Hands-on Workshop on Computational Biophysics

by

The Theoretical and Computational Biophysics Group (TCBG)

and

The National Center for Multiscale Modeling of Biological Systems (MMBioS)

Workshop Program

Thu, May 22: Collective Dynamics of Proteins Using Elastic Network Models - *Iv*

Bahar, Tim Lezon and Chakra Chennubhotla

Fri, May 23: Druggability Simulations, and Analyzing Sequence Patterns and Structural Dynamics - *Ivet Bahar , Chakra Chennubhotla, Indira Shrivastava*

Workshop Program

Thu, May 22: Collective Dynamics of Proteins Using Elastic Network Models - *Bahar, Tim Lezon and Chakra Chennubhotla*

~~**Fri, May 23:** Druggability Simulations, and Analyzing Sequence Patterns~~ and Structural Dynamics - *Ivet Bahar , Chakra Chennubhotla, Indira Shrivastava*

9:45 – 10:15 am: Applications and Comparison with Ensembles of Experimental Structures [2] *Ivet Bahar*

Coffee Break

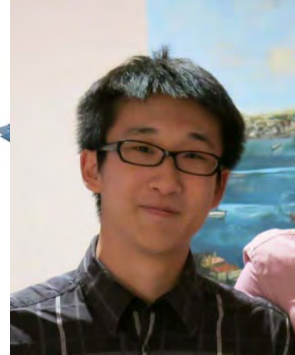**10:30 –12:00pm** ProDy Overview and Applications [3] *Tim Lezon, Chakra Chennubhotla*

1. **Bahar, Lezon, Yang & Eyal (2010)** *Annu Rev Biophys* **39**: 23-42;
2. **Bakan & Bahar** (2009) *Proc Natl Acad Sci* **106,** 14349-54; 3. **Bakan** et al. (2011) *Bioinformatics* **27**:1575-77.

ProDy
Protein Dynamics Analysis in Python

Wenzhi Mao

Dr. Indira Shrivastava
Assist Prof, DCSB, Pitt

Dr. Ying Liu

Dr. Timothy R Lezon
Assistant Prof, DCSB, Pitt

Drs. Ahmet Bakan and Anindita Dutta

Dr. Chakra Chennubhotla
Assist Prof, DCSB, Pitt

Reference:

Bakan A, Meireles LM, **Bahar I**. (2011) ProDy: Protein dynamics inferred from theory and experiments *Bioinformatics* **27**:1575-7
Bakan, A., Dutta, A., Whenzi, M., Liu, Y., Chennubhotla, C., Lezon, T.R., & Bahar, I. (2014) *Bioinformatics* in press.

ProDy for exploring conformational space

Protein Dynamics Analysis in Python

User inputs a protein sequence

>1A9U:A|PDBID|CHAIN
GSSHHHHHHSSGLVPRGSHMSQER
PTFYRQELNKTIWEVPERYQNLSPV
GSGAYGSVCAAFDTKTGLRVAVKK
LSRPFQSIIHAKRTYRELRLLKHMKH
ENVIGLLDVFT......

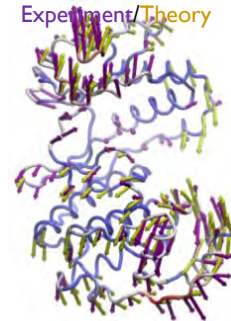1A06 1A9U 1AD5
1BKX 1BL6 1BLZ
1CM8 1CMK 1CSN

ProDy *identifies*, *retrieves*, *aligns,* and *analyzes (PCA)* structures that match the input sequence
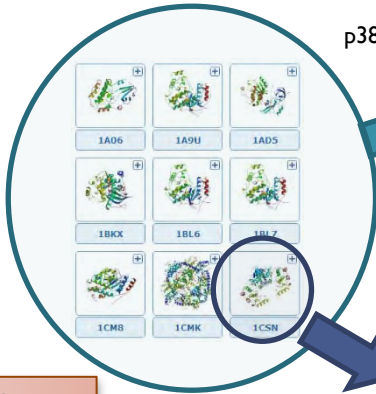
p38 ensemble (PCA)
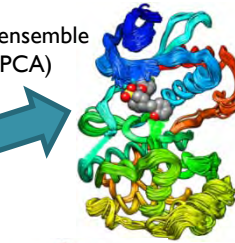
p38 network model (ANM)
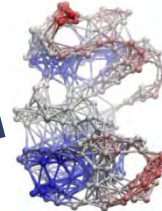
Experiment/Theory
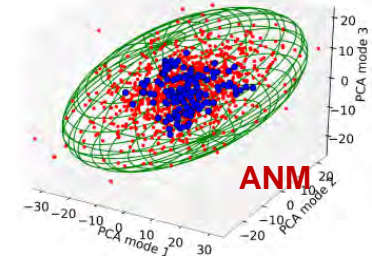
User can compare experimental and theoretical models

Overlap table
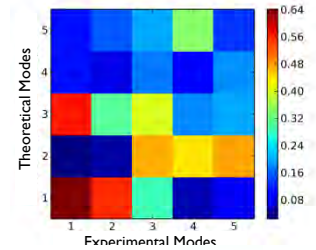
Theoretical Modes / Experimental Modes

ANM

A Sim2

MD

PCA mode 1 / PCA mode 2 / PCA mode 3

Growth of Source Code and Usage

	Releases	Downloads	Visits[2]	Unique[3]
Nov '10 - Oct '11	19	8,530	8,678	2,946
Nov '11 - Oct '12	15	35,108	16,472	6,414
Nov '12 - Oct '13	8*	87,909	19,888	8,145
Total	42	131,547	45,038	17,505

2011   2012

130,000+ downloads

Source http://www.google.com/analytics/

Tutorials

Days 1-3

http://www.ks.uiuc.edu/Training/Tutorials/

NAMD

VMD

Days 4-5

http://www.csb.pitt.edu/prody/#tutorials

ProDy

NMWiz
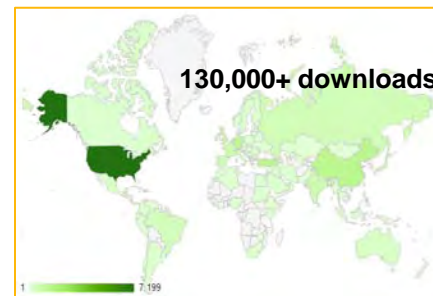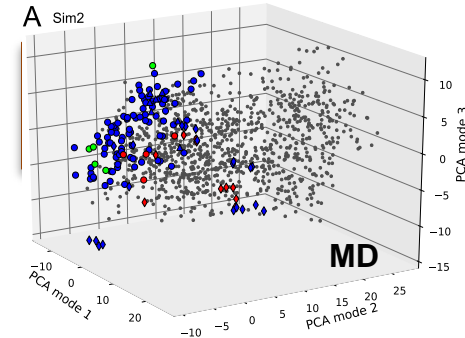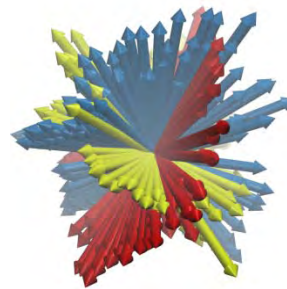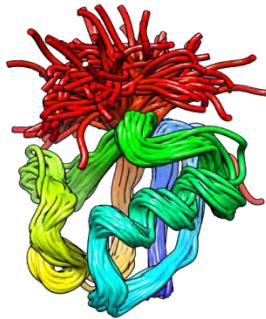
Biomedical Technology Research Center (BTRC)

High Performance Computing

for

Multiscale Modeling of Biological Systems

Overarching biological theme:
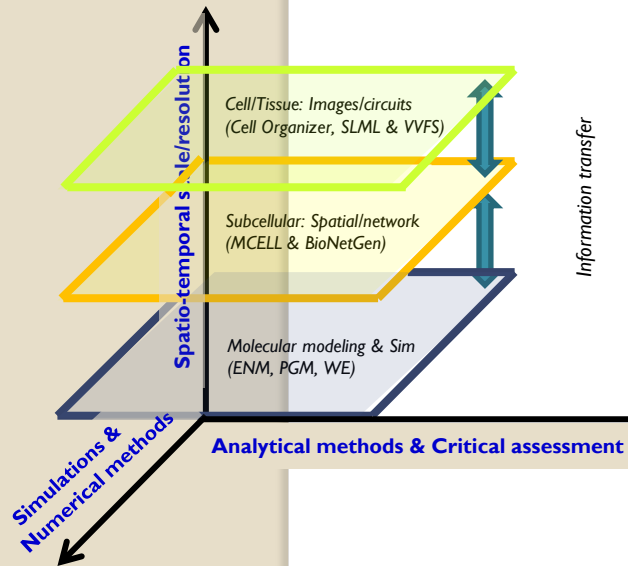
- Spatial organization
- Temporal evolution

of (neuro)signaling systems/events

Salk

PSC

PITT

CMU

GOAL: TO GENERATE DATA FOR MESOSCOPIC SCALE

Microphysiological simulations

Developing integrated methodology to enable information transfer across scales

to subcellar events

Spatio-temporal scale/resolution

Cell/Tissue: Images/circuits
(Cell Organizer, SLML & VVFS)

Subcellular: Spatial/network
(MCELL & BioNetGen)

Molecular modeling & Sim
(ENM, PGM, WE)

Information transfer

Simulations & Numerical methods

Analytical methods & Critical assessment

from molecules

13nm

Axon in Neuropil Reconstruction
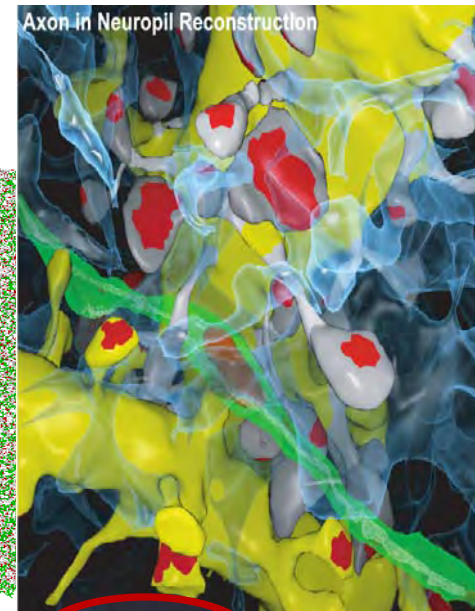
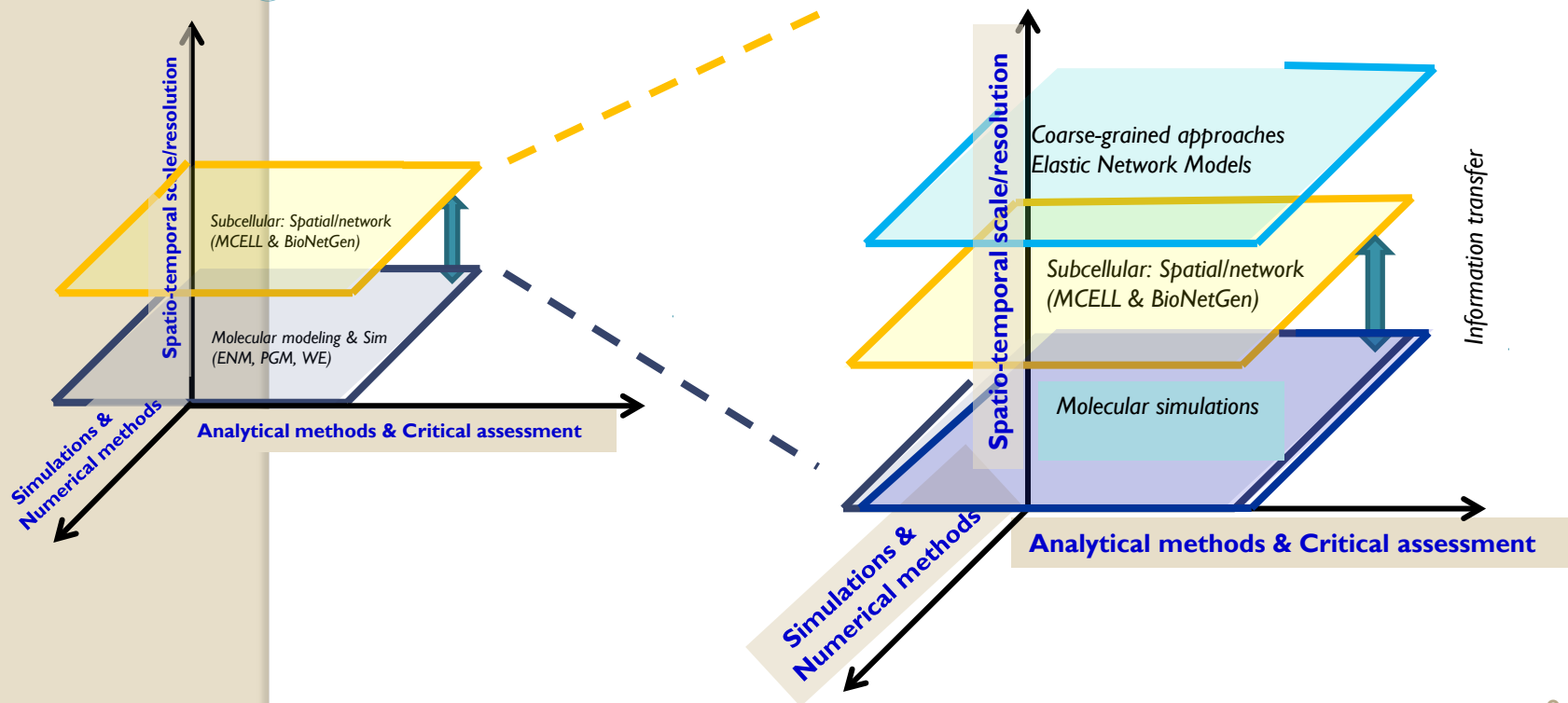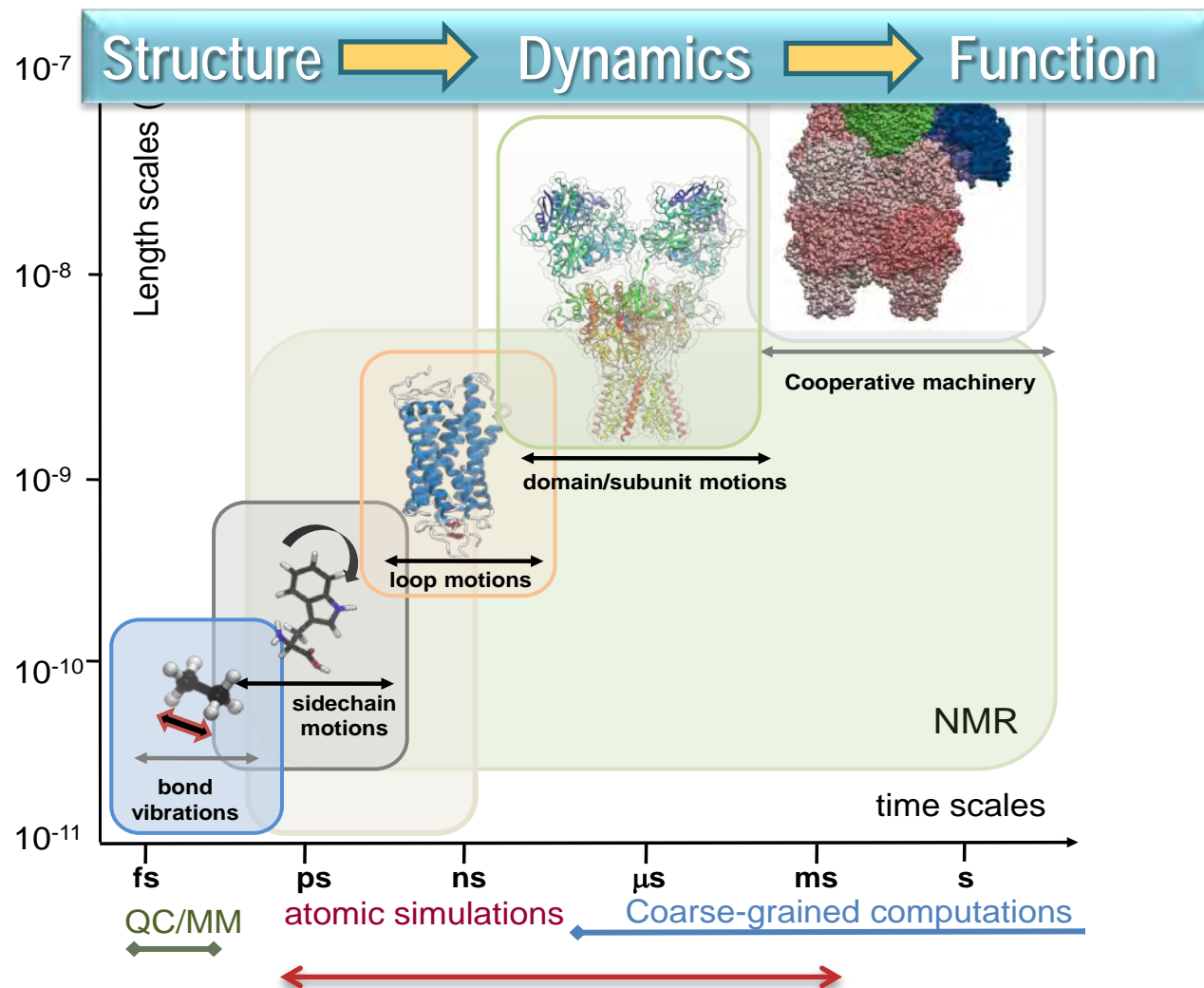from 6 x 6 x 5 μm³ sample of adult rat hippocampal stratum radiatum neuropil

Goal: to generate data for mesoscopic scale

Developing integrated *methodology for complex systems dynamics, to enable information transfer across scales*
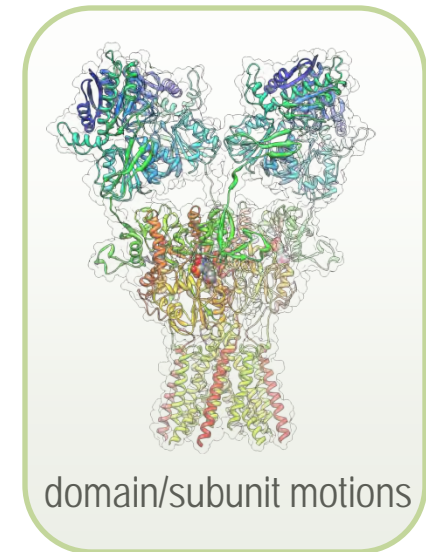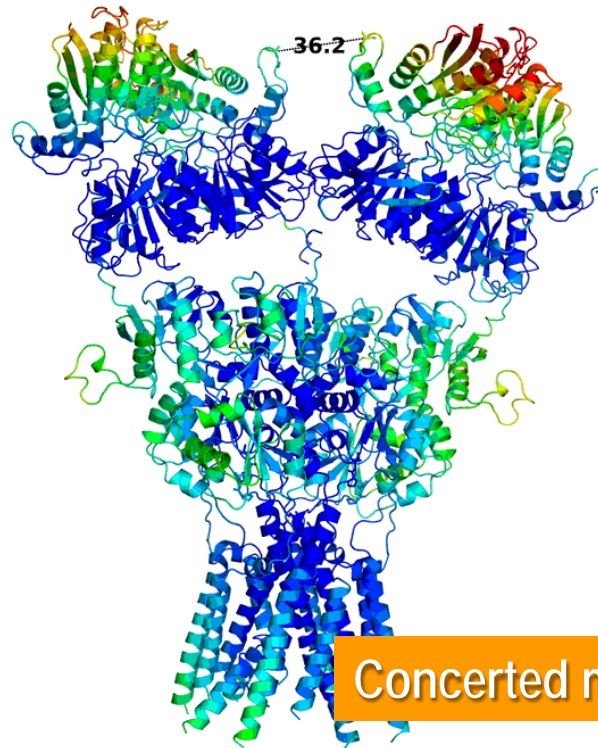
Each structure encodes a unique dynamics

Structure ➡ Dynamics ➡ Function

Length scales

- 10^{-7}
- 10^{-8}
- 10^{-9}
- 10^{-10}
- 10^{-11}

Cooperative machinery

domain/subunit motions

loop motions

sidechain motions

bond vibrations

NMR

time scales

fs   ps   ns   μs   ms   s

QC/MM

atomic simulations

Coarse-grained computations

Each structure encodes a unique dynamics

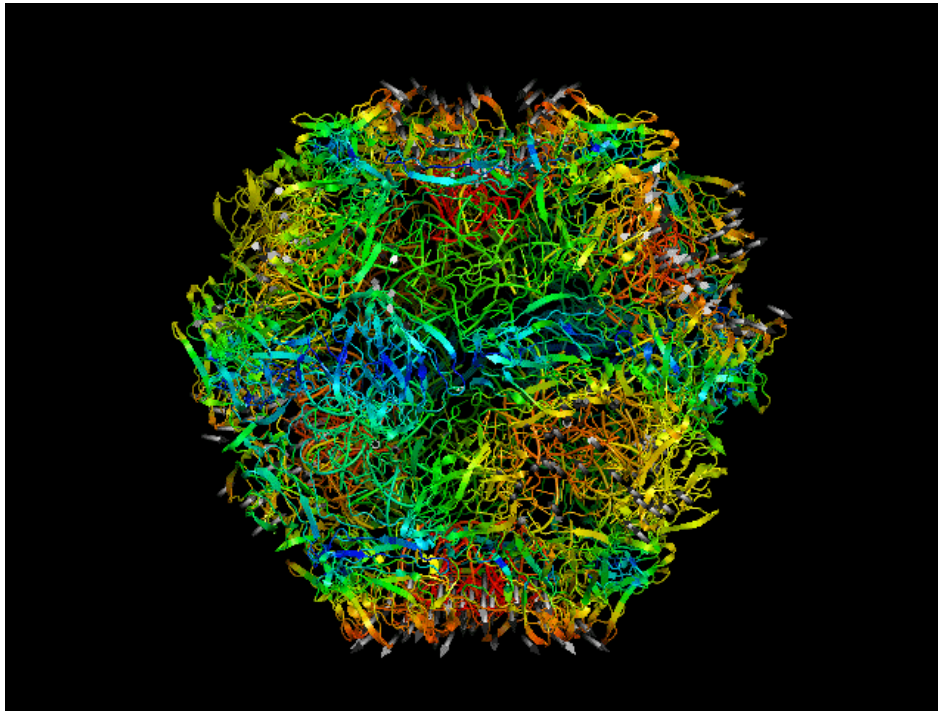Structure ➡ Dynamics ➡ Function

Signaling dynamics of AMPARs and NMDARs

36.2

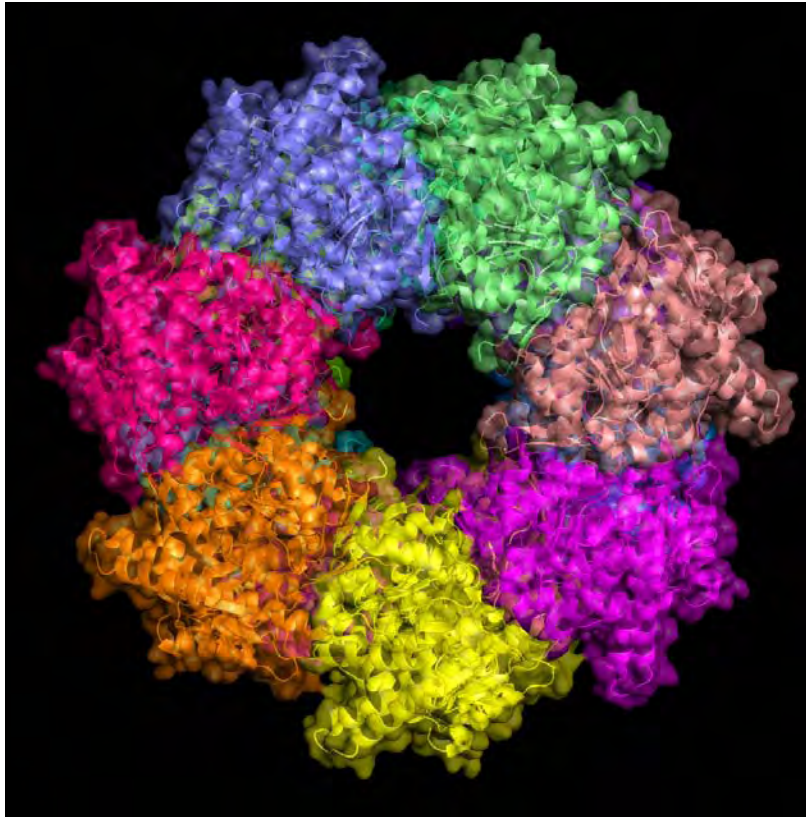domain/subunit motions

Concerted movements of signaling molecules

Many proteins are molecular machines

And mechanical properties become more important in complexes/assemblies
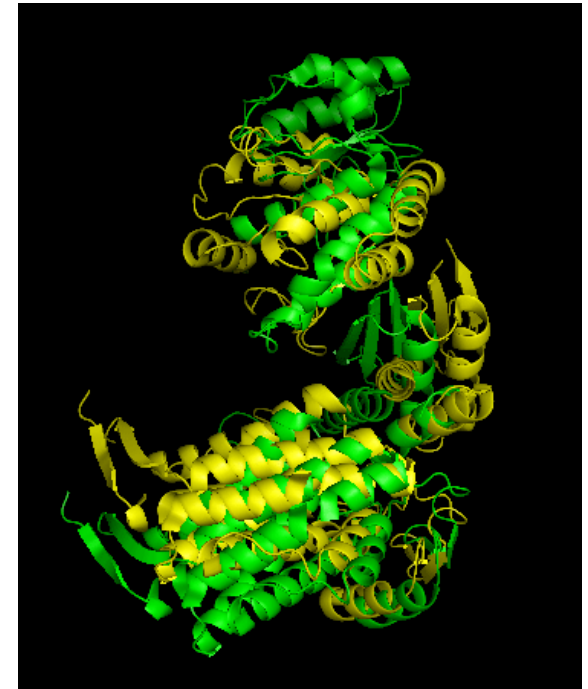
STMV dynamics (Zheng Yang)

Representation of structure as a network

Why network models?

- for large systems' collective motions & long time processes beyond the capability of full atomic simulations

- to incorporate structural data in the models – at multiple levels of resolution

- to take advantage of theories of polymer physics, spectral graph methods, etc.

Physics-based approach

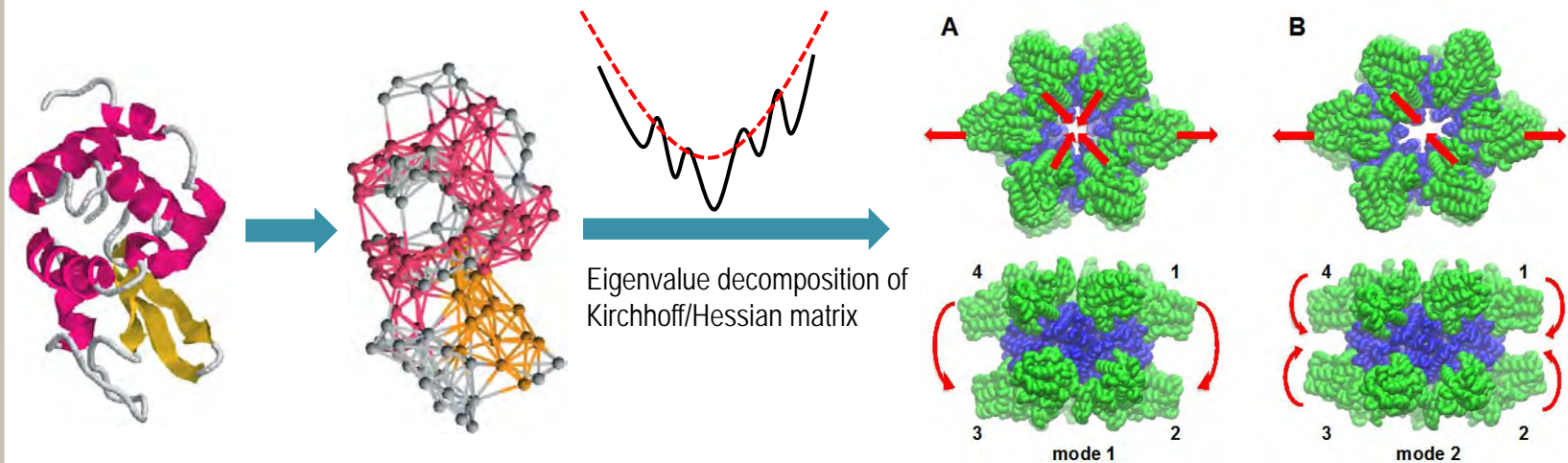- Statistical Mechanics of Polymers
- Theory of Rubber Elasticity

↓

Elastic Network Model for Proteins

Paul J. Flory (1910-1985)
Nobel Prize in Chemistry 1974

And Pearson (1976), Eichinger (1980), Klockzkowski, Erman & Mark (1989)…

Collective motions
using elastic network models (ENM)

Eigenvalue decomposition of Kirchhoff/Hessian matrix

A   B

4   1   4   1

3   2   3   2

mode 1   mode 2

GNM: Bahar et al *Fold & Des* 1996; Haliloglu et al. *Phys Rev Lett* 1997
ANM: Doruker et al. *Proteins* 2000; Atilgan et al, *Biophys J* 2001

Based on theory of elasticity for polymer networks by **Flory, 1976**

Gaussian network model (GNM)
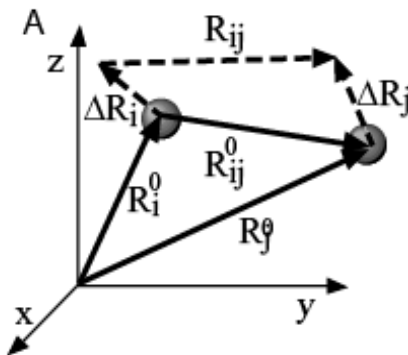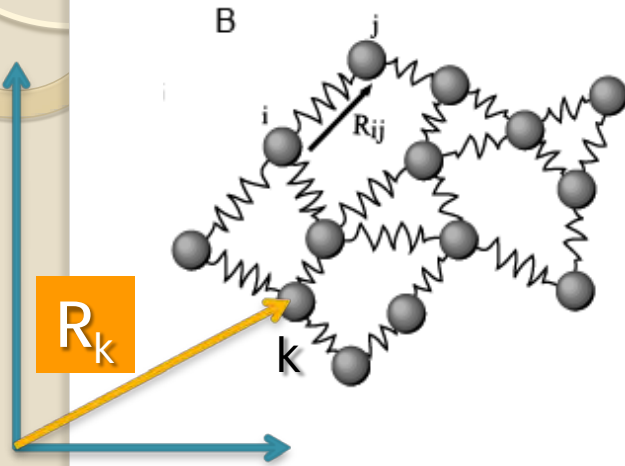
- Each node represents a residue

- Residue positions, R_i, identified by their α-carbons' coordinates

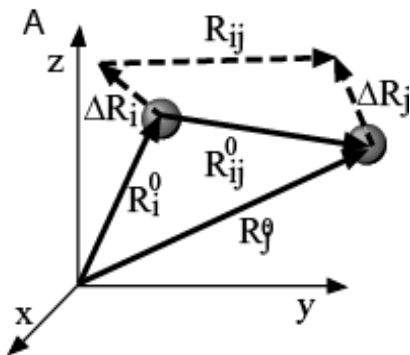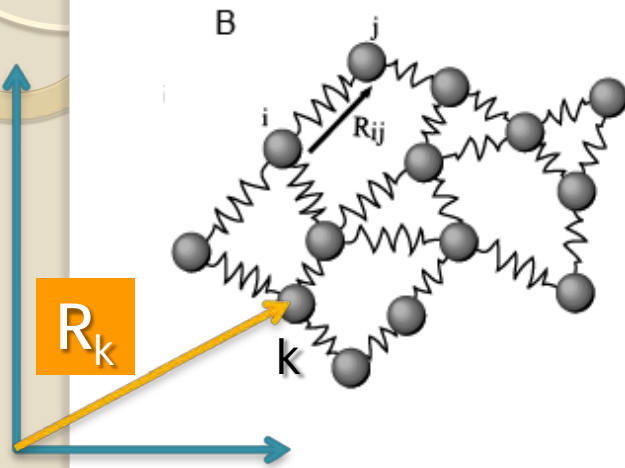- Springs connect residues located within a cutoff distance (e.g., 10 Å)

→ Nodes are subject to **Gaussian fluctuations** ΔR_i

→ Inter-residue distances R_{ij} also undergo Gaussian fluctuations

$$\rightarrow \quad \Delta R_{ij} = \Delta R_j - \Delta R_i$$

Fluctuations in residue positions
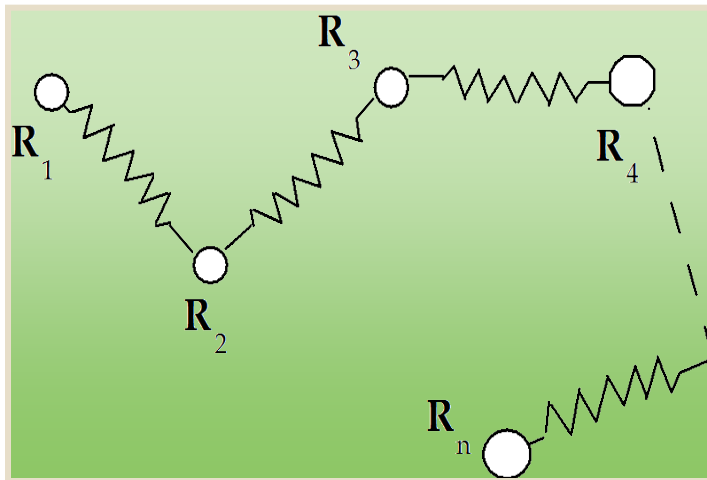
Gaussian network model (GNM)

Fluctuation vector:

$$\rightarrow \Delta \mathbf{R} = \begin{bmatrix} \Delta \mathbf{R}_1 \\ \Delta \mathbf{R}_2 \\ \Delta \mathbf{R}_3 \\ \Delta \mathbf{R}_4 \\ .. \\ .. \\ .. \\ .. \\ \Delta \mathbf{R}_N \end{bmatrix}$$

Fluctuations in residue positions

Bahar, Atilgan & Erman, *Fold & Des* 1997

Rouse model for polymers

Classical bead-and-spring model

Kirchhoff matrix

$$\Delta R_{int} = R_{12} - R_{12}^0$$

Force constant

$$\Gamma = \begin{bmatrix} 1 & -1 & & & & & \\ -1 & 2 & -1 & & & & \\ & -1 & 2 & -1 & & & \\ & & & .. & ... & & \\ & & & & -1 & 2 & -1 \\ & & & & & -1 & 1 \end{bmatrix}$$

$$V_{tot} = (\gamma/2) \left[(\Delta R_{12})^2 + (\Delta R_{23})^2 + ... (\Delta R_{N-1,N})^2 \right]$$

$$= (\gamma/2) \left[(\Delta R_2 - \Delta R_1)^2 + (\Delta R_3 - \Delta R_2)^2 + ... \right]$$

Rouse model for polymers

Kirchhoff matrix

$$\Gamma = \begin{bmatrix} 1 & -1 & & & & & \\ -1 & 2 & -1 & & & & \\ & -1 & 2 & -1 & & & \\ & & & \ddots & \ldots & & \\ & & & & -1 & 2 & -1 \\ & & & & & -1 & 1 \end{bmatrix}$$

Force constant

$$V_{tot} = (\gamma/2) \, [\, (\Delta R_{12})^2 + (\Delta R_{23})^2 + \ldots\ldots (\Delta R_{N-1,N})^2 \,]$$

$$= (\gamma/2) \, [\, (\Delta R_2 - \Delta R_1)^2 + (\Delta R_3 - \Delta R_2)^2 + \ldots\ldots$$

Rouse model for polymers

Fluctuation vector   Kirchhoff matrix

$(\gamma/2)\ [\Delta R_1\quad \Delta R_2\ \ \Delta R_3\ ...\ \ \Delta R_N]$
$\begin{bmatrix} 1 & -1 & & & & & \\ -1 & 2 & -1 & & & & \\ & -1 & 2 & -1 & & & \\ & & & & & & \\ & & .. & ... & & & \\ & & & -1 & 2 & -1 & \\ & & & & 1 & 1 & \end{bmatrix}$
$\begin{bmatrix} \Delta R_1 \\ \Delta R_2 \\ \Delta R_3 \\ \\ \end{bmatrix} =$
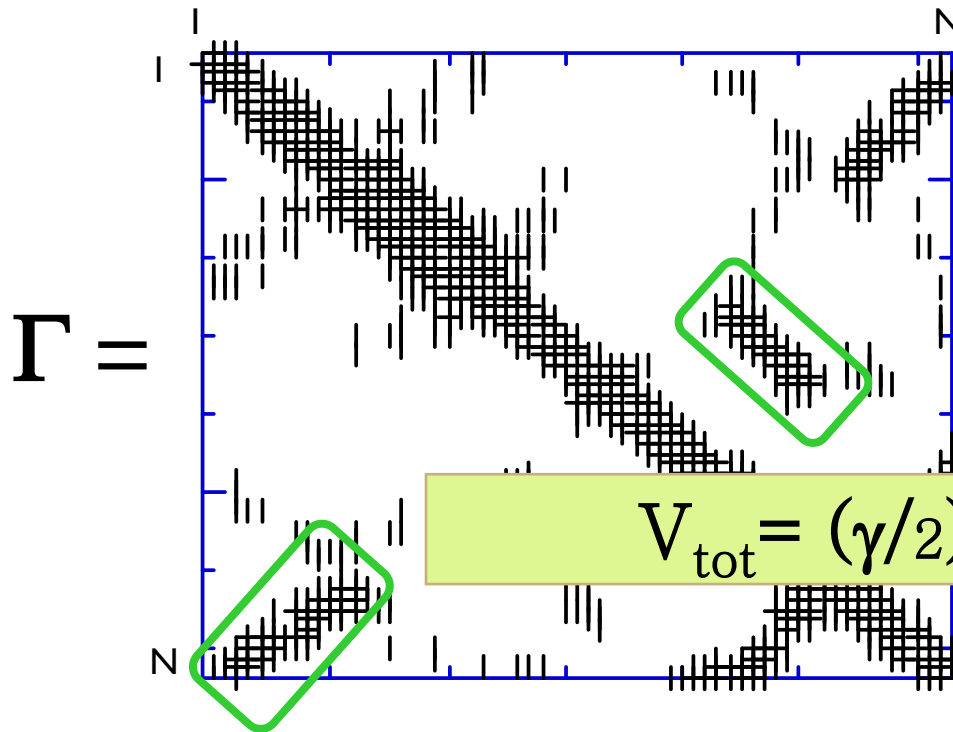
$$V_{tot} = (\gamma/2)\ \Delta R^T\ \Gamma\ \Delta R$$

Force constant

$V_{tot} = (\gamma/2)\ [\ (\Delta R_{12})^2\ +\ (\Delta R_{23})^2\ +\ ....\ (\Delta R_{N-1,N})^2\ ]$

$\qquad = (\gamma/2)\ [\ (\Delta R_2 - \Delta R_1)^2\ +\ (\Delta R_3 - \Delta R_2)^2\ +\ ....$

Kirchhoff matrix for inter-residue contacts

For a protein of N residues

$$\Gamma =$$

$$\Gamma_{ik}=\begin{cases} -1 & \text{if } r_{ik} < r_{cut} \\ 0 & \text{if } r_{ik} > r_{cut} \end{cases}$$

$$\Gamma_{ii} = - \sum_k \Gamma_{ik}$$

$$V_{tot}= (\gamma/2) \, \Delta R^T \, \Gamma \, \Delta R$$

Γ provides a complete description of contact topology!

Statistical mechanical averages

For a protein of N residues

$$<\Delta \boldsymbol{R}_i \cdot \Delta \boldsymbol{R}_j> \quad = (1/Z_N)\int (\Delta \boldsymbol{R}_i \cdot \Delta \boldsymbol{R}_j)\, e^{-V/k_B T} d\{\Delta \boldsymbol{R}\}$$

$$= (3\,k_B\,T/\gamma)\left[\boldsymbol{\Gamma}^{-1}\right]_{ij}$$

$\boldsymbol{\Gamma}$ provides a complete description of contact topology!

Kirchhoff matrix determines the mean-square fluctuations

$$[\mathbf{\Gamma}^{-1}]_{ii} \sim \langle (\Delta\mathbf{R}_i)^2 \rangle$$

And cross-correlations between residue motions

$$[\mathbf{\Gamma}^{-1}]_{ij} \sim \langle (\Delta\mathbf{R}_i . \Delta\mathbf{R}_j) \rangle$$

1. Application to hemoglobin
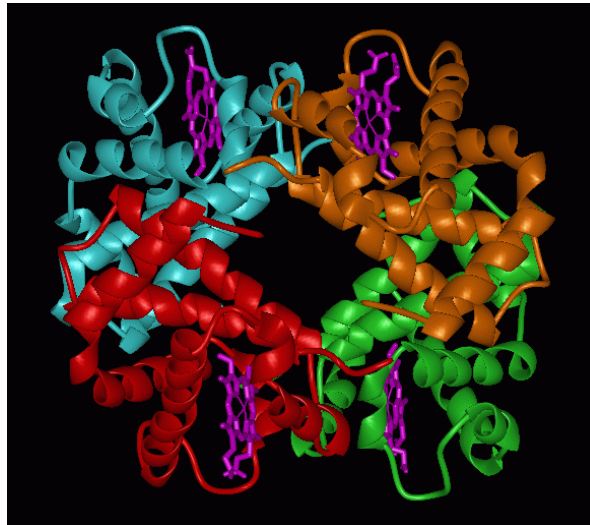
$$B_i = 8\pi^2/3 \langle(\Delta R_i)^2\rangle$$
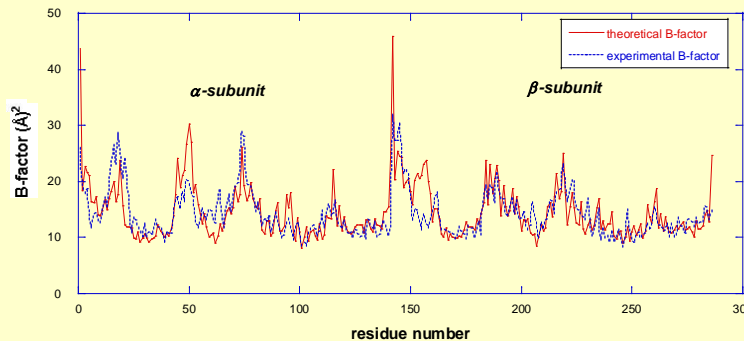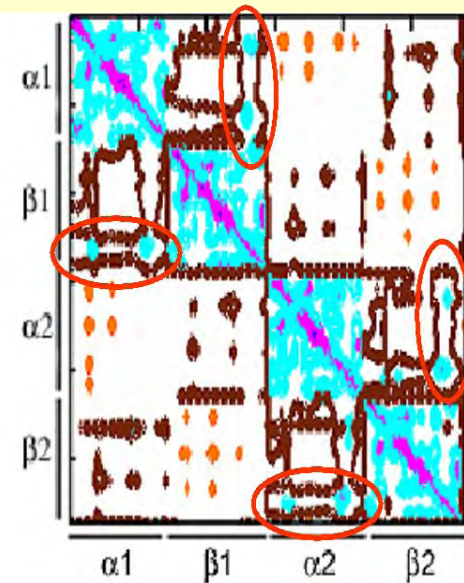
B- factors – Comparison with experiments

Intradimer cooperativity – Symmetry rule (Yuan et al. JMB 2002; Ackers et al. PNAS 2002.)

C. Xu, D. Tobi and I. Bahar (2003) *J. Mol. Biol.* 2003, 153-168

B-factors are affected by crystal contacts
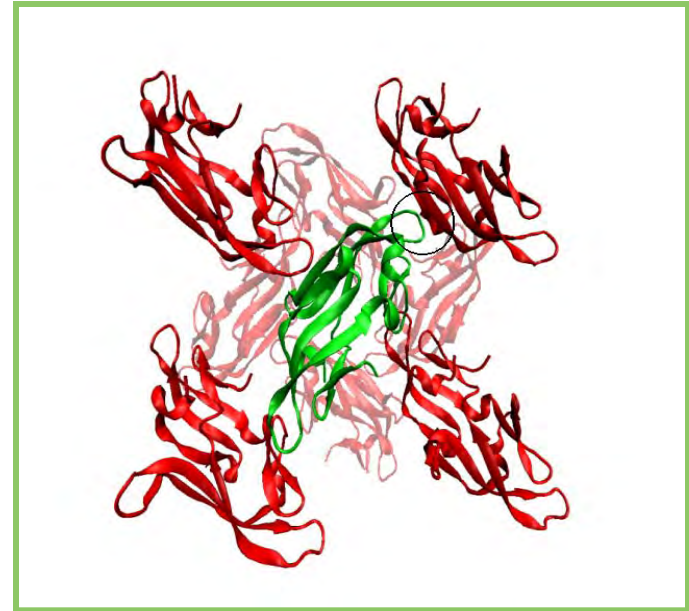
Two X-ray structures for a designed sugar-binding protein LKAMG
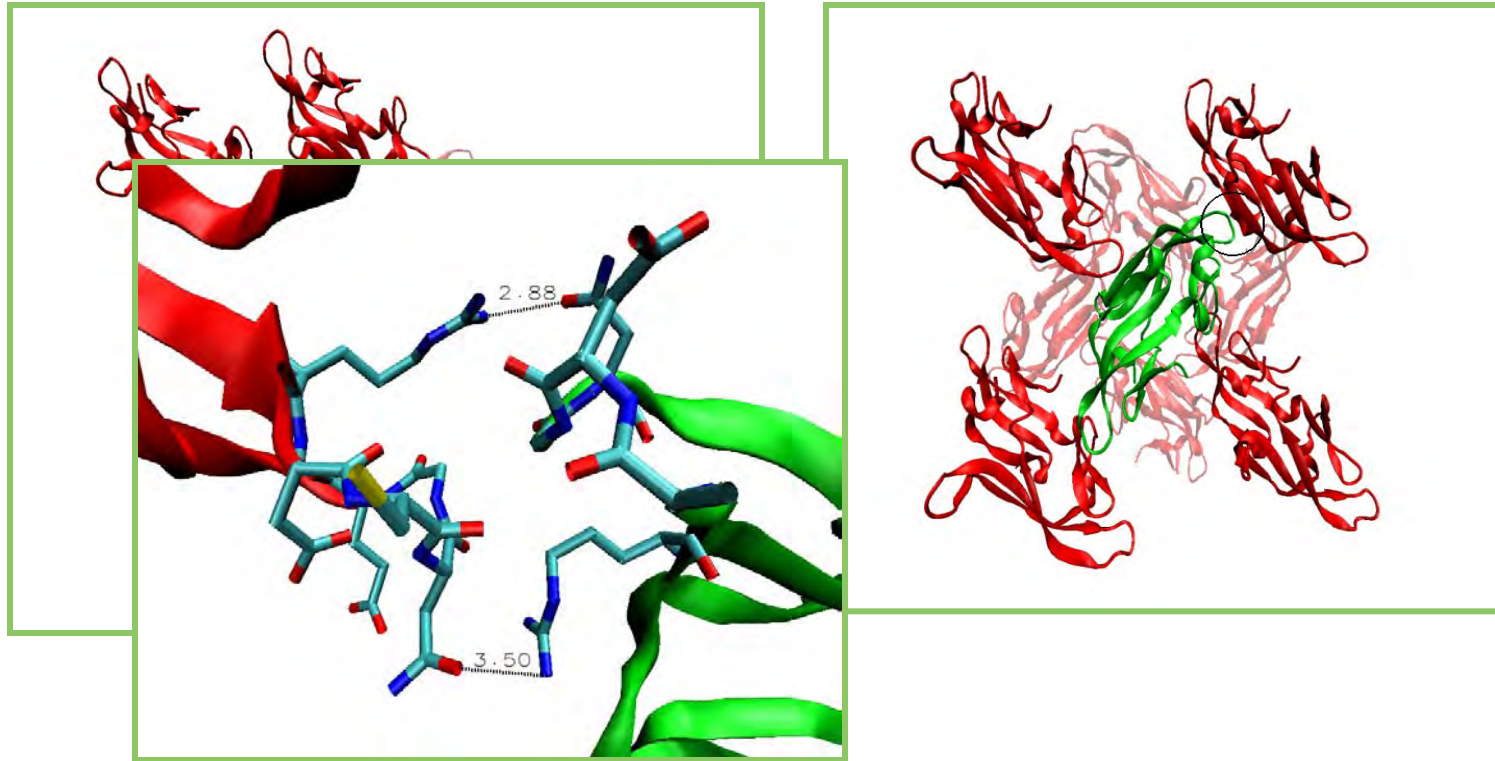
B-factors are affected by crystal contacts

Particular loop motions are curtailed by intermolecular contacts in the crystal environment causing a discrepancy between theory and experiments

Liu, Koharudin, Gronenborn & Bahar (2009) *Proteins 77, 927-939.*

Agreement between theory and experiments upon inclusion of crystal lattice effects into the GNM
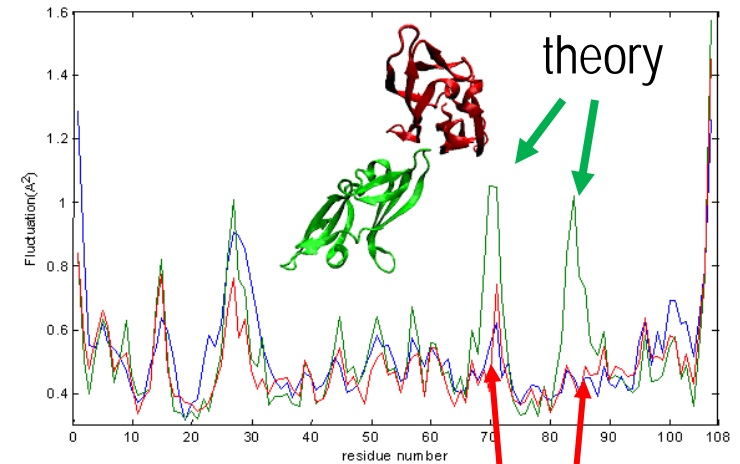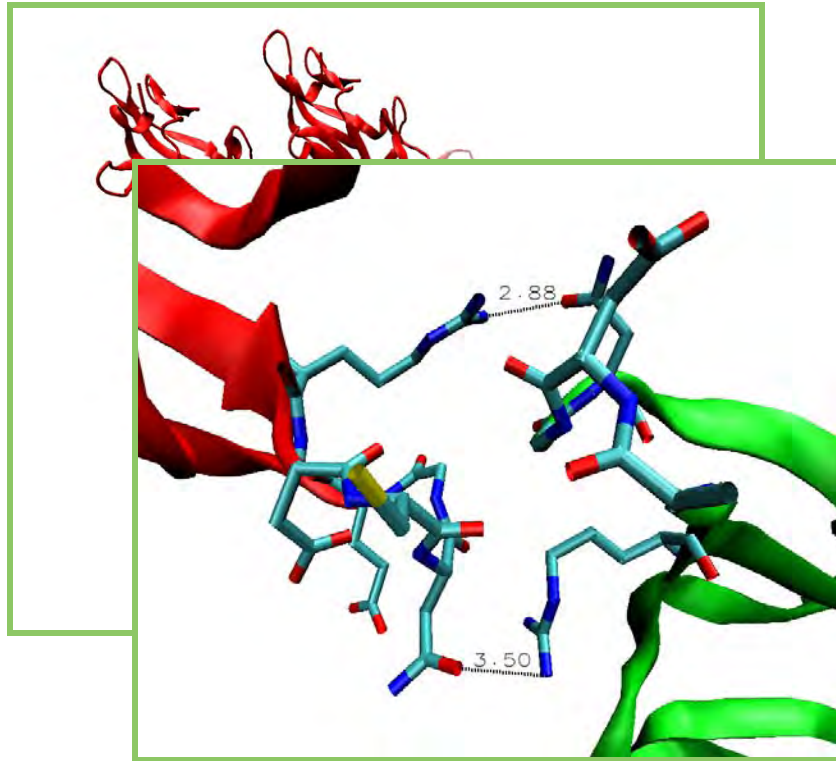
theory

Crystal contacts

Particular loop motions are curtailed by intermolecular contacts in the crystal environment causing a discrepancy between theory and experiments

Collective Motions Encoded by the Structure: Normal Modes

Several modes contribute to dynamics

Contribution of mode k

$$< \Delta \mathbf{R}_i \bullet \Delta \mathbf{R}_j > = \sum_k [\Delta \mathbf{R}_i \bullet \Delta \mathbf{R}_j]_k$$

$$< \Delta \mathbf{R}_i \bullet \Delta \mathbf{R}_j > = (3 k_B T / \gamma) \left[\mathbf{\Gamma}^{-1} \right]_{ij}$$
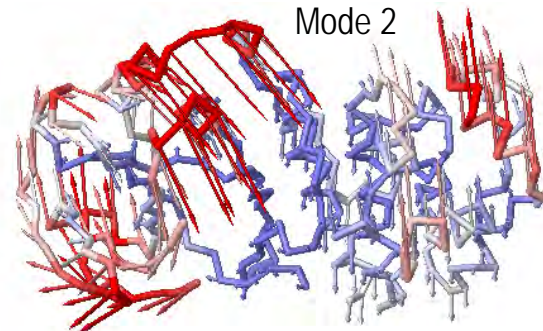
Contribution of mode k

$$[\Delta \mathbf{R}_i \bullet \Delta \mathbf{R}_j]_k = (3 k_B T / \gamma) \left[\lambda_k^{-1} \mathbf{u}_k \mathbf{u}_k^{T} \right]_{ij}$$

expressed in terms of kth eigenvalue λ_k and kth eigenvector $\mathbf{u}_k$ of Γ

FOR MORE INFO...

Bahar et al. (1998) Phys Rev Lett. 80, 2733

Several modes contribute to dynamics
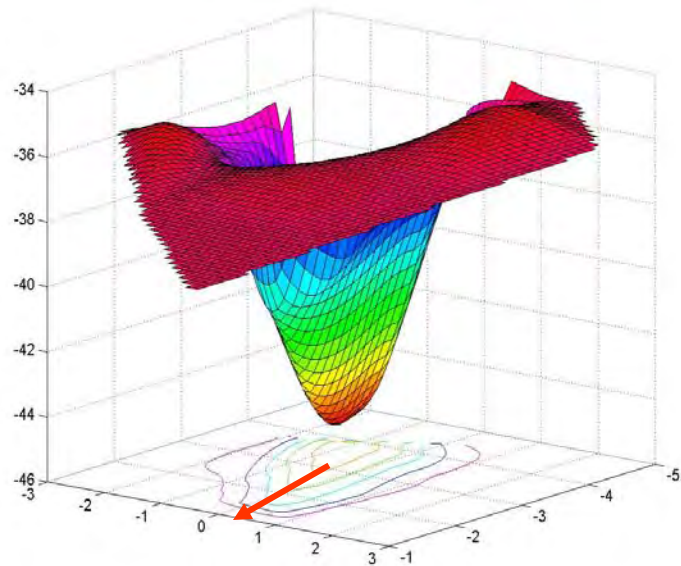
The first mode selects
the 'easiest' collective motion
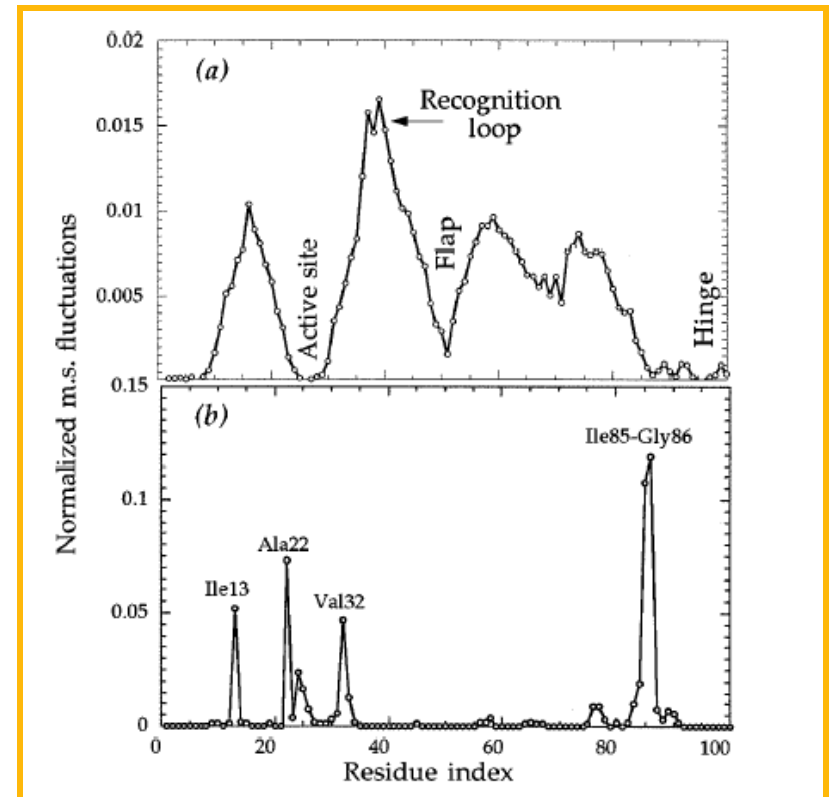
Bahar et al. (1998) Phys Rev Lett. 80, 2733

Gaussian network model (GNM)

$$[\Delta \mathbf{R}_i \bullet \Delta \mathbf{R}_i]_k = (3k_B T / \gamma)\left[\lambda_k^{-1}\mathbf{u}_k\mathbf{u}_k^{T}\right]_{ii}$$

Kirchhoff matrix for inter-residue contacts

$$\Gamma =$$
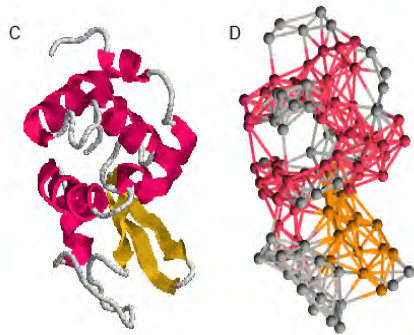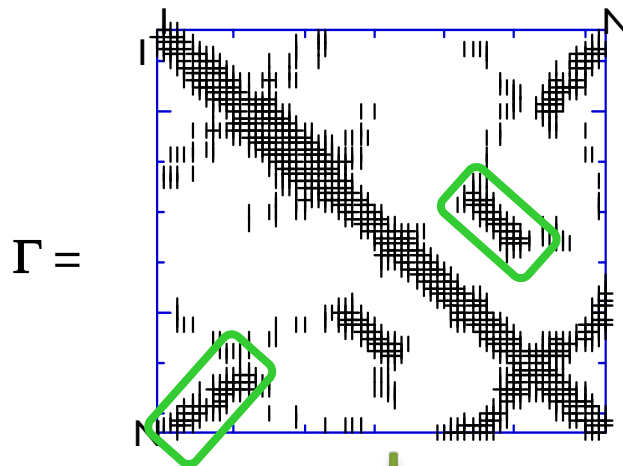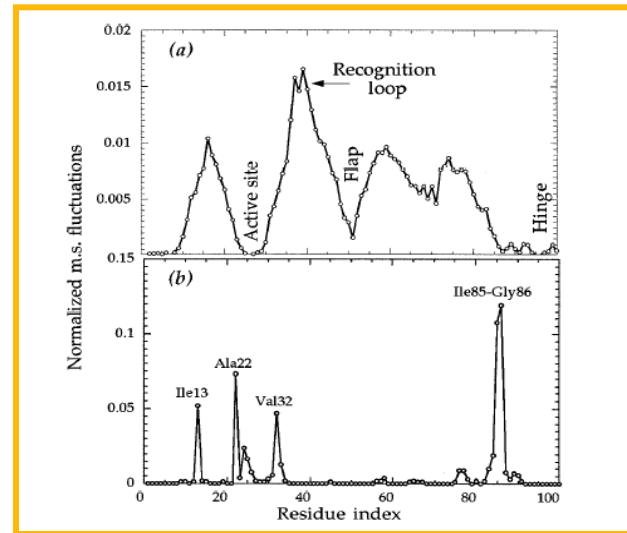
Several modes of motion contribute to dynamics

$$< \Delta \boldsymbol{R}_i \bullet \Delta \boldsymbol{R}_j > = (1/Z_N)\int (\Delta \boldsymbol{R}_i \bullet \Delta \boldsymbol{R}_j)e^{-V/k_B T}d\{\Delta \boldsymbol{R}\} = (3k_B T / \gamma)\left[\Gamma^{-1}\right]_{ij}$$

Anisotropic Network Model (ANM)

ANM mode 1 (u_1)

low-frequency ~ global modes

$\sim\lambda_1$

Energy — Displacement

Mode 2

$\sim\lambda_2$
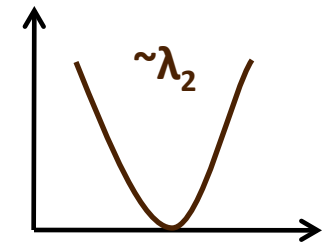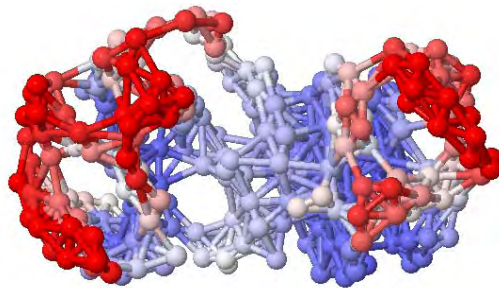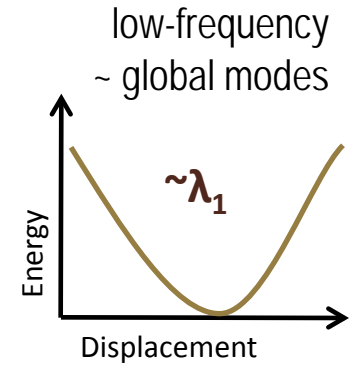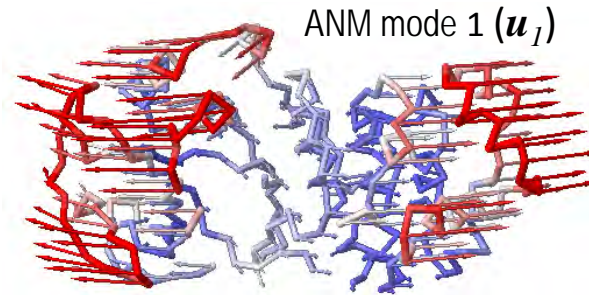
$$\mathbf{H} = \sum_{i=1}^{3N-6} \lambda_i \, u_i \, u_i^{\mathbf{T}}$$

$$\mathbf{H}^{(ij)} = \frac{\gamma\,\mathbf{\Gamma}_{ij}}{\left(R_{ij}^0\right)^2} \begin{bmatrix} X_{ij}X_{ij} & X_{ij}Y_{ij} & X_{ij}Z_{ij} \\ Y_{ij}X_{ij} & Y_{ij}Y_{ij} & Y_{ij}Z_{ij} \\ Z_{ij}X_{ij} & Z_{ij}Y_{ij} & Z_{ij}Z_{ij} \end{bmatrix}$$
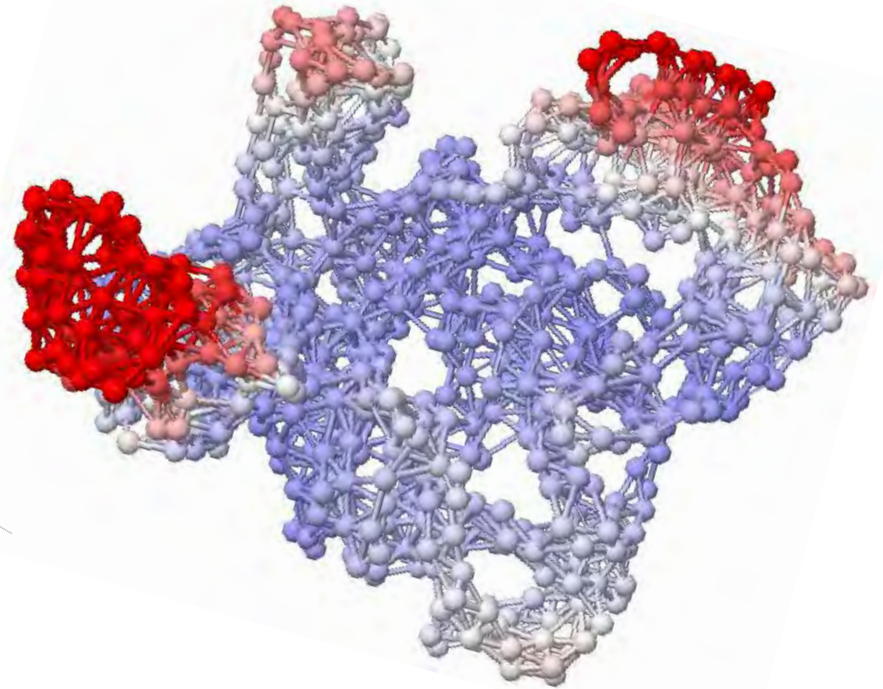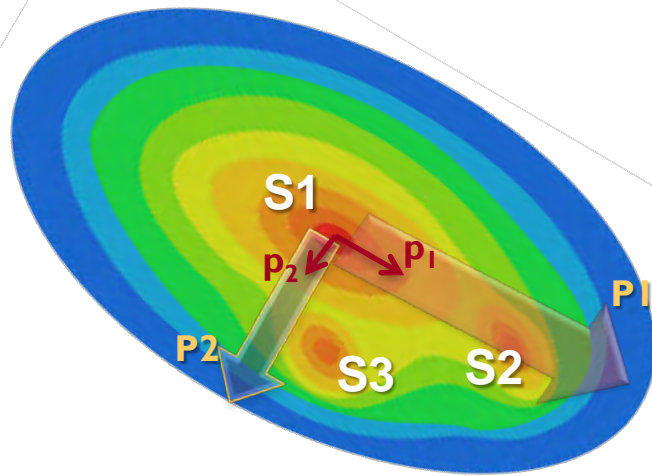
Mode 20

$\lambda_1 < \lambda_2 < \lambda_3 < \ldots$

$\sim\lambda_{20}$

higher-frequency

Doruker et al. (2000) *Proteins*; Atilgan AR et al. (2001) *Biophys J.*; Eyal et al. (2006) *Bioinformatics* 22, 2619
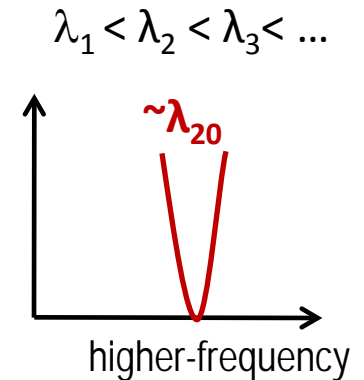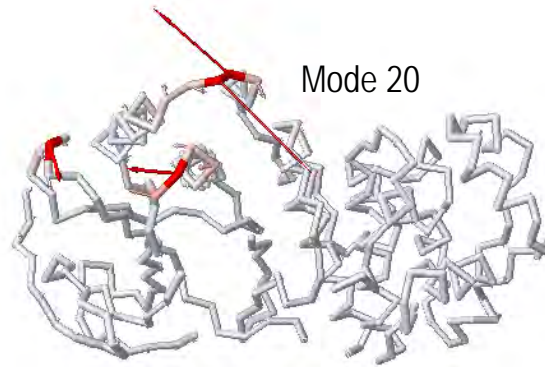
Allosteric changes in conformation

ANM (anisotropic network model)

Elastic Network Models are particularly useful for exploring the allosteric dynamics of large multimeric structures

Comparison with experimental data shows that **the functional movements are those predicted by the ENM to be intrinsically encoded by the structure**

Session 1: Plotting $<(\Delta R_i)^2>$ and contributions of selected modes

```
from prody import *
anm = calcANM('1cot', selstr='calpha')
anm, cot = calcANM('1cot', selstr='calpha')
anm
cot
figure()
showProtein(cot)

figure()
showSqFlucts(anm)

figure()
 showSqFlucts(anm[0])
showSqFlucts(anm[:10])

figure()
showSqFlucts(anm[:10], label='10 modes')
legend()
```

Application to cytochrome c
PDB: 1cot
A protein of 121 residues

Session 2: Viewing color-coded animations of individual modes

- writeNMD('cot_anm.nmd', anm, cot)

- *Start VMD*
- *select* Extensions → Analysis →Normal Mode Wizard
- *Select* 'Load NMD File'

Session 3: Cross-correlations $<(\Delta \mathbf{R}_i . \Delta \mathbf{R}_j)>$ between fluctuations

- cross_corr = calcCrossCorr?
- cross_corr = calcCrossCorr(anm[0])
- figure()
- showCrossCorr(anm[0])
- writeHeatmap('anm_cross1.hm', cross_corr)

Session 4:
Viewing cross-correlations using VMD

- *VMD – Load file*
- *Select cot_anm.nmd (from your local folder)*
- *Load HeatMap*
- *open anm_cross1.hm (from your local folder)*

Ensembles of structures

- Structural changes accompanying substrate (protein) binding

- Structural changes induced by, or stabilized upon, ligand binding

Ubiquitin
140 structures
1732 models

Ensembles of structures

- Structural changes accompanying substrate (protein) binding

- Structural changes induced by, or stabilized upon, ligand binding

Ubiquitin
140 structures
1732 models

p38 MAP kinase
(**182 structures**)
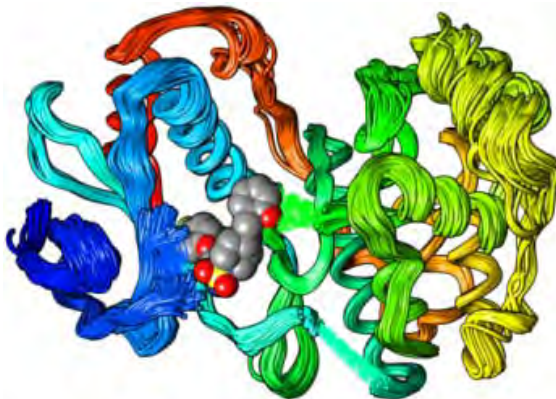
p38 inhibitors
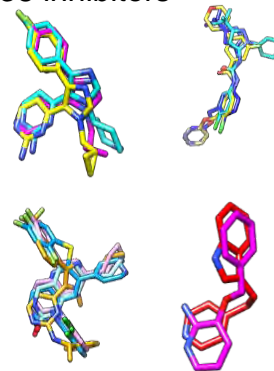
Ensembles of structures

- Structural changes accompanying substrate (protein) binding

- Structural changes induced by, or stabilized upon, ligand binding

- Alternative conformations sampled during allosteric cycles
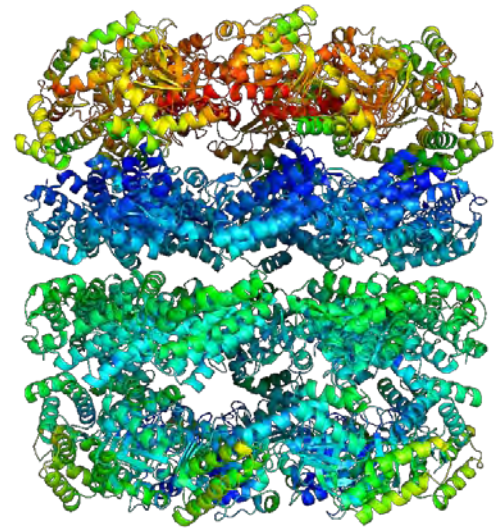
Yang et al. *PLoS Comp Biol* 2009

Redistribution of interactions at interfaces
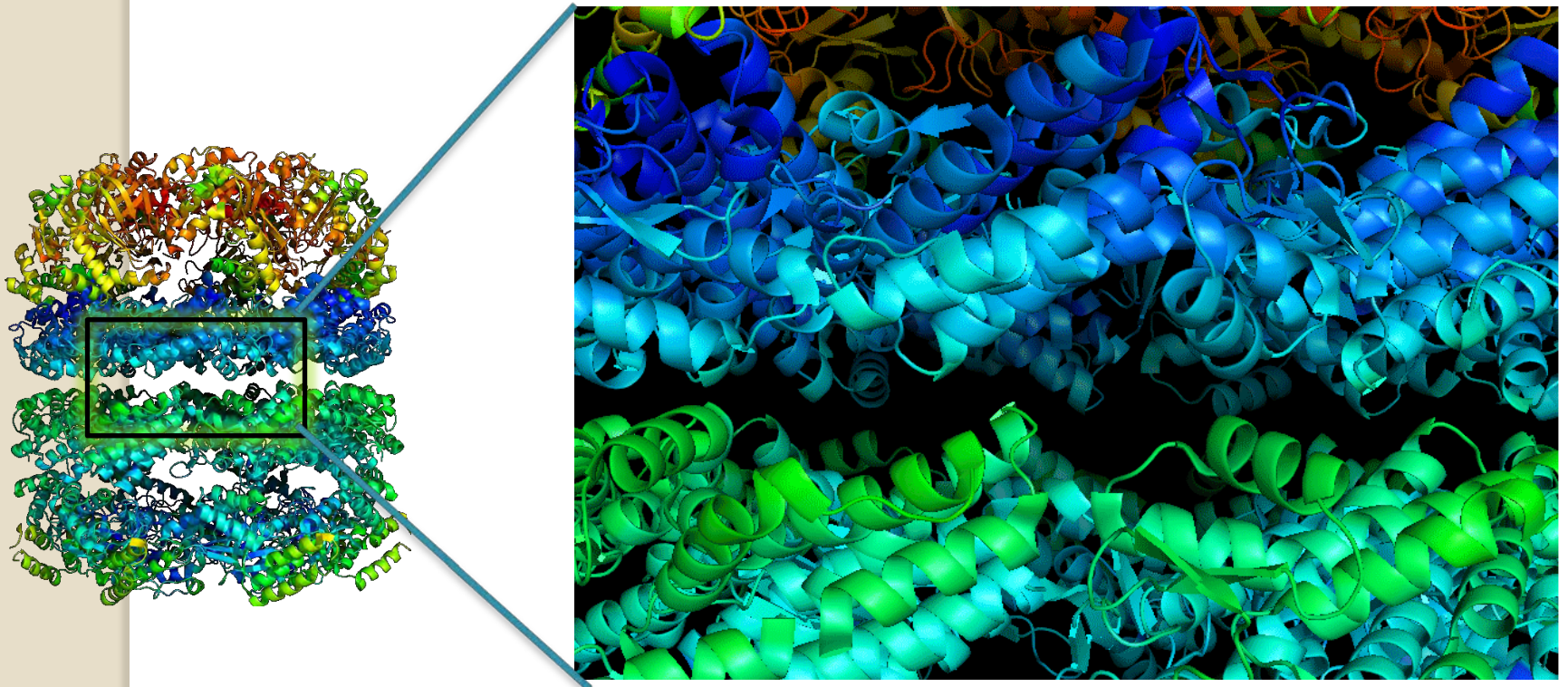
Mutations may stabilizate conformers along soft modes – which may be dysfunctional
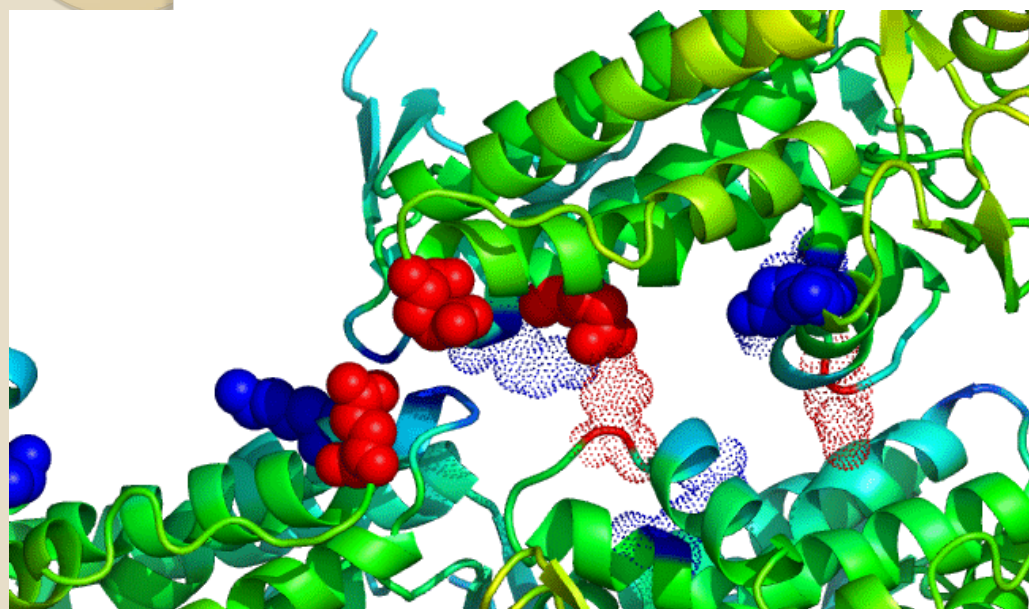
E461 mutant is a deformed structure along mode 1
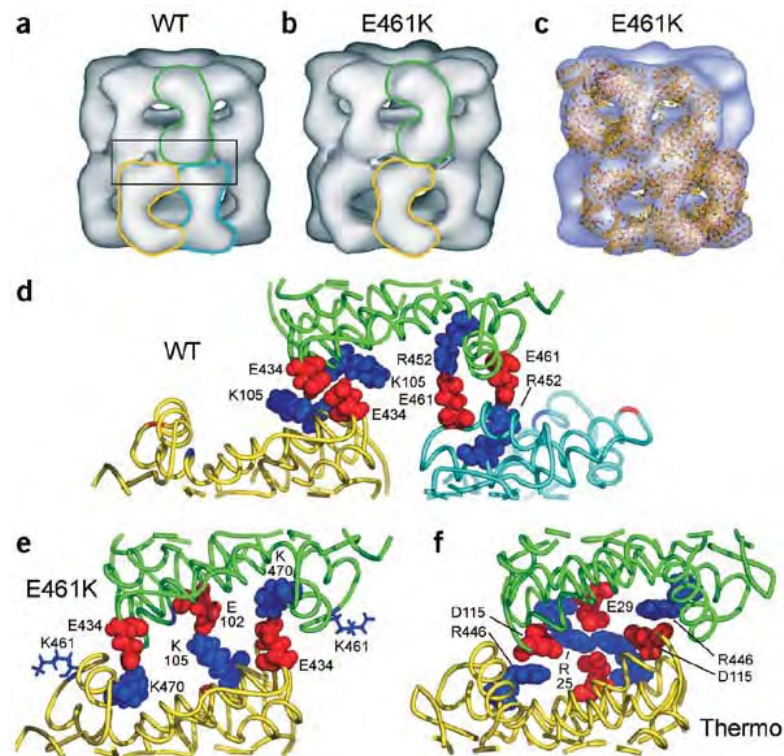
E461K mutation causes disruption of inter-ring transfer of ATP-induced signal (Sewell et al NSB 2004)
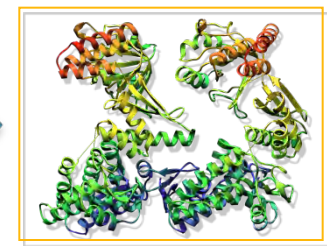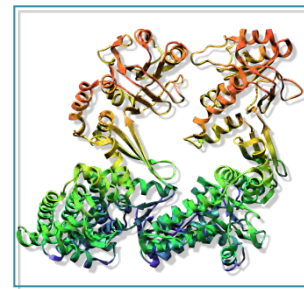
Yang et al. *Mol Biosyst* 2008

Passage between the R and T state of GroEL
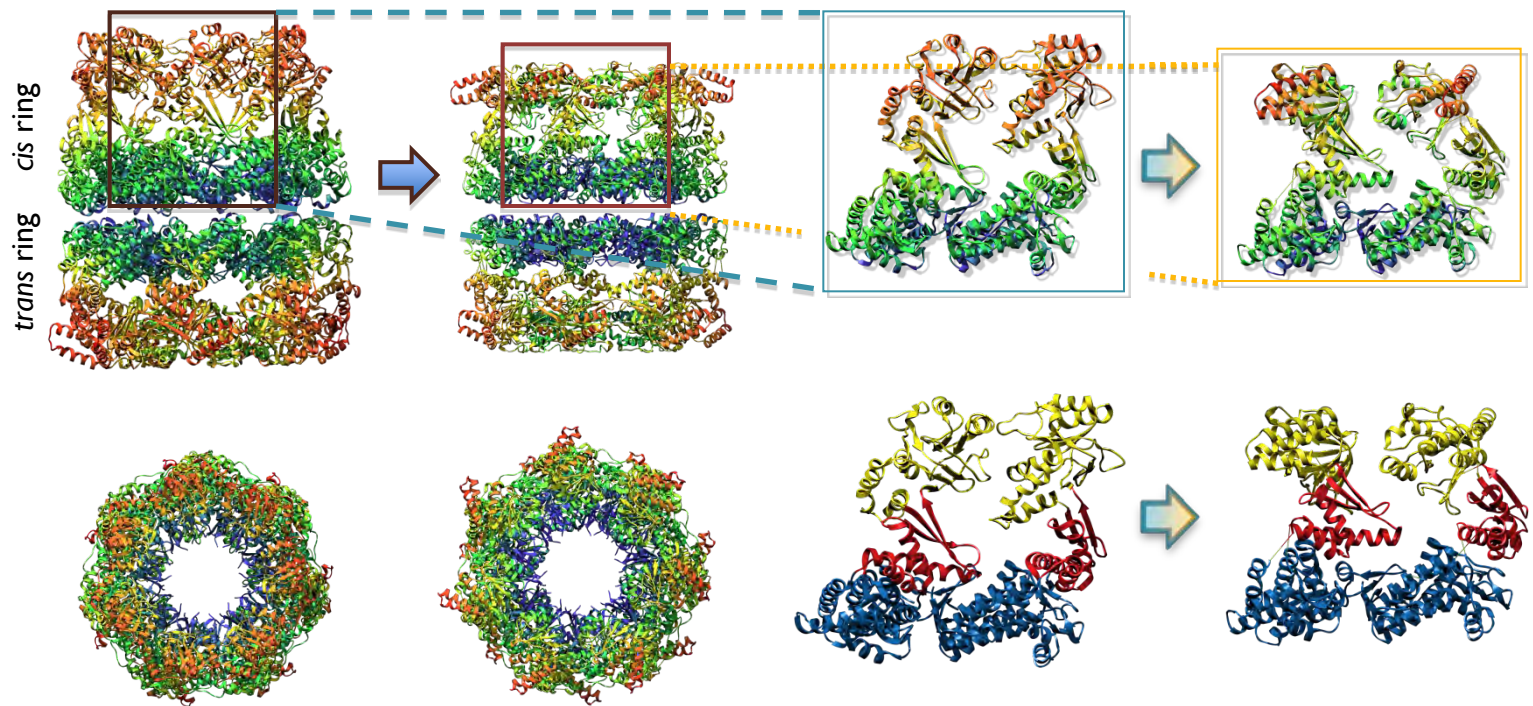
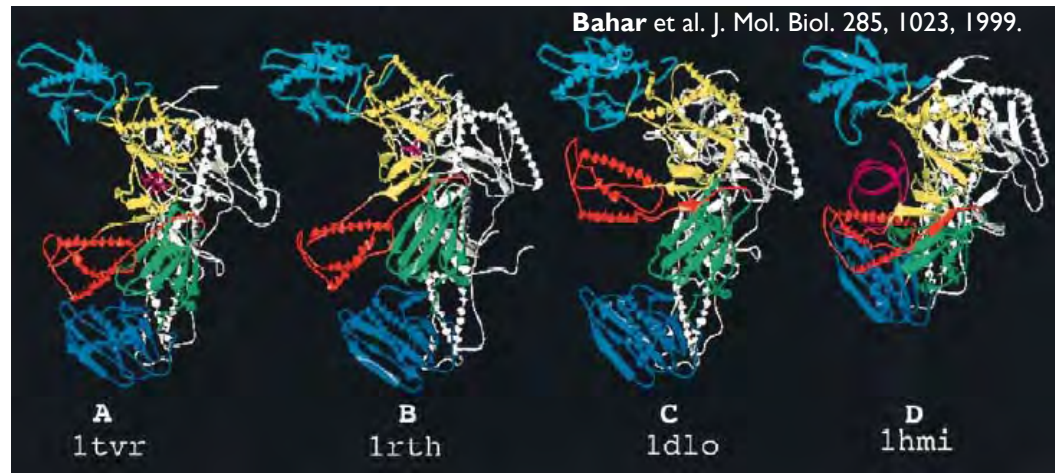# The softest mode enables the passage R →T
(with a correlation of 0.81)

$$d = [\Delta x_I \quad \Delta y_I \quad \Delta z_I \ldots \qquad \Delta z_N]^T$$
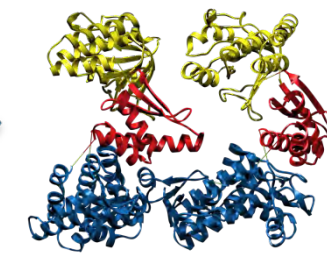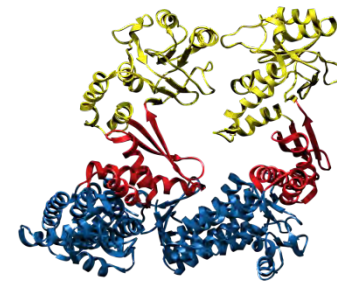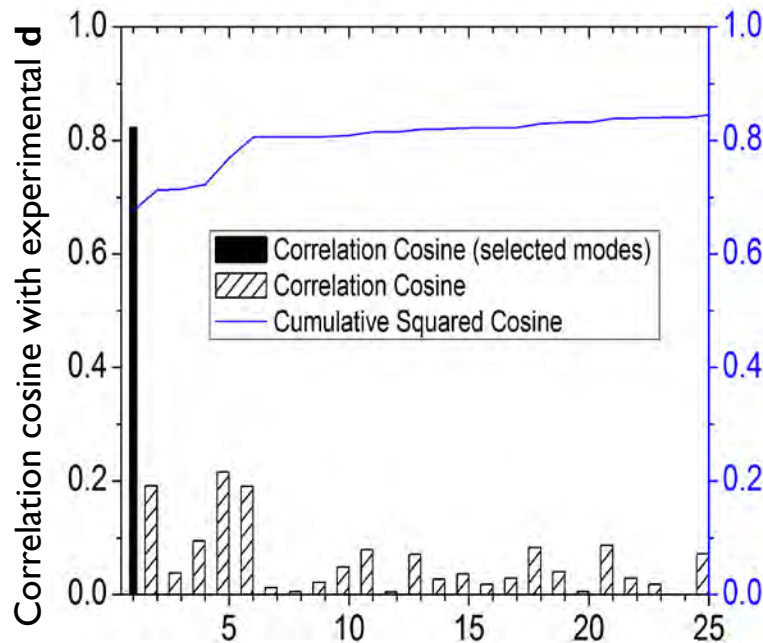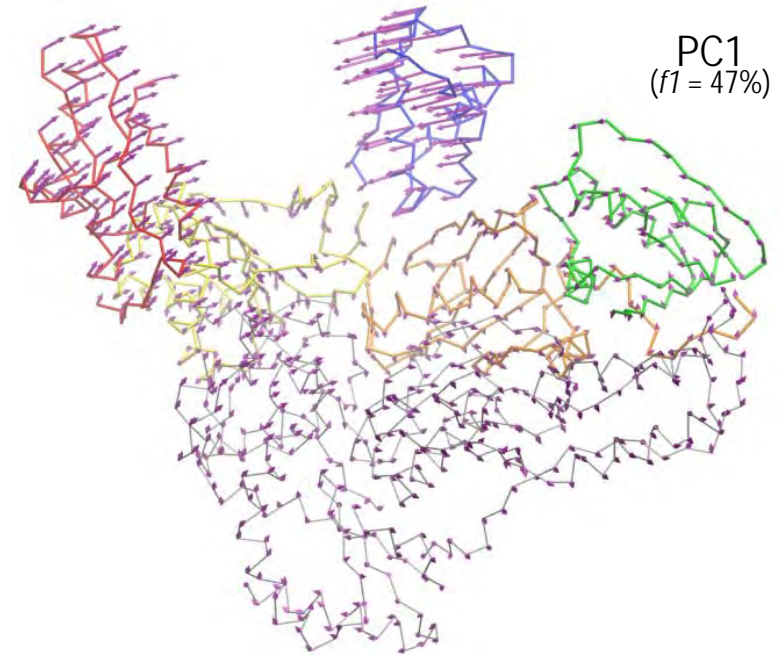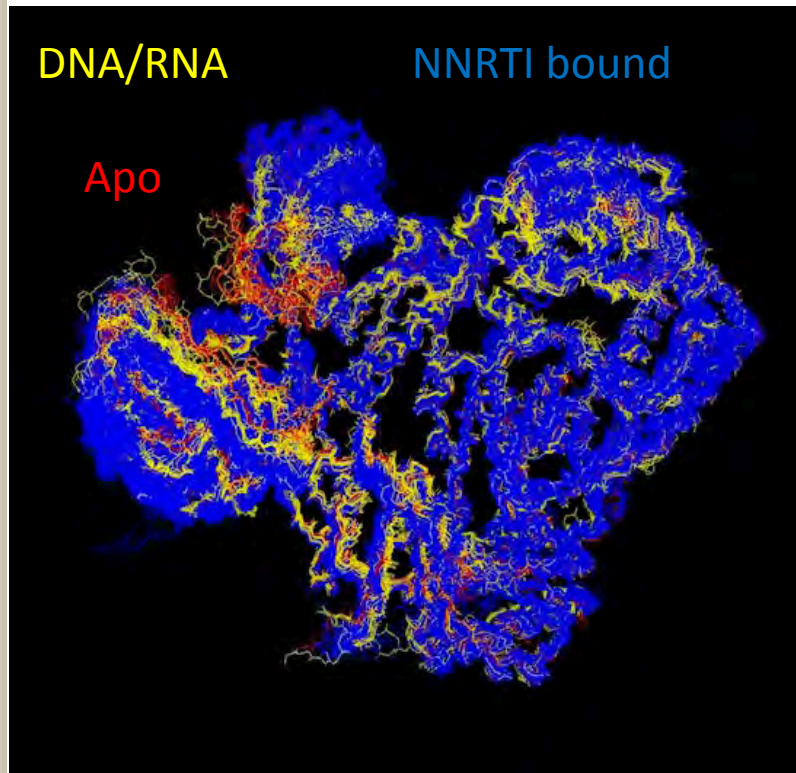
Dynamics inferred from known structures

Comparison of static structures available in the PDB for the same protein in different form has been widely used is an **indirect** method of inferring dynamics.

Different structures resolved for HIV-1 reverse transcriptase (RT)

Principal Component Analysis (PCA)

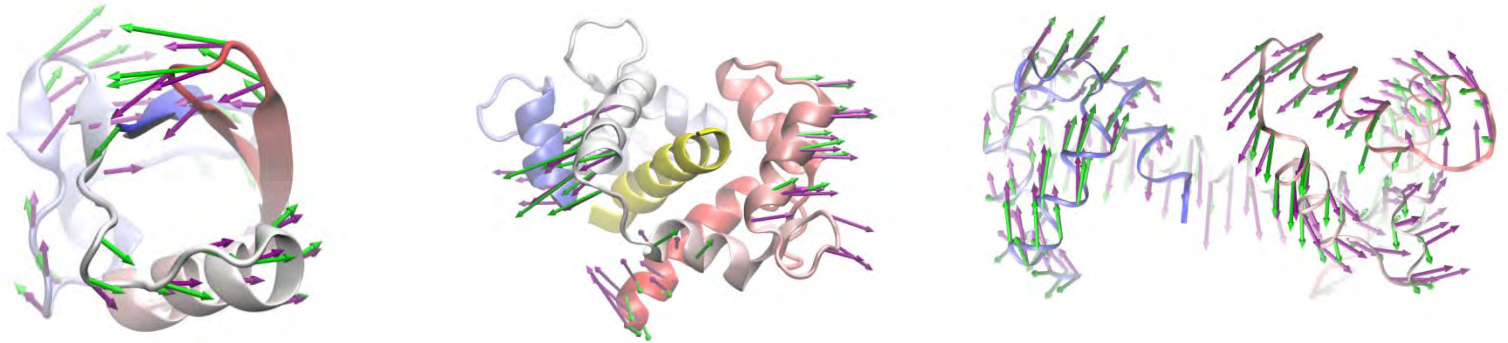DNA/RNA   NNRTI bound

Apo

PC1
($f1 = 47\%$)

$$\mathbf{C}^{(ij)} = \begin{bmatrix} \langle \Delta x_i \Delta x_j \rangle & \langle \Delta x_i \Delta y_j \rangle & \langle \Delta x_i \Delta z_j \rangle \\ \langle \Delta y_i \Delta x_j \rangle & \langle \Delta y_i \Delta y_j \rangle & \langle \Delta y_i \Delta z_j \rangle \\ \langle \Delta z_i \Delta x_j \rangle & \langle \Delta z_i \Delta y_j \rangle & \langle \Delta z_i \Delta z_j \rangle \end{bmatrix}$$

$$\mathbf{C} = \mathbf{PSP}^T = \sum_{i=1}^{3N} \sigma_i \, \boldsymbol{p}^i \, \boldsymbol{p}^{iT}$$
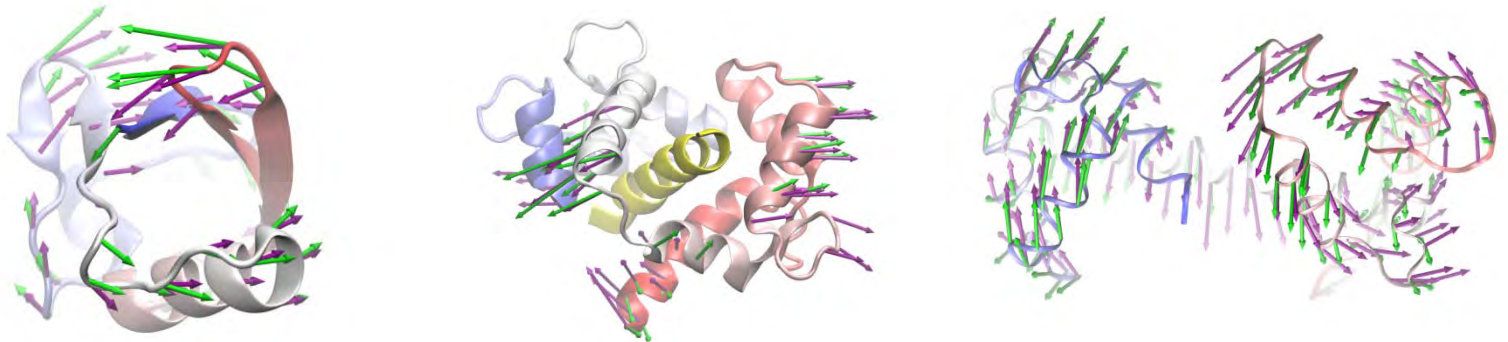
Global motions inferred from theory and experiments

→ PCA of the ensemble of resolved structures
→ ANM analysis of a single structure from the ensemble

Global motions inferred from theory and experiments

The intrinsic dynamics of enzymes plays a dominant role in determining the structural changes induced upon inhibitor binding

Ahmet Bakan and Ivet Bahar[1]

Department of Computational Biology, School of Medicine, University of Pittsburgh, 3064 BST3, 3501 Fifth Avenue, Pittsburgh, PA 15213
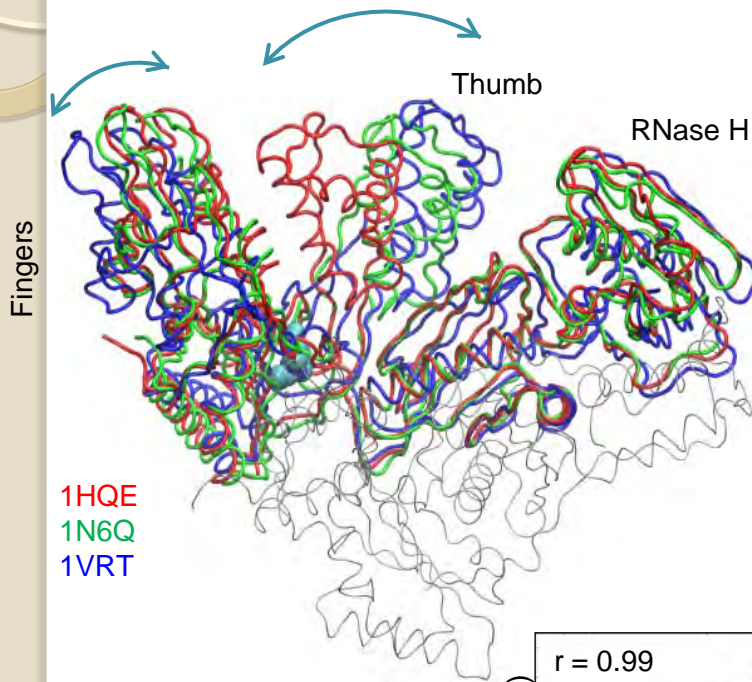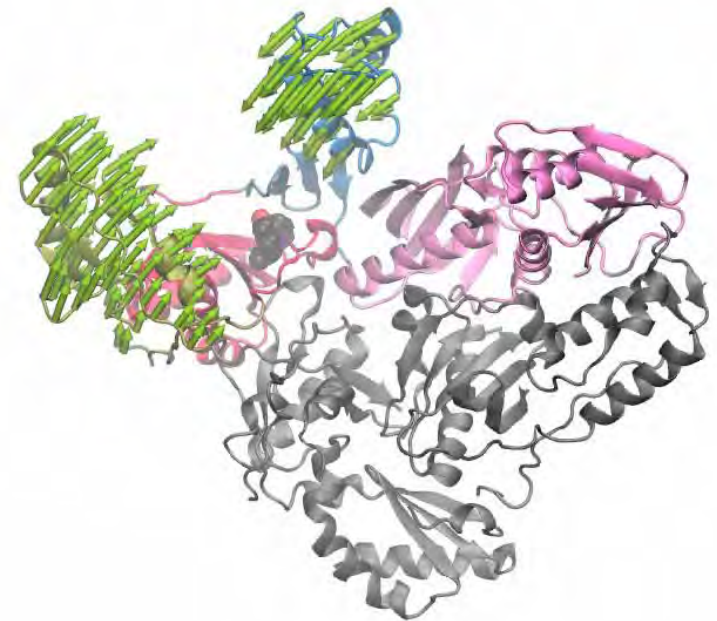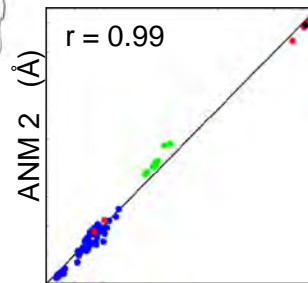
Reference:

*Bakan & Bahar (2009) PNAS **106**, 14349-54*

Induced Dynamics or Intrinsic Dynamics?

Fingers

Thumb

RNase H

1HQE
1N6Q
1VRT

r = 0.99

ANM 2 (Å)

PC1 (Å)

Experiments

Theory

http://www.youtube.com/watch?v=1OUzdzm68YY

References:

*Bakan & Bahar (2009) PNAS **106**, 14349-54.*

Soft modes enable functional movements

Thumb

RNase H

Fingers

1HQE
1N6Q
1VRT

r = 0.99

ANM 2 (Å)

PC1 (Å)

Experiments
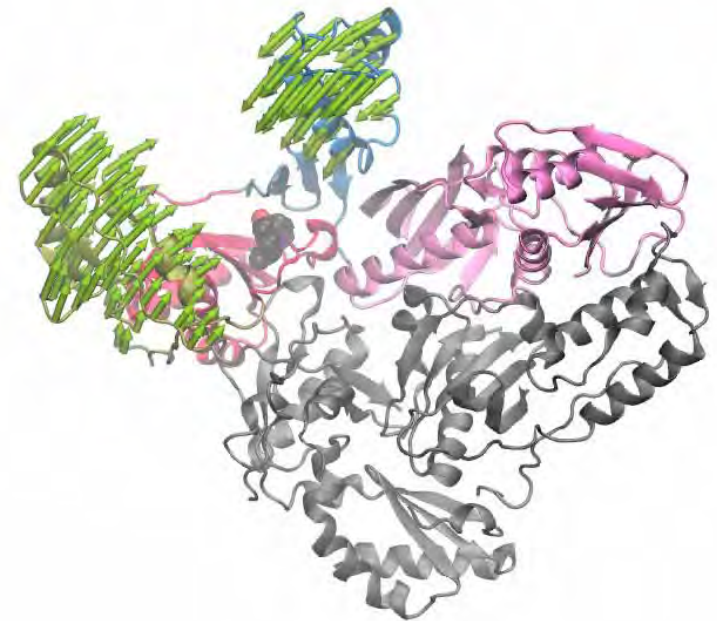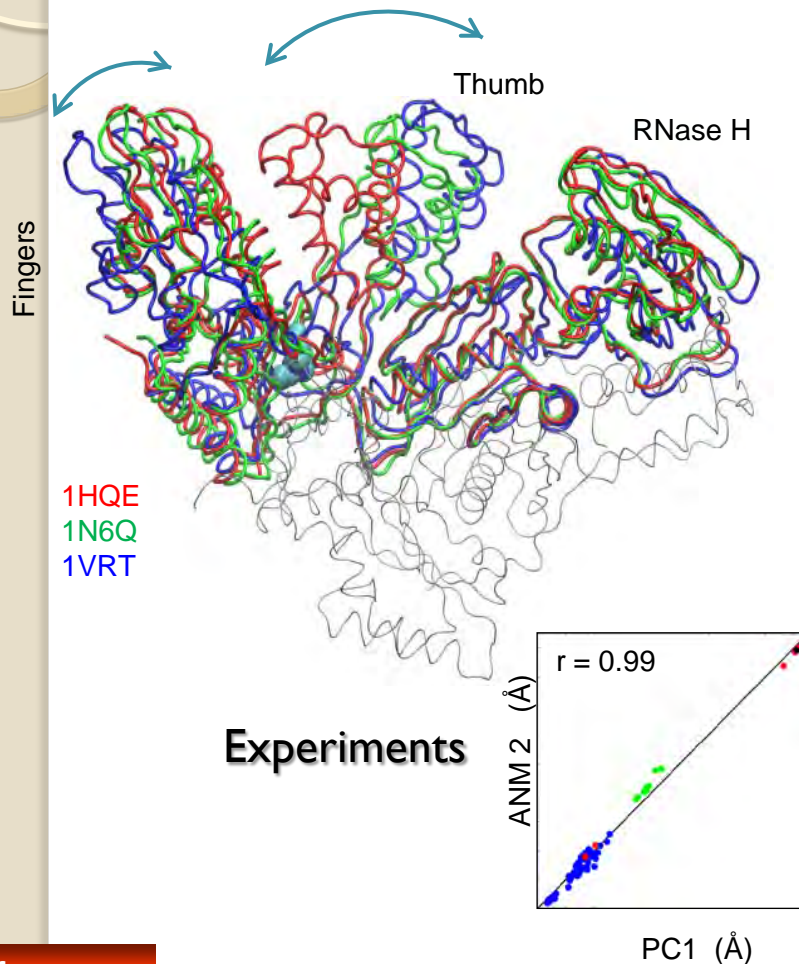
Theory

References:

Bakan & Bahar (2009) PNAS **106**, 14349-54.

Intrinsically accessible motions enable
Optimal binding of substrate or drugs

Conformational flexibility + sequence variability mediates **substrate selectivity**

- Two conformations of P450-CYP2B4:

  open (orange) with a large substrate (bifonazole, red), and

  closed (light blue) with the smaller substrate 4-(4-chlorophenyl) imidazole (blue)
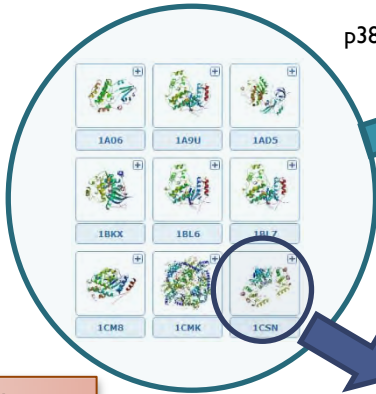
See...

ProDy for exploring conformational space
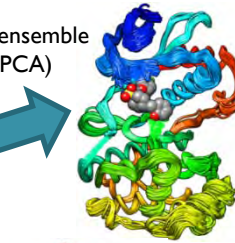
Protein Dynamics Analysis in Python

User inputs a protein sequence

>1A9U:A|PDBID|CHAIN
GSSHHHHHHSSGLVPRGSHMSQER
PTFYRQELNKTIWEVPERYQNLSPV
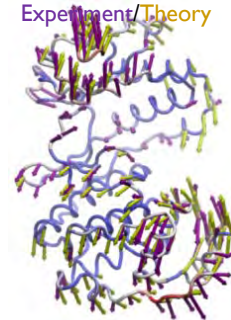GSGAYGSVCAAFDTKTGLRVAVKK
LSRPFQSIIHAKRTYRELRLLKHMKH
ENVIGLLDVFT......

ProDy *identifies*, *retrieves*, *aligns*,
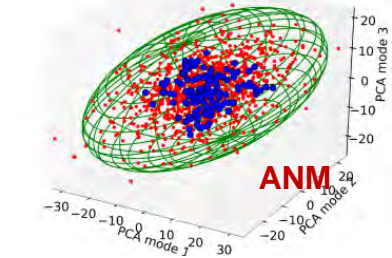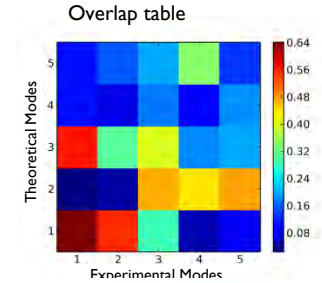and *analyzes (PCA)* structures that
match the input sequence

p38 ensemble
(PCA)

p38 network model
(ANM)

Experiment/Theory

Overlap table

User can compare
experimental and
theoretical models

ANM

MD

Growth of Source Code and Usage

	Releases	Downloads	Visits[2]	Unique[3]
Nov '10 - Oct '11	19	8,530	8,678	2,946
Nov '11 - Oct '12	15	35,108	16,472	6,414
Nov '12 - Oct '13	8*	87,909	19,888	8,145
Total	42	131,547	45,038	17,505

130,000+ downloads

Source http://www.google.com/analytics/

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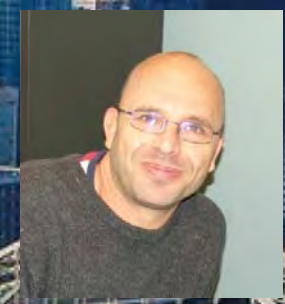- Klaus Schulten
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Acknowledgment

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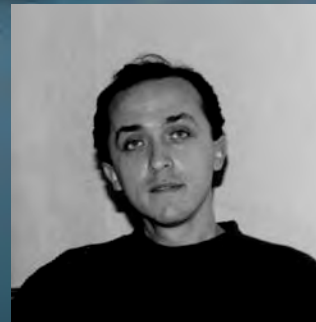
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