TLS and Normal Modes

IUCr Crystallographic Computing School
IISc Bangalore, 19-Aug-2017

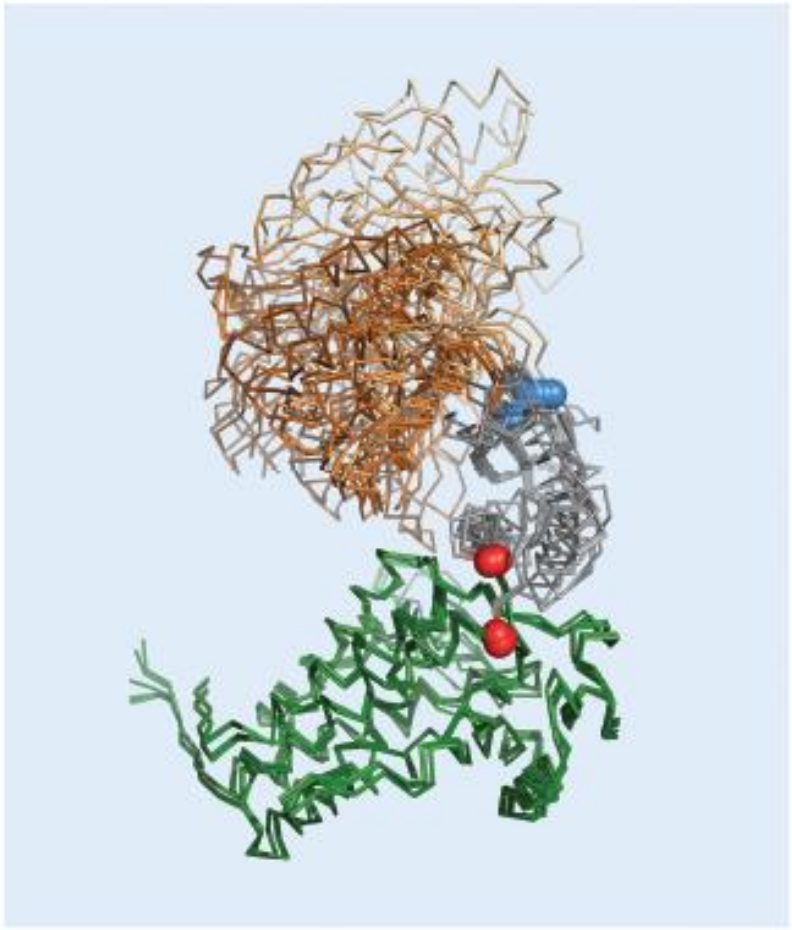
Shekhar C. Mande

National Center for Cell Science
Pune

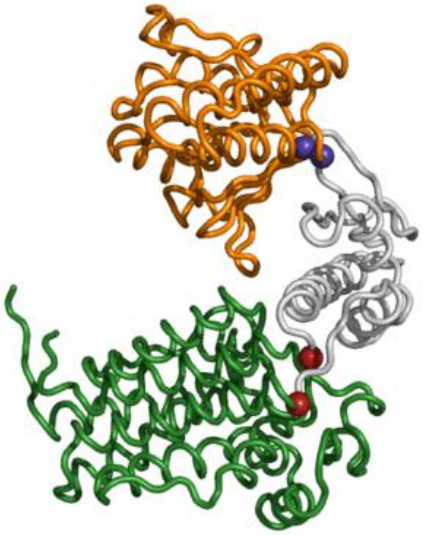
Mass migration of Animals/ birds/ insects

Functional Aspects of Humming Bird feeding:

- Vision
- Nectar drawing
- Flight modulation



Conformational transitions in GroEL



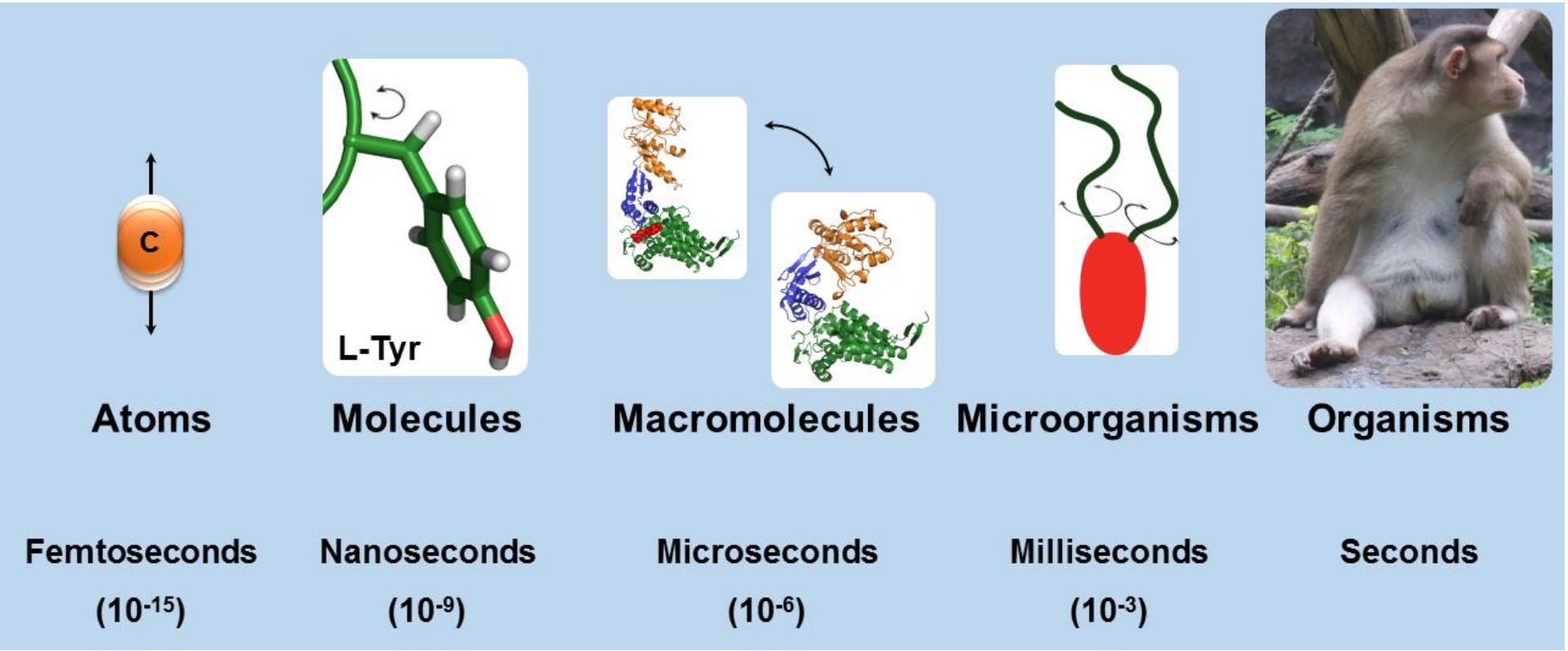
February 2016
Volume 198
Number 3
Published Twice Monthly

AMERICAN SOCIETY FOR MICROBIOLOGY

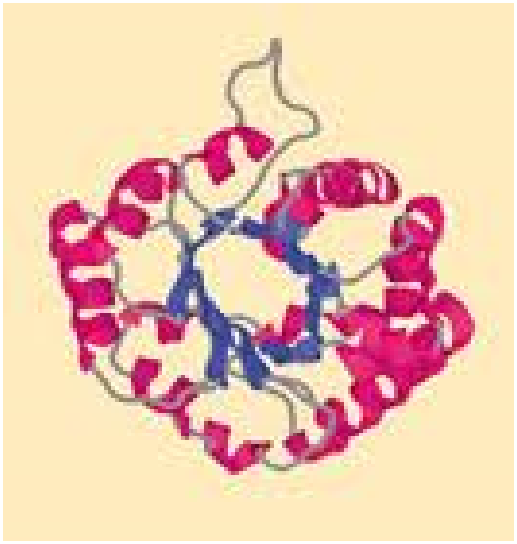
JB
Journal of Bacteriology

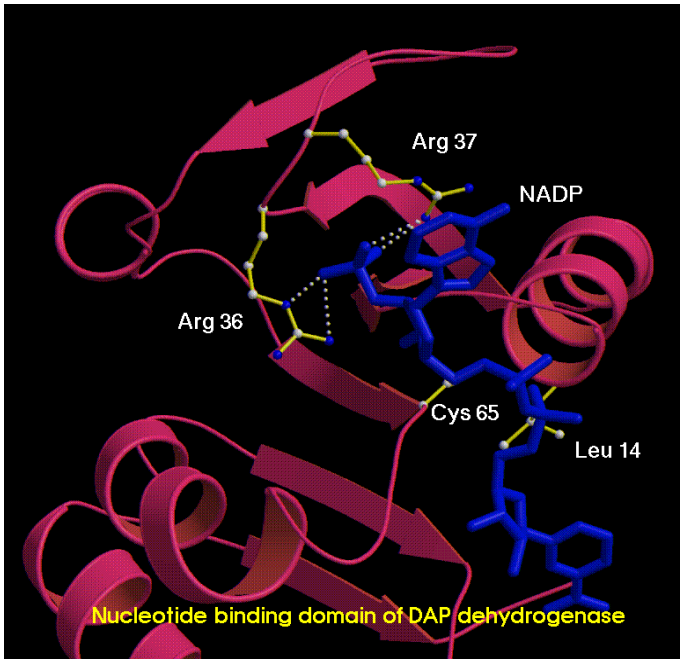
Chilukoti, Kumar and Mande, *J. Bacteriol*., 2016

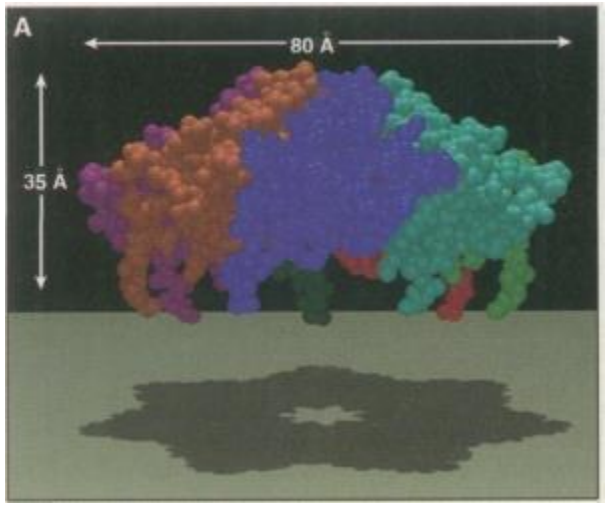
Scale of Biological Motions



Protein Structures:
Beyond pretty pictures







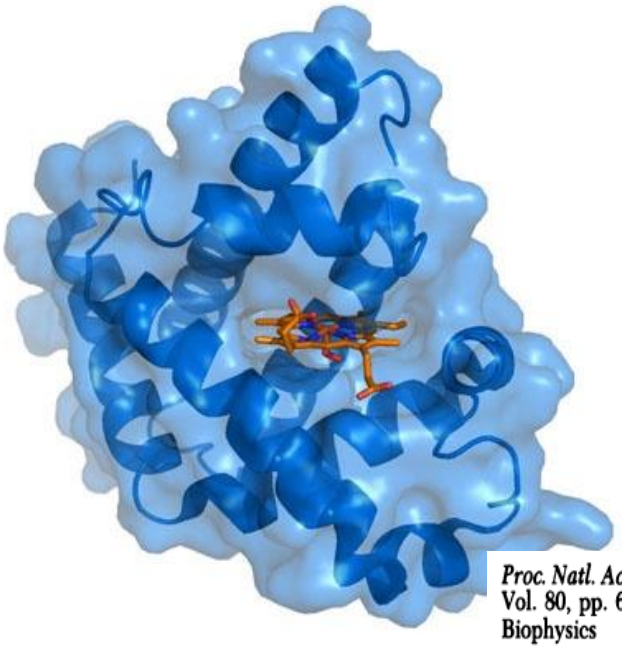
Need to account for dynamic behaviour of proteins: computational approaches

(Reprinted Nature, Vol. 253, No. 5494, pp. 6 94-6 98, February 2 7, 19 75)

Computer simulation of protein folding

Michael Levitt* & Arieh Warshel*

Department of Chemical Physics, Weizmann Institute of Science, Rehovoth, Israel



Dynamics of ligand binding to heme proteins

D. A. Case and M. Karplus

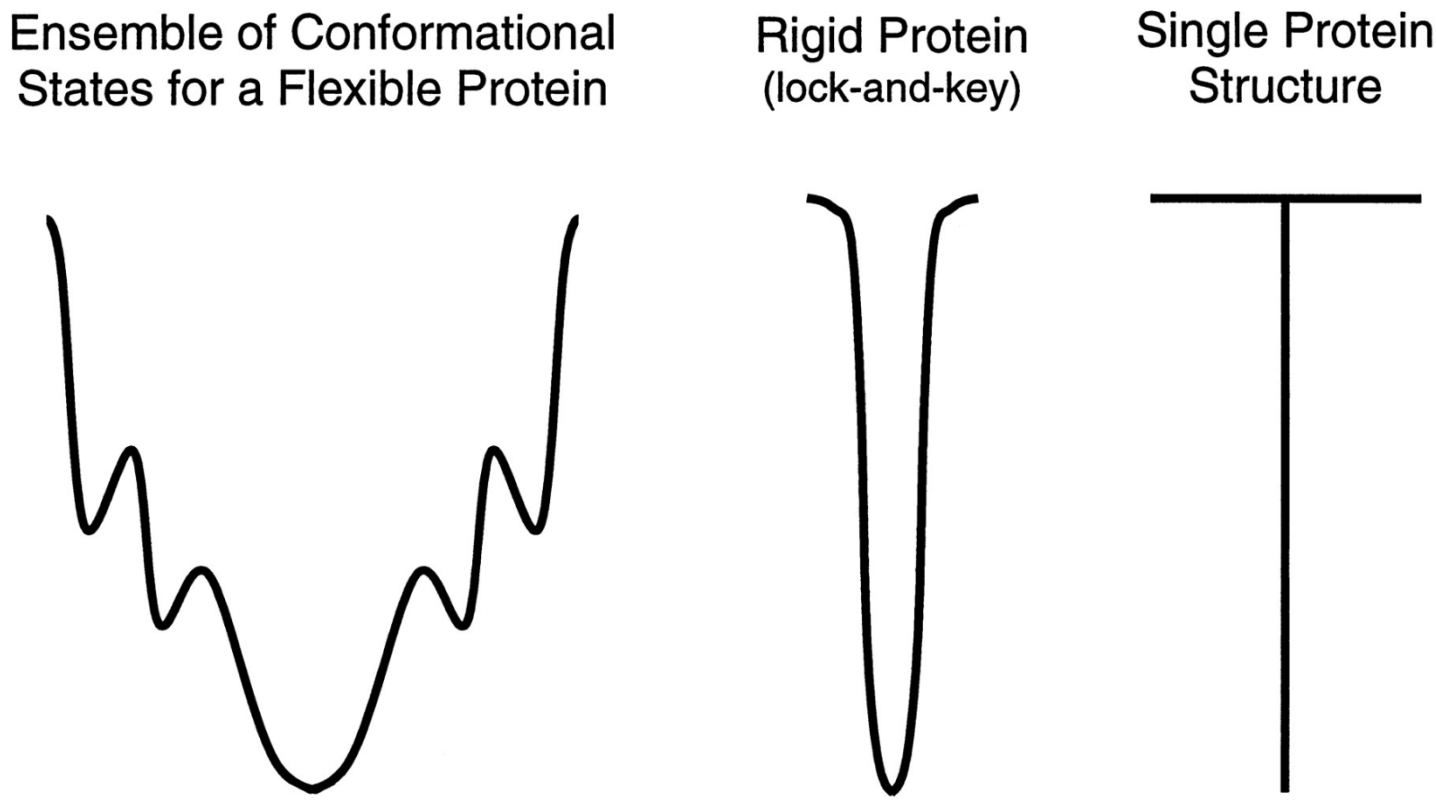
J Mol Biol (1979) **132,** 343- 368

Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 6571–6575, November 1983
Biophysics

Harmonic dynamics of proteins: Normal modes and fluctuations in bovine pancreatic trypsin inhibitor

(vibrational analysis/protein dynamics/temperature factors/thermodynamic properties/frequency spectrum)

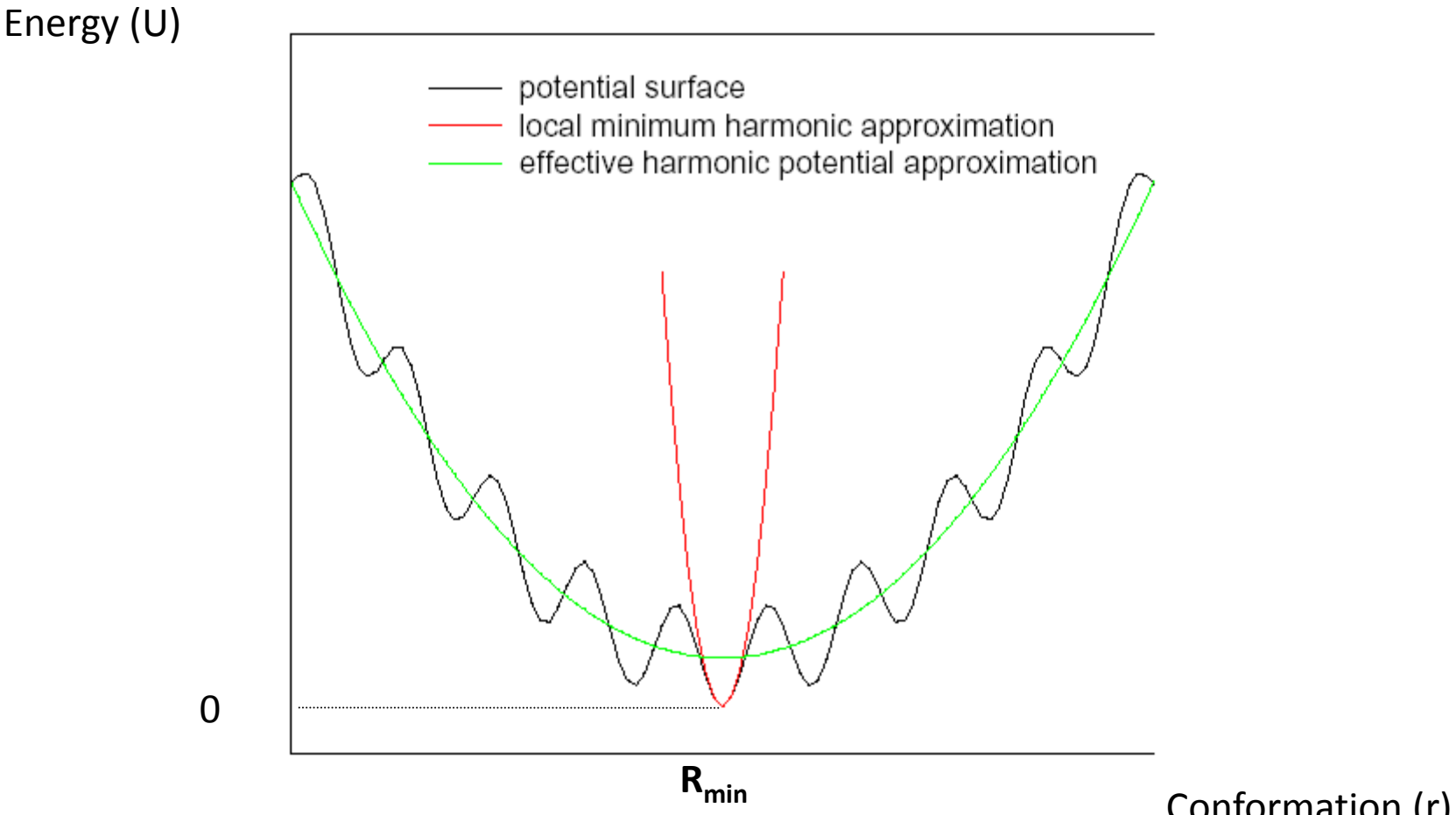
BERNARD BROOKS AND MARTIN KARPLUS



A single structure of a protein implies an all-or-nothing folding funnel, perhaps best described with the mathematic singularity shown on the right. The folding funnels on the left and in the center demonstrate the conformational flexibility of a “standard” flexible protein and a rigid protein, respectively. Although the rigid system may be described adequately by a single structure (depending on the degree of rigidity as reflected by the narrowness of the funnel), a typical system is not.

From Carlson and McCammon, *Mol. Pharmacol*., 2000

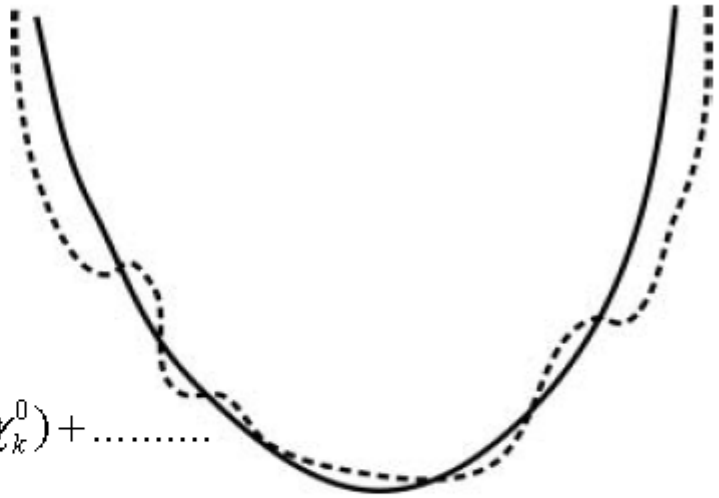
Harmonic approximation



$$U(\chi) = \sum_i (\chi_i - \chi_i^0) \left.\frac{\partial U}{\partial \chi_i}\right|_{\chi=\chi_o} +$$

$$\frac{1}{2!} \sum_{ij} \left.\frac{\partial^2 U}{\partial \chi_i \partial \chi_j}\right|_{\chi=\chi_o} (\chi_i - \chi_i^0)(\chi_j - \chi_j^0) +$$

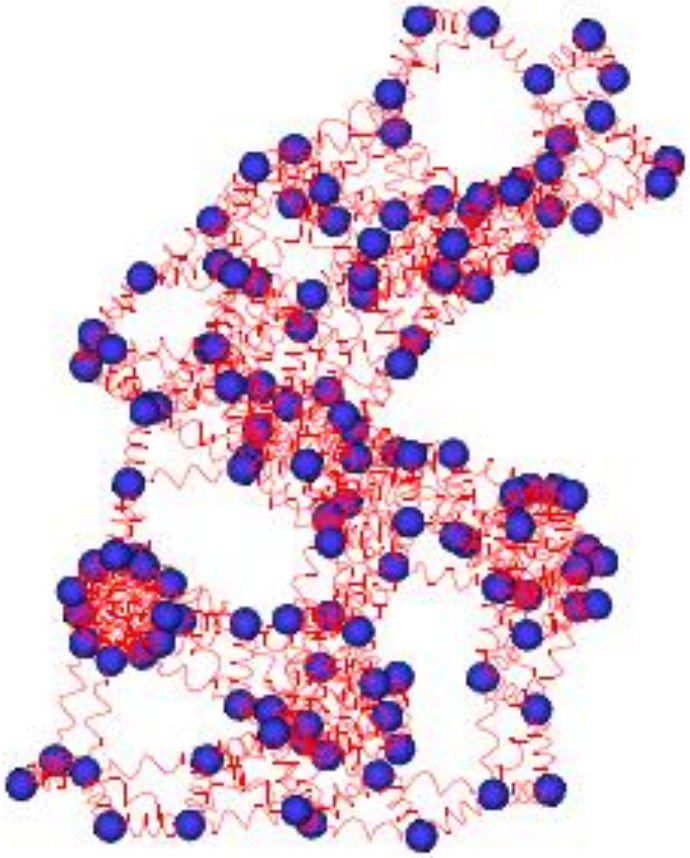
$$\frac{1}{3!} \sum_{ijk} \left.\frac{\partial^3 U}{\partial \chi_i \partial \chi_j \partial \chi_k}\right|_{\chi=\chi_o} (\chi_i - \chi_i^0)(\chi_j - \chi_j^0)(\chi_k - \chi_k^0) + \ldots\ldots\ldots$$



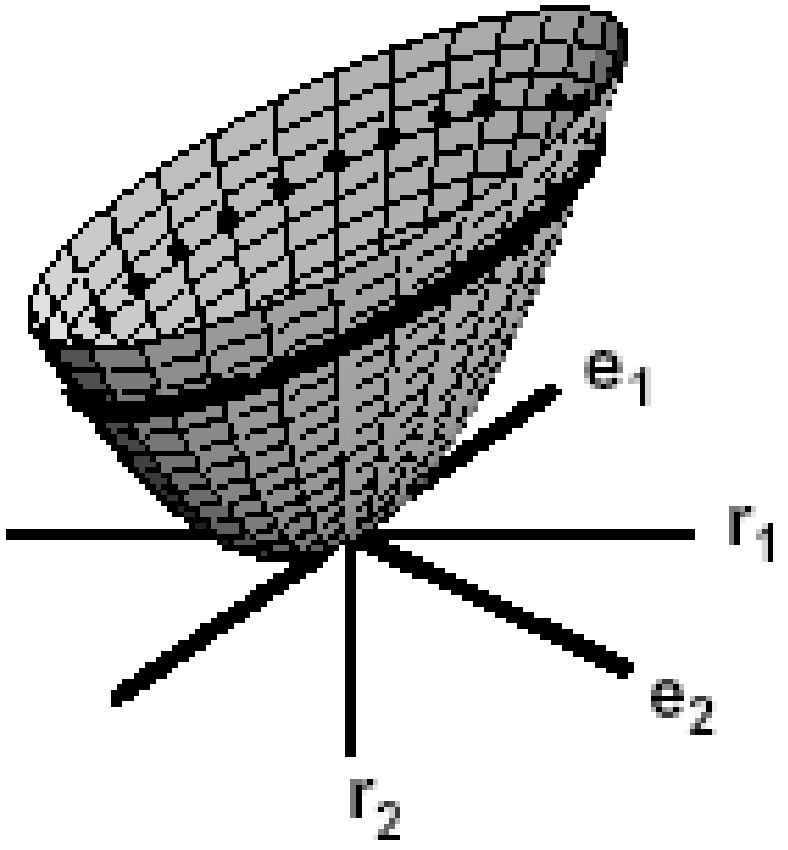
Harmonic approximation

$$U(\chi) \cong \frac{1}{2} \sum_{ij} \left.\frac{\partial^2 U}{\partial \chi_i \partial \chi_j}\right|_{\gamma=\gamma_o} (\chi_i - \chi_i^0)(\chi_j - \chi_j^0)$$

Elastic Network Model

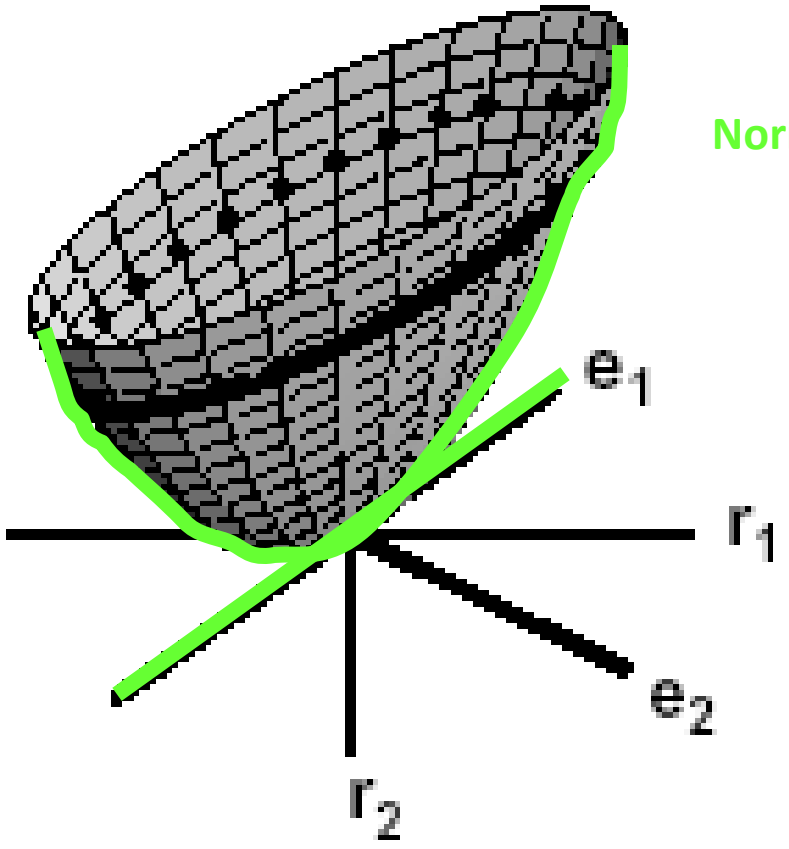


NMA



$$U(r) = 0.5\,(r - R_{min})' \cdot K(R_{min}) \cdot (r - R_{min})$$

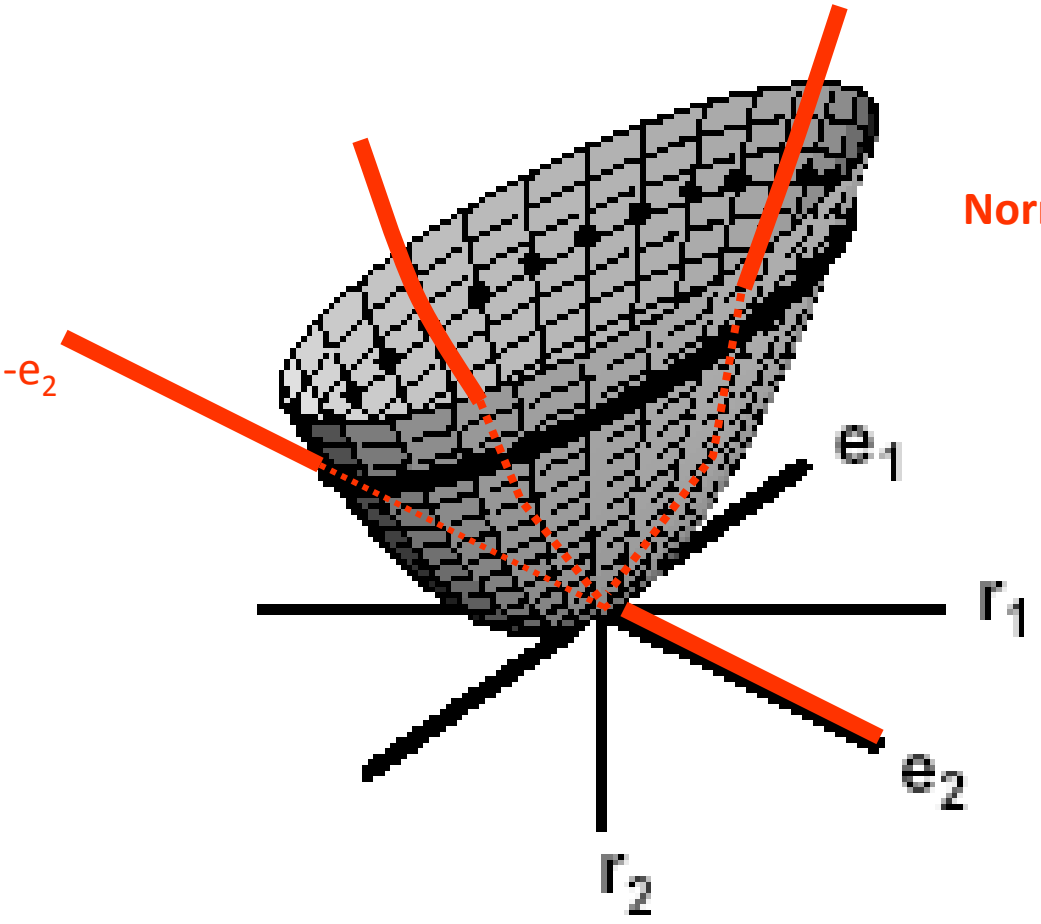
NMA



Normal mode direction 1

$$U(r) = 0.5\,(r - R_{min})' \cdot K(R_{min}) \cdot (r - R_{min})$$

NMA

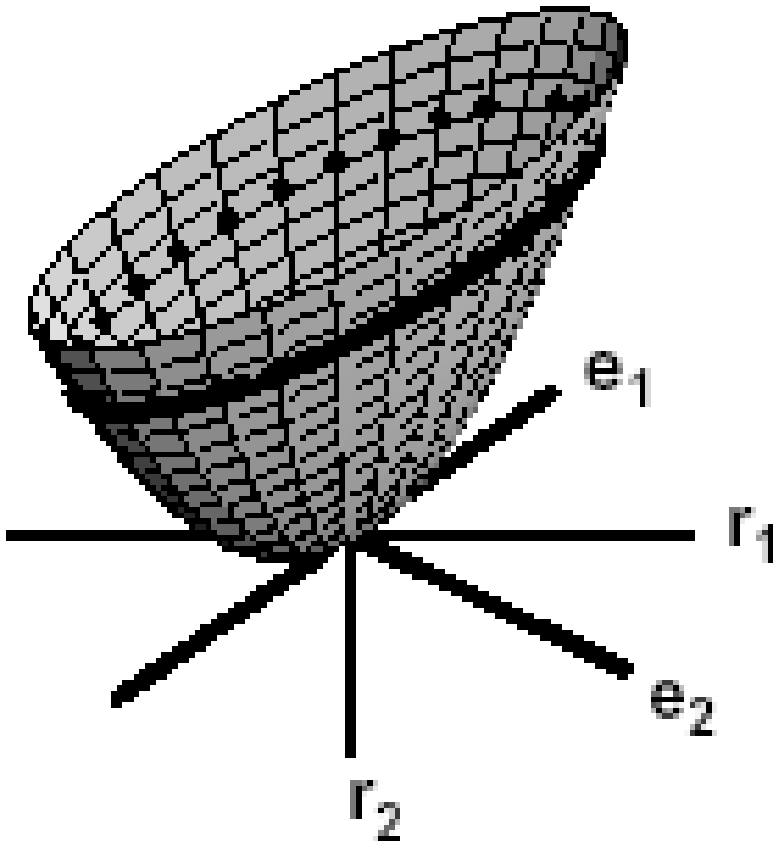


Normal mode direction 2

$$U(r) = 0.5\,(r - R_{min})' \cdot K(R_{min}) \cdot (r - R_{min})$$

Properties of NMA

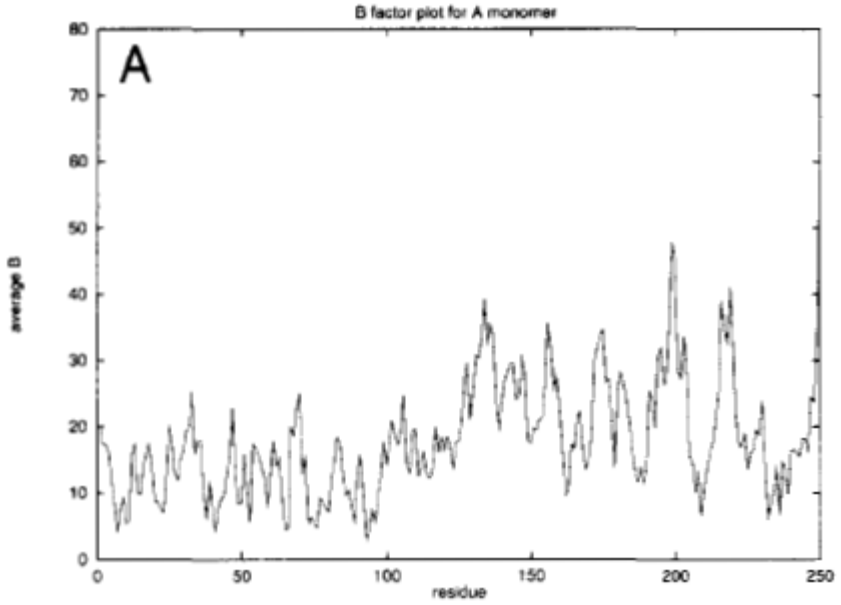
- The eigenvalues describe the energetic cost of displacing the system by one length unit along the eigenvectors.
- For a given amount of energy, the molecule can move more along the low frequency normal modes
- The first six eigenvalues are 0, corresponding to rigid body movements of the protein

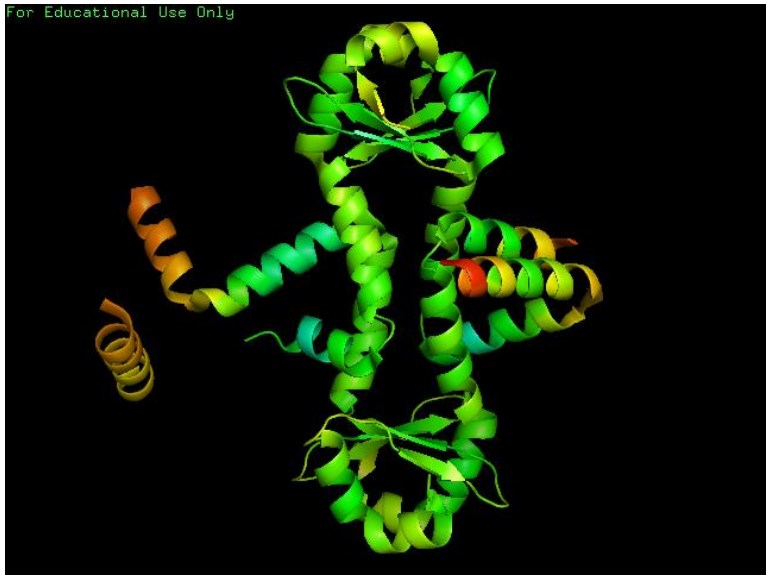


Need to account for dynamic behaviour of proteins: crystallographic approaches

Thermal fluctuations in proteins:
B-factor

$$B = 8 \pi^2 \langle u^2 \rangle$$





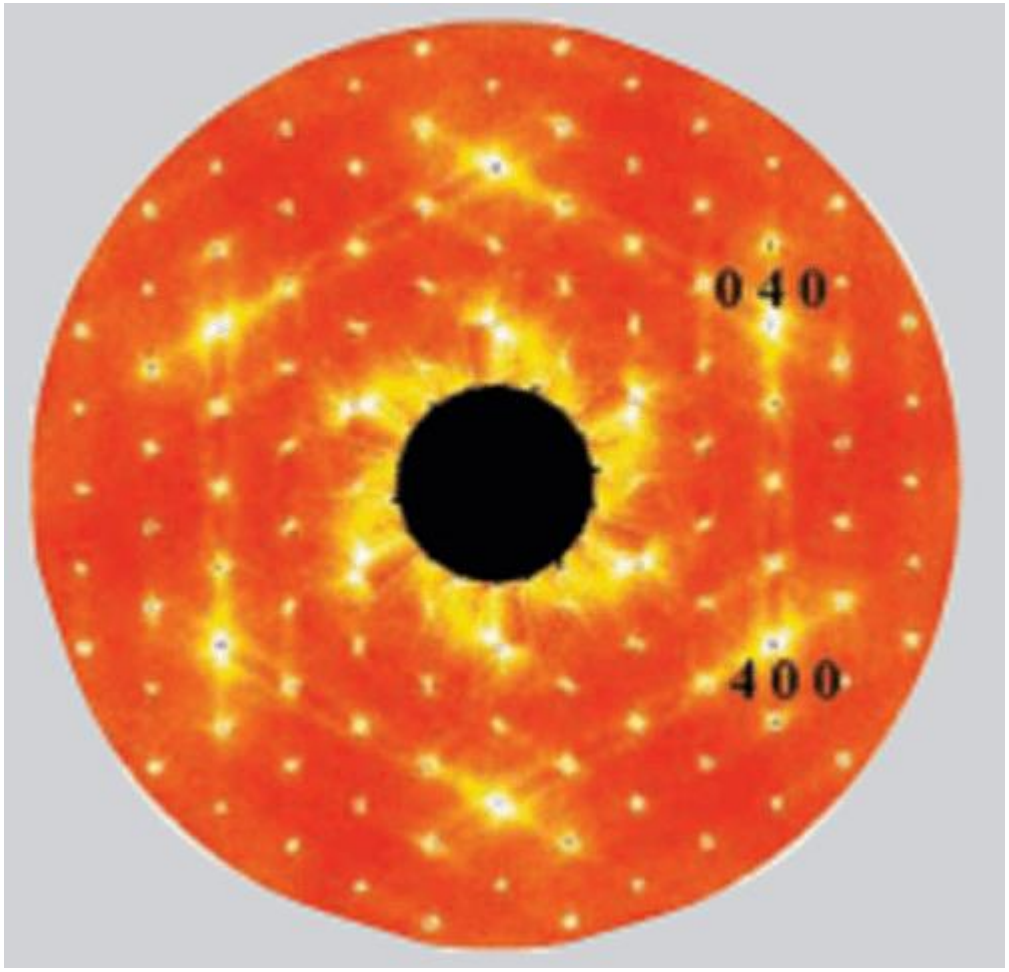
A typical B-factor represents isotropic movement of an atom around its mean position

Molecules are in motion, even when they are packed

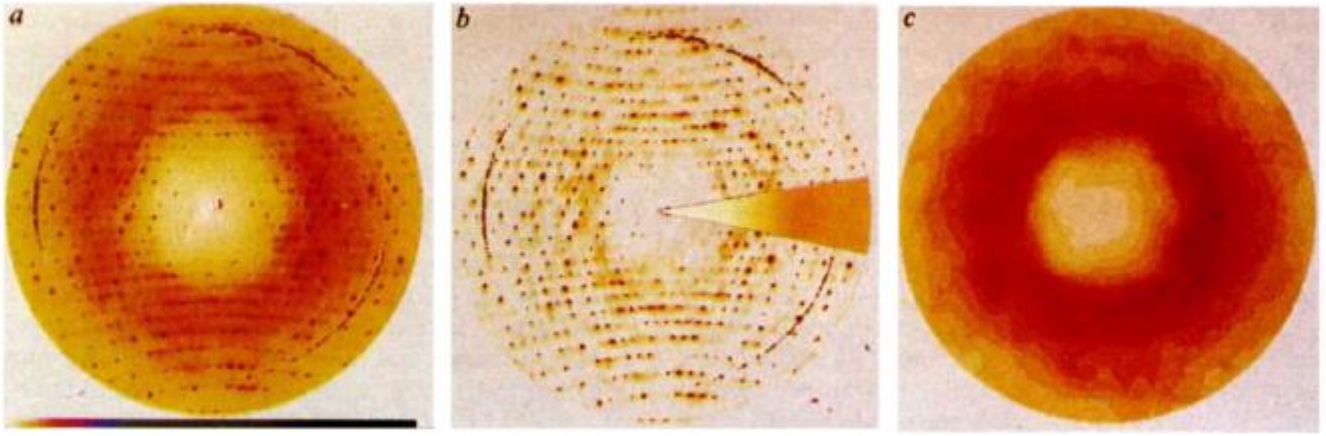
- In a crystal lattice, even though the molecules have restricted motions due to packing effects, they still show considerable flexibility similar to that in a solution.
- This flexibility is defined by Atomic Displacement Parameters (ADPs).
- Anisotropic refinement of ADPs gives both magnitude as well as direction of the atomic flexibility.
- ADPs Can be visualized as Thermal ellipsoids.

- Thus, diffraction data can be used to decipher the dynamics within a macromolecule, providing biologically relevant information.
- However, individual ADPs can be attributed to a model only in case of a high-resolution data (better than 1.2 Å).
- For low resolution data collective contribution of a group of atoms (rigid domains) can be used to deduce the dynamical behavior of a molecule.

Dynamics information from diffuse scattering



Long range and short range couplings of molecular motions in crystallographic lattice



Very diffuse: variations in atomic positions locally correlated in molecule

Liquid-like: water and Compton scattering

Haloes around Bragg peaks: Coupled displacements of neighbouring molecules in the lattice

Liquid like movements in crystalline insulin
Caspar, Clarage, Salunke and Clarage
Nature (1988) **332**, 669-662.

Acta Cryst. (1968). B**24**, 63

On the Rigid-Body Motion of Molecules in Crystals*

BY VERNER SCHOMAKER

Department of Chemistry, University of Washington, Seattle, Washington 98105, *U.S.A.*

AND K. N. TRUEBLOOD

Department of Chemistry,† University of California, Los Angeles, California 90024, *U.S.A.*

(*Received* 15 *May* 1967)

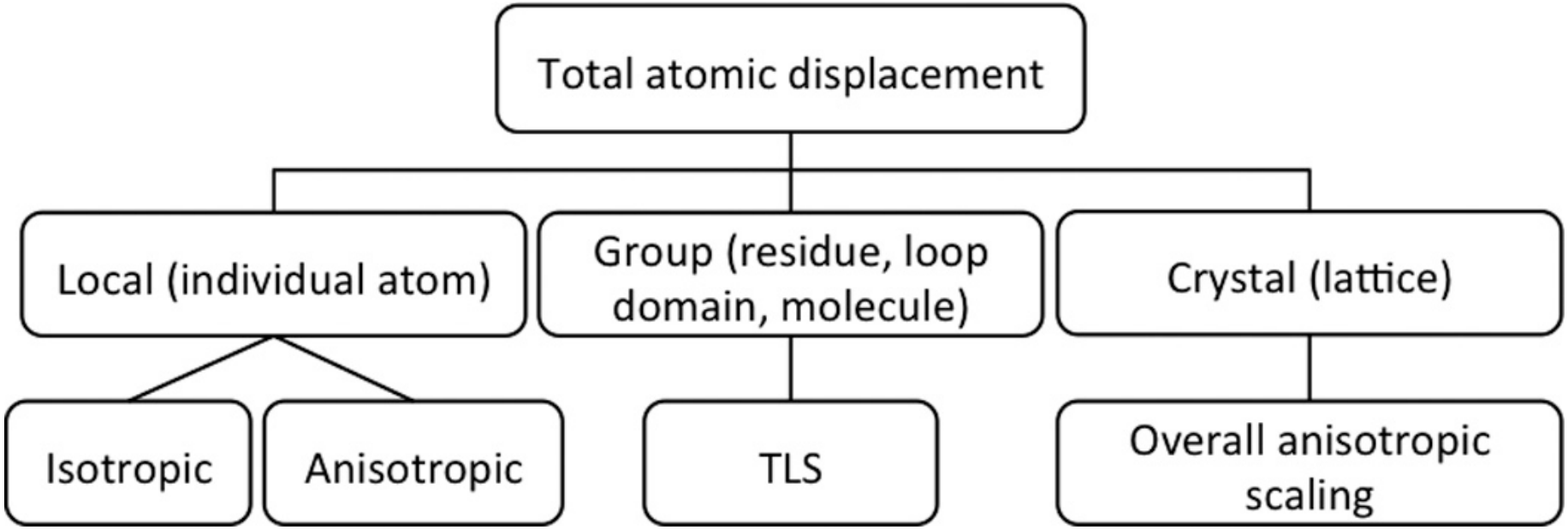
Cruickshank's analysis of the rigid-body motion of molecules in crystals in terms of two symmetric tensors, one for libration and one for translation, has been widely adopted in the interpretation of the results of anisotropic refinements of the structures of molecular crystals. In recent years it has been recognized by several people that this treatment is inadequate when there is no pre-ordained center of libration (*e.g.* a center of symmetry), but in each instance the two-tensor description of molecular motion has been retained, an effective center of libration has been assumed to exist, and its location has been sought by one means or another.

Actually, an additional tensor (which we call **S**) is needed to account for correlations of libration and translation. For a molecule at a sufficiently unsymmetrical site, **S** has eight independent components, one of its diagonal elements being arbitrary, and the contribution to the anisotropic displacement tensors by the rigid-body part of the enormously various actual motions can be described in terms of six uncorrelated simple motions: the three familar principal mean-square translations plus three *screw* (*helical*) motions about and along three mutually perpendicular, *non-intersecting* axes.

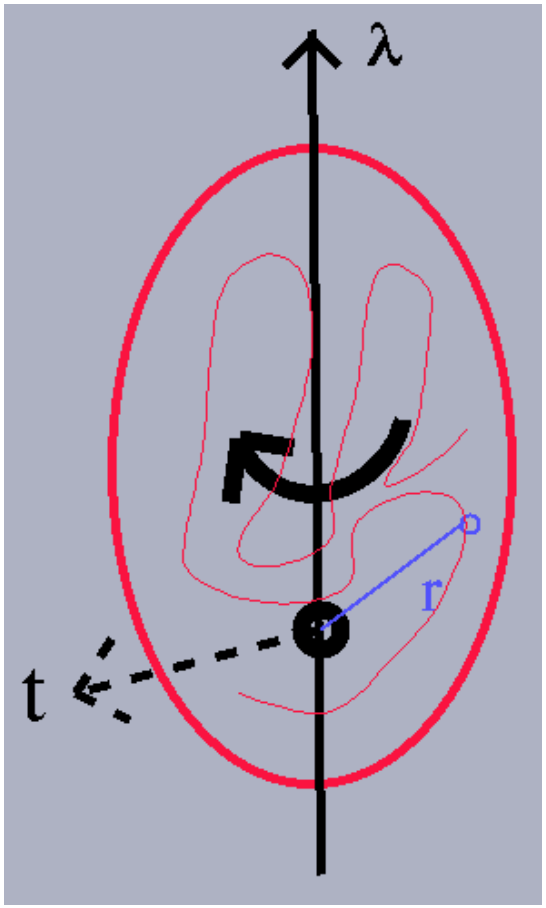
The problem of fitting the observed atomic displacement tensors in terms of rigid-body translation and screw motion involves in the general case a least-squares fit of twenty independent parameters. If the molecule is at a symmetrical site, some or all of the components of **S** are subject to special restrictions; for example, if the site-symmetry is $\bar{1}$, **S** vanishes completely and the treatment is identical with Cruickshank's. In any event, the fit is always independent of the origin assumed in the description of the motion and is found by a straightforward linear least-squares process. Corrections to intramolecular interatomic distances foreshortened by rigid-body motion are shown to depend only on the libration tensor, which is independent of the assumed origin.

Examples of the application of this analysis are given.

Hierarchy of contributions to atomic displacement parameters and possible ways to model these contributions



***TLS* from fundamentals to practice**
Alexandre Urzhumtsev, Pavel V. Afonine and Paul D. Adams
Crystallogr Rev. 2013 Jul 1; 19(4): 230–270



General displacement of an atom (position ***r*** w.r.t. origin O) in rigid body**:**

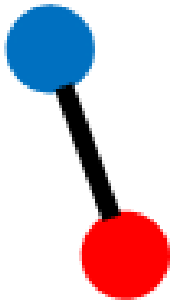
$$\mathbf{u} = \mathbf{t} + \mathrm{D}.\mathbf{r}$$

For small libration **λ**,

$$\mathbf{u} \approx \mathbf{t} + \boldsymbol{\lambda} \times \mathbf{r}$$

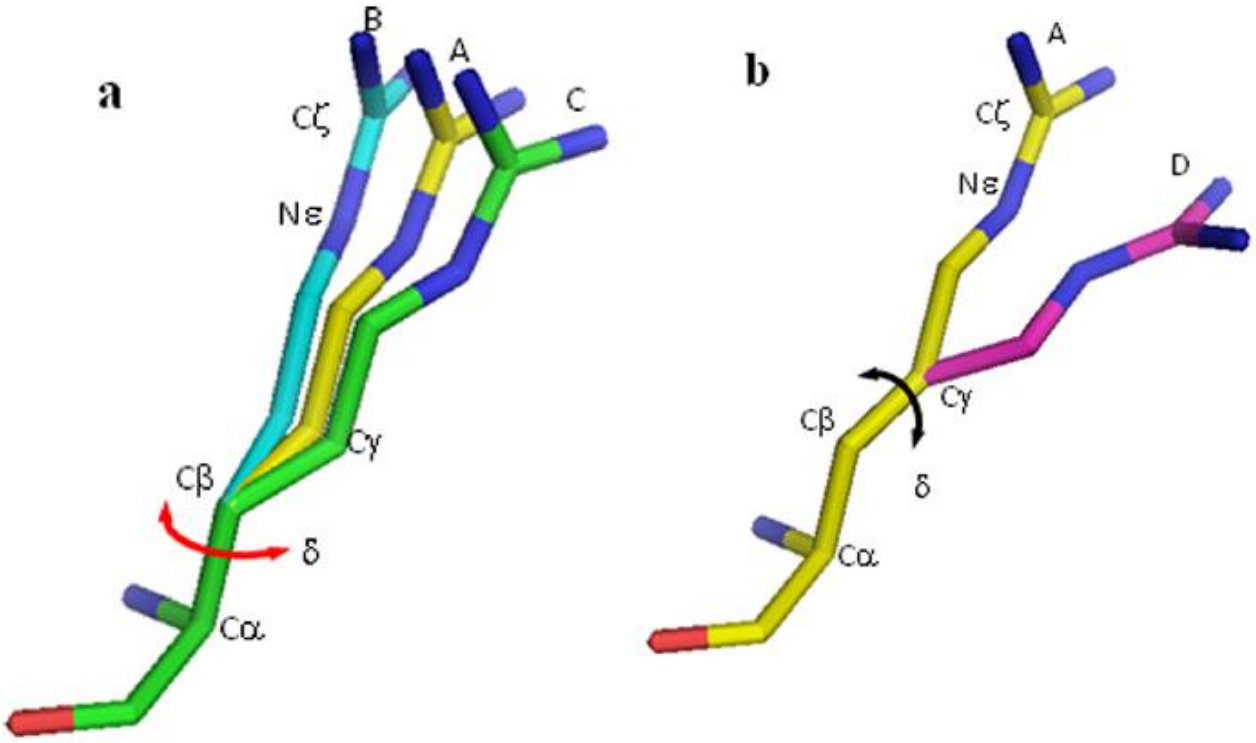
Libration

- Derived from the Latin verb “*Librare*” meaning “to balance, to sway”.
- Rotation/oscillation of a rigid body
- In crystals, molecules undergo libration motions within confinements of certain preferred orientations due to neighboring molecules.



A diatomic molecule
Undergoing libration

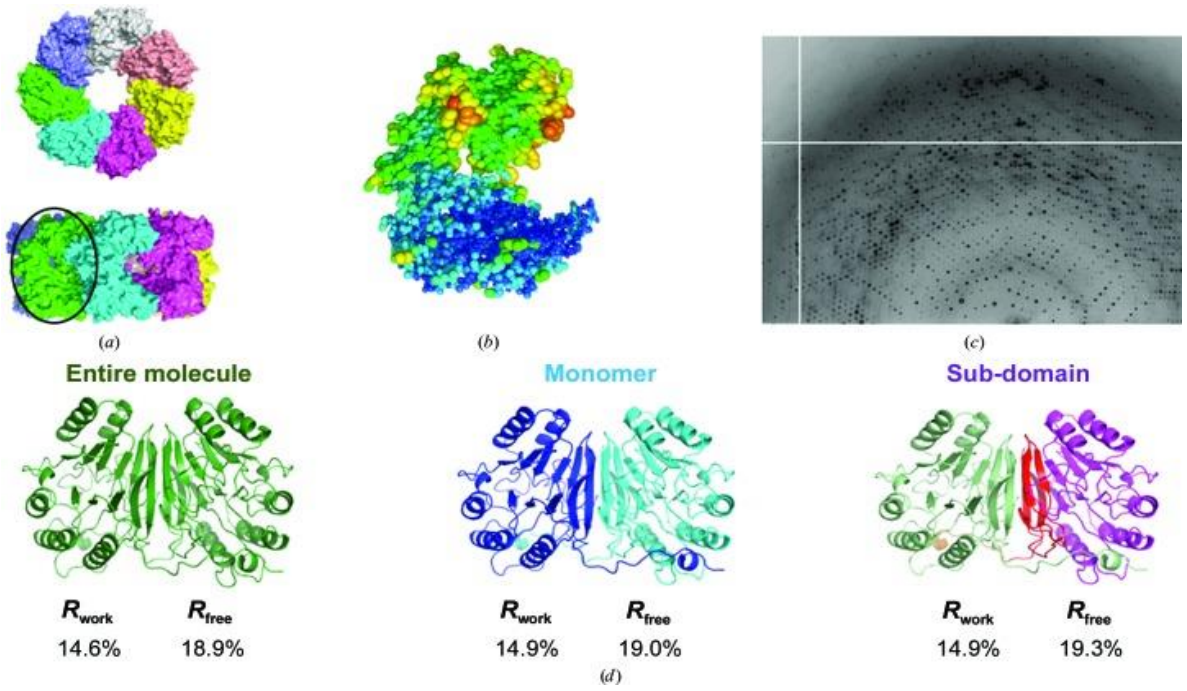
Libration around a bond



a. Oscillation about the fixed C_α-C_β bond (Rotation axis), leading to translation of N_ε-C_ζ bond.
b. Rotation about C_β-C_γ bond changes orientation of N_ε-C_ζ, leading to conformations A and D.

Adapted from Urzhumtsev and Adams, *Crystallogr. Rev., 2013*

Can crystallographic refinement capture biologically relevant motions?



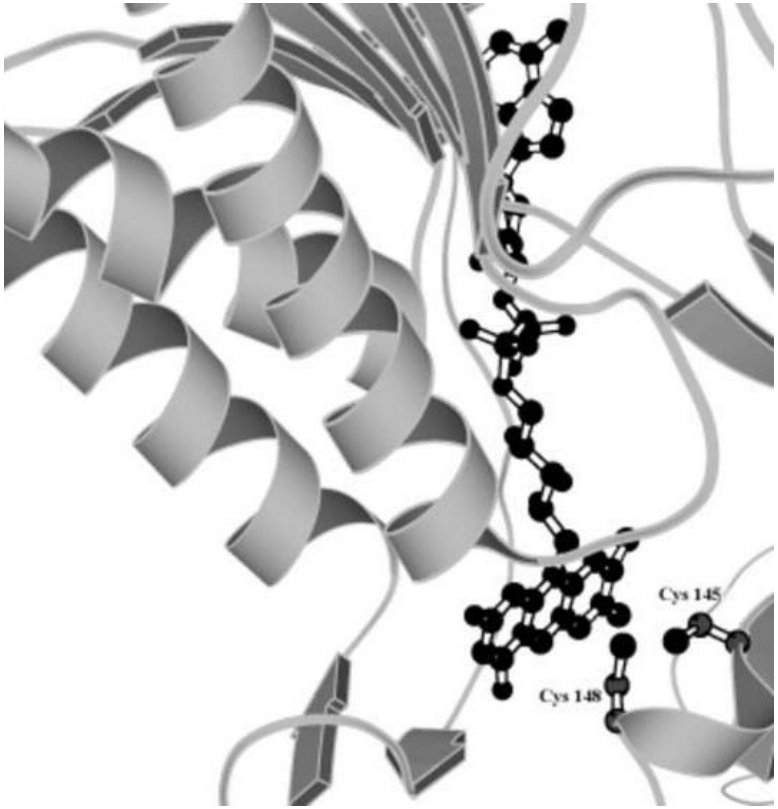
TLS refinement suggests macromolecular motions linked to function. (*a*) Top and side view of GroEL. Each color denotes a unique chain. (*b*) TLS refinement of GroEL subunits reveals a 'tilting' motion around the center of the subunit. (*c*) GpdQ diffraction image showing significant diffuse scattering features. (*d*) Refinement of GpdQ fails to produce substantial changes in R_{work} and R_{free} values between alternate TLS groups. TLS refinement significantly improves the overall R_{free} (23.1% pre-TLS).

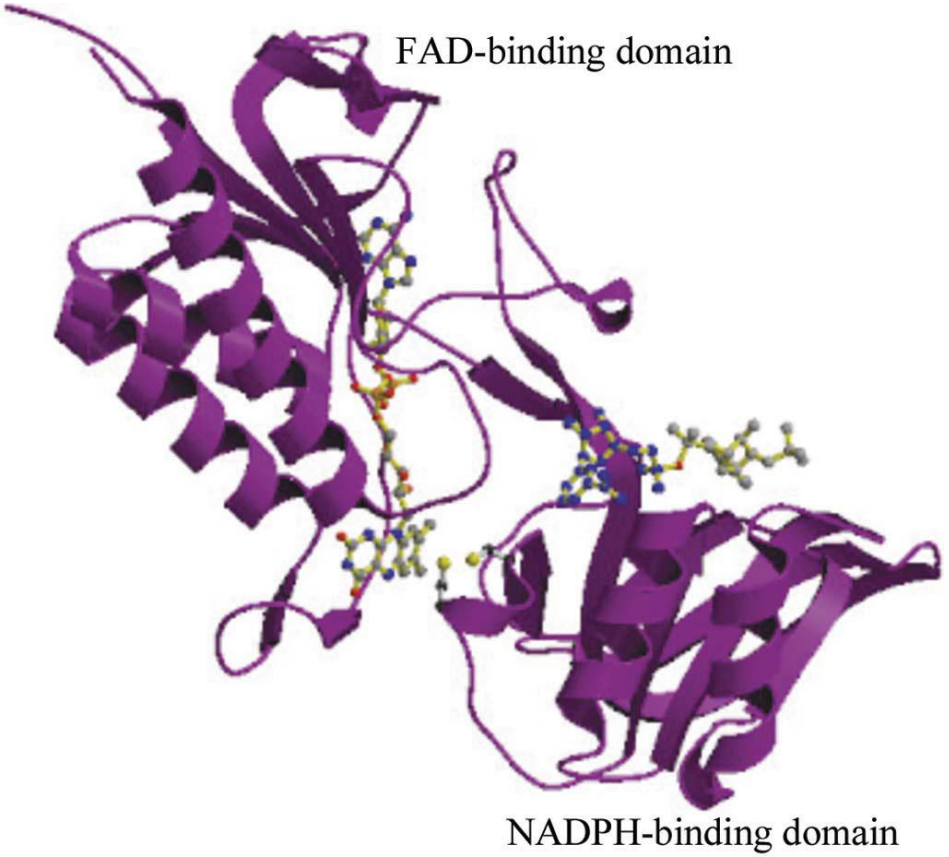
Predicting X-ray diffuse scattering from translation–libration–screw structural ensembles

Andrew H. Van Benschoten, Pavel V. Afonine, Thomas C. Terwilliger, Michael E. Wall, Colin J. Jackson, Nicholas K. Sauter, Paul D. Adams, Alexandre Urzhumtsev, and James S. Fraser

Acta Crystallogr D Biol Crystallogr. 2015 Aug 1; 71(Pt 8): 1657–1667.

Structure of *M. tuberculosis* Thioredoxin reductase

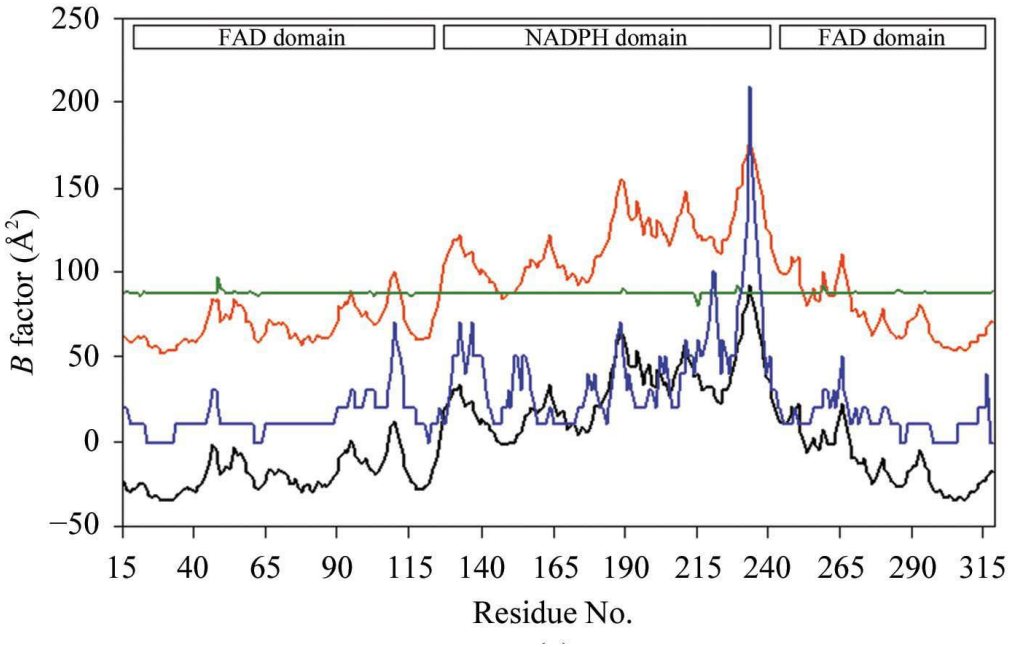




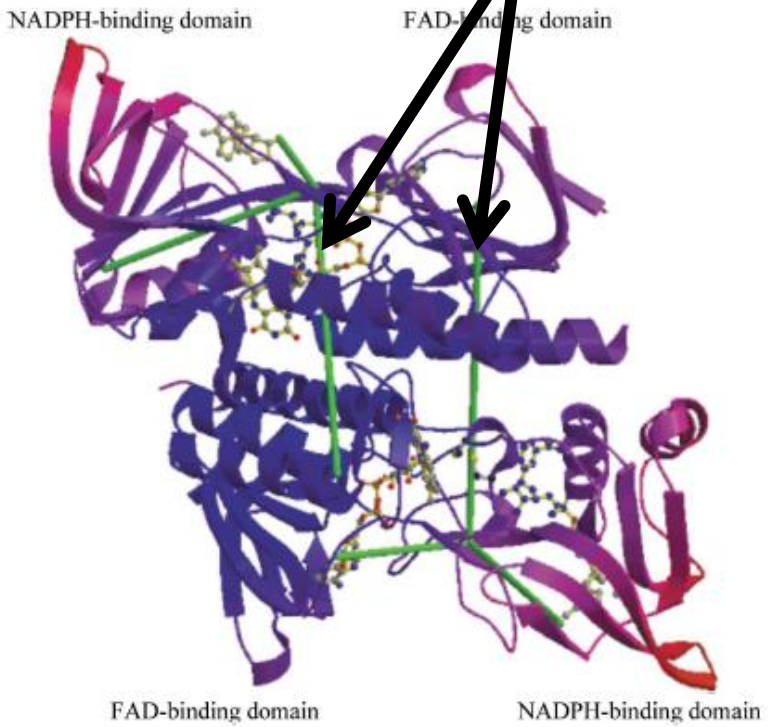
Akif *et al.*, *Acta Cryst D*, 2005

Analysis of B-factors

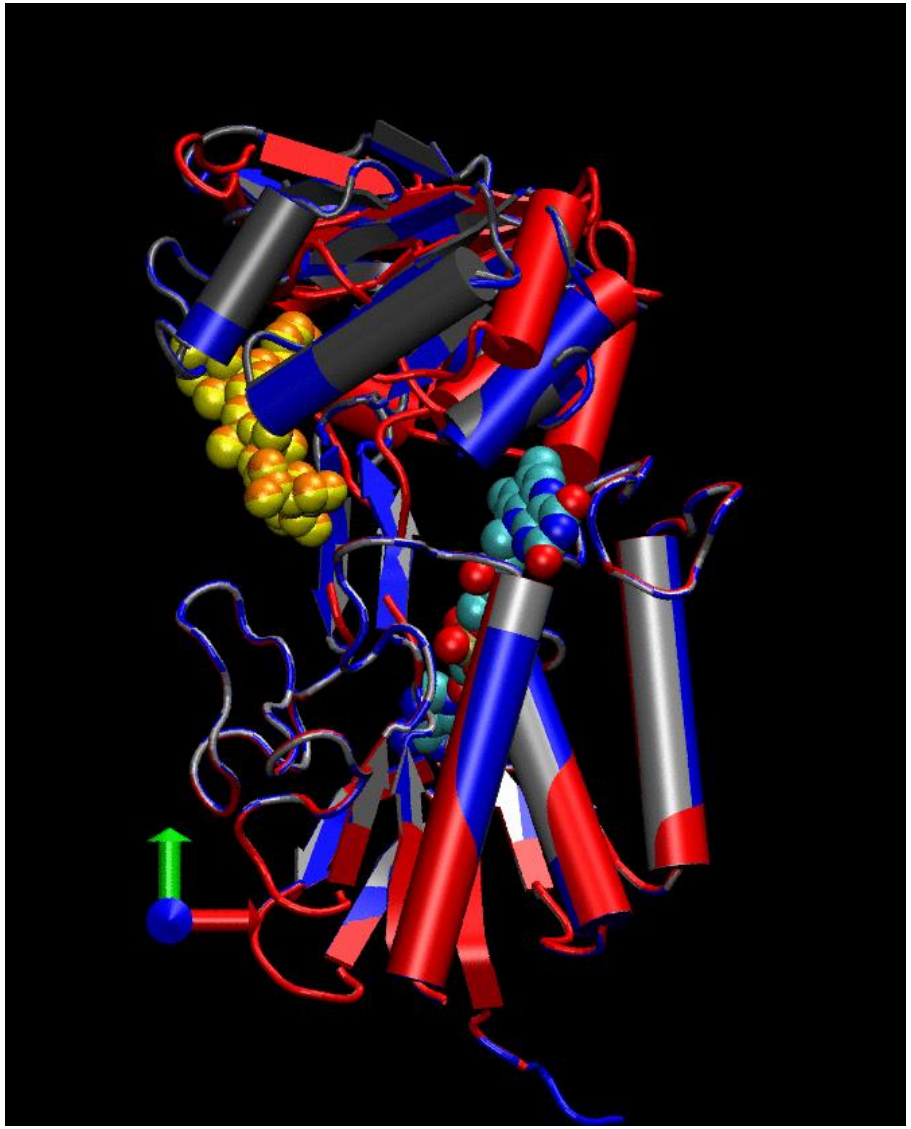
Static or dynamic disorder?



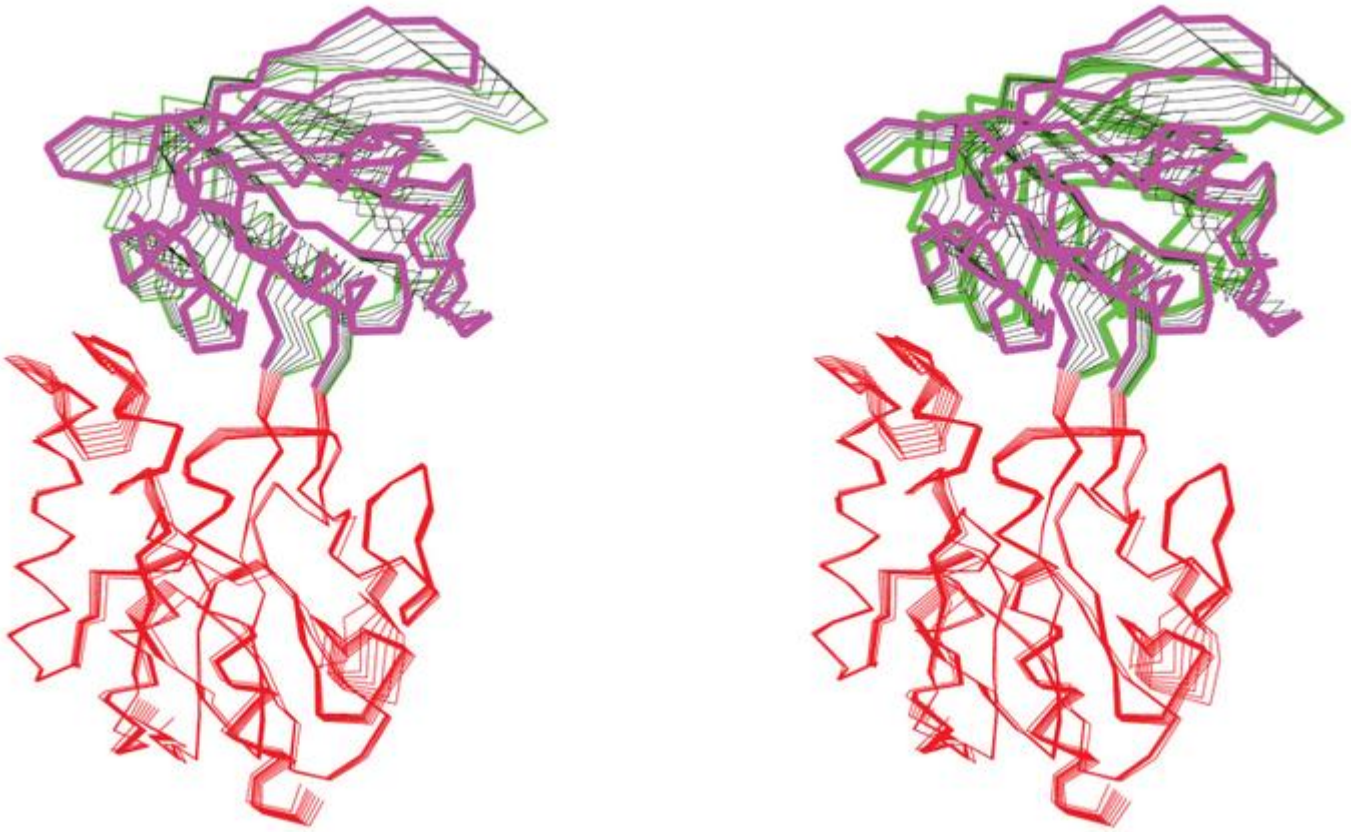
Principal axes of libration tensor



Flexibility of domains analyzed by NMA



Normal Mode 8 represents conformational change between F_O and F_R



Mechanism of RNR

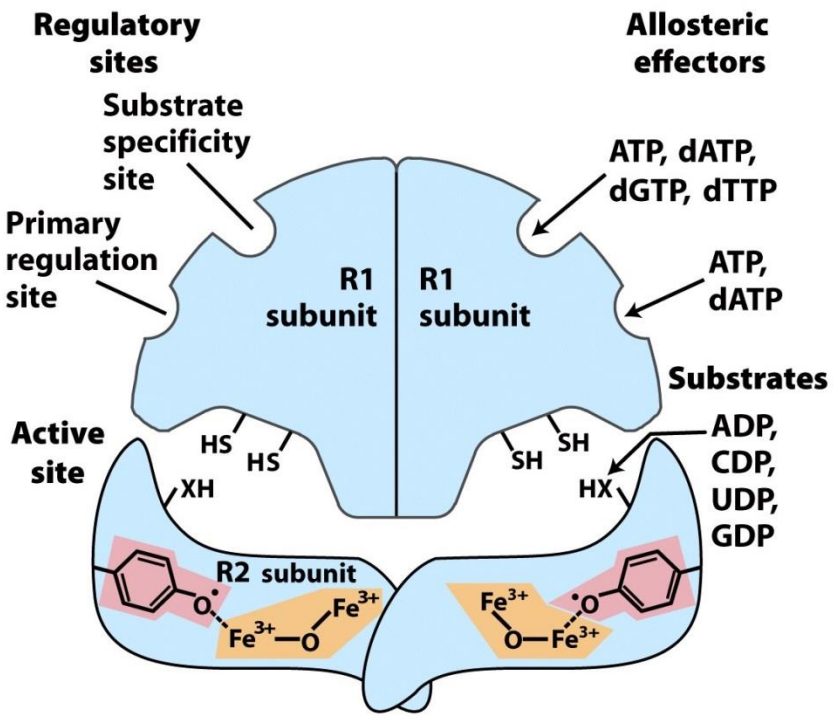


Figure 22-40a
Lehninger Principles of Biochemistry, Fifth Edition

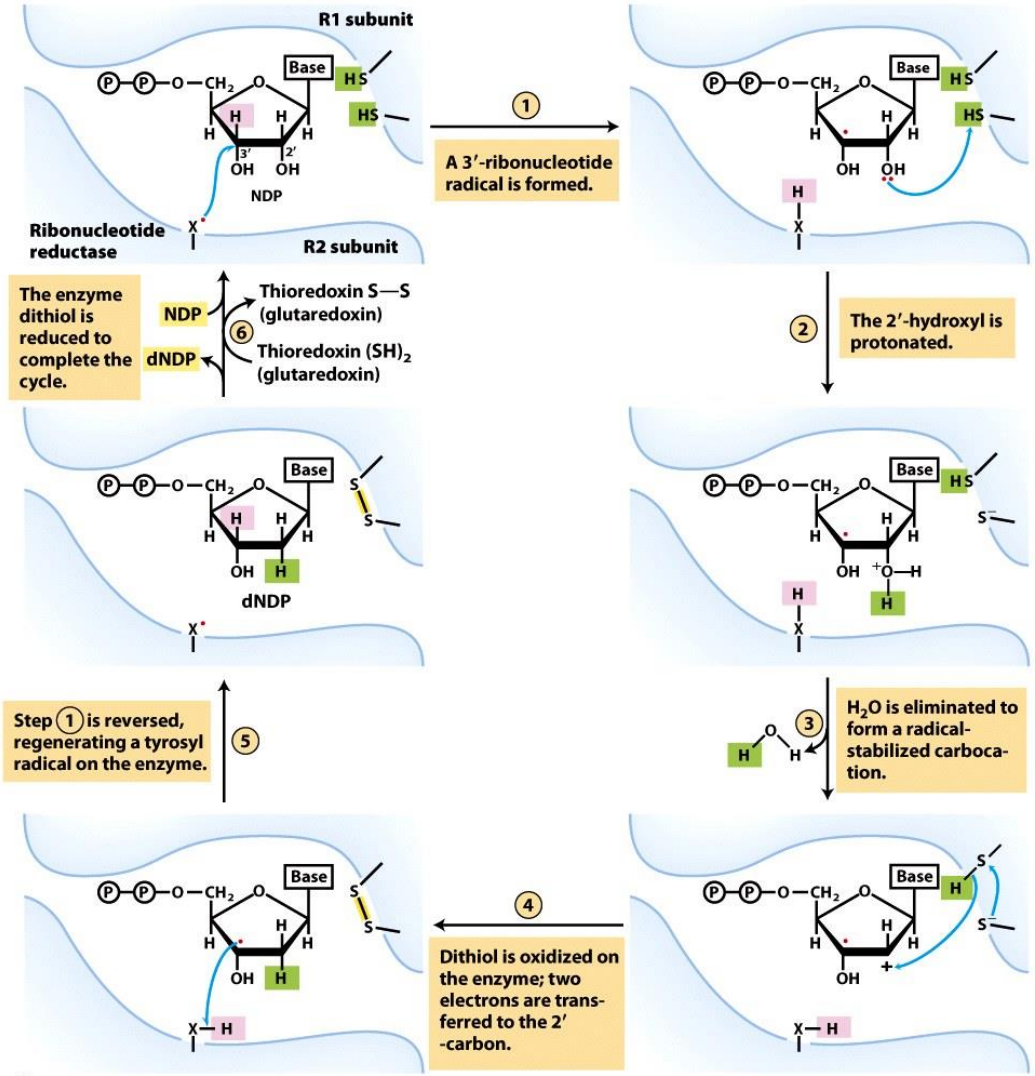


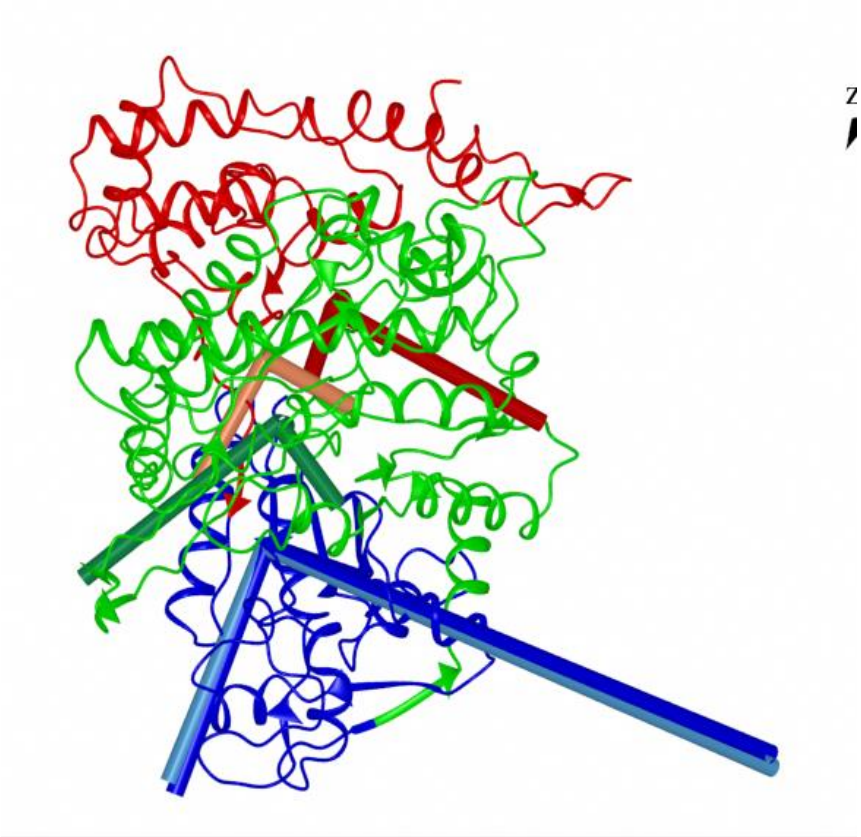
Figure 22-41
Lehninger Principles of Biochemistry, Fifth Edition

Ribonucleotide Reductase Protein R1E from *Salmonella typhimurium*

Data refinement statistics	1PEM_post_TLS	1PEM
Resolution range (A)	19.96 - 2.993 (3.1 - 2.993)	19.96 - 2.99 (3.1 - 2.993)
Space group	P 4_3 2_1 2	P 4_3 2_1 2
Unique reflections	29951 (2904)	29951 (2904)
Completeness (%)	99.86 (99.05)	99.86 (99.05)
Wilson B-factor	66.61	66.61
R-work	0.1588 (0.2347)	0.2180 (0.2763)
R-free	0.2052 (0.3064)	0.2430 (0.3696)
RMS(bonds)	0.007	0.015
RMS(angles)	1.08	1.89
Ramachandran outliers (%)	0.59	1.2
Clashscore	4.45	16.24
Average B-factor	64.60	57.50

Structure of the large subunit of class Ib ribonucleotide reductase from Salmonella typhimurium and its complexes with allosteric effectors.
Uppsten, M., Farnegardh, M., Jordan, A., Eliasson, R., Eklund, H., Uhlin, U. (2003) J.Mol.Biol. **330**: 87-97

Ribonucleotide Reductase Protein R1E from *Salmonella typhimurium*



The eigen values of the Libration tensor represent the magnitude of the rotation of the rigid body around the orthogonal axes

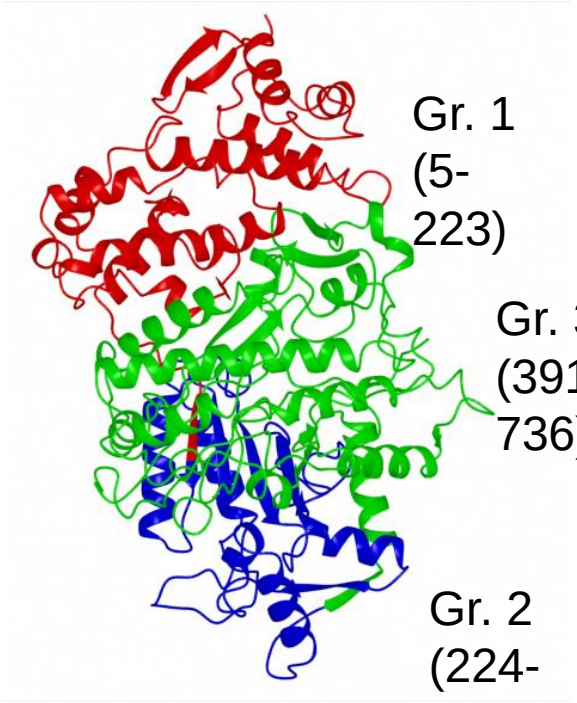
Starting structures used for TLS refinement

Class	PDB ID	Organism	Oligomeric status	Resolution (Å)	Space Group	*Dimer Interface Surface Area (Å^2)	**Surface Area (Å^2) >500 Å^2	Ligands
Ia	1R1R	*E. coli*	A6B6	3	H 3 2	1185.1	853.1, 532.4	None
	2R1R	*E. coli*	A2	3		1324.2	634.2, 501.1	dTTP
	3R1R	*E. coli*	A2	3		1428.4	628.1, 539.7	AMP-PNP
	4R1R	*E. coli*	A2	3.2		1366.5	872.9, 562.6	GDP, dTTP
	5CNS	*E. coli*	A4B4	2.98	C 1 2 1	1596.7	-	dATP, CDP
Ib	1PEM	*S. typhimurium*	E	2.99	P 4_3 2_1 2	1407	858.3	None
	1PEO	*S. typhimurium*	E	3		1407.7	885.1	dCTP
	1PEQ	*S. typhimurium*	E	2.8		1216.4	1189.5	TTP
	1PEU	*S.typhimurium*	E	3.2		1481.3	1181.5	dATP
	2BQ1	*S. typhimurium*	E2F2	4	P 4_1 3 2	1166.7	779.5	None

*Biologically relevant dimer surface area

**Crystal contacts that do not make the dimer. Values >500 Å^2 have been shown here

TLS groups

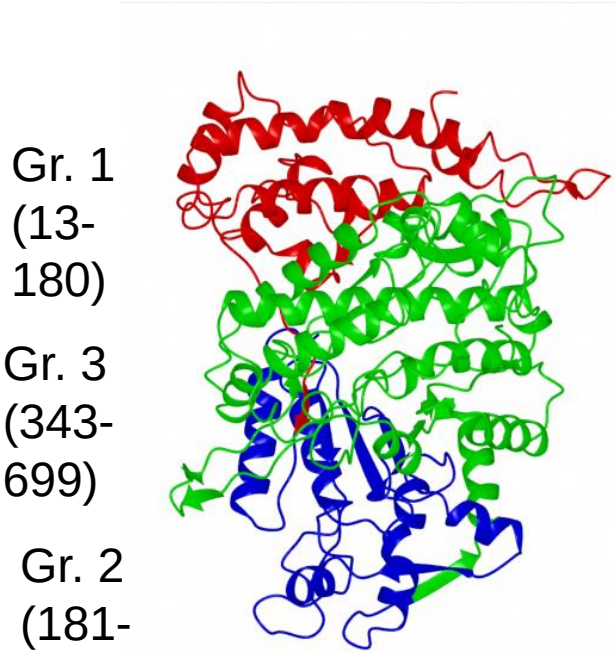


Gr. 1 (5-223)

Gr. 3 (391-736)

Gr. 2 (224-390)

4R1R
Class Ia



Gr. 1 (13-180)

Gr. 3 (343-699)

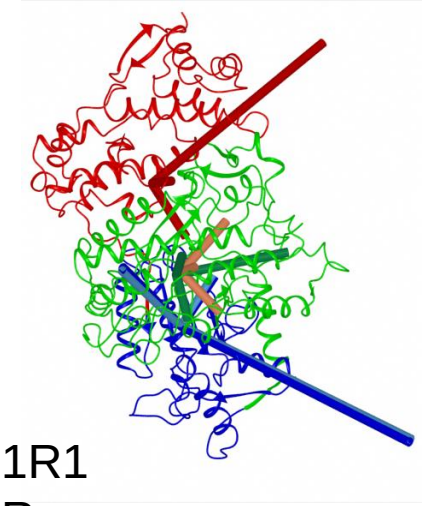
Gr. 2 (181-342)

1PEM
Class Ib

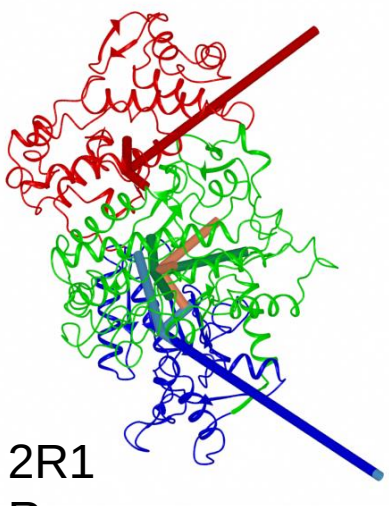
R/R_{free} after TLS refinement of large subunit of Class Ia and Ib RNR structures when partitioned into 3 groups and after merging groups 1 & 3.

PDB ID	TLS groups	Resolution range	R/ R_{free}	3 groups	2 groups (gr. 1,3 merged)
			Initial	Final	Final
1R1R	1 (4-227) , 2 (228-390) 3 (391-737)	20.0-2.9	0.26/0.27	0.14/0.19	0.14/0.19
2R1R	1 (5-223), 2 (224-390) 3 (391-736)	20.0-3.02	0.26/0.26	0.20/0.24	0.20/0.24
3R1R		39.7-2.99	0.28/0.30	0.18/0.25	0.18/0.25
4R1R		64.67-3.01	0.30/0.32	0.19/0.25	0.19/0.25
5CNS		143.9-2.97	0.24/0.25	0.16/0.22	0.16/0.22
1PEM	1 (13-180), 2 (181-342) 3 (343-699)	19.96-2.99	0.24/0.27	0.16/0.22	0.16/0.22
1PEO		39.69-3.0	0.24/0.27	0.17/ 0.22	0.17/0.22
1PEQ		39.75-2.8	0.25/0.28	0.18/0.23	0.18/0.23
1PEU		42.36-3.2	0.24/0.28	0.16/0.22	0.16/0.22
2BQ1		39.86-3.99	0.27/0.32	0.22/0.29	0.22/0.29

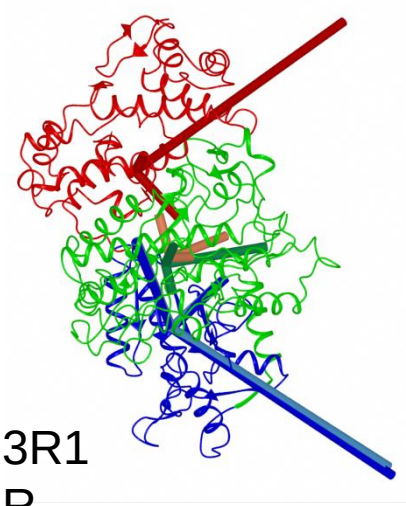
Class Ia



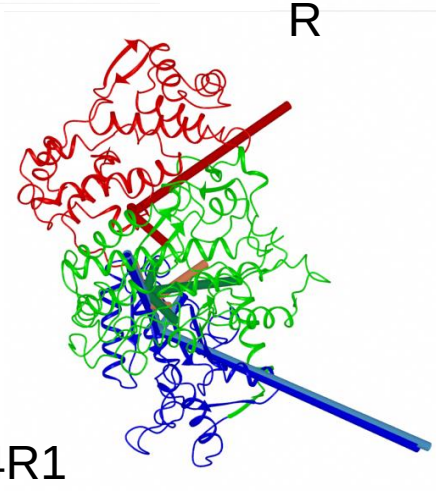
1R1R



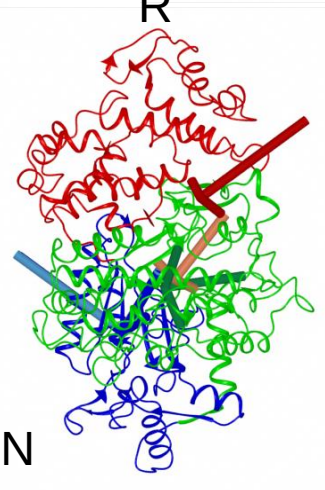
2R1R



3R1R

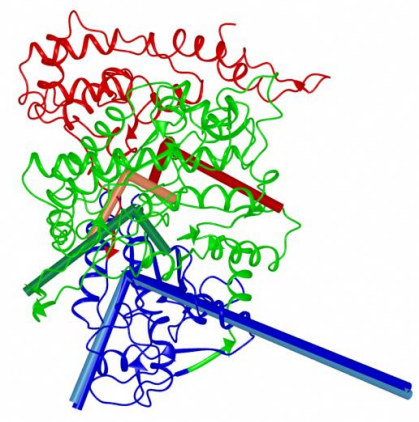


4R1R

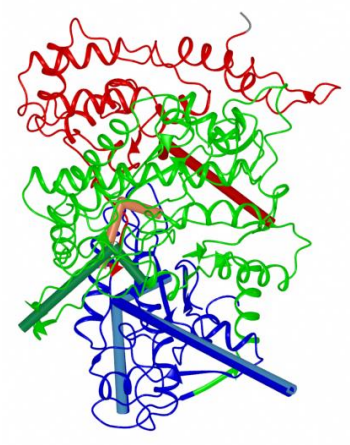


5CNS

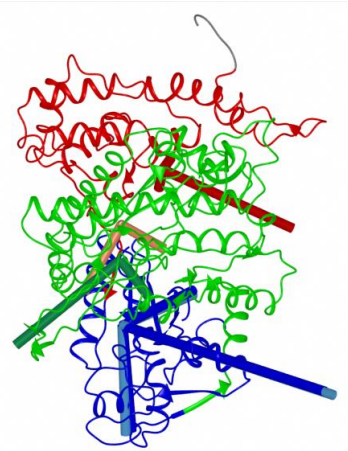
Class Ib



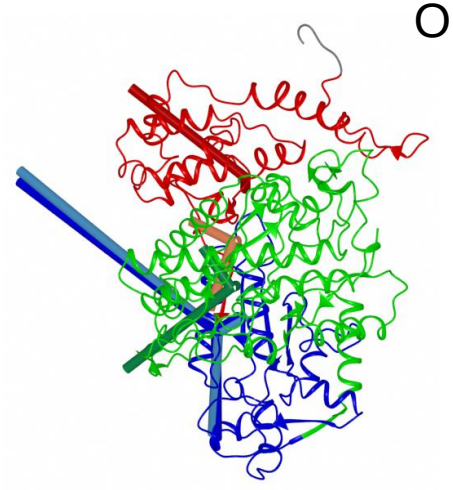
1PEM



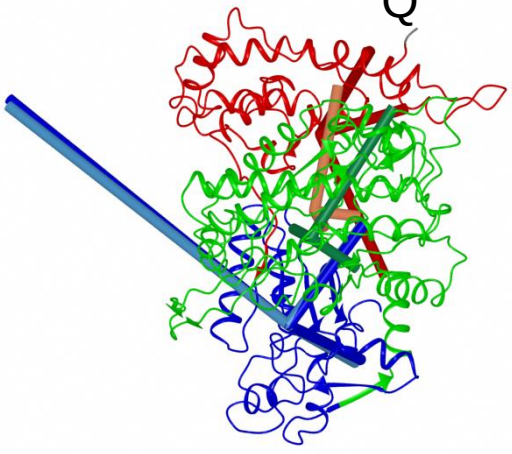
1PEO



1PEQ

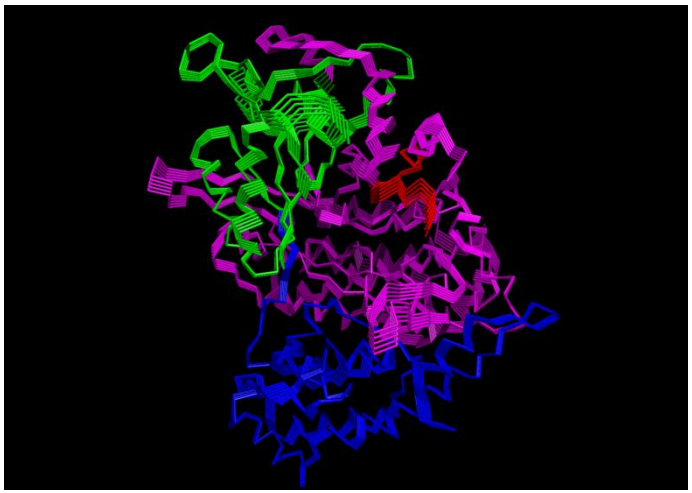


1PEU

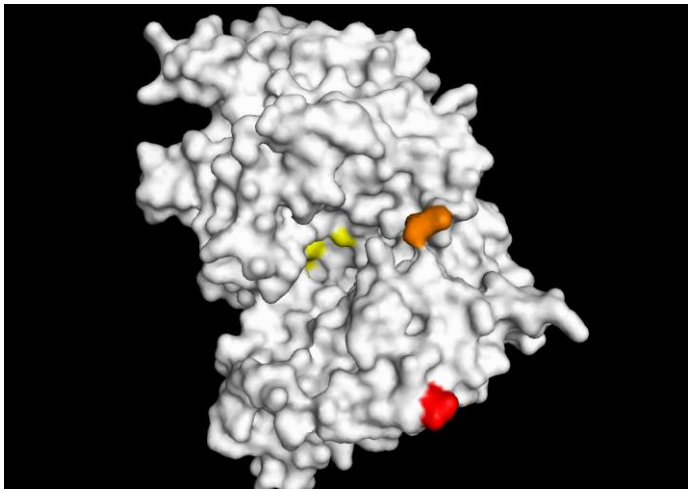


2BQ1

TLS refinement



Normal modes



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