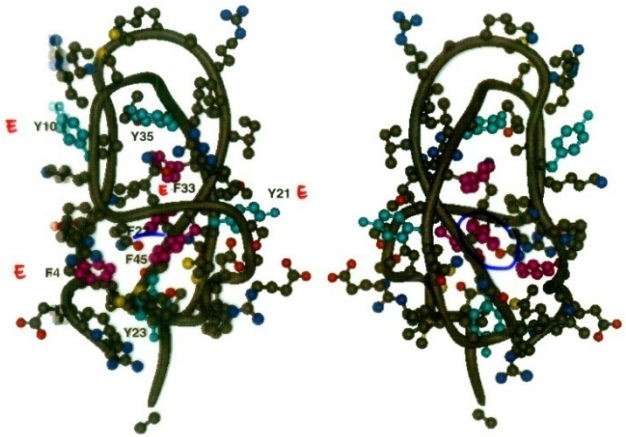
Protein Dynamics

10/1/14 and 10/3/14



Valerie Daggett

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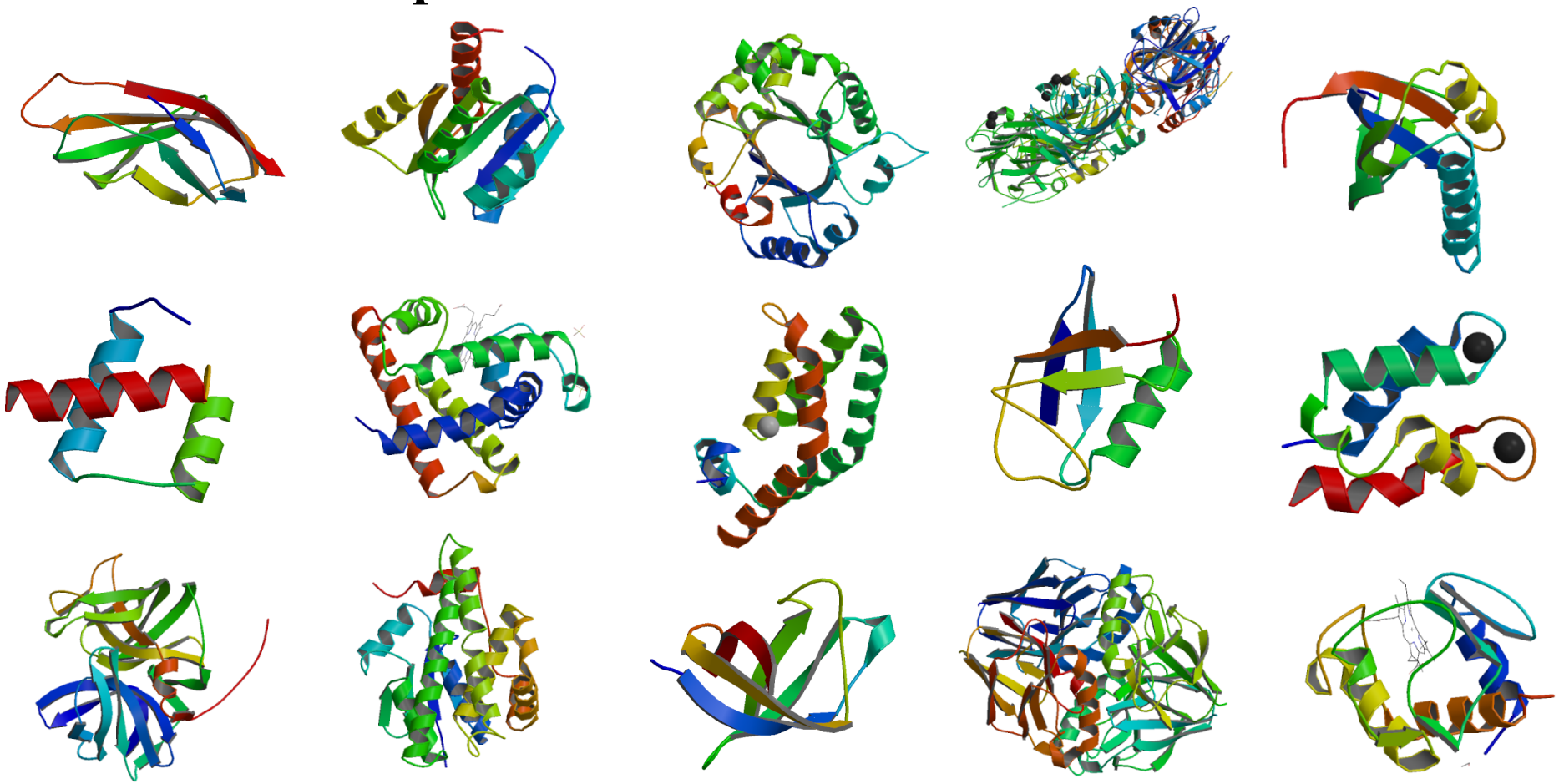
Proteins

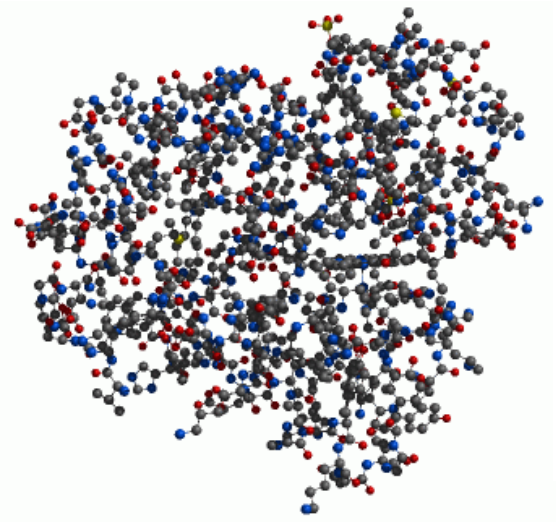
Many shapes, many sizes, >100,000 in PDB ➔ 807 unique autonomous protein folds

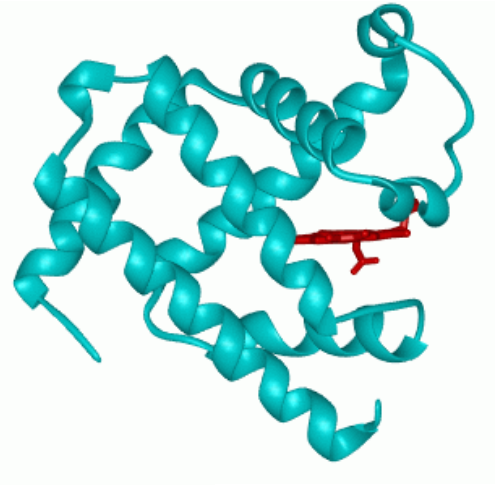


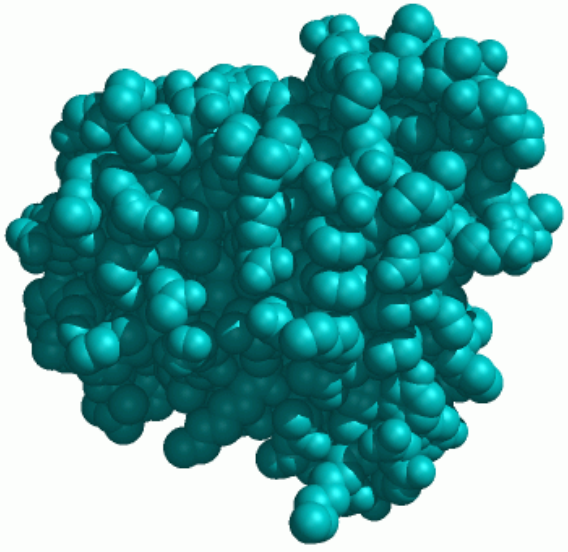
Protein Structure

- First protein crystal structure solved in the 1950s
- Myoglobin
- Nobel prize to Perutz and Kendrew

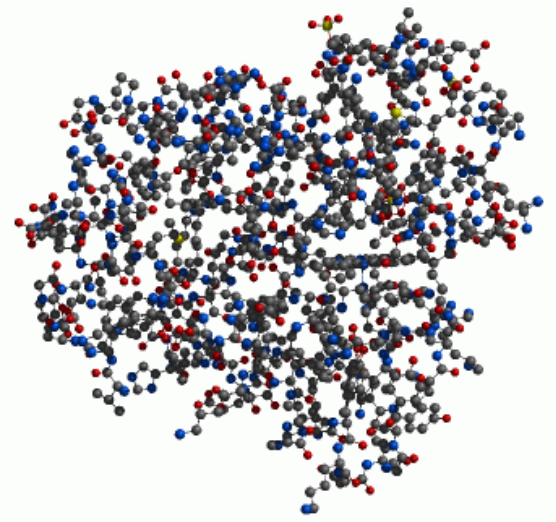
Myoglobin

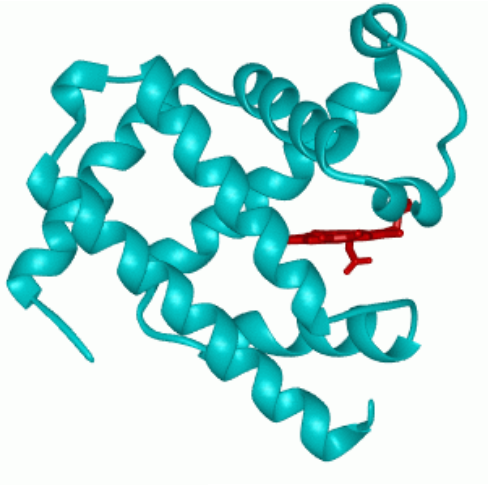


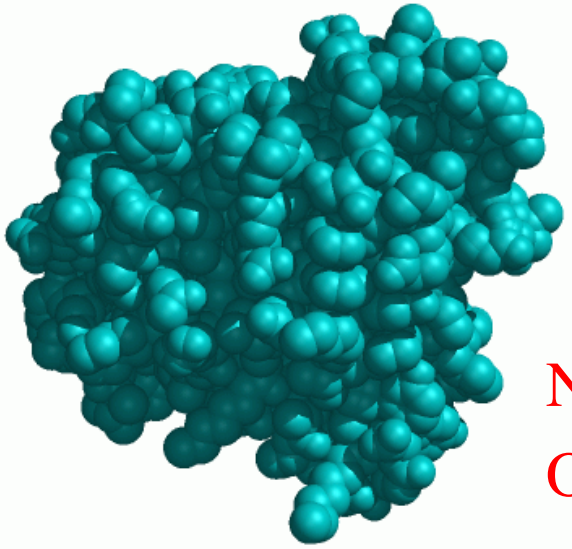




Myoglobin







Tightly packed!

No pathway for $O_2 \rightarrow$ heme!

Protein Dynamics

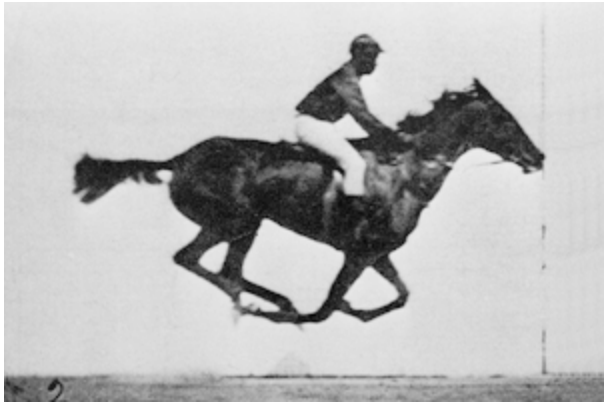
- Proteins are not static
- Motion is an incontrovertible consequence of existing @ room temperature (or any T > 0 K)
- Kinetic energy per atom is ~ 1 kcal/mole @ 298K (25°C) $\Rightarrow$ several Å/ps
- Motion recognized to be important since first crystal structure solved

...everything that living things do can be understood in terms of the jigglings and wigglings of atoms.

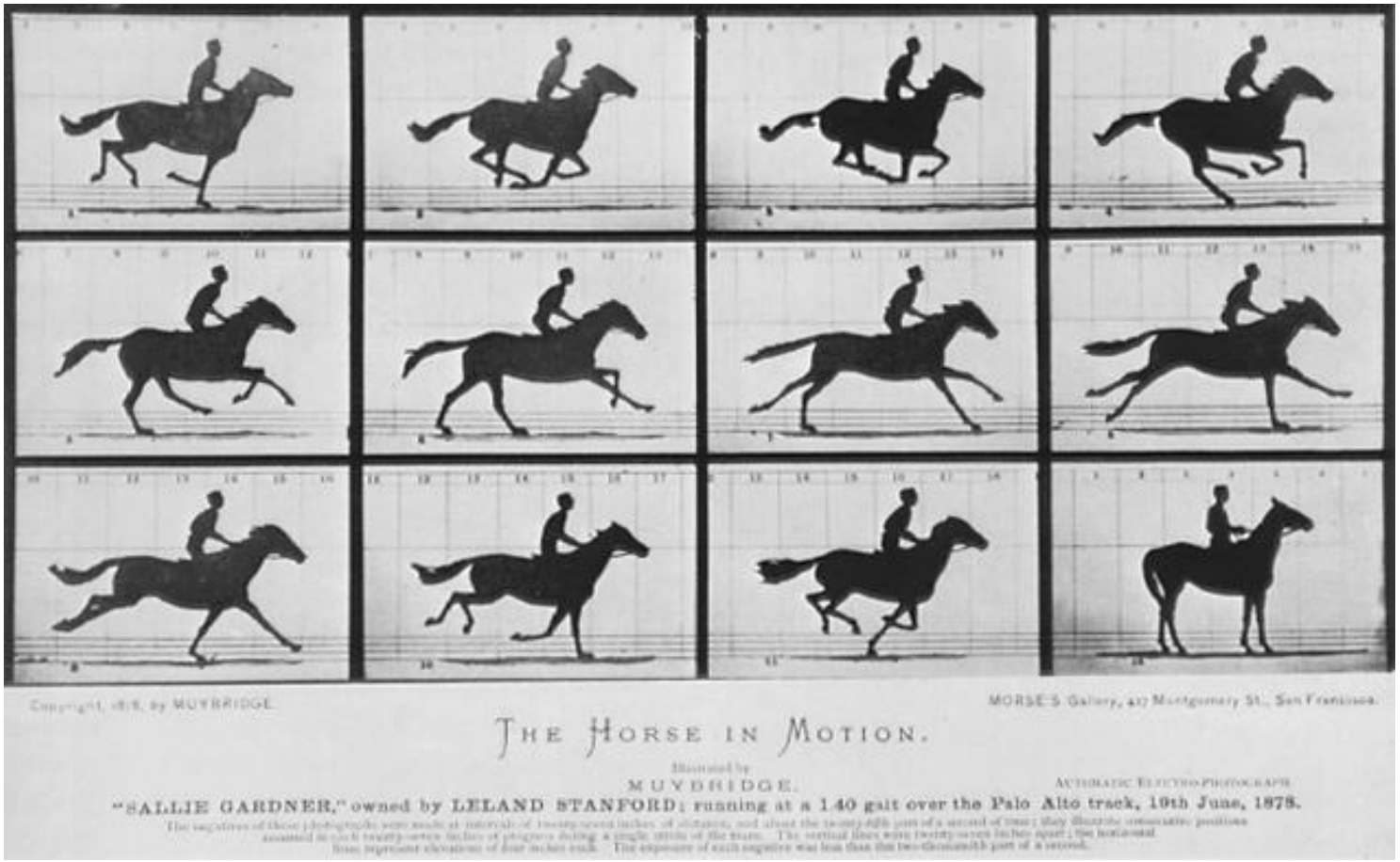
Richard Feynman

Function from Static Structure

Dynamics necessary for function



Dynamics Necessary for Function



Outline of Lecture

- Prevalent Protein Motions
  - Experiment
  - Theory
  - Biological Relevance
    - » Is motion just a result of thermal energy and weak interactions, or is such motion functionally important and designed into a protein?

Problems w/extracting detailed structural information regarding dynamics from expt

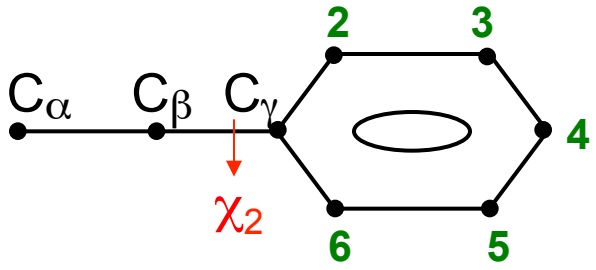
Prevalent Protein Motions

Type of Motion	Extent of Motion (Å)	Time Scale	
Bond Vibration	0.01-0.1	0.01-0.1 ps	
Side Chain Rotation	5-10 E 5 B	10-100 ps E 10^8-10^{12} ps B (0.1-10^3 ms)	1
Breathing	0.5-2	10 ps-1 ns	2
Relative Motion - hinge bending - allosteric tran.	1-15	10 ps-1 s	3
Local Denaturation	5-10	< ms, μs, ns deadtime	

10^{12} ps/s 10^9 ns/s

1 Side Chain Rotations

- Ring flips
- 1H NMR
  - Rotational freedom of Tyr and Phe



- Isotropic - Rapid rotation on NMR time scale
  - 2 pairs of closely spaced doublets 10^4-10^5/s

2=6
3=5

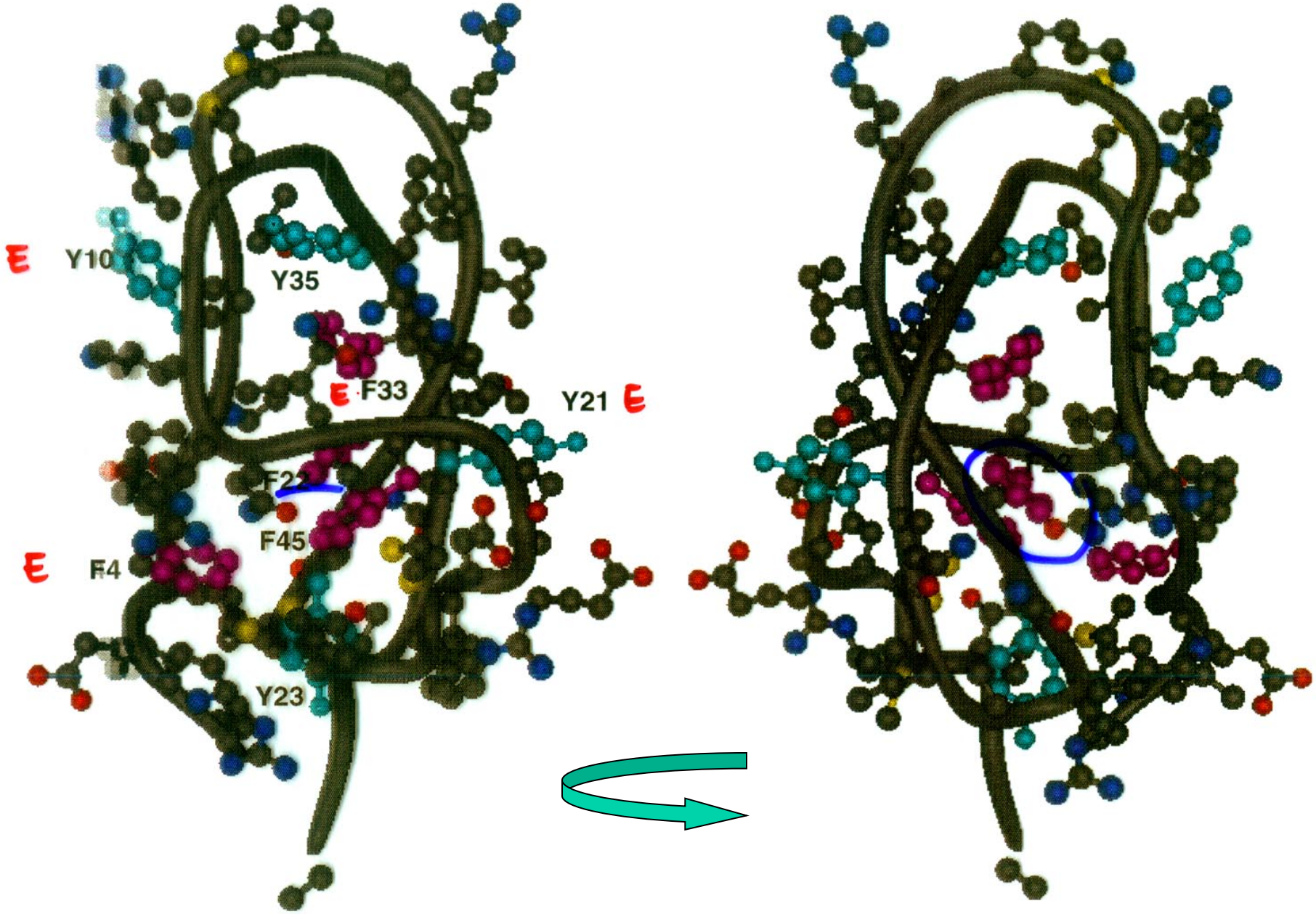
- Anisotropic - Slow rotation
  - 4 separate resonances
    1-10/s

Table 7.6 Rotation of Aromatic Rings in BPTI

Residue	Frequency of 180° Rotations (s^{-1}) at Temperature of 4°C	40°C	80°C	Activation Parameters: Enthalpy $\Delta H^{\ddagger}$ (kcal/mol)	Entropy $\Delta S^{\ddagger}$ [cal/(mol · °C)]	Volume $\Delta V^{\ddagger}$ (Å^3)
Tyr 10	Rotating rapidly at all temperatures					
Tyr 21	Rotating rapidly at all temperatures					
Tyr 23	<5	3×10^2	5×10^4	26	35	
Tyr 35	<1	50	5×10^4	37	68	60
Phe 4	Rotating rapidly at all temperatures					
Phe 22	Rotating rapidly at all temperatures					
Phe 33	Rotating rapidly at all temperatures					
Phe 45	30	1.7×10^3	5×10^4	17	11	50

From G. Wagner et al., *Biophys. Struct. Mech.* 2:139–159 (1976); *J. Mol. Biol.* 196:227–231 (1987).

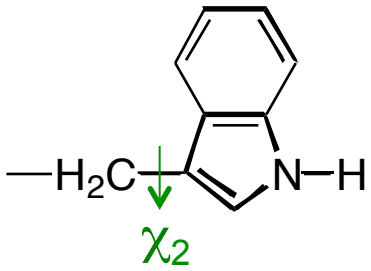
P↑ flips ↓



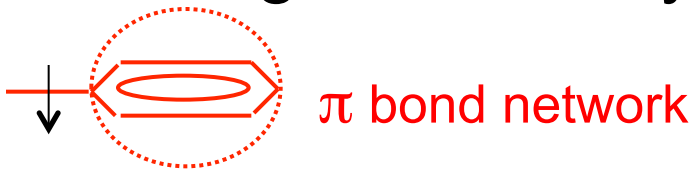
BPTI---Aromatic Groups displayed. Phe in magenta. Tyr in cyan.

Side Chain Rotations (cont)

- w/ NMR
  - Jumps of 180° flips - not continuous rotation
- Trp usually is immobile - bulky hard to flip



- Y & F - on edge, almost cylindrical shape



- Methyl groups usually have equivalent protons - spinning rapidly

Side Chain Rotations (cont)

- X-ray crystallography
  - *Smith et al. (1986) Biochem 25:5018*
    4 high resolution Xtal structures
  - Found that 6-13% of the side chains have multiple discrete conformations
    - » Usually on surface
    - » Some inside
  - Preference for discrete substates rather than giving an unresolved continuous smear of electron density but some lack unique conformation

Side Chain Rotations (cont)

- So, multiple conformational substates sampled @ RT

- Slightly different conformations can perform the same function but @ different rates

- *Frauenfelder et al. Nature 280:559 (1979)*
- *Hong et al. Biophys J. 58:429 (1990)*

2 Breathing

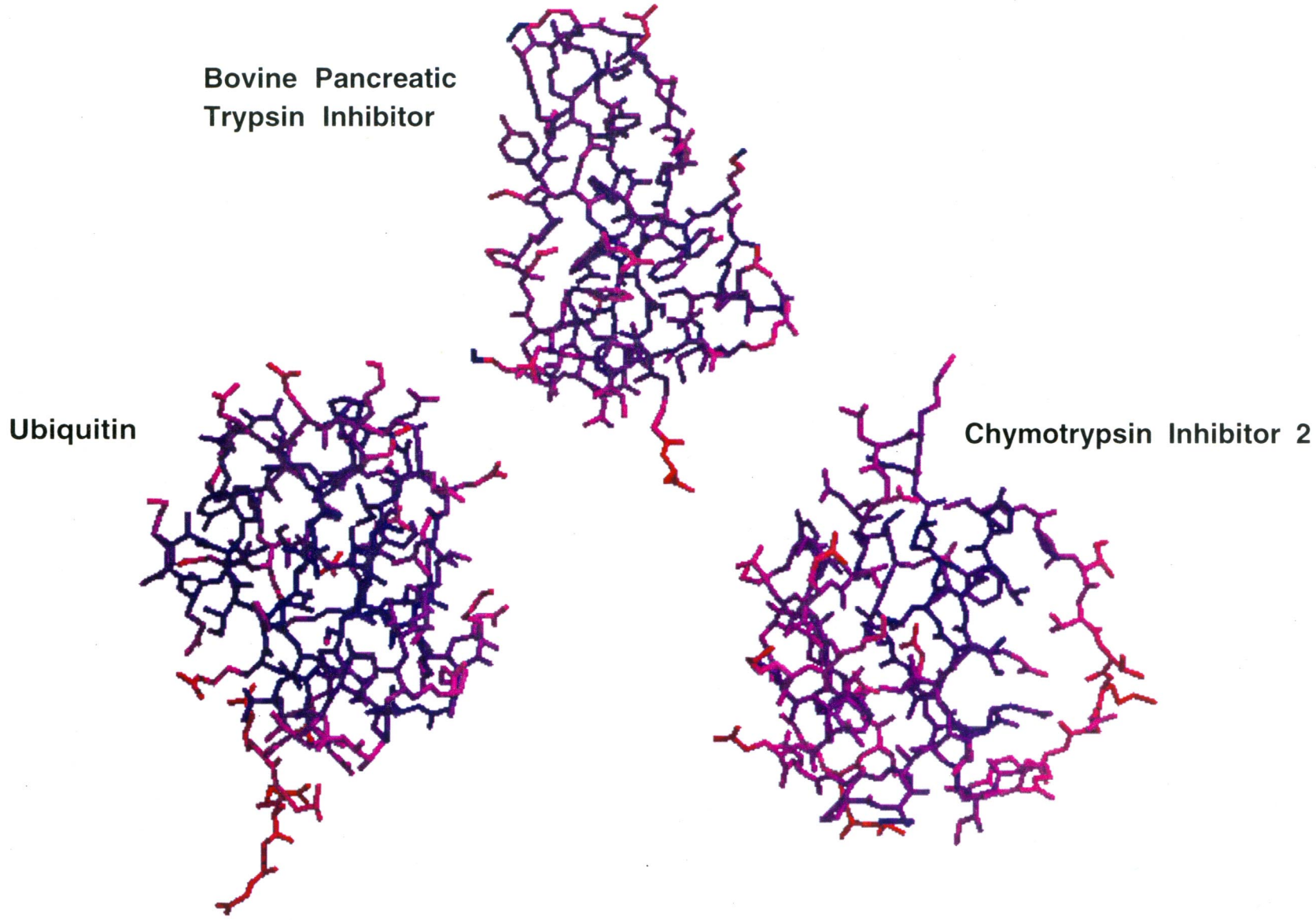
- Crystallography - not just static information
- In addition to position from e^- density, the spread of the density reflects the mobility of the atoms
- B-factor, B-value, Debye-Waller isotropic temperature factor, temperature factor

$$\langle \Delta x^2 \rangle^{1/2} = (3B/8\pi^2)^{1/2}$$

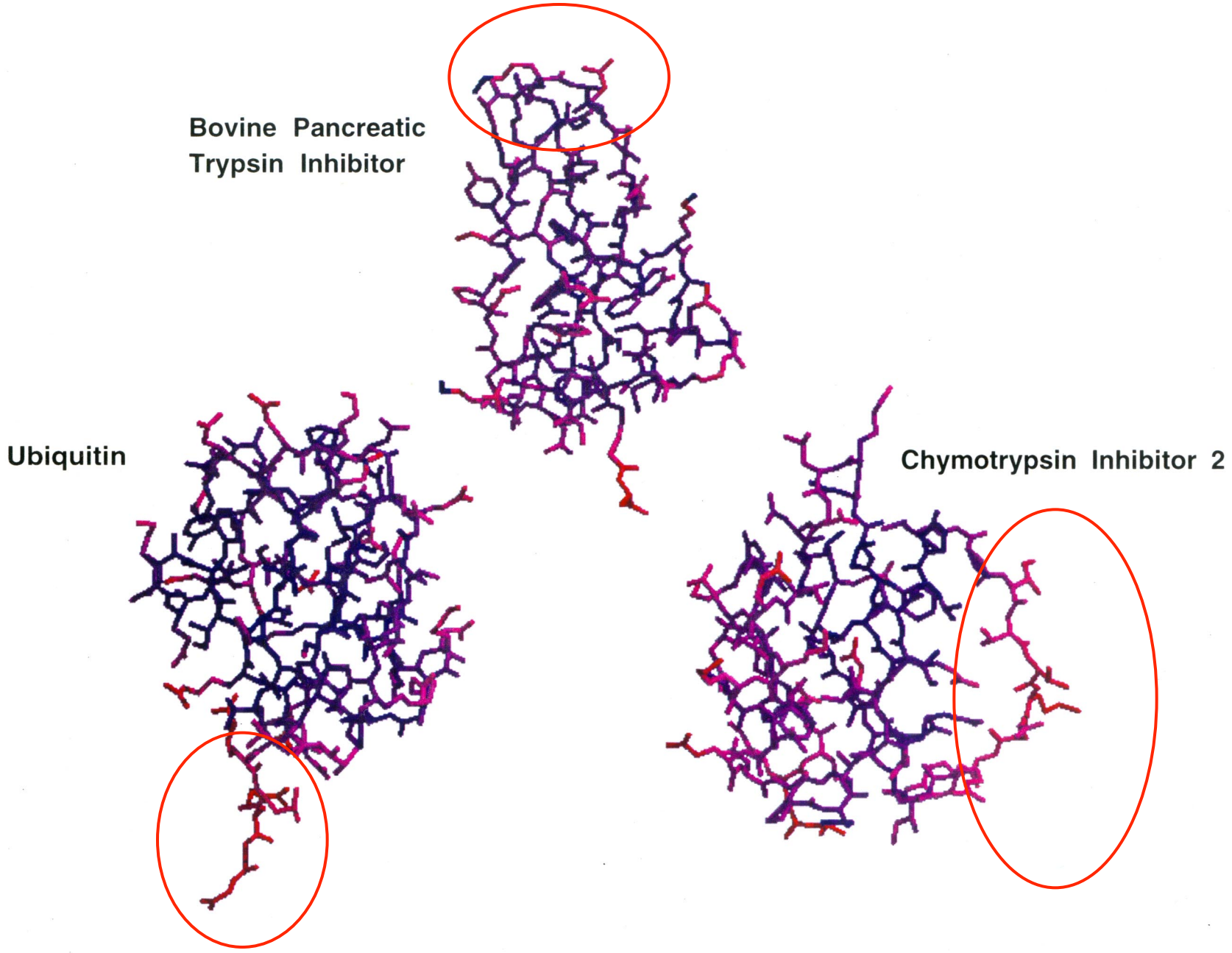
displacement ↑ B-factor ↑ Å^2

when atoms move they occupy space → spread ↑

- B factors distributed differently along the sequence
  - Lower in core
  - Higher at surface
- Typical values
  - 0.5-0.7 Å for mainchain
  - 0.5-7.0 Å for side chains



Colored according to B–factors: blue, least mobile, – red, most mobile.



Colored according to B–factors: blue, least mobile, – red, most mobile.

CI-2 Free vs. Bound to Subtilisin

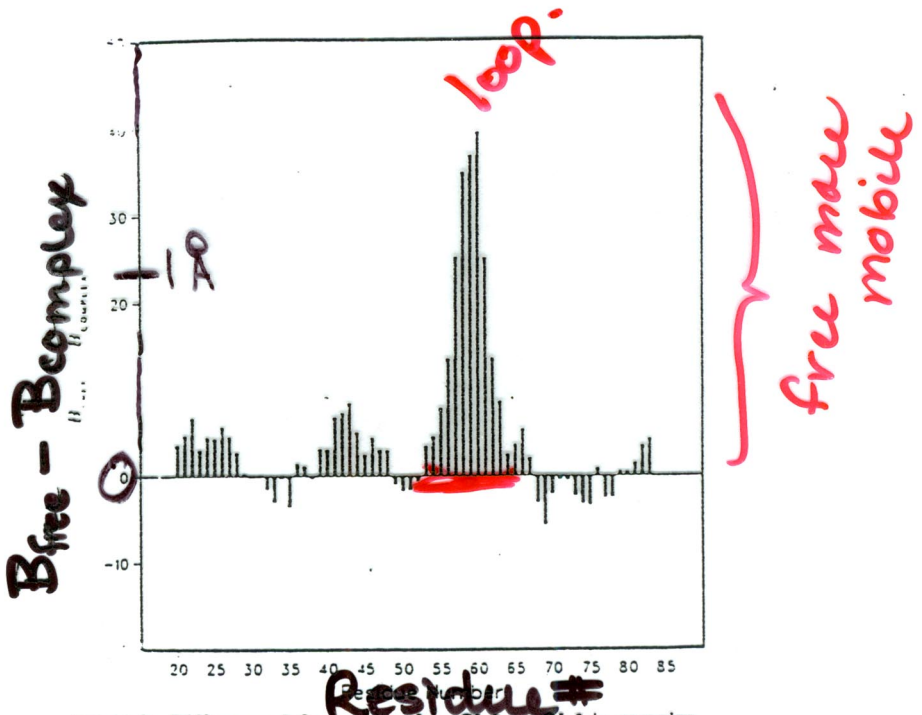


FIGURE 9: Difference *B* factor plot, free CI-2 vs. CI-2 in complex. The mean *B* factor of the main-chain atoms was calculated for each residue in free CI-2 and CI-2 from the complex with subtilisin Novo. The difference in mean *B* factor is plotted vs. residue number. No value is given for Asn-19I; no density is seen for this residue in the CI-2 structure from the complex.

McPhalen & James (1987) Biochem 26:261

Another Complication

- B-factor
  - Static Xtal Disorder Phase ambiguities, etc. [1]
  - Internal Molecular Motion [2]

[1] Should be T independent

[2] T dependent

- Ribonuclease A 124 aa.
  - Determine B-factors as a function of T

    (98 - 320K)

    (-175°- 47°C)

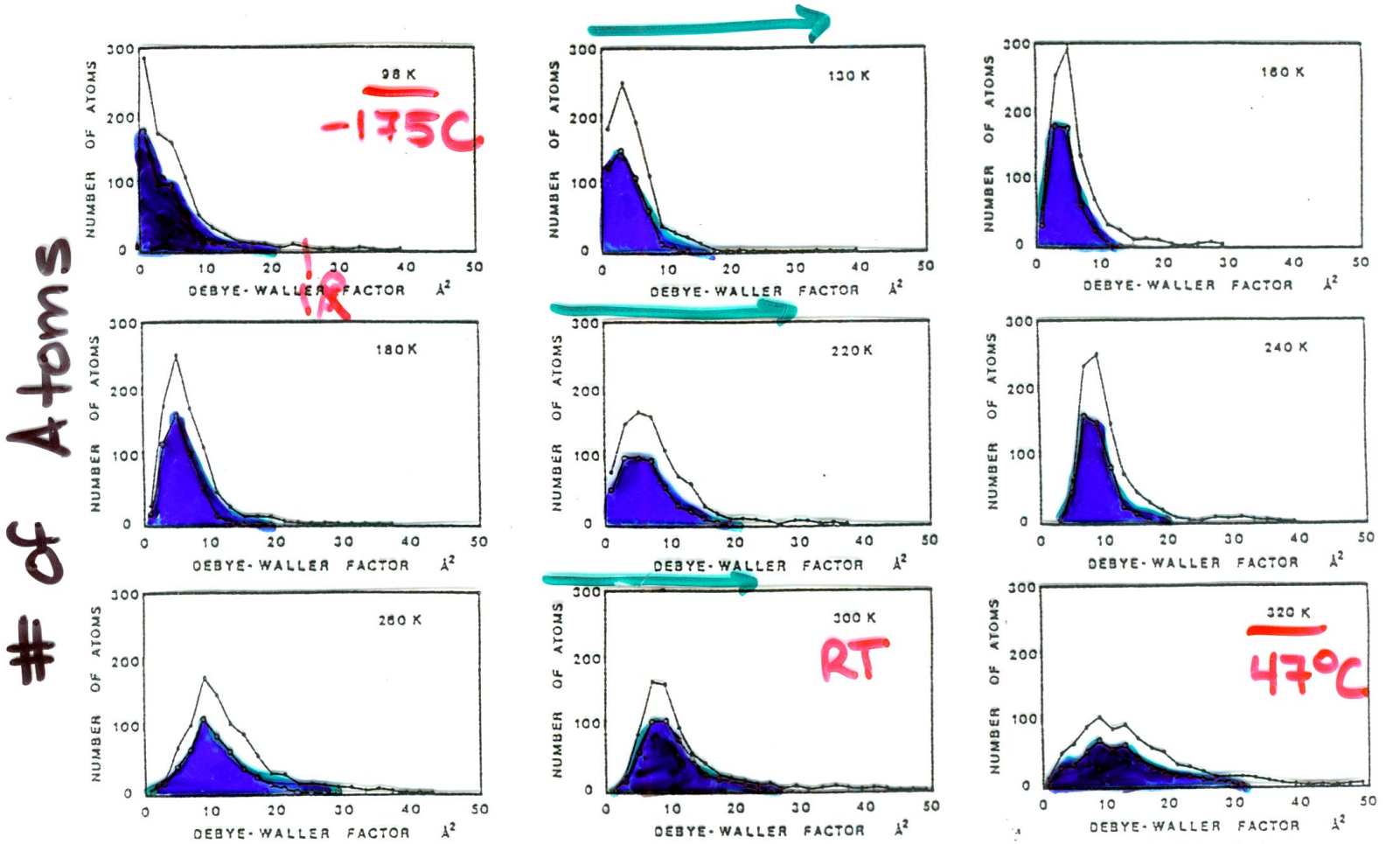


FIGURE 8: Histograms of protein atomic Debye–Waller factors for all atoms (●) and for main-chain atoms (○) at each of the nine temperatures.

Tilton, Dewan & Petsko (1992) Biochem 31:2469

- Distribution narrow @ low T - harmonic vibration
- Shifts and broadens @ higher T - anharmonic

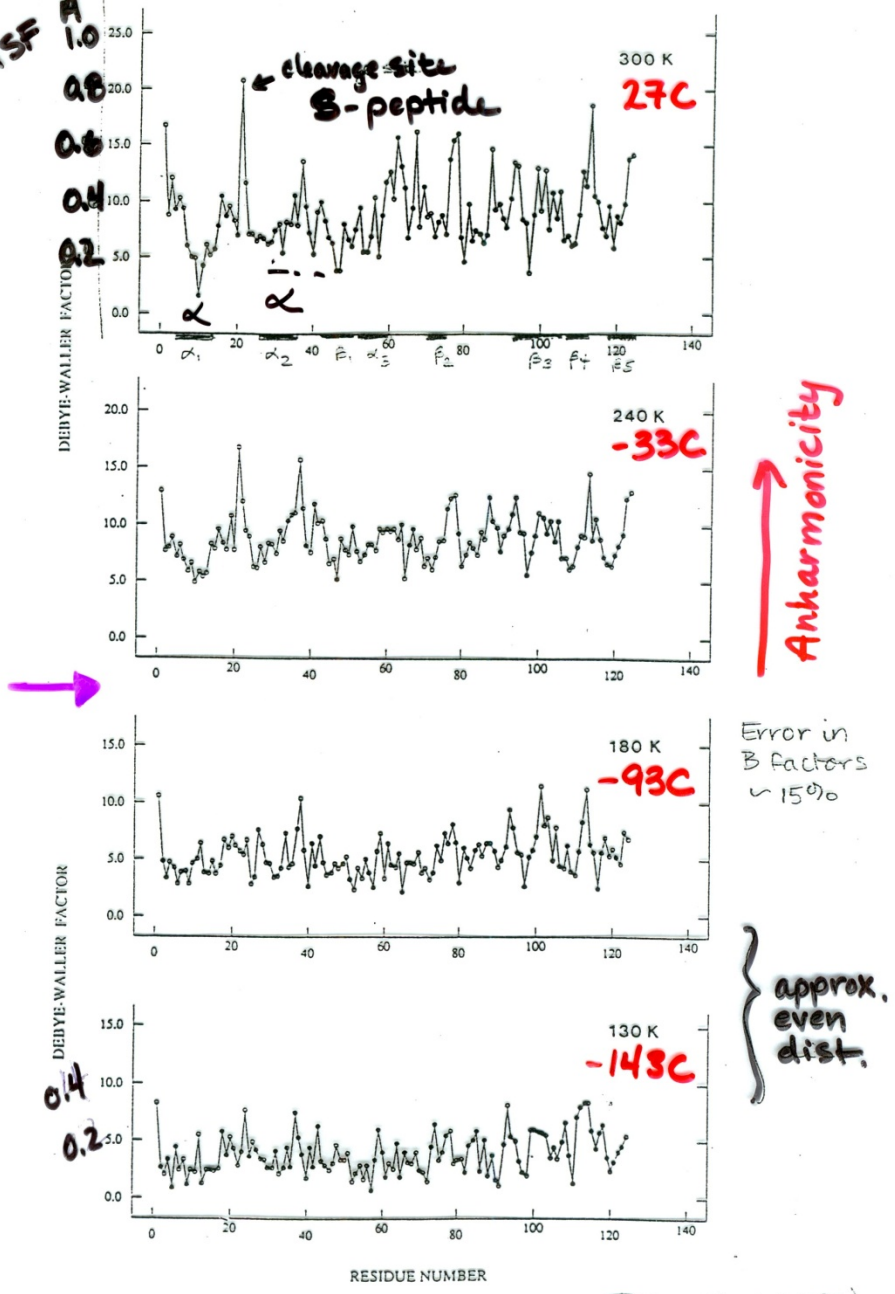
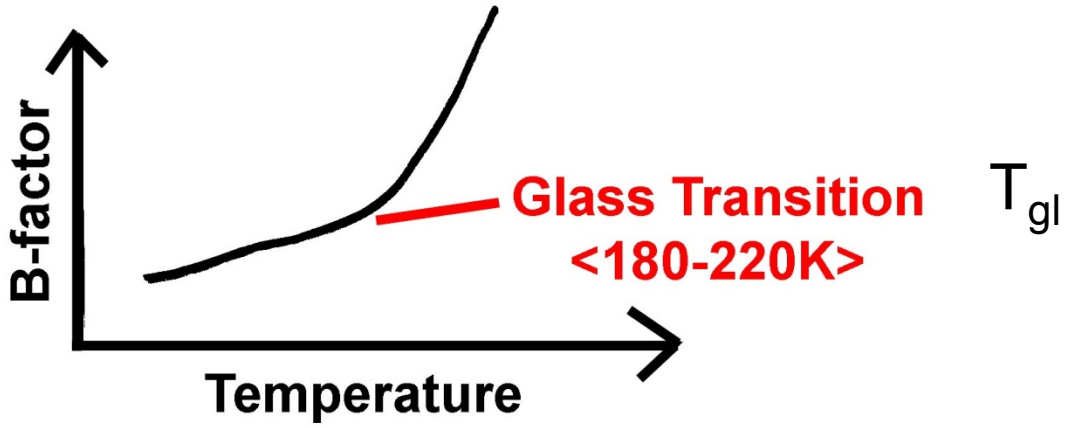


FIGURE 9: Average main-chain (Cα, C, N) Debye–Waller factors as a function of residue number for temperatures of 130, 180, 240, and 300 K. An overall increase as well as a greater range in the Debye–Waller factor profile is observed. Residue 21, the site of proteolytic cleavage in the production of ribonuclease-S, has the largest absolute Debye–Waller factor at elevated temperatures and exhibits the greatest temperature sensitivity.

Biphasic Behavior of B w/ T



- Individual amino acids display different behavior w/ T ↑
  - Independent of T
  - Linear change w/ T
  - Different biphasic behavior w/ T (Most)
- Even when protein @ 80K, some atoms retain some ability to move.

Other findings:

- The smaller the B-factor, the smaller the effective volume
  - » Low B, higher local atomic packing density
- V_{prot} ↑ linearly w/ T↑
- Greatest motion in turns and loops
  - Correlates with ligand-induced conformational changes
    - » α-helix and loops
    - » not β-structure

Link to Function?

- RNase

  @ 212K does not bind substrate nor inhibitor (-61°C, below T_{gl})

  @ 228K - reversible binding (-45°C, above T_{gl})

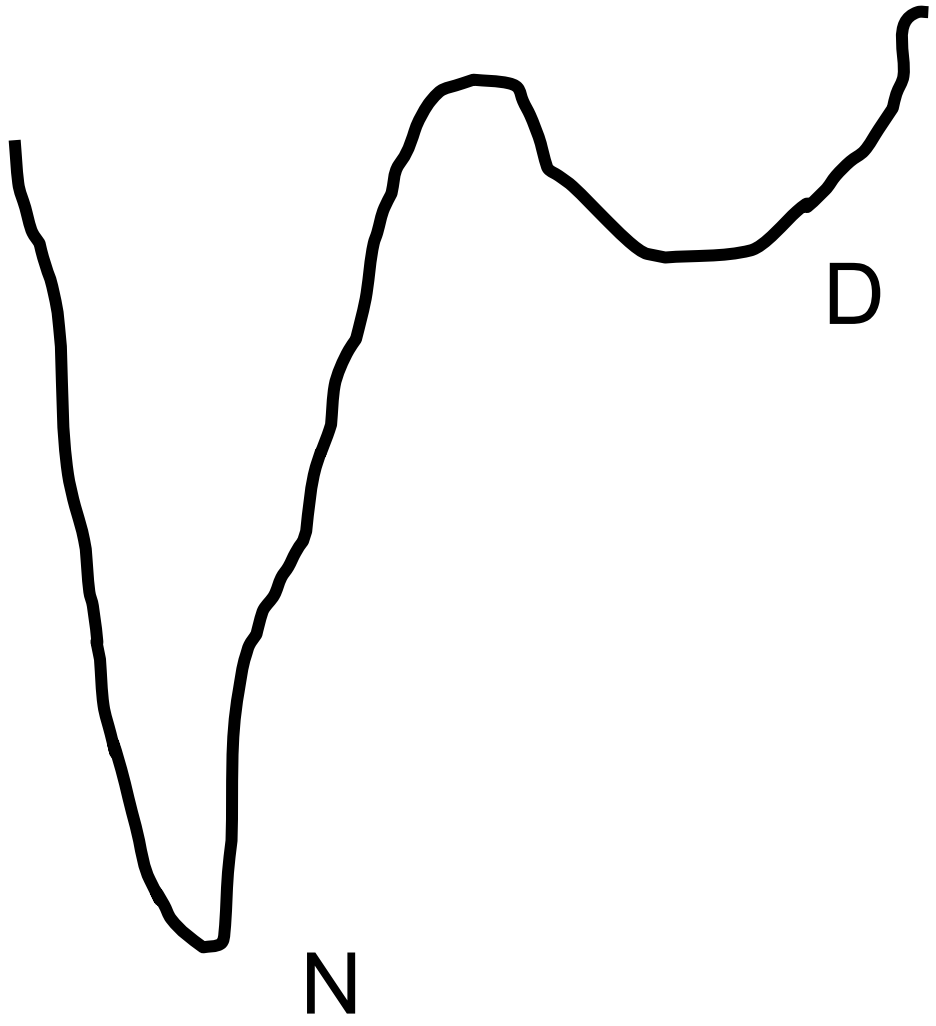
  ↓ 228K w/ substrate cooled to 212K

  No longer reversible

∴ flexibility (correlated, anharmonic motion) important for binding and enzymatic activity

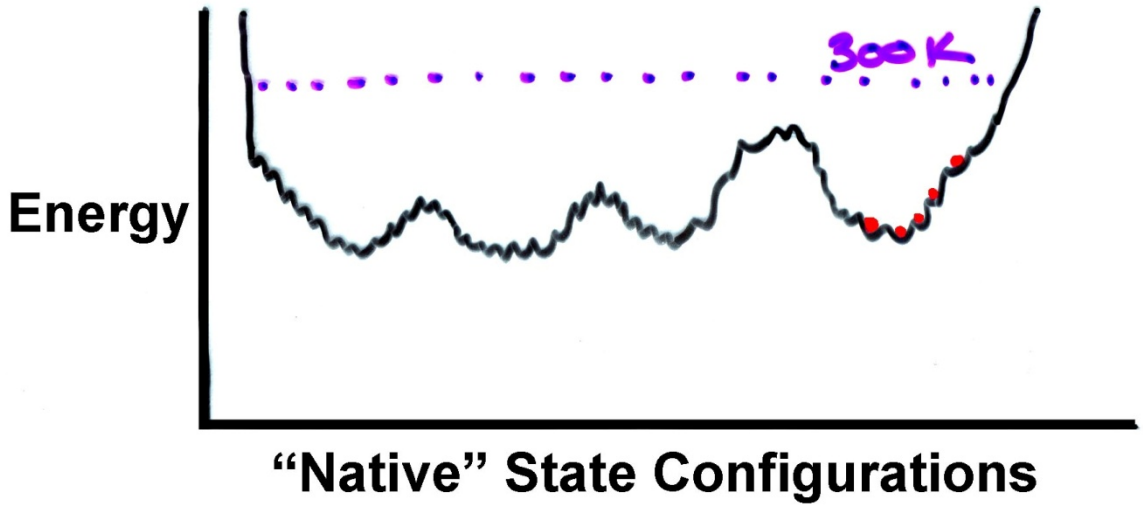
Petsko and co-workers, Nature 357:423

Conformational States



Conformational Substates

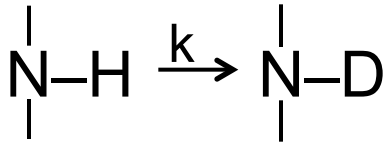
- Below the glass transition, proteins "frozen" into particular conformational states
- At room temperature, proteins rapidly convert between substates



- Low T, $<T_{gl}$, bond vibrations - local
- Higher T, $>T_{gl}$, coupled, correlated motion

Hydrogen Exchange

- Measure rate of exchange



NMR

- In general, exposed NH groups exchange rapidly
- Majority of interior NH groups also exchange but more slowly
- 2 Models for slow exchange
  - Solvent Penetration 1
  - Local Unfolding 2
- *Englander & Kallenbach (1984) Quart. Rev. of Biophys. 16:521*

1 Support for Penetration Model

- H/D Exchange in crystalline myoglobin using neutron diffraction

- Mb Xtals $\xrightarrow[\text{in } D_2O]{\text{soak}}$ 95% of HN → DN

- No unfolding occurs, the protein is constrained by the crystal lattice

2 Support for Local Unfolding Model

- H/D exchange in the S peptide-S protein complex
- All HN in S peptide exchange at similar rates yet some are buried and some are on the surface

- Local unwinding of helix?

- Re. folding see:
  Woodward. Curr. Opin. Struct. Biol. 4:112 (1994)
- Re. sequence effects see:
  Englander, Proteins, 17: 75, 87 (1993)

NMR

- Advantages
  - –In solution
  - –Sensitive to time scale & magnitude of motion
- Relaxation Experiments

  See Wagner (1993) Curr Opin Struct Biol 3:748

  model free approach $\longrightarrow$ S^2 generalized order parameters
- Extent of angular motion

  $S^2 = 0$ freely rotating bond

  $S^2 = 1$ totally rigid
- Also, effective correlation time, τ_e for reorientation of vector
- For methyl groups in BPTI τ_e = 19-70 ps

  $S^2 = 0.6$

Main Chain Dynamics of Ubiquitin

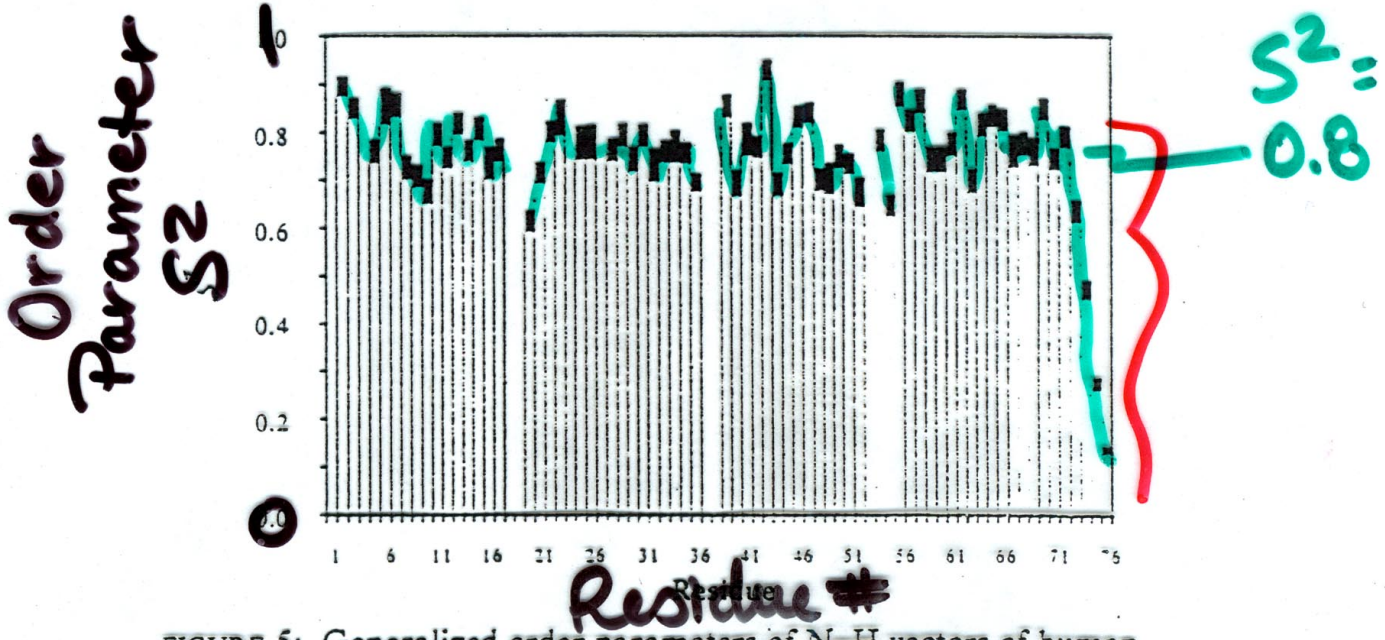
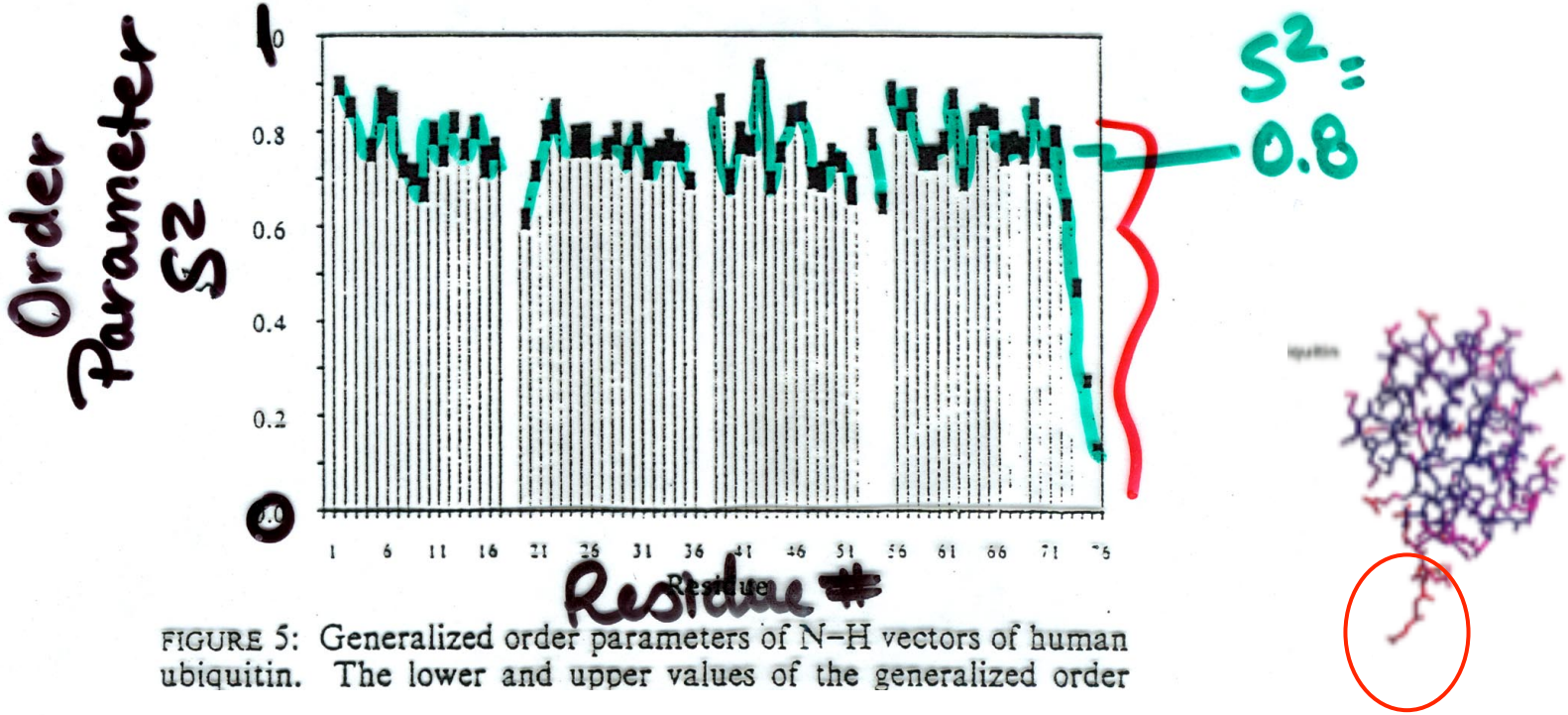


FIGURE 5: Generalized order parameters of N–H vectors of human ubiquitin. The lower and upper values of the generalized order

- S^2 not correlated w/ 2° structure
- Correlated w/ HB e.g. when Hbonds formed ~0.8, when no HB S^2 ~ 0.6
- Correlation time < 150ps (still fast, slower than Me)
- *Schneider, Dellwo & Wood (1992) Biochem 31:3645*

Main Chain Dynamics of Ubiquitin



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3 Relative Motion

- X-ray

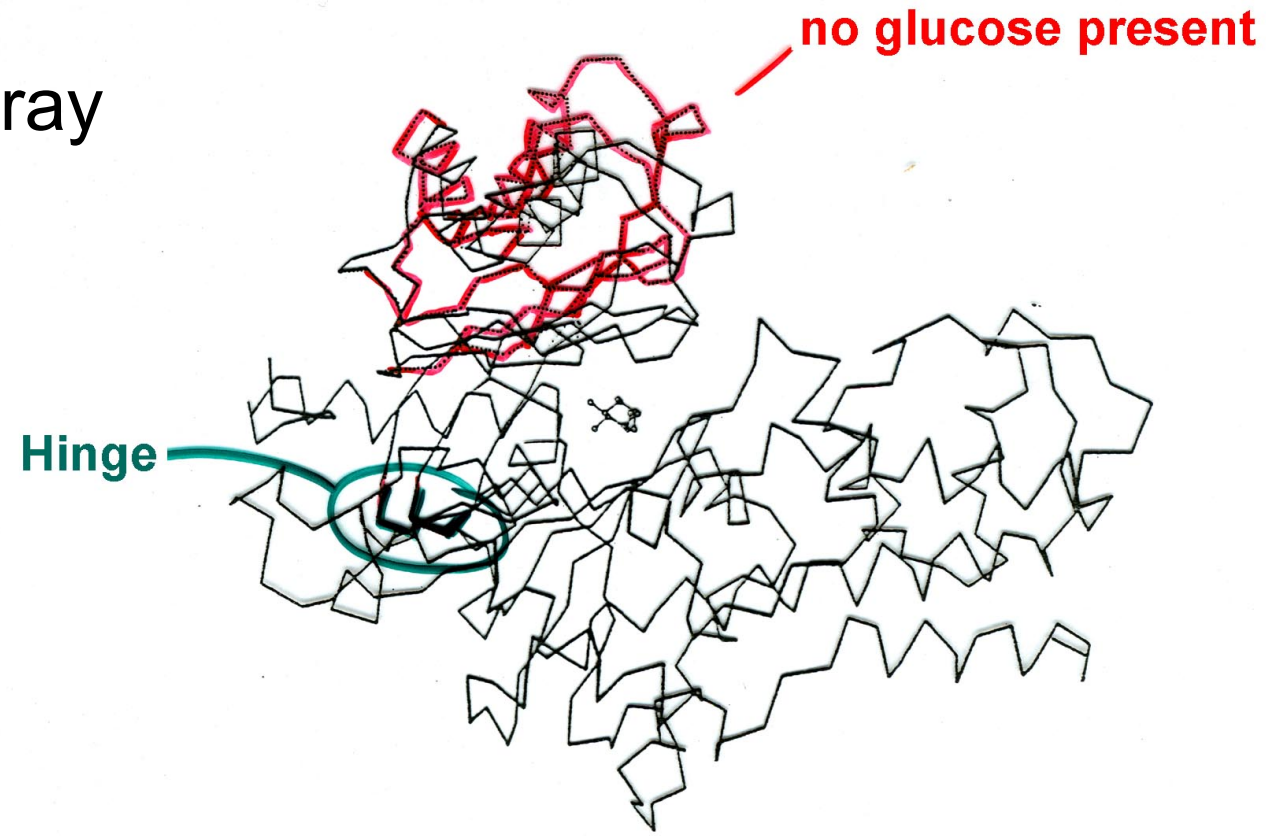


FIGURE 12·13. The conformational changes induced in hexokinase by glucose binding. The solid lines show the α-carbon backbone of the A isozyme crystallized in the presence of glucose. The dotted lines show the backbone of the part of the B isozyme that has a different structure when crystallization occurs in the absence of glucose. [From W. S. Bennett and T. A. Steitz, *Fedn. Proc. Abstr.* (1977).]

- Conformational Changes in Hexokinase

Theory

- Experiment clearly demonstrates that proteins are mobile, but no single experiment or combination of experiments can provide an all-inclusive view of the dynamic behavior of all atoms in a protein.

- Computer simulations can however
  - ea. atom as a function of time

- *Review: Karplus & Petsko (1990) Nature 347:631*

Why Molecular Dynamics?

- System Size: *n*=# of atoms
  - Ab initio QM n^4
  - Semiempirical QM n^3
  - MM/Empirical force field methods n^2, or n w/ truncation
- Most realistic simulation method available
- Can provide structural and dynamic information unobtainable by experiment, but is experimentally testable
- 4^{th} dimension to PDB, 3D structures moving through time

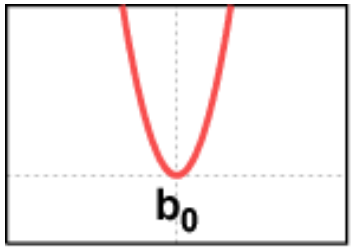
But

- Sampling is limited, the goal is to sample experimentally relevant regions of conformational space, not all of conformational space

Methods

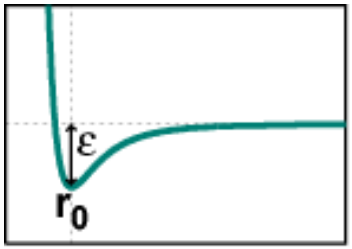
- **Molecular mechanics force field**

  ***U* = Bond + Angle + Dihedral + van der Waals + Electrostatic**



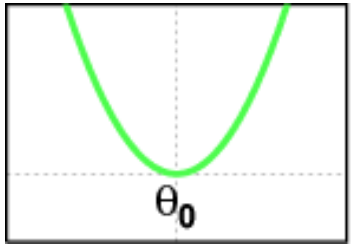
Bond

$$\sum_{i}^{bonds} K_{b,i}(b_i - b_{0,i})^2$$



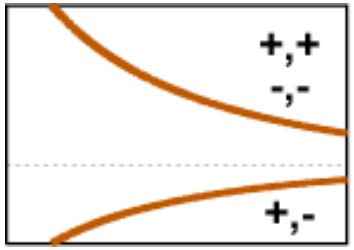
van der Waals

$$\sum_{pairs\text{-}i,j} [\varepsilon_{ij}\left(\frac{r_{0,ij}}{r_{ij}}\right)^{12} - 2\varepsilon_{ij}\left(\frac{r_{0,ij}}{r_{ij}}\right)^{6}]$$



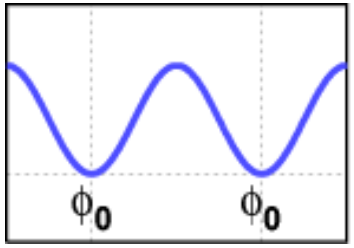
Angle

$$\sum_{i}^{\substack{bond \\ angles}} K_{,i}(\theta_i - \theta_{0,i})^2$$



Electrostatic

$$332 \sum_{pairs\text{-}i,j} \left(\frac{q_i q_j}{r_{ij}}\right)$$



Dihedral

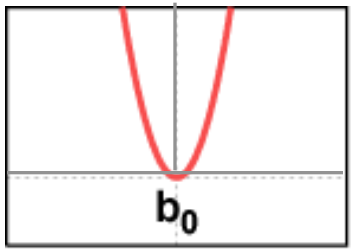
$$\sum_{i}^{\substack{torsion \\ angles}} K_{\phi,i}\{1 - \cos[n_i(\phi_i - \phi_{0,i})]\}$$

Levitt, Sharon, Hirshberg, & Daggett, 1995, J. Comp. Phys. Water Model: J Phys Chem,1997

Molecular Dynamics

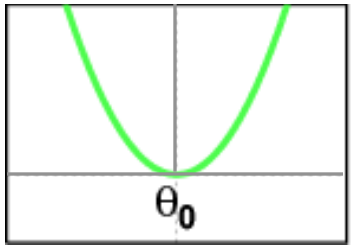
- **Potential function for MD[1,2]**

***U* = Bond + Angle + Dihedral**



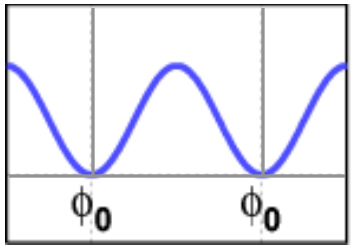
Bond

$$\sum_{i}^{bonds} K_{b,i}(b_i - b_{0,i})^2$$



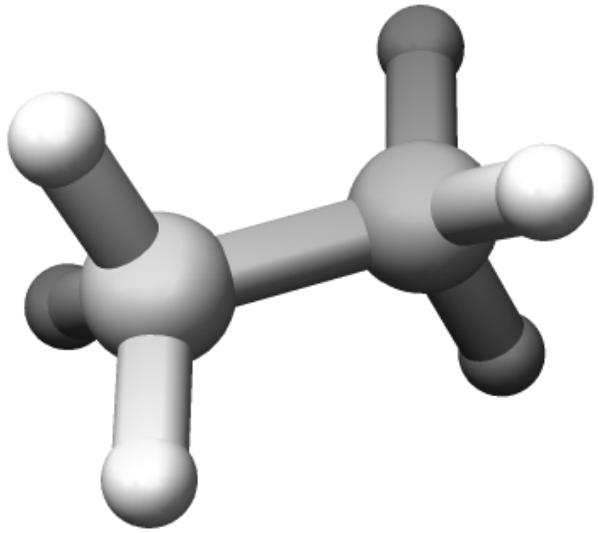
Angle

$$\sum_{i}^{\substack{bond \\ angles}} K_{\theta,i}(\theta_i - \theta_{0,i})^2$$



Dihedral

$$\sum_{i}^{\substack{torsion \\ angles}} K_{\phi,i}\{1 - \cos[n_i(\phi_i - \phi_{0,i})]\}$$

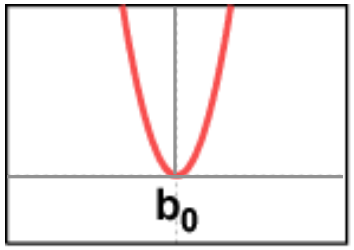


1. Levitt M. Hirshberg M. Sharon R. Daggett V. Comp. Phys. Comm. (1995) 91: 215-231
2. Levitt M. *et al.* J. Phys. Chem. B (1997) 101: 5051-5061

Molecular Dynamics

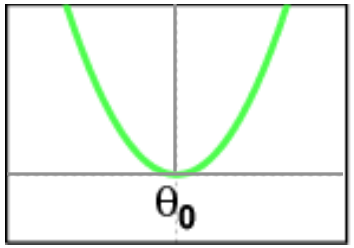
- **Potential function for MD[1,2]**

***U* = Bond + Angle + Dihedral + van der Waals +**



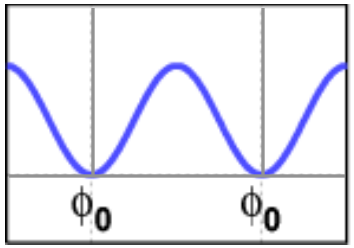
Bond

$$\sum_{i}^{bonds} K_{b,i}(b_i - b_{0,i})^2$$



Angle

$$\sum_{i}^{\substack{bond \\ angles}} K_{\theta,i}(\theta_i - \theta_{0,i})^2$$



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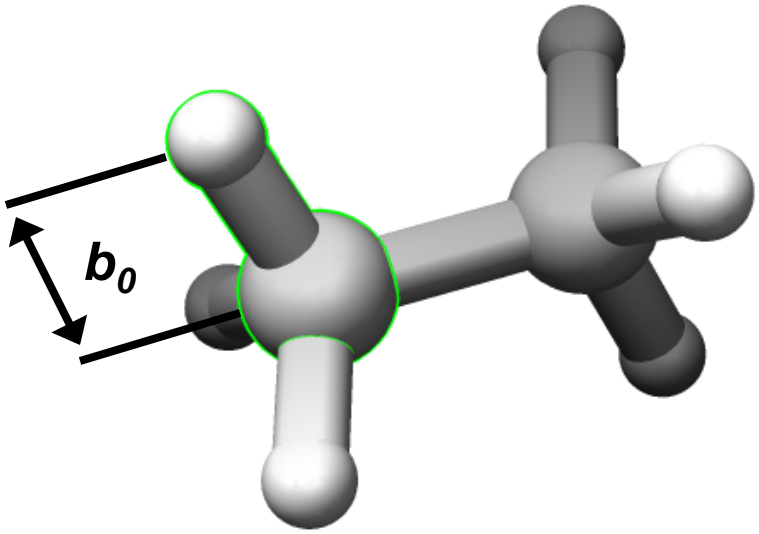

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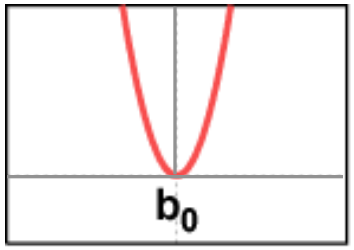

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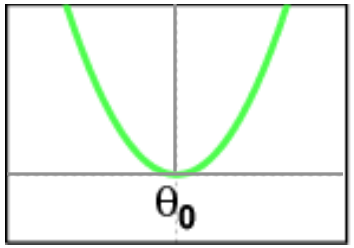

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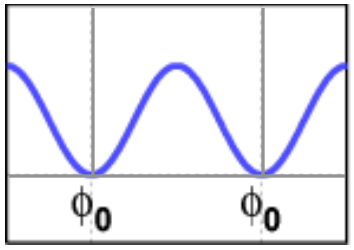

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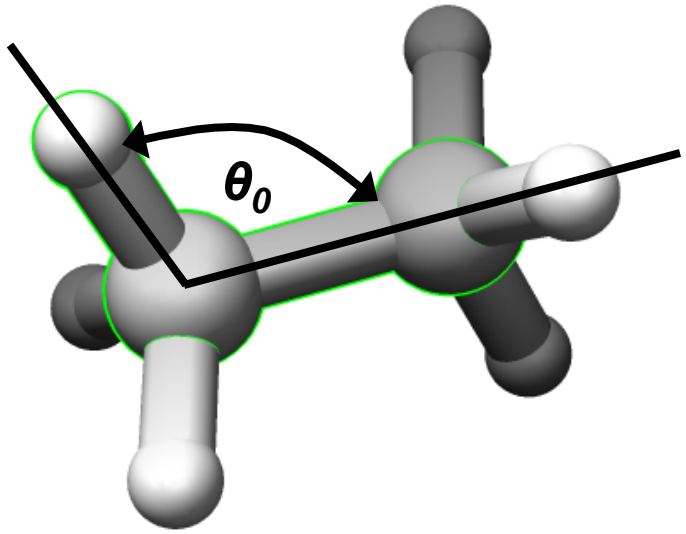

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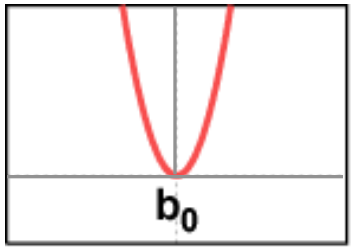

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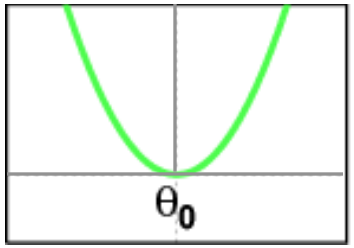

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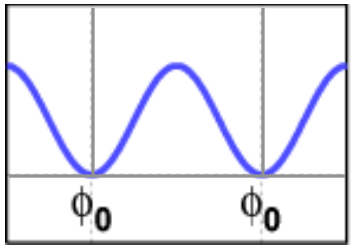

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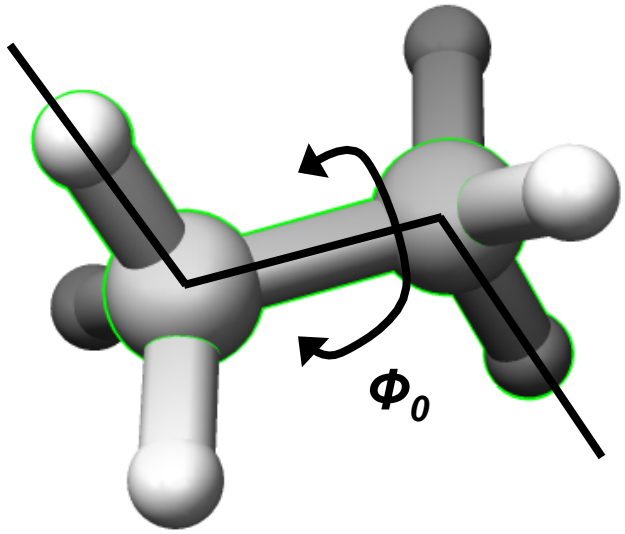

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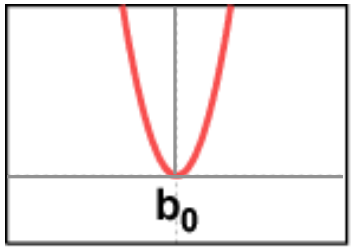
Molecular Dynamics

- **Potential function for MD[1,2]**

U **= Bond + Angle + Dihedral + vdW+ Electrostatic**



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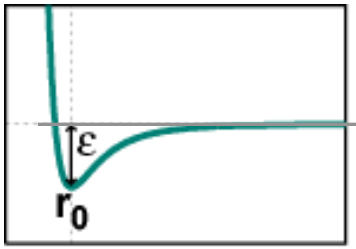
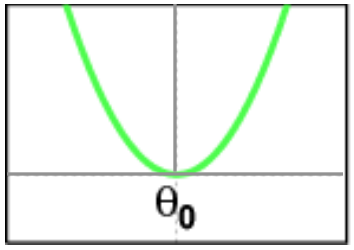


van der Waals

$$\sum_{pairs\cdot i,j} [\varepsilon_{ij}\left(\frac{r_{0,ij}}{r_{ij}}\right)^{12} - 2\varepsilon_{ij}\left(\frac{r_{0,ij}}{r_{ij}}\right)^{6}]$$



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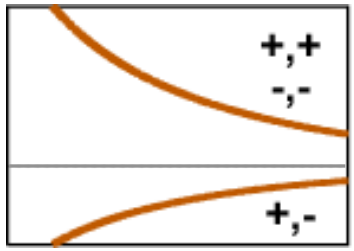
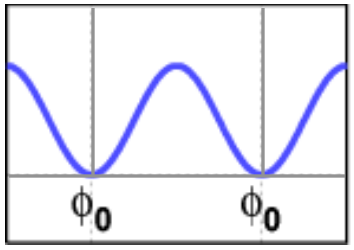


Electrostatic

$$332 \sum_{pairs\cdot i,j} \left(\frac{q_i q_j}{r_{ij}}\right)$$



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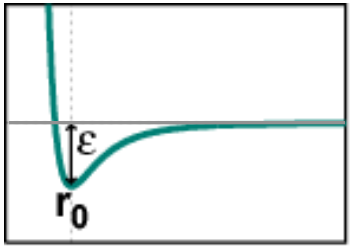
Molecular Dynamics

- **Non-bonded components of potential function**

  U_{nb} = van der Waals + Electrostatic



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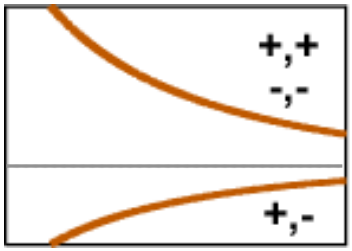

Electrostatic

$$332 \sum_{pairs\cdot i,j} \left(\frac{q_i q_j}{r_{ij}} \right)$$

- ***To a large degree, protein structure is dependent on non-bonded atomic interactions***

Molecular Dynamics

- **Non-bonded components of potential function**

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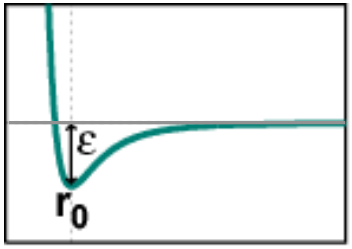

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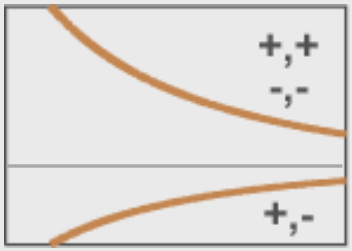

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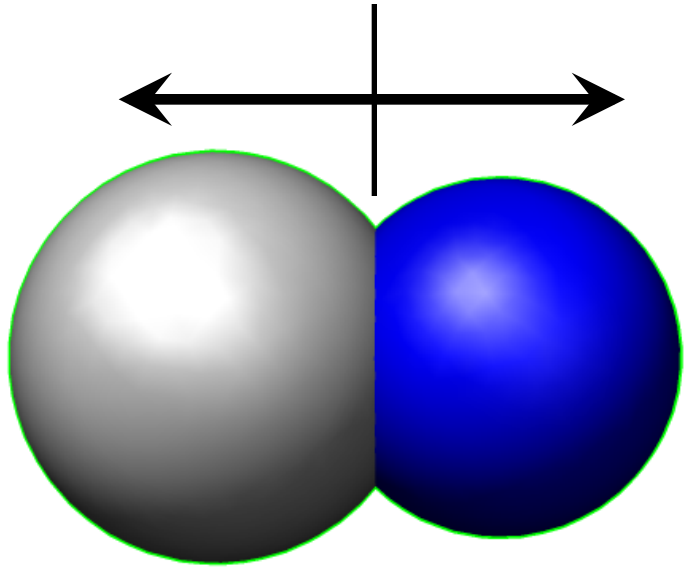

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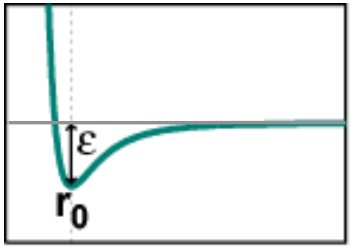

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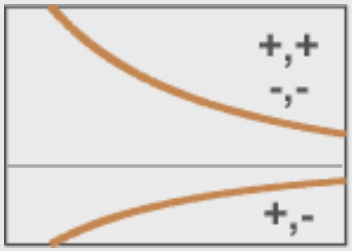

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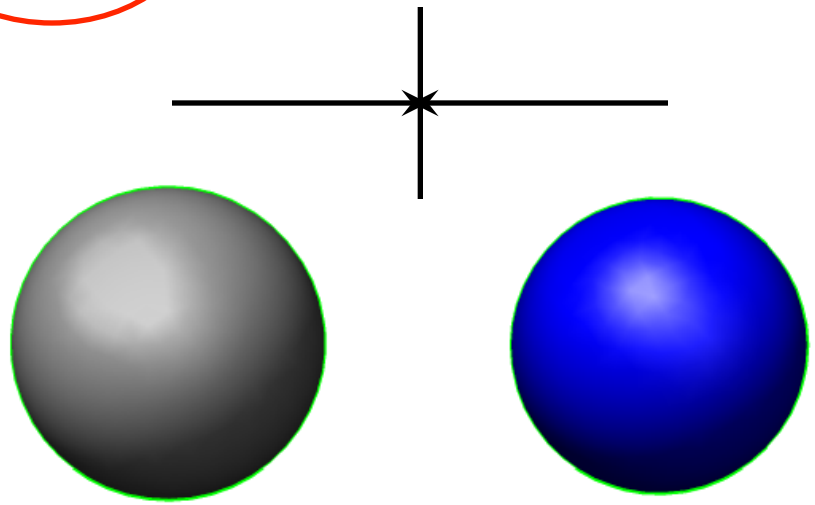

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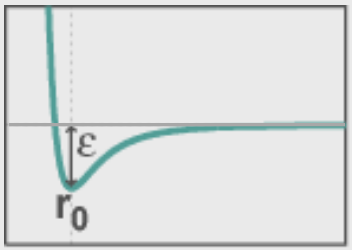

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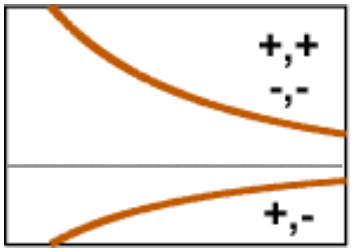

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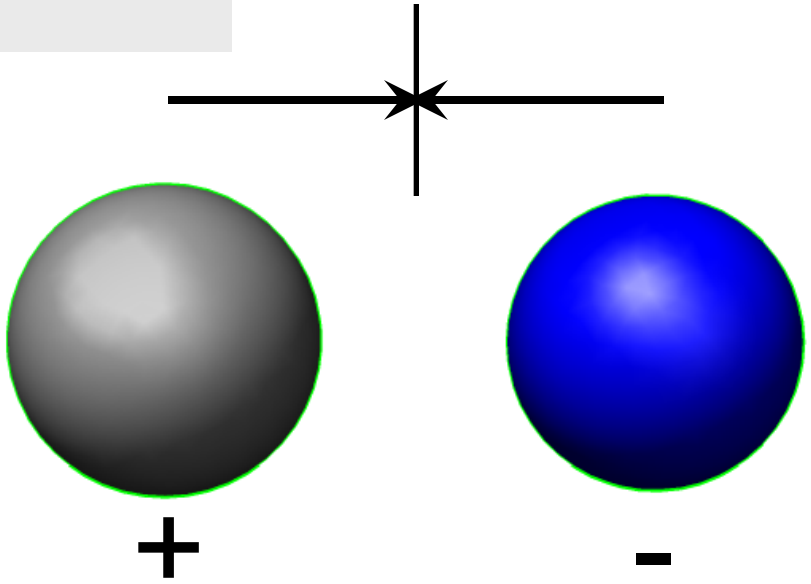

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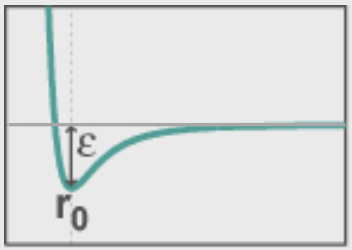

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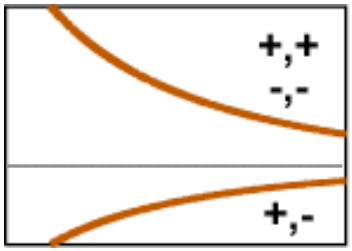

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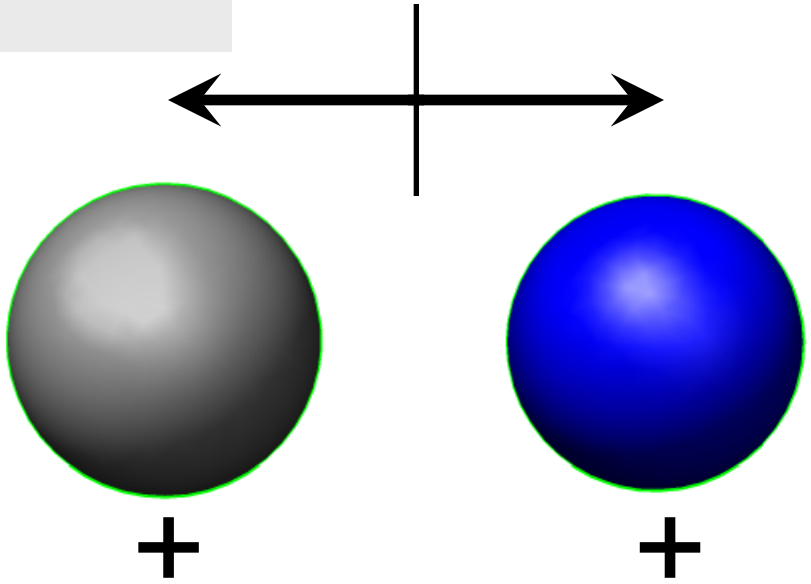

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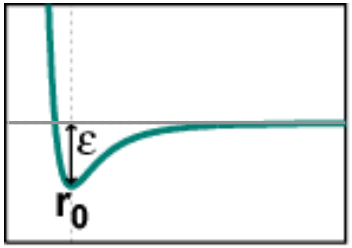

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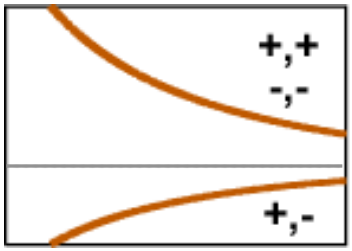

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NOTE:

Sum over all pairs of N atoms, or

$$\frac{N * N - 1}{2}$$ **pairs**

N is often between $5x10^5$ to $5x10^6$

For $5x10^5$ that is $1.25x10^{11}$ pairs

THAT IS A LOT OF POSSIBLE PAIRS!

What can you do with a force field?

- Relax your structure
- Refine your structure
- Determine your structure
- Score structures
- Etc.

Methods

- **Molecular dynamics (MD)**
  - **time dependent integration of classical equations of motion**

$$F = -\frac{\partial U}{\partial x}$$

$$F = ma$$

$$a = \frac{v_2 - v_1}{\partial t}$$

$$v = \frac{x_2 - x_1}{\partial t}$$

$$\partial t = 2 \text{ fs}$$

Methods

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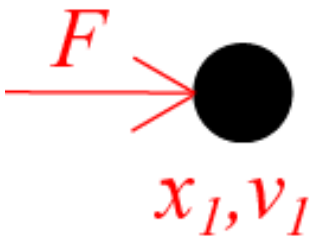

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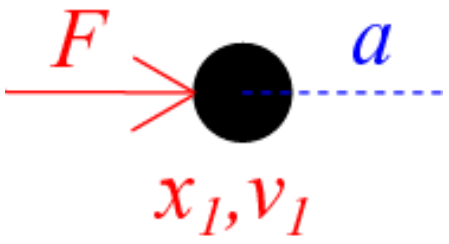

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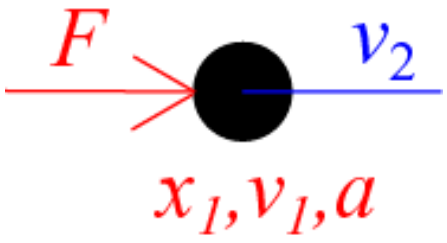

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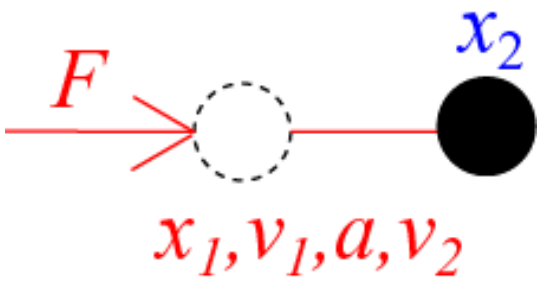

$$\partial t = 2 \text{ fs}$$

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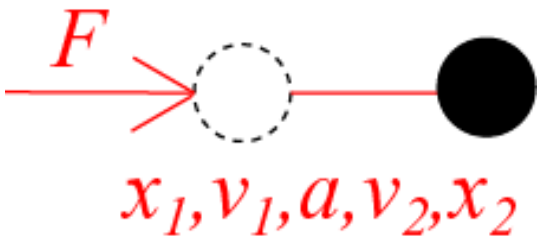


$$\partial t = 2 \text{ fs}$$

Methods

- **Molecular dynamics (MD)**

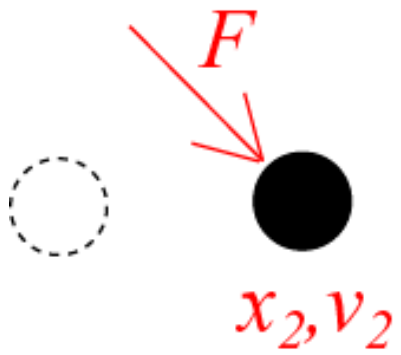
$$F = -\frac{\partial U}{\partial x}$$

$$F = ma$$

$$a = \frac{v_3 - v_2}{\partial t}$$

$$v = \frac{x_3 - x_2}{\partial t}$$

$$\partial t = 2 \text{ fs}$$

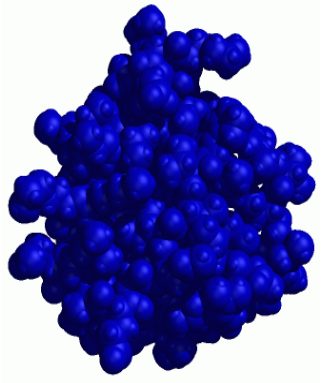


Do it all over again and again and again

50,000 atoms, each ps involves 25,000,000 evaluations

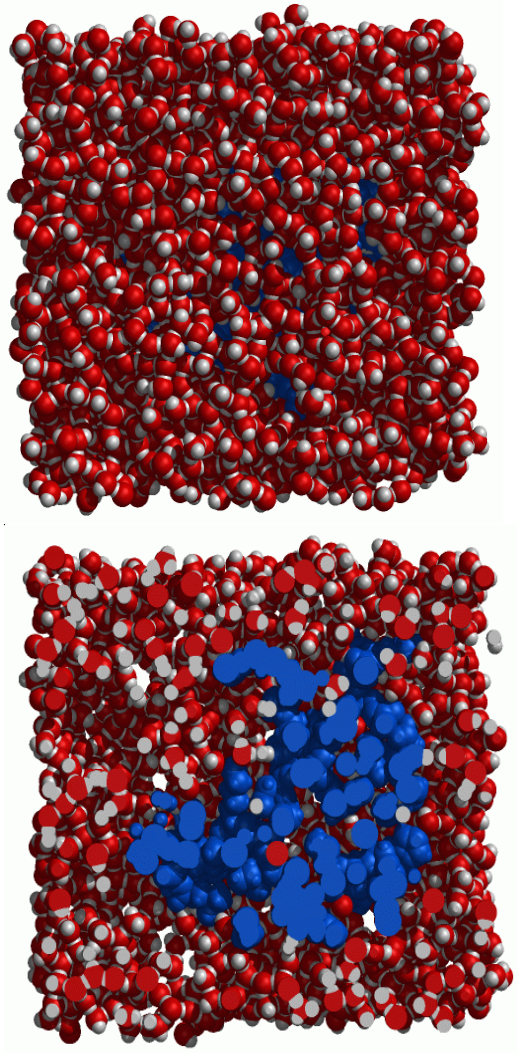
20 ns (20,000 ps) requires 5×10^{11} evaluations

Starting a Molecular Dynamics Simulation



Crystal or
NMR Structure

Solvate with
water or
other solvent
8 - 14 Å
from protein



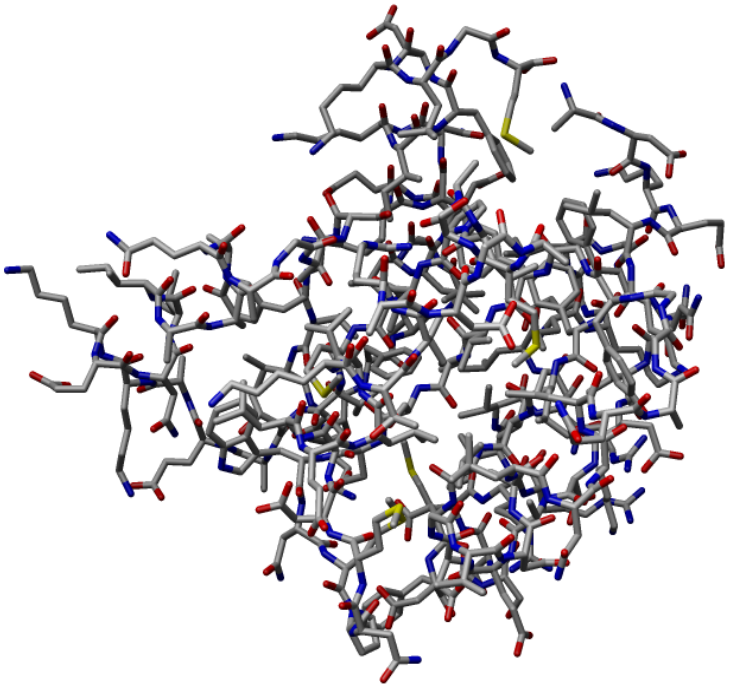
Heat to desired
temperature and
allow motion to
evolve over time

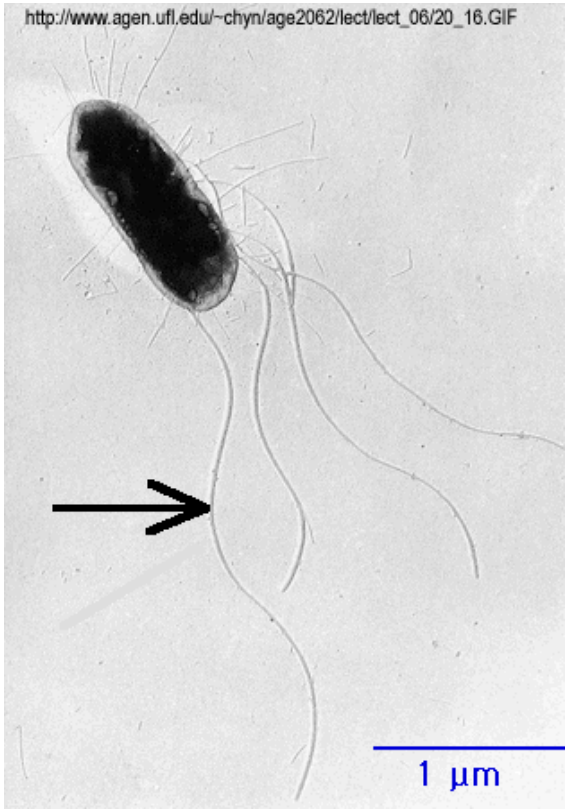
T = 25 ºC
ρ = 0.997 gm/ml

T = 60 ºC
ρ = 0.983 gm/ml

Molecular Dynamics

- **MD provides atomic resolution of native dynamics**

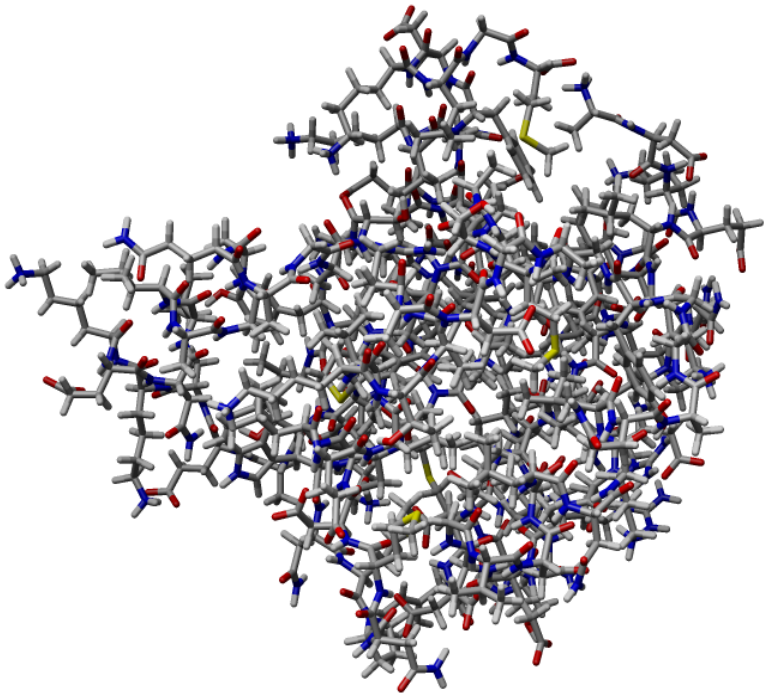




PDB ID: 3chy, *E.* coli CheY 1.66 Å X-ray crystallography

Molecular Dynamics

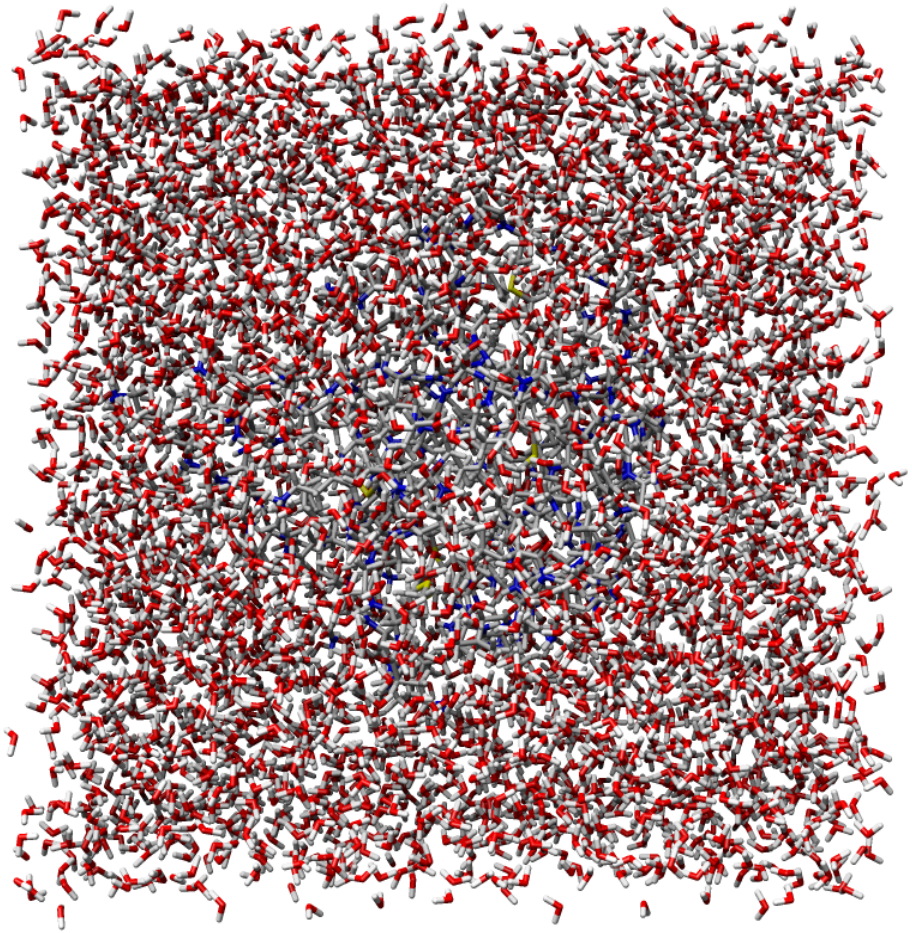
- **MD provides atomic resolution of native dynamics**



3chy, hydrogens added

Molecular Dynamics

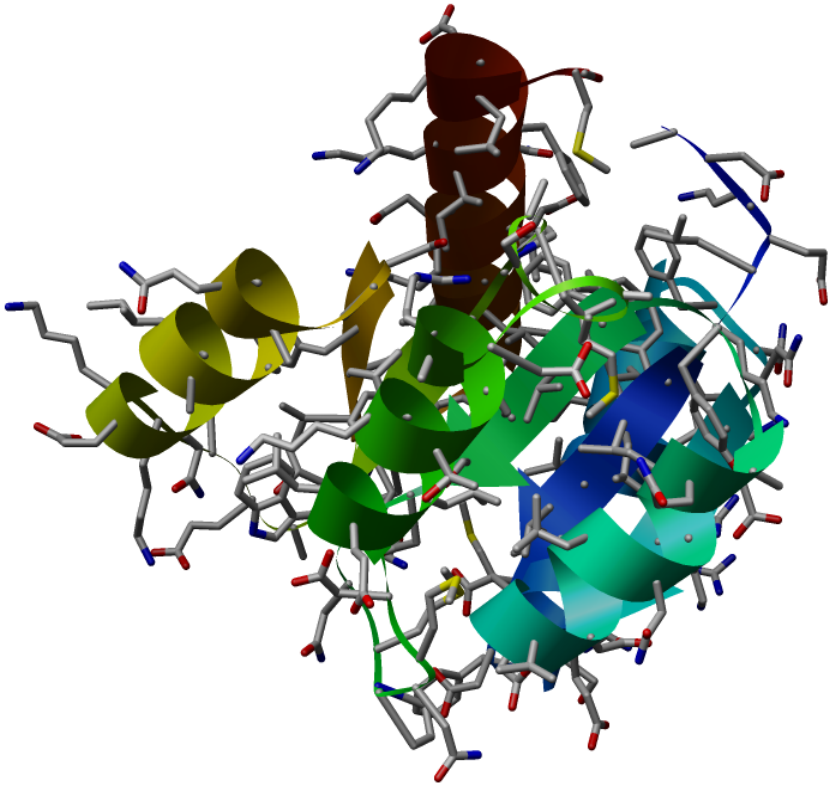
- **MD provides atomic resolution of native dynamics**



3chy, waters added (i.e. solvated)

Molecular Dynamics

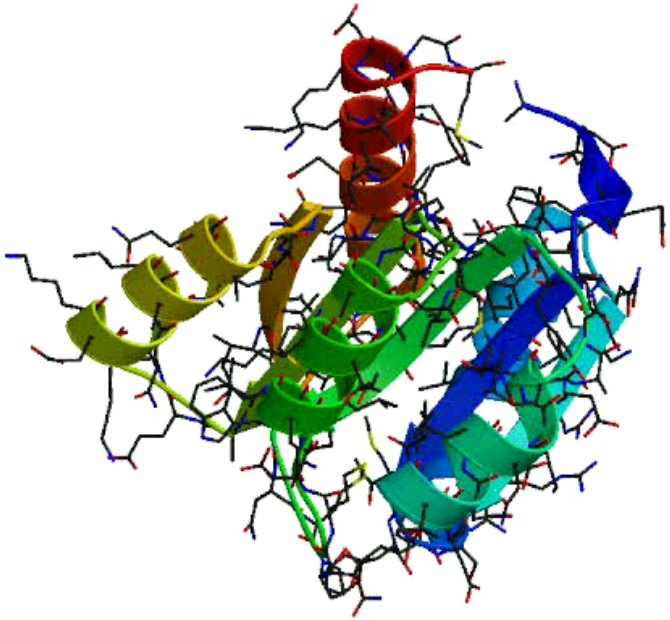
- **MD provides atomic resolution of native dynamics**



3chy, waters and hydrogens hidden

Molecular Dynamics

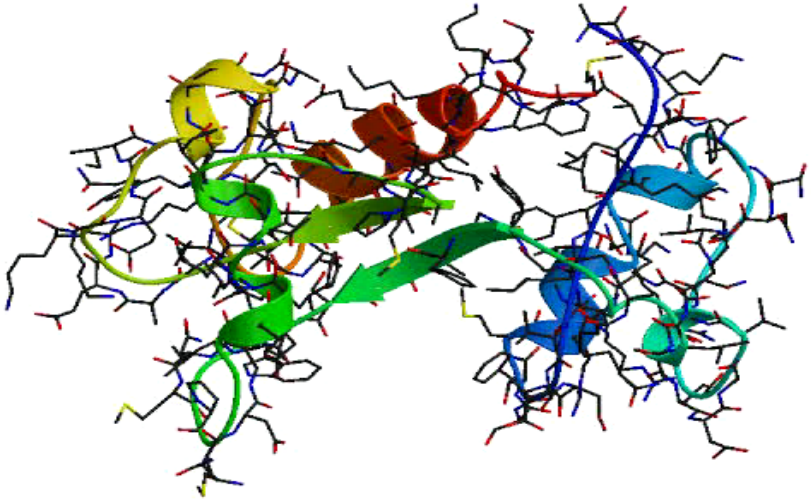
- **MD provides atomic resolution of native dynamics**



native state simulation of 3chy at 298 Kelvin, waters and hydrogens hidden

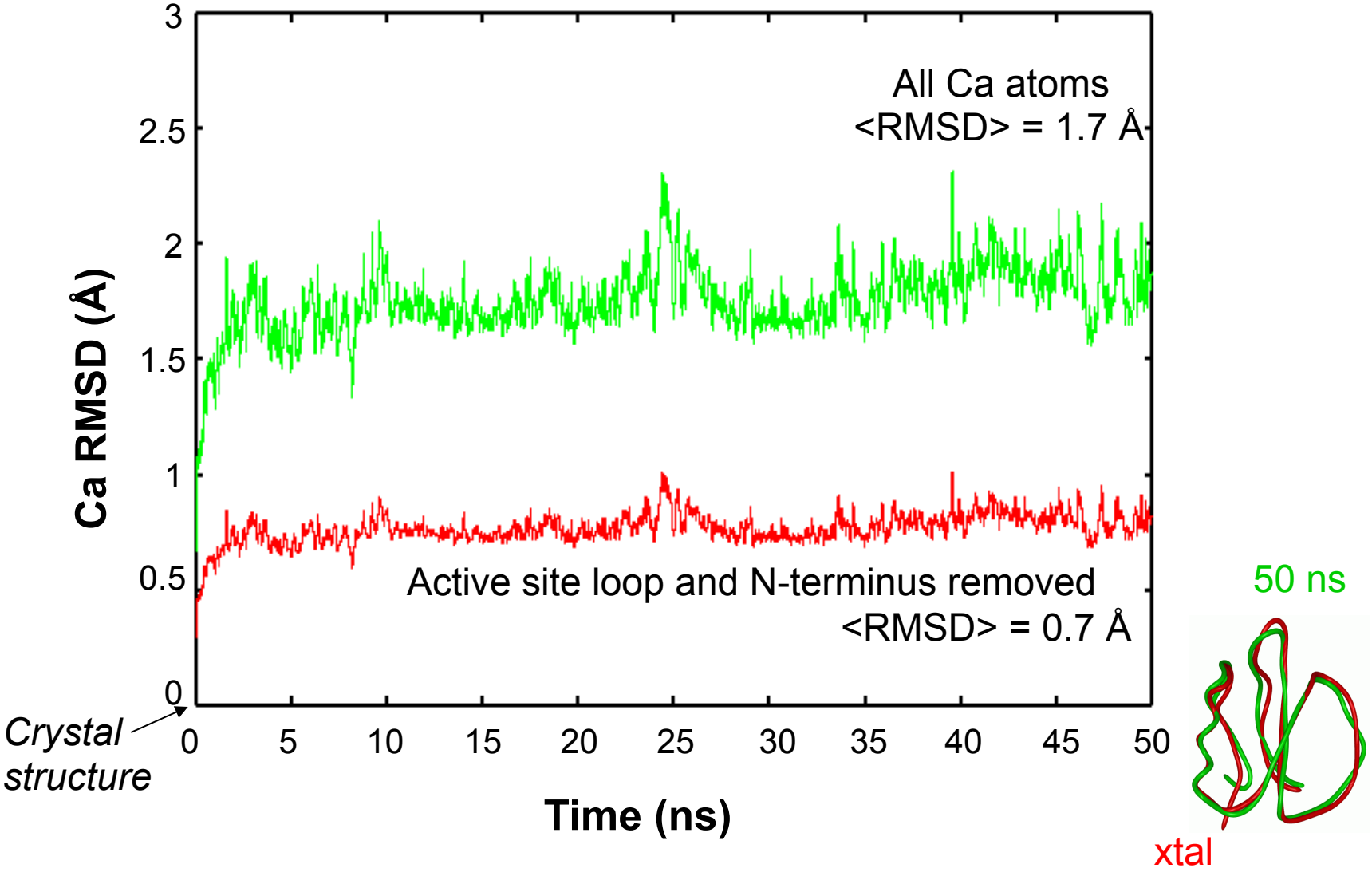
Molecular Dynamics

- **MD provides atomic resolution of folding / unfolding**

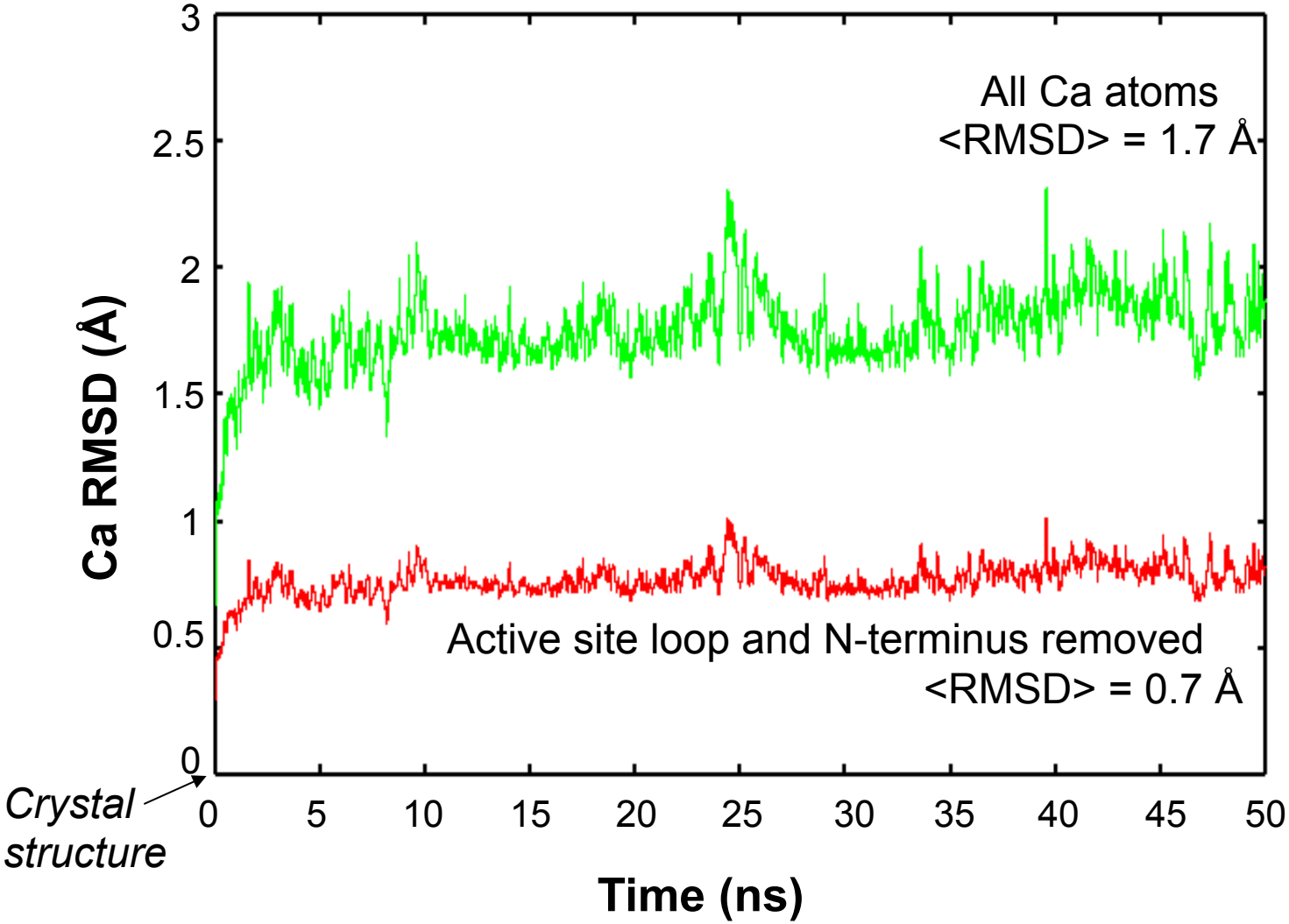


unfolding simulation (reversed) of 3chy at 498 Kelvin, waters & hydrogens hidden

Native Dynamics at 25 ºC

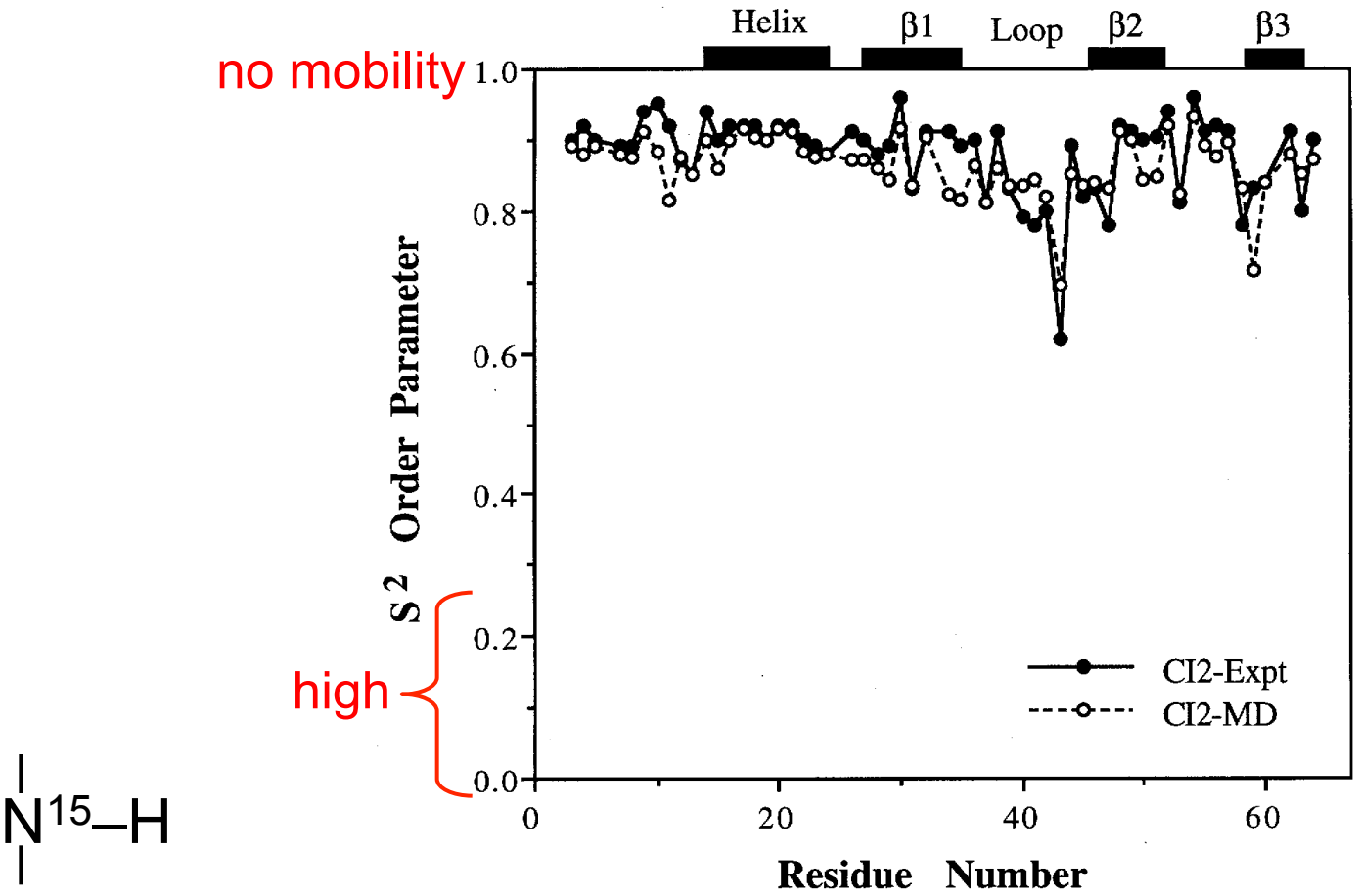


Native Dynamics at 25 ºC



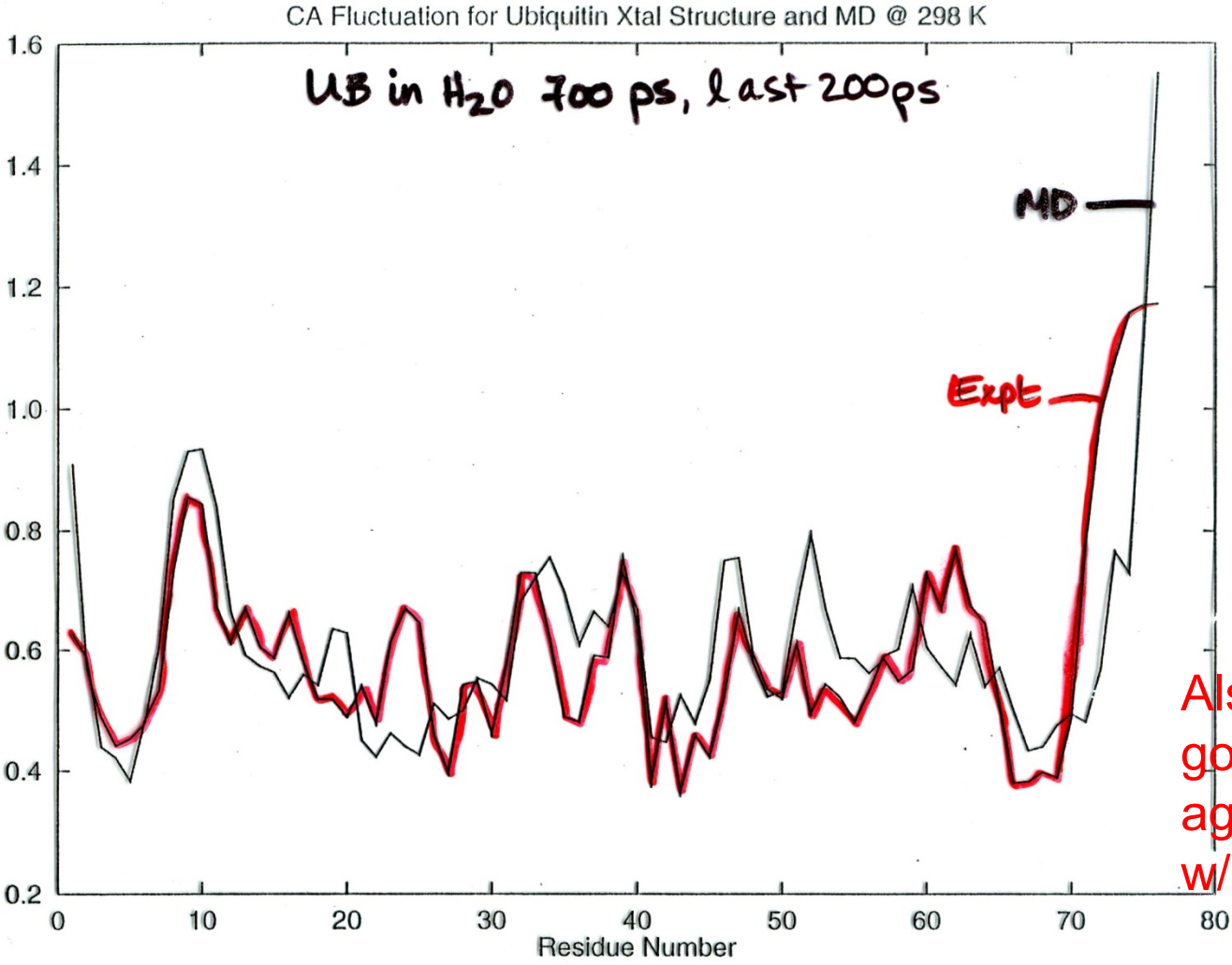
NOEs reproduced

Chymotrypsin Inhibitor 2



- *Expt = Fersht and co-workers (1995) Biochem 34:2225*
- *MD = Li & Daggett (1995) Prot. Eng. 8(11)*

RMSF - B Value (Å)



Also, S^2 in good agreement w/ expt.

- *Alonso & Daggett (1995) J. Mol. Biol. 247:501*

Greg Petsko, “Not just your average structures”, Nat. Struct. Biol., 1996

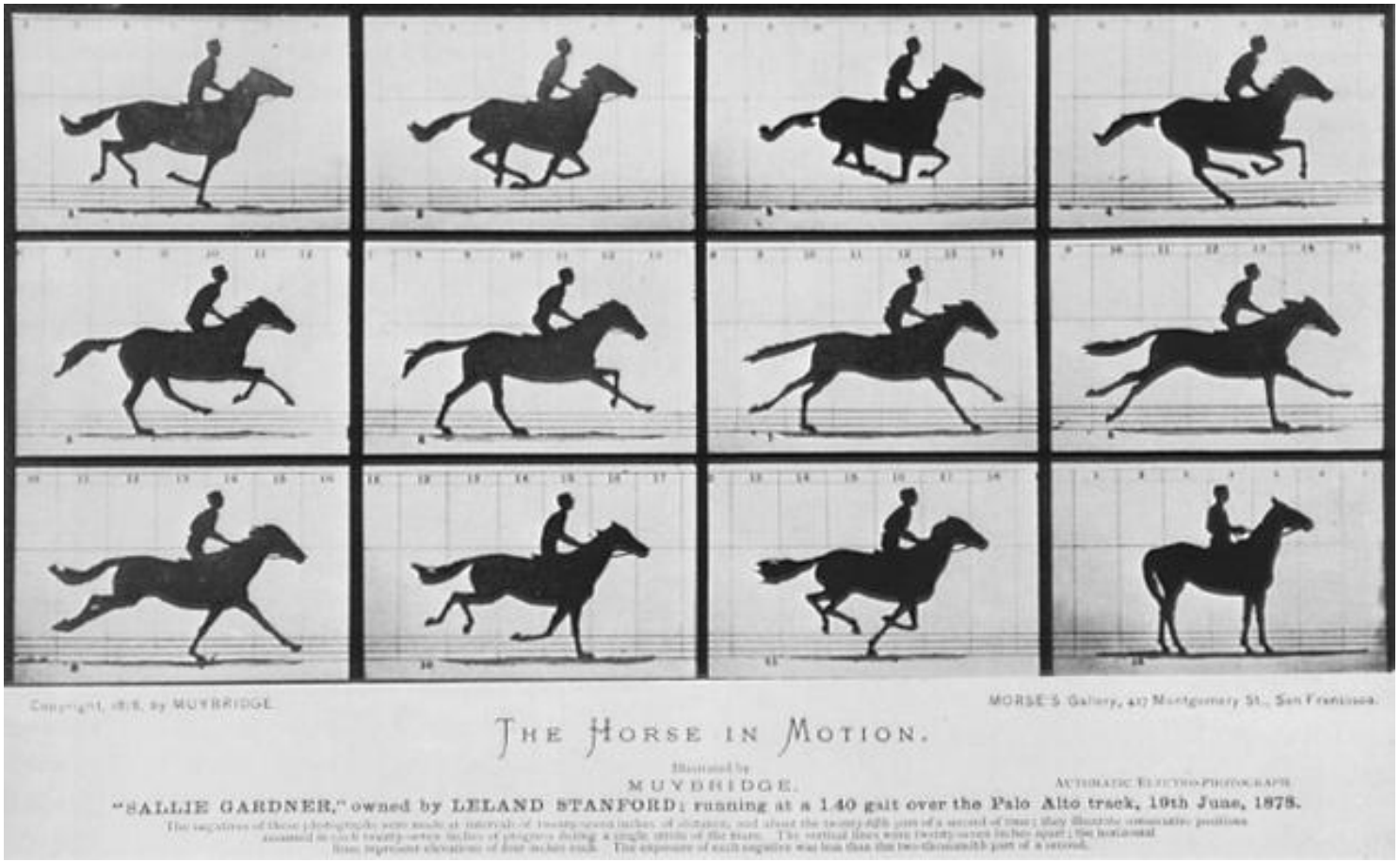
Law of averages repealed → chaos

…..”We have designed our civilization around averages, but we understand that reality consists of fluctuations about those averages.”

Proteins no exception. Beautiful static, time and ensemble averaged structures from NMR and crystallography only part of the story.

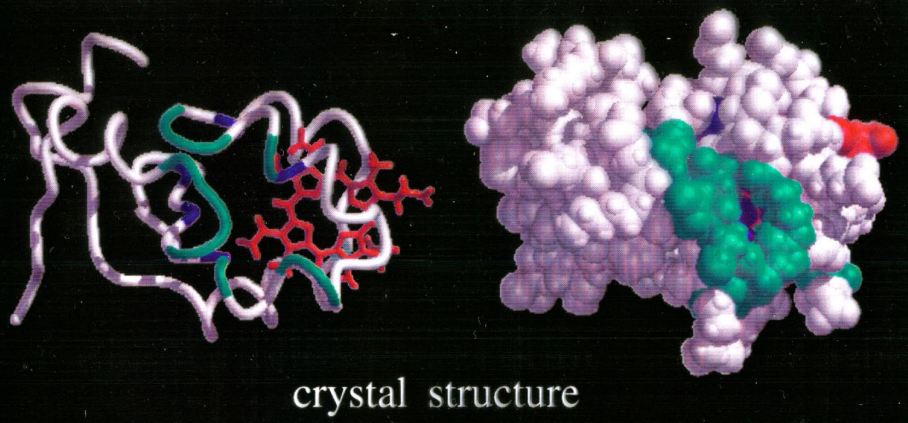
Protein function can depend on what we don’t see: the excursions from average.

Dynamics Necessary for Function

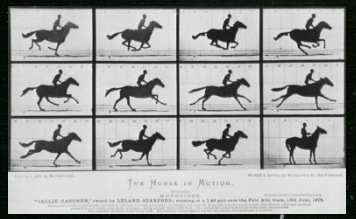


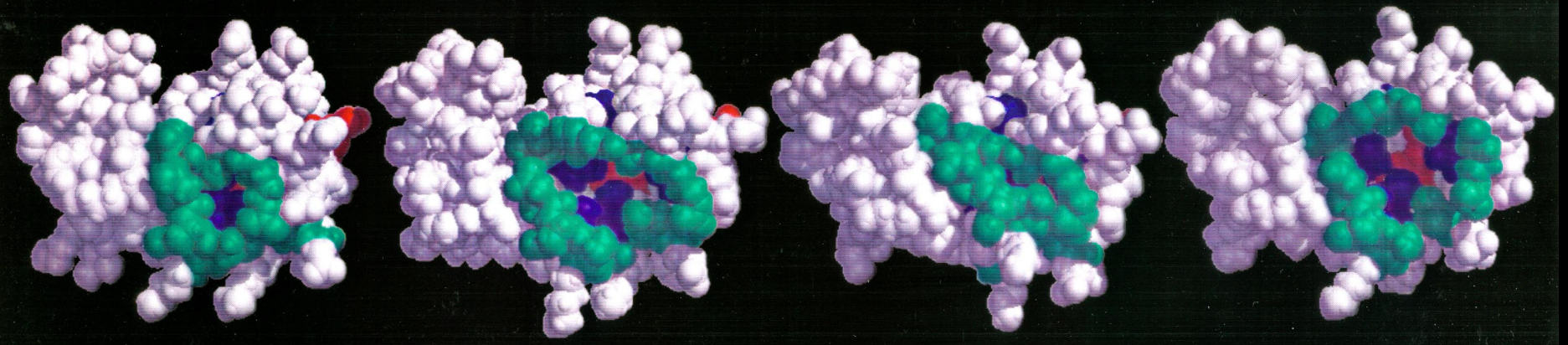
Conformational Substates

Cytochrome b_5



crystal structure



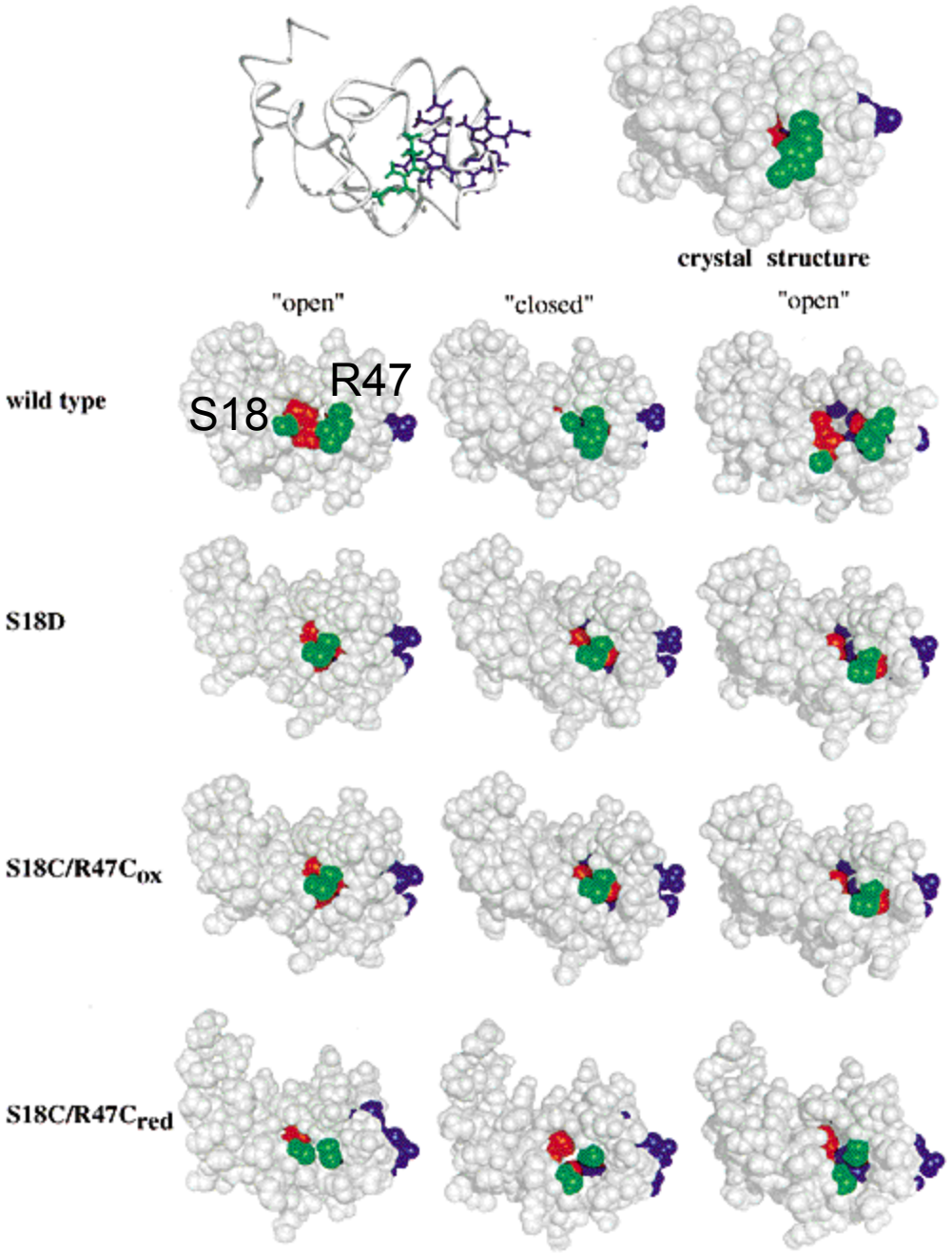


360 ps 800 ps 1480 ps 1600 ps

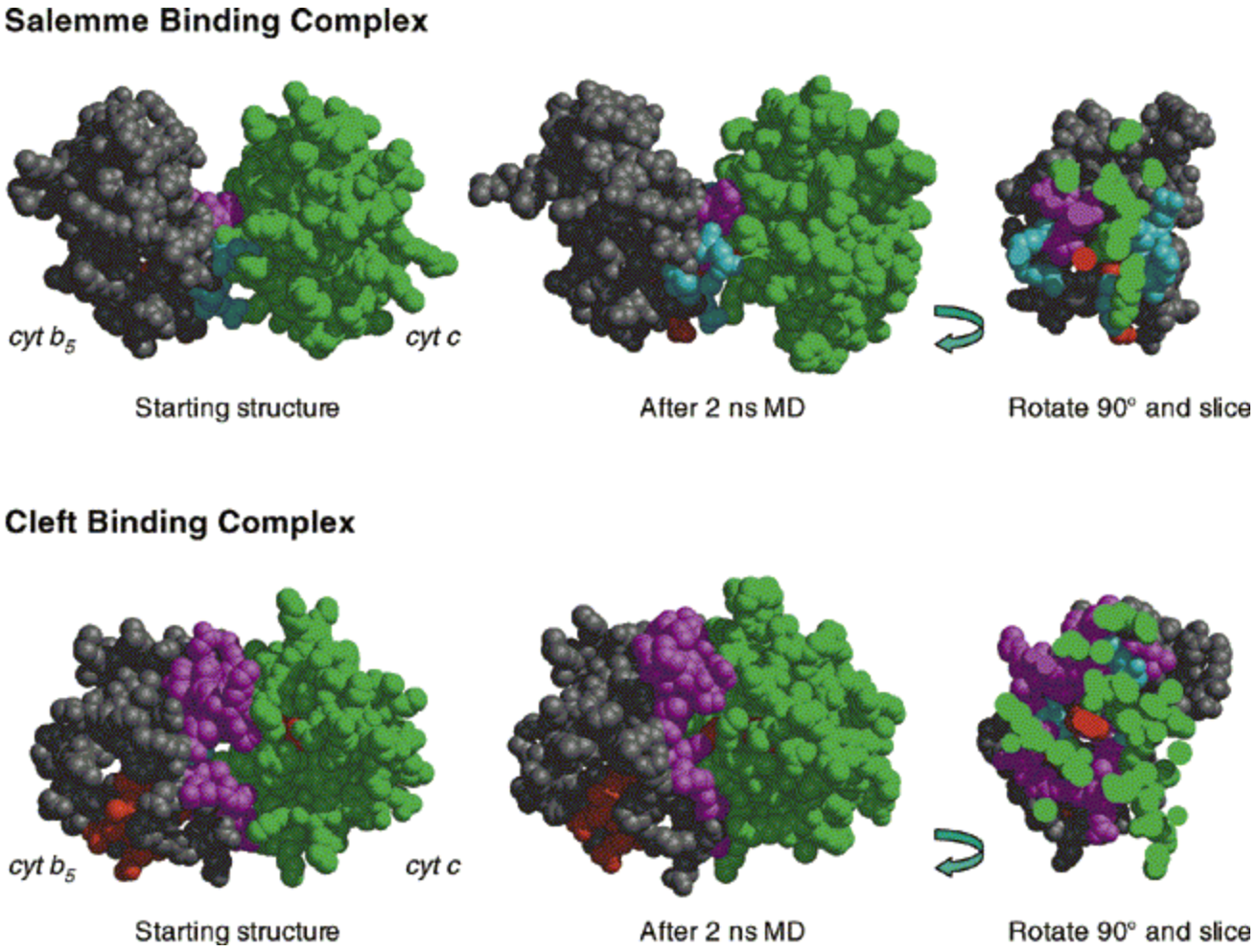
Storch & Daggett, Biochem, 1995

Construction of mutants to test whether cleft forms

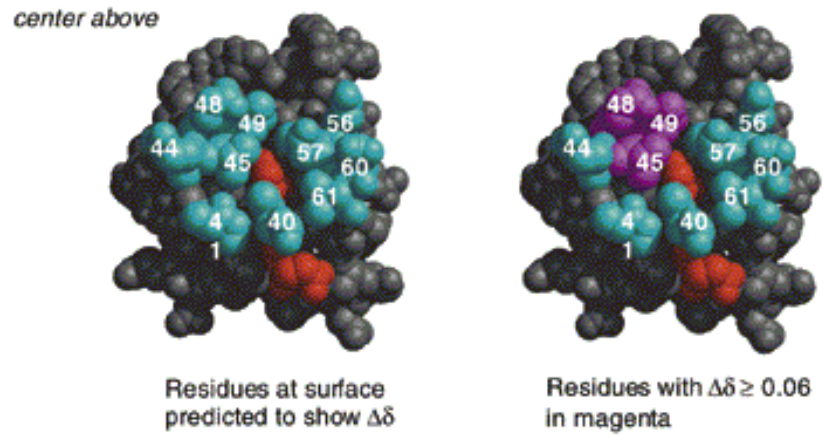
Storch et al., Biochem, 1999

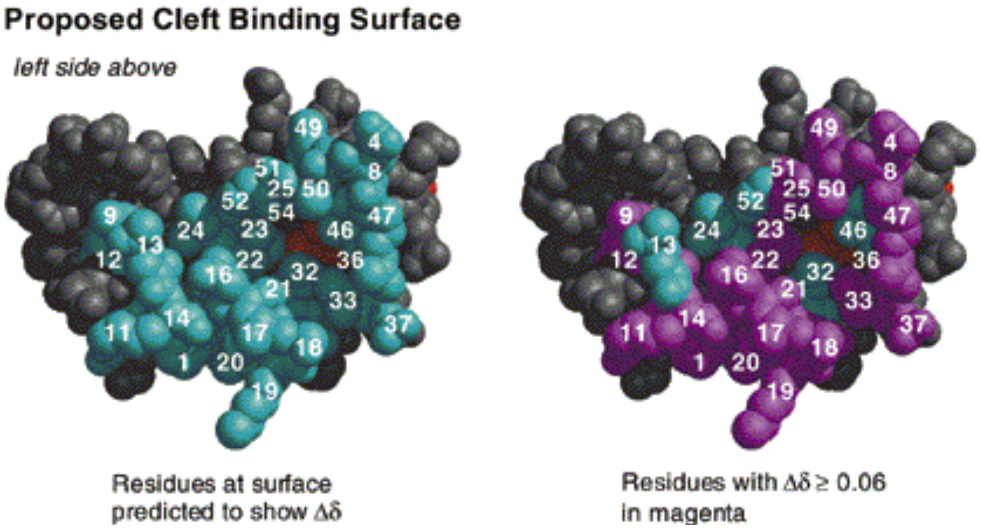


Construction of cyt c – cyt b_5 complexes



Changes in cyt b_5 upon binding cyt c



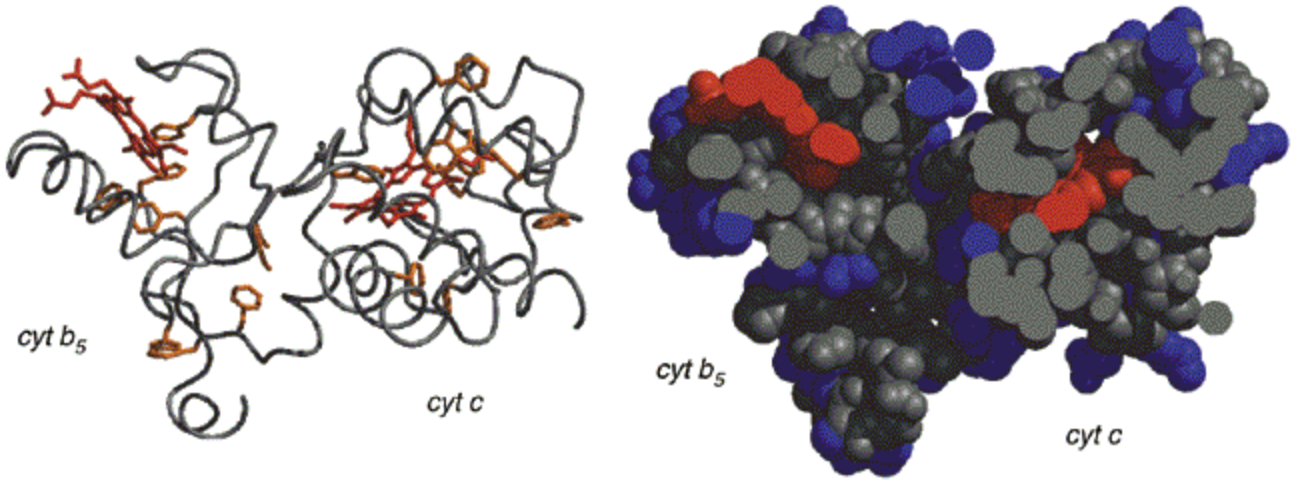


= Predicted binding surface

= Change in chemical shift

Hom et al., Biochem, 2000

Cleft allows for electron transfer through the protein in channel lined with aromatics



= Nonpolar

= Polar

Native States

- Not rigid
- Not unique
- Diverse motion
  - Time scale
  - Amplitude
- > average motion - why?
  - Important for function

Native States (cont)

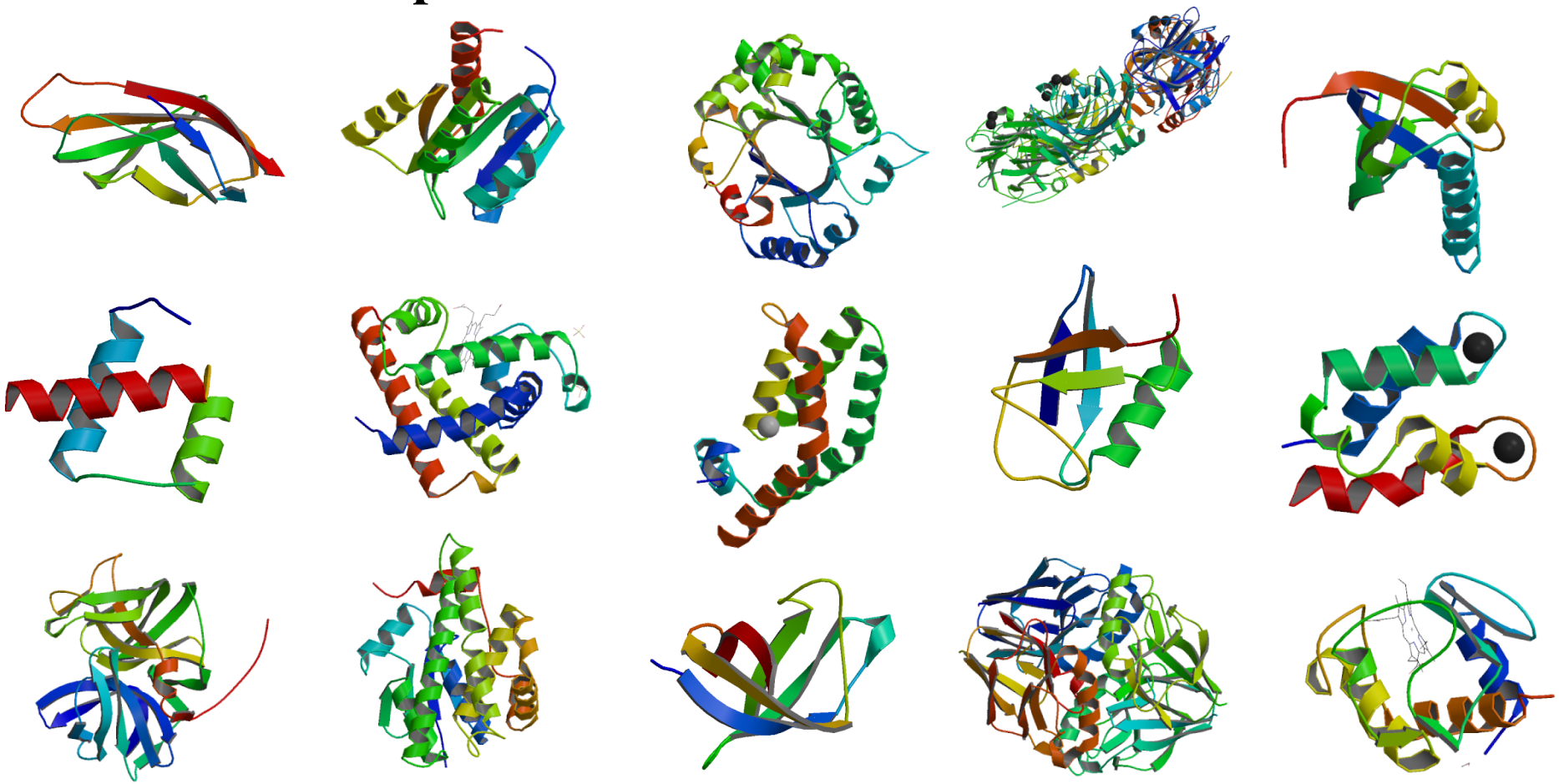
- Functionally important motion & alternate states may not be well represented by an average structure
  - Conformational substates
    - » Mb - ligand binding
    - » Cyt b5 – recognition and electron transfer
    - » Rnase - binding
  - Catalysis
    - » RNase
    - » CI2 (inhibition of catalysis)
  - Evolution, adaptation to mutation
    - » Globins

Relevance of Protein Dynamics

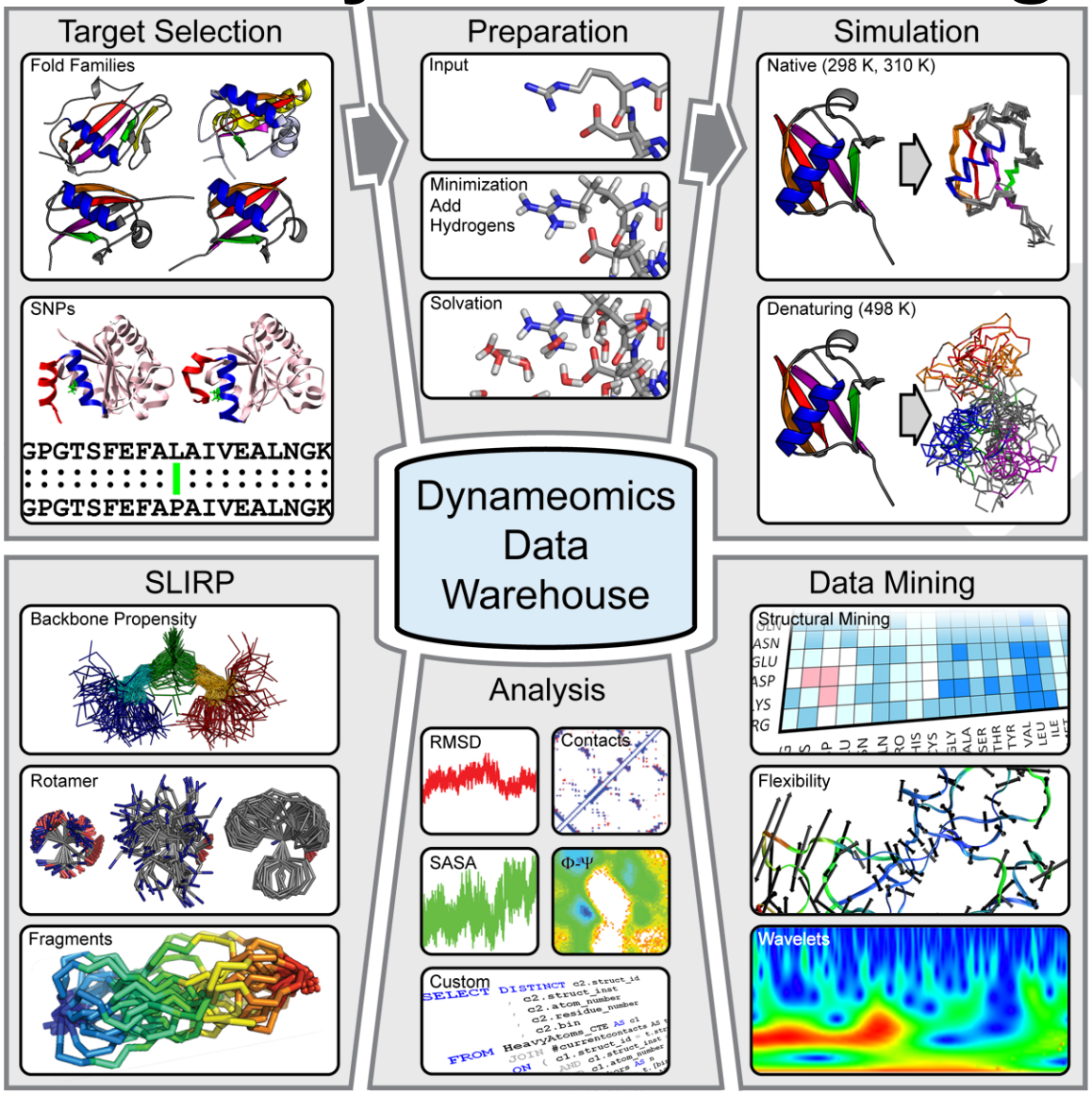
- Native State
  - Adaptation to environment---solvent
  - Binding of ligands, ions, etc.
  - Binding to other proteins – recognition
  - Catalysis
  - Flexibility important for activity (thermophilic proteins)
  - Signal transduction
  - Protein translocation
  - etc

Proteins

Many shapes, many sizes, >100,000 in PDB ➔ 807 unique autonomous protein folds



www.dynameomics.org



View standard results:

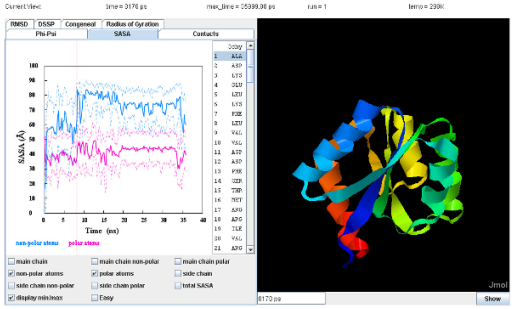


Figure 7.

View movies

Create your own results:
Request a login

Top 100 targets
and libraries

Daggett and co-workers
Structure, 14 April 2010