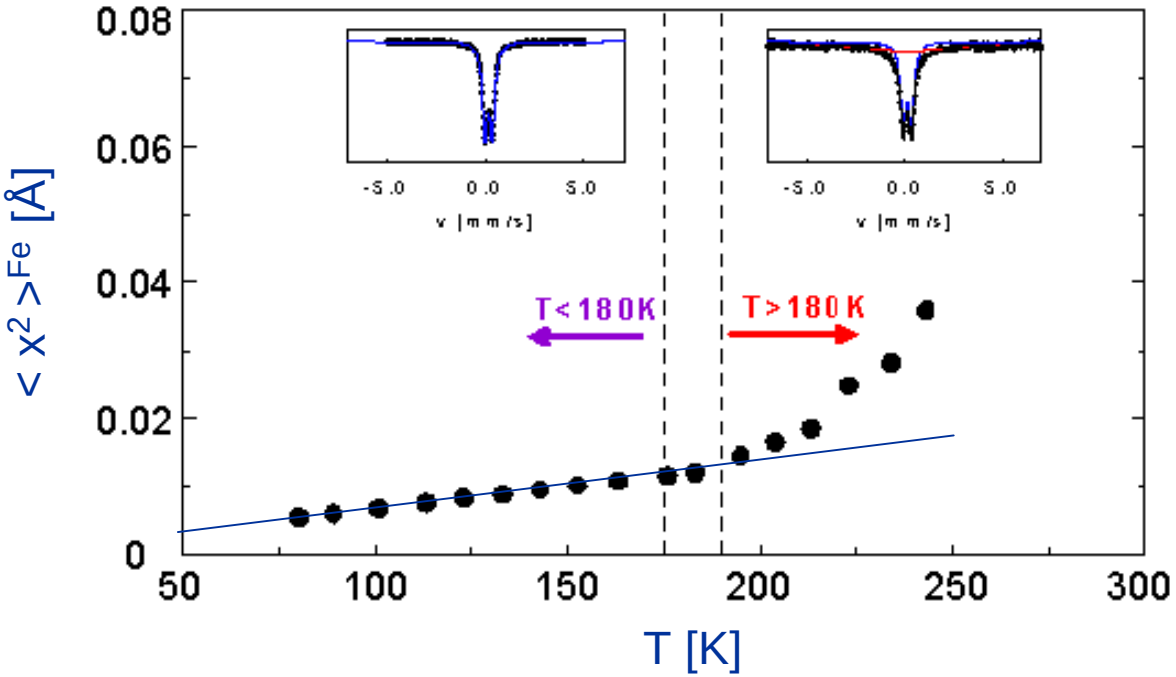
Arrhenius **Ferry's law**



$$E^{\neq} = \overline{E} - \langle E\rangle$$

$$S^{\neq} = S(\overline{E}) - S(\langle E\rangle)$$

Dynamical Transition at T ≈ 180 K

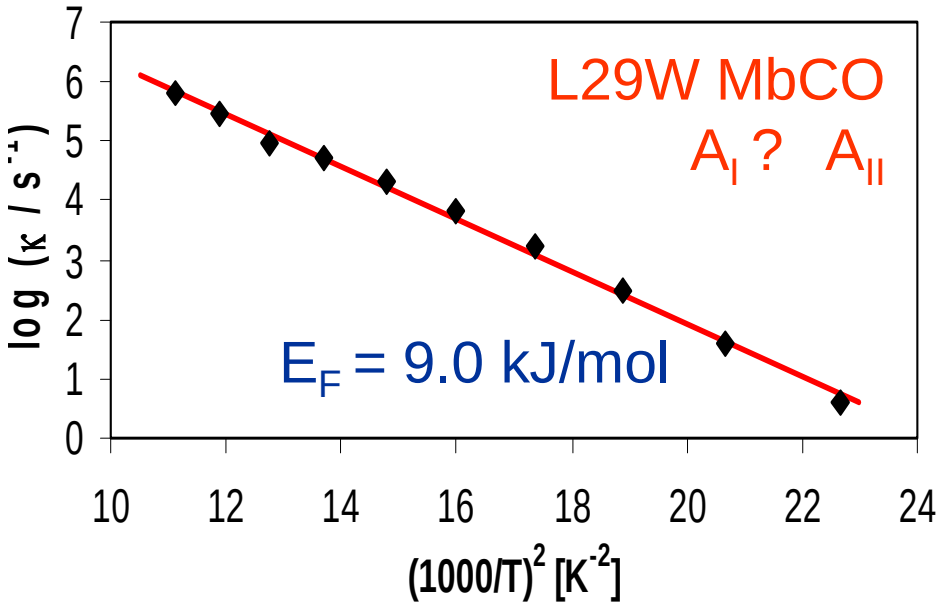


Mössbauer spectroscopy on myoglobin

T < 180 K: harmonic vibrations
T > 180 K: large-scale fluctuations

- Mössbauer spectroscopy — Parak et al., J. Mol. Biol. 161 (1982) 177.
  Nienhaus et al., Nature 338 (1989) 665.
- Neutron scattering — Doster et al., Nature 337 (1989) 754.
- X-ray crystallography — Hartmann et al., PNAS 79 (1982) 4967.
  Parak et al., Eur. Biophys. J. 15 (1987) 237.
  Rasmussen et al., Nature 357 (1992) 423.
- Spin transition in Hb — Perutz, Nature 358 (1992) 548.

Conformational Changes - Temperature Dependence



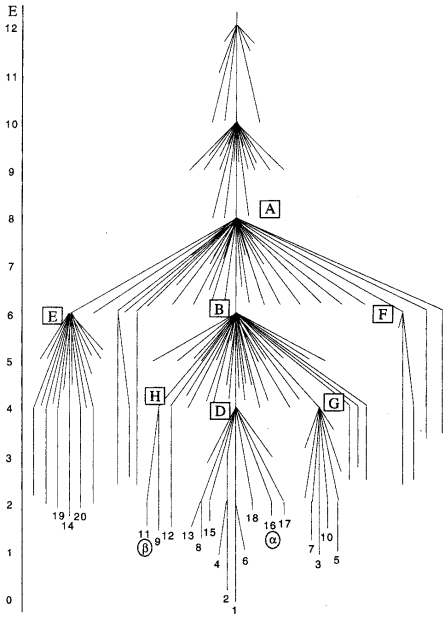
System	Process	logA_A [s^{-1}]	E_A [kJ/mol]	logA_F [s^{-1}]	E_F [kJ/mol]	T range [K]
MbCO L29W	$A_I \leftrightarrow A_{II}$	20	74	12.5	9.0	210-300
MbCO native[1]	$A_0 \leftrightarrow A_1 + A_3$	22.7	94	11.7	9.3	180-300
	$A_1 \leftrightarrow A_3$	30.7	114	16.2	9.8	
RC[2]	CS0	25	110	11.7	9.9	180-300
	CS1	21	78	10.6	7.8	
BPTI	Tyr 35 ring flip[3]	24.3	138	12.6	13.3	277-350
	W 122 res. time[4]	19.4	90	11.7	10.7	

[1] Johnson et al., Biophys. J. 71, 1563 (1996).
[2] McMahon et al., Biophys. J. 74, 2567 (1998).
[3] Otting et al., Biochemistry 32, 3571 (1993).
[4] Denisov et al., Nature Struct. Biol. 3, 505 (1996).

Arrhenius $$\kappa = A_A \exp\left[-\frac{E_A}{k_B T}\right]$$

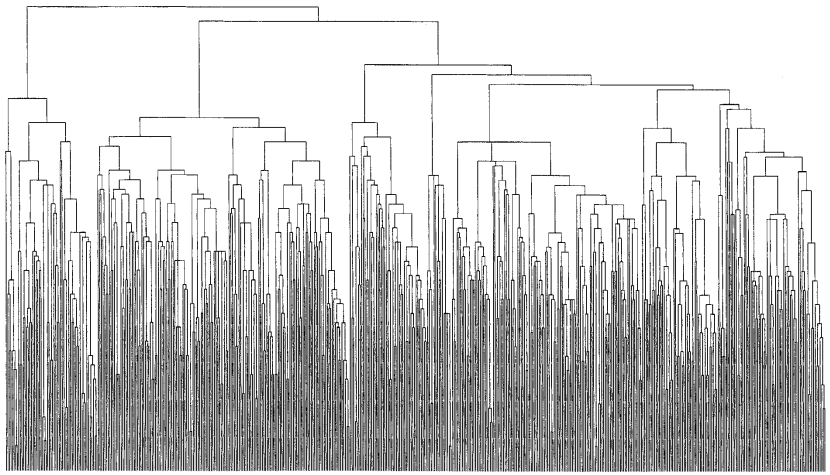
Ferry $$\kappa = A_F \exp\left[-\left(\frac{E_F}{k_B T}\right)^2\right]$$

Computational Approaches



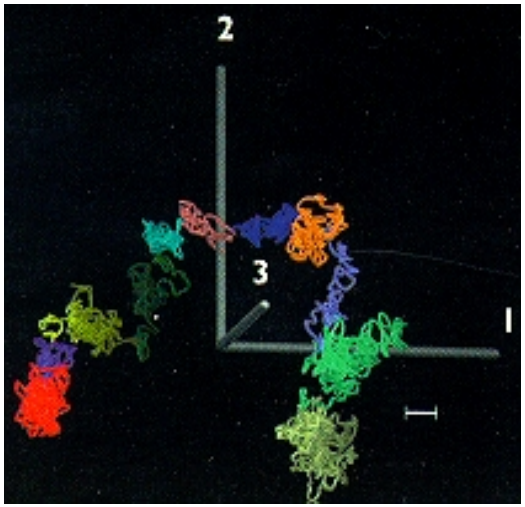
Conformational analysis of isobuturyl-Ala_3-NH-CH_3 (IAN, flexible tetrapeptide)

Czerminski & Elber, PNAS 86 (1989) 6963.
Becker & Karplus, JCP 106 (1997) 1506.



Hierarchical tree of conformations from a 5.1 ns MD simulation of crambin

Garcia et al., Physica D 107 (1997) 225.

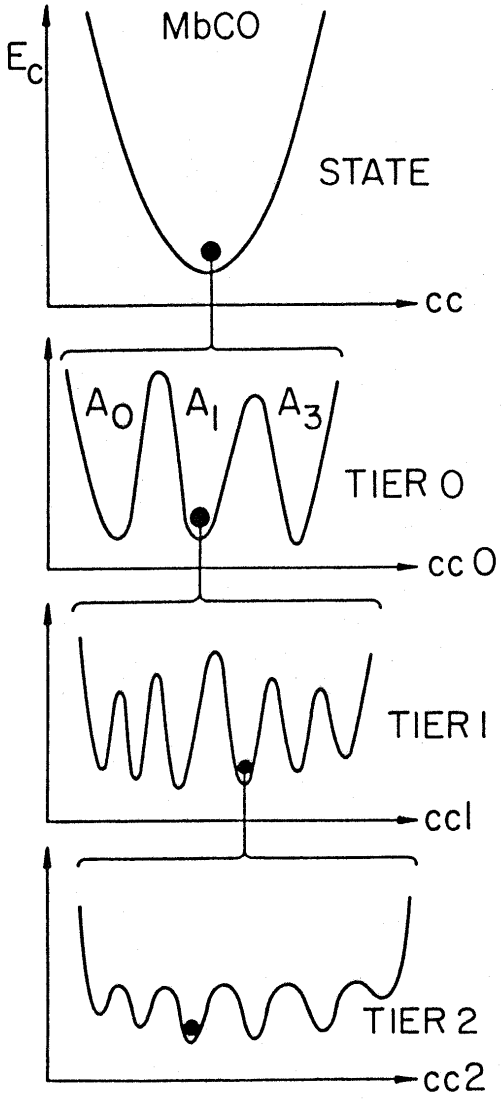


Configuration space projections from SVD analysis of 1 ns myoglobin MD trajectory

Andrews et al., Structure 6 (1998) 587.

Go
Berendsen
Di Nola

Experimental Studies



Hans Frauenfelder, UIUC (1985)

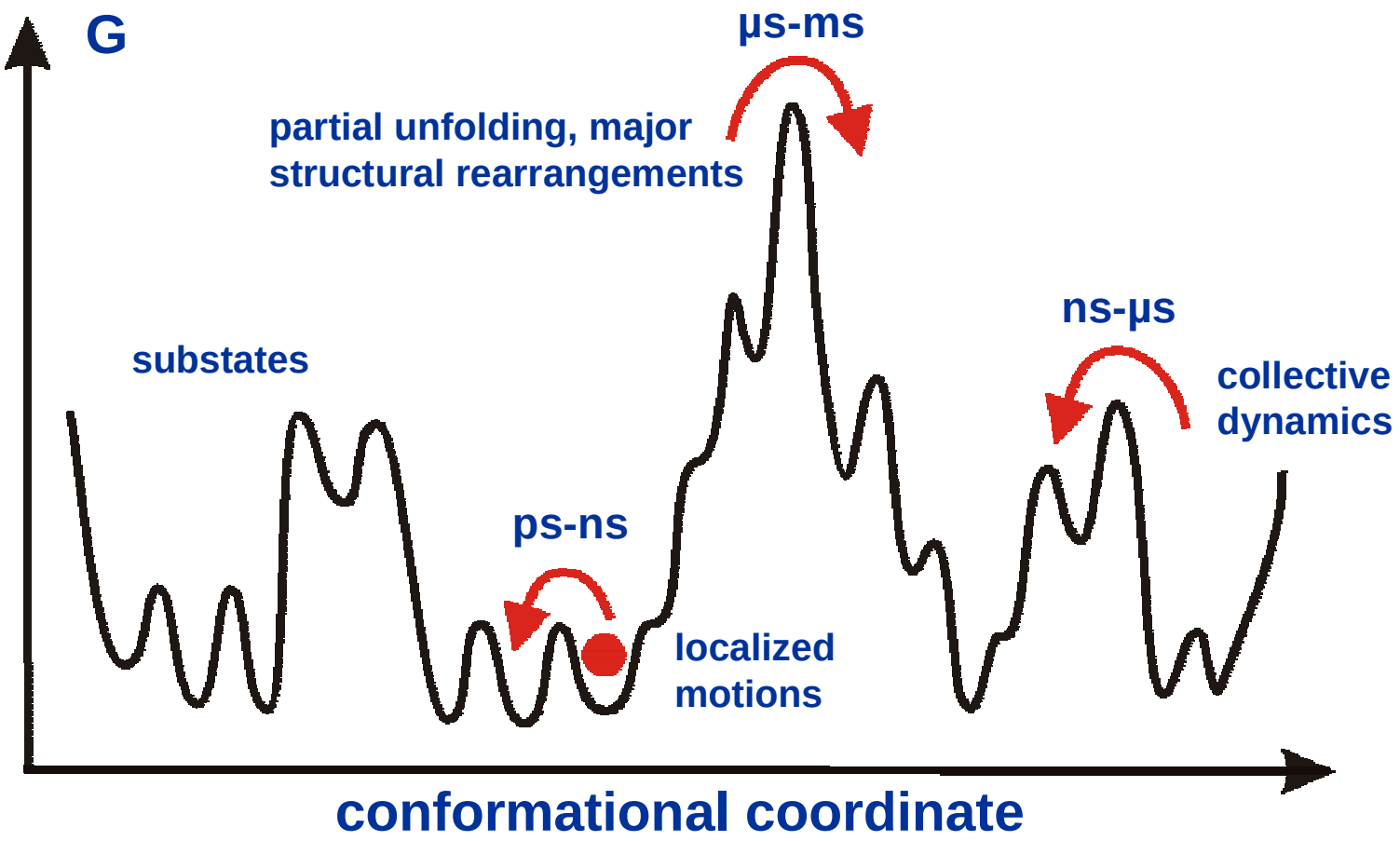
Hierarchical energy landscape

Taxonomic substates
(IR, Raman)

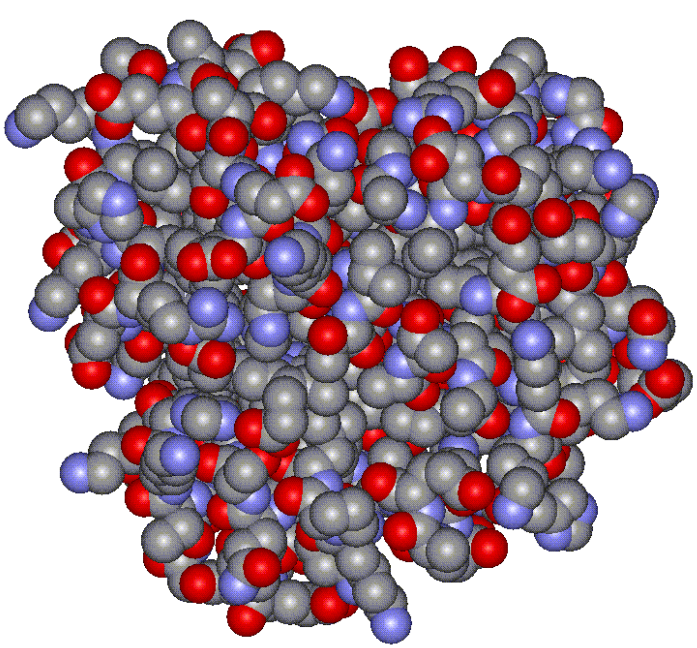
Statistical substates
(nonexponential ligand binding)

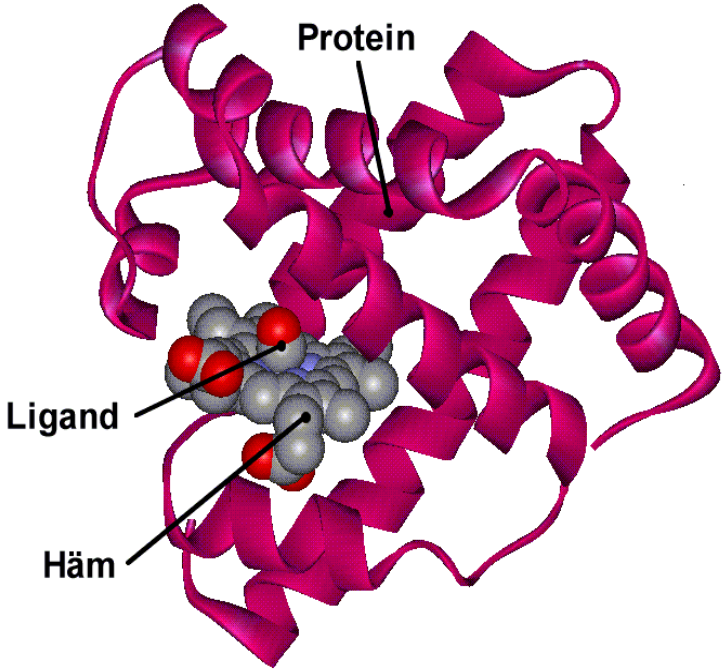
(Hole burning, specific heat)

Conformational Dynamics at Physiological Temperature



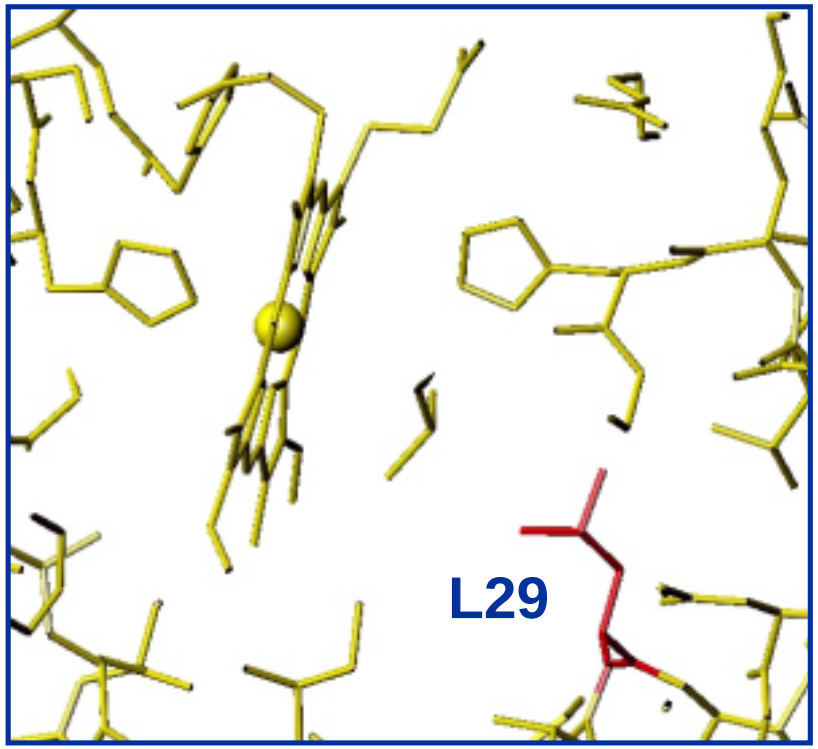
Myoglobin



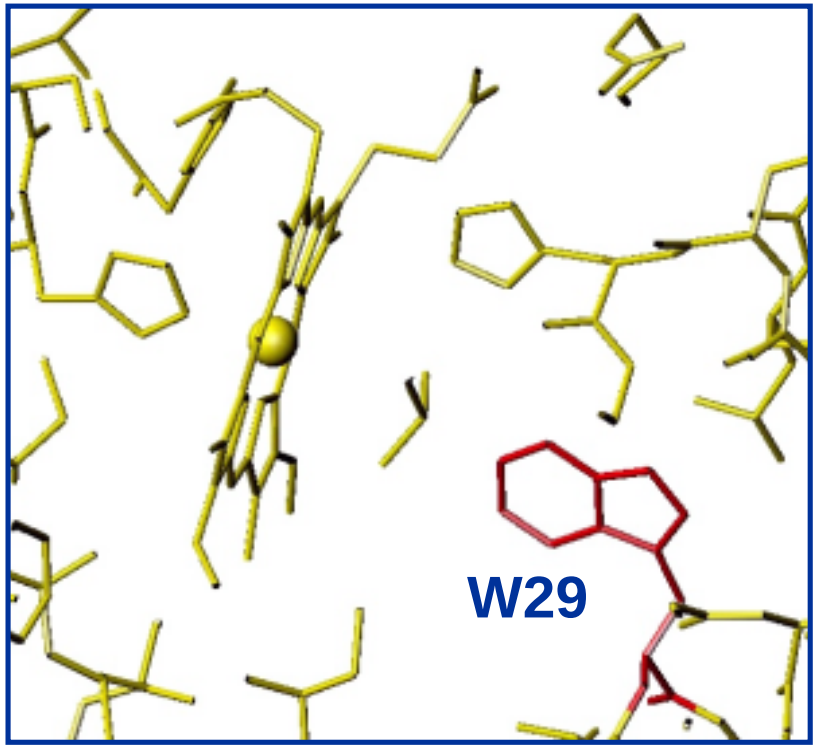


- Function: storage and transport of oxygen
- Molecular weight: 17.8 kD
- Size: ~45 × 35 × 25 Å^3
- Structure: 153 aa, 8 α helices, 1 heme group

Mutant Myoglobins



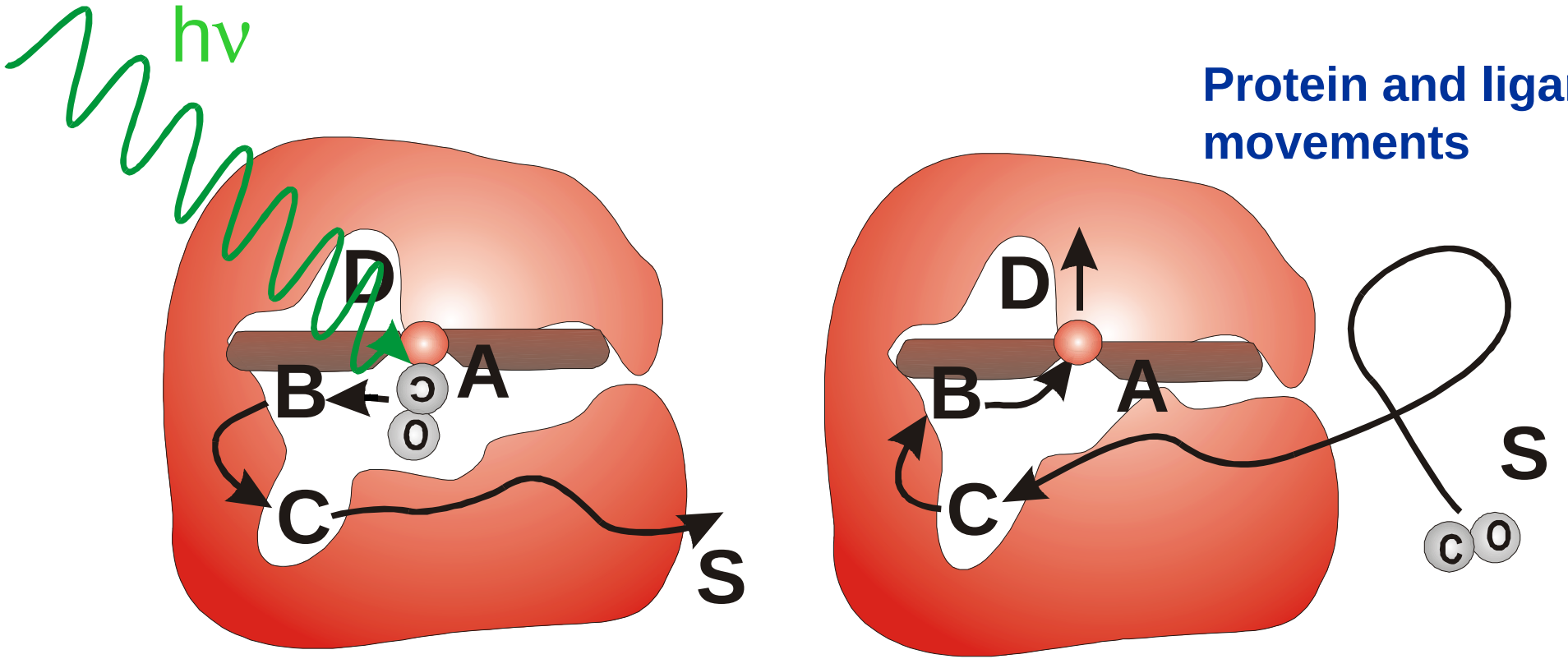
wild type myoglobin



mutant myoglobin L29W

L29W myoglobin binds ligands extremely slowly at room temperature

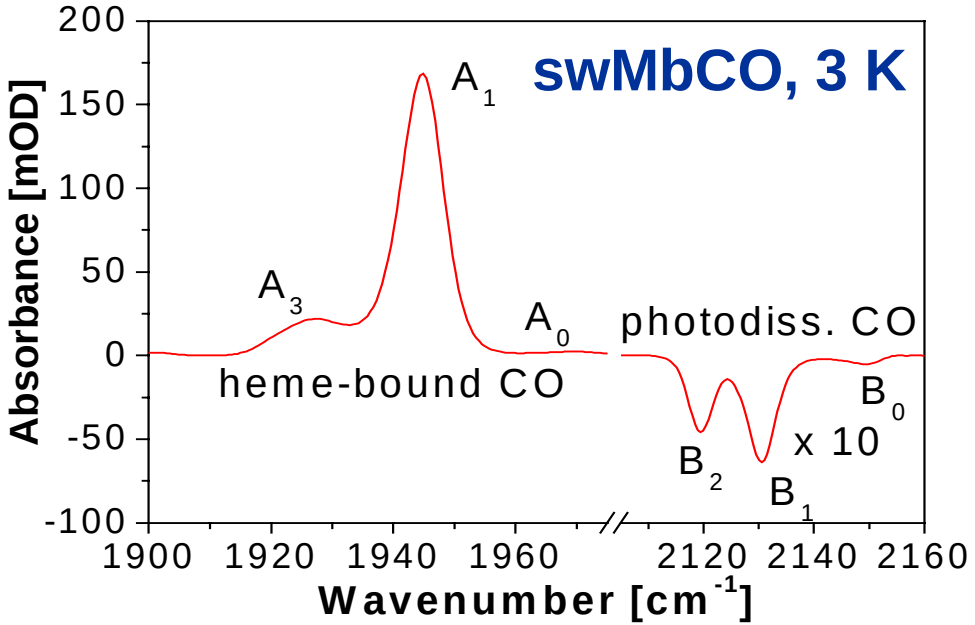
Flash Photolysis

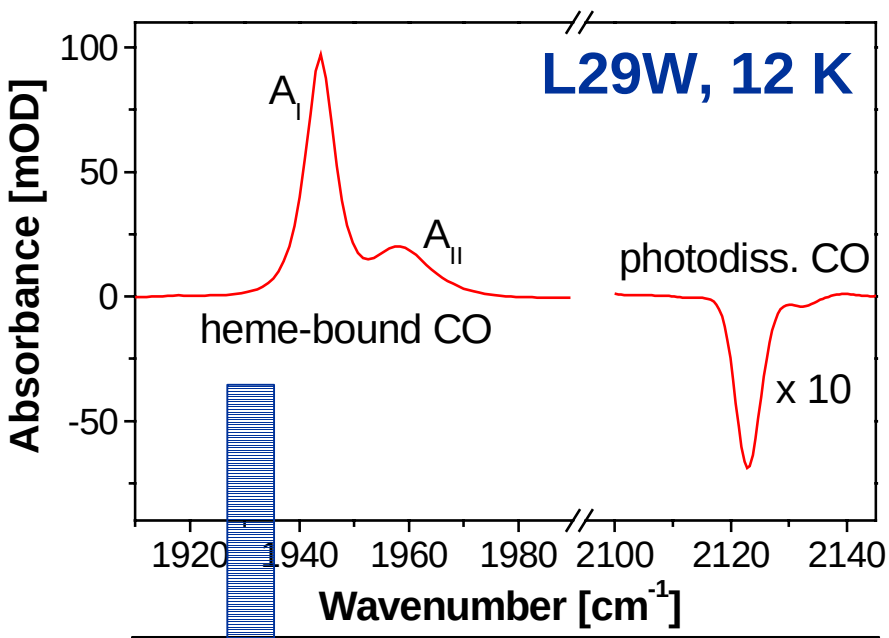


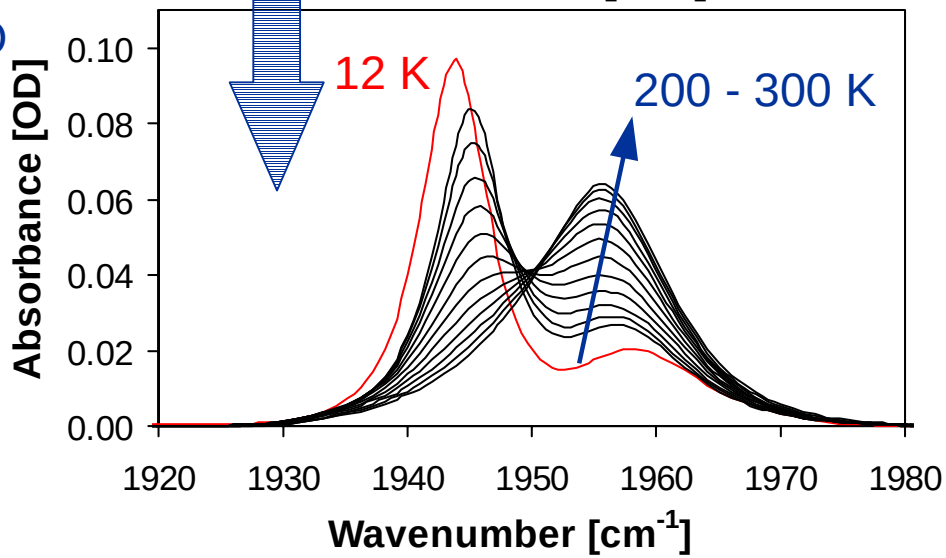
Photodissociation

Rebinding

CO Infrared Bands – Taxonomic Substates



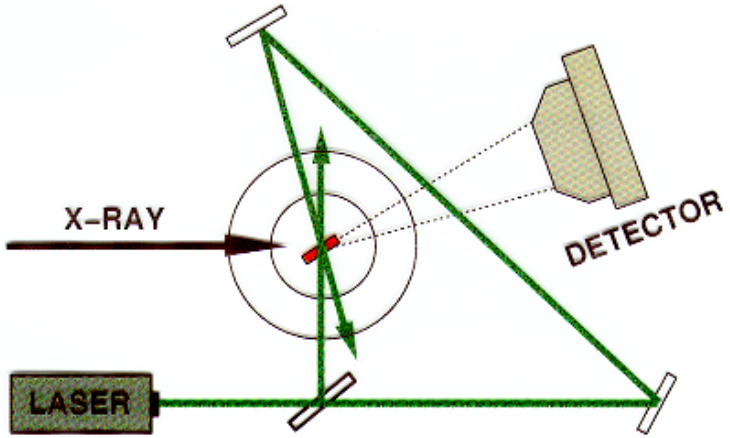




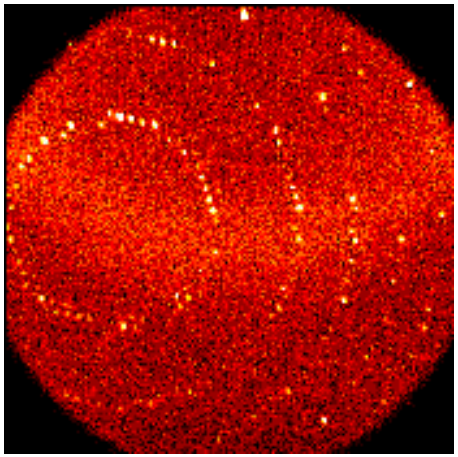
E-field interactions (Stark effect) between the CO dipole and its environment

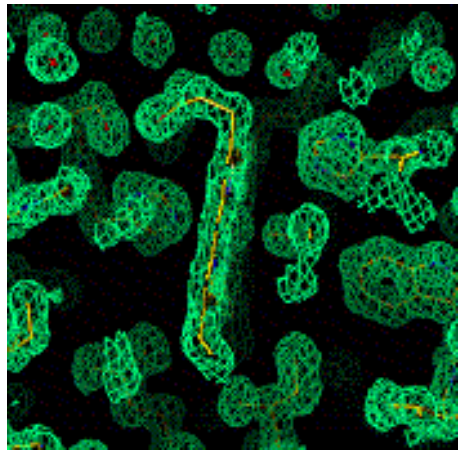
- Multiple ‘A’ bands (CO bound)
  - **multiple conformations (T, p, pH dep.)**
- Multiple ‘B’ bands (CO dissociated)
  - **multiple ligand docking sites**
- Heterogeneity within each band
  - **statistical substates**

X-Ray Cryo-Crystallography

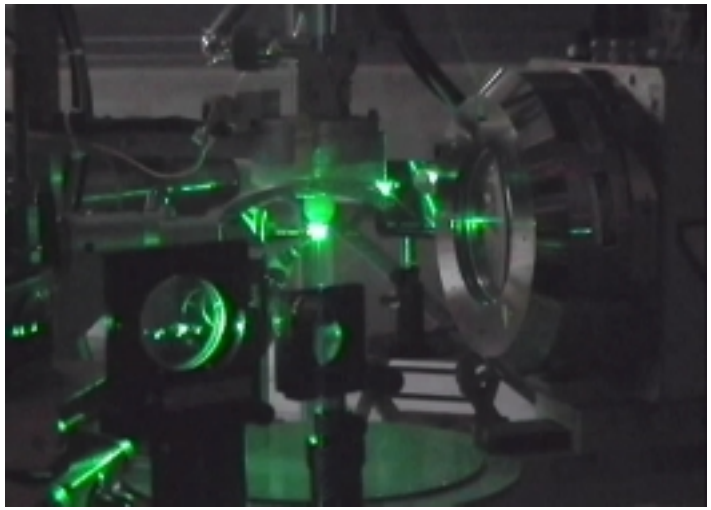


Nd:YAG CW LASER; 532nm; max. 300mW



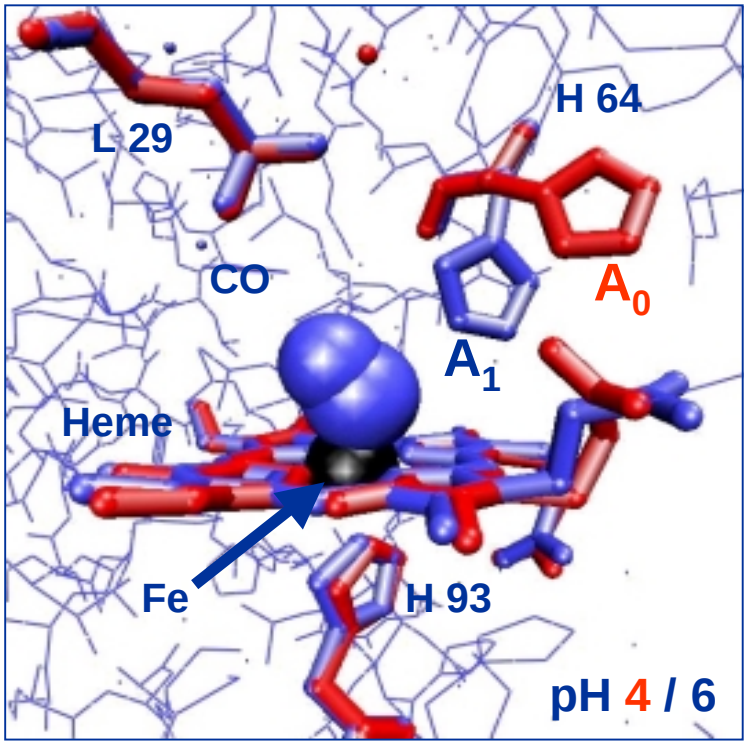


Fourier Transform

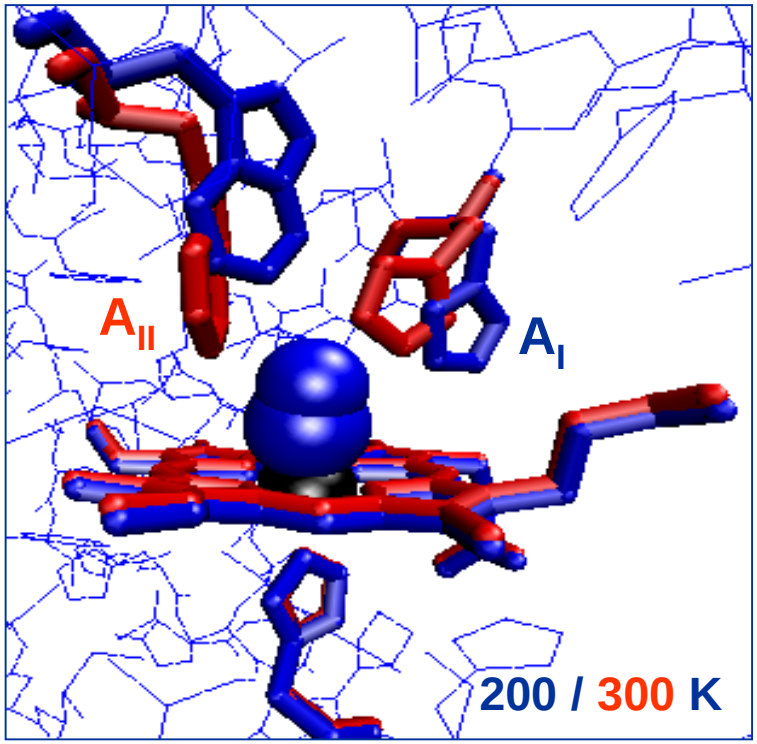


Ostermann, A., Waschipky, R., Parak, F. G. & Nienhaus, G. U., Nature 404 (2000) 205-208.

Structures of A Substates



wild type MbCO

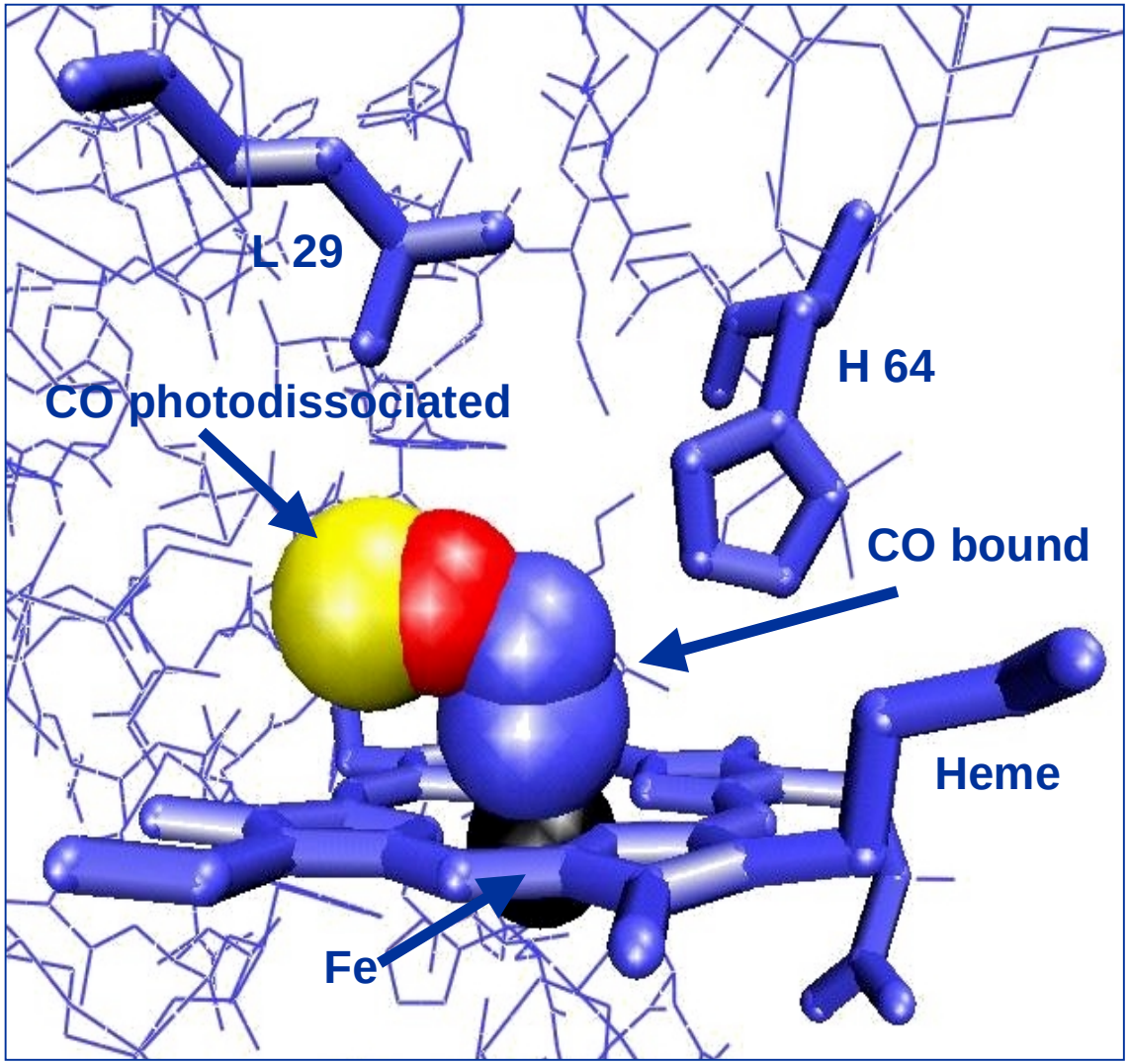


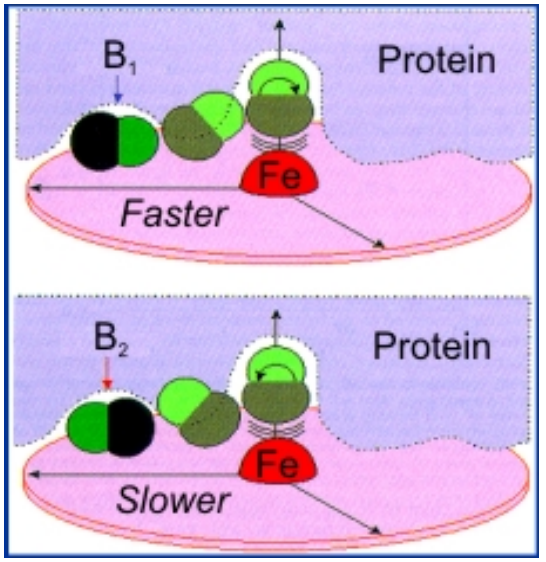
mutant MbCO L29W

Yang & Phillips, J. Mol. Biol. 256 (1996) 762 – 774.
Johnson et al., Biophys. J. 71 (1996) 1563-1573.
Müller et al., Biophys. J. 77 (1999) 1036 – 1051.

Ostermann et al., Nature 404 (2000) 205-208.

CO Primary Docking Site (T < 40 K)





fs IR

Femtosecond IR spectroscopy

Lim et al., Nature Struct. Biol. 4 (1997) 209 – 214.

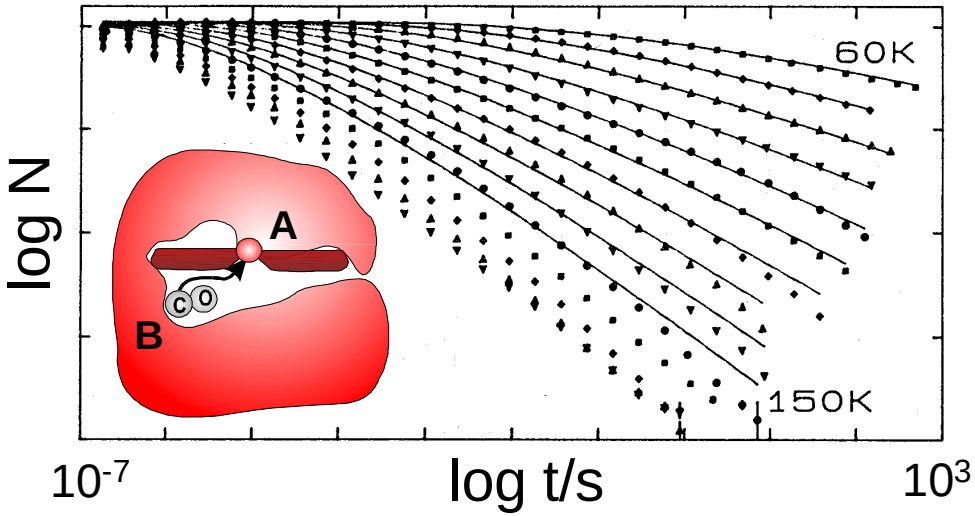
Photoproduct x-ray structures:

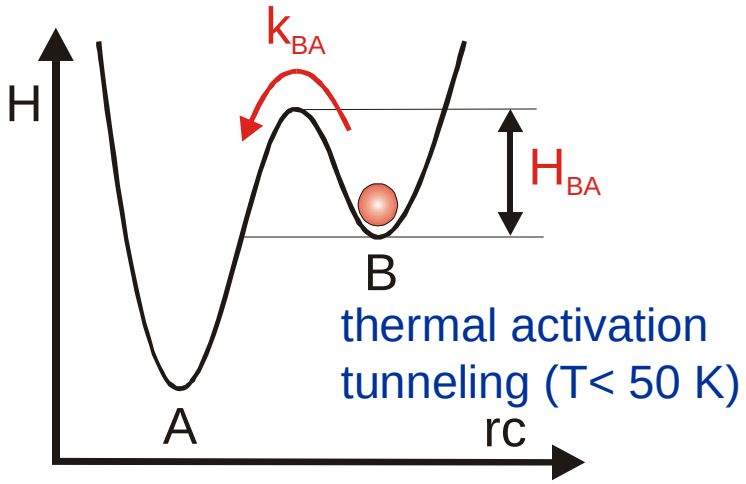
Schlichting et al., Nature 317 (1994) 808.
Teng et al., Nature Struct. Biol. 1 (1994) 701.
Hartmann et al., PNAS 93 (1996) 7013.

MD simulations:

Vitkup et al., Nature Struct. Biol. 4 (1997) 202.
Ma et al., JACS 119 (1997) 2541.
Meller & Elber, Biophys. J. 75 (1998) 789.

Low Temperature CO Rebinding





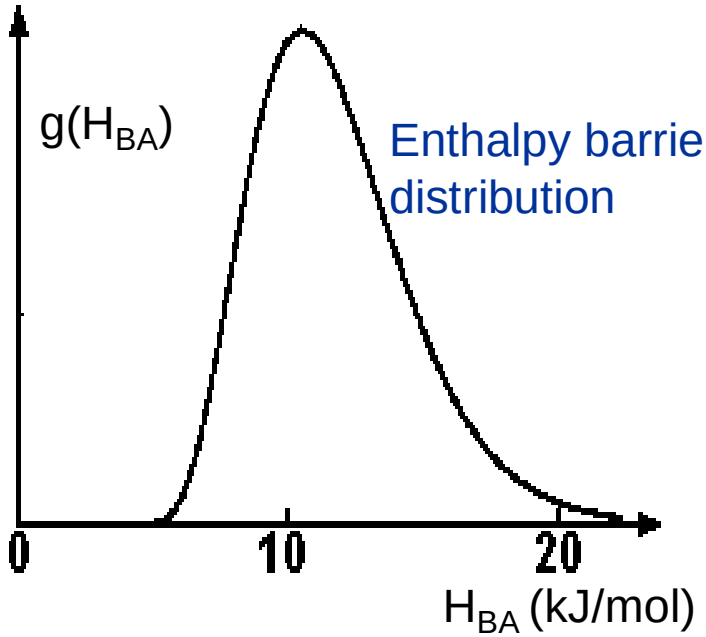
$$N(t) = \int g(H_{BA}) \exp[-k_{BA} t]\, dH_{BA}$$

with $k_{BA} = A_{BA} \exp[-H_{BA}/(RT)]$

(Arrhenius)

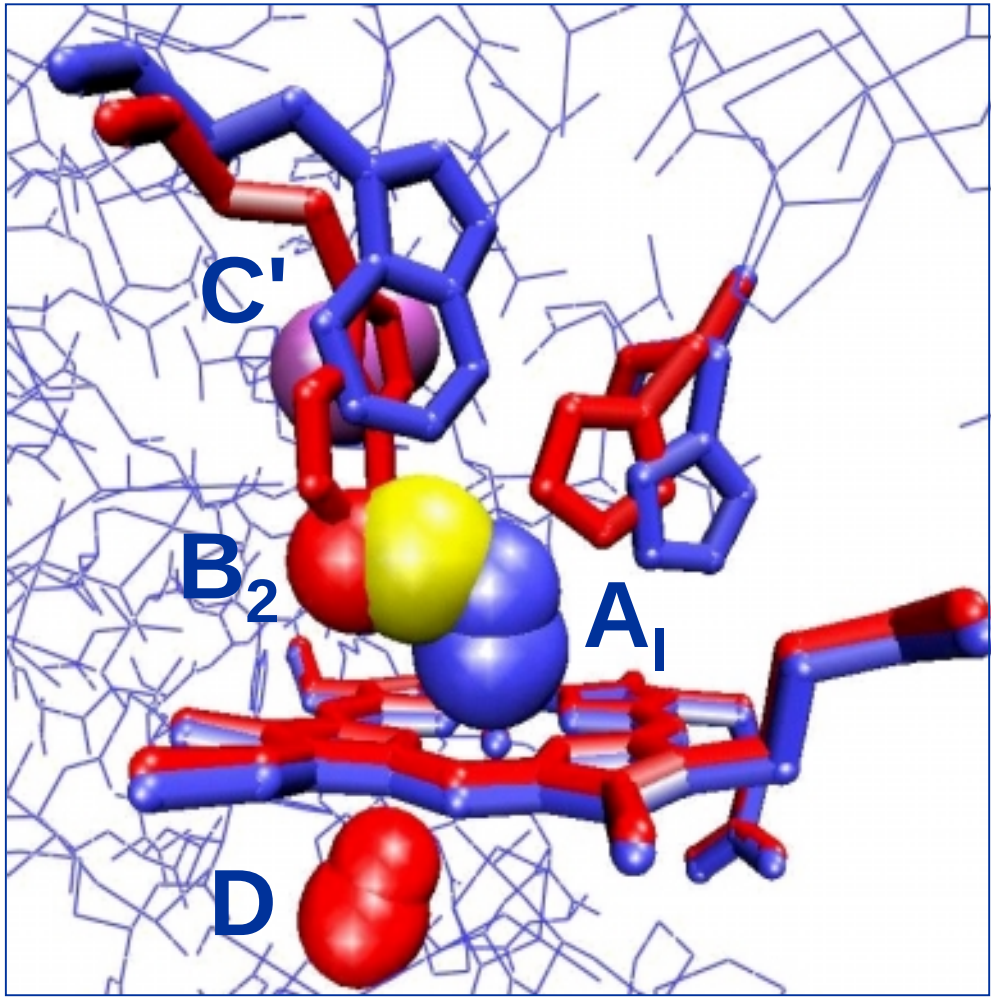
functional heterogeneity

structural heterogeneity



Austin et al., Biochemistry 14 (1975) 5355-5373.

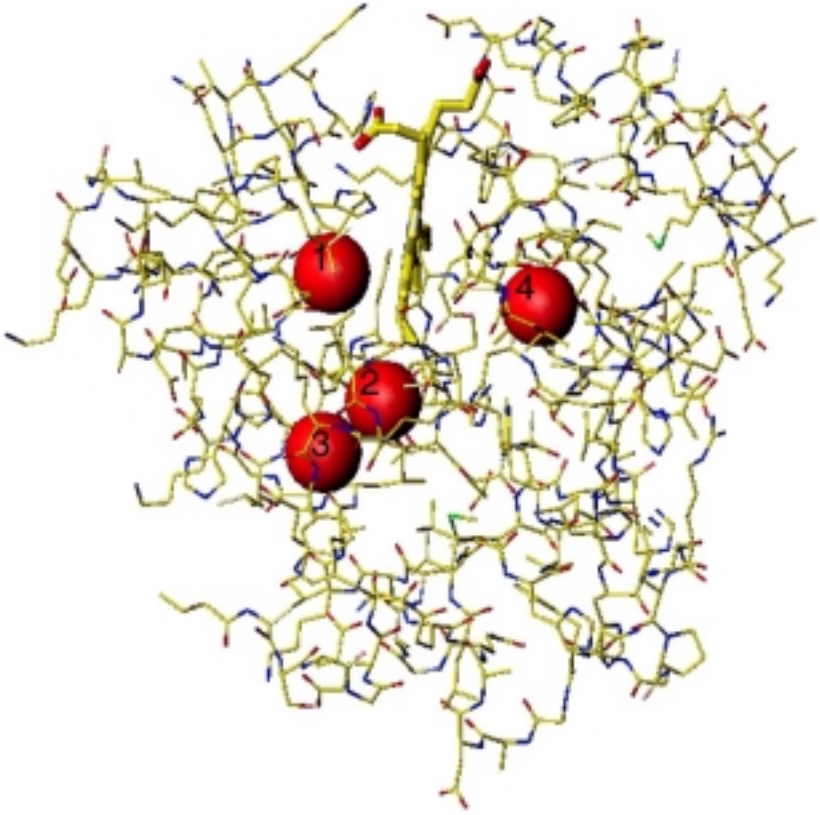
Ligand Migration to Alternative Docking Sites in L29W

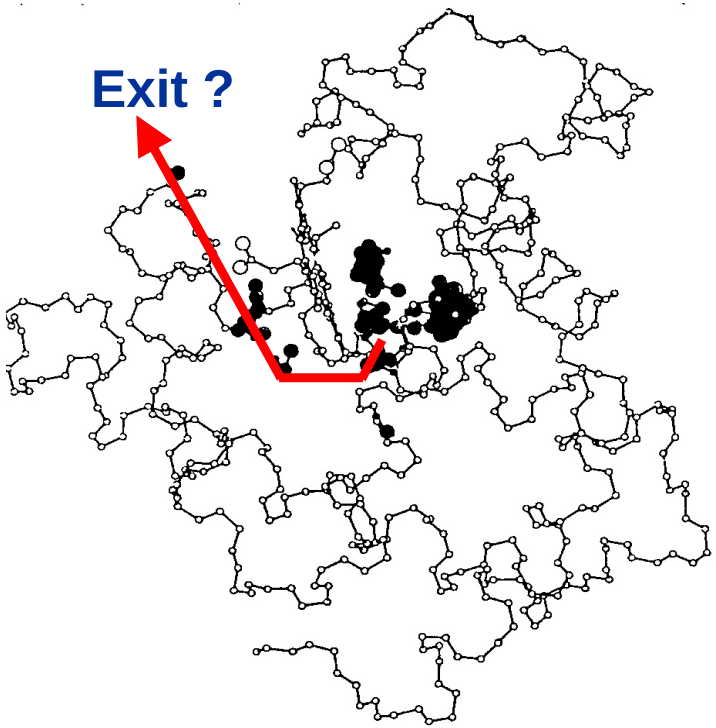


T < 180 K:
Ligand movements in the frozen protein.

T > 180 K:
Ligand migration and concomitant structural changes in the protein.

Secondary Docking Sites – Xenon Cavities



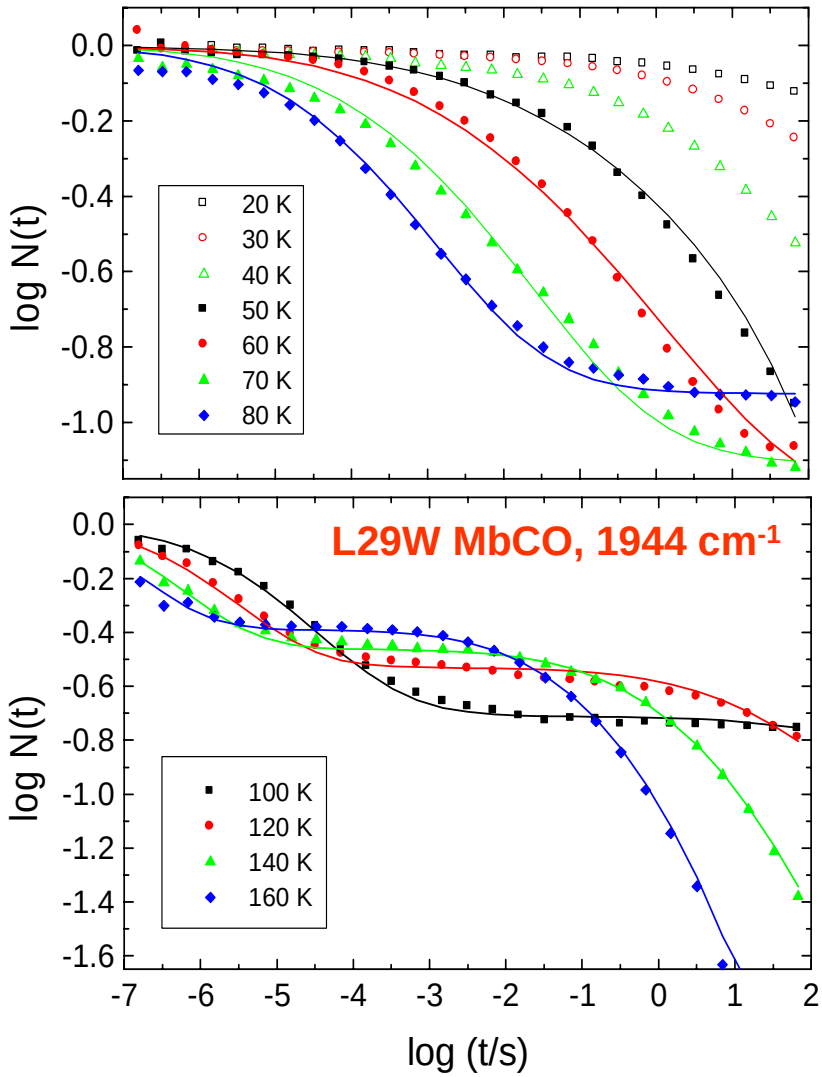


Xenon cavities:
Tilton et al., Biochemistry
23 (1984) 2849

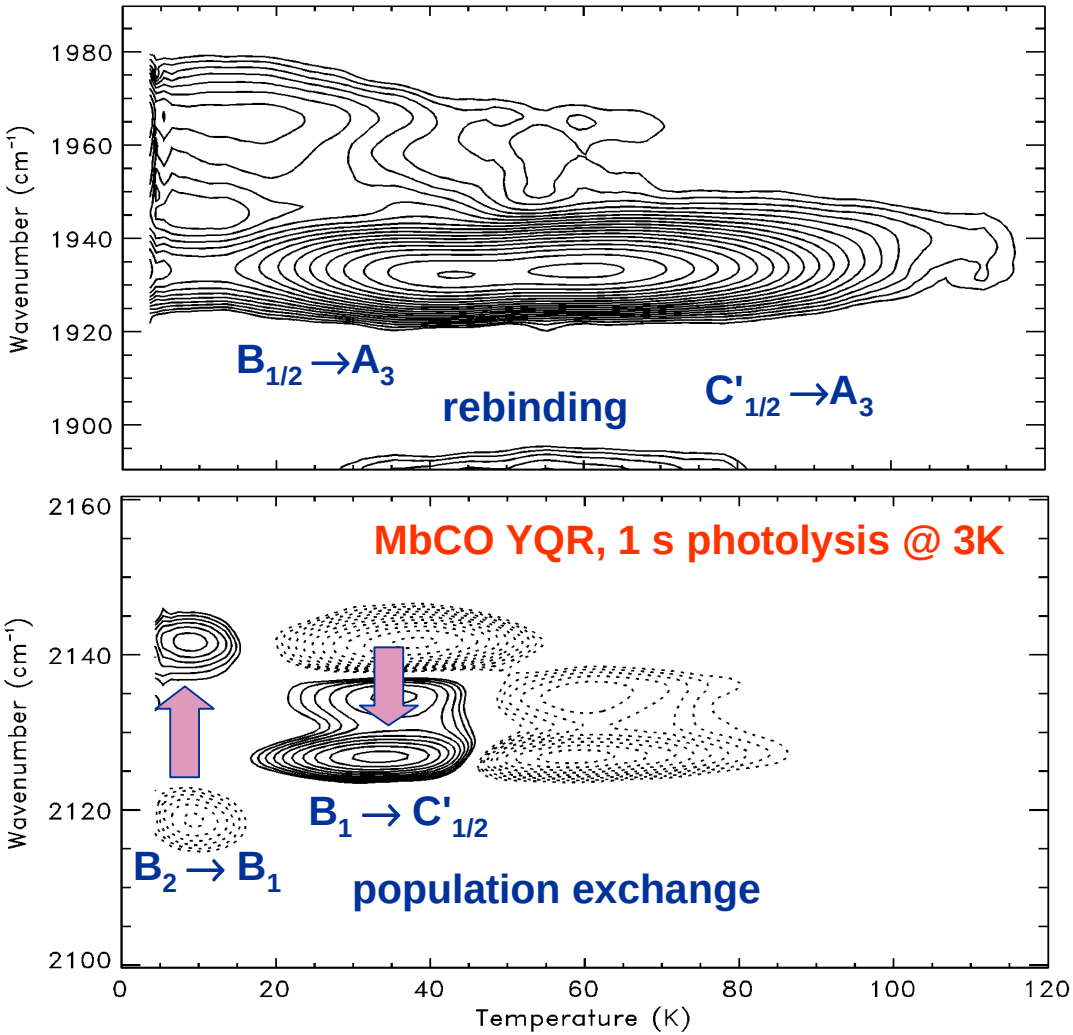
MD simulations:
Elber and Karplus, J. Am. Chem. Soc.
112 (1990) 9161

Ligand Migration Studies

Infrared Kinetics

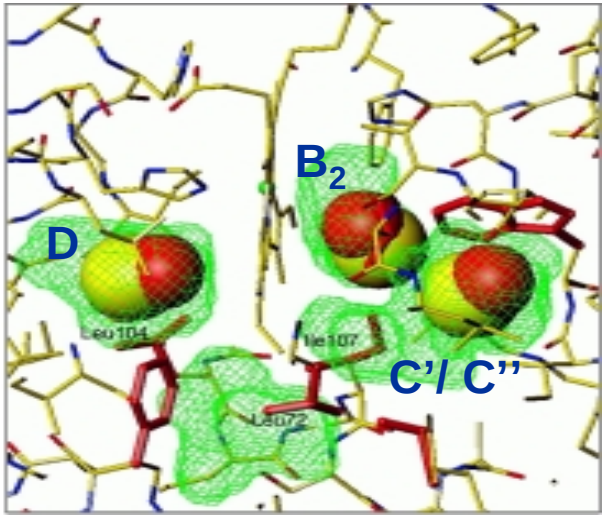


Temperature Derivative Spectroscopy

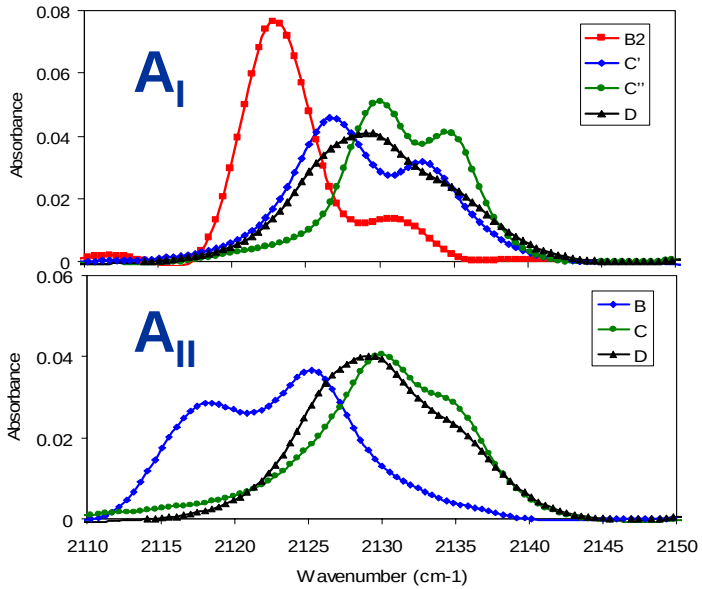


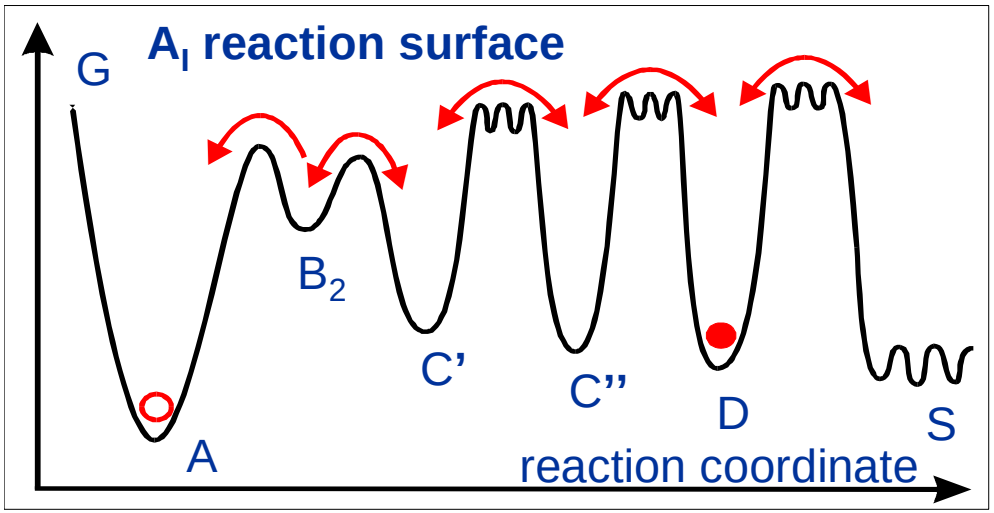
Lamb et al., J. Biol. Chem. 277 (2002) 11636-11644.

Characterization of Reaction Intermediates



- Structure
- IR Spectra (E-fields, CO dynamics)
- Energetics of ligand binding
- Role of A substate interconversions





Summary

- Proteins possess a rugged energy landscape
  - Huge number of ~isoenergetic conformational substates
  - Energy barriers distributed in height
    - Dynamics on essentially all time scales
    - Dynamics over wide temperature range
    - Non-Arrhenius temperature dependencies
  - Hierarchy of Substates
    - Taxonomic substates
    - Statistical substates
- Model protein myoglobin
  - Energy landscape exploration by
    - Cryocrystallography
    - IR spectroscopy
    - Kinetics

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