Dynamics of proteins in crystals or "Please hold still so we can take your picture!"

George N. Phillips Jr.

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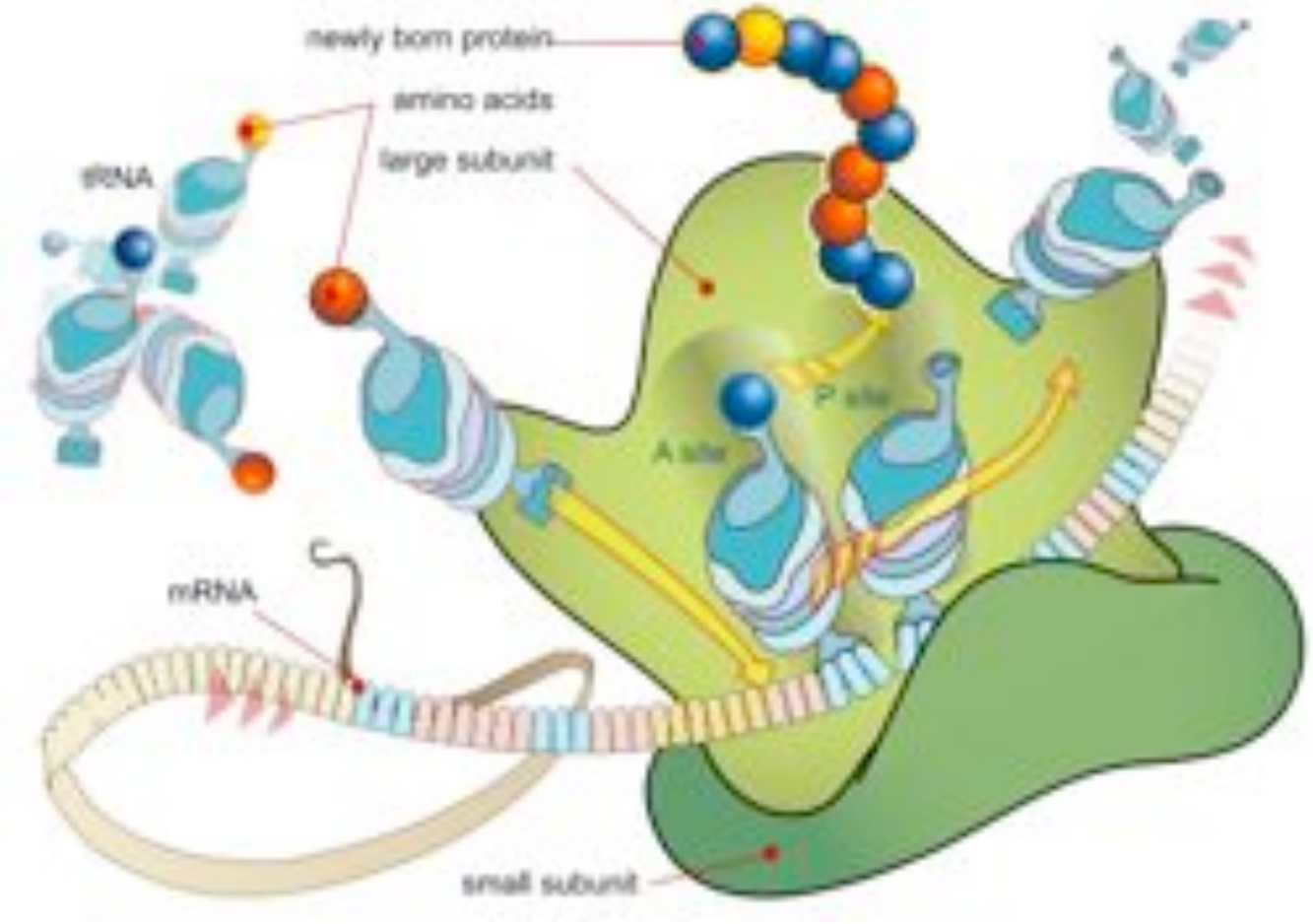
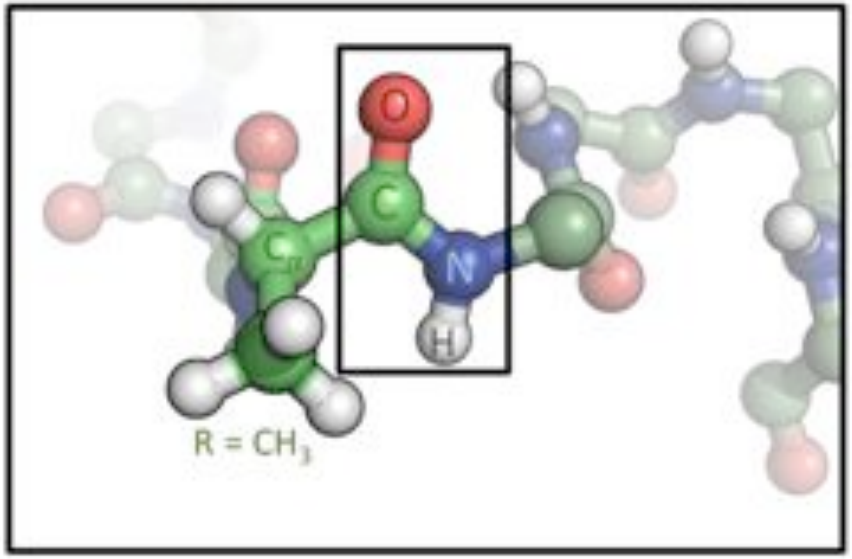
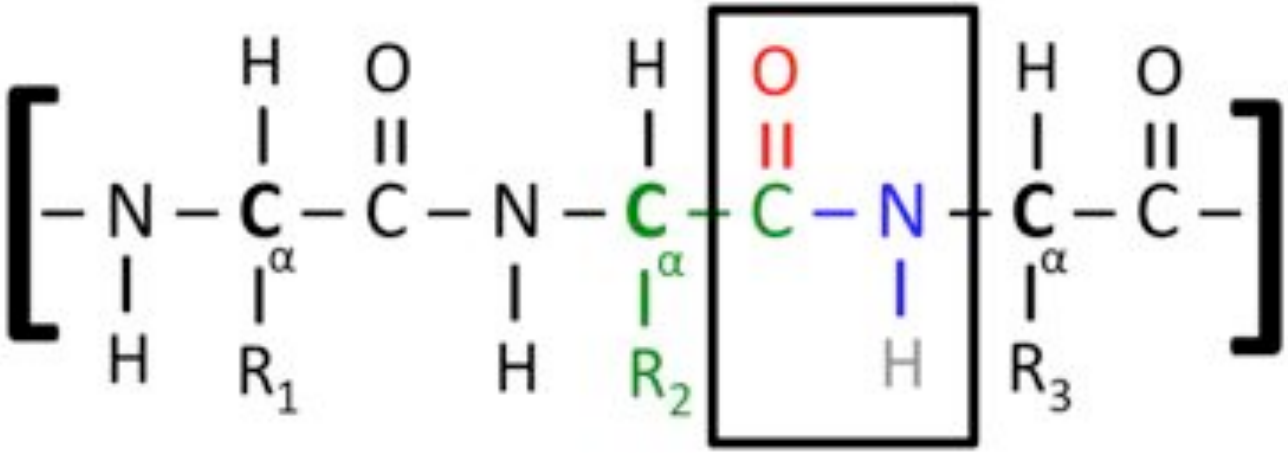
Rice University

Molecular Biology 101



Covalent structure of Proteins





Our Nobel Prize-Winning Founders

1915 WH Bragg and WL Bragg

Use of X-rays to determine crystal structure

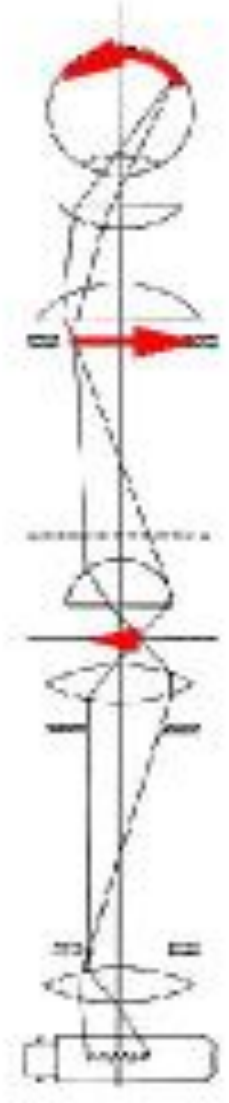
1914 M von Laue

Diffraction of X-rays by crystals

1901 WC Röntgen

Discovery of X-rays

Light microscope

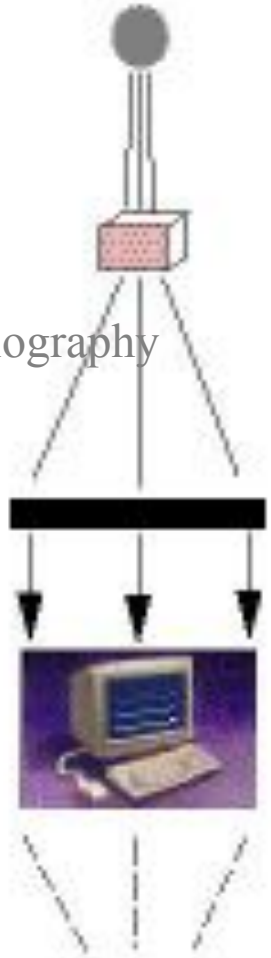


2,000 X

Electron microscope

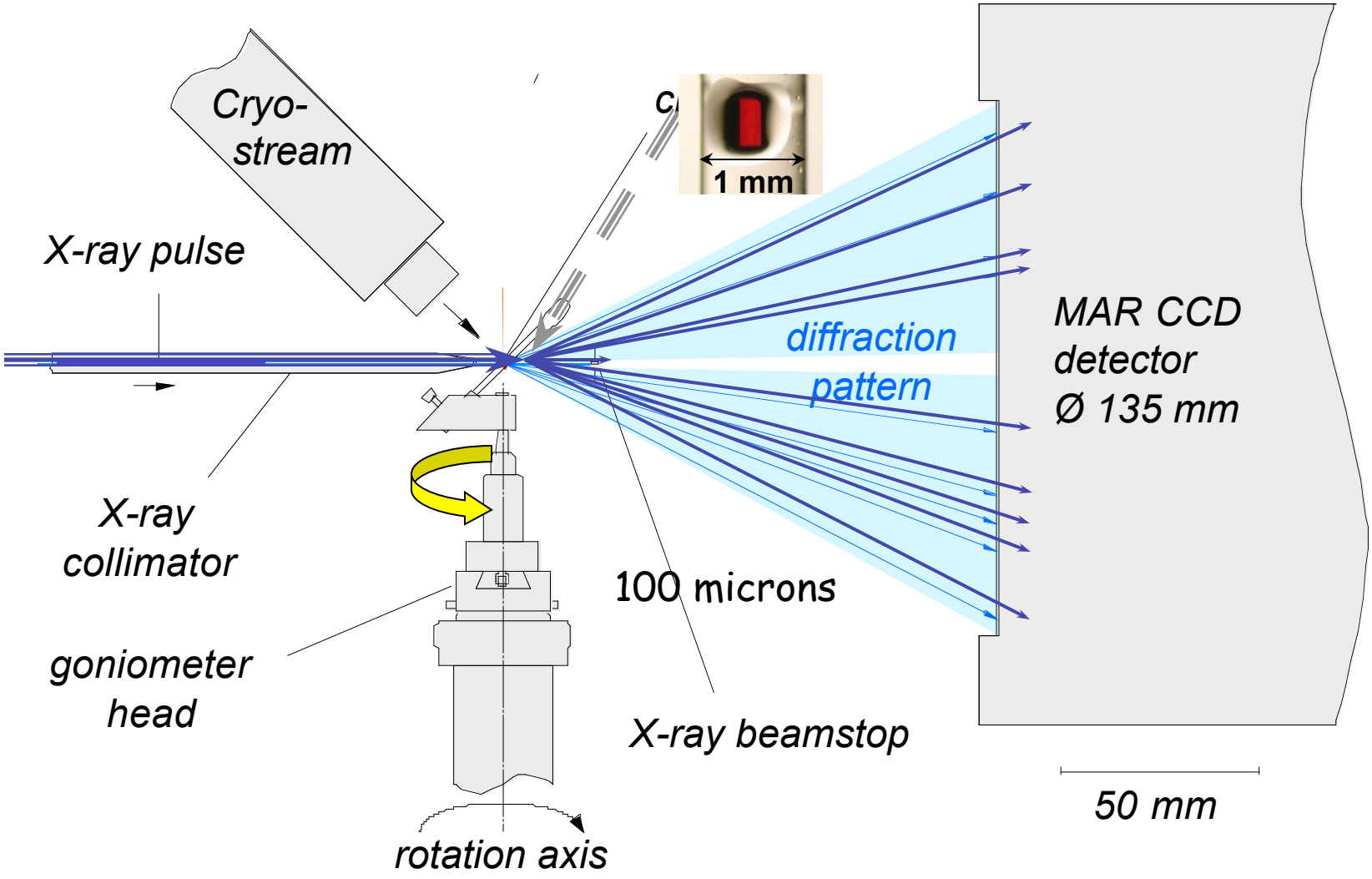
1,000,000 x

X-ray
Crystallography

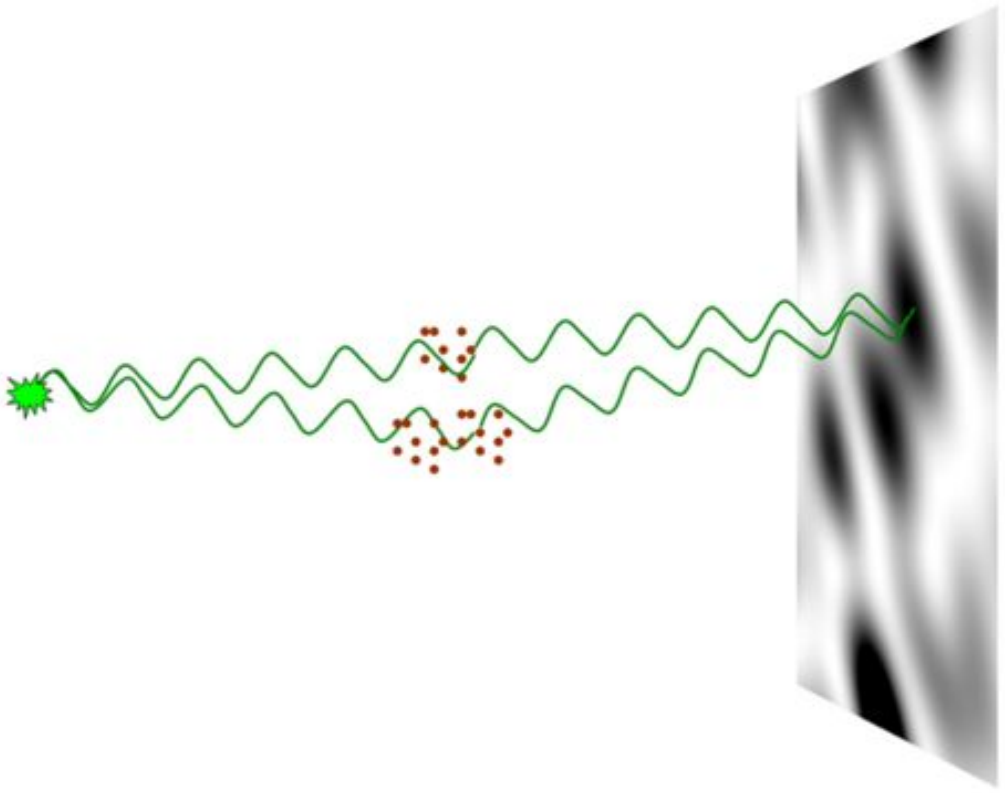


100,000,000 X

Intro to Crystallography

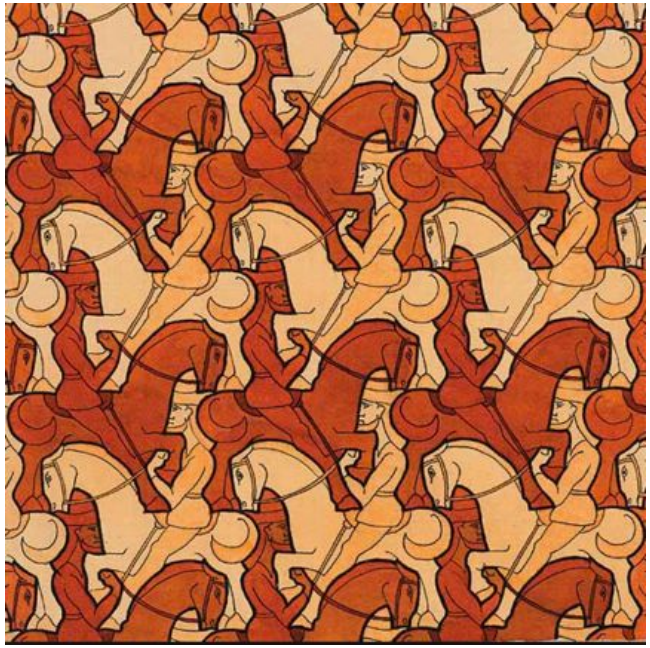


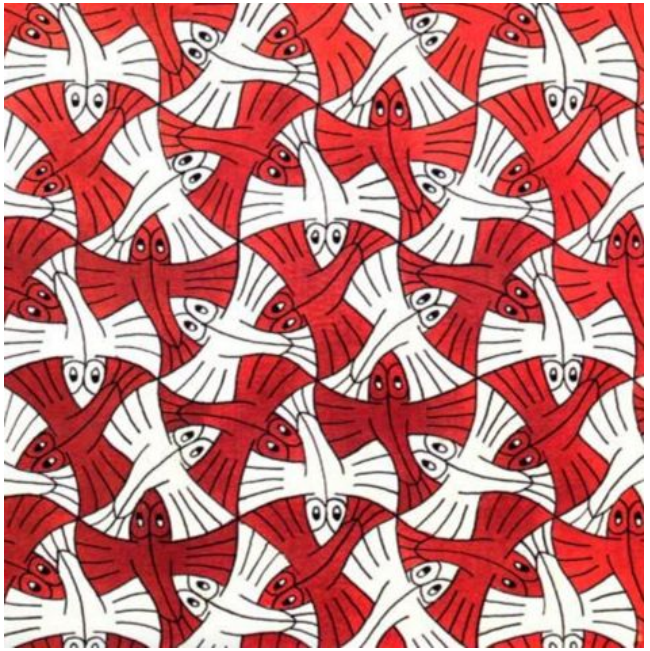
Scattering by several electrons



James Holton

Periodicity and Symmetry

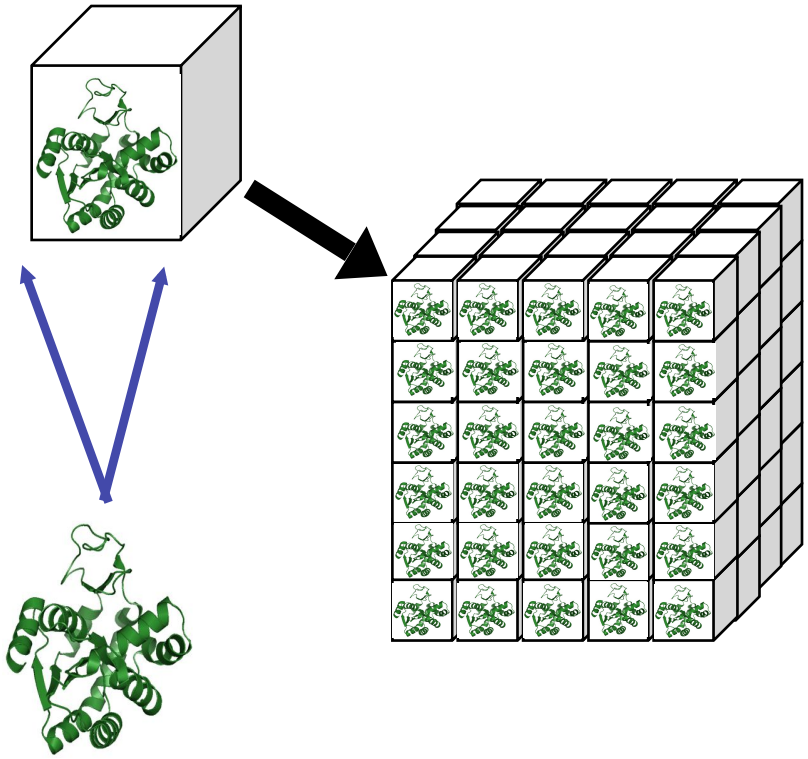


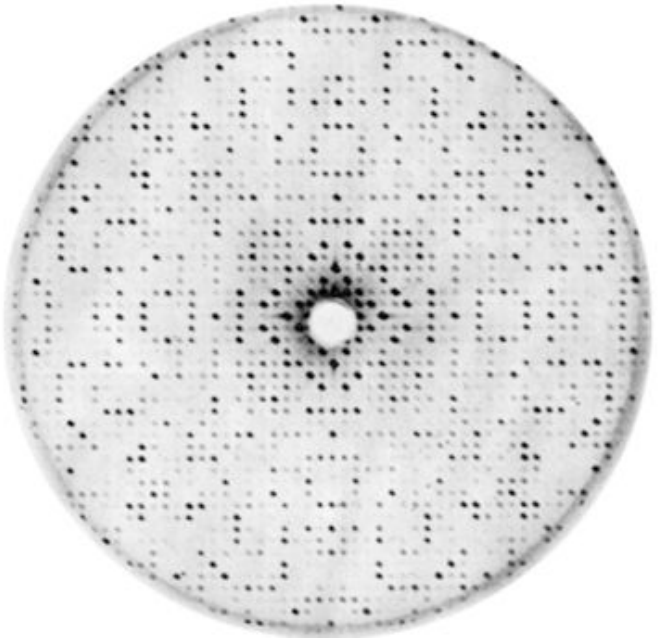


M.C. Escher

Convolution Theorum

$$FT(\rho_{molecule} \otimes L_{\inf}) = FT(\rho_{molecule}) \times FT(L_{\inf})$$





Kinematic Level Theory

General diffraction expression

$$I(\mathrm{Q}) = \sum_{lk} \sum_{l'k'} f_{kQ} e^{-(\mathbf{Q}^T \langle \mathbf{u}_{lk} \mathbf{u}_{lk}^T \rangle \mathbf{Q})/2} f^*_{kQ} e^{-(\mathbf{Q}^T \langle \mathbf{u}_{l'k'} \mathbf{u}_{l'k'}^T \rangle \mathbf{Q})/2} e^{i\mathbf{Q}\cdot(\mathbf{r}_{lk} - \mathbf{r}_{l'k'})} e^{\mathbf{Q}^T \langle \mathbf{u}_{lk} \mathbf{u}_{l'k'}^T \rangle \mathbf{Q}}$$

By application of periodicity and with isotropic displacements of the atoms

$$I(\mathrm{H}) = \sum_{k} \sum_{k'} f_{kH} e^{-(2\pi H)^2 \langle u_k^2 \rangle /2} f^*_{k'H} e^{-(2\pi H)^2 \langle u_{k'}^2 \rangle /2} e^{i2\pi \mathbf{H}\cdot(\mathbf{r}_k - \mathbf{r}_{k'})} e^{(2\pi H)^2 \langle u_k u_{k'} \rangle}$$

A nasty inverse problem

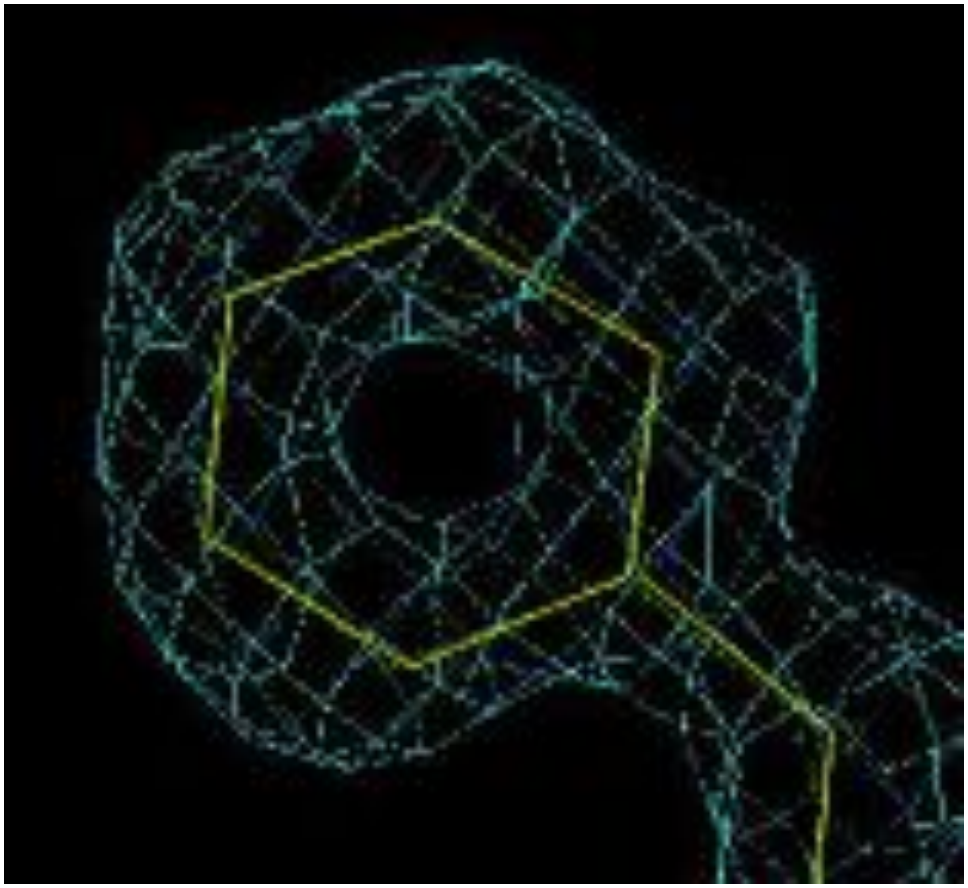
Requires experimental or other estimation of the real versus complex parts of thousands of measured structure factor amplitudes.

Electron density equation

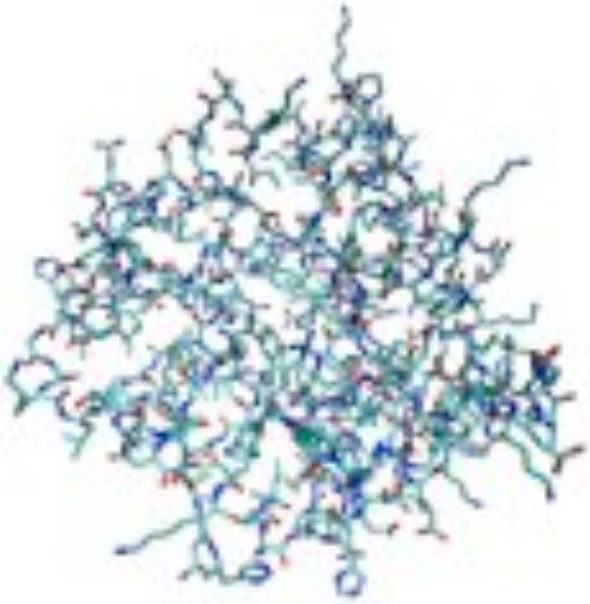
$$\rho(\mathbf{x}) = \frac{1}{V}\sum_{\mathbf{h}} \mathbf{F}(\mathbf{h}) \exp(-2\pi i \mathbf{h}\cdot\mathbf{x})$$

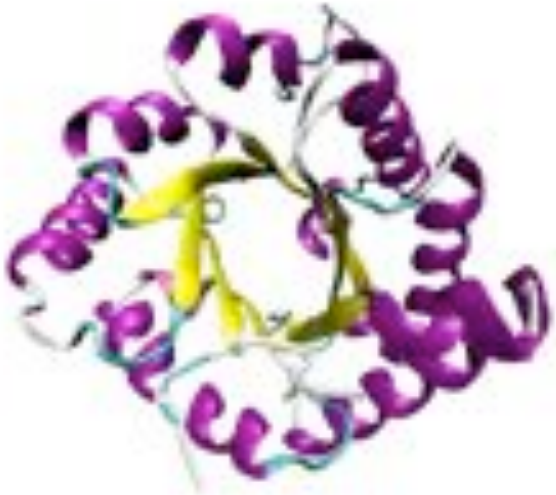
x is a vector with x,y,z fractional components in real space
h is a vector with h,k,l components in reciprocal space
F(h) is the complex structure factor
V is the unit cell volume

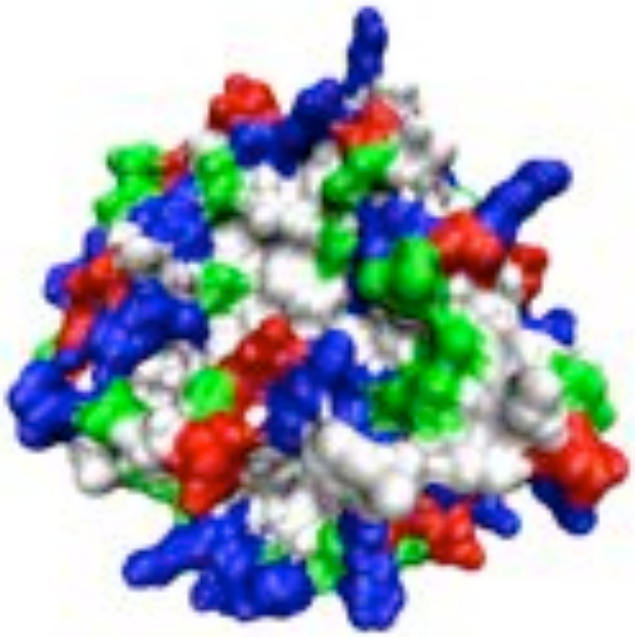
Electron density map



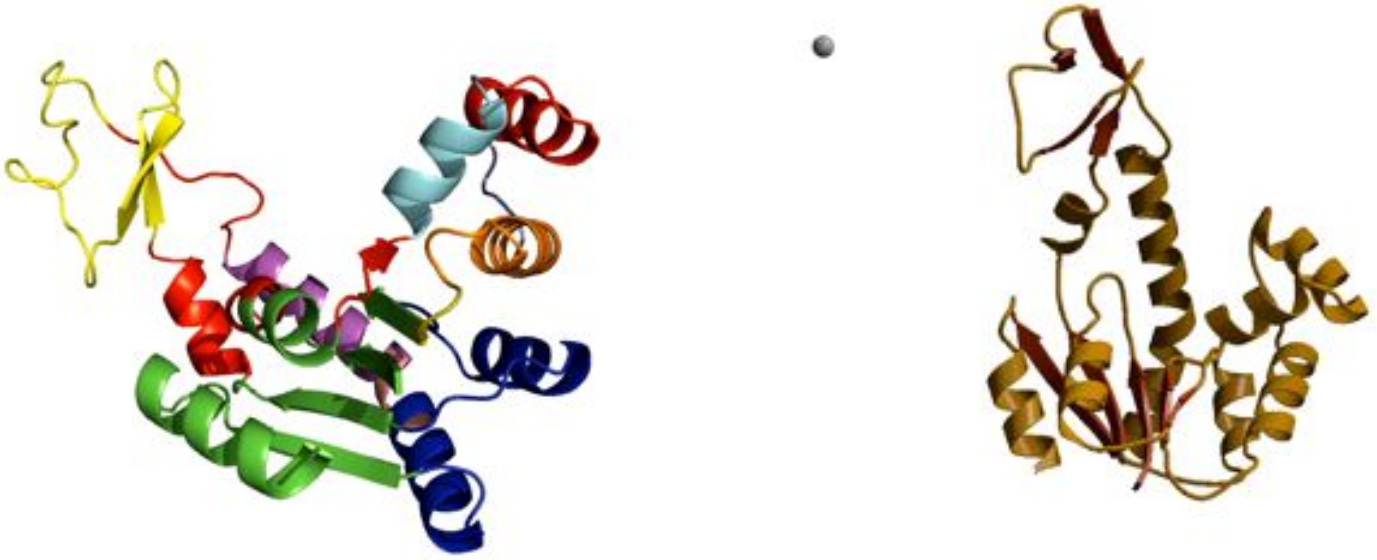
Representations of protein molecules





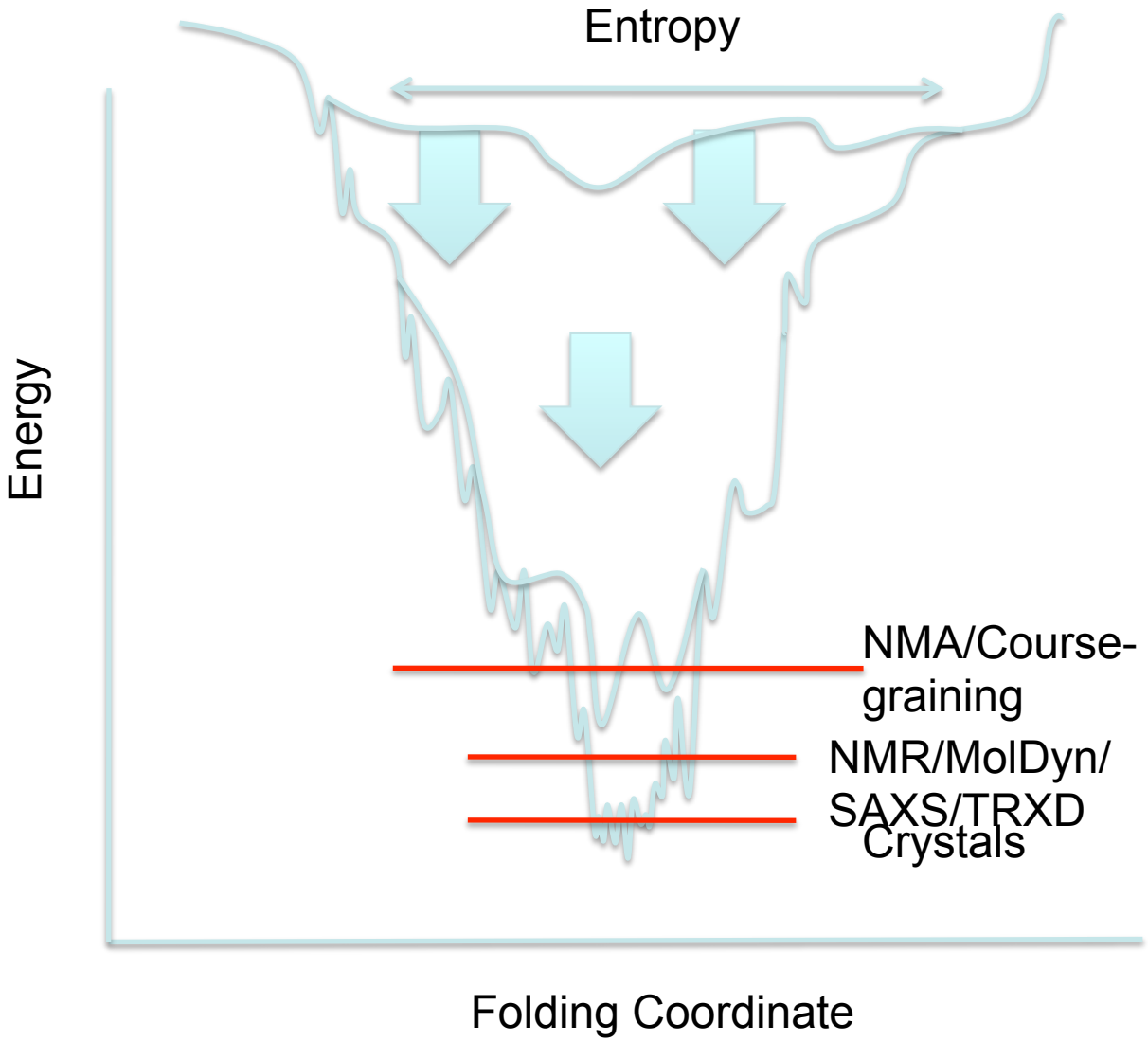


Adenylate kinase motions



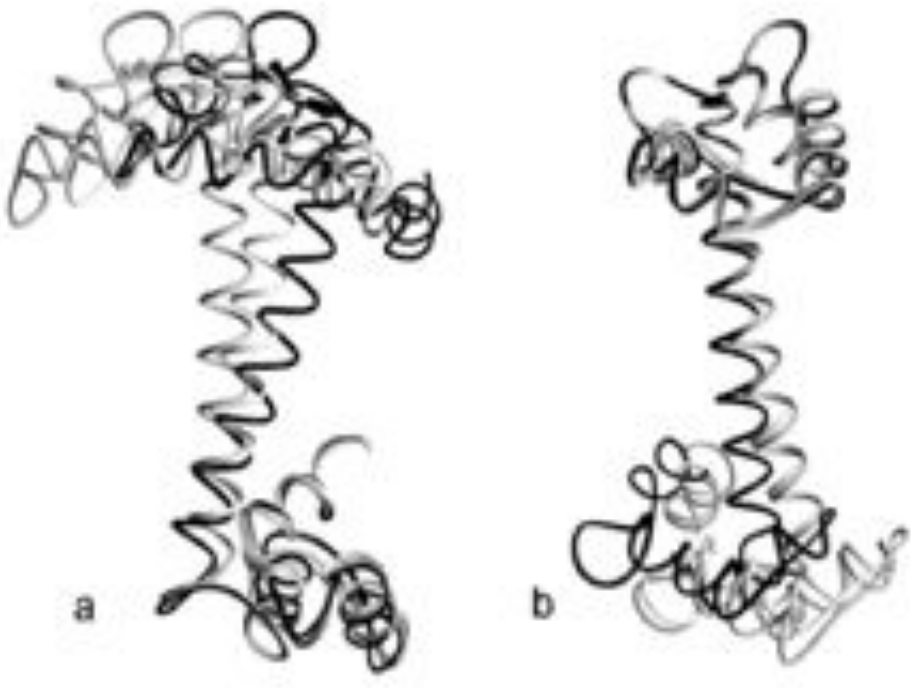
Schulz et al. and Berry and Phillips *Proteins* 1998

Ensembles at Multiple Levels



Crystal's effect on Structure?

Troponin C

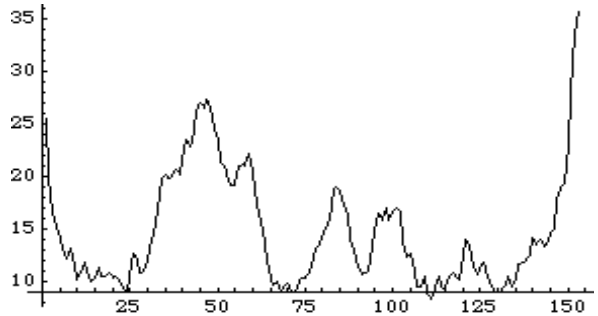


Soman, Tao, Phillips *Proteins* 1999

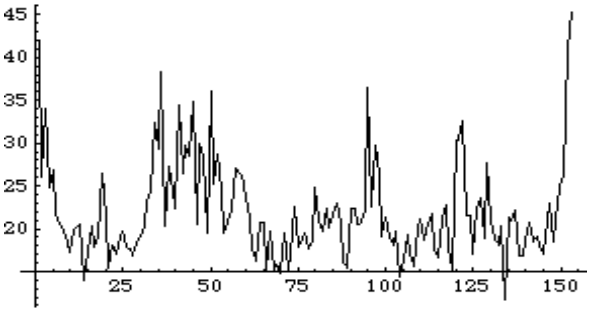
The protein is variable in structure

- Crystallography (usually) confuses the space and time averages.
- Dynamic behavior remains--There IS temperature dependence, both kT-ish and landscapes more shallow
- The crystal lattice constrains the ‘dynamics’ to varying degrees

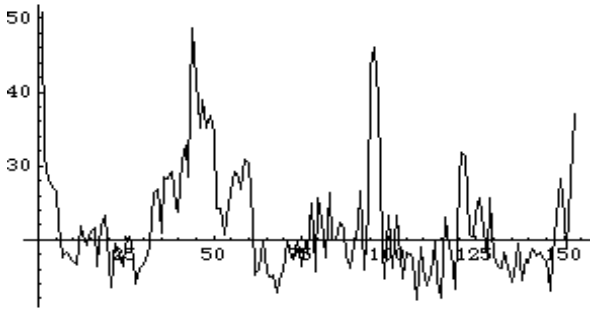
Experimental B-factors of myoglobin in five crystal forms



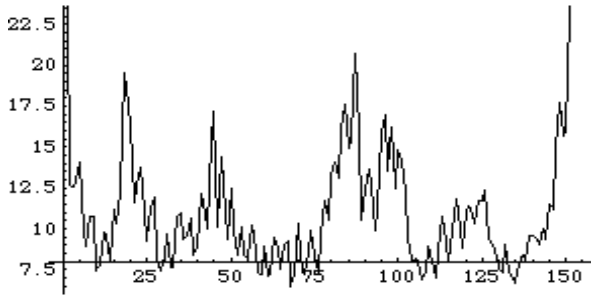
P6 AmSulfate pH 9



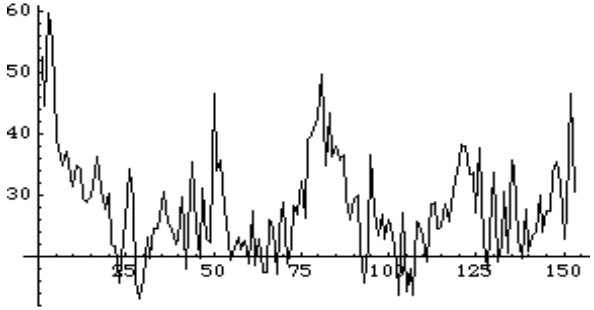
$P2_12_12_1$ - PEG
Imd pH 7



$P2_12_12_1$
2.5 M AS pH 8



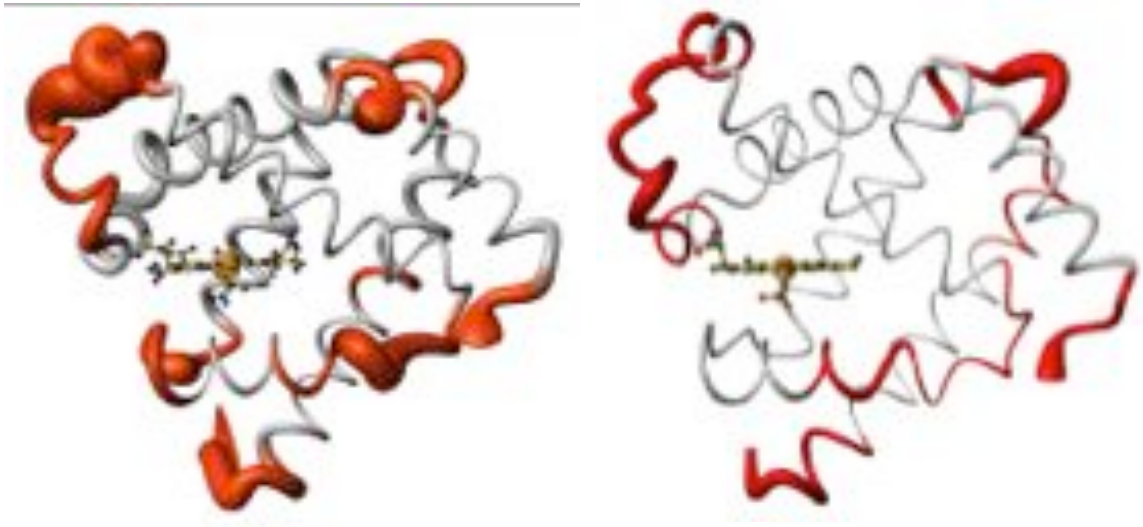
$P2_1$ AmSulphate pH 7



$P6_122$ citrate

Phillips Biophys J. 1990
Kondrashov, Zhang, Aranda, Stec, Phillips *Proteins* 2007

NMR and Crystallography: comparison of backbone dynamics

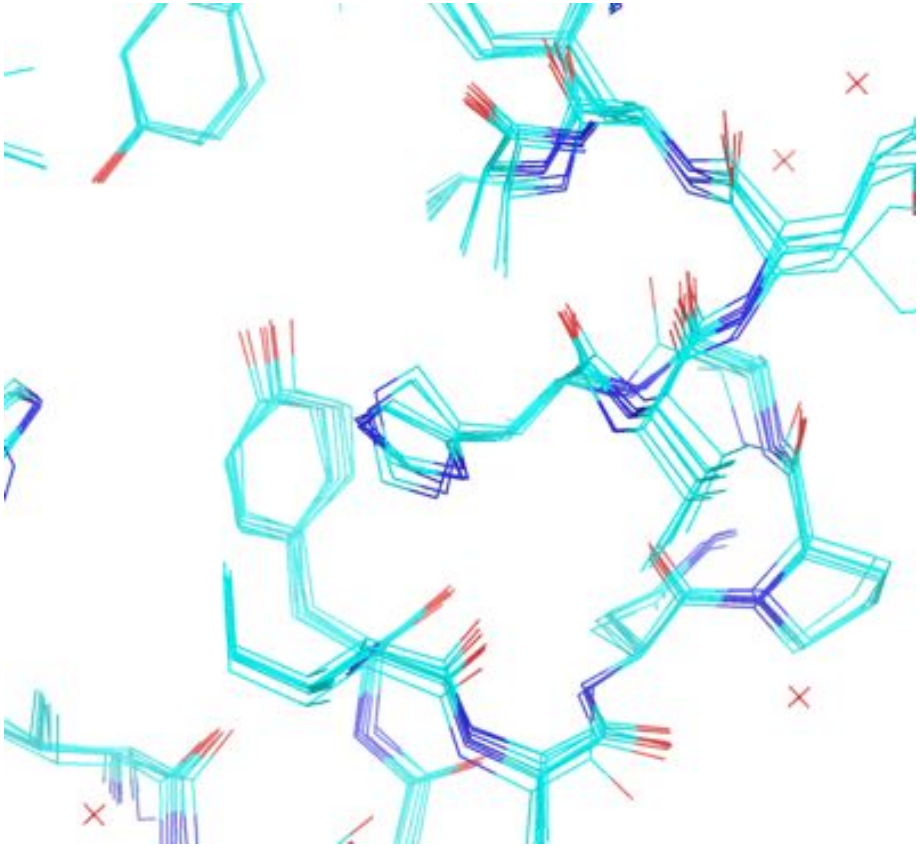


Main chain variations from NMR ensemble and various crystal forms of myoglobin.

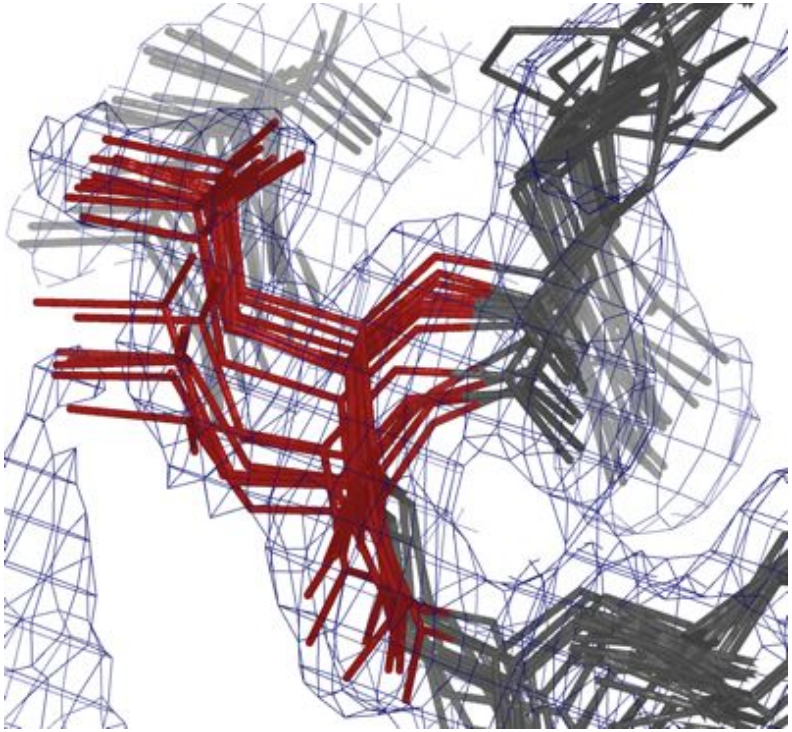
Kondrashov, Zhang, Aranda, Stec, and Phillips *Proteins* 2008

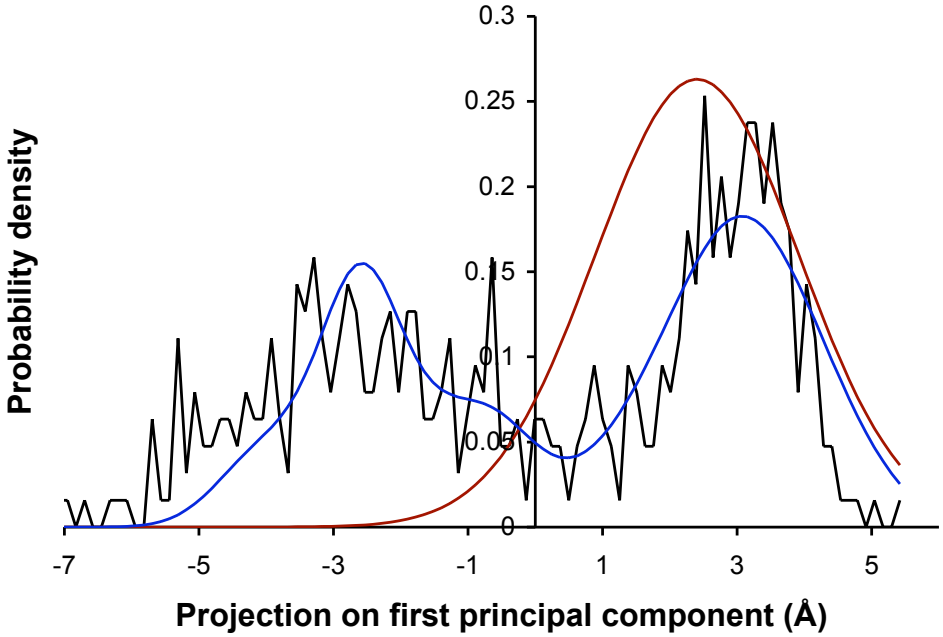
Ensemble Refinement

- Refine several copies of the entire protein simultaneously.
- Each copy has a fractional occupancy and does not interact with the other copies.

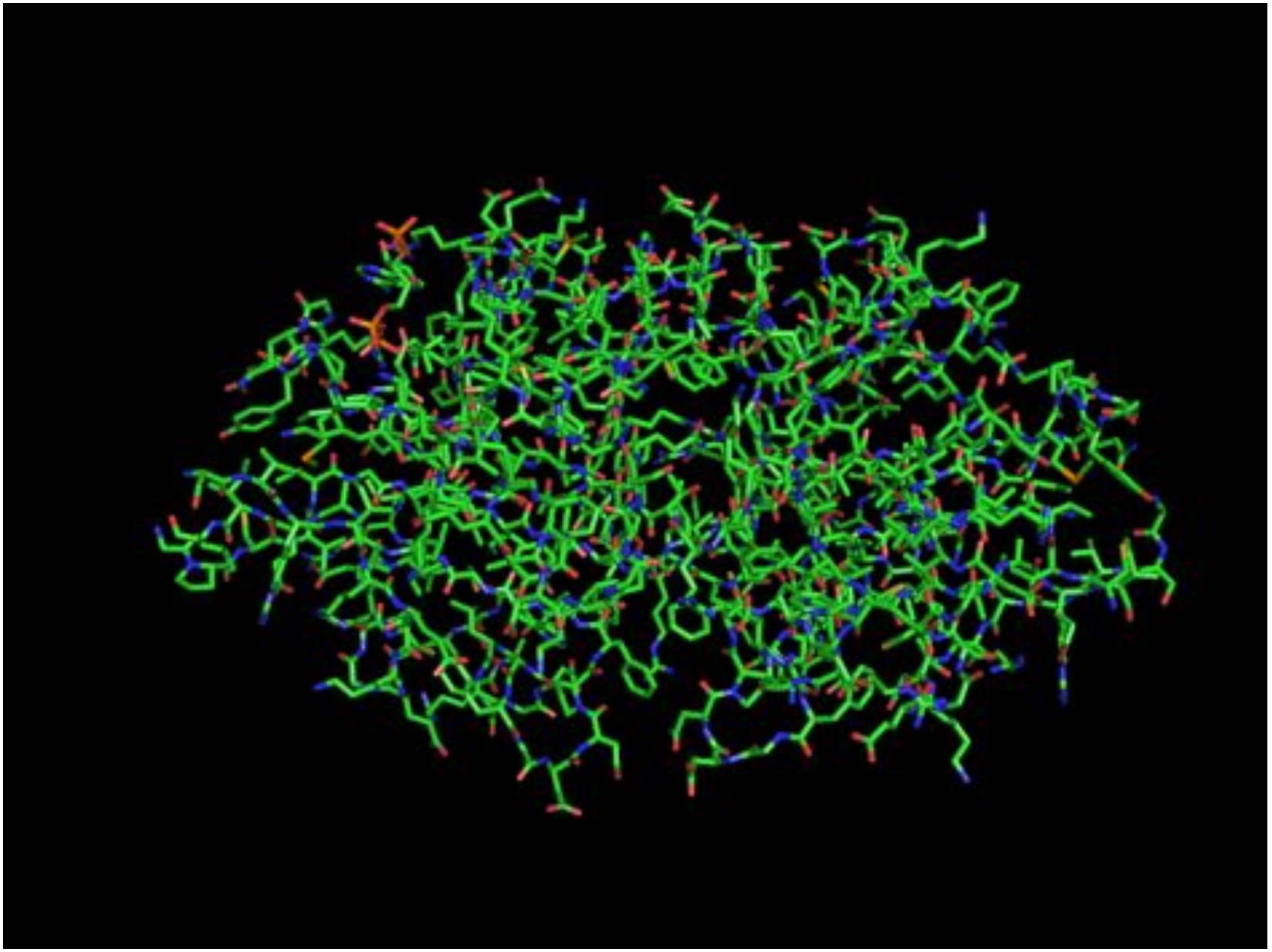


Levin, Kondrashov, Wesenberg, Phillips, Structure, 2007

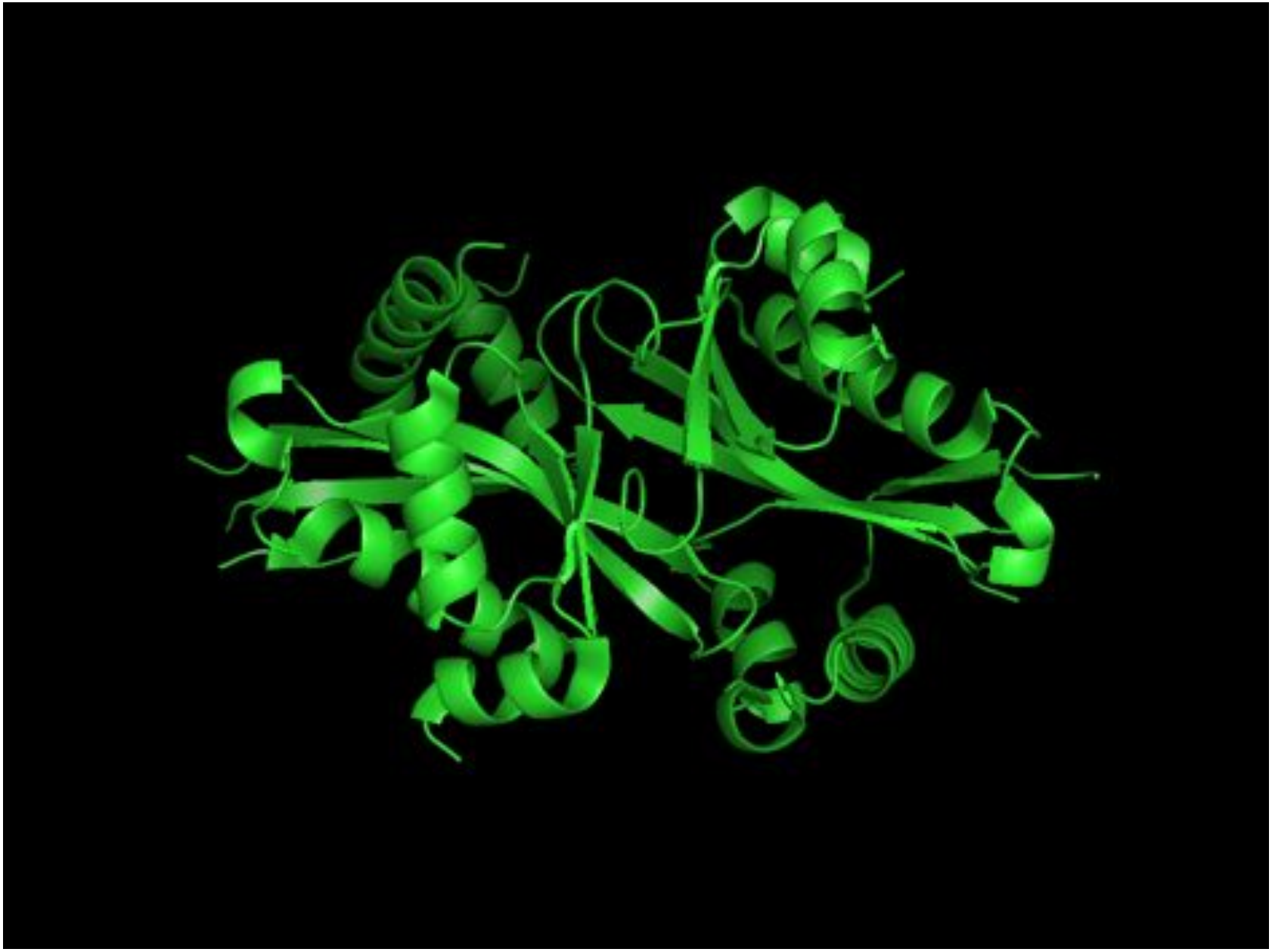



Probability density
0.3
0.25
0.2
0.15
0.1
0.05
0
-7
-5
-3
-1
1
3
5
Projection on first principal component (Å)

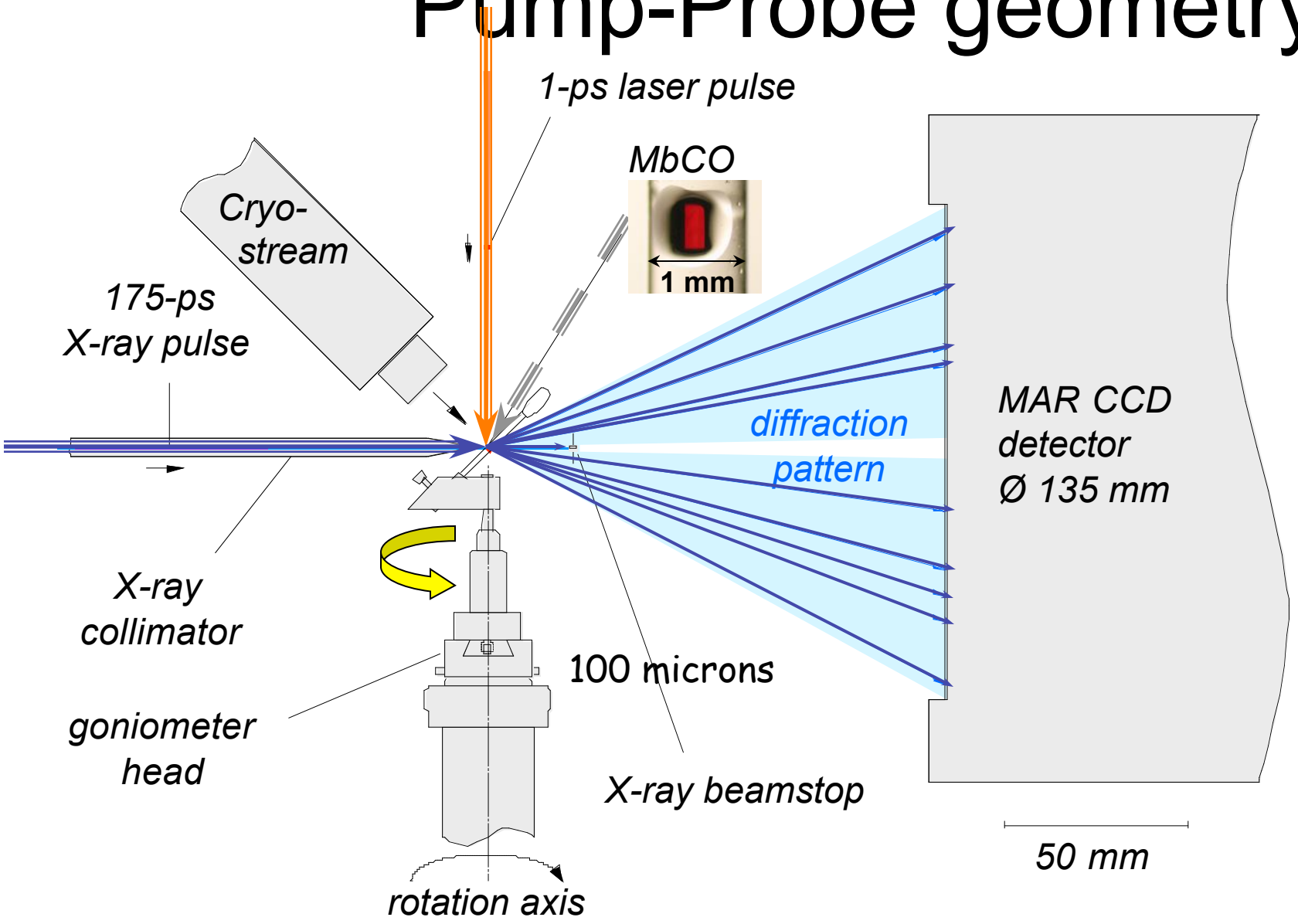
Entire Dimeric Protein



Protein Cartoon with Larger Scale Variations

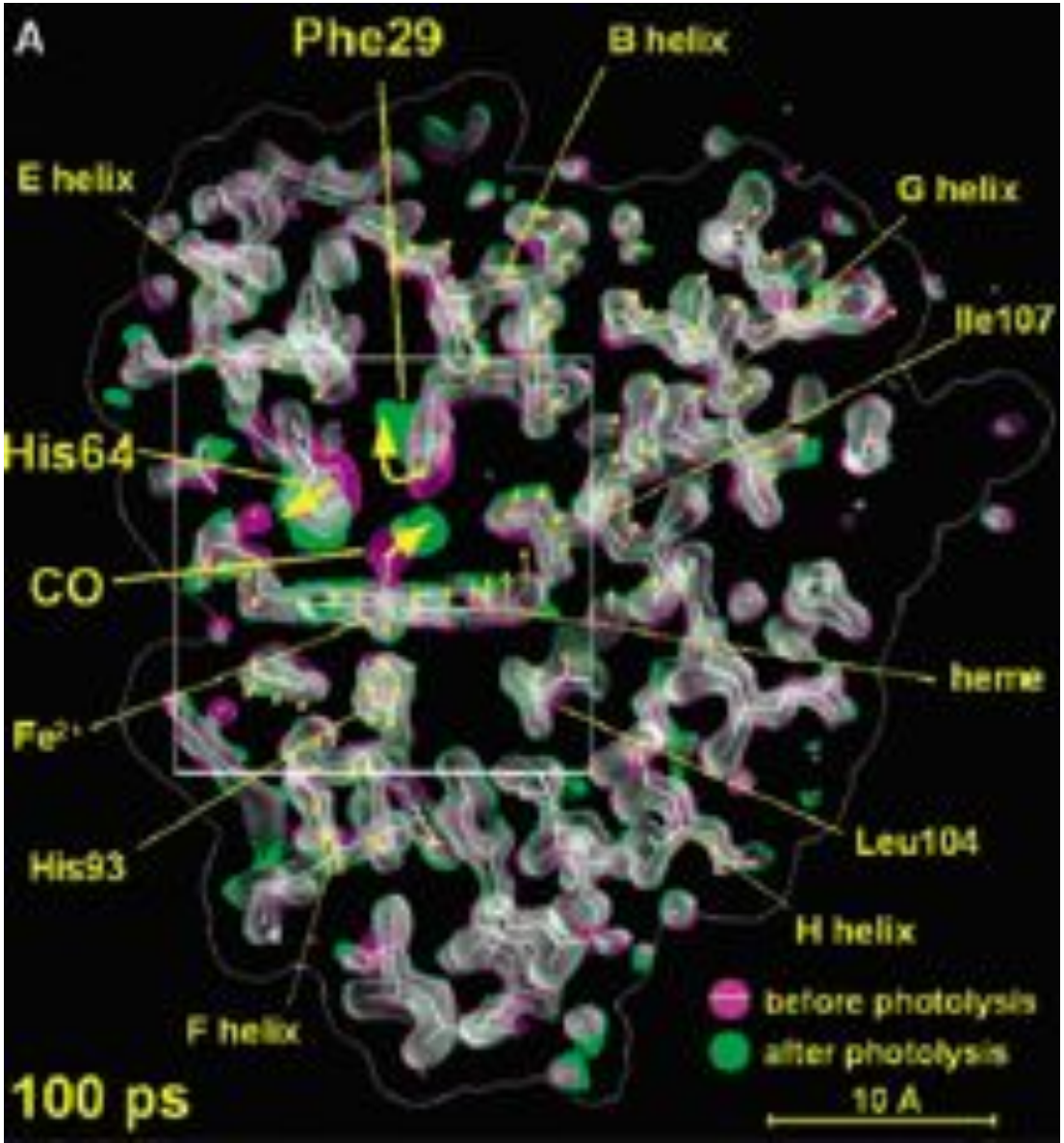


Pump-Probe geometry

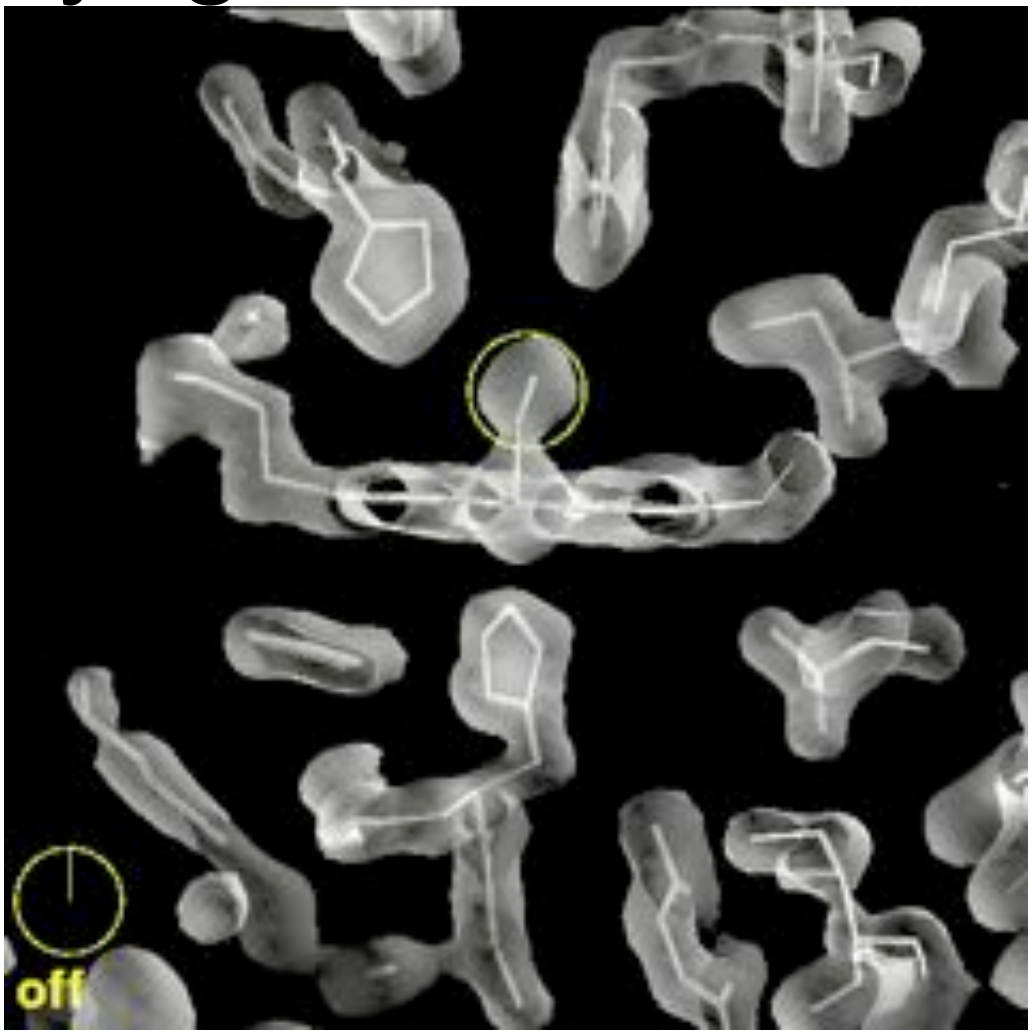


Schotte, Lim, Jackson, Smirnov, Soman, Olson, Phillips, Wulff, Anfinrud, *Science* 2003

Guide to the “actors”

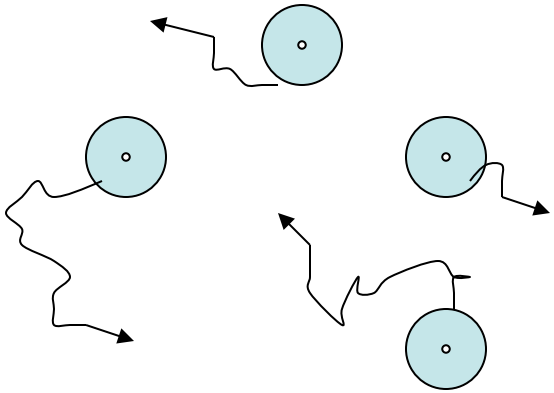


Myoglobin: The movie



Molecular Dynamics Simulations

F = m a = - grad V, where V is the potential



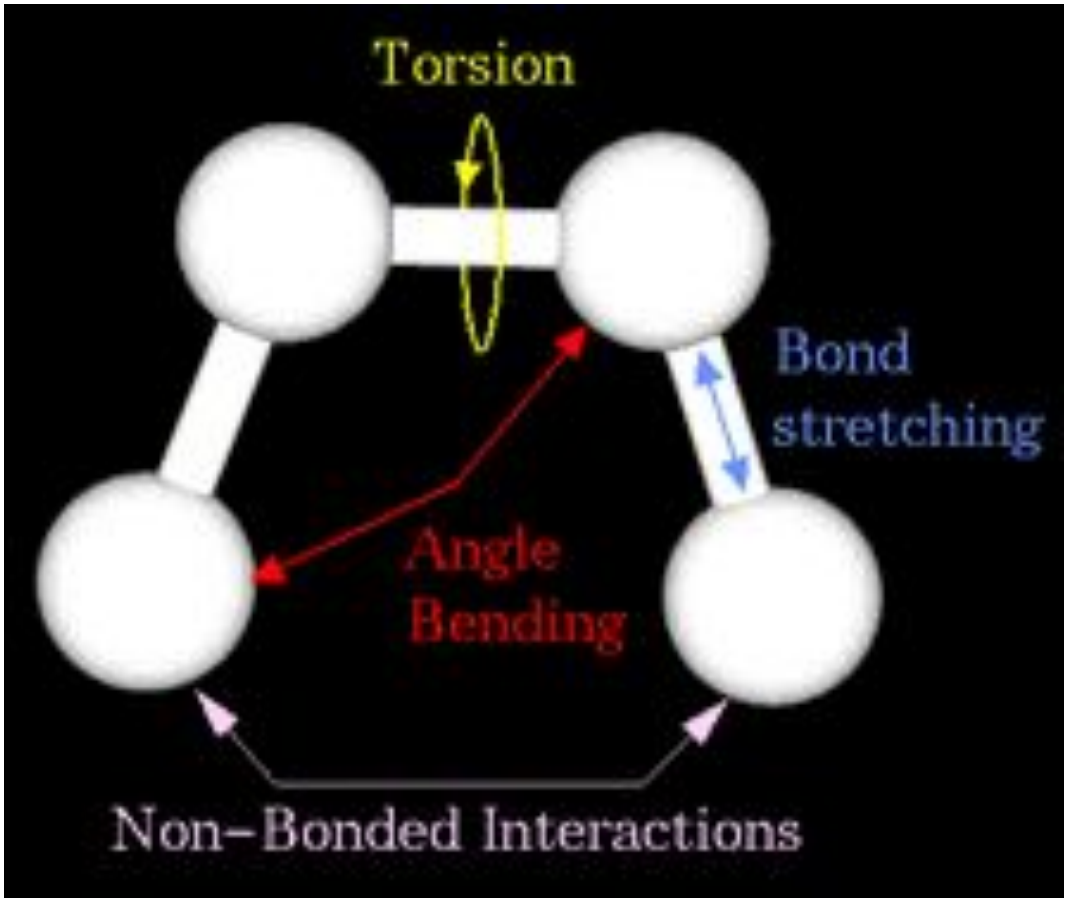
All atoms are moving

Forces between atoms are complicated functions of time

ANALYTICAL solution of x(t) and v(t) is impossible!
This is an N-body problem.

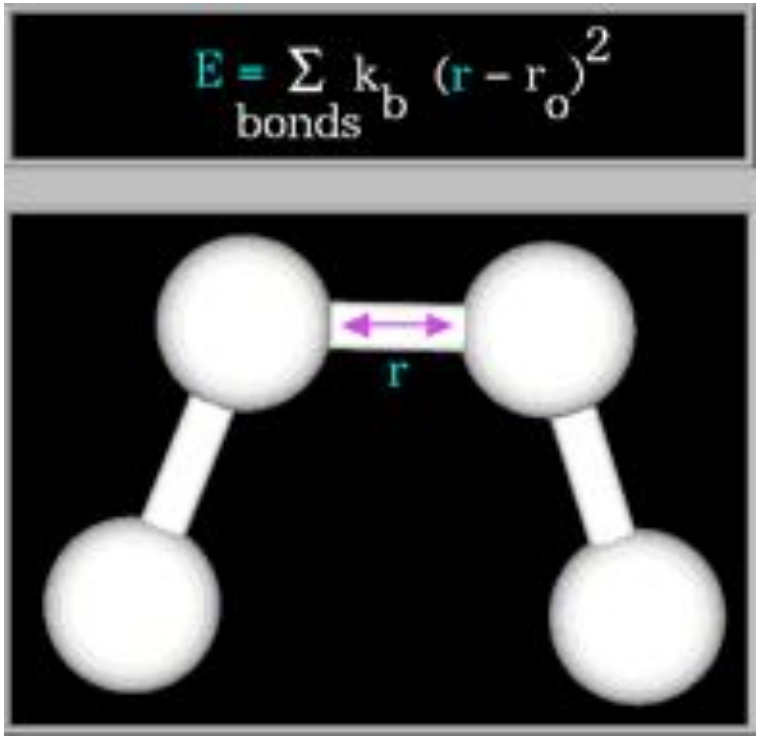
NUMERICAL solution is possible but expensive.
(use short time steps and assume independence)

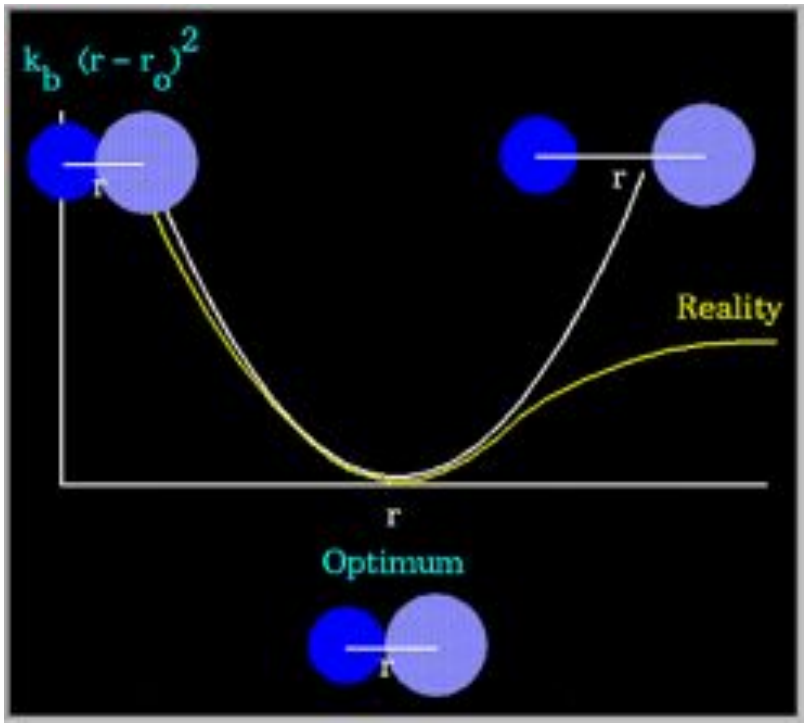
Force field



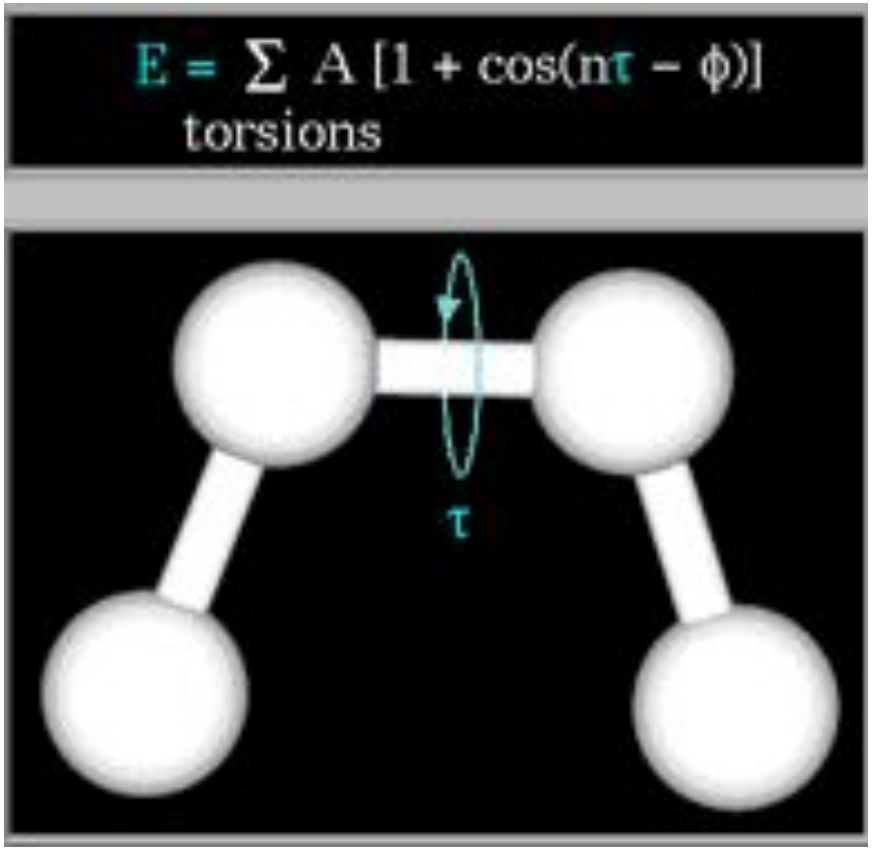
http://cmm.info.nih.gov/modeling/guide_documents/molecular_mechanics_document.html

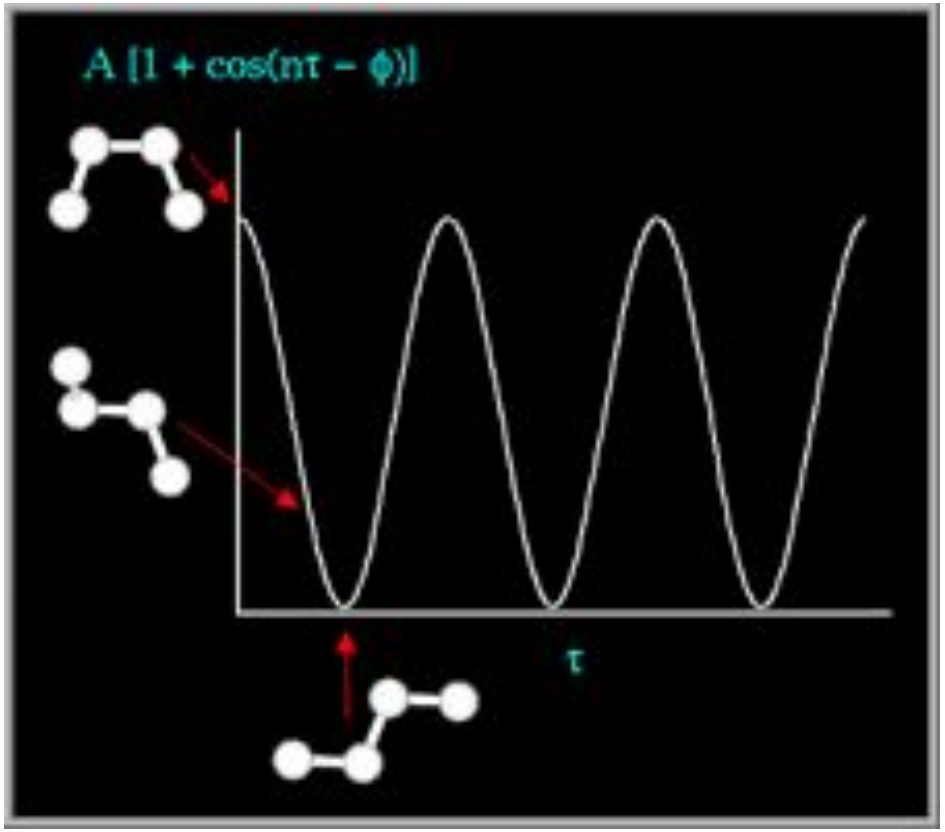
Bonds





Dihedrals

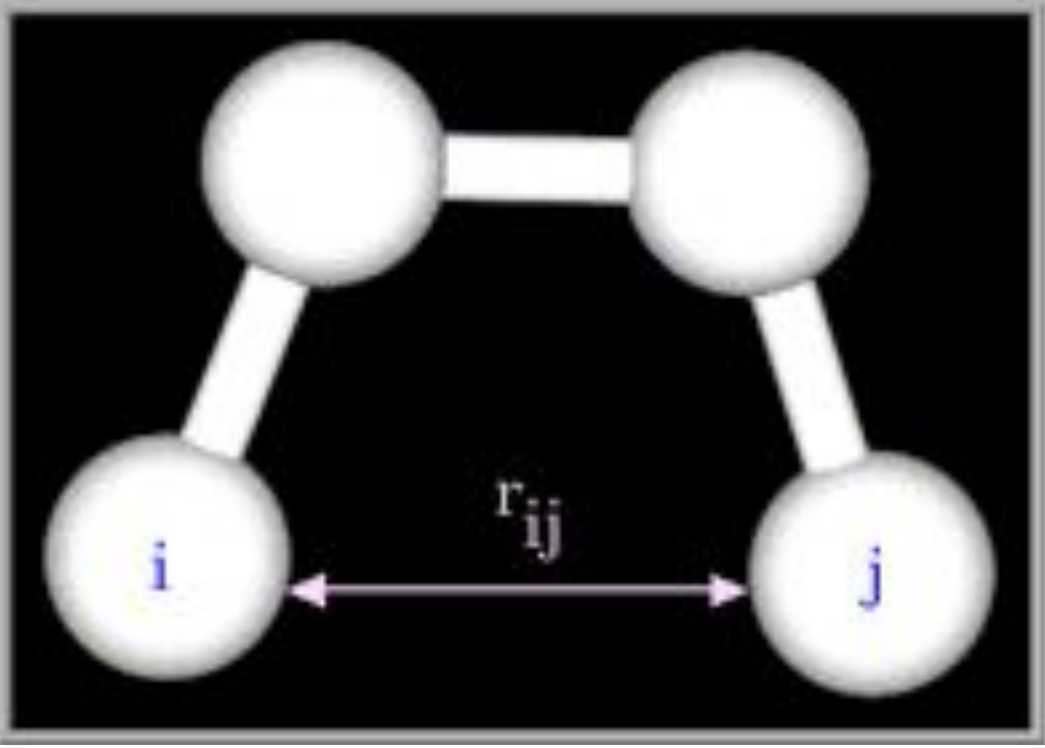




Non-bonded interactions

$$E = \sum_i \sum_j \frac{-A_{ij}}{r_{ij}^6} + \frac{B_{ij}}{r_{ij}^{12}} + \sum_i \sum_j \frac{q_i q_j}{r_{ij}}$$

van der Waals term Electrostatic term



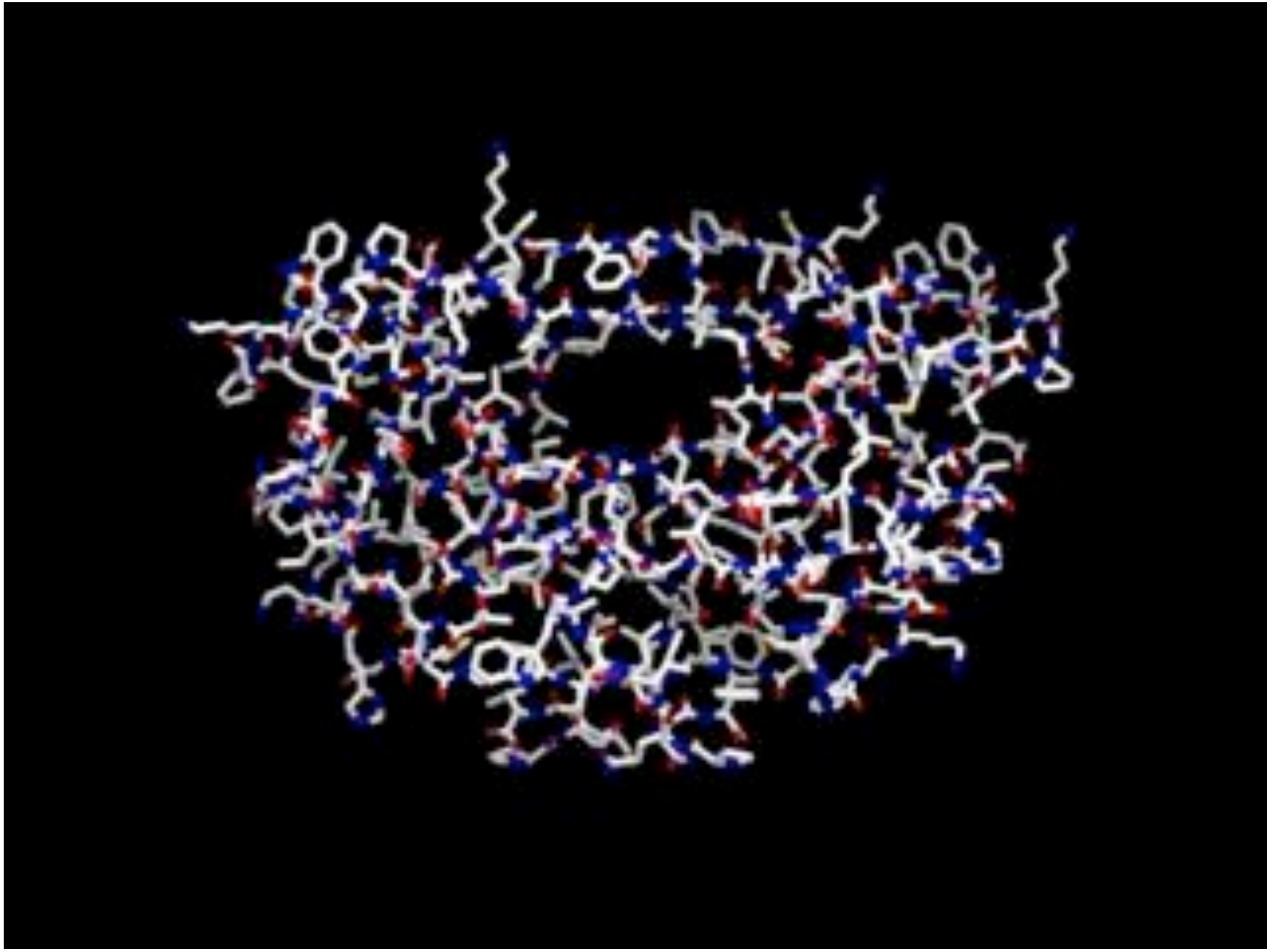
Time component

1 solve for a_i at t using: $-\frac{dE}{dr_i} = F_i = m_i\, a_i(t)$

2 update v_i at $t + \Delta t/2$ using: $v_i(t + \Delta t/2) = v_i(t - \Delta t/2) + a_i(t)\, \Delta t$

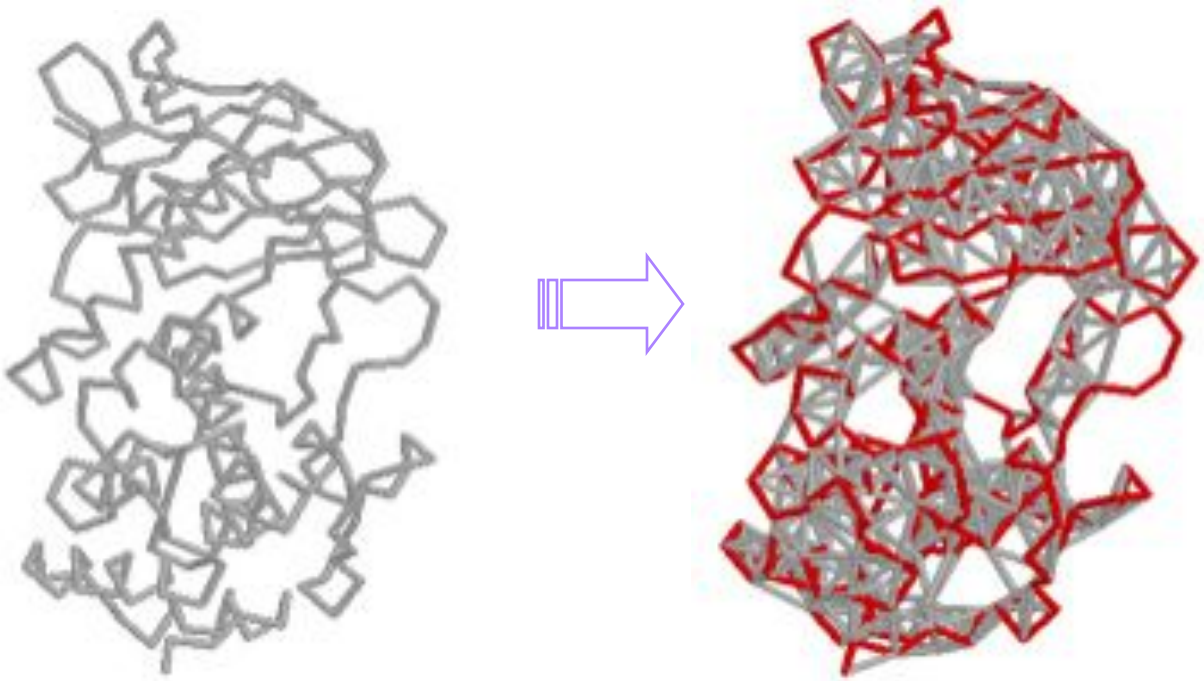
3 update r_i at $t + \Delta t$ using: $r_i(t + \Delta t) = r_i(t) + v_i(t + \Delta t/2)\, \Delta t$

Leap frog algorithm



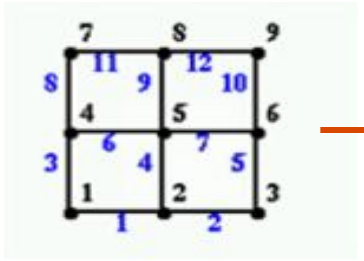
Gaussian Network Model

- Model assumes harmonic “springs” between segments (represented by Cα locations) within a certain cutoff distance (~7 Å), forming an elastic network



- Each Cα atom forms a node in the network and represent a single residue. Edges correspond to the springs.
- (After M.M. Tirion and I. Bahar et al, who popularized the method)

Formulation of GNM



$$\Gamma = \begin{cases} -1 & \text{if } i \neq j \text{ and } R_{ij} \leq r_c \\ 0 & \text{if } i \neq j \text{ and } R_{ij} > r_c \\ -\sum_{i,i\neq j} \Gamma_{ij} & \text{if } i = j \end{cases}$$

- Build a matrix (Kirchhoff, from graph theory, or Laplacian matrix)
- Mobility of Cα atom depends on the inverse of the matrix, which is related to the number of neighboring Cα atoms i.e, their connectivity and contact map
- Being an “elastic network” of springs, the model provides dynamic information from static crystal structures

Relating GNM to atomic displacements

- Eigen analysis or SVD to get psuedo-inverse

$$\Gamma^{-1} = \sum_{k=1}^{n-1} \lambda^{-1} q_k q_k^T \qquad \Gamma^{-1} = V^T M_D^{-1} S$$

- Mean square fluctuation (variance and co-variance)

$$< u_i u_j >= (3k_B T / \gamma)[\Gamma^{-1}]_{ij}$$

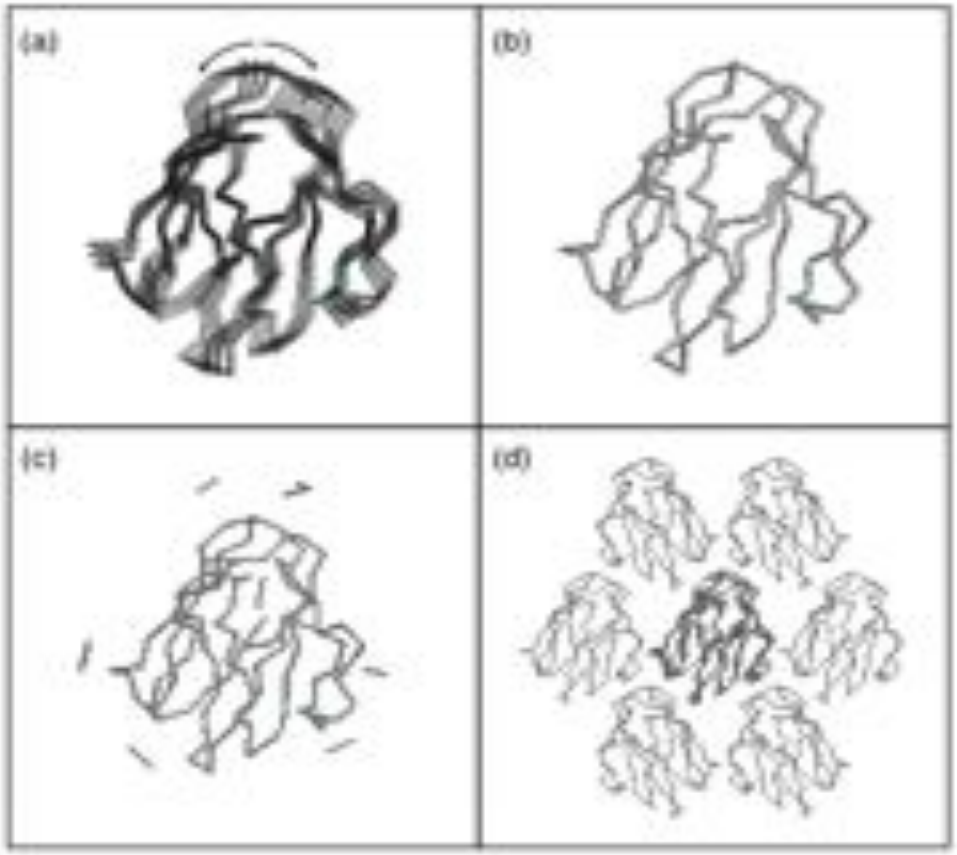
- Calculation of crystallographers' B-factors

$$B_i = 8\pi^2 \langle u_i^2 \rangle$$

Visual description of different model systems

Libration

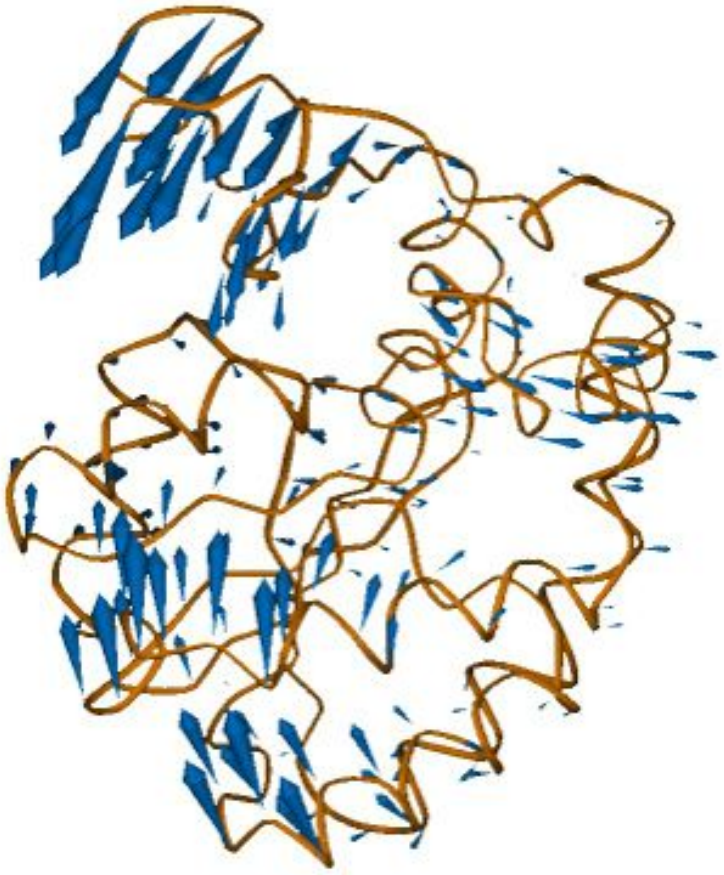
Contact atoms



Isolated molecule

Neighbor molecules

Normal mode analysis with elastic network models



Adenylate Kinase

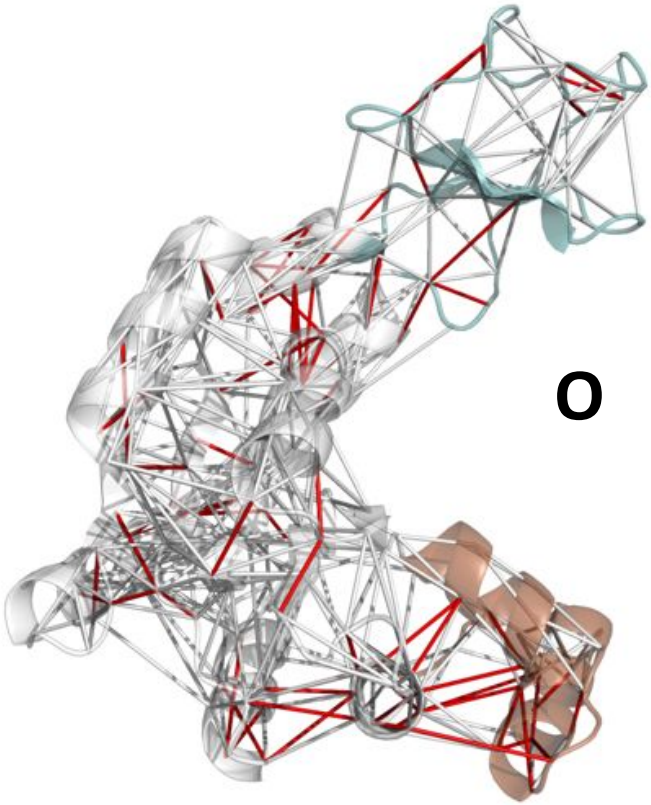
- One of adenylate kinase's major motions can be seen in its lowest mode
  - Orange = α-carbon backbone
  - Blue = Movement vector

Other Coarse-grained Gō-like models

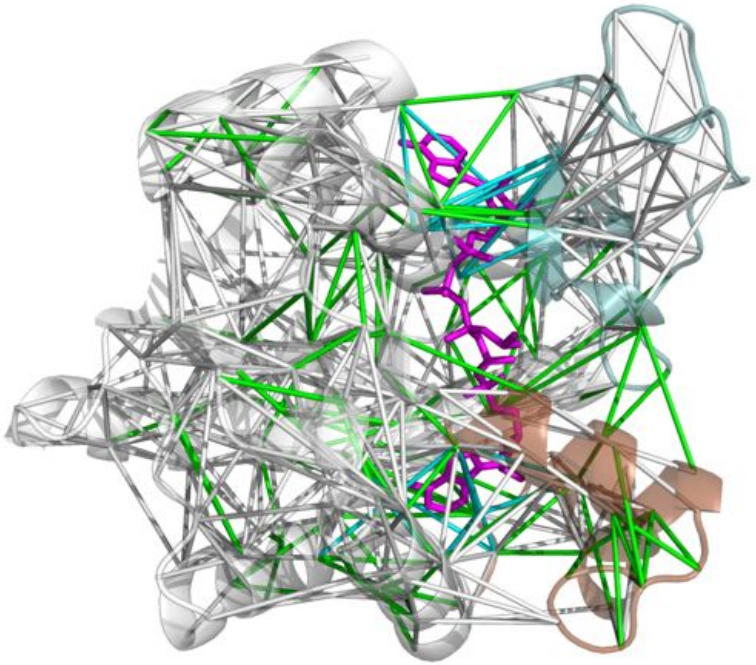
- Can simulate large-scale structural transitions without constraints
- One bead (C_α) per residue
- Harmonic bond potential
- Dihedrals
  - statistical based on sequence of residues *i*-1,*i* , no structural info
- Bond angles (some implicit φ,ψ)
  - generic: allow both α-helix and β-sheet
- Contacts
  - native: Lennard-Jones 12-10 potential (increase curvature)
  - non-native: LJ repulsion only

refs: Karanicolas & Brooks (2002), Best et al. (2005)
Daily, Phillips, Cui, J. Mol. Biol. (2010)

AKmeso O and C native contacts



O



C

LID

NMP

Common contacts

Unique to O

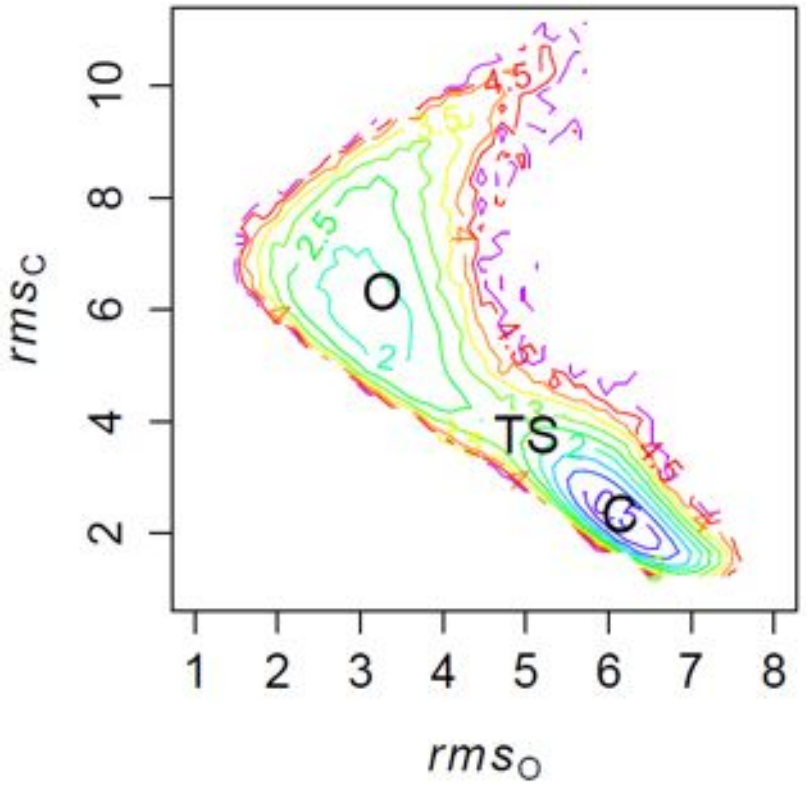
Unique to C

Substrate

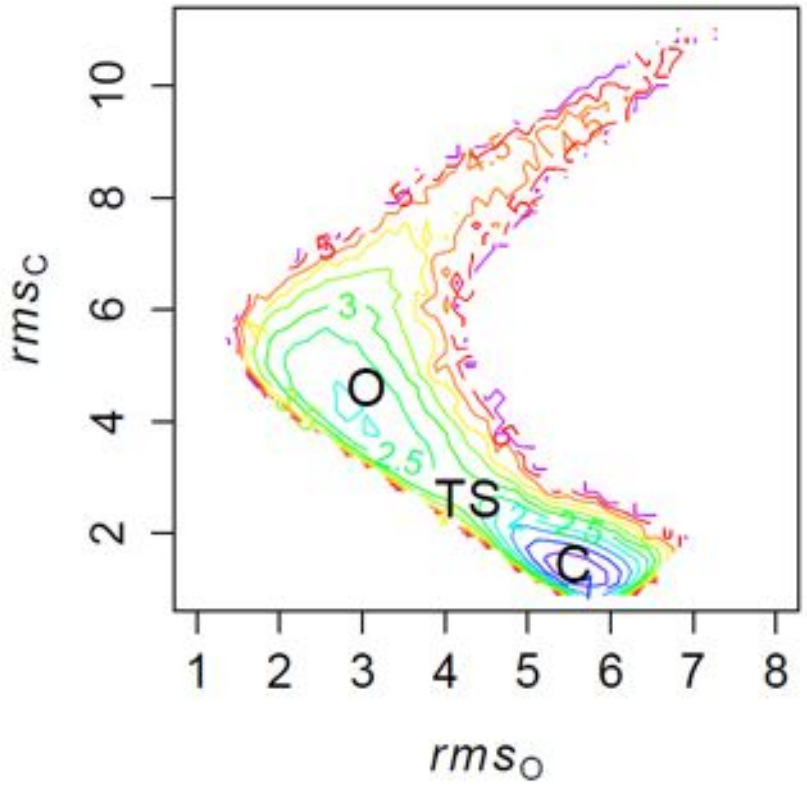
Ligand-mediated contacts

AKmeso and AKthermo simulations in rmsd space

AKmeso



AKthermo



Very similar PMFs, thermo slightly more stable in rms_C

Summary

- Crystals allow average structures of large molecules to be determined
- The crystal symmetry is only an approximation, however
- Motions of proteins are critical parts of their fitness for their functions
- While we can start to make ‘movies’ of proteins, to understand the motions, they are primitive

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