Network Visualization of Conformational Sampling during Molecular Dynamics Simulation

Osamu Miyashita
Computational Structural Biology Unit

ADVANCED INSTITUTE FOR COMPUTATIONAL SCIENCE

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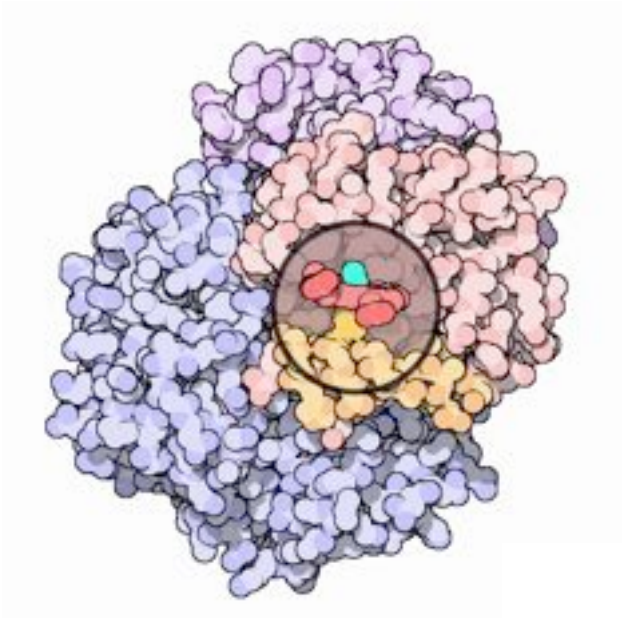
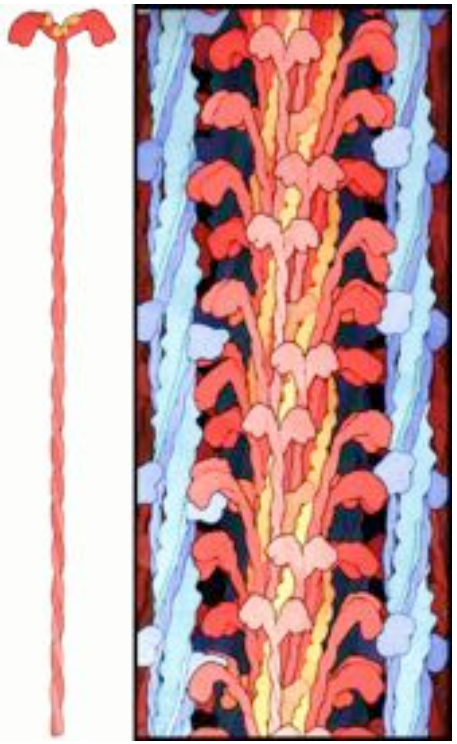
Proteins and Nucleotides (DNA/RNA)

- Players of cell life activities

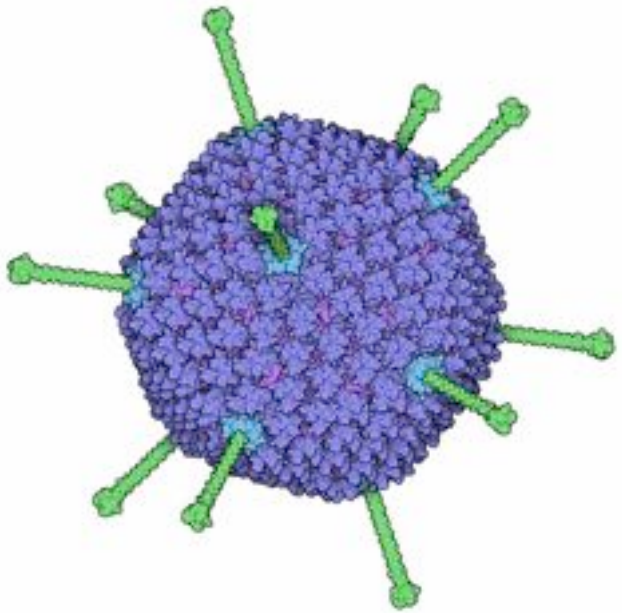
Hemoblogin

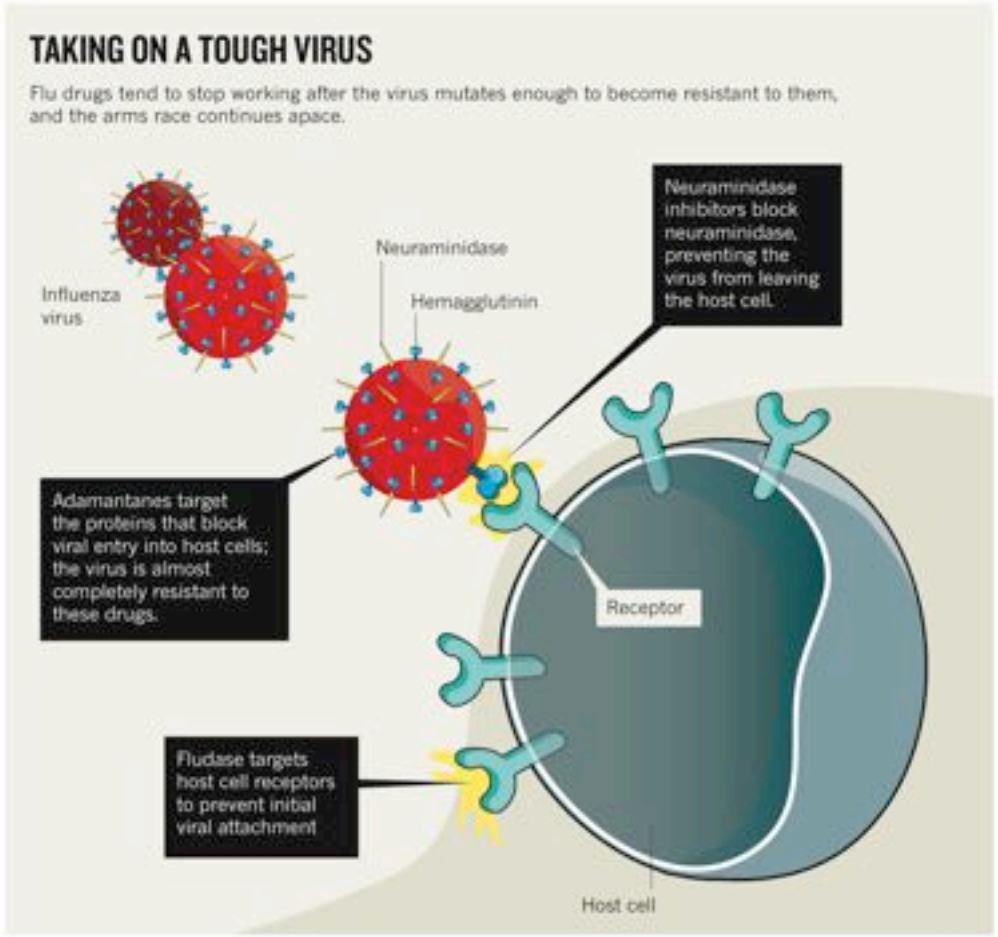


Myosin



Virus





DRUGS

Lines of defence

Antiviral treatments are a critical component of an effective healthcare response to influenza, but drug resistance to the treatment-of-choice has public health officials searching for other options.

Zachary Taylor, an infectious disease fellow at the Kaiser Permanente Fontana Medical Center in Sacramento, California. In part to safeguard against the possibility of such game-changing developments, drug developers are slowly filling the pipeline with alternative therapies (see 'Drugs to treat influenza infection'). Each drug come with side effects, which make them only worthwhile for those whom the flu could be potentially lethal — the elderly and the immunocompromised.

Given the wily history of the influenza virus, any sudden appearance of drug resistance is certain to concern public health officials. The first antiviral drugs to combat the disease — the adamantanes, which target the M2 channel protein to block virus entry into host cells — are now essentially useless. The US Centers for Disease Control and Prevention (CDC) found that 100 % of seasonal H3N2 flu in the 2009–2010 season and 99.8% of 2009 pandemic H1N1 flu were resistant to adamantanes.

Oseltamivir belongs to a class of drugs called neuraminidase inhibitors. These agents block the active site of a viral protein called neuraminidase (N), thereby arresting the influenza virus' ability to leave the host cell after it proliferates. The most common way for the influenza virus to evade oseltamivir is via the H275Y mutation (also known as H274Y) of neuraminidase, which replaces a single histidine amino acid with a tyrosine. This alteration interferes with the drug's ability to bind to the protein — a problem acknowledged by the maker of oseltamivir. "There remains a medical need and room for additional treatment options, especially for the management of severe infections and for improved pandemic preparedness," says Klaus Klumpp, Roche's top virologist. Klumpp says the Roche is supporting research into new therapies targeting viral replication as well as other mechanisms, but notes that these efforts are preclinical.

Fortunately, viruses with the H275Y mutation are still susceptible to a different neuraminidase

R. Palmer, Nature 2011

NAs catalyze the hydrolysis of sialosides with net retention of stereochemistry at the site of substitution. A mechanism involving an ion-pair intermediate has long been suggested for the GH34 196 min^{-1} mM^{-1} could be measured. Turnover of the covalent intermediate (k_{hydr}) also occurred rapidly, with a $t_{1/2} < 1$ min. Confirmation of the formation of a covalent species and identification optimize the ratio of k_i/k_{hydr}. Versions of **1** bearing amine (Am) and guanidine (Gu) substituents at C-4 were of interest because these cationic substituents might improve the initial affinity and

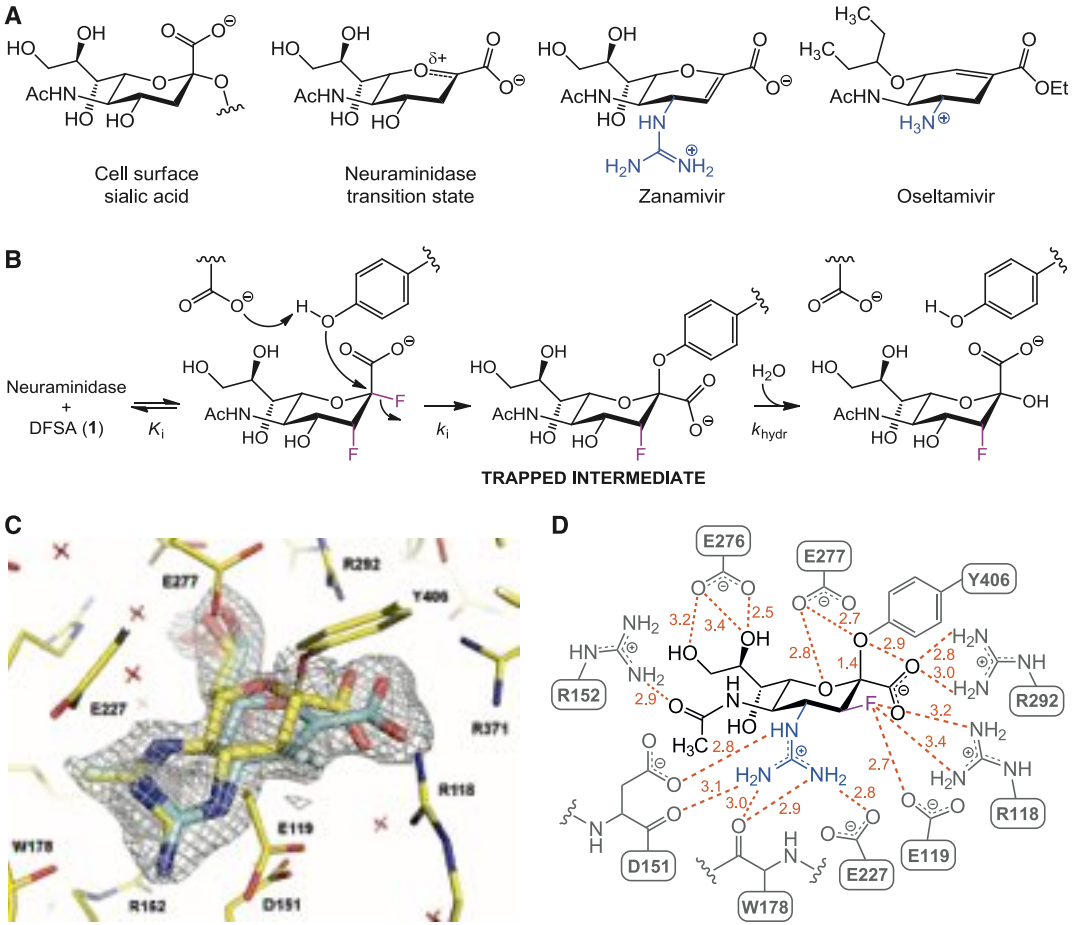
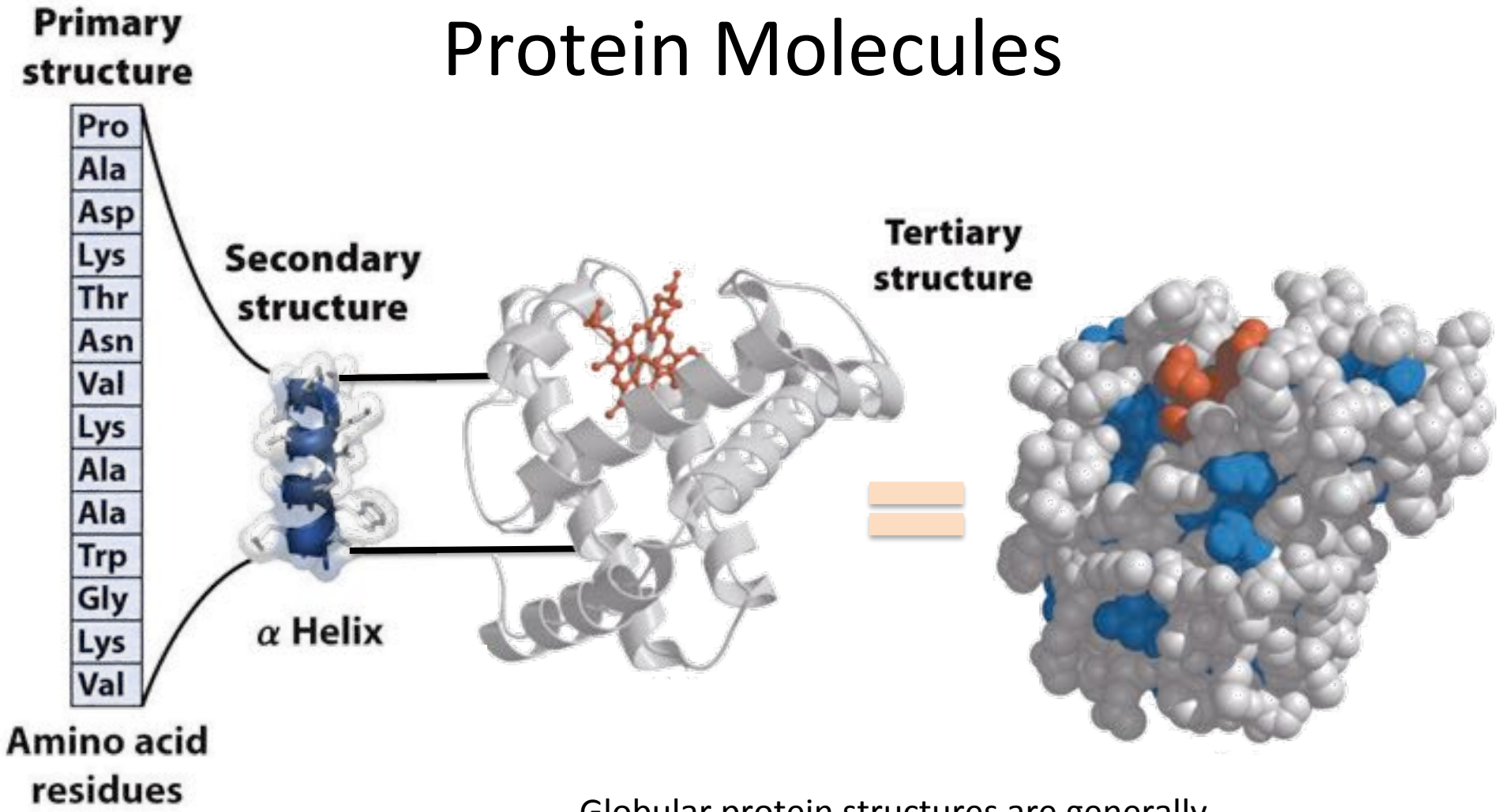
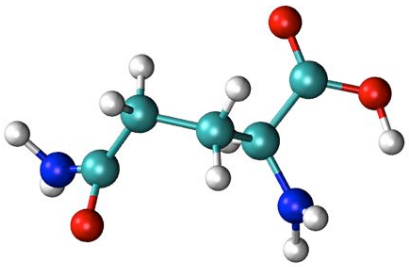


Fig. 1. Structures of key influenza therapeutics, mechanism of action of DFSAs, and x-ray structure of inhibited enzyme. (**A**) Chemical structures of cell surface sialic acids, the neuraminidase transition state, zanamivir (Relenza), and oseltamivir (Tamiflu). (**B**) Mechanism of action of the DFSAs. (**C**) X-ray crystallographic structure of the active site of the enzyme trapped as its 3-fluoro(eq)-4-guanidino-sialyl-enzyme intermediate (elimination product is in pale cyan) overlaid with omit (*22*) electron density map shown as a gray mesh contoured at 1σ within 1.6 Å of ligands. The electron density extends from the ligand molecule to Y406, suggesting a covalent link between the inhibitor's C-2 atom and the OH of Y406. (**D**) Diagram of interactions (orange dashed lines; distances in Å) with the sialic acid in the covalently inhibited enzyme. The corresponding diagram of interactions for the elimination product is shown in fig. S4.

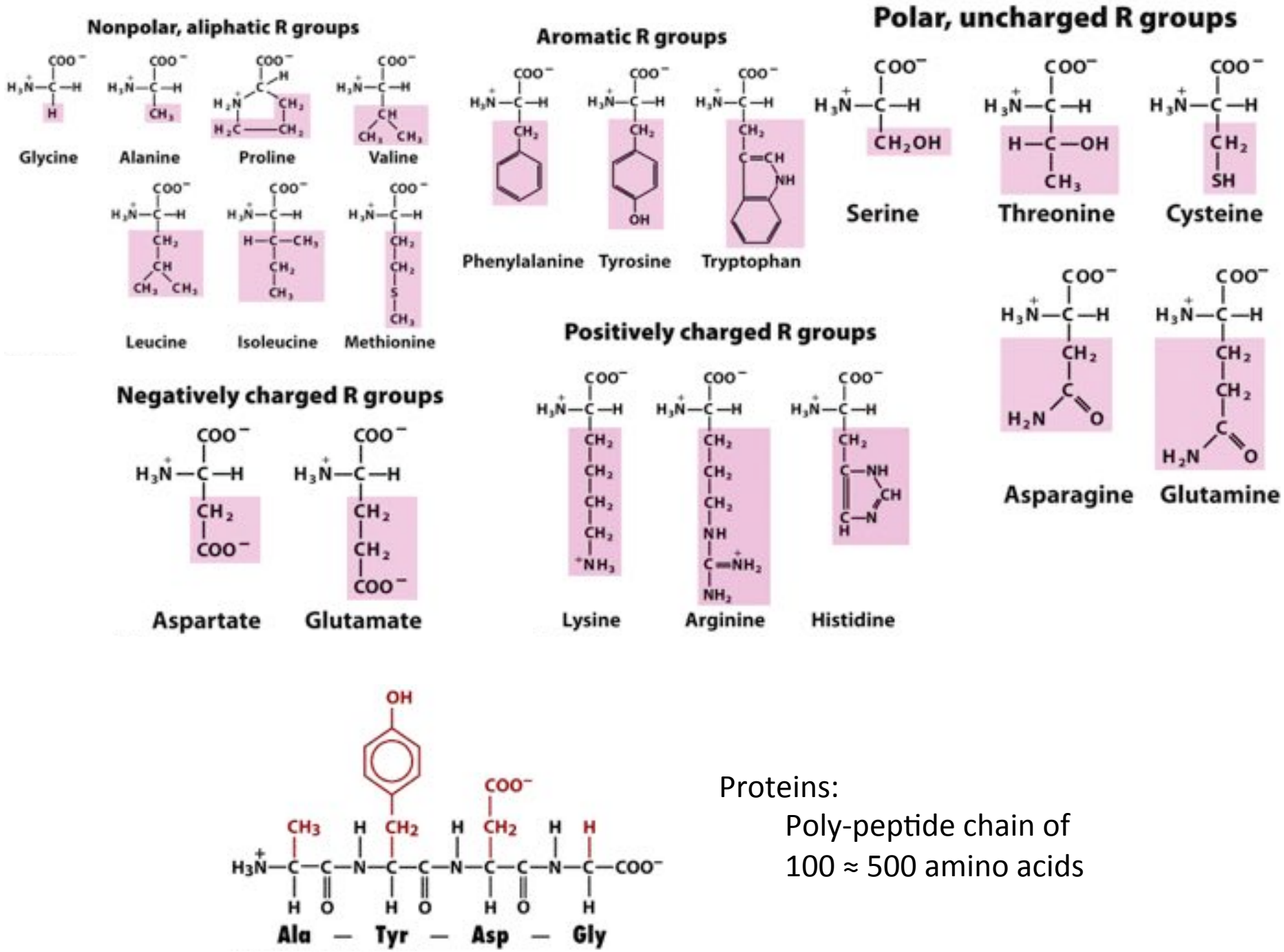
Protein Molecules





Globular protein structures are generally tightly packed, compact units

1 amino acid → 10 ≈ 30 atoms



Proteins:

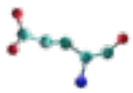
Poly-peptide chain of

100 ≈ 500 amino acids

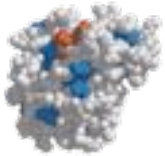
Biological Assemblies

Protein:
amino acids linked together

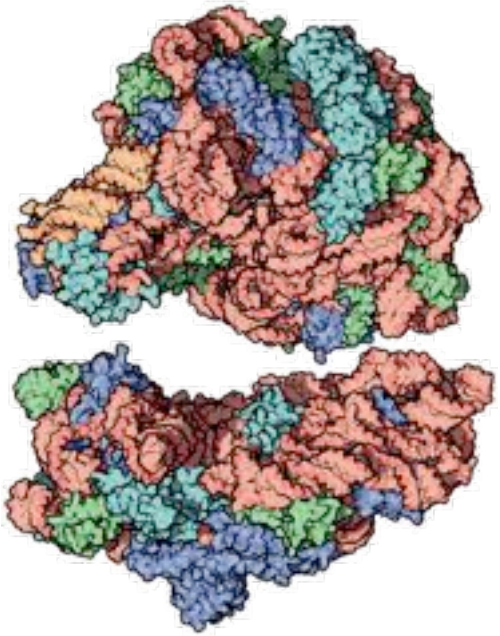
supramolecular complexes of
proteins / RNA



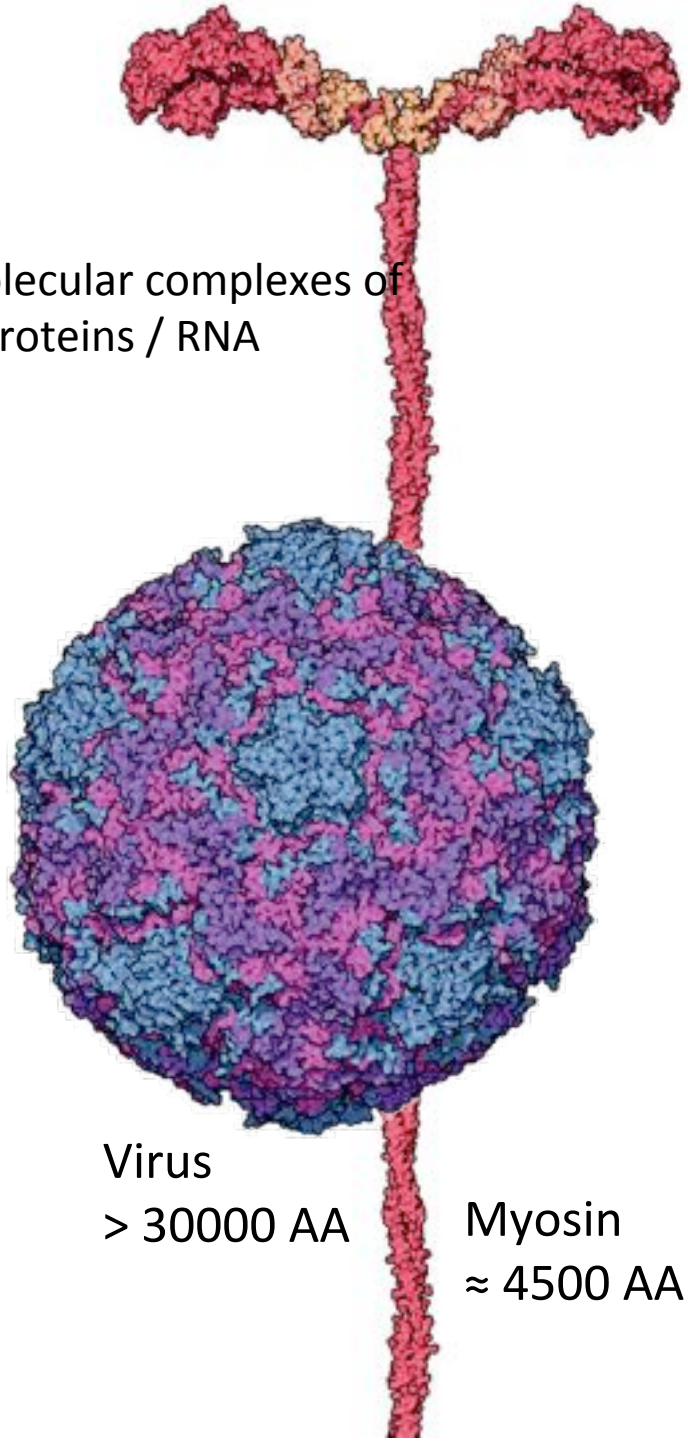
amino
acid (AA)



myoglobin
≈ 130 AA



Ribosome
≈ 11000 nucleotides/
AA

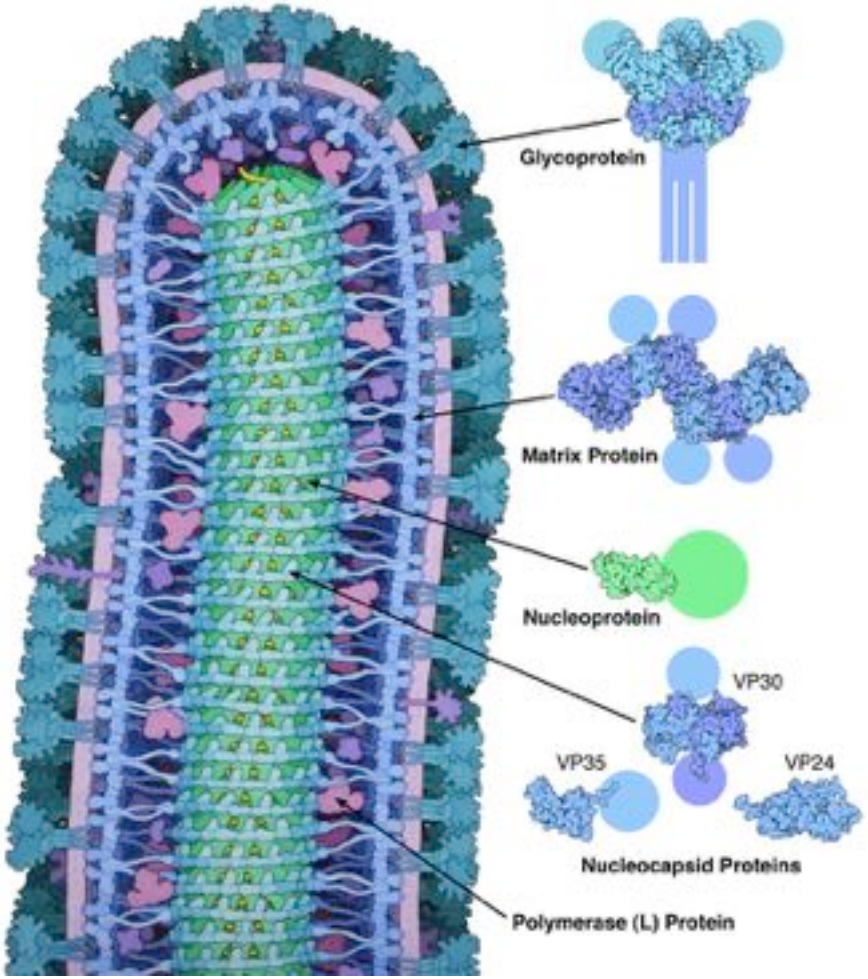


Virus
> 30000 AA

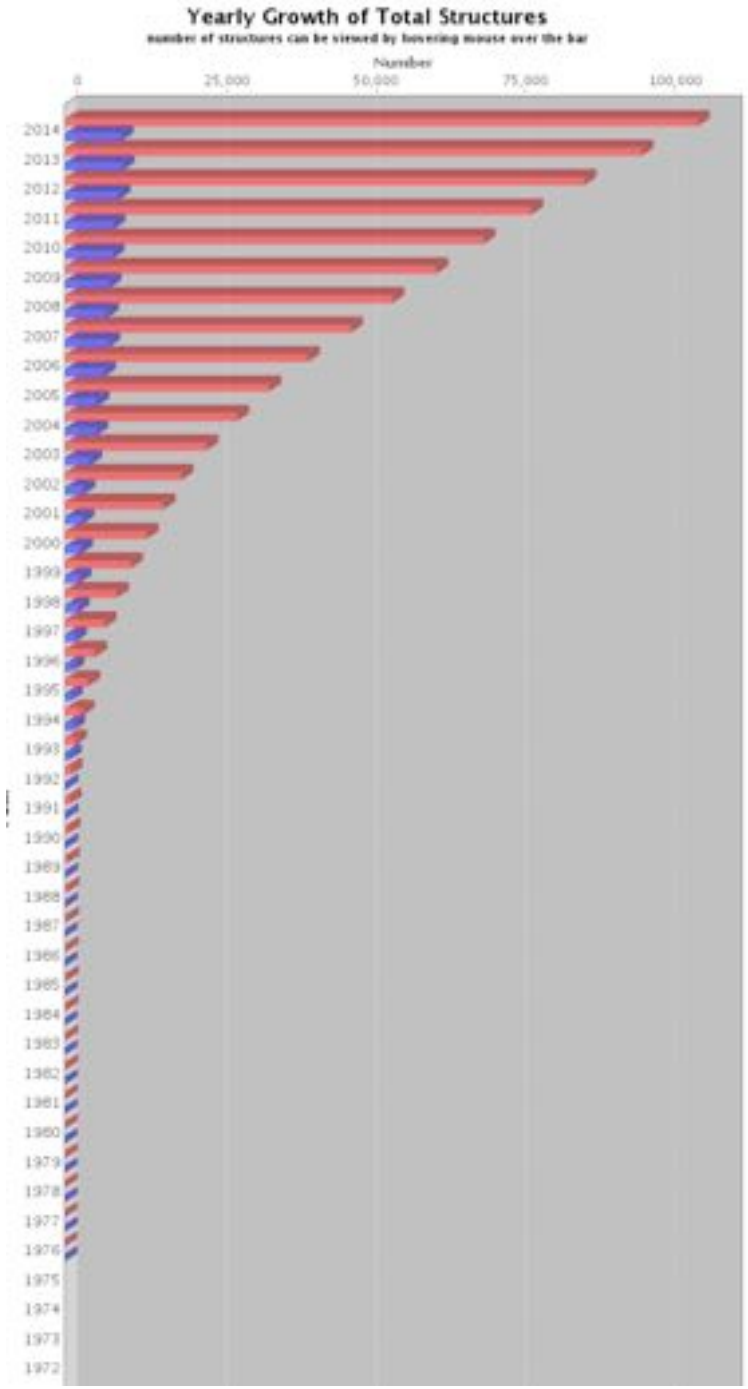
Myosin
≈ 4500 AA

PDB – protein structure database

www.pdb.org



Ebola virus proteins



http://multimedia.mcb.harvard.edu

Computer Simulations

Molecular Dynamics Simulations

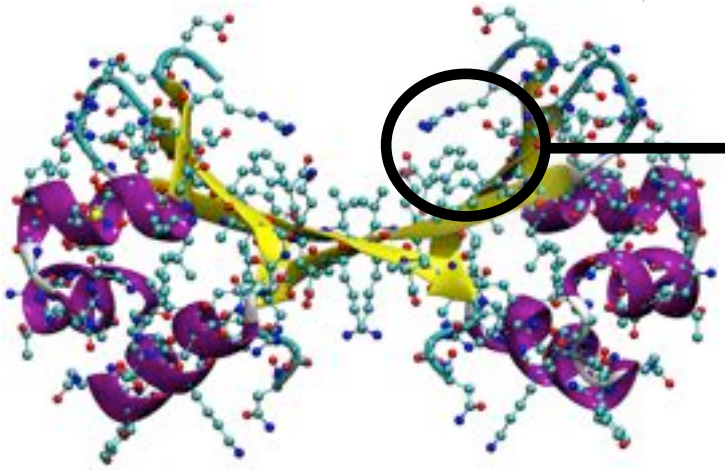
- Simulate the motions of atoms within biological molecules using physics theories
- Newton Equation

$$F_n = -\nabla_n U(r_n) = m_n a_n = m_n \frac{d^2 r_n}{dt^2}$$

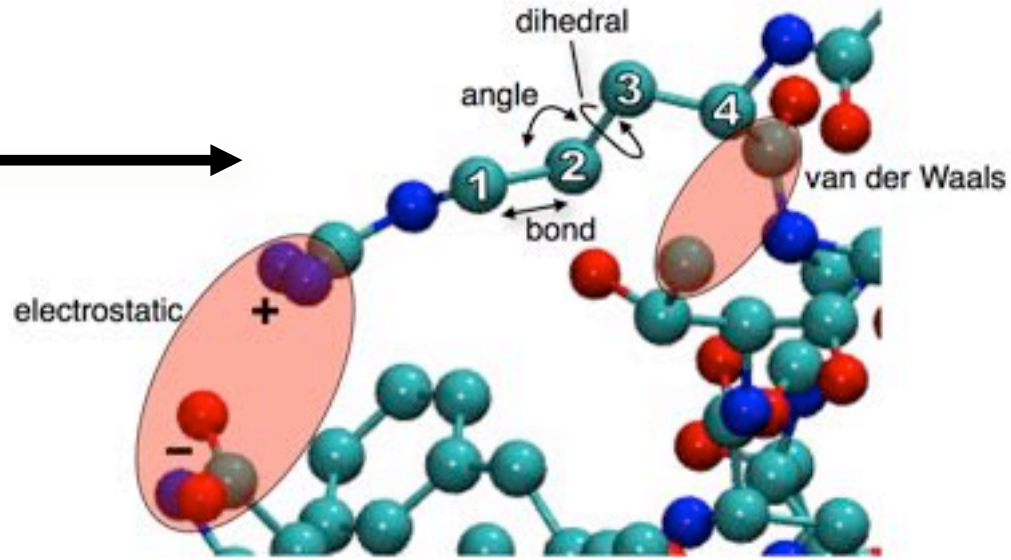
F_n = force on particle n
m_n = mass
a_n = acceleration
$U(r_n)$ = potential energy
r_n = coordinate of particle n

1. Numerical procedure to integrate the differential equation
2. Energy function for the biological molecules

Molecular Dynamics (MD) Simulation



starting coordinates = X-ray, NMR structure



Molecular Mechanics Potential ("Force Field")

Bonded

$$U(\vec{r}_n) = \sum_{bonds} \frac{k_i}{2}(l_i - l_0)^2 + \sum_{angles} \frac{k_i}{2}(\theta_i - \theta_0)^2 + \sum_{dihedrals} \frac{V_n}{2}(1 + \cos(n\omega - \gamma))$$

Non-bonded

$$+\sum_{i=1}^{N}\sum_{j=i+1}^{N}\left(\frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} + 4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]\right)$$

Newton's second law

$$\vec{F}_n = -\vec{\nabla}U(\vec{r}_n) = m\vec{a}_n$$

Use the accelerations to numerically integrate the equations of motion

Integration Algorithms

- Use a finite-difference approach: molecular coordinates and velocities at time $t=t+\Delta t$ are obtained from coordinates and velocities at time t
- A time-reversible integrator can be generated from Taylor expansions:

$$r_n(t+\Delta t) = r_n(t) + v_n(t)\Delta t + \frac{1}{2}a_n(t)\Delta t^2 + O(\Delta t^3)... \qquad v_n = \frac{dr_n}{dt}$$

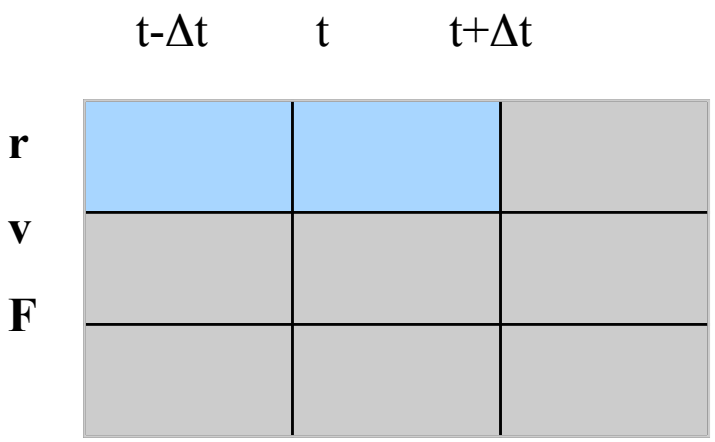
+/-

$$r_n(t-\Delta t) = r_n(t) - v_n(t)\Delta t + \frac{1}{2}a_n(t)\Delta t^2 - O(\Delta t^3)... \qquad a_n = \frac{dv_n}{dt} = \frac{d^2 r_n}{d^2 t}$$

$$r_n(t+\Delta t) = 2r_n(t) - r_n(t-\Delta t) + a_n(t)\Delta t^2 + O(\Delta t^4)...$$

$$v_n(t) = \frac{r_n(t+\Delta t) - r_n(t-\Delta t)}{2\Delta t} + O(\Delta t^2)...$$

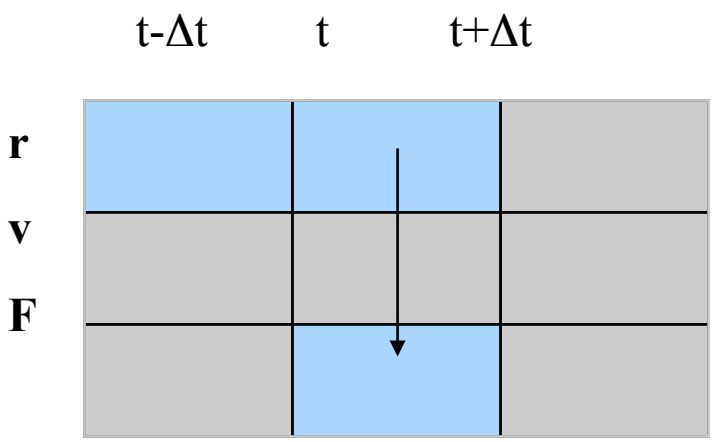
Verlet Integrator



Start with current position and position at end of previous time step

$$r_n(t+\Delta t) = 2r_n(t) - r_n(t-\Delta t) + a_n(t)\Delta t^2 + O(\Delta t^4)\ldots$$

Verlet Integrator

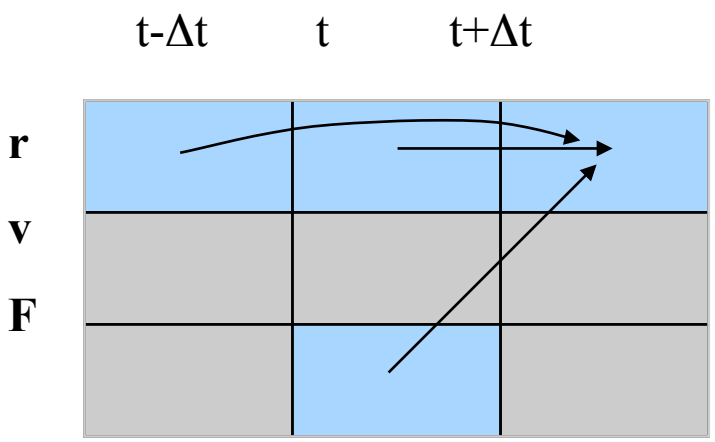


Compute forces at the current position using current positions

$$a_n = F_n / m_n$$

$$r_n(t + \Delta t) = 2r_n(t) - r_n(t - \Delta t) + a_n(t)\Delta t^2 + O(\Delta t^4)\ldots$$

Verlet Integrator



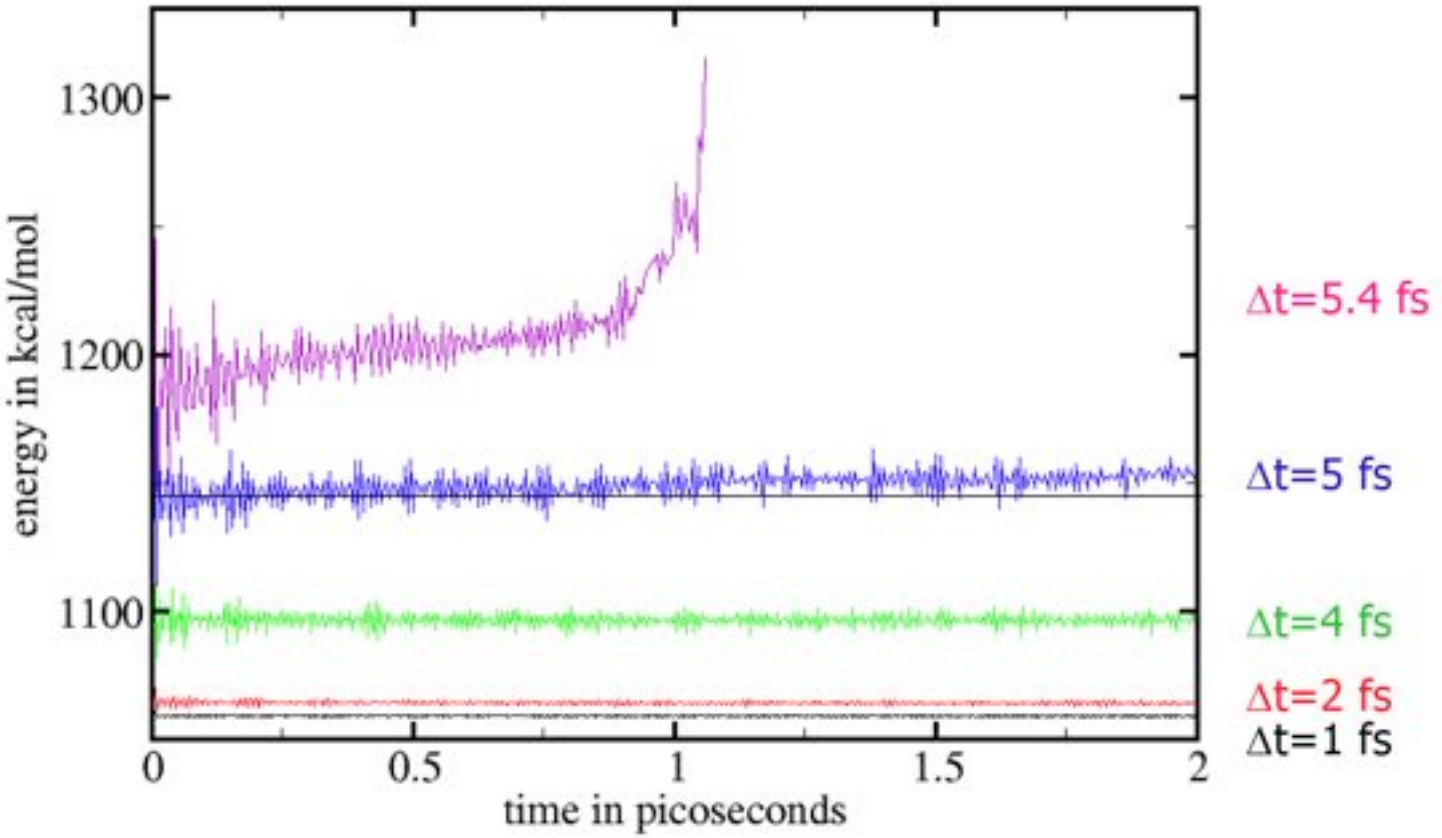
Compute new position from present and previous positions, and present force

$$r_n(t+\Delta t) = 2r_n(t) - r_n(t-\Delta t) + a_n(t)\Delta t^2 + O(\Delta t^4)\ldots$$

➔ Advance to next step and repeat

Time Step in MD simulation

- If time step is too large, some energies (and forces) may suddenly get too large (e.g. because of hard sphere repulsion or bond over-stretching) and simulation "explodes".



Temperature Control

- Langevin Dynamics

$$m_n \frac{d^2 r_n}{dt^2} = F_n - \gamma_n m_n \frac{dr_n}{dt} + R_n(t)$$

Friction and random force are added to the systematic forces.

Motivation: representation of a simple heat bath by accounting for molecular collisions

γ: collision parameter

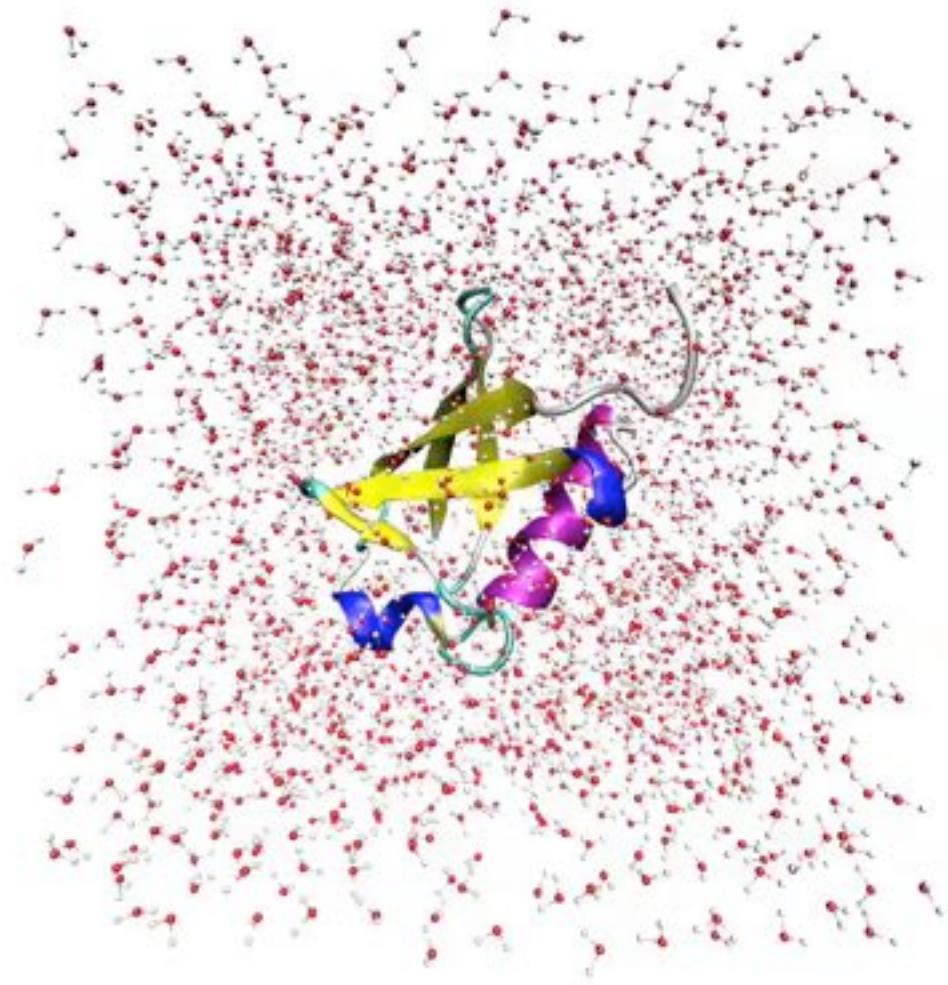
R: random force vector

The strengths of random forces should follow

$\langle R(t) \rangle = 0$, $\langle R(t) R(t')^T \rangle = 2\gamma k_B T_m \delta(t-t')$

Solvent Box

- Protein in a box of water
- Used with *periodic boundary condition*
- No interface artifact
- But artifact of *mirror images*



Periodic Boundary Condition

- Protein and solvent are placed in the unit cell, surrounded by infinitely many copies in space
- Atoms go out, then come back from opposite side
- Cell has to be large enough to minimize artifacts
  - At least a 10Å layer of water molecules

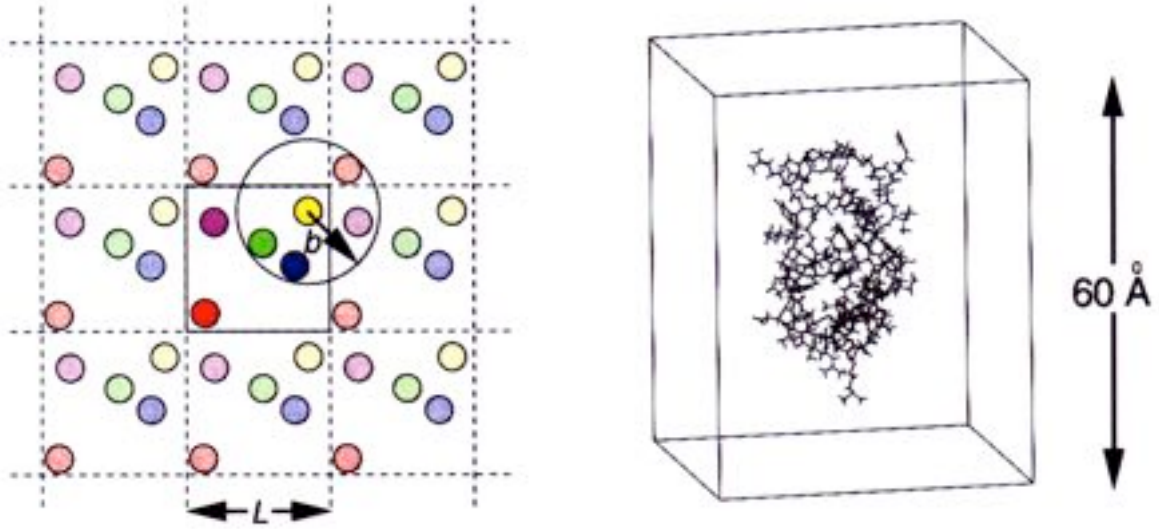
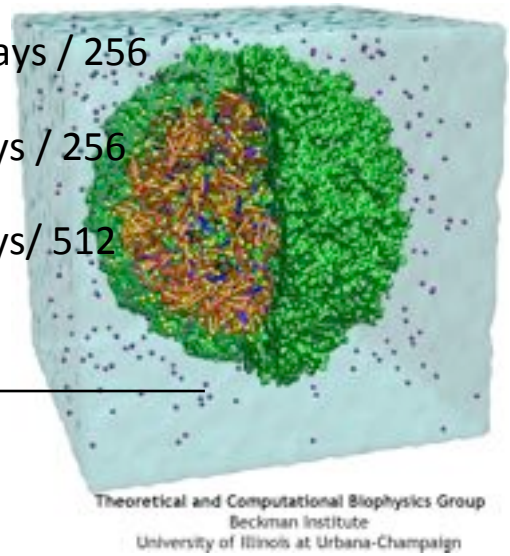


Figure 9.4. Periodic domain in 2D, showing the unit cell (center) and its images (surrounding replicas), and in 3D (rectangular), as used for a solvated protein (BPTI) system [609].

MD Simulation History

$$F_n = m_n a_n = -\nabla_n U(r_n) = m_n \frac{d^2 r_n}{dt^2}$$

Year	Protein	Atoms	Simulation (ns)	CPU time / processors
1977	BPTI	885	0.01	
1985	Myoglobin	1,423	0.3	50 days / 1
1992	HIV protease	25,000	0.1	4 days / 1
1998	Villin headpiece	12,000	1000	120 days / 256
2006	STM Virus	1,000,000	10	10 days / 256
2014	Protein*	24,000	2.5×10^6 (= 2.5 ms)	30 days/ 512
2020	Protein	40,000	10^9 (=1 sec)	?????



1974: Rahman & Stillinger - simulation of liquid water

* Anton 2 – DE Shaw

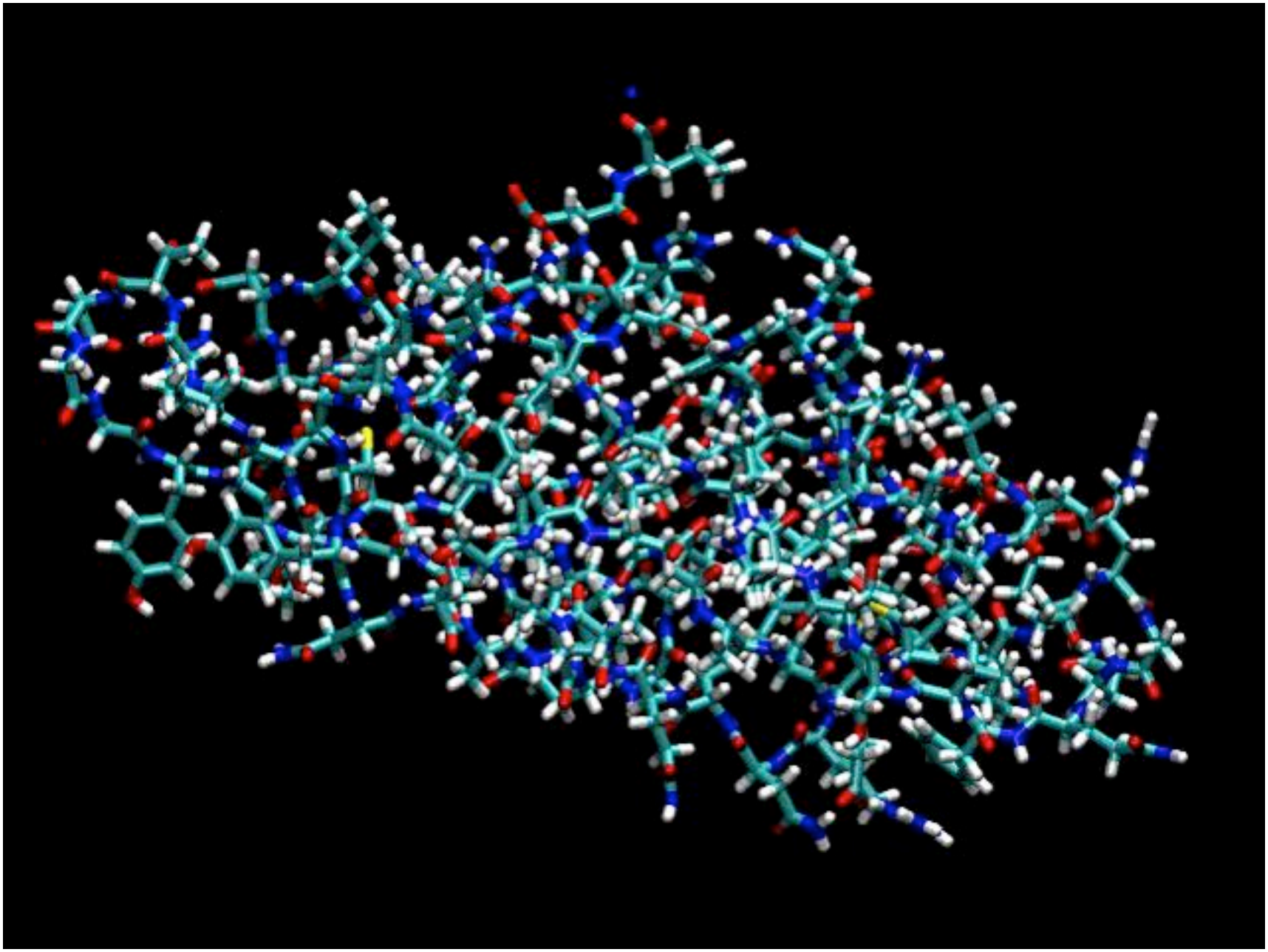
What can Molecular Dynamics Simulation Do ?

- Atomic detail information of dynamics
- Complete control
  - Output is created solely by inputs (parameters, structure, equations, algorithms, sampling etc…)

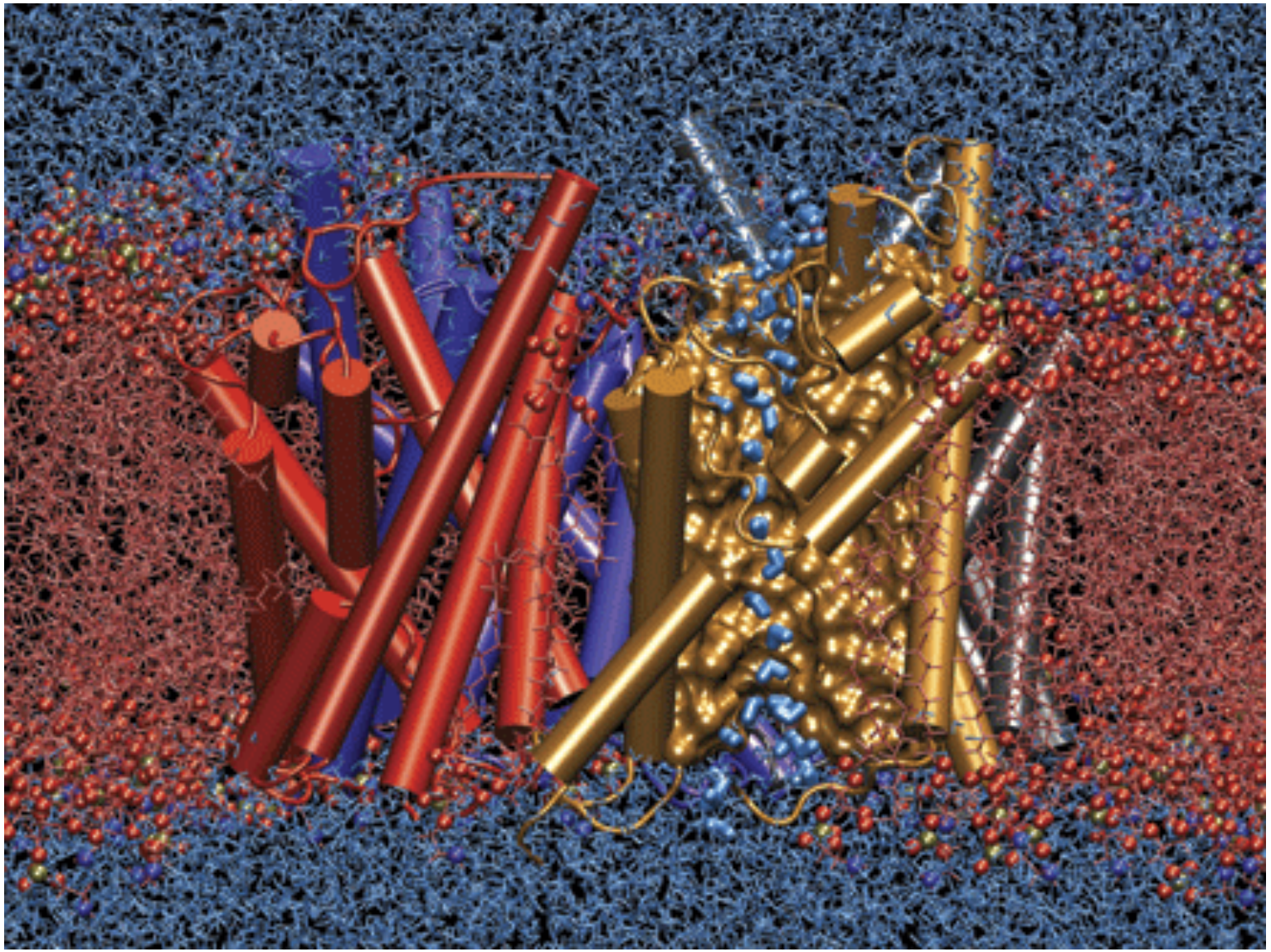
- But there are some limitations …

Molecular Dynamics Limitations

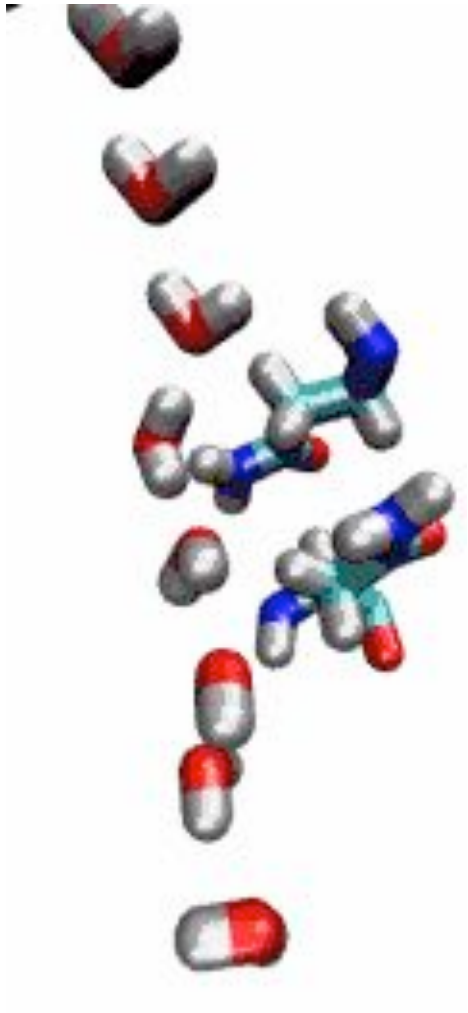
- Limited time scale
  - 1ns is easy, 1μs is difficult to reach
- Limited system size
  - Larger system needs more computation
- No chemical reactions
  - No bond breaking
- Statistical error due to insufficient sampling
  - Statistical accuracy needs to be checked

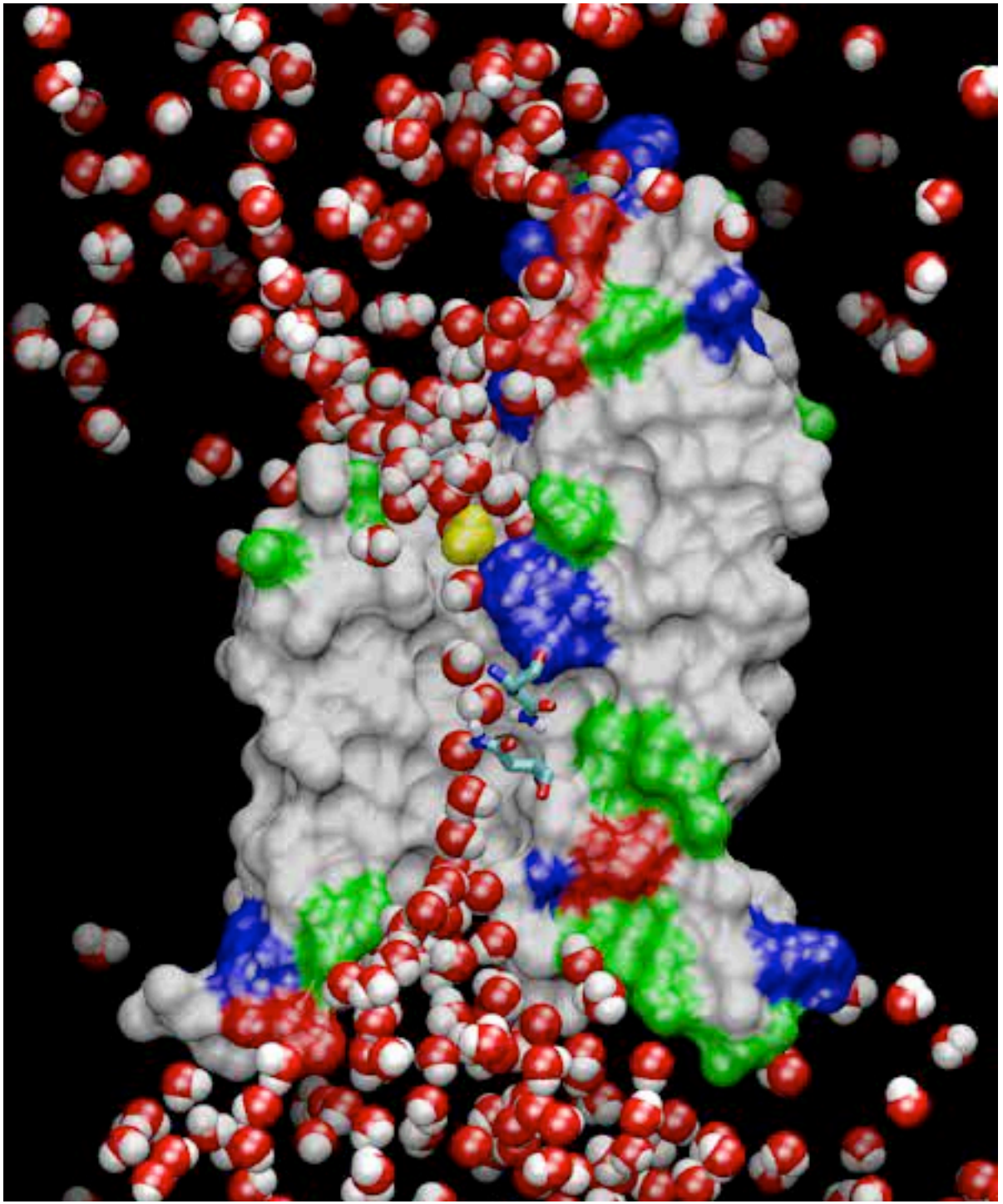


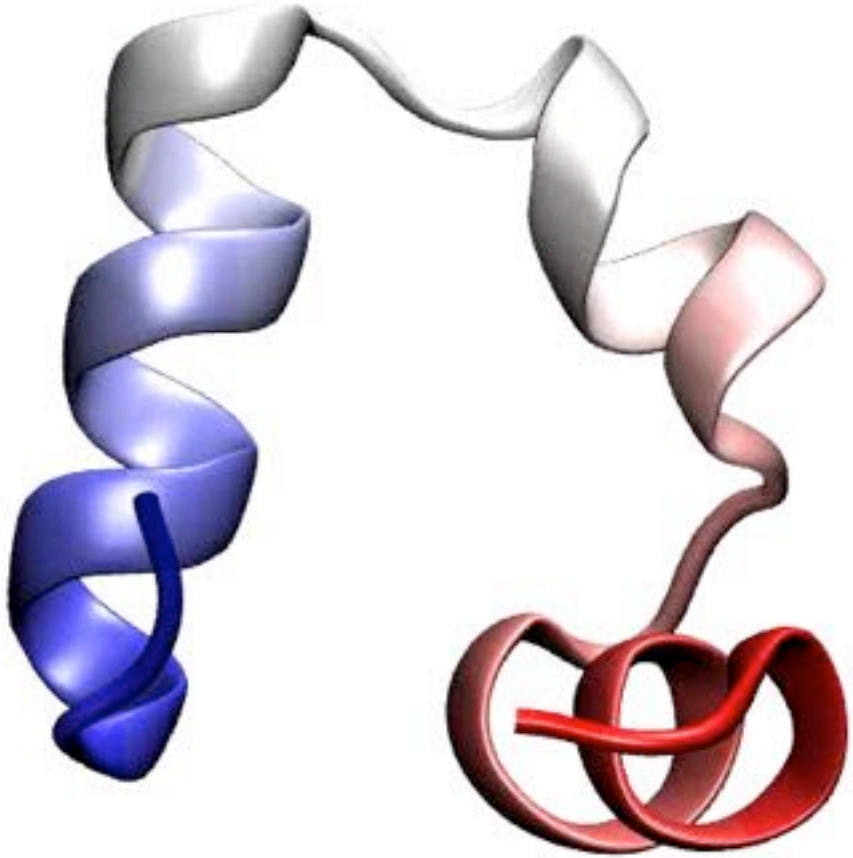
Aquaporin Water Channel



Tajkhorshid et al, Science 296, 525-530 (2002)





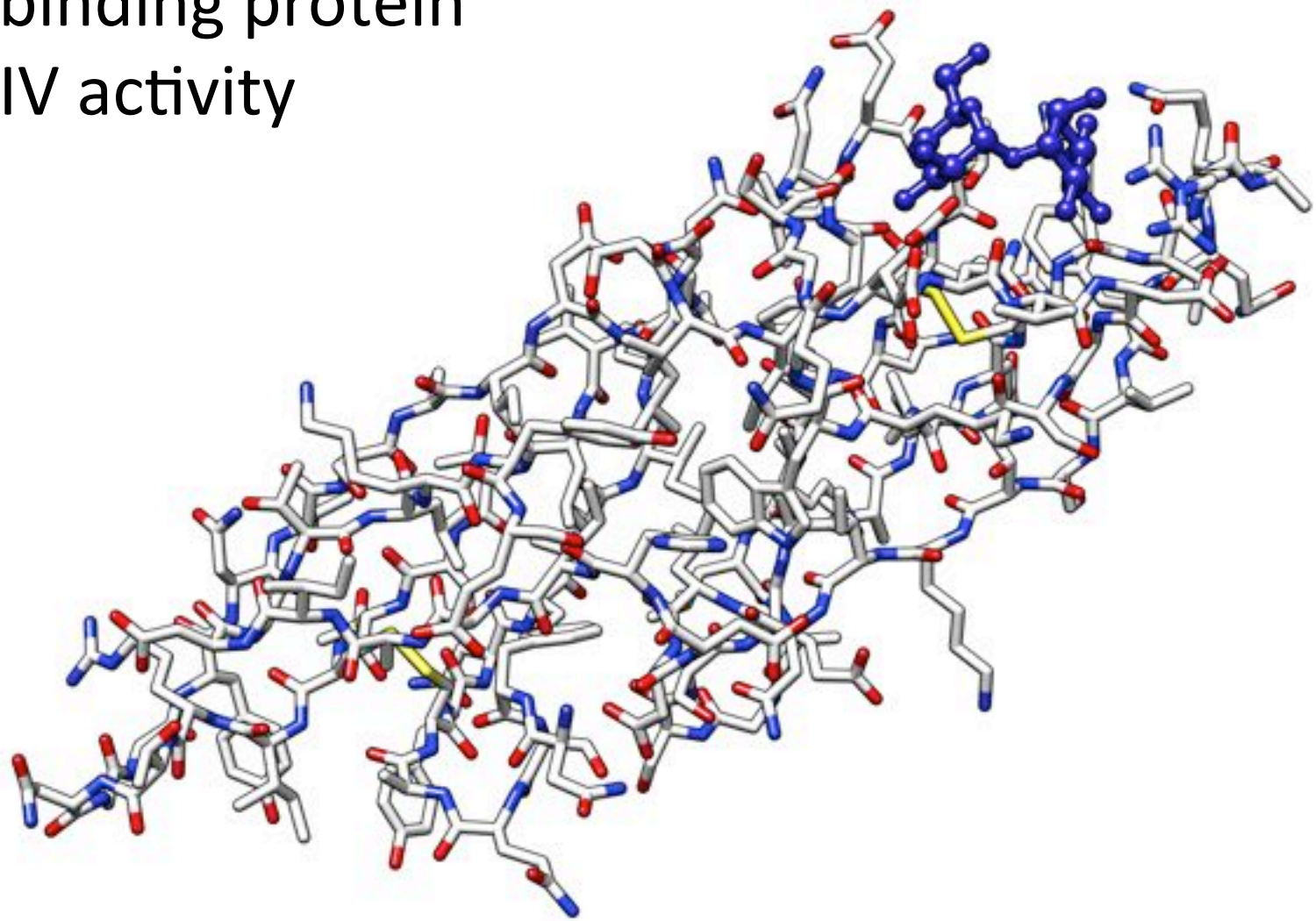


Information from Simulation

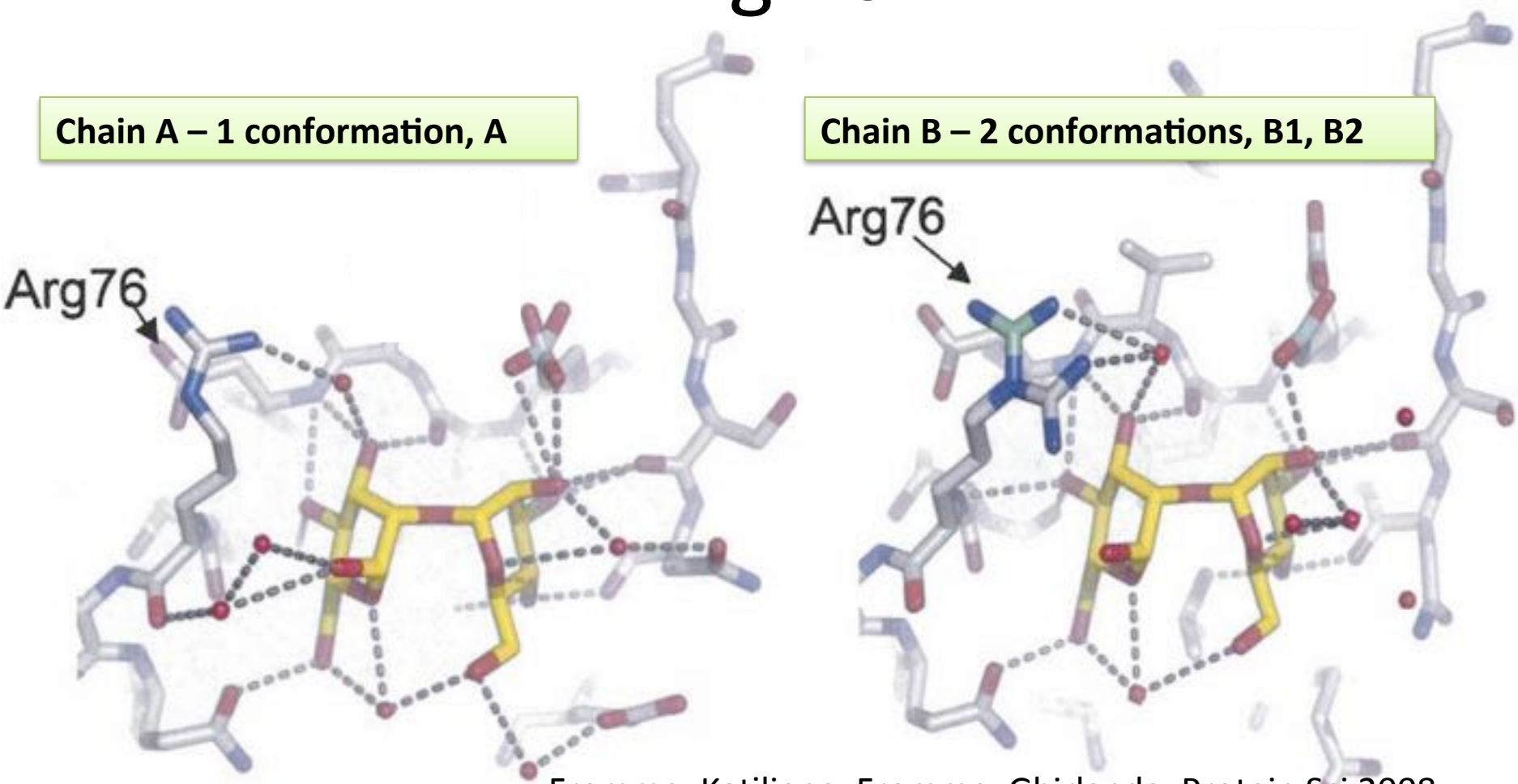
- Detail information inaccessible by experiments
  - Time course of biological events
  - Motion of each atom
  - Interaction energy
  - RMSD, energy, volume…
  - There are too many information…
  - ***We need to analyze huge number of sampled structures***
    - ***Clustering of the structures***
    - ***Network visualization of conformational space***

Cyanovirin

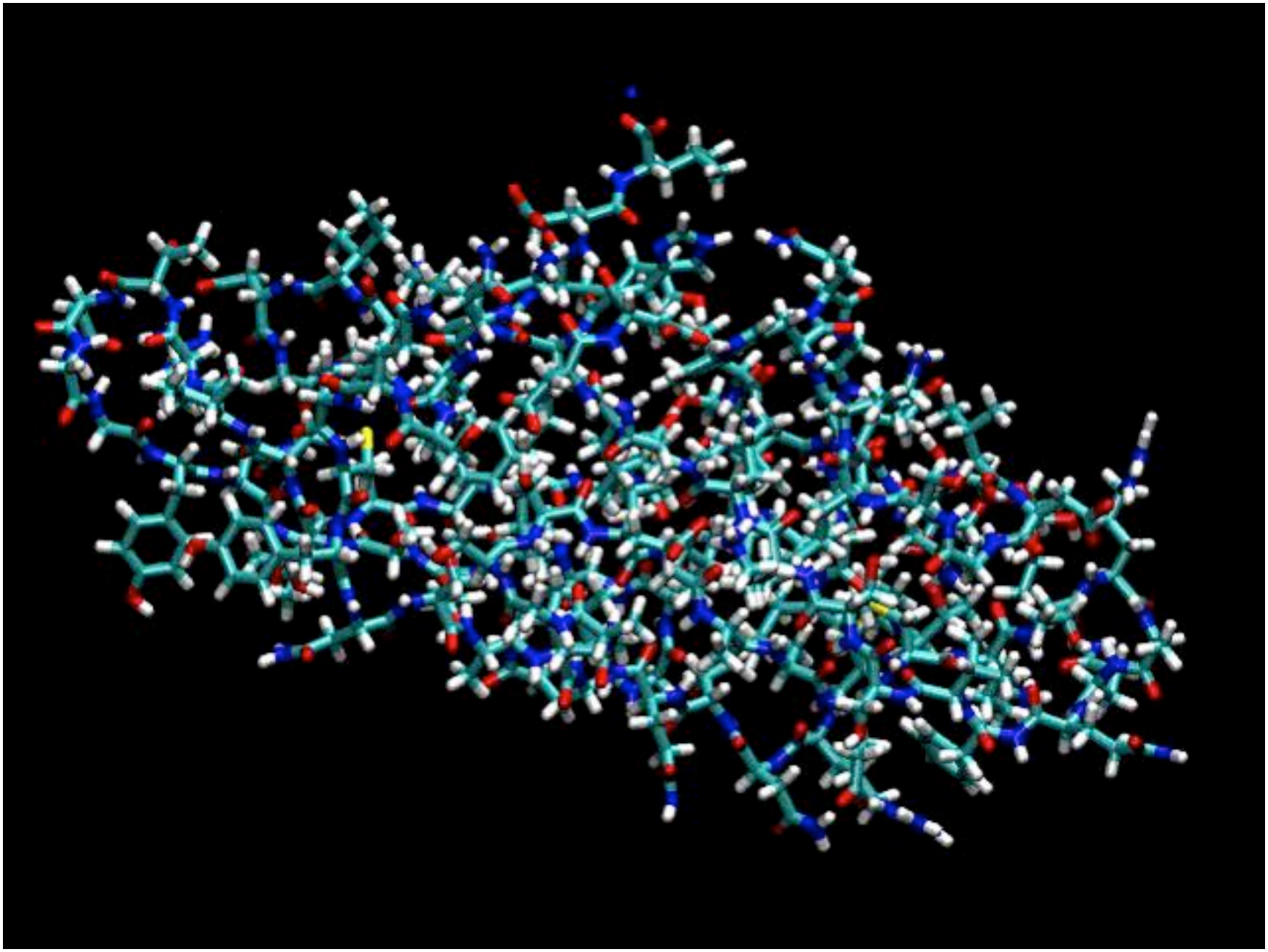
- Sugar binding protein
- Anti HIV activity

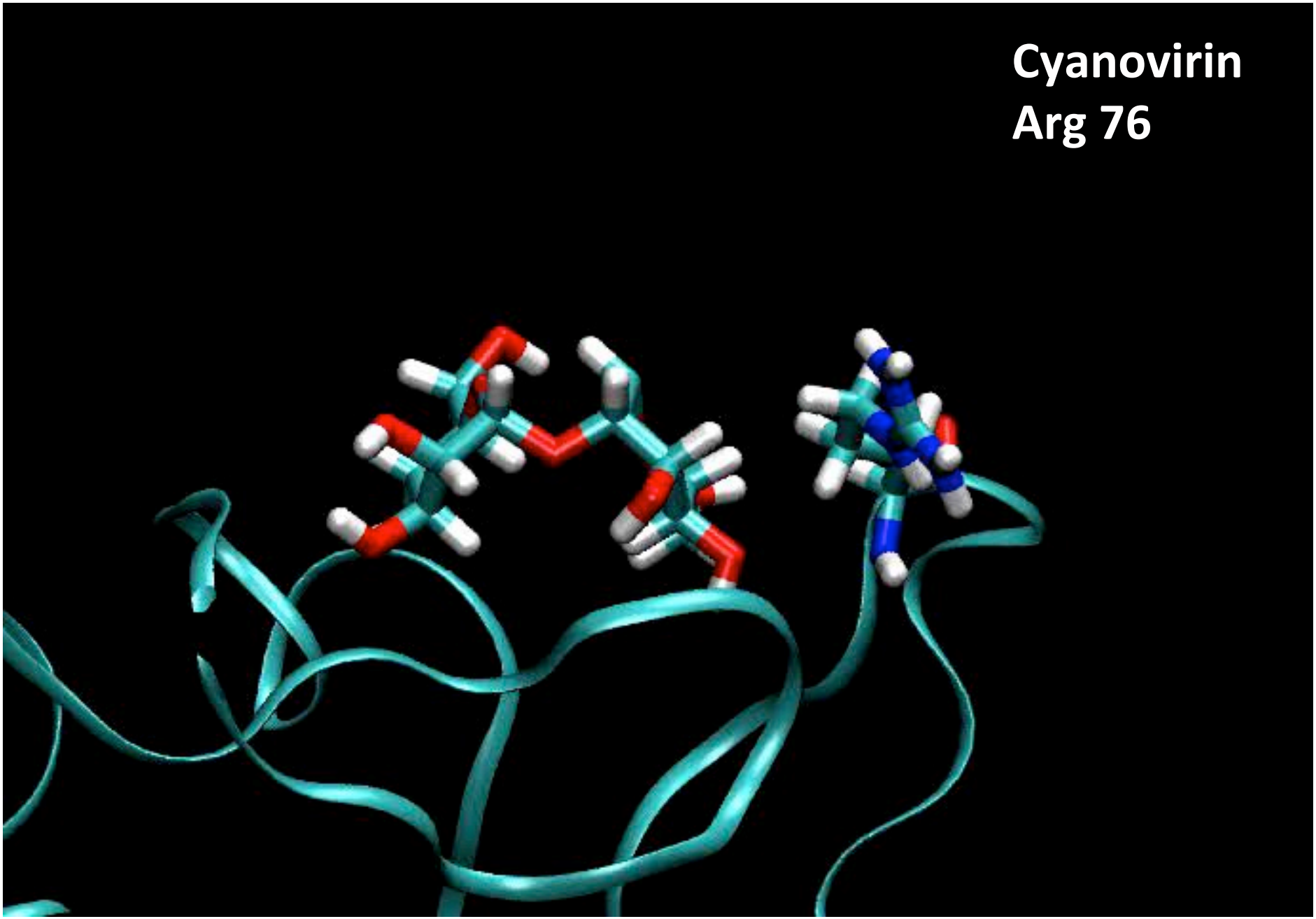


1.3Å high resolution structure of Cyanovirin reveals the dynamics of Arg 76

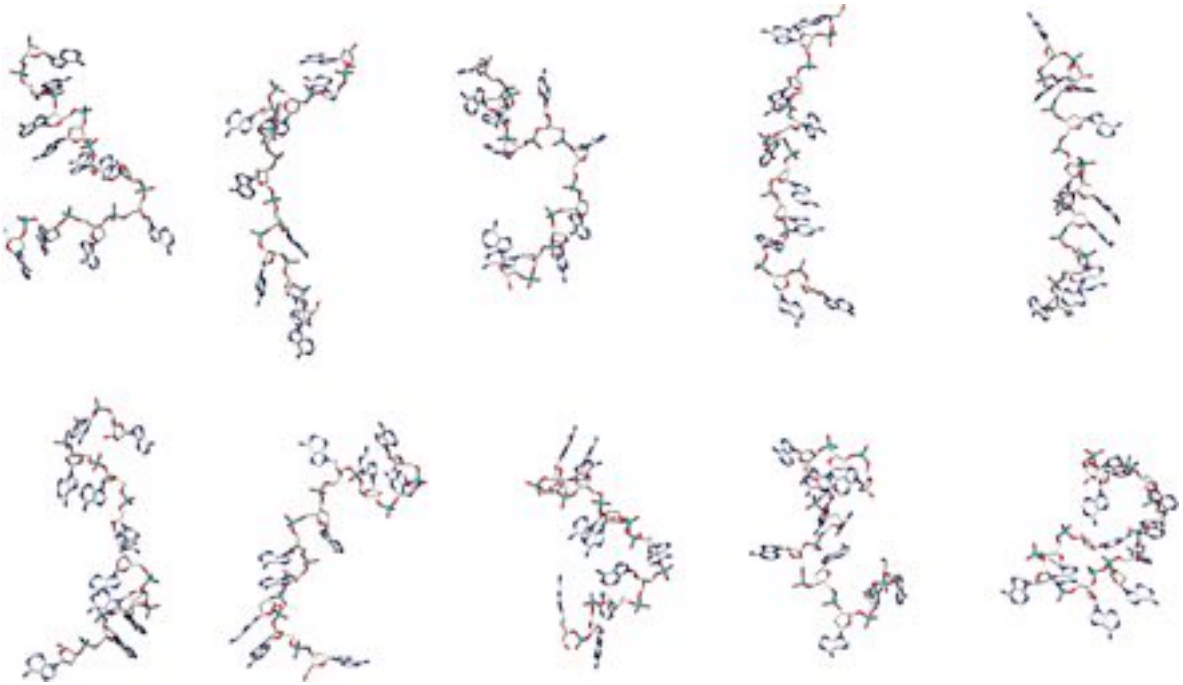


Fromme, Katiliene, Fromme, Ghirlanda, Protein Sci 2008




Cyanovirin
Arg 76

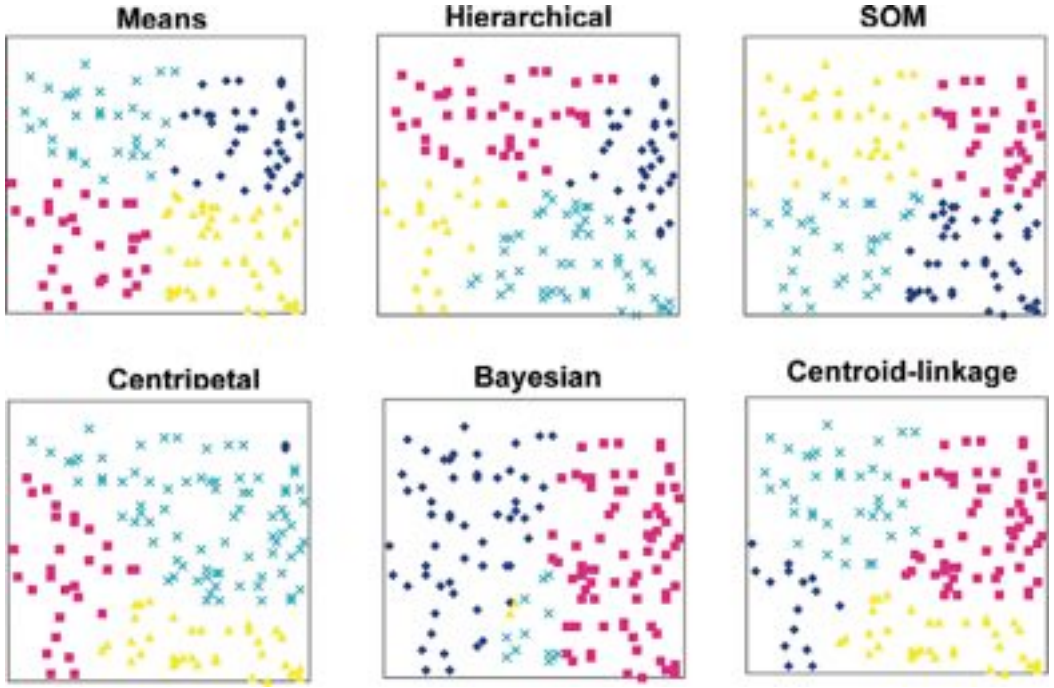
Snapshots of protein dynamics from MD simulations



Some examples of clustering methods

- "Similar" conformations are grouped together
- Different algorithms use different criteria to join groups.
- "Similar" needs to be defined.

$$\mathrm{RMSD} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\delta_i^2}$$

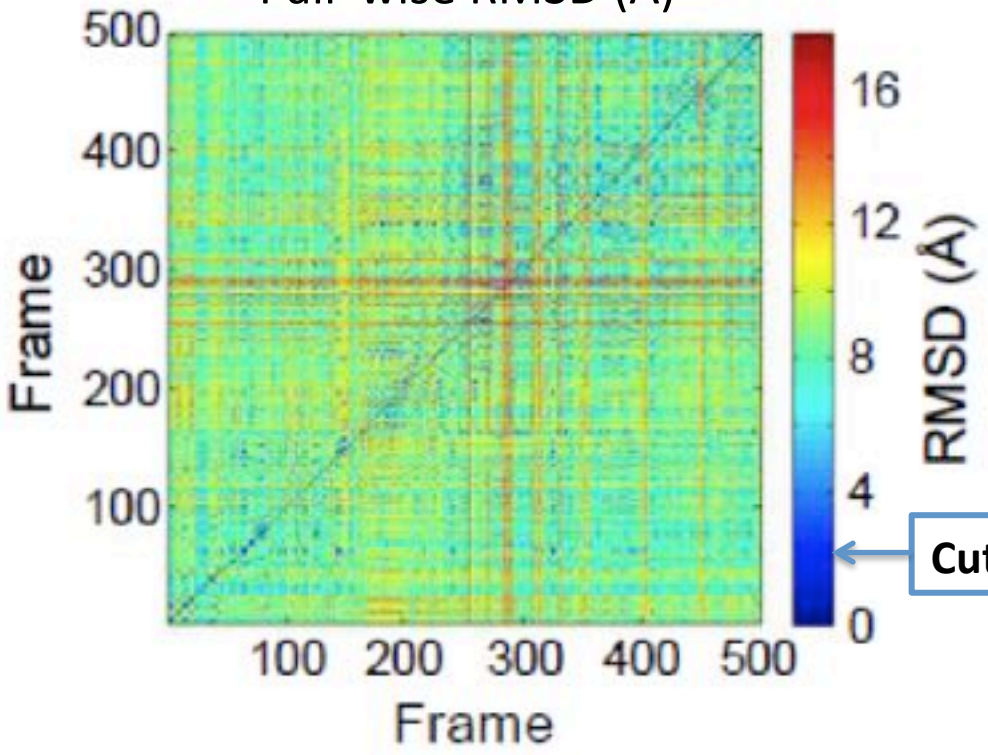


Shao, J. Y., Tanner, S. W., Thompson, N. & Cheatham, T. E. (2007)

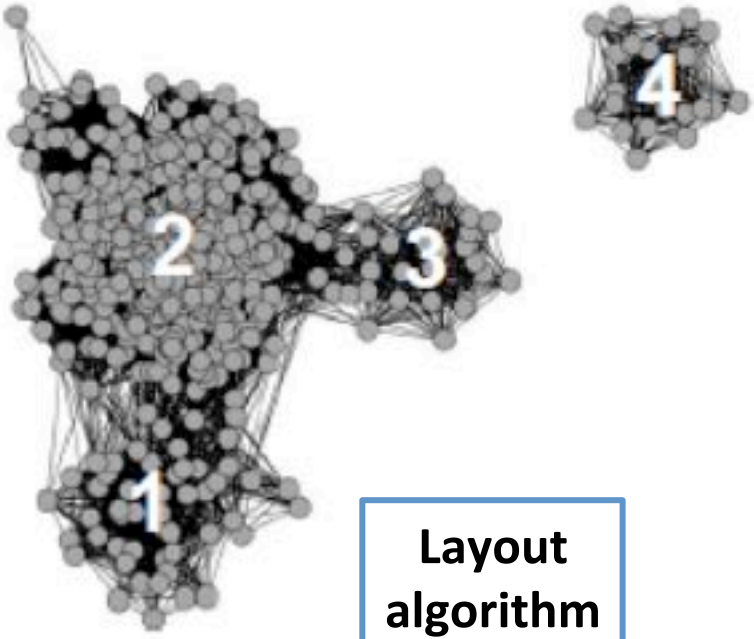
Network visualization of conformational ensemble

Conformational snapshots from MD simulation

Pair-wise RMSD (Å)



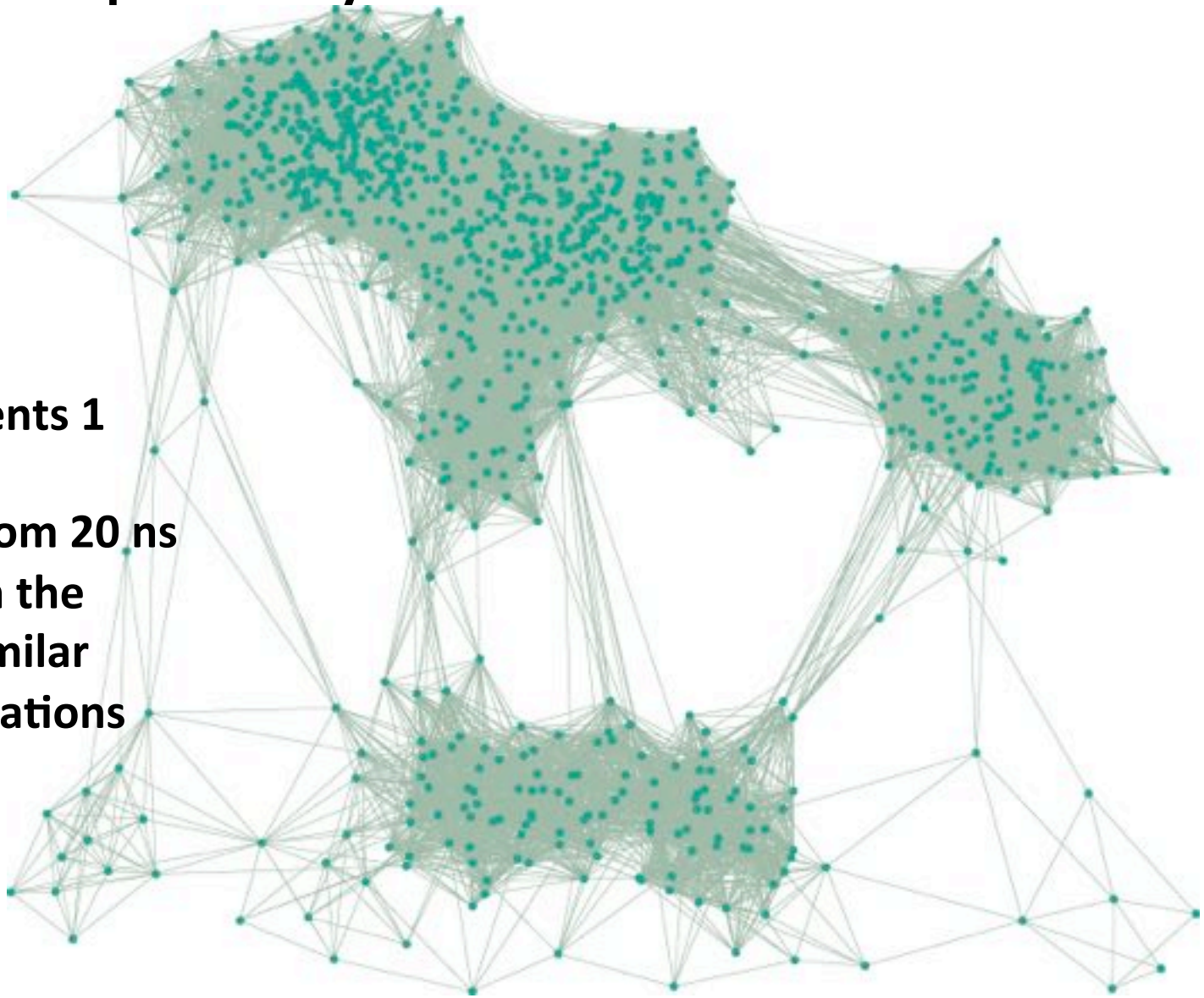
Network connectivity = Conformational similarity



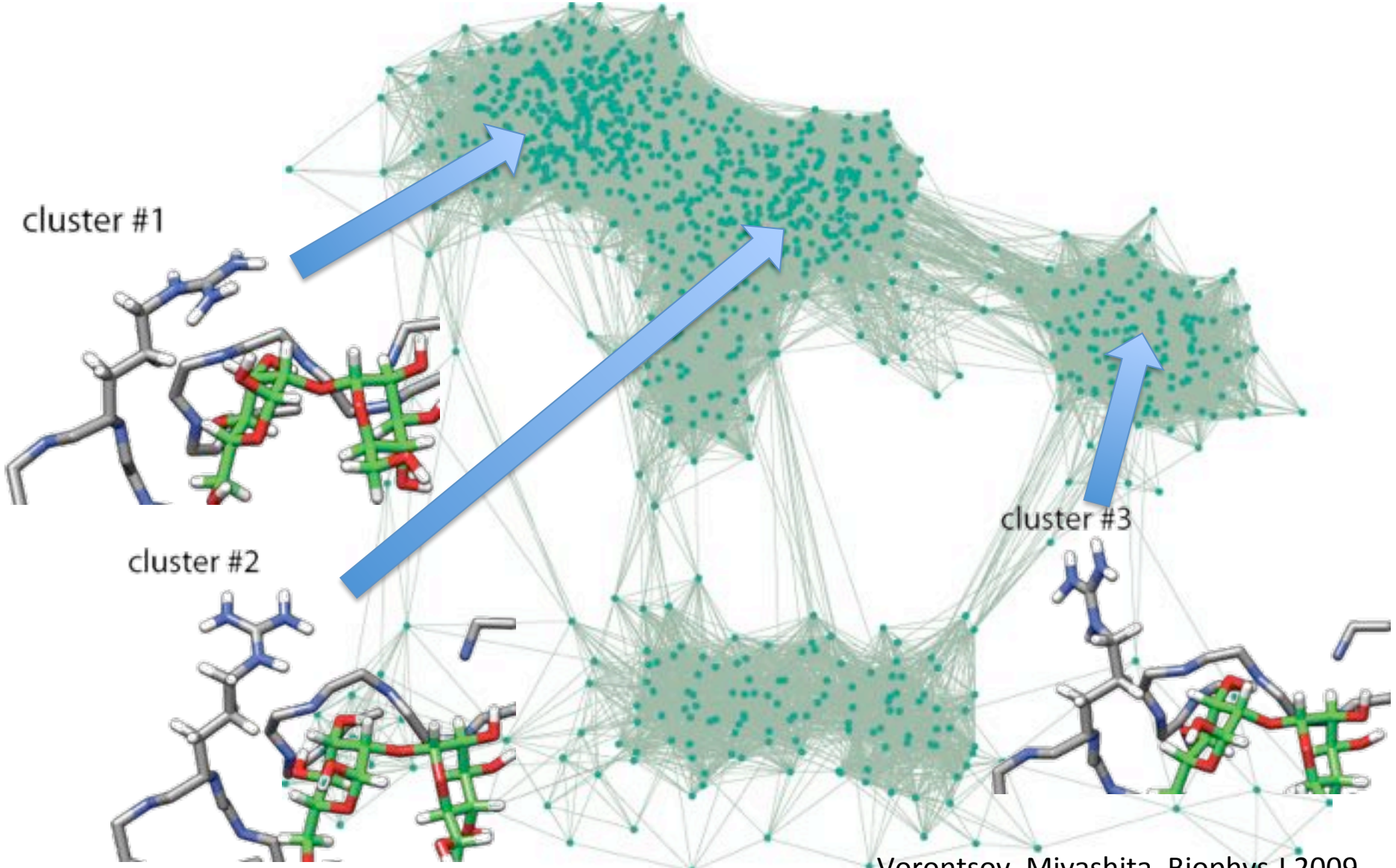
Layout algorithm

Conformational Space of Arg76 sampled by MD simulation

- **1 point represents 1 conformation**
- **1000 frames from 20 ns**
- **Edges between the frames with similar Arg76 conformations**

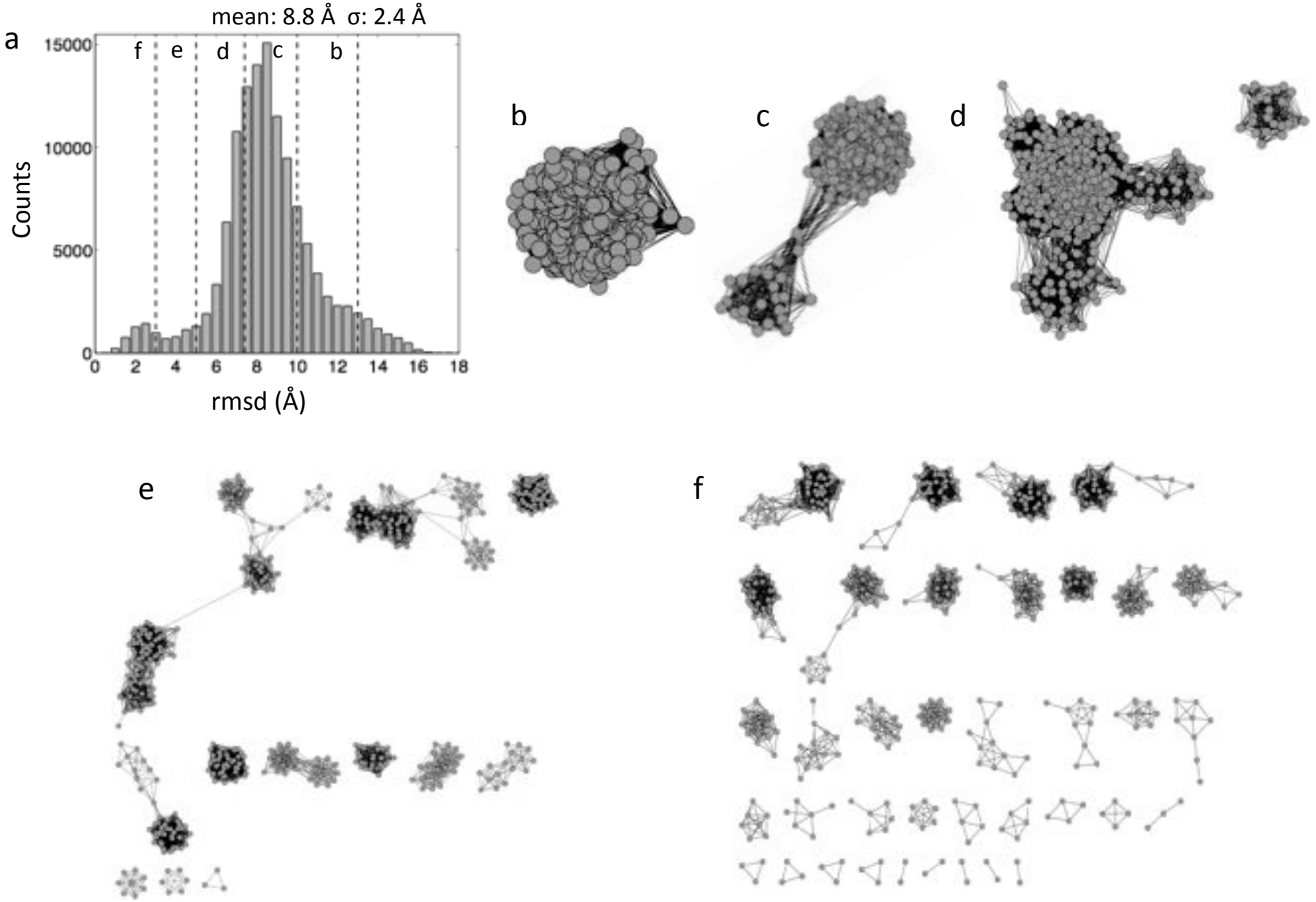


Representative Structures

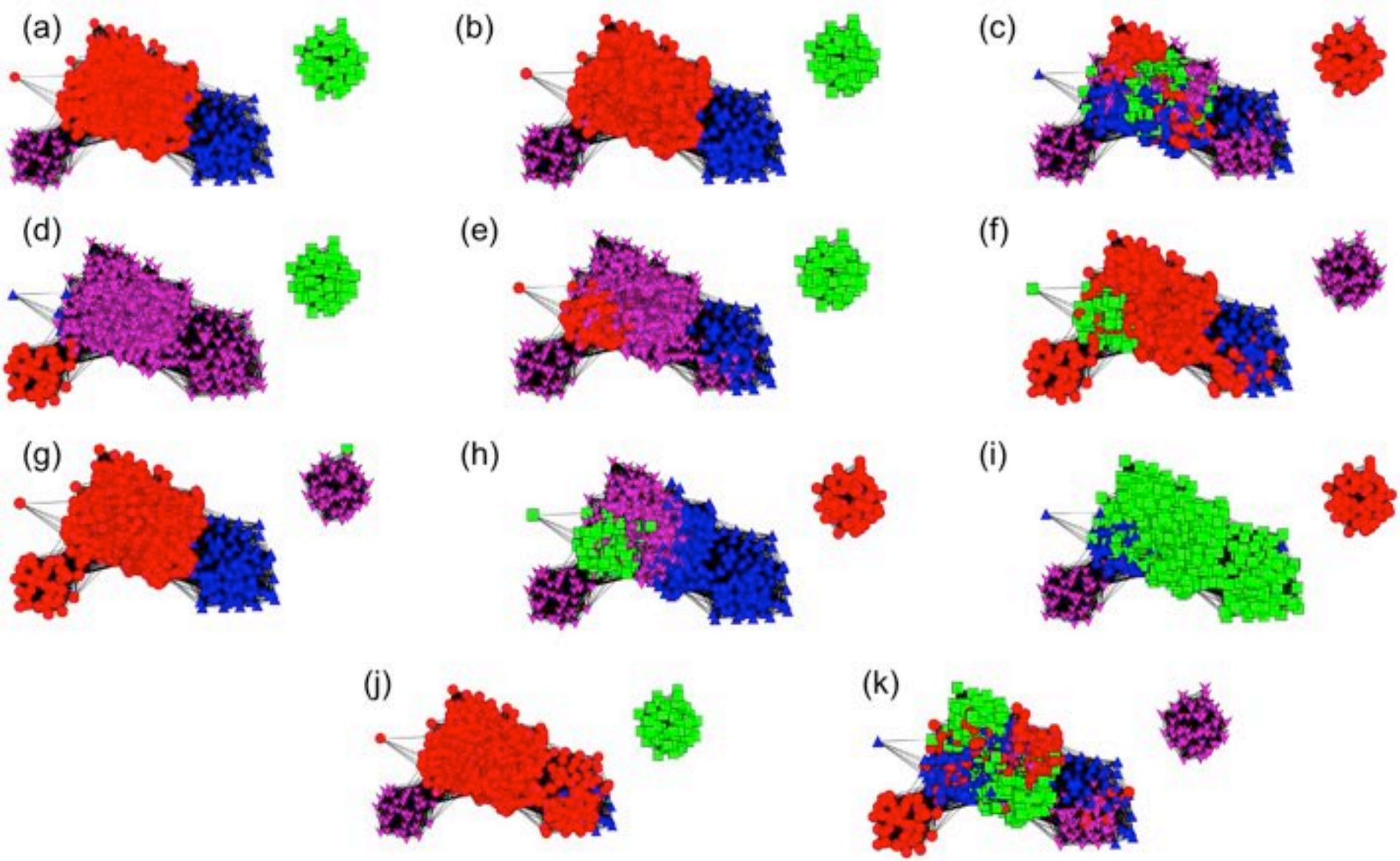


Vorontsov, Miyashita, Biophys J 2009

Cut-off for Network Connectivity



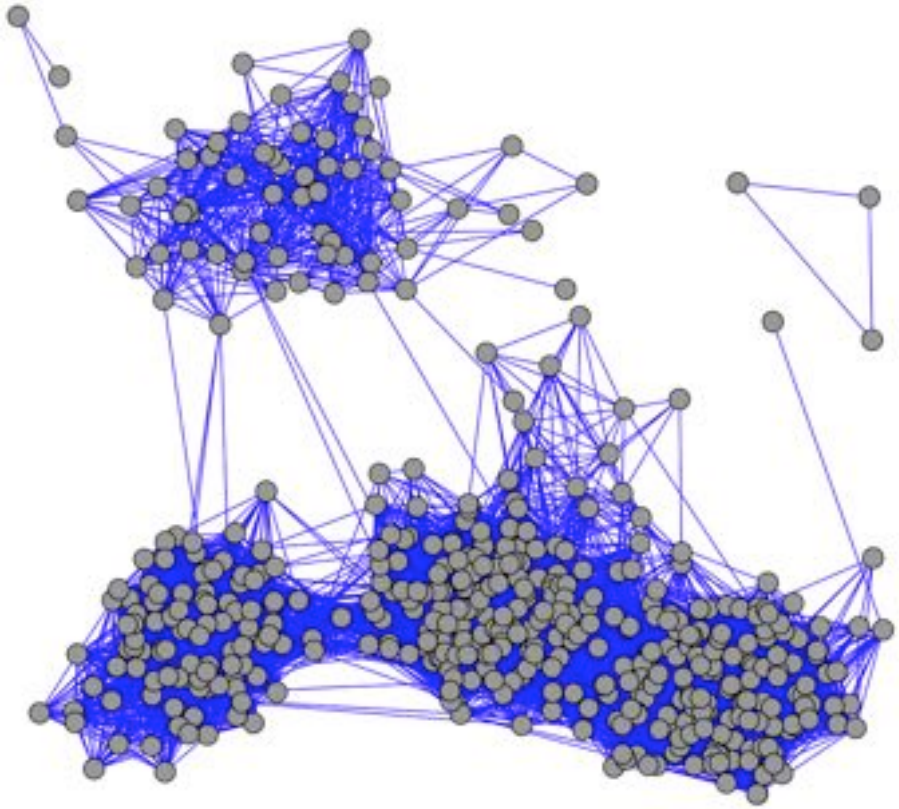
Network v.s. Clustering



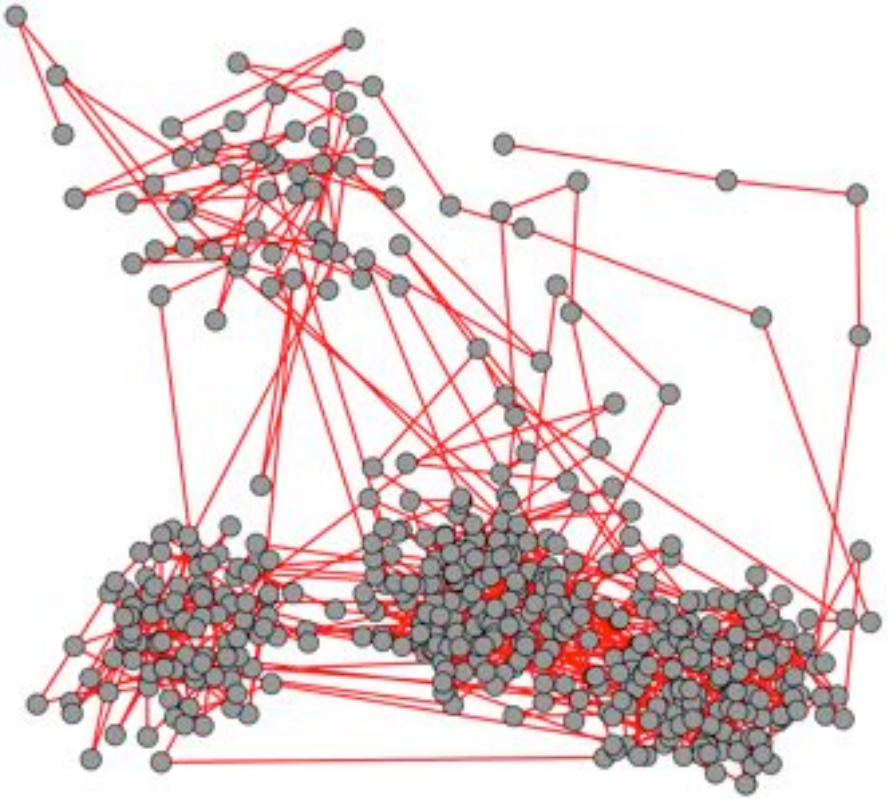
Baker, Ahlstrom, Ehrlich, Vorontsov, Patel, Campbell, Tama, Miyashita, 2013

Conformational Transitions

Network based on Structure Similarity

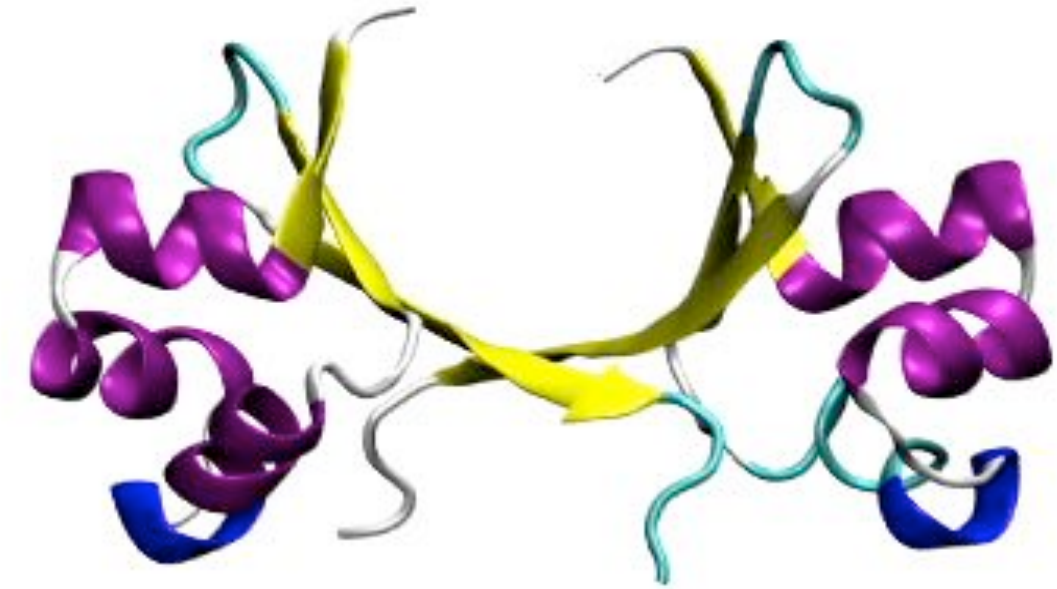


Edges showing Actual Transitions

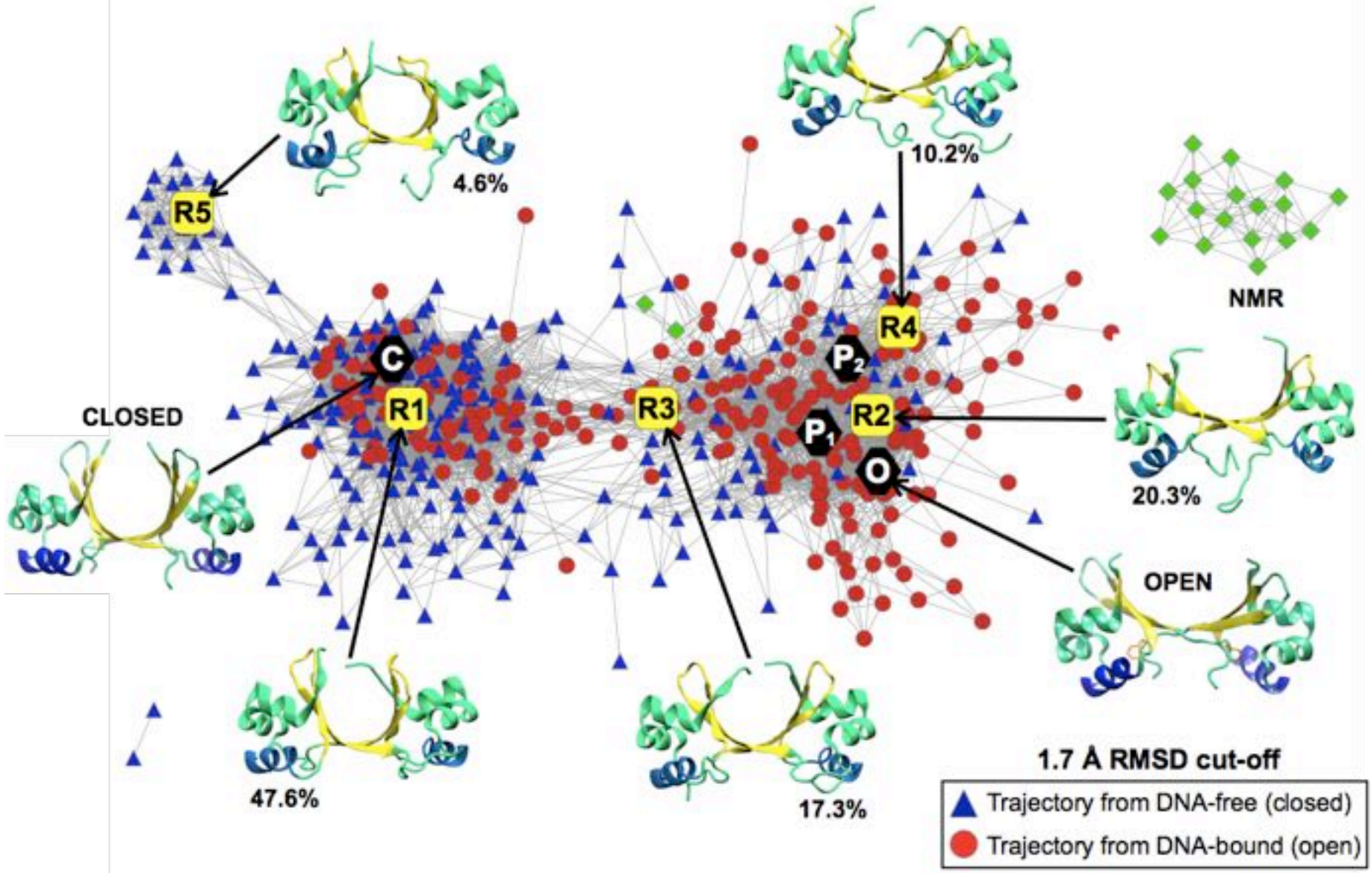


Replica Exchange Molecular Dynamics Simulation of λ Cro Protein

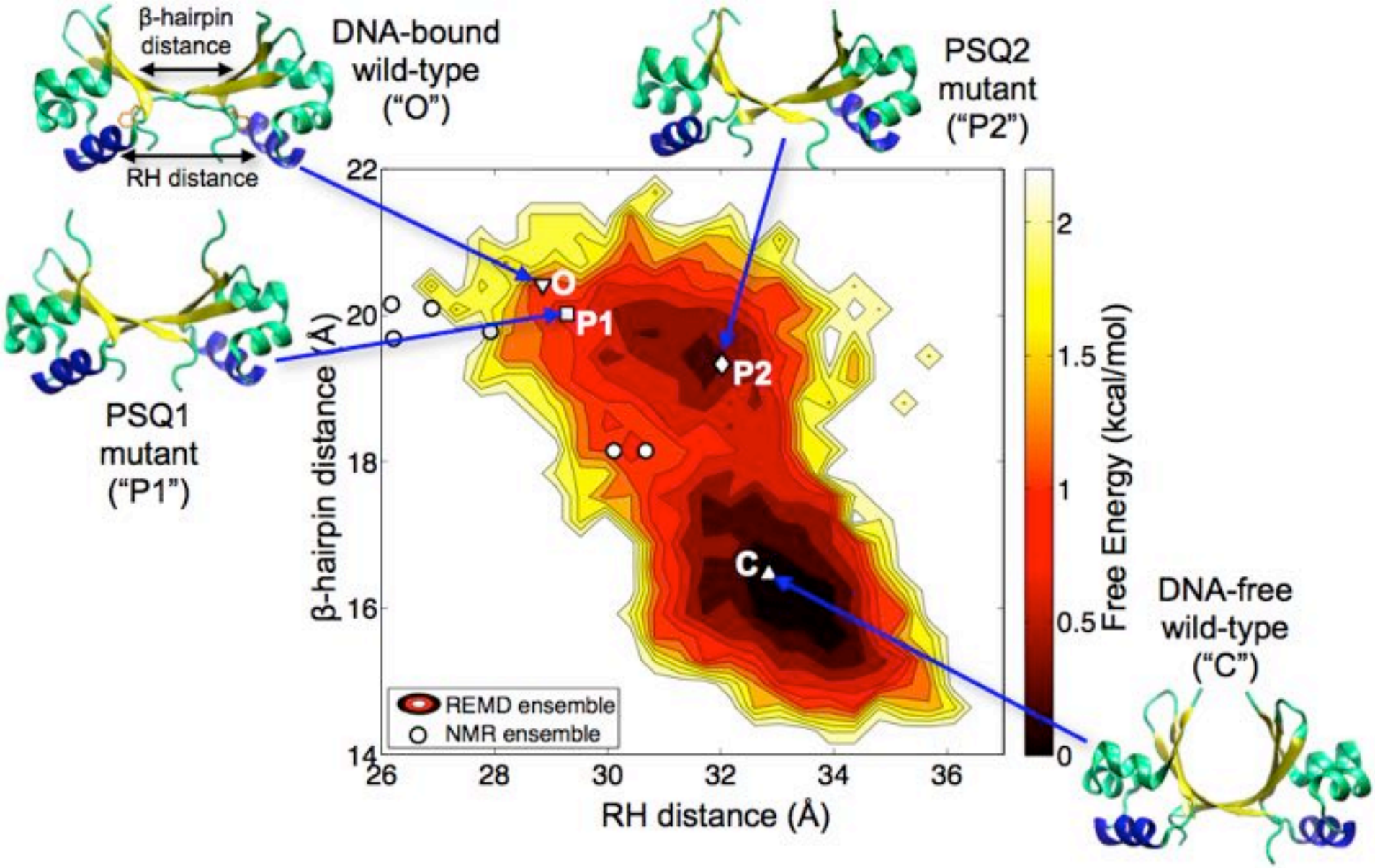
- 132 amino acids, 14,000 waters
- 15 ns equilibration, 15 ns production



Two Stable Conformation in Solution

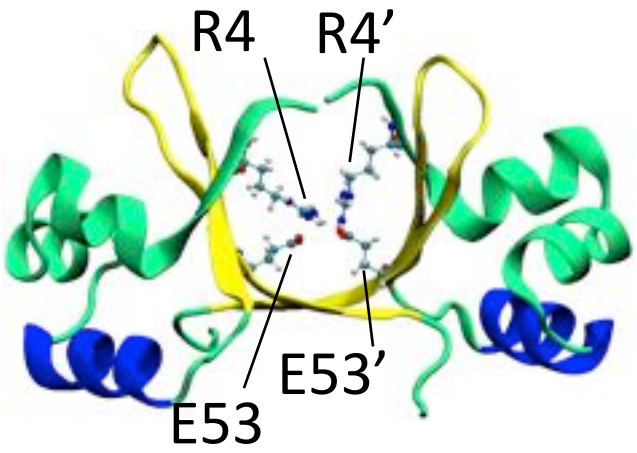


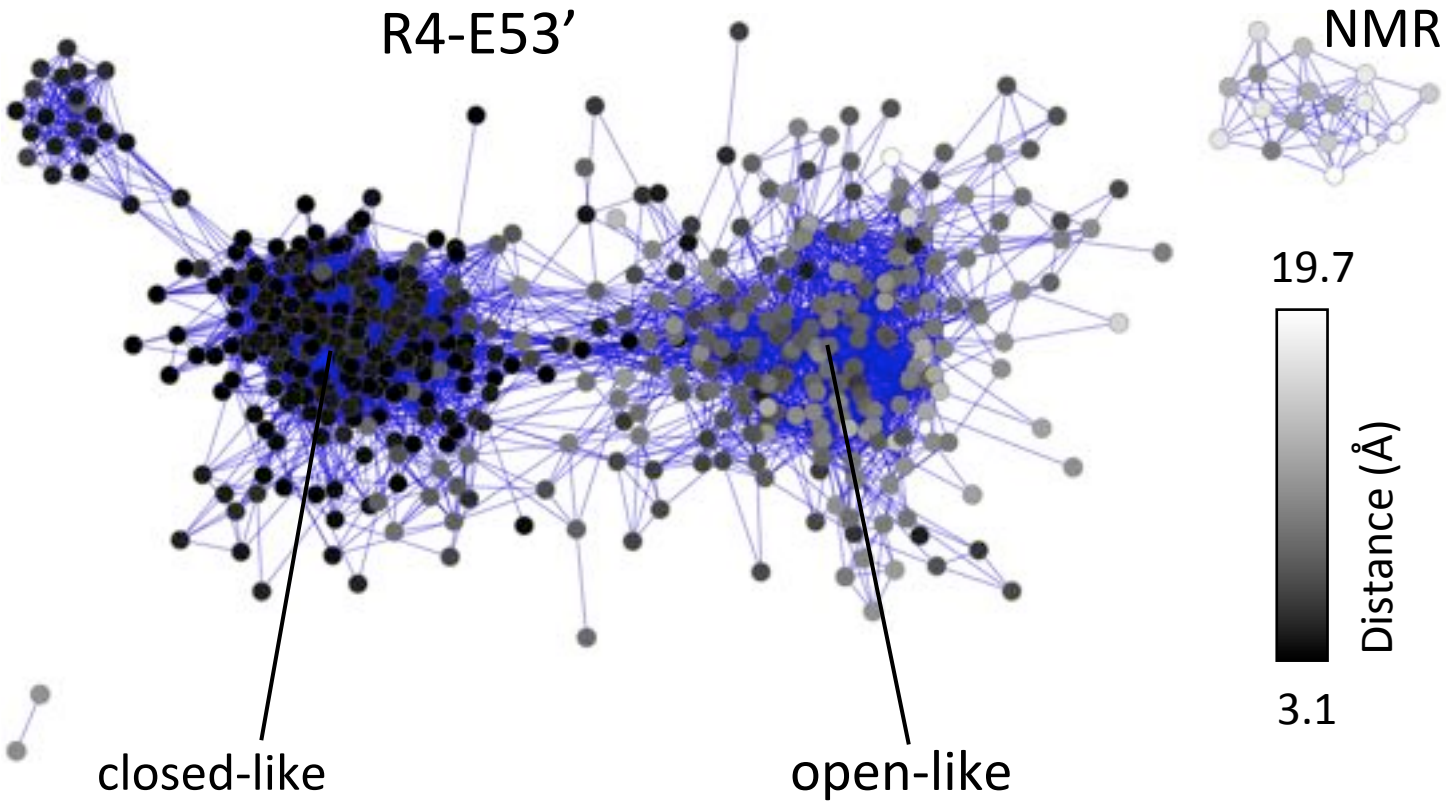
2D Energy Landscape of Cro Conformational Space



Ahlstrom, L.S. and Miyashita, O. *Biophys J 2011*

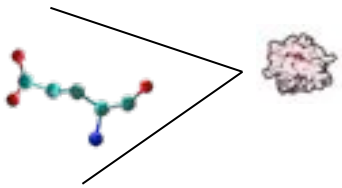
Conformational Transitions Controlled by Salt-bridges



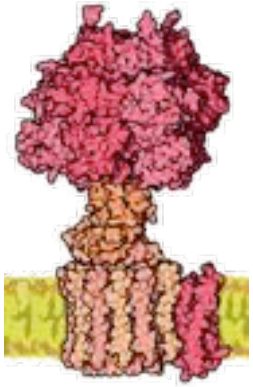


Toward Larger and Larger Biological Systems

protein → supramolecular complexes

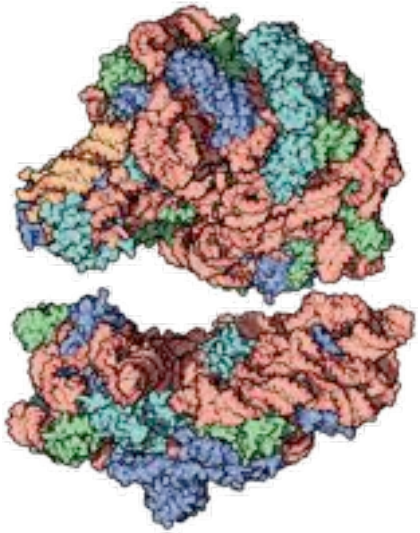


amino acid

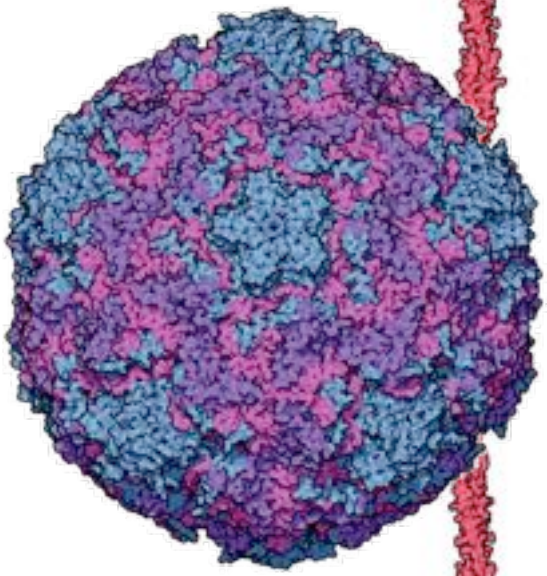


cytochrome ~ 100 aa

ATP synthase ~ 5000 aa



Ribosome ~11000 nucleotides/aa

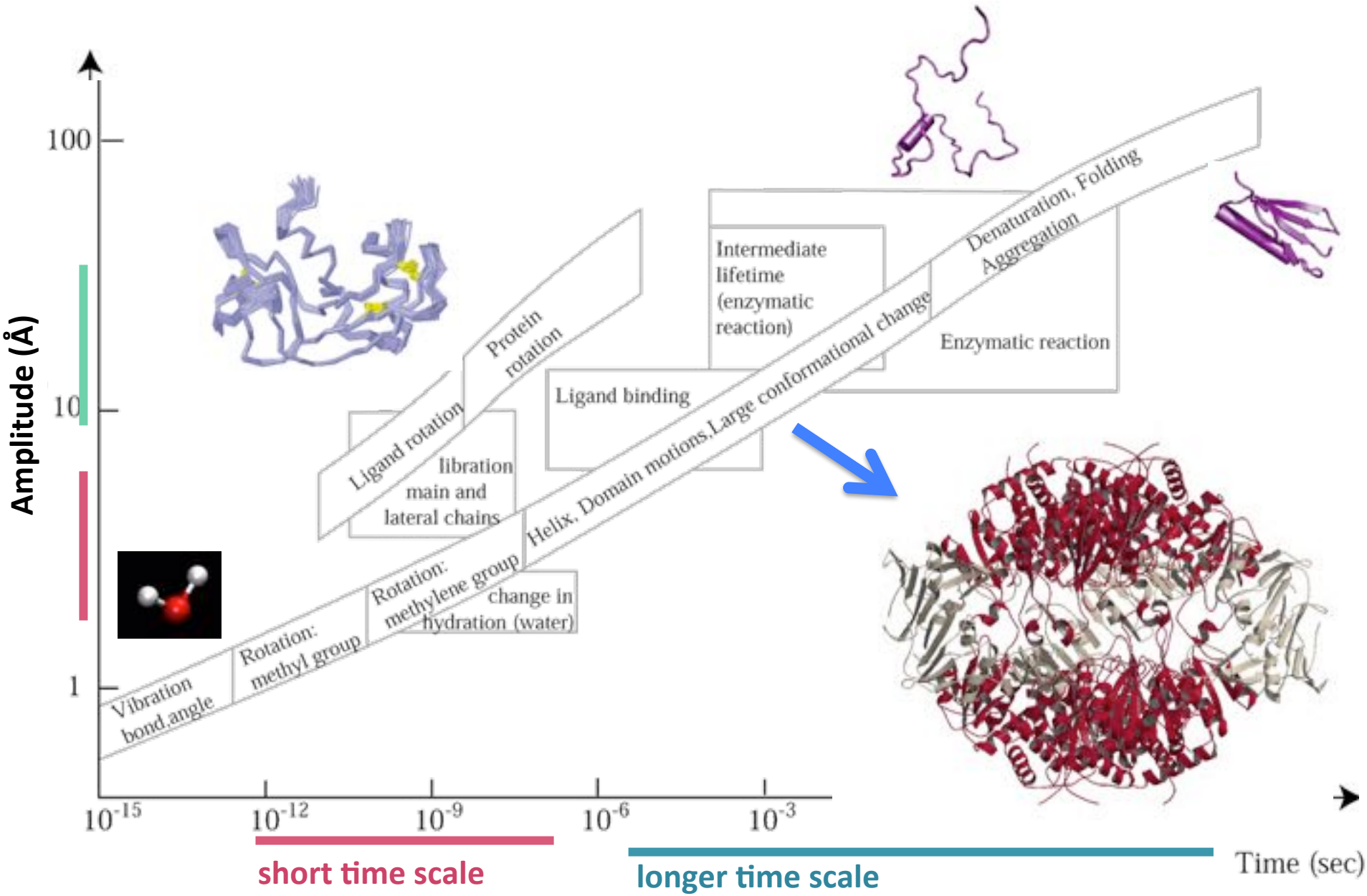


Virus > 30000 aa

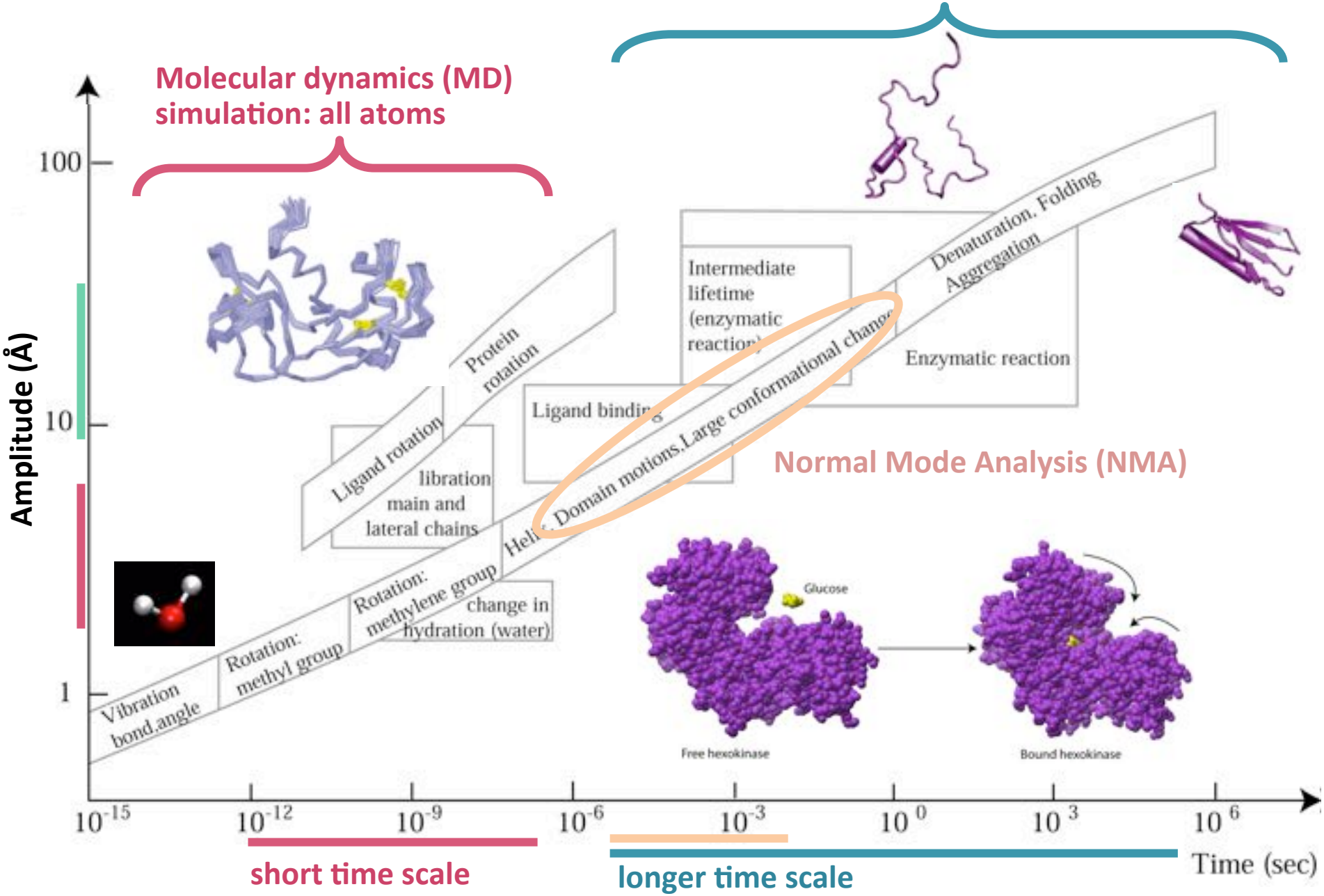
Myosin ~ 4500 aa

http://www.pdb.org/

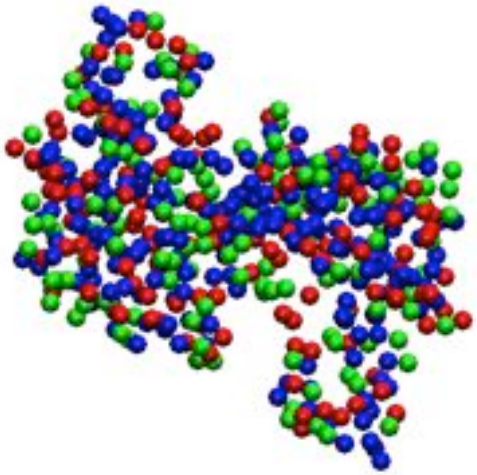
Dynamics in Biological Systems

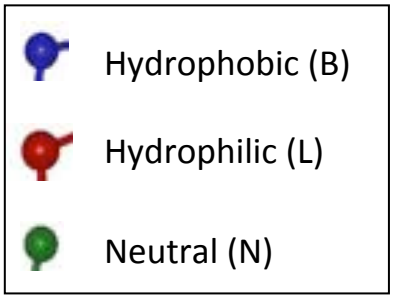


Computation

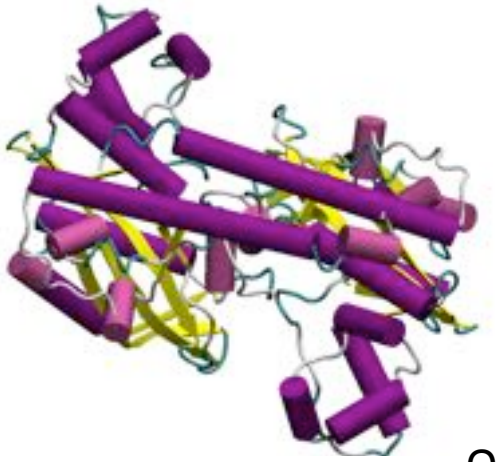


Coarse Grained Models

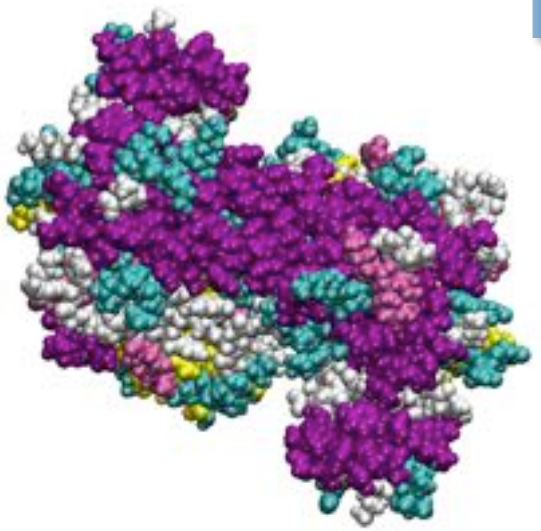


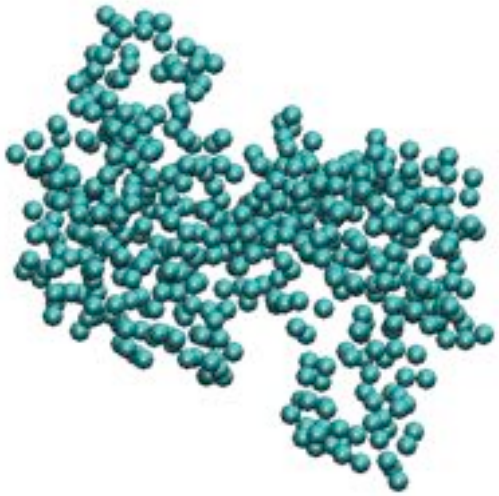


POTENTIAL: $V_{HH} + V_{px} + V_{NX} + V_{bond\ angle} + V_{dihedral}$



One point per residue





POTENTIAL: $V_{CA\text{-}CA}$

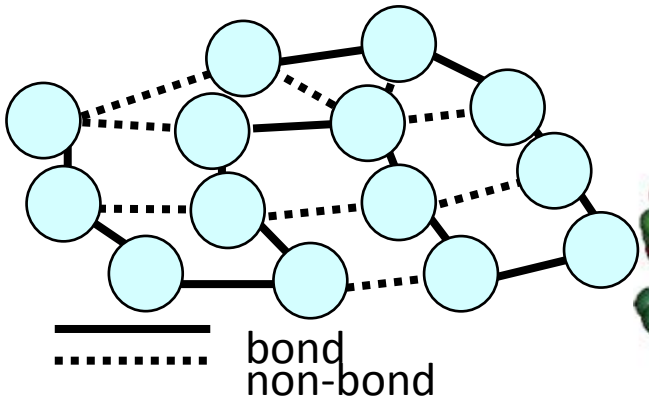
Coarse grained model: Go Model

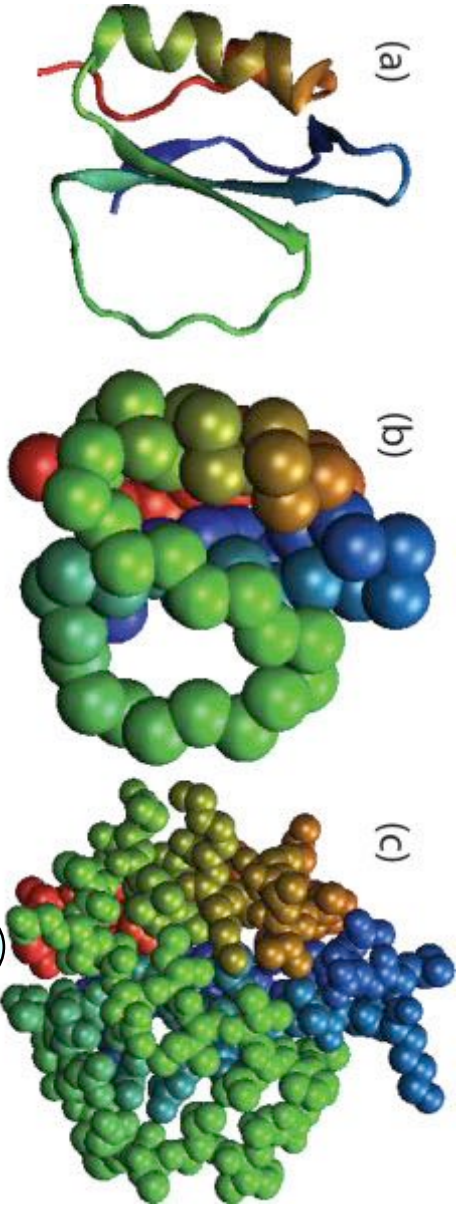
Each atom is represented as a single bead of unit mass.

Bond lengths, bond angles, improper dihedrals, and planar dihedrals are maintained by harmonic potentials.

Nonbonded atom pairs that are in contact in the native structure are given a Lennard-Jones contact potential.

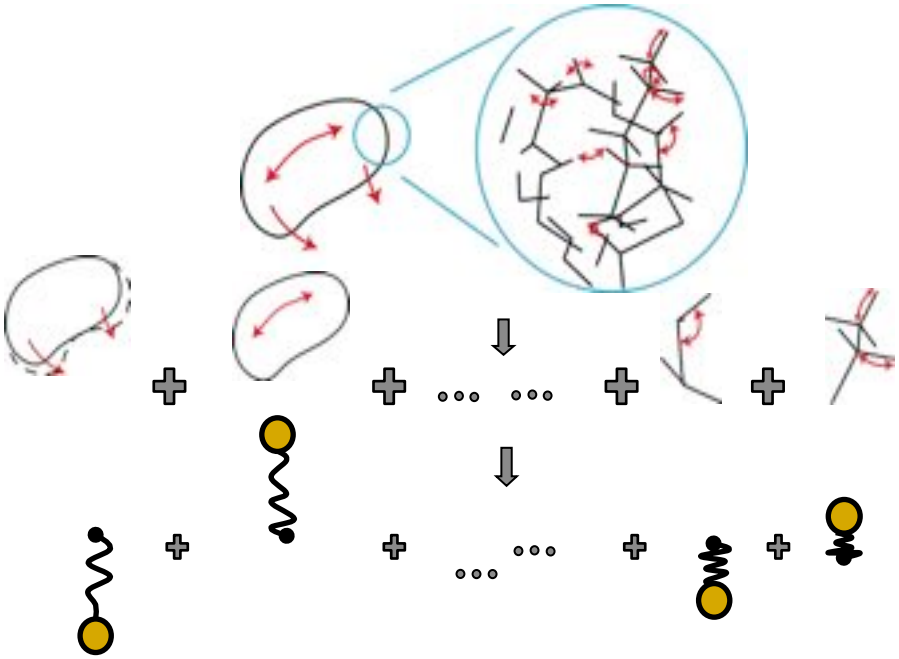
all other nonlocal interactions are repulsive.





Protein Model: Whitford PC et al. Proteins, 2009
RNA Model: Whitford PC et al. Biophys. J. 2009

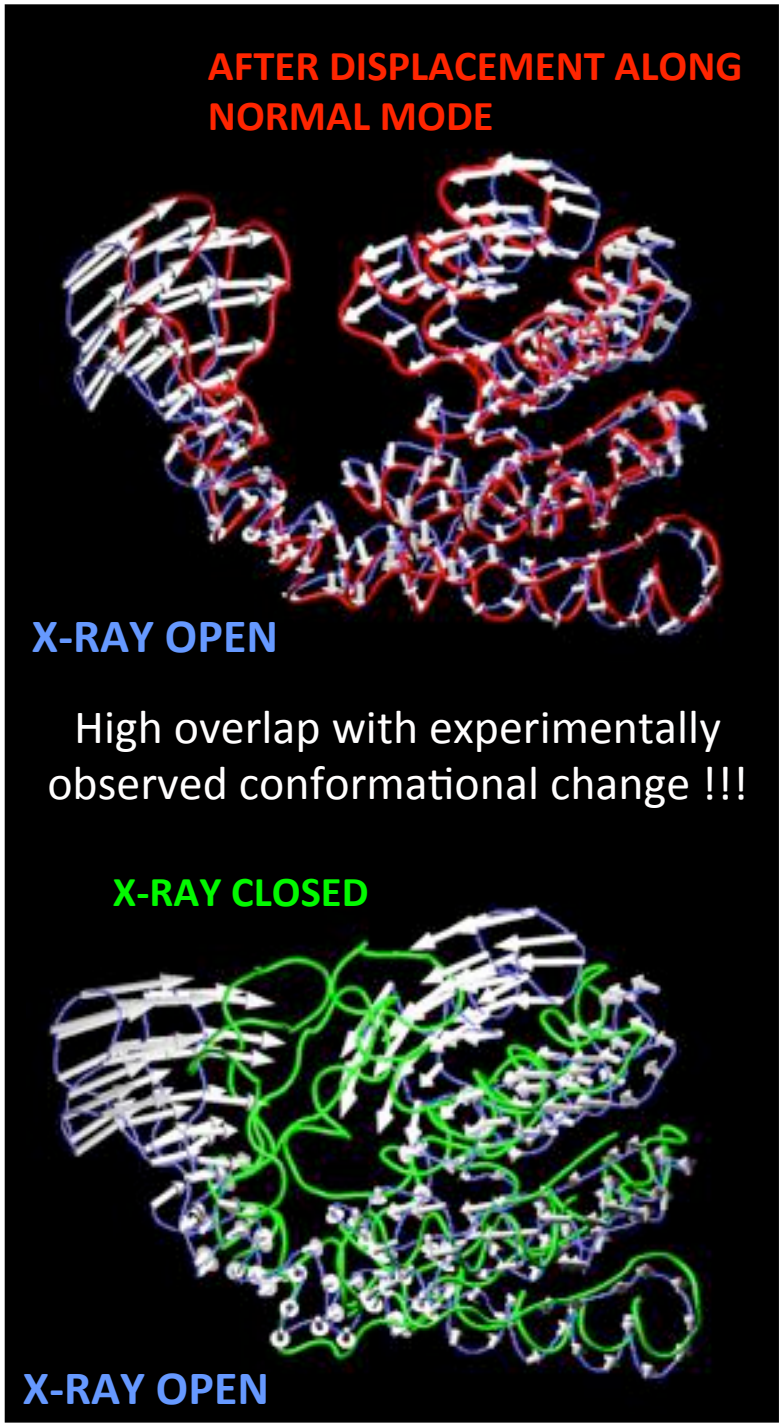
Normal mode analysis



Low frequencies ⟷ High frequencies

Related to conformational changes of biological systems

Tama & Sanejouand (2001)

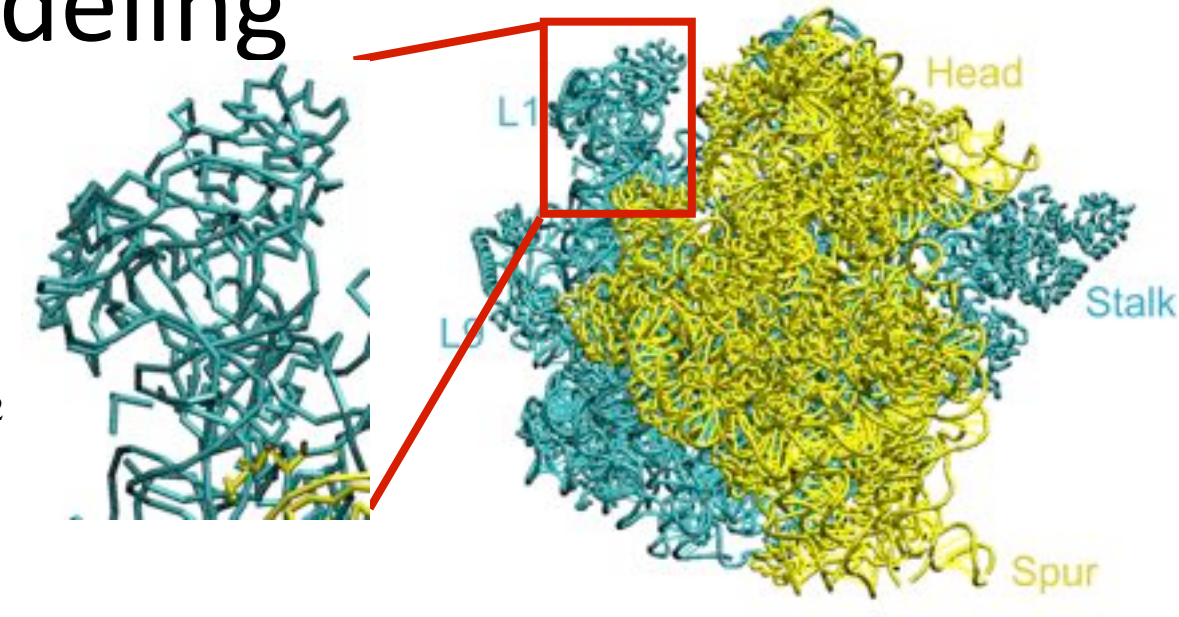


Multi-scale modeling

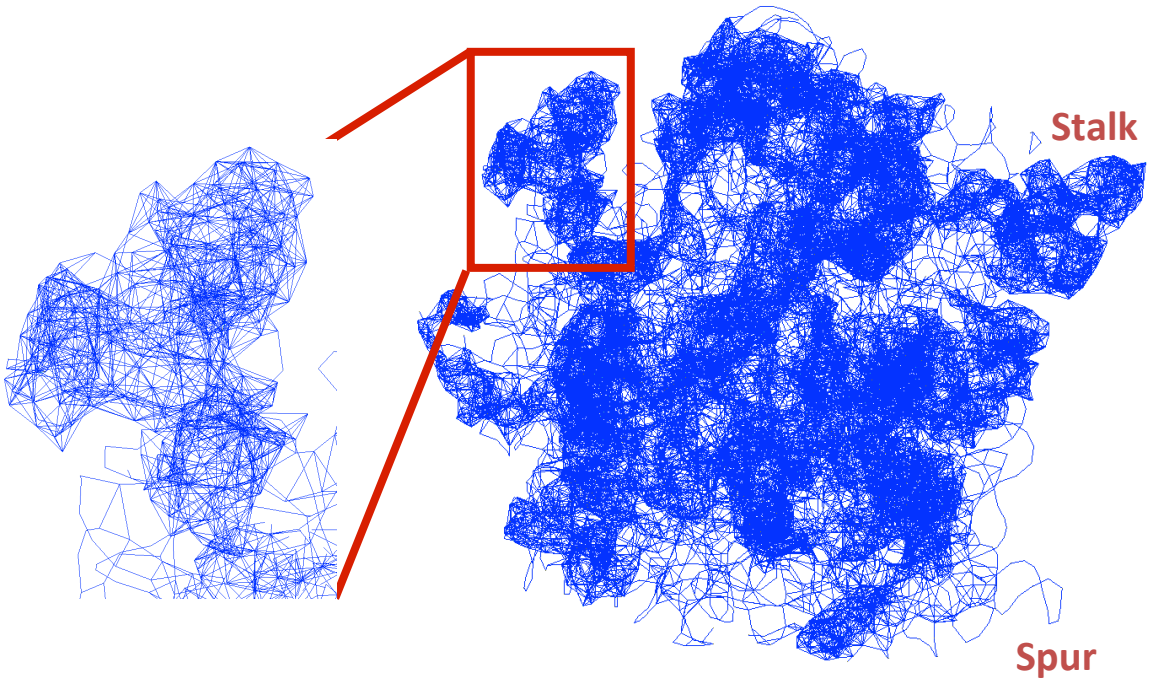
Coarse-Grained Elastic network model:

$$E(r_a, r_b) = \frac{C}{2}\left(|r_{a,b}| - |r^0_{a,b}| \right)^2$$

$$E_p = \sum_{r^0_{a,b} < R_c} E(r_a, r_b)$$



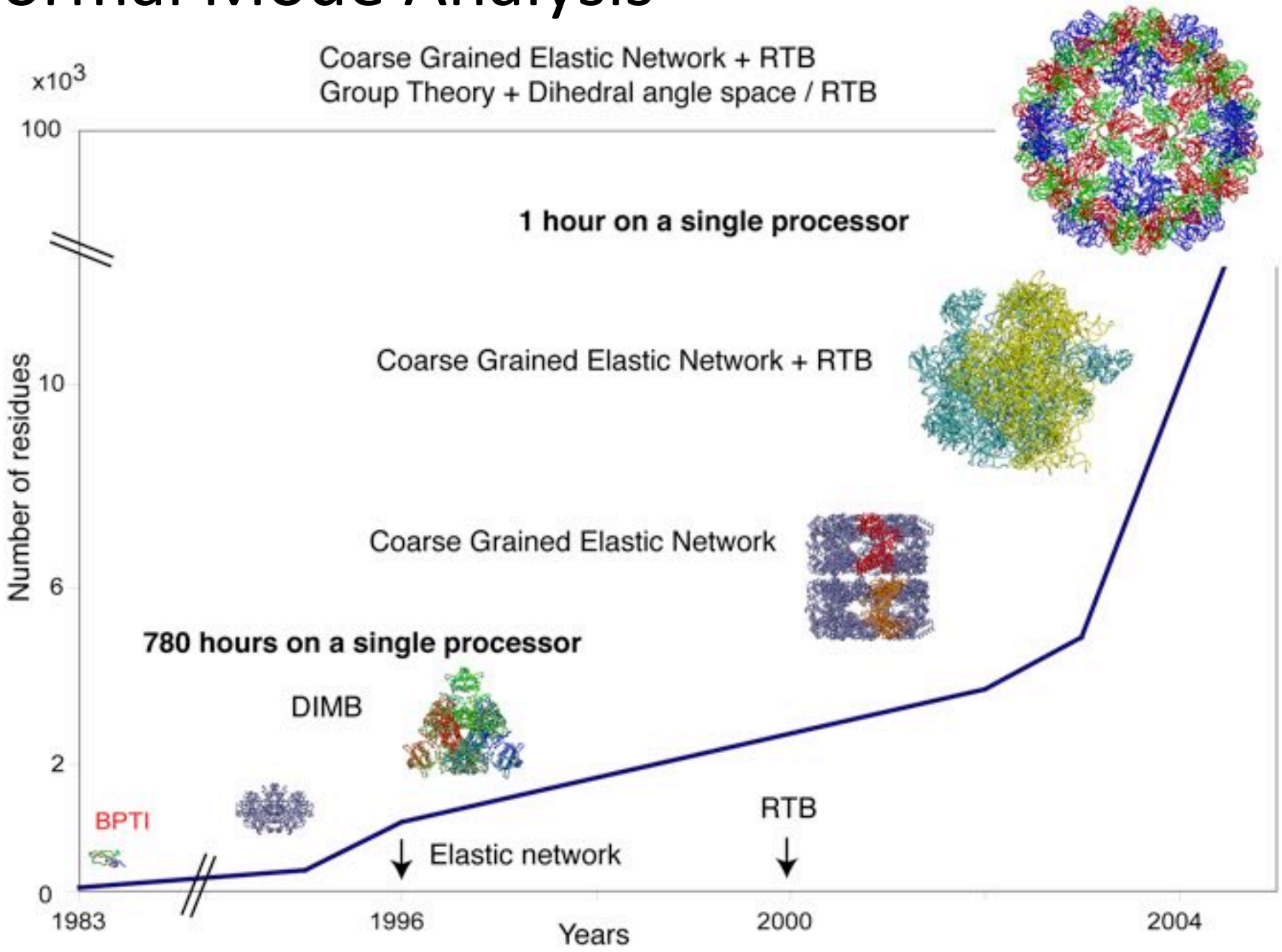
capture the shape of the ribosome



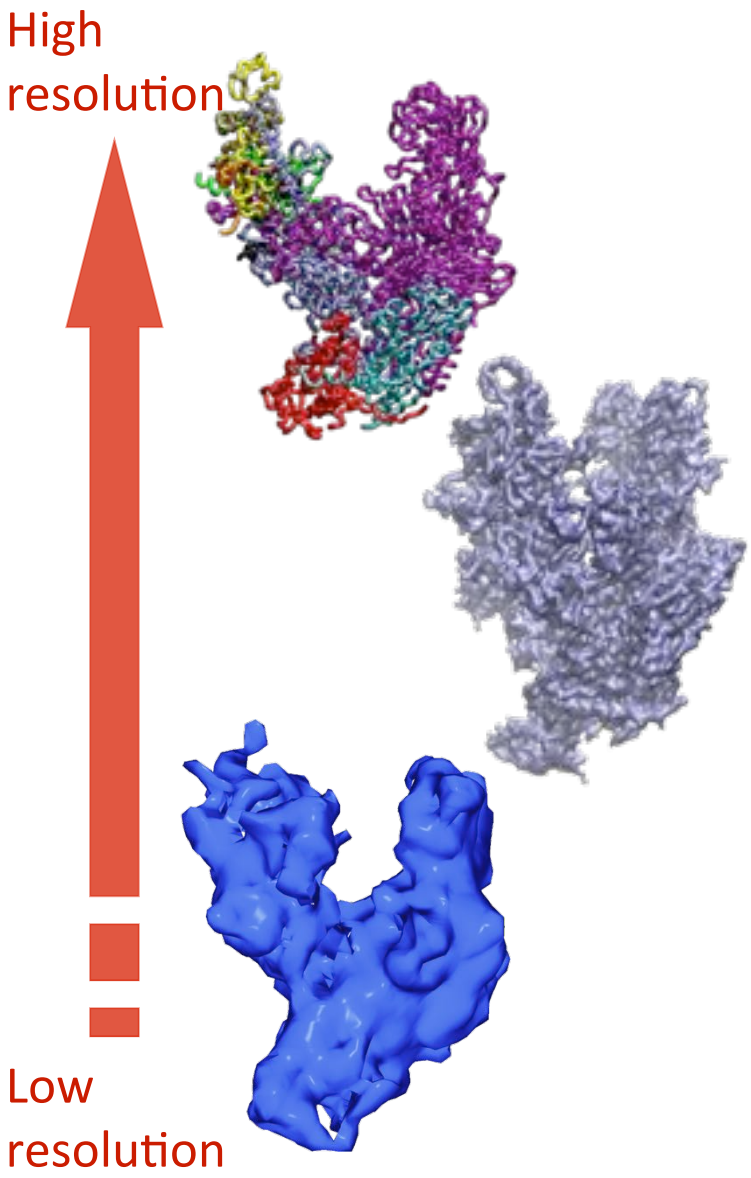
functional motions predicted

Tirion MM (1996) *Phys Rev Lett*.

Normal Mode Analysis



Experimental Techniques for Structure Analysis



X-ray crystallography/NMR

＋ atomic level detail

— large systems are difficult to crystalize

— NMR is only for small systems

X-ray Free Electron Laser (XFEL)

＋Single molecule measurement

＋No crystallization

＋Higher resolution

Cryo-Electron Microscopy

— low resolution, only rough shape

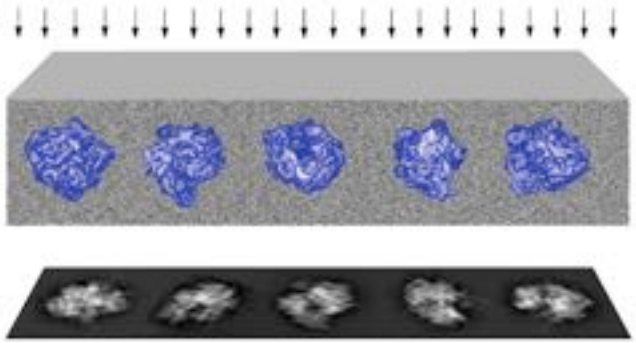
＋ applied to large important systems

＋ can capture different conf states

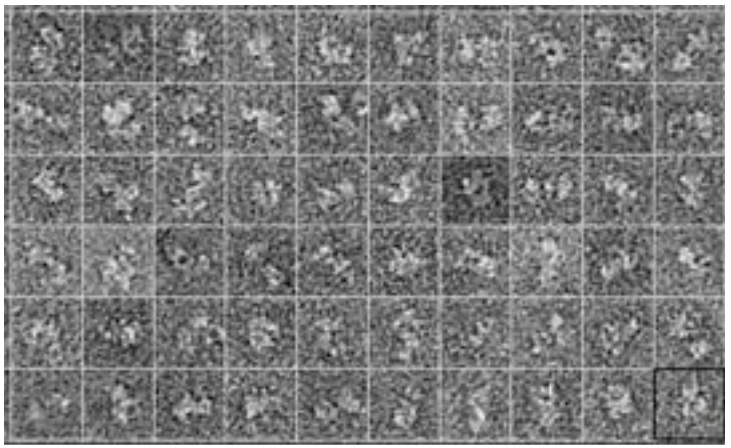
Other approaches: SAXS, FRET etc

Cryo Electron Microscopy – Single Particle Analysis

Arrows => impact of the parallel electron beam upon the specimen

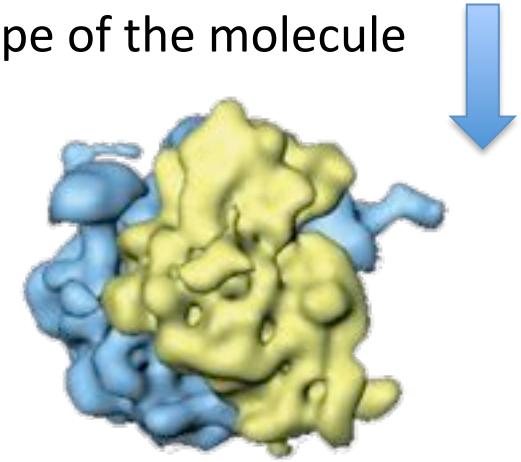


Many "copies" of the molecule are lying in random orientations.

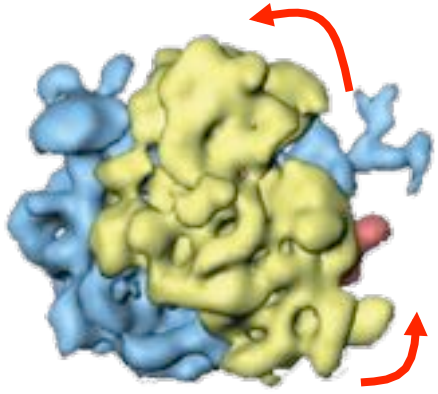


Reconstruction of the 3D structure from thousands of 2D images / Image processing

shape of the molecule



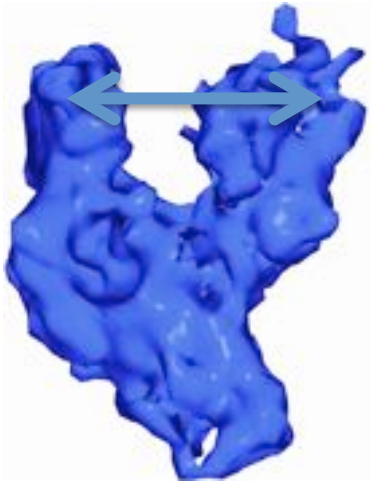
no atomic positions



- Low-resolution compared to X-ray (5 – 30 A)
- Easier to catch molecules in different conformational states

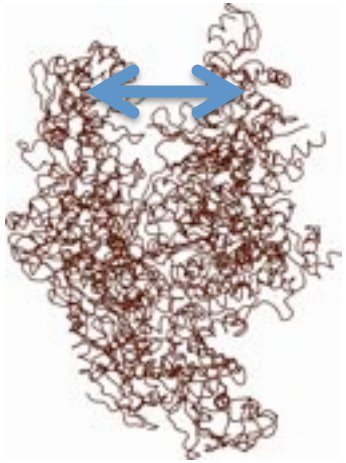
Structure Analysis using Cryo Electron Microscopy Data

Cryo-EM: low resolution



OPEN

X-ray: high resolution



CLOSED

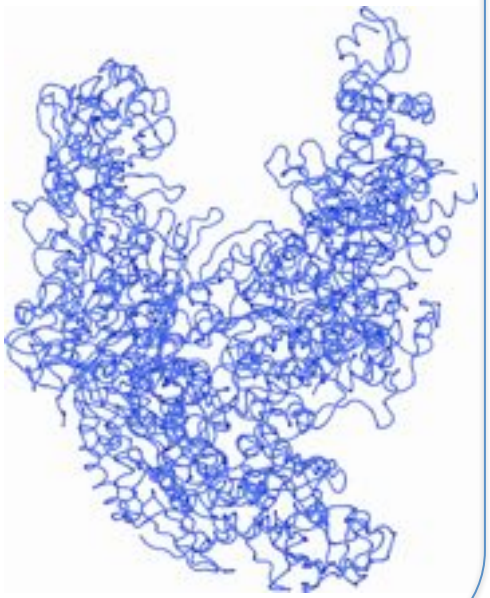
Flexible fitting

MD simulation
Normal Mode

$$f_{\mathrm{EM}}$$

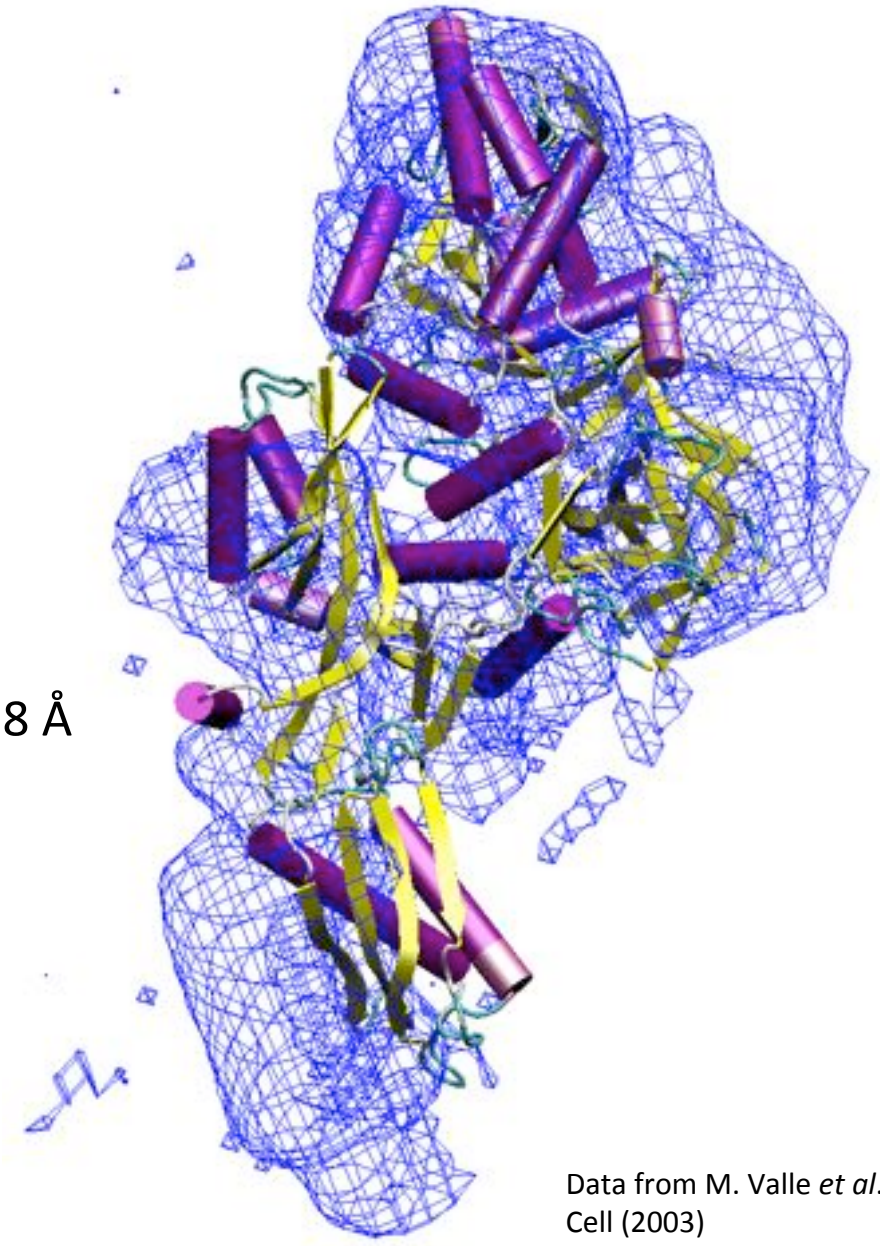
Optimization function

Atomic model



Flexible fitting using NMA

Resolution = 10.8 Å



F. Tama *et al.*
J. Struct. Biol. (2004)

Data from M. Valle *et al*.
Cell (2003)

Medium resolution: works well

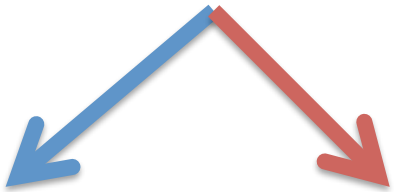
High resolution: limitations as more localized motions become evident

Some conformational changes are not well described

Flexible fitting using targeted MD

$$U = U_{\text{molecule}} + k(1 - f_{EM})$$

$$f_{EM} = \frac{\sum_{ijk} \rho^{\exp}(i,j,k)\rho^{\text{sim}}(i,j,k)}{\sqrt{\sum_{ijk} \rho^{\exp}(i,j,k)^2 \sum_{ijk} \rho^{\text{sim}}(i,j,k)^2}}$$



Lower resolution

Higher resolution

Coarse-grained

All atoms

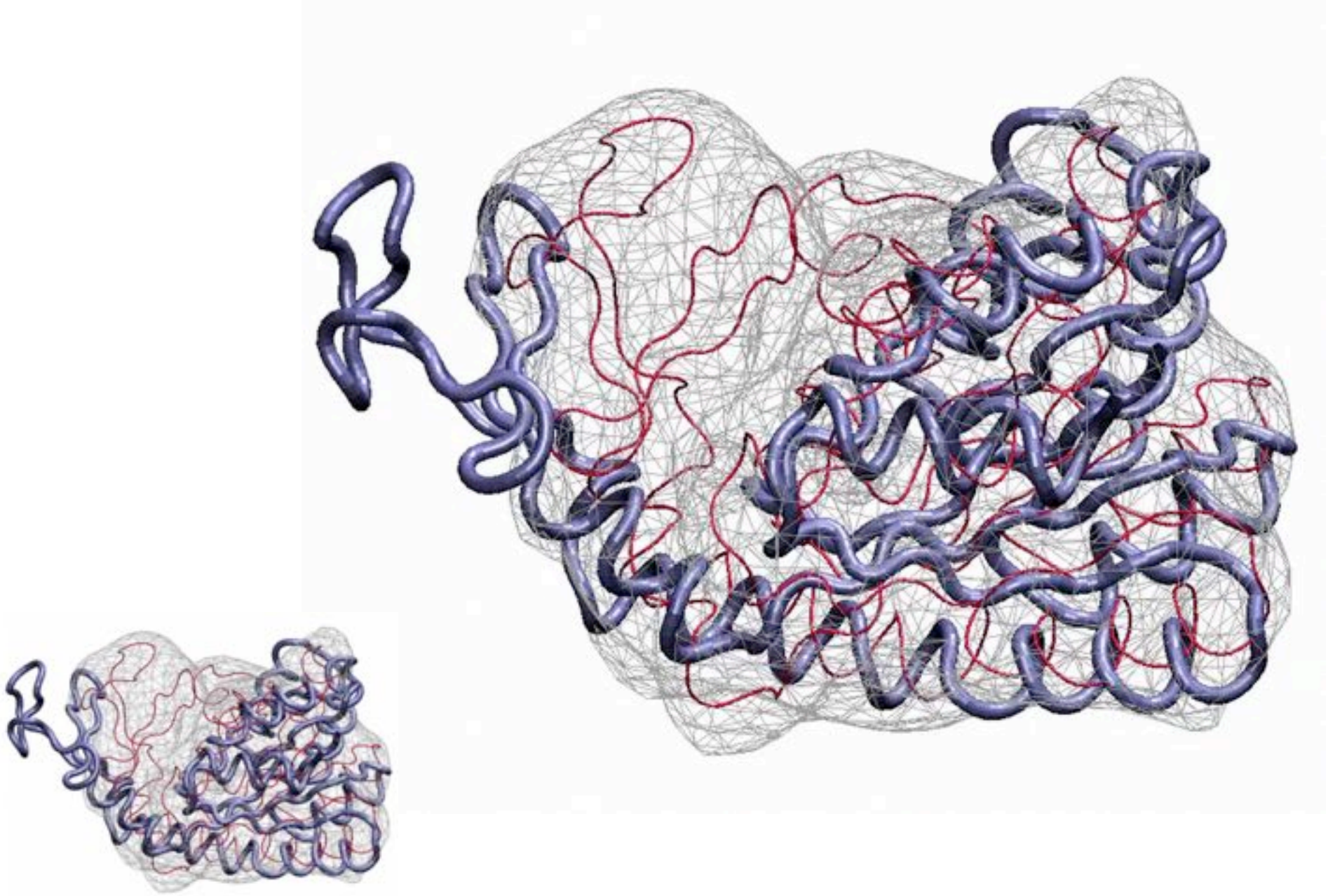
+ Fast, Easy to set up
- Only Cα atoms (Go-model)

- Slow, cumbersome
+ Full atomic description (amber, all atom Go model*)

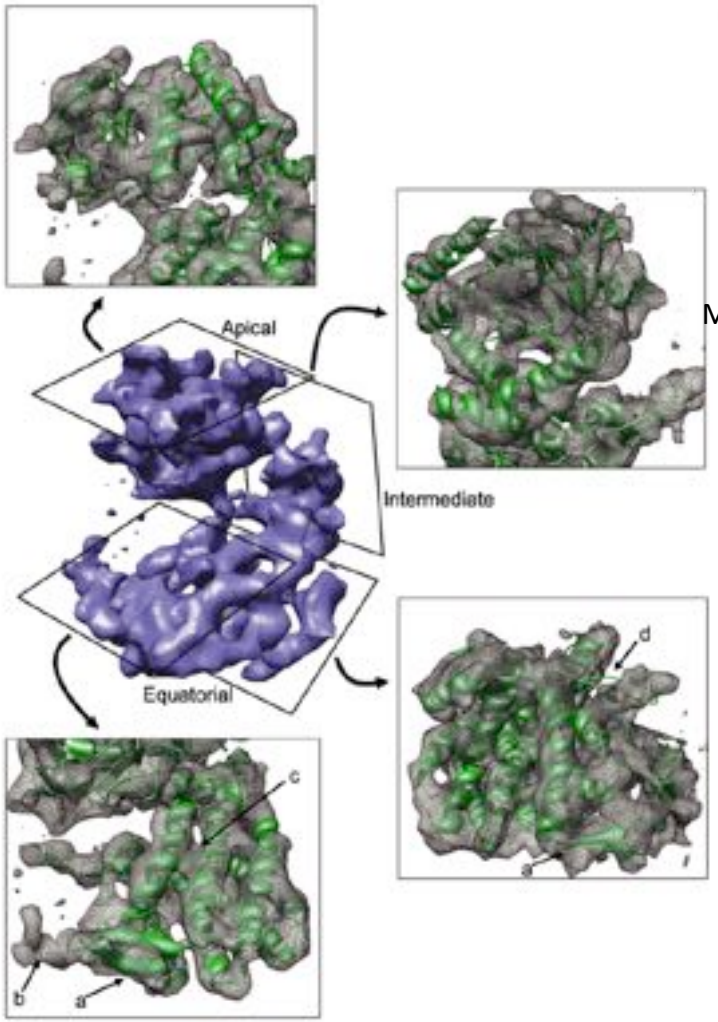
M Orzechowski and F. Tama, Biophys. J. 2008
I. Grubisic et al., JSB, 2010

*Protein Model: Whitford PC et al. Proteins, 2009
*RNA Model: Whitford PC et al. Biophys. J. 2009

Flexible fitting using targeted MD



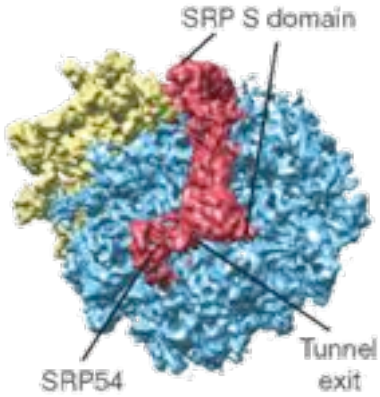
GroEL at 4.2 Å



SJ. Ludtke et al., Structure 2004

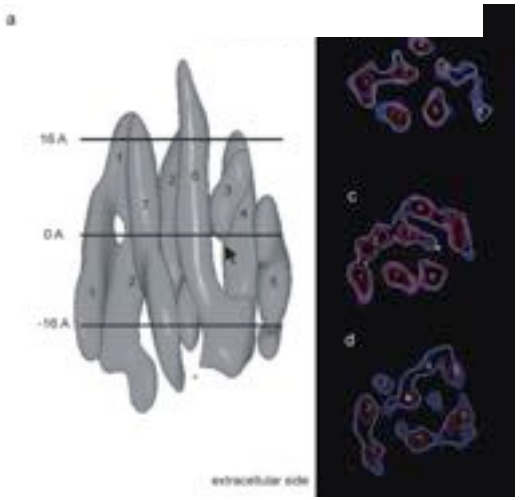
SJ. Ludtke et al., Structure 2008

Ribosome at 8.6 Å



M. Halic et al. Nature. 2006

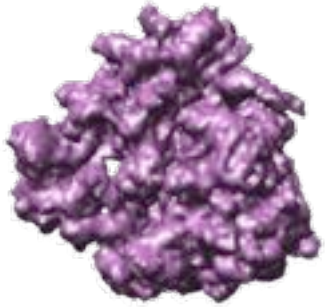
Rhodopsin at 5.5 Å



A Krebs et al., JBC 2003

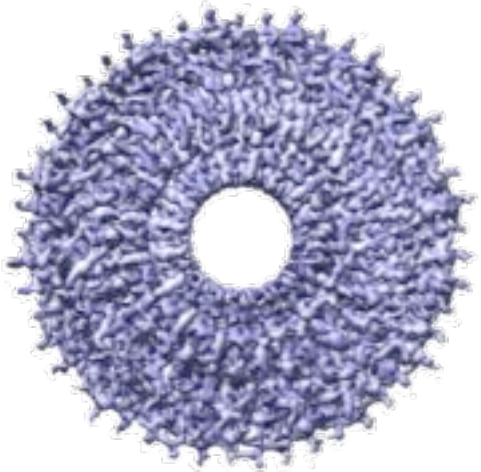
Toward higher resolution data

Ribosome at 7.3 Å



Schueler M, Nat Struct Biol 2006

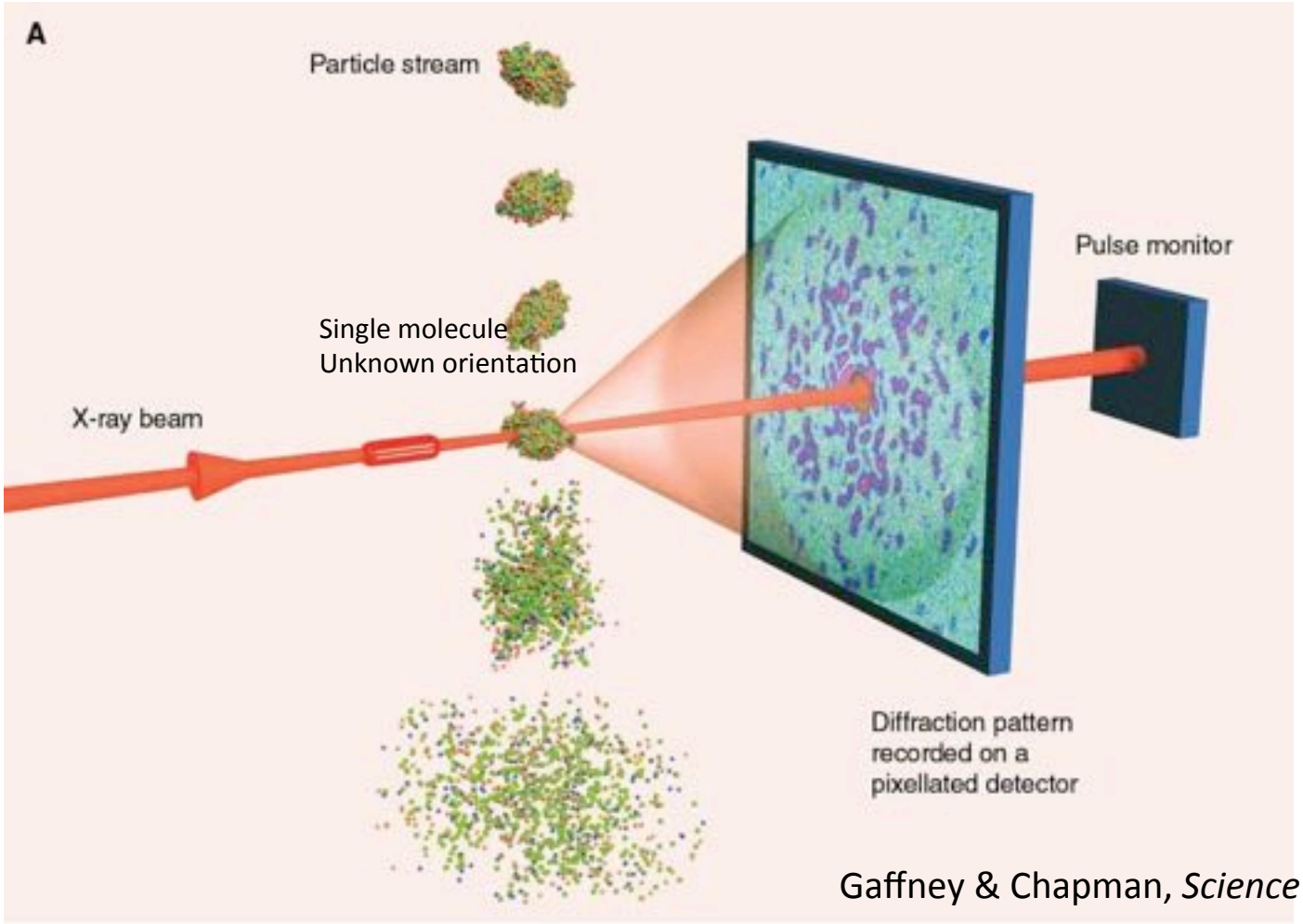
TMV at 4.4 Å



C.Sachse, J. Mol. Biol. 2007

X-ray Free Electron Laser (XFEL)

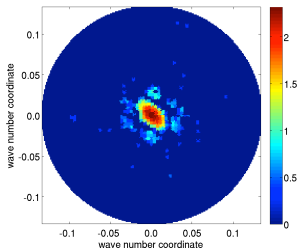
- High intensity X-ray laser, SACLA: June 2011
- Single molecule measurement, No crystallization
- Higher resolution (in theory) but techniques not established



Gaffney & Chapman, *Science* (2007)

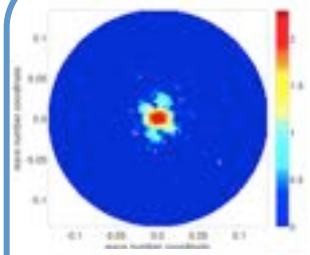
Structure / Dynamics Study by XFEL

There is not enough data to construct 3D model. Needs Computational Modeling

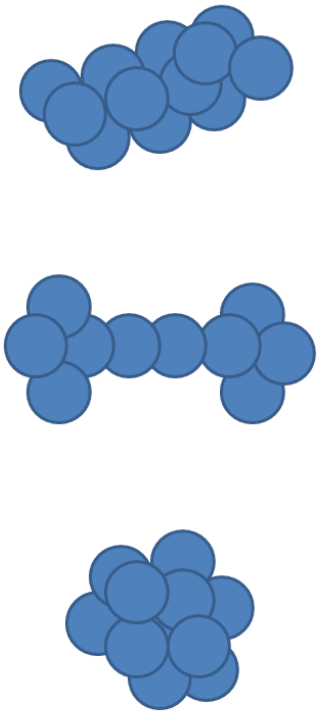


Diffractions from the samples in different orientations and (probably) different conformations

Find pairs



Simulated diffractions



Hypothetical conformations in different orientations

Ab initio

Deformed low resolution models

Other experimental data

etc…

Acknowledgements

- Logan Ahlstrom
- Joe Baker
- Kent Ehrlich
- Ivan Vorontsov
- Zack Campbell
- Sunita Patel
- Florence Tama
- Aqeel Ahmed
- Marek Orzechowski
- Ivan Grubisic
- Max Shokhirev
- Christian Gorba
- Slavica Jonic (CNRS)

- FOCUS Establishing Supercomputing Center of Excellence
- University of Arizona High Performance Computing Center