Experimental restraints on molecular shape for protein structure calculations

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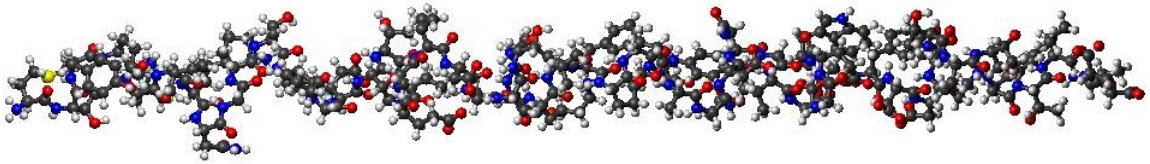
OUTLINE

- Global and local restraints for protein structure calculations
- Global restraints on overall shape from NMR relaxation data
- Combination of global and local restraints in NMR relaxation data
- NMR relaxation data as the source of dynamic information about protein domain motions

Ultimate Goal of protein structure prediction

Sequence of amino acid residues



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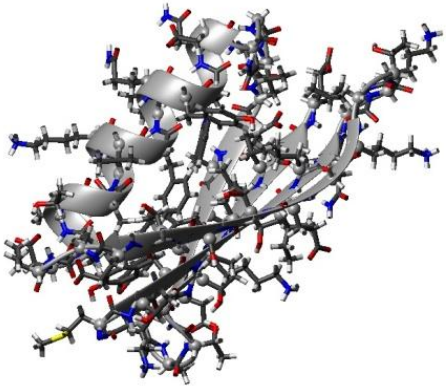
Model inter atomic forces

=



Ultimate Goal of protein structure prediction

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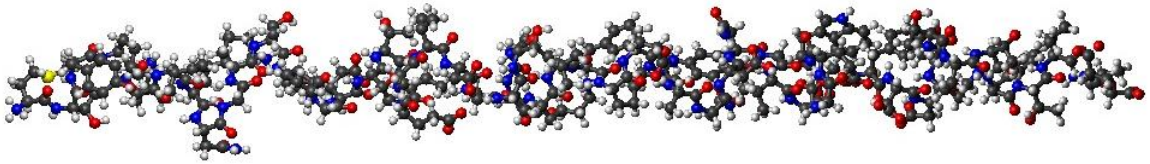

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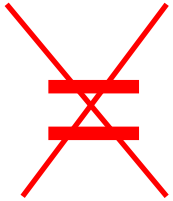
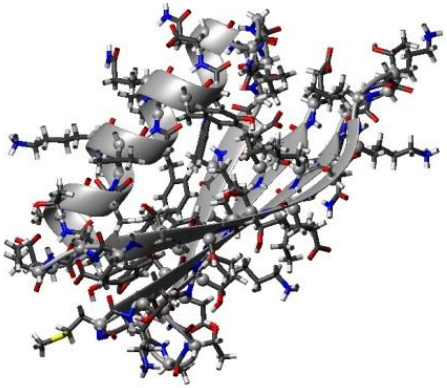
Model inter atomic forces

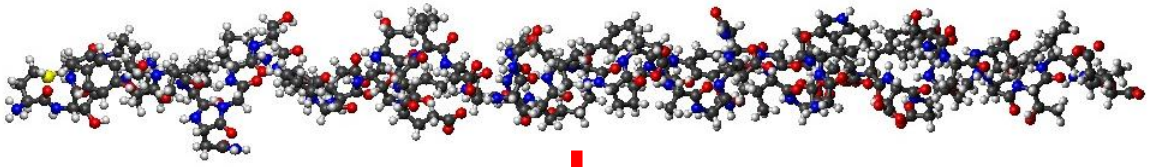




Is not yet accomplished

NMR Protein structure determination

Sequence of amino acid residues



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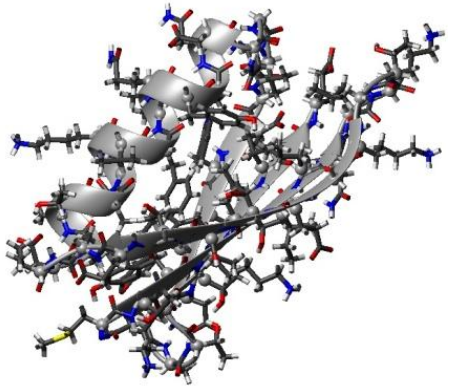
Experimental restraints: NOE, RDC, SAXS and etc.

+

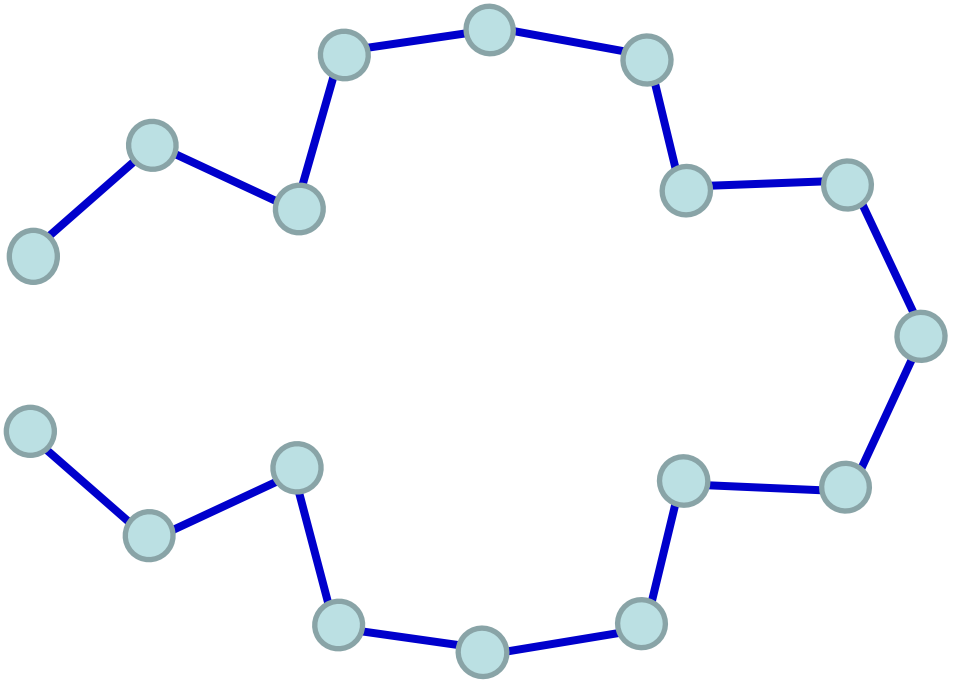
***a priori* restraints from database of known structures**

=



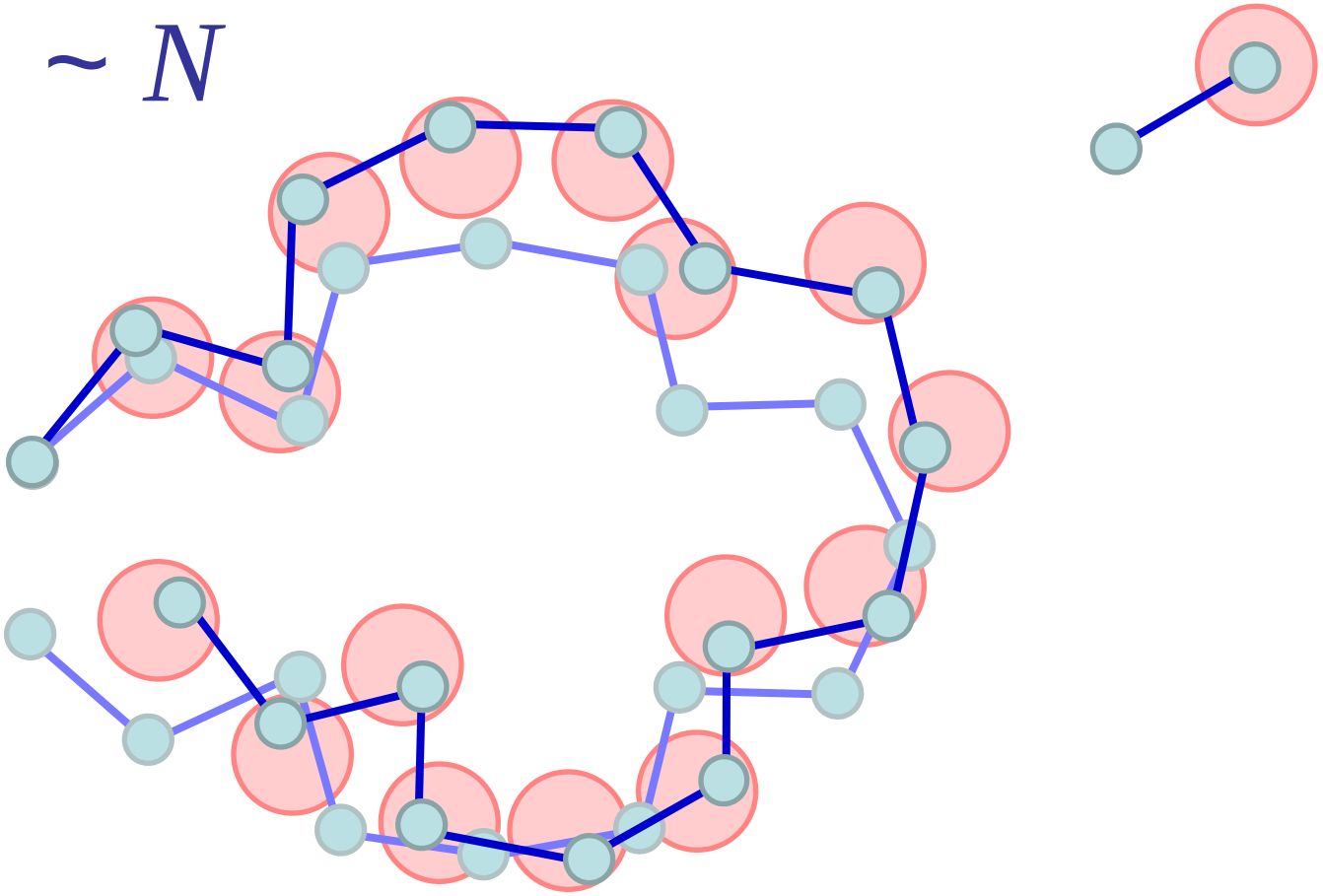
Local and Global restraints

Ideal structure



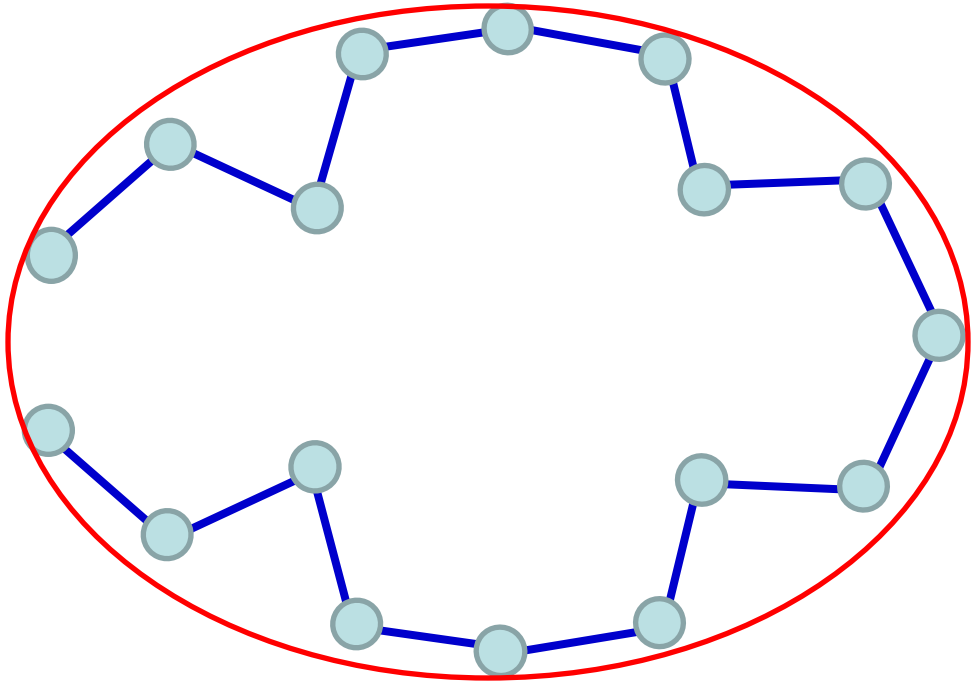
Local restraints

$$\langle \Delta^2 \rangle \sim N$$

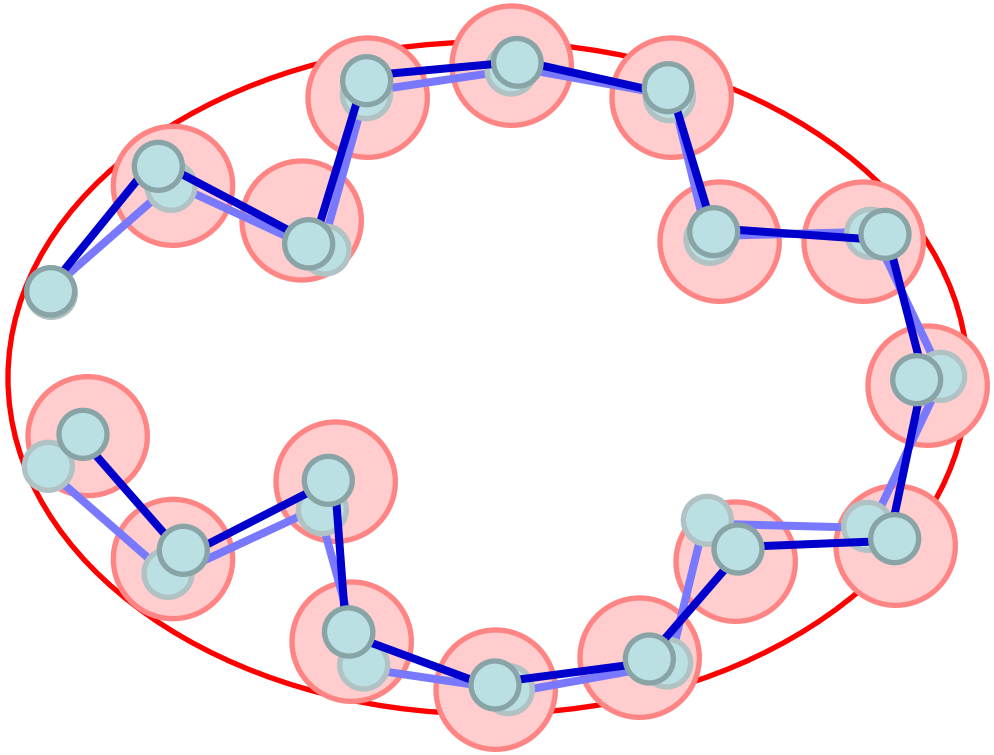


Global restraints

Overall shape



Local and Global restraints



Some possible experimental sources of global restraints

- **Radius of gyration**

could be obtained form empiric relationship between protein size and radius of gyration

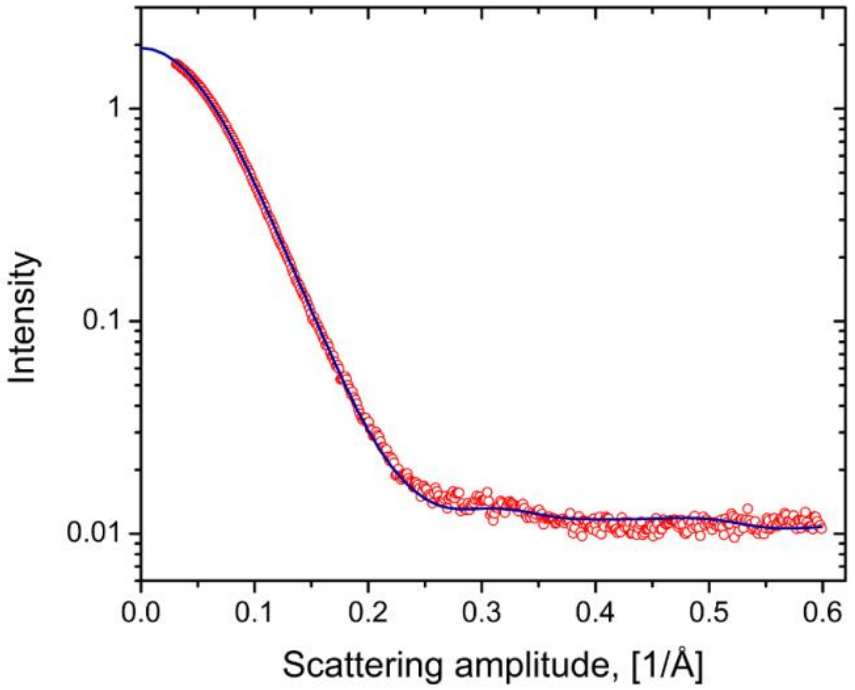
$$R_g = 2.2\, N^{0.38}\ \text{Å}$$

or directly form SAXS cure at the limit at the of small scattering angles (Guinier limit)

$$\ln I(Q) = \ln I(0) - \frac{1}{3} R_g^2 Q^2$$

Some possible experimental sources of global restraints

- **Radius of gyration**
- **SAXS curve**



~ 10 Å resolution

Some possible experimental sources of global restraints

- Radius of gyration
- SAXS curve
- NMR relaxation data

Sampling protein surface

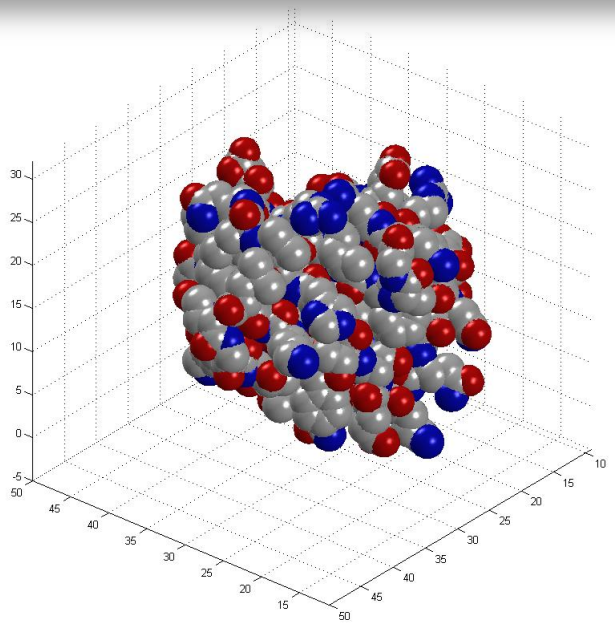
Implemented into specific Xplor NIH module and already used for

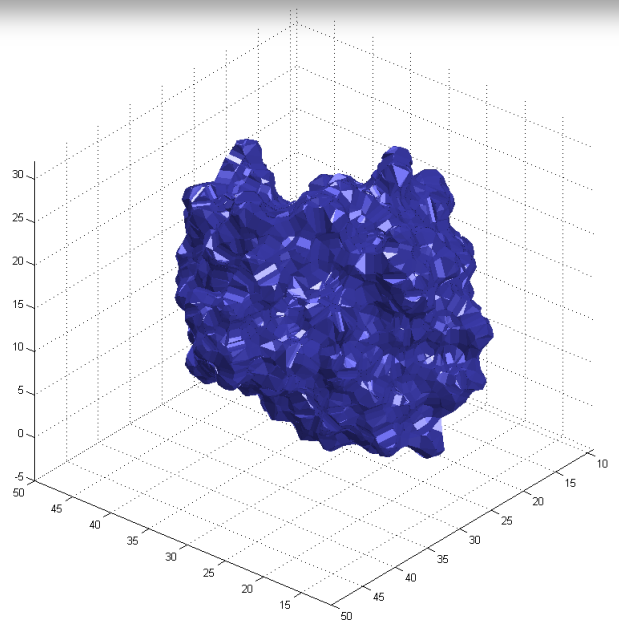
- Evaluation protein *Rotation Diffusion* properties
- Evaluation of *NMR relaxation* rates
- Simulation *SAXS scattering curve*

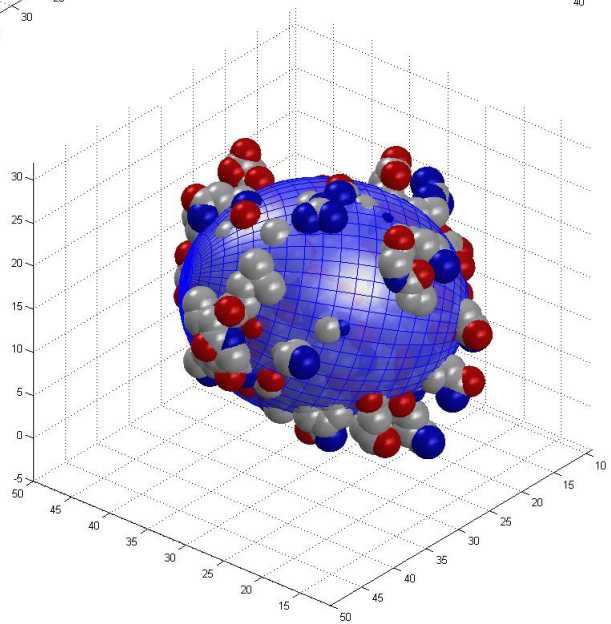
and **potentially can be used for evaluation of**

- *Translational Diffusion* coefficients
- *Residual Dipolar Coupling* originated from steric hindrances
- etc.

Sampling protein surface



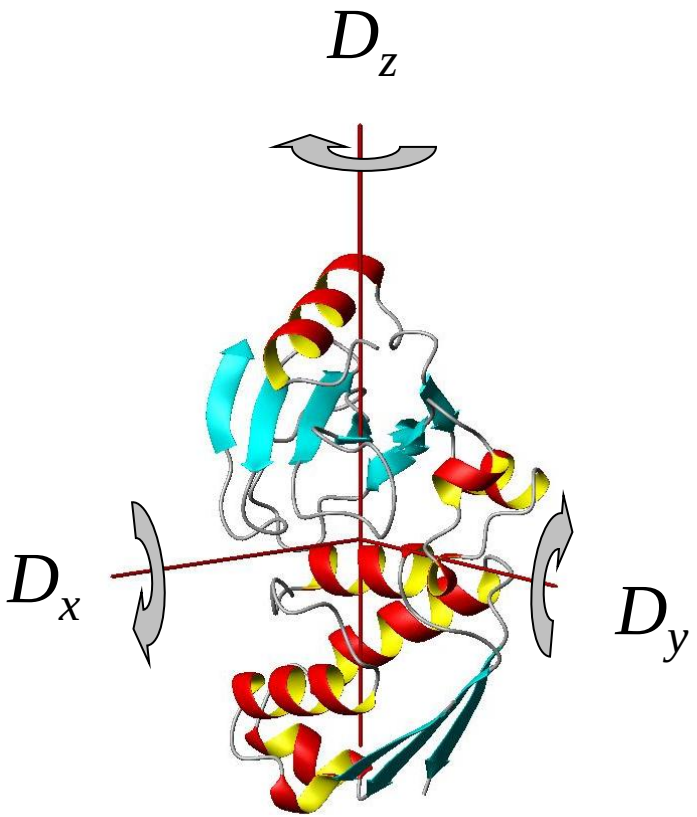




Overall shape restraints from Diffusion tensor

Diffusion Tensor

$$\begin{bmatrix} D_x & 0 & 0 \\ 0 & D_y & 0 \\ 0 & 0 & D_z \end{bmatrix}$$



3 Euler angles for
Diffusion Tensor PAF

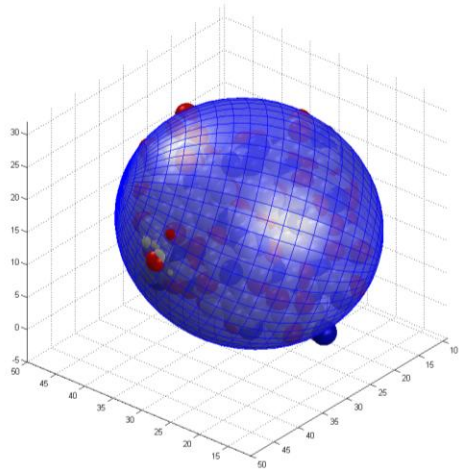
Effect of overall protein shape on NMR relaxation data

Rotation Diffusion tensor

$$R_2 \leftarrow J(\omega) \leftarrow C(t) \sim \sum_{n,pq} e^{-E_n t} A_{np} A_{nq} Y_2^p(\Omega) Y_2^{q*}(\Omega)$$

$$R_1 \leftarrow$$

Schematic Procedure



I) Build equivalent ellipsoid

II) Evaluate components of diffusion tensor

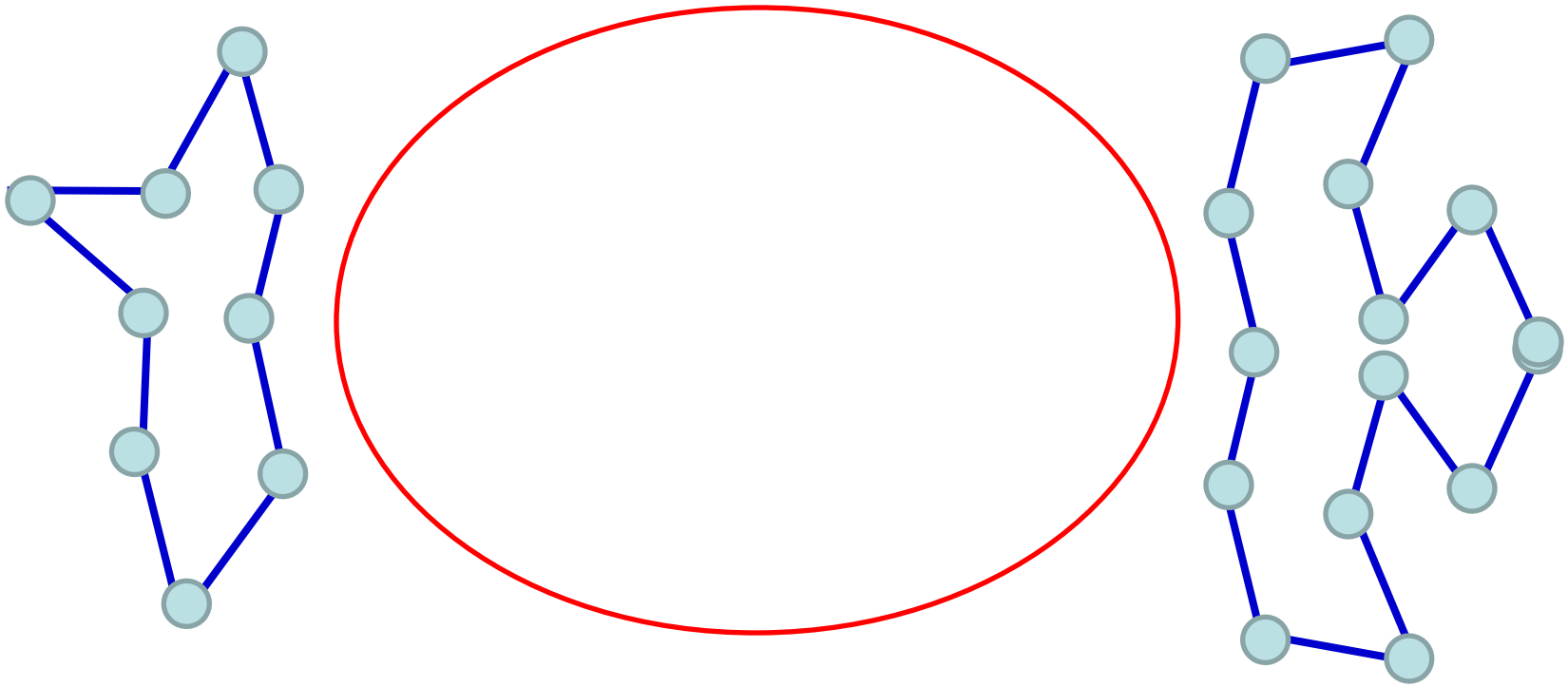
$$\begin{bmatrix} D_x & 0 & 0 \\ 0 & D_y & 0 \\ 0 & 0 & D_z \end{bmatrix}$$

III) Compare evaluated components of Diffusion tensor with experimental values obtained form NMR

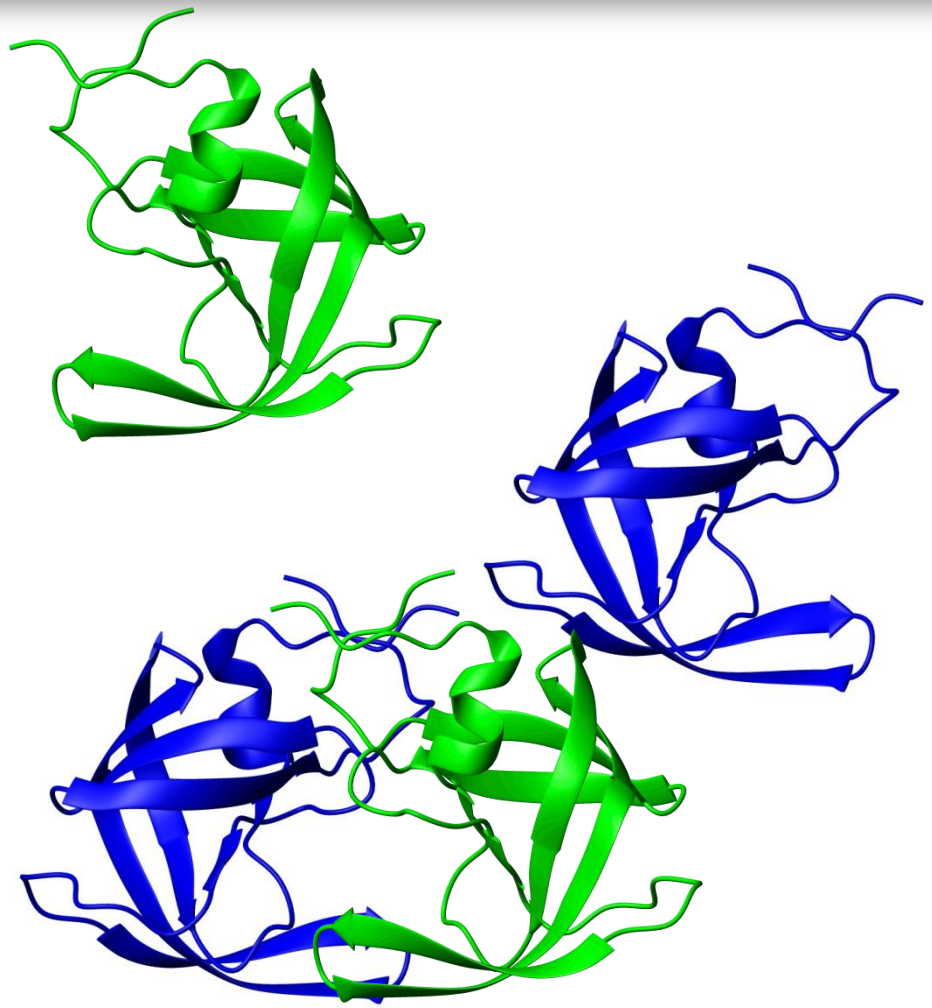
$$\chi^2 = \sum_{\substack{i=1,3 \\ j=i,3}} \left(D_{i,j}^{calc} - D_{i,j}^{exp} \right)^2$$

Assembling structures of multi domain proteins

Global restraints on Overall shape



Assembling structure of a symmetric protein homo dimer



Generic docking protocol

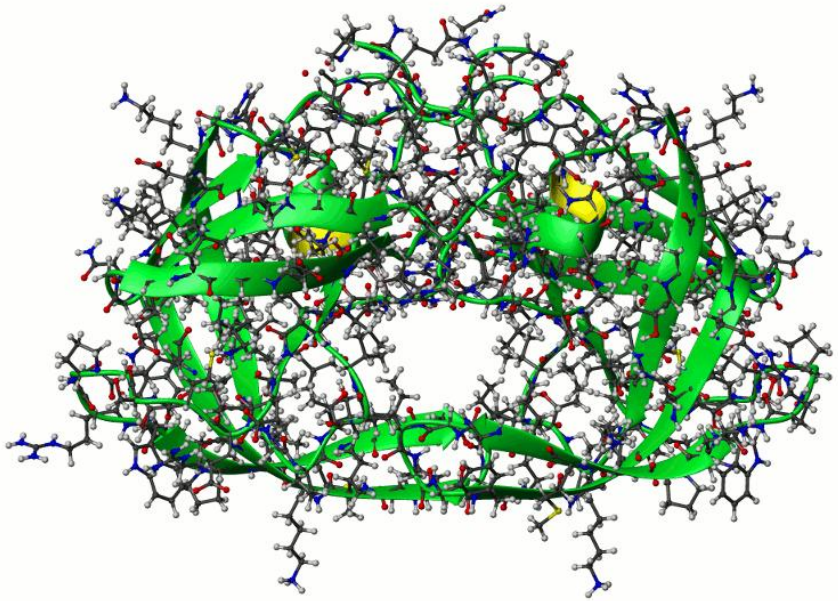
Part I:
Rigid body dynamics
for raw domain positioning.

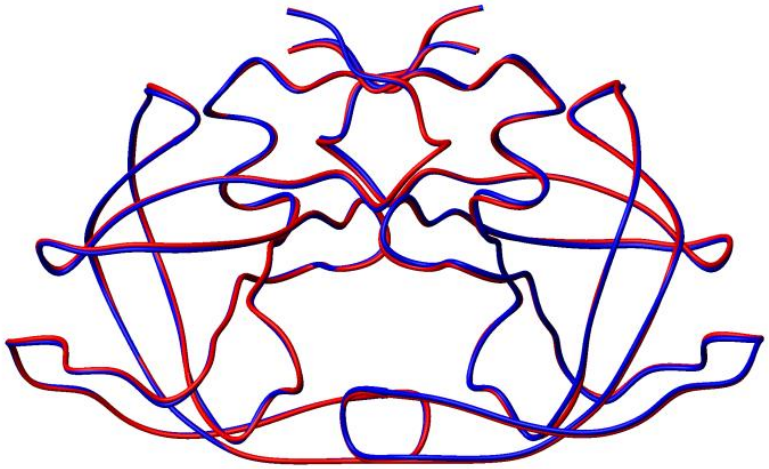
Part II:
Simulated annealing
with flexible side chains
for final adjustment.

Assembling structure of a symmetric protein homo dimer

HIV -1 protease

Shape restraints from
Components of diffusion tensor





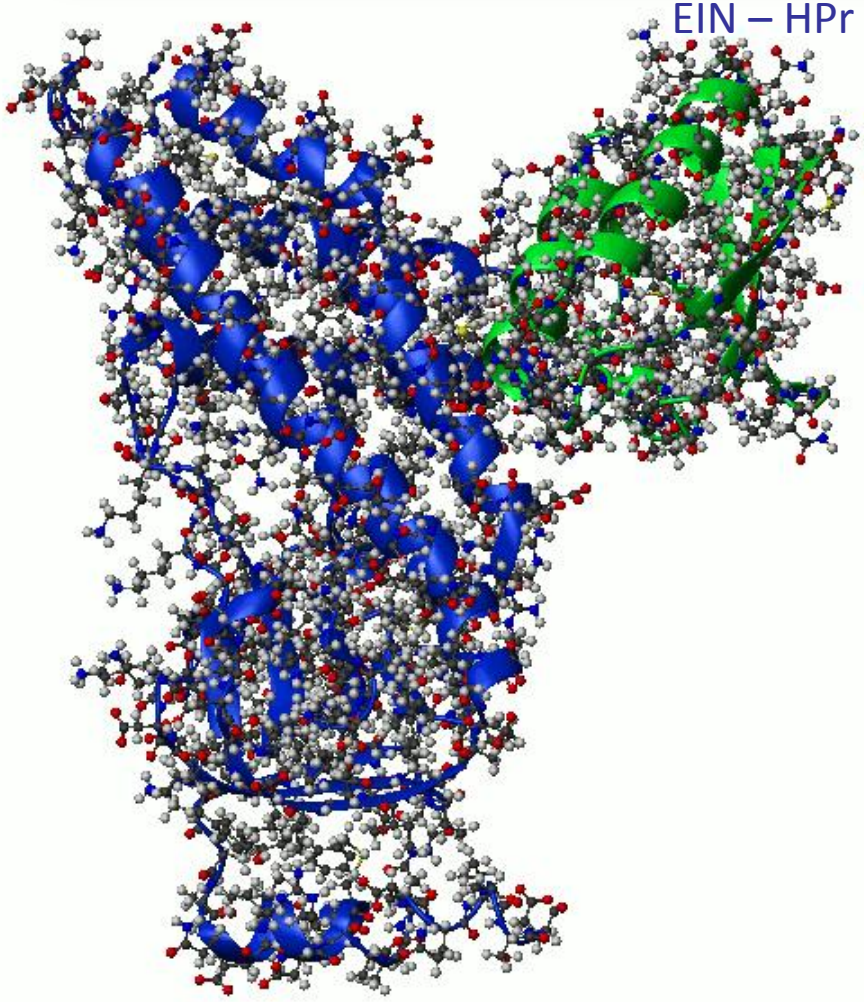
10 lowest energy structures

Averaged over 10 lowest energy structures (blue) versus reference (red)

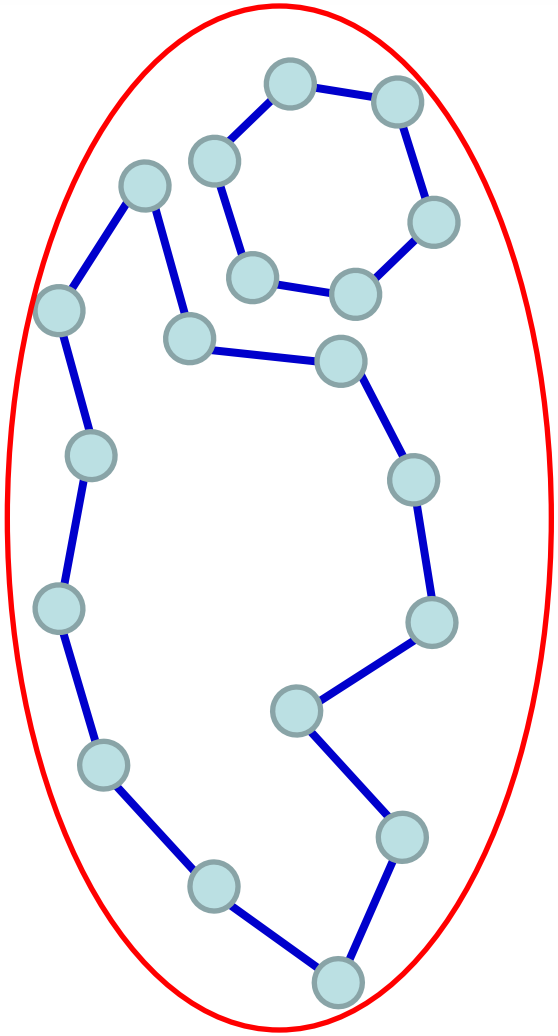
Cα RMSD 0.35 ±0.09 [Å]

Application to an asymmetric complex

EIN – HPr complex

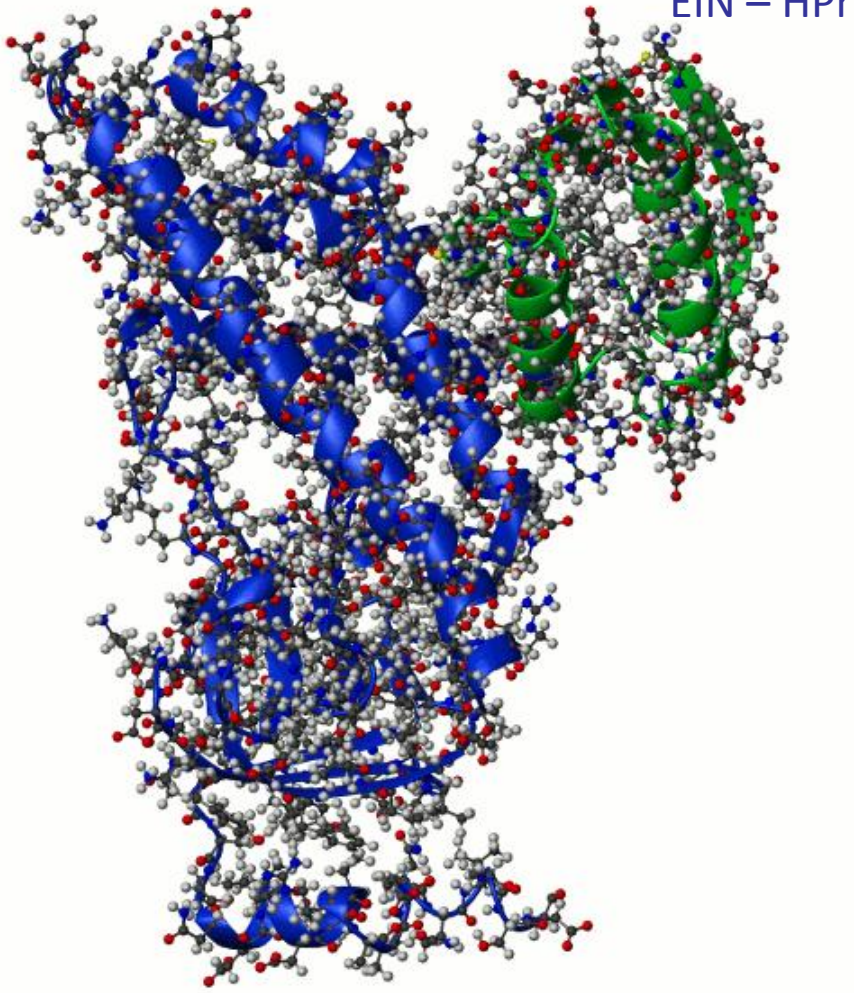


10 lowest energy structures

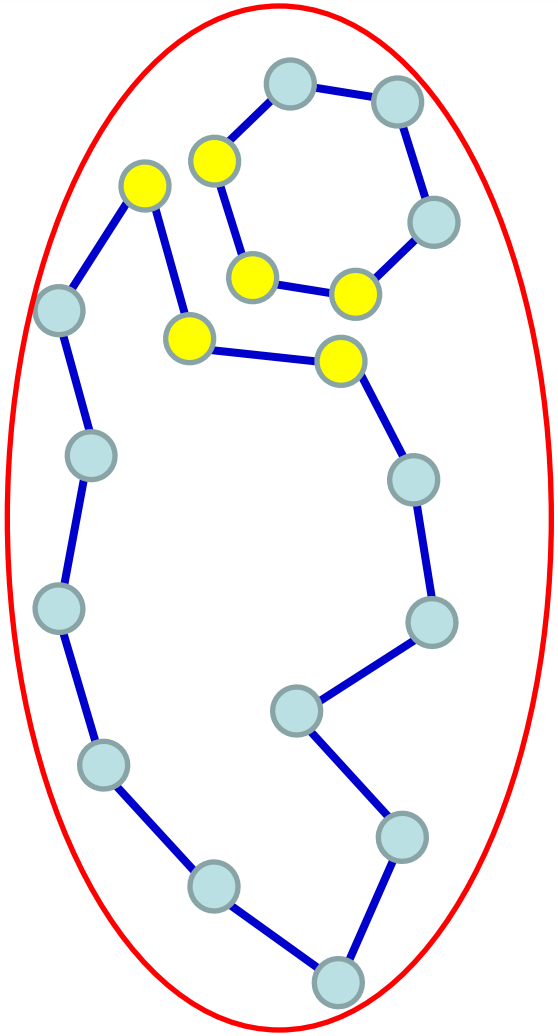


Application to an asymmetric complex

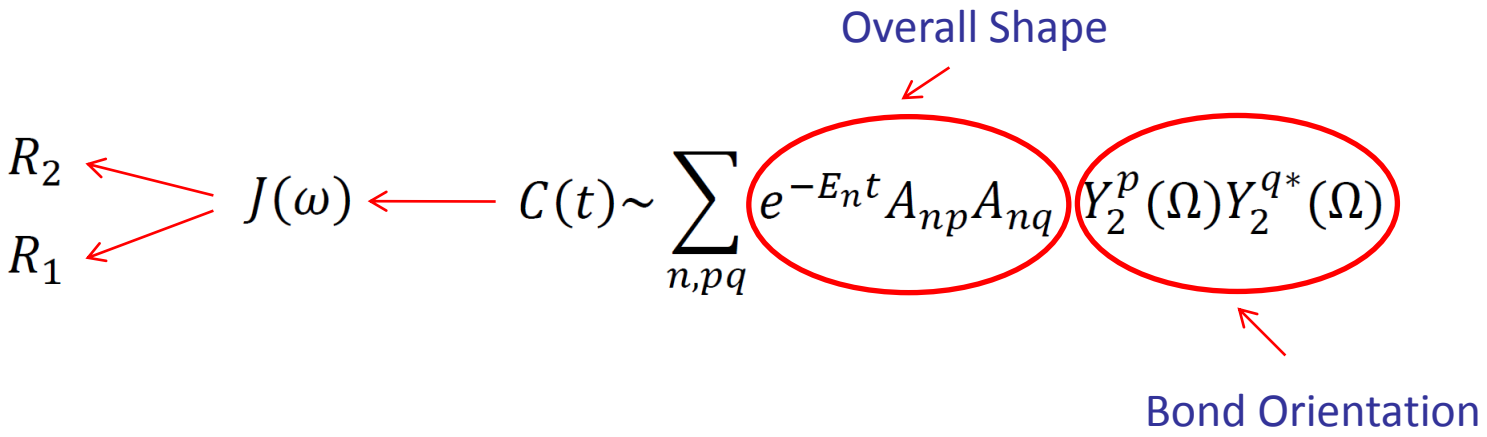
EIN – HPr complex



10 lowest energy structures



Using both shape and orientation restraints

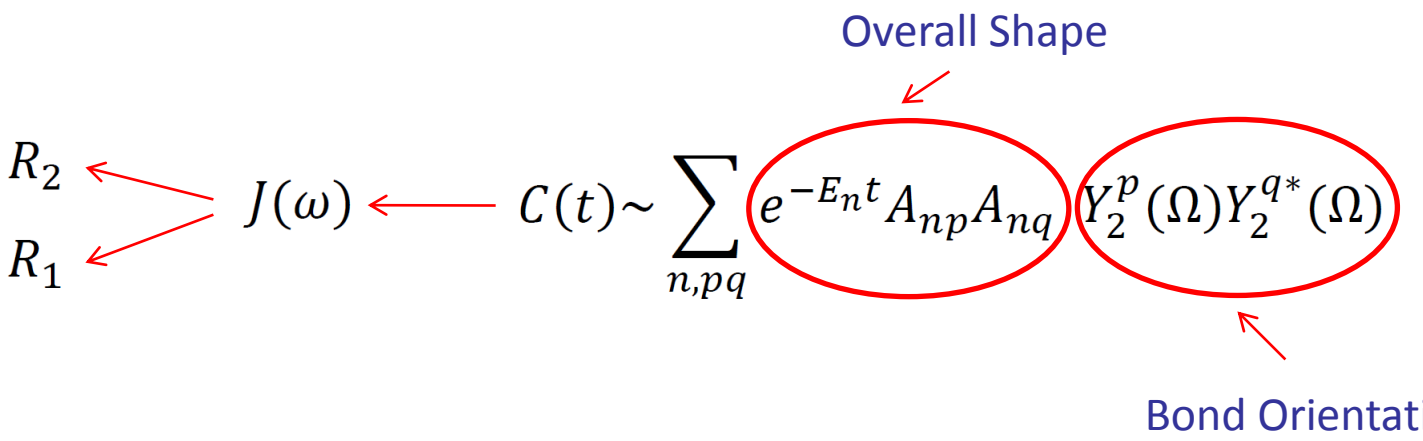


Ratio of relaxation rates "**almost**" **independent of fast local motions**

$$\frac{R_2}{R_1} = \frac{4J(0) + 6J(\omega_H - \omega_N) + J(\omega_H + \omega_N) + 6J(\omega_H) + 3J(\omega_N)}{2[6J(\omega_H - \omega_N) + J(\omega_H + \omega_N) + 3J(\omega_N)]}$$

Tjandra N and Bax A, **1995**

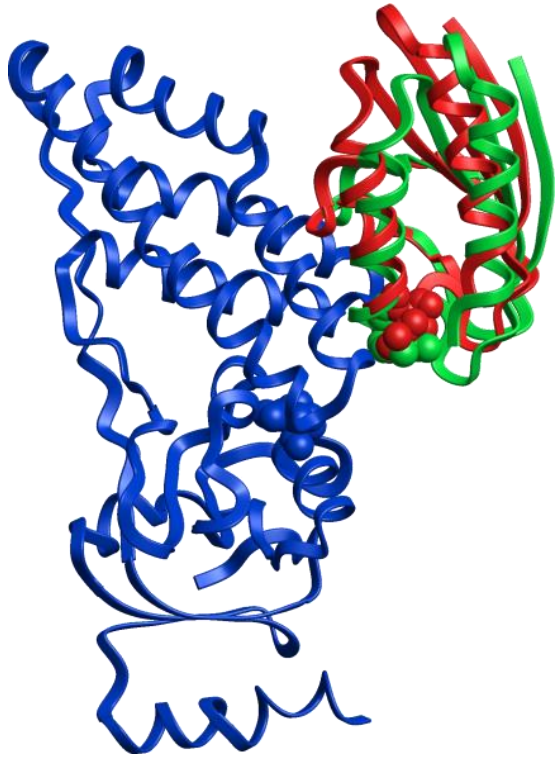
Using both shape and orientation restraints

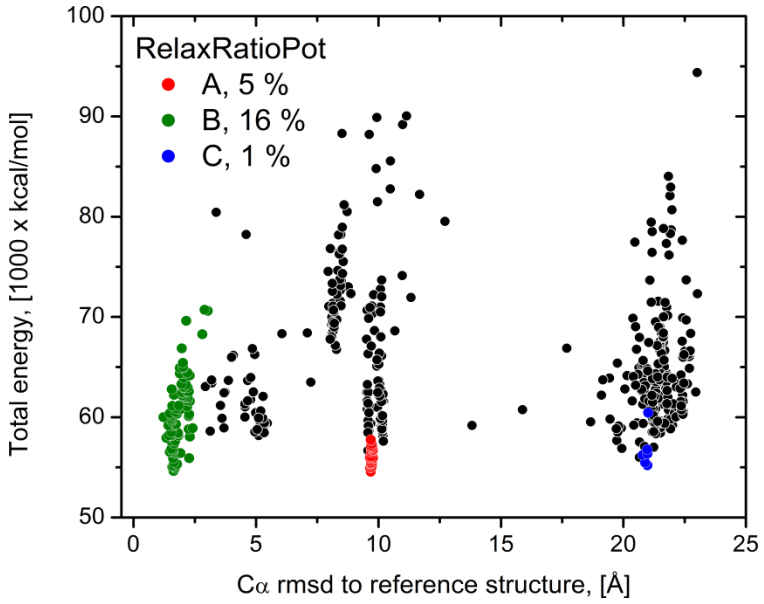


Energy of a potential term

$$\chi^2 \sim \sum_i \left(\frac{(R_2/R_1)_i^{calc} - (R_2/R_1)_i^{exp}}{\sigma_i^{err}} \right)^2$$

Using both shape and orientation restraints



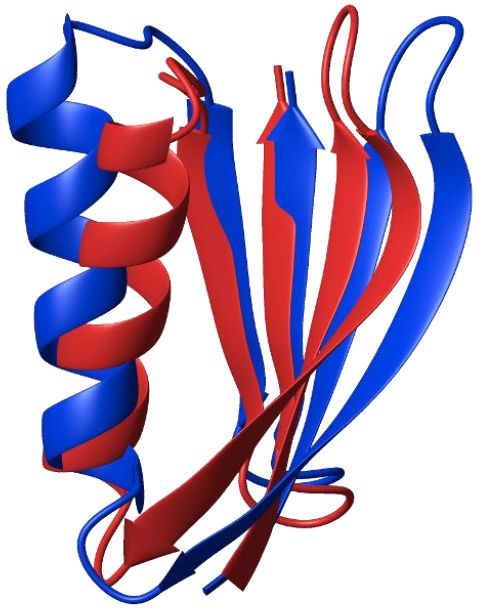


10 lowest energy structures
from the most populated cluster B

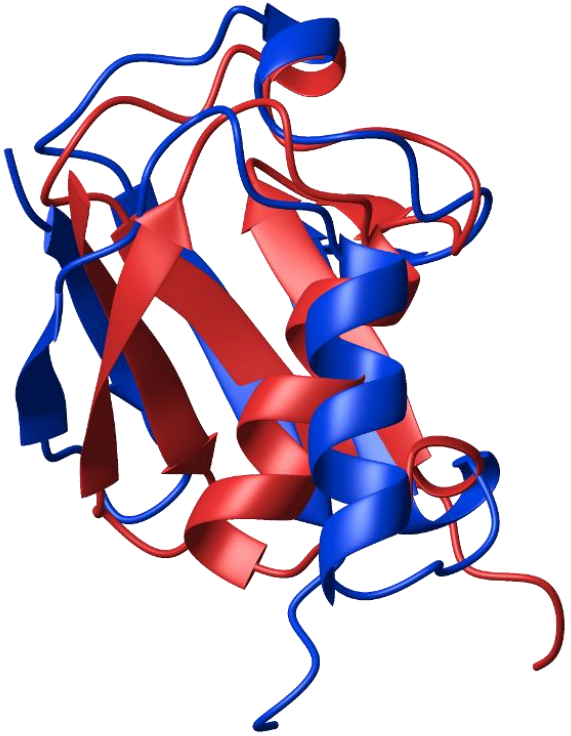
Cα RMSD 1.73 ±0.20 [Å]

Structure determination of globular proteins

Gb 3: b.b.Cα RMSD 3.2 [Å]



Ubiquitin: b.b.Cα RMSD 3.5 [Å]



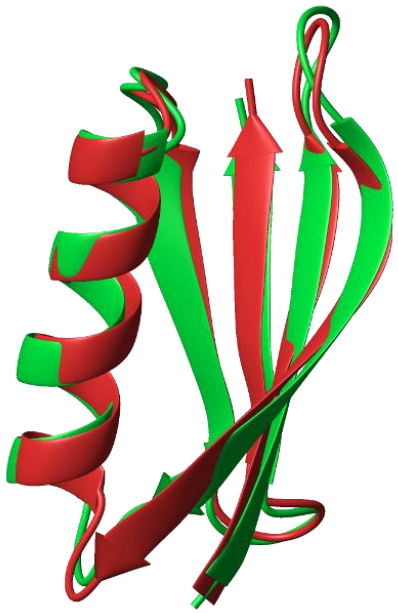
Experimental restraints:

Dihedral angles from TALOS+ predictions

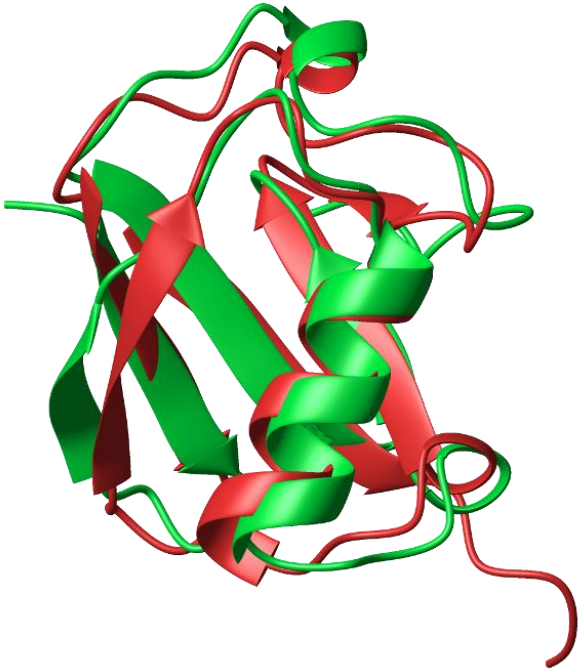
Back Bone Hydrogen bonds connectivity

Structure determination of globular proteins

Gb 3: b.b.Cα RMSD 1.1 [Å]



Ubiquitin: b.b.Cα RMSD 1.8 [Å]



Experimental restraints:

Dihedral angles from TALOS+ predictions

Back Bone Hydrogen bonds connectivity

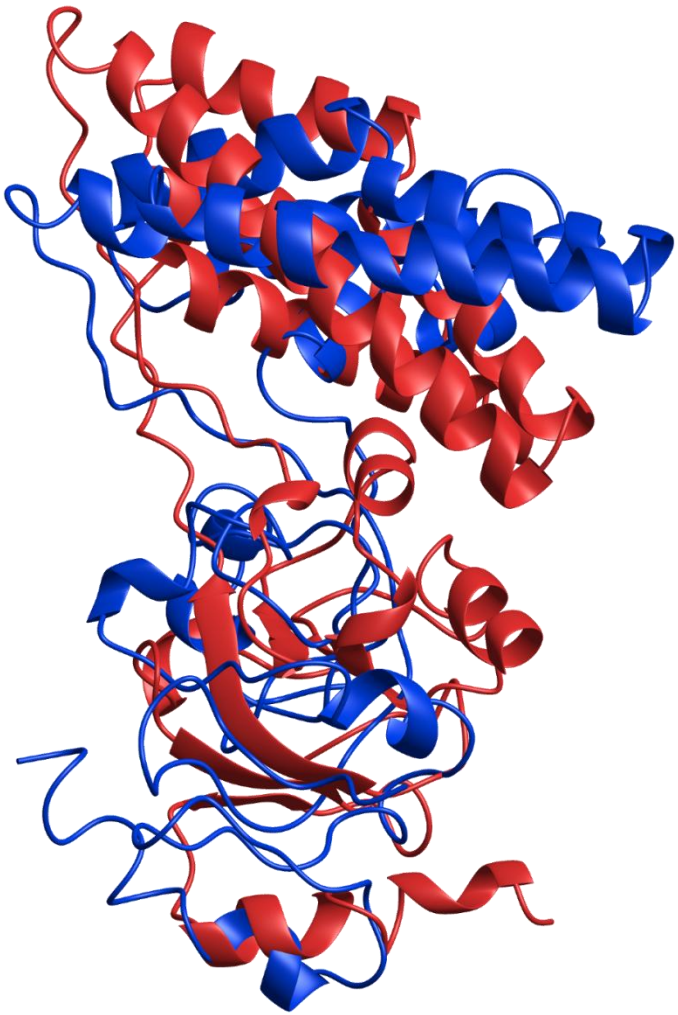
+

R_2/R_1 ratios

of ^{15}N relaxation rates

Structure determination of globular proteins

EIN: b.b.Cα RMSD 14.7 [Å]



Experimental restraints:

Dihedral angles from TALOS+ predictions

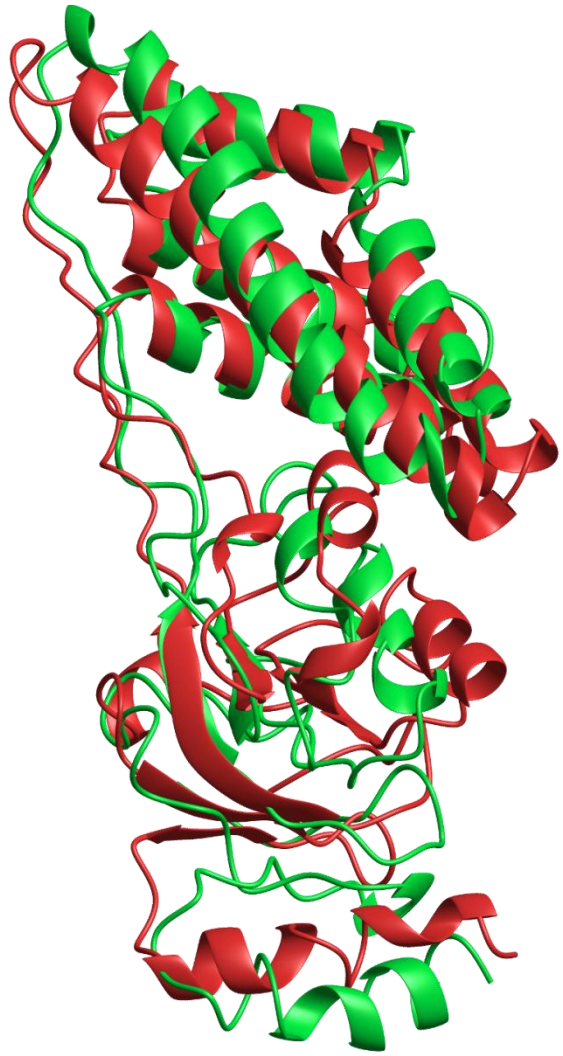
Back Bone Hydrogen bonds connectivity

and

limited set of 804 NOEs
for methyl and HN protons

Structure determination of globular proteins

EIN: b.b.Cα RMSD 4.1 [Å]



Experimental restraints:

Dihedral angles from TALOS+ predictions

Back Bone Hydrogen bonds connectivity

and

limited set of 804 NOEs
for methyl and HN protons

+

***R_2/R_1* ratios**
of ^{15}N relaxation rates

Xplor-NIH facilities

Examples and Helps

in every **Xplor-NIH** installation

~/xplor/eginput/

Example scripts for using DiffPot and RelaxaRatioPot

Sample scripts for

- Docking
- Refinement
- Structure determination

with all necessary sample data files

Helper scripts for data fitting and filtering

Future challenges

Experimental / Spectroscopic

- Temperature control and calibration
- Viscosity measurements
- Better spectroscopic techniques

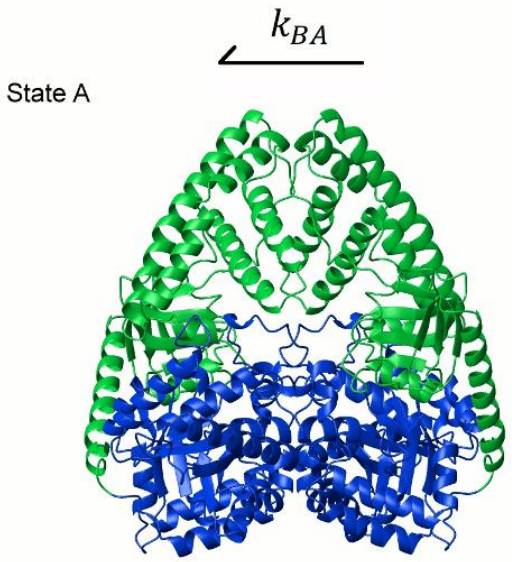
Computational / Theoretical

- Inhomogeneous hydration layer
- Treatment of internal motions
- Better models for diffusion tensor predictions

Theoretical Modeling of internal protein motions

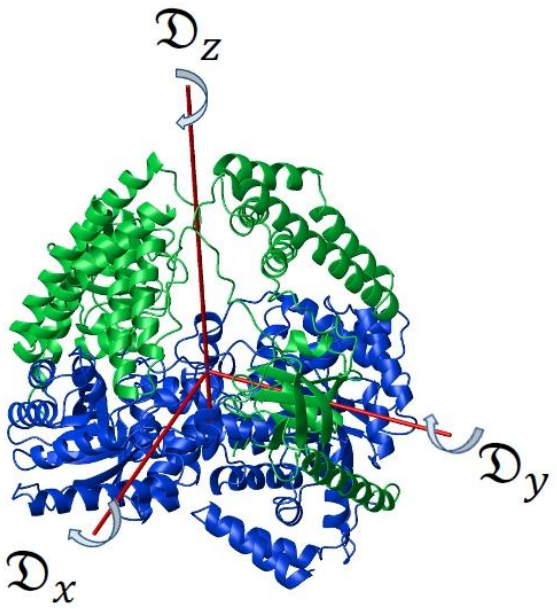
- Local mobility of small protein subunits
- Motions of Large protein domains

Time scale of Conformational transitions

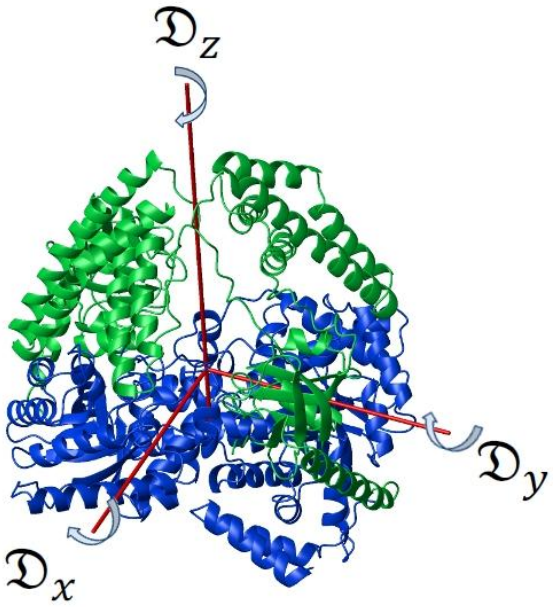


$$\tau_c = \frac{1}{k_{AB} + k_{BA}}$$

Time scale of Rotational diffusion

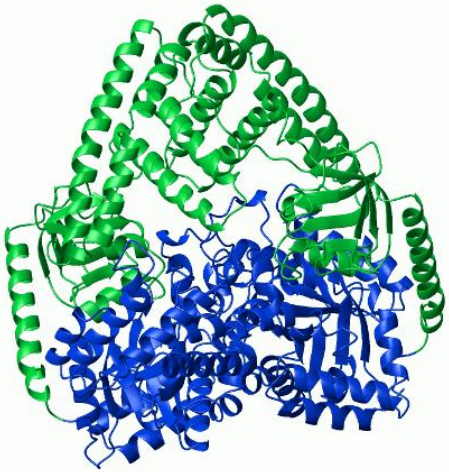


$$\tau_{\mathfrak{D}} = \frac{1}{2\left(\mathfrak{D}_x + \mathfrak{D}_y + \mathfrak{D}_z\right)}$$



Conformation transitions on different time scales

State A



Slow Exchange

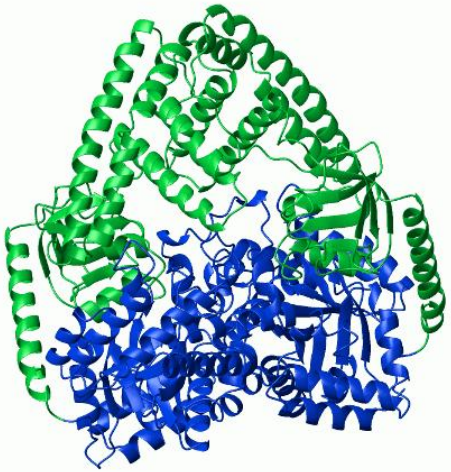
$$\tau_C \gg \tau_{\mathfrak{D}}$$

Approximation of **two species** with **two different structures** and **two diffusion tensors**

$$\mathfrak{D}^A \quad \text{and} \quad \mathfrak{D}^B$$

Conformation transitions on different time scales

State A



Fast Exchange

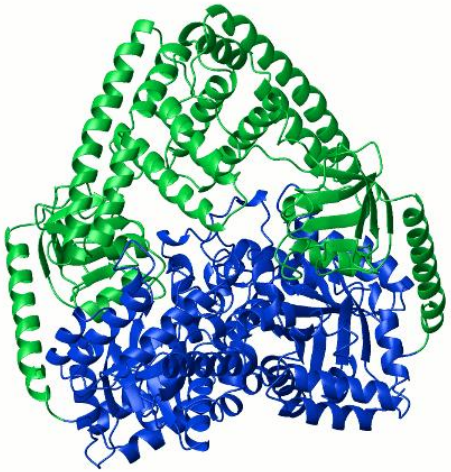
$$\tau_c \ll \tau_{\mathfrak{D}}$$

Approximation of **single conformer** with **one averaged structure** and **one averaged diffusion tensor**

$$\bar{\mathfrak{D}}$$

Conformation transitions on different time scales

State A



Intermediate Exchange

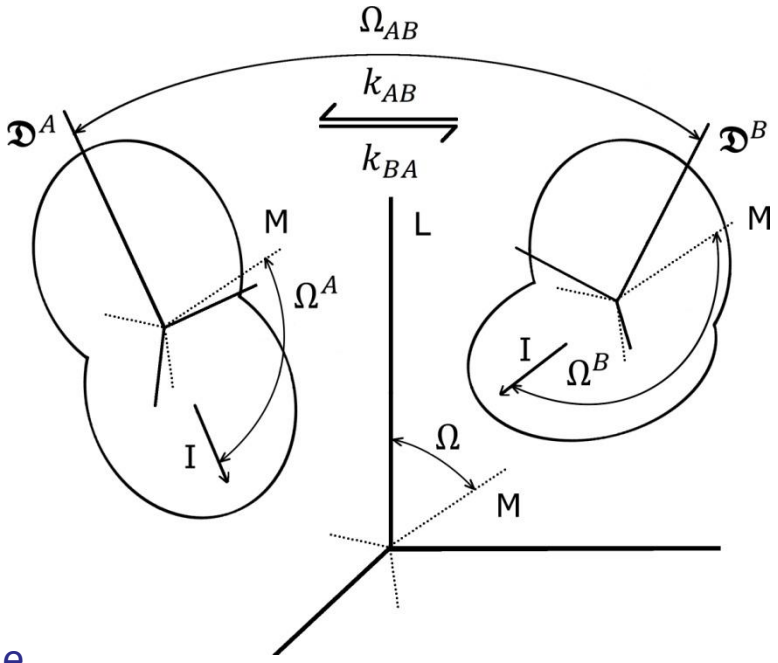
$$\tau_c \sim \tau_{\mathcal{D}}$$

?

Rotational diffusion of semi-Rigid molecule

Conformation transition between discrete set of states

- Molecule tumbles in isotropic solvent
- Molecule exchanges between discrete conformations $\varepsilon = A, B, \ldots$
- In each conformation state molecule is rigid and have diffusion tensor $\mathfrak{D}^{\varepsilon}$
- The transition time is much shorter than the time which molecule spends in any conformation



Rotational diffusion of semi-Rigid molecule

Correlation function in frequency domain (no restrictions)

$$\tilde{C}_l(\sigma) = \frac{4\pi}{2l+1} \sum_{\varepsilon,\eta} \mathbf{Y}_l^T(\Omega_{\varepsilon \mathrm{I}}) \mathbf{A}^{\varepsilon,l\dagger} \mathbf{R}^{\varepsilon\eta}(\sigma) \mathbf{A}^{\eta,l} \mathbf{Y}_l^*(\Omega_{\eta \mathrm{I}}) P_{eq}^{\eta}$$

Ryabov, Clore, Schwieters (2012)

Rotational diffusion of semi-Rigid molecule

Correlation function in frequency domain (no restrictions)

$$\tilde{C}_l(\sigma) = \frac{4\pi}{2l+1}\sum_{\varepsilon,\eta} \mathbf{Y}_l^T(\Omega_{\varepsilon \mathrm{I}})\mathbf{A}^{\varepsilon,l\dagger}\mathbf{R}^{\varepsilon\eta}(\sigma)\mathbf{A}^{\eta,l}\mathbf{Y}_l^*(\Omega_{\eta \mathrm{I}})P_{eq}^{\eta}$$

Spectral density

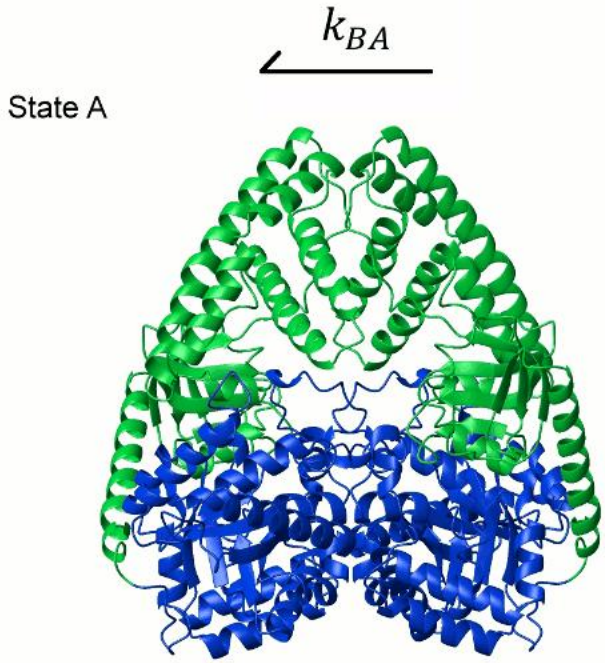
$$J_l(\omega) = Re\left\{\tilde{C}_l(\sigma)\big|_{\sigma\to i\omega}\right\}$$

Experimental observables: R1, R2 etc.

Ryabov, Clore, Schwieters (2012)

Illustrative calculations

EI dimer



Estimations of XplorNIH @ 300 K

$\mathfrak{D}_x^A = 29.16 \times 10^7\ [s^{-1}]$ $\quad \tau_{\mathfrak{D}}^A = 53.73\ \ [ns]$

$\mathfrak{D}_y^A = 31.47 \times 10^7\ [s^{-1}]$

$\mathfrak{D}_z^A = 32.43 \times 10^7\ [s^{-1}]$

$\mathfrak{D}_x^B = 15.71 \times 10^7\ [s^{-1}]$ $\quad \tau_{\mathfrak{D}}^B = 79.99\ \ [ns]$

$\mathfrak{D}_y^B = 15.82 \times 10^7\ [s^{-1}]$

$\mathfrak{D}_z^B = 30.99 \times 10^7\ [s^{-1}]$

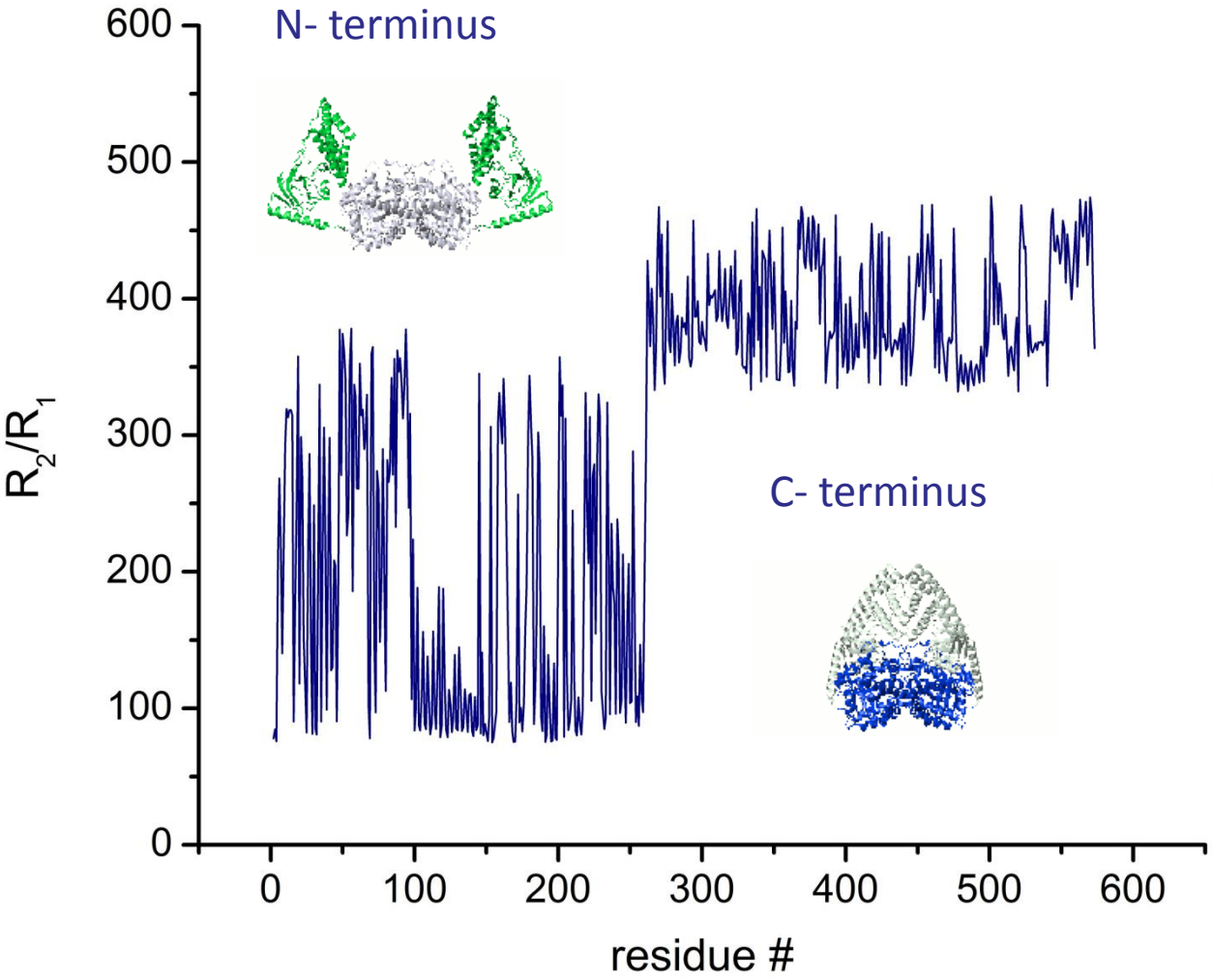
Assumptions

Symmetric motions $\quad \Omega_{\mathrm{AB}}: \{\alpha_{\mathrm{AB}} = 0, \beta_{\mathrm{AB}} = 0, \gamma_{\mathrm{AB}} = 0\}$

Equal occupation $\quad P_{eq}^A = P_{eq}^B = 1/2 \quad k_{AB} = k_{BA}$

Illustrative calculations

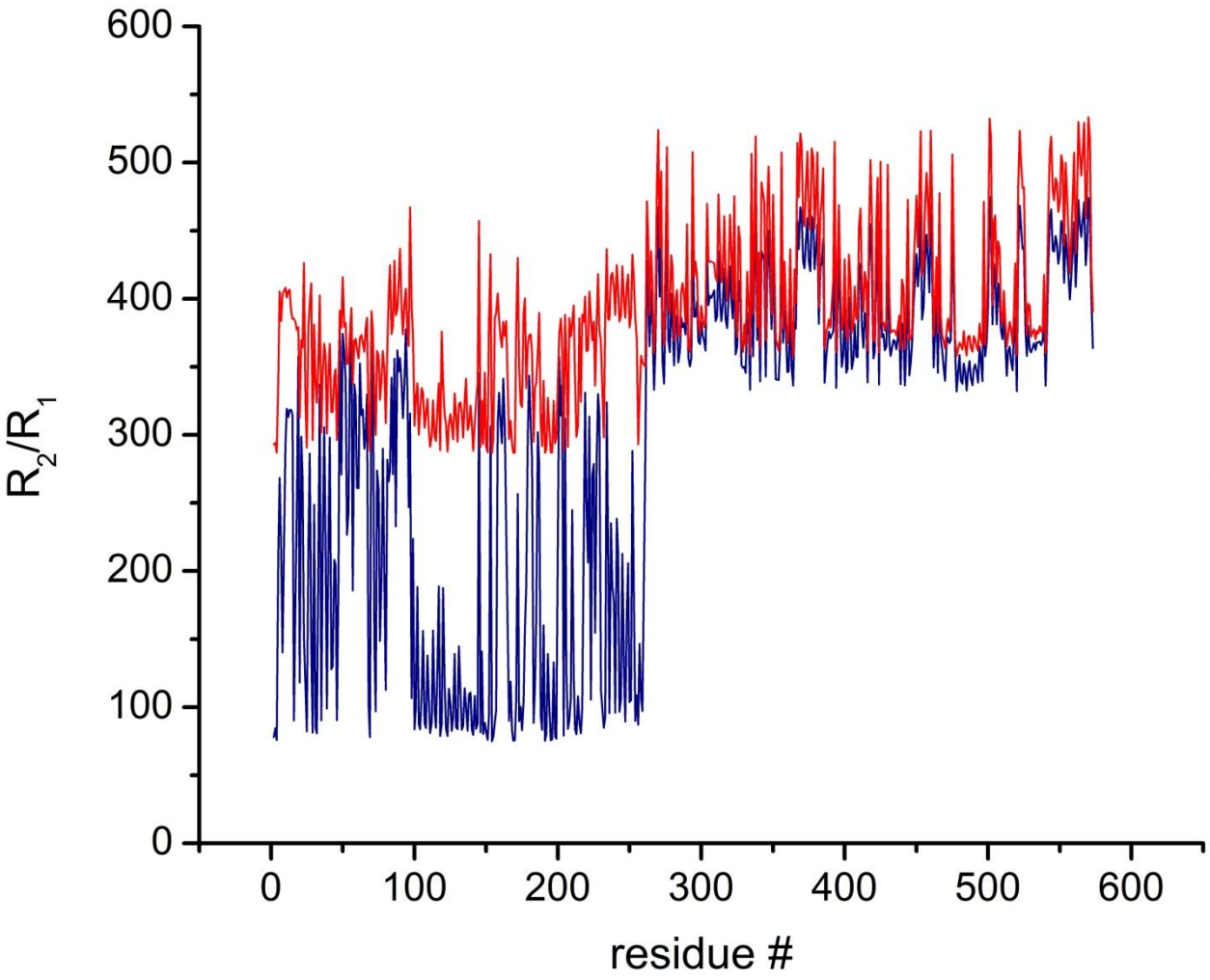
NMR relaxation rates for 600 MHz @ 300 K



$$k = \frac{2}{\tau_{\mathfrak{D}}^{A} + \tau_{\mathfrak{D}}^{B}} = 155.57 \ [ns^{-1}]$$

Illustrative calculations

NMR relaxation rates for 600 MHz @ 300 K

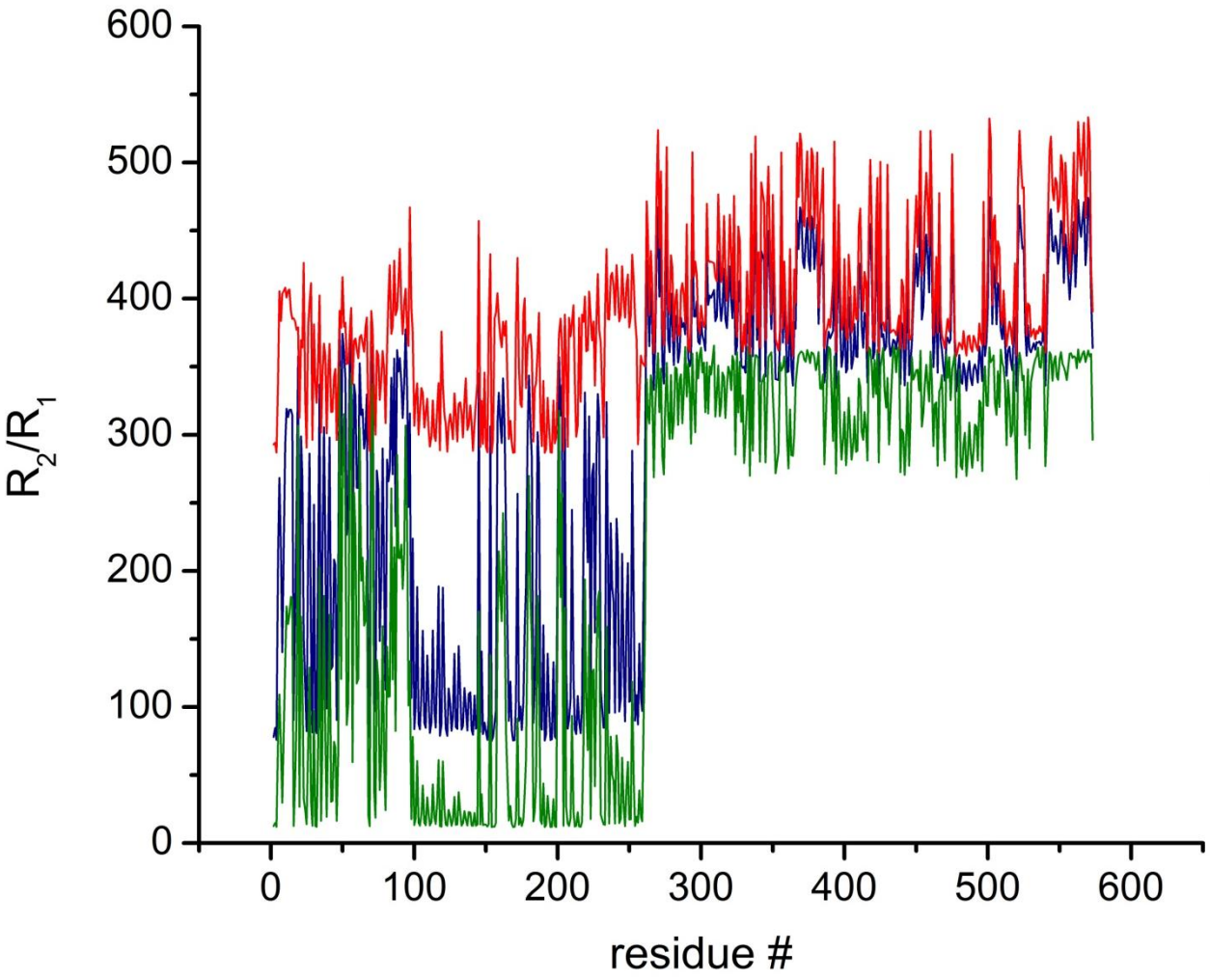


$$k_{slow} = 0.1 \times k$$

$$k = \frac{2}{\tau_{\mathfrak{D}}^{A} + \tau_{\mathfrak{D}}^{B}} = 155.57 \ [ns^{-1}]$$

Illustrative calculations

NMR relaxation rates for 600 MHz @ 300 K



$$k_{slow} = 0.1 \times k$$

$$k = \frac{2}{\tau_{\mathfrak{D}}^{A} + \tau_{\mathfrak{D}}^{B}} = 155.57 \ [ns^{-1}]$$

$$k_{fast} = 10 \times k$$

CONCLUSIONS

- Global restrains on overall molecular shape are useful for protein structure calculations. However, to achieve the best performances global restrains need to be supplemented by local restrains.
- Xplor-NIH is equipped with specific module for sampling molecular surfaces which opens the possibly to use the experimental restraints related to molecular surface topology for molecular structure computations.
- NMR relaxation data can be used as source of both global and local restrains simultaneously.
- Recent theoretical developments in combination with computational facilities of Xplor-NIH open the ways for new methods of simultaneous characterization of molecular structure and internal dynamics.

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