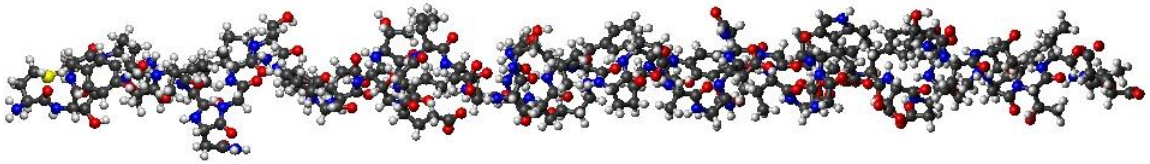
Yaroslav Ryabov

Making use of random tumbling

or deriving information about protein structures
from the way they move

Ultimate Goal of protein structure prediction

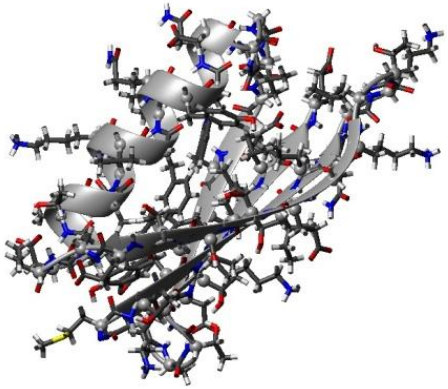
Sequence of amino acid residues



+

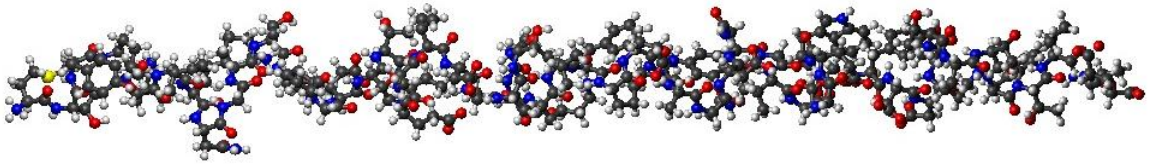
Model inter atomic forces

=



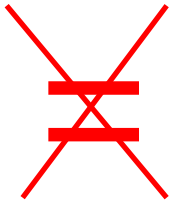
Ultimate Goal of protein structure prediction

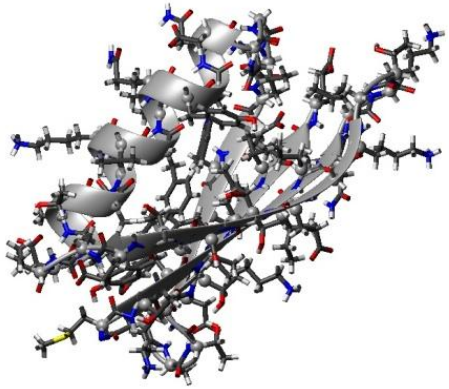
Sequence of amino acid residues



+

Model inter atomic forces

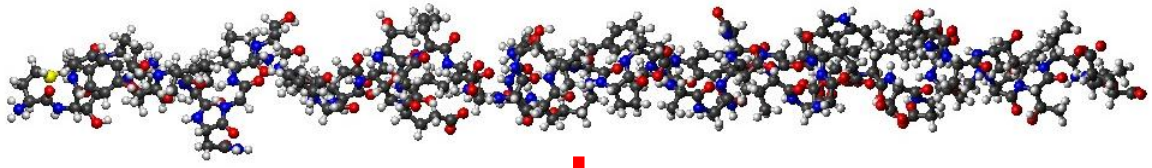




Is not yet accomplished

NMR Protein structure determination

Sequence of amino acid residues



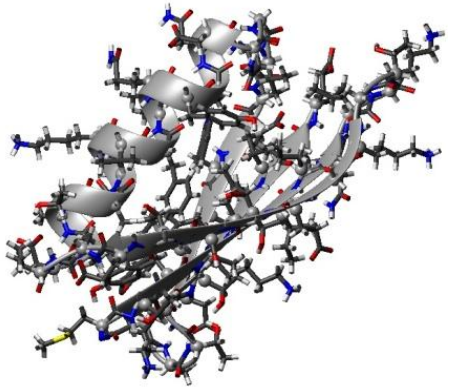
+

Experimental restraints: NOE, RDC, and etc.

+

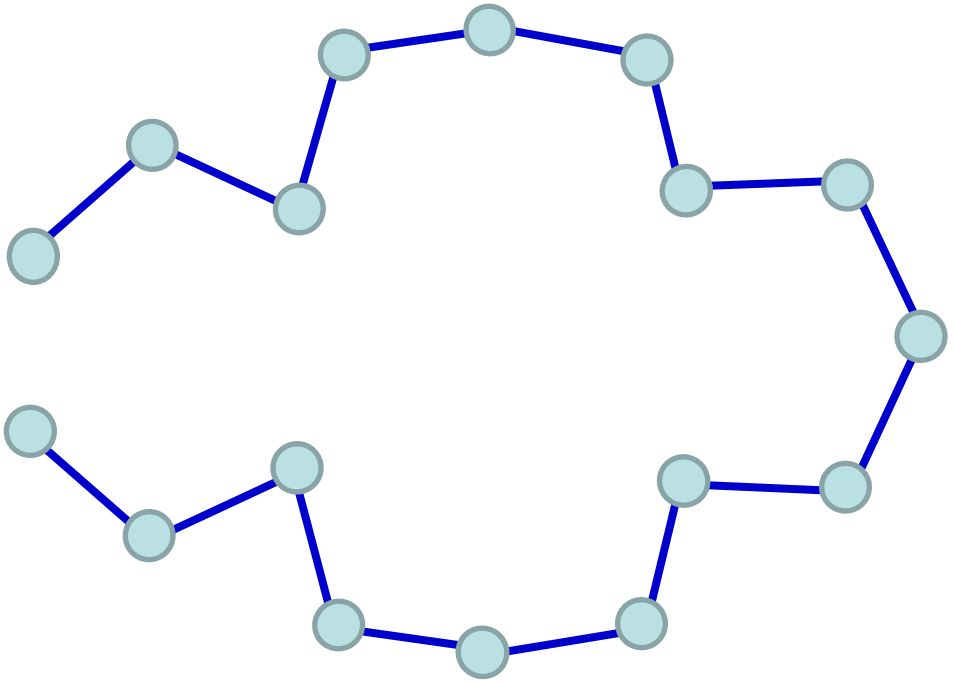
a priori **restraints from database of known structures**

=



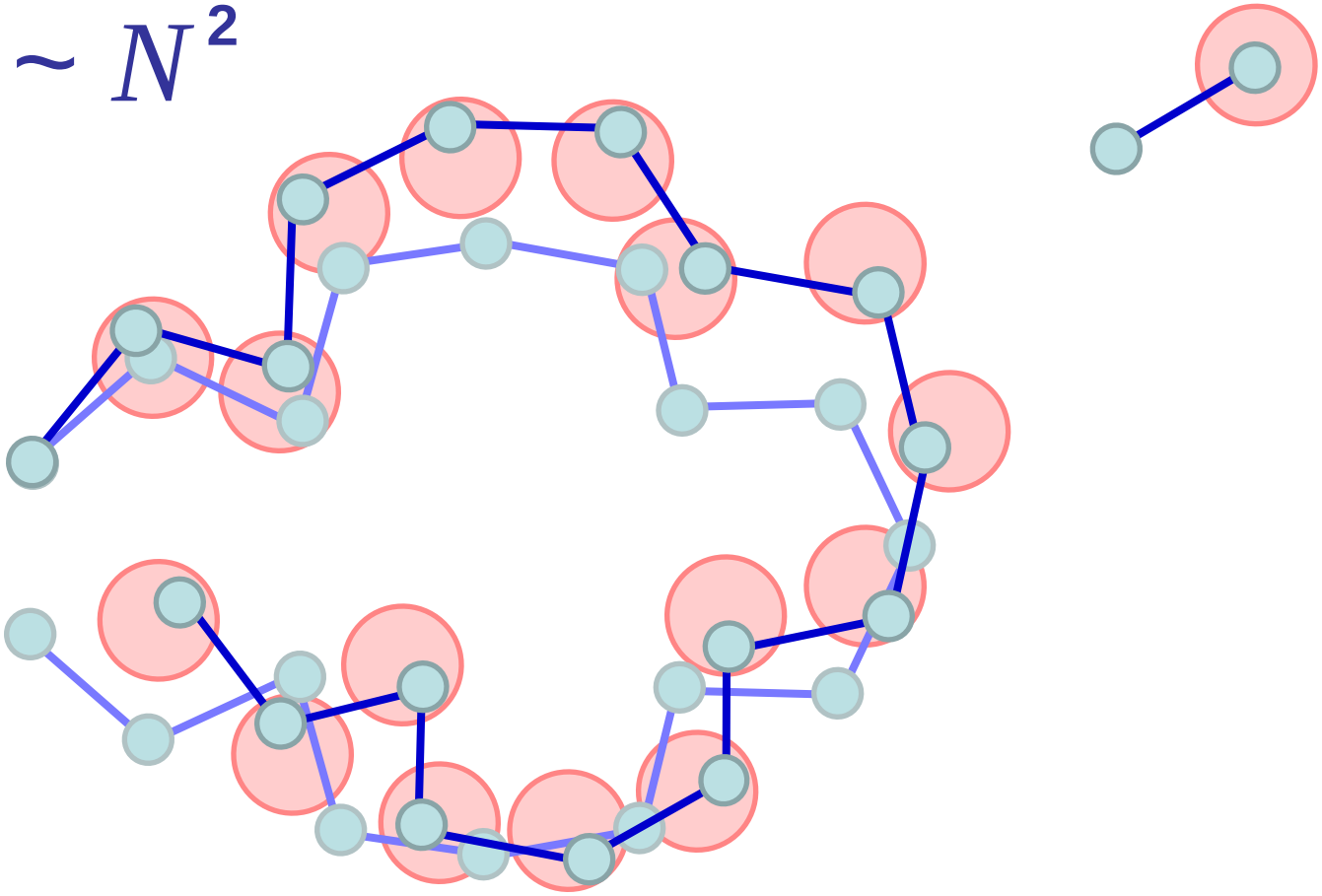
Local and Global restraints

Ideal structure



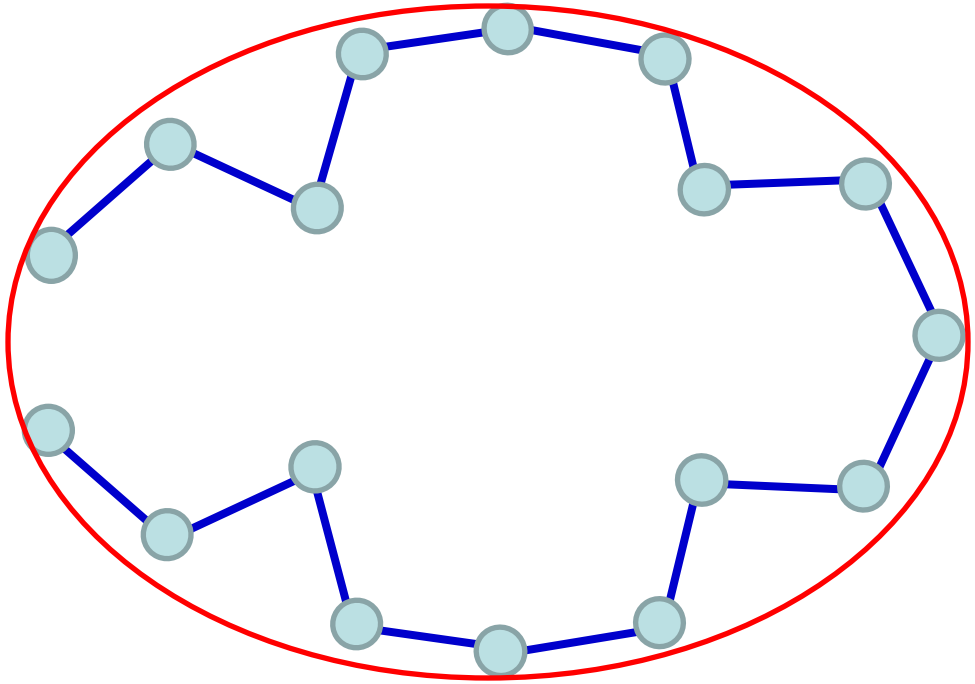
Local restraints

$$\Delta \sim N^2$$

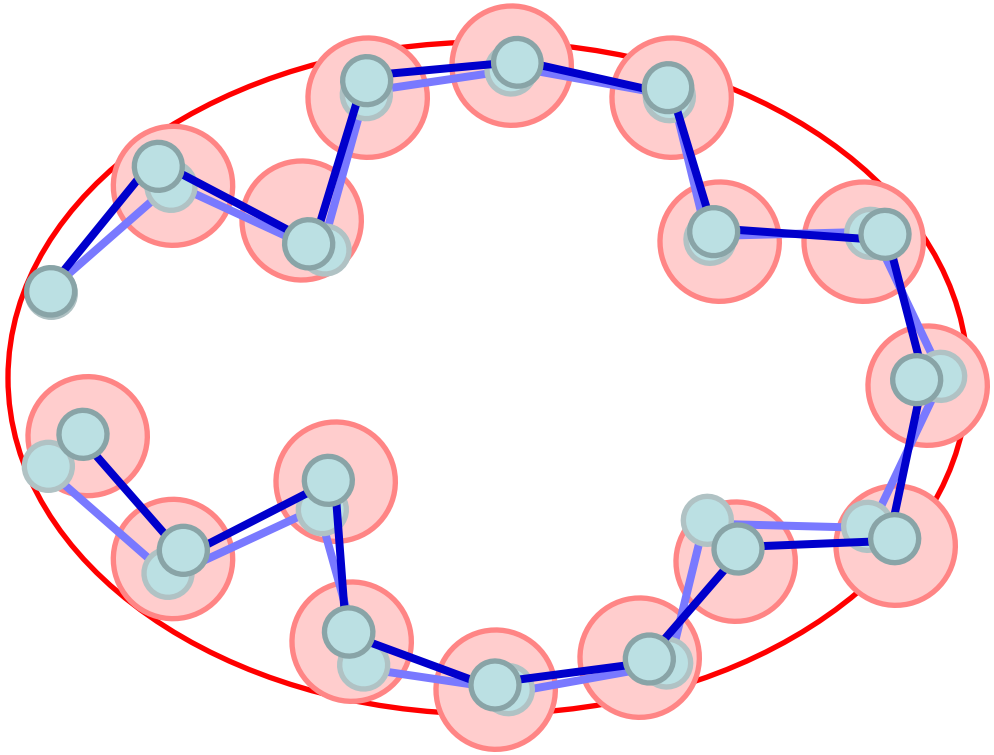


Global restraints

Overall shape

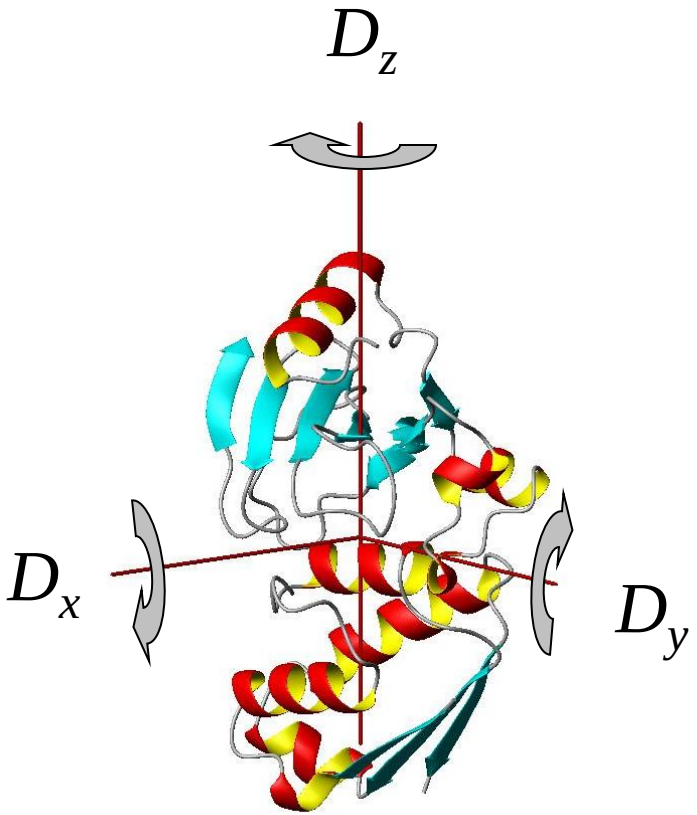


Local and Global restraints



Overall shape restraints from protein dynamics

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



3 Euler angles for
Diffusion Tensor PAF

Diffusion Properties of Proteins

from ellipsoid model

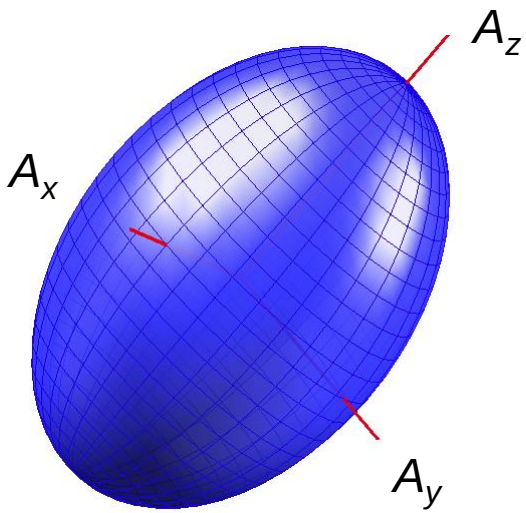
Why an ellipsoid model ?

Diffusion Tensor

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

One-to-**One**
mapping

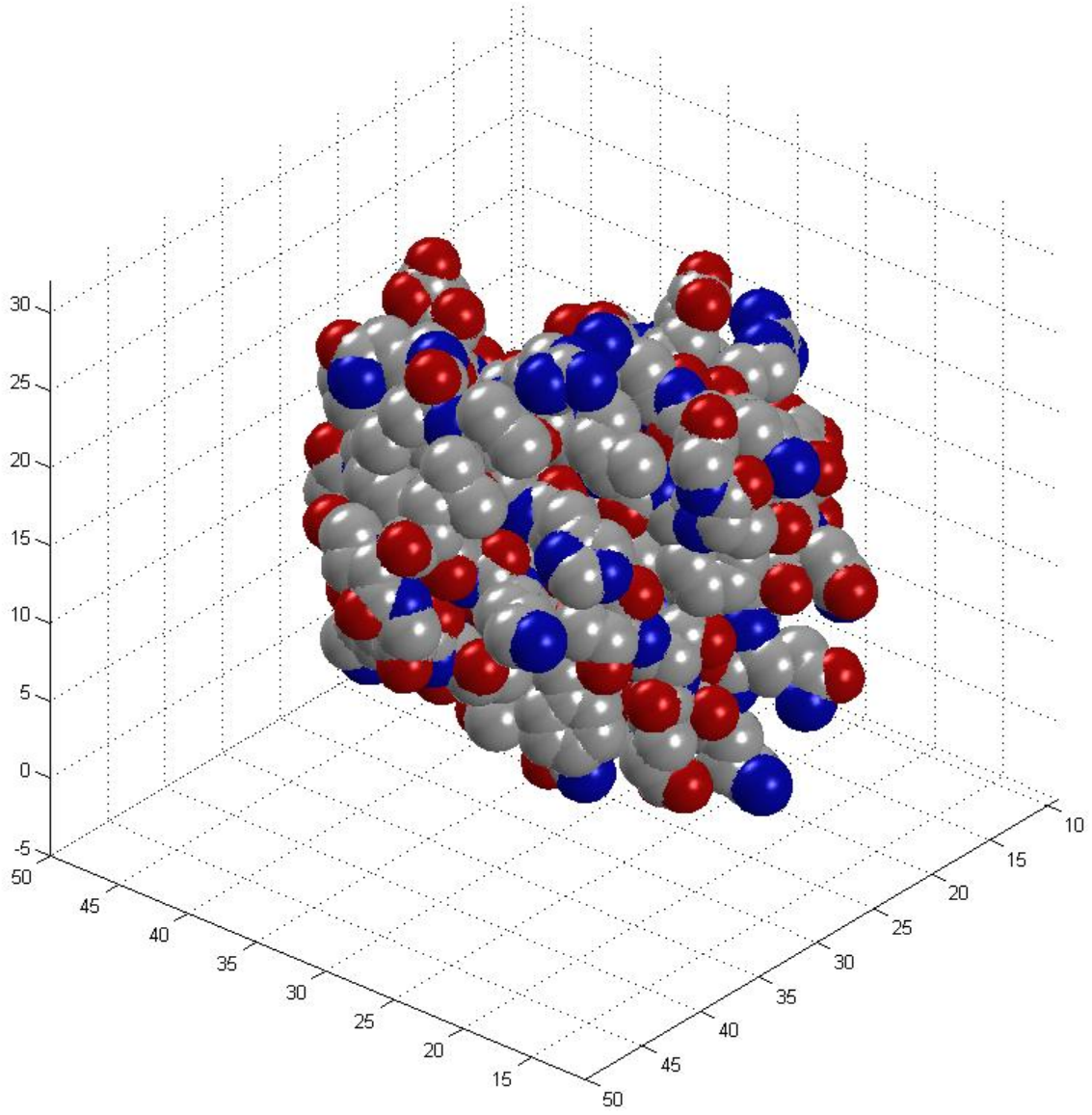
Ellipsoid Shell



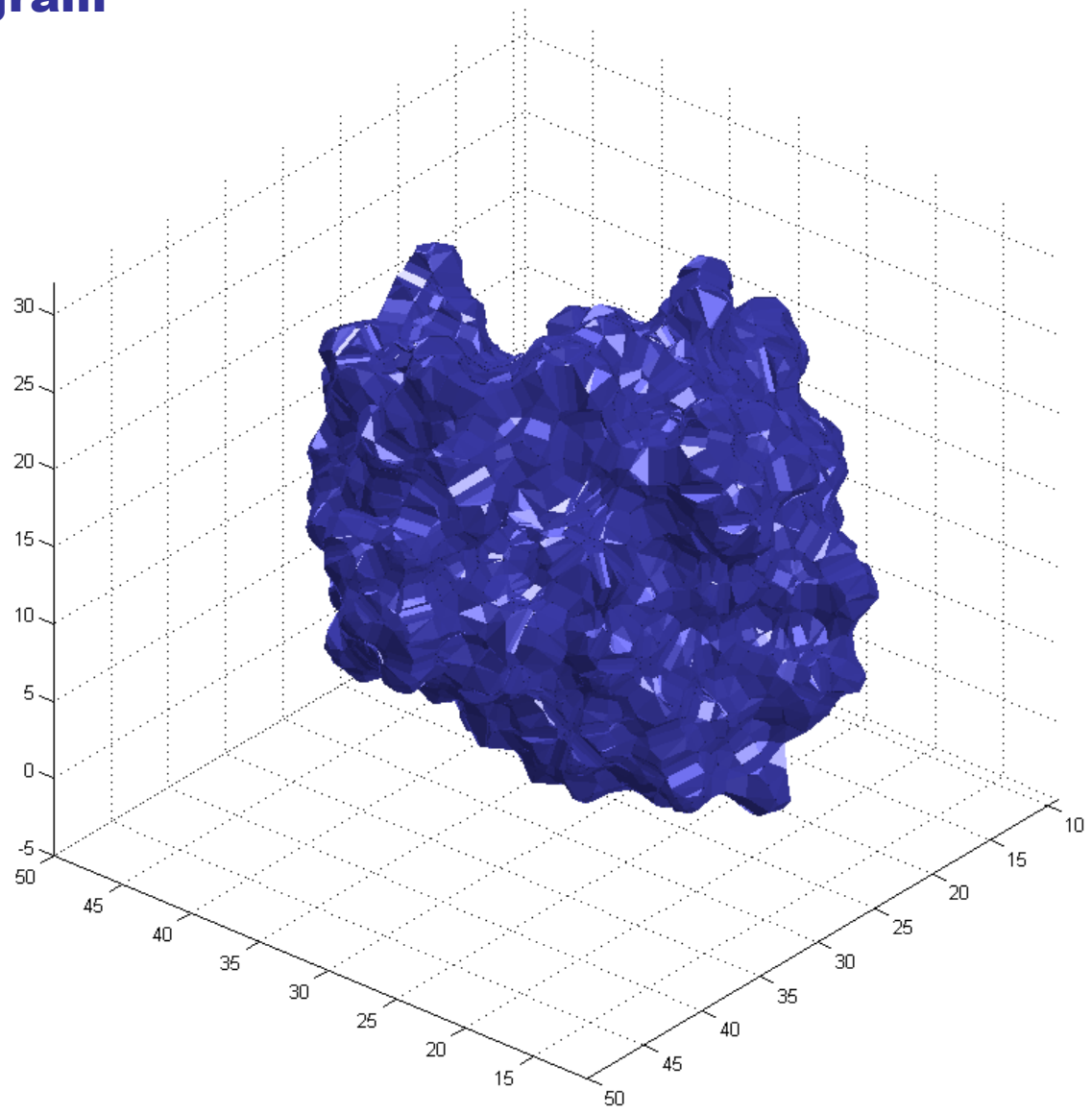
3 Euler angles for
Diffusion Tensor PAF

3 Euler angles for
Ellipsoid orientation

Mapping protein surfaces



Mapping protein surfaces
SURF program



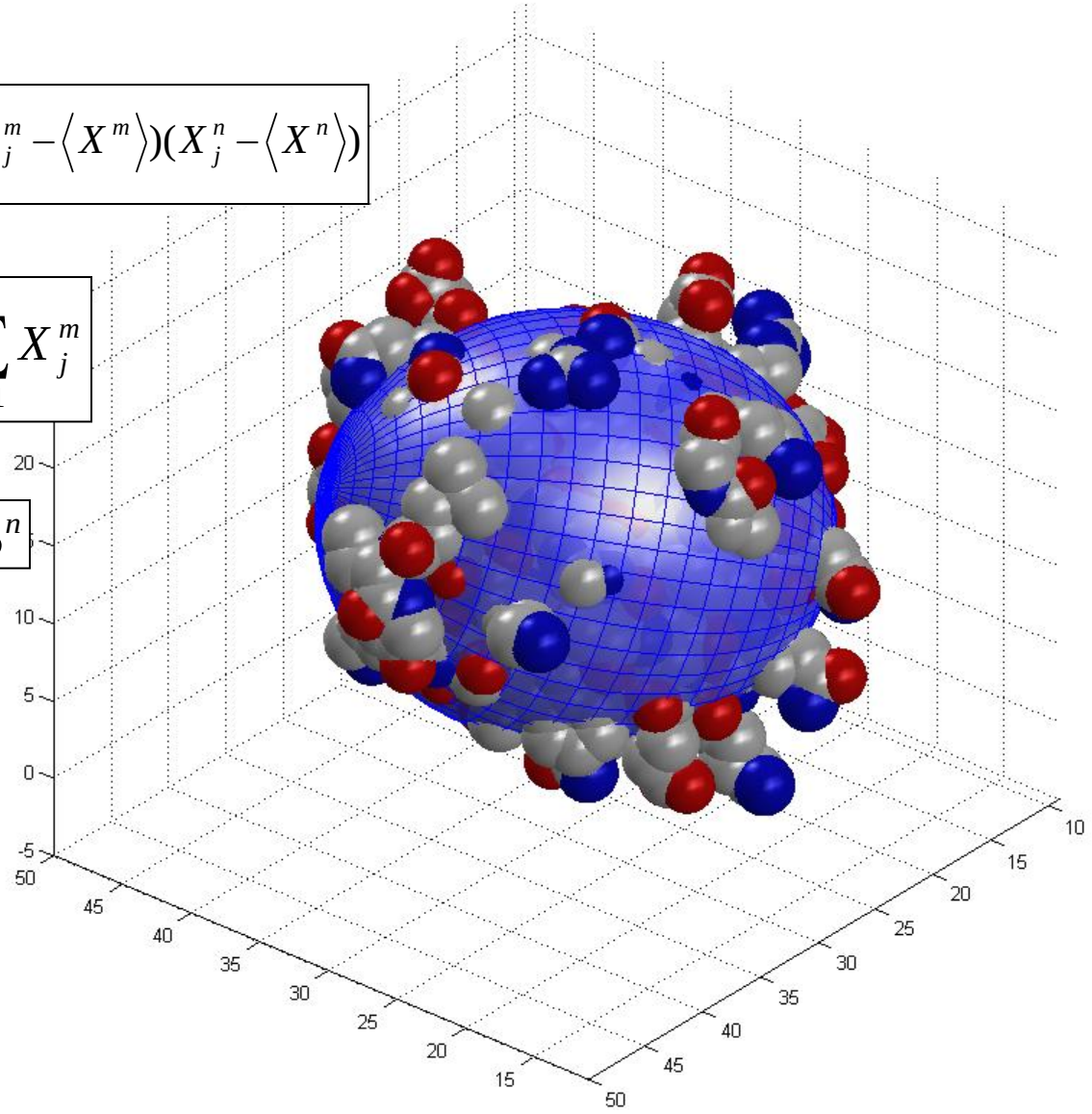
Build equivalent ellipsoid
Principal Component Analysis (PCA)

$$Cov_{m,n} = \frac{1}{N}\sum_{j=1}^{N}(X_j^m - \langle X^m \rangle)(X_j^n - \langle X^n \rangle)$$

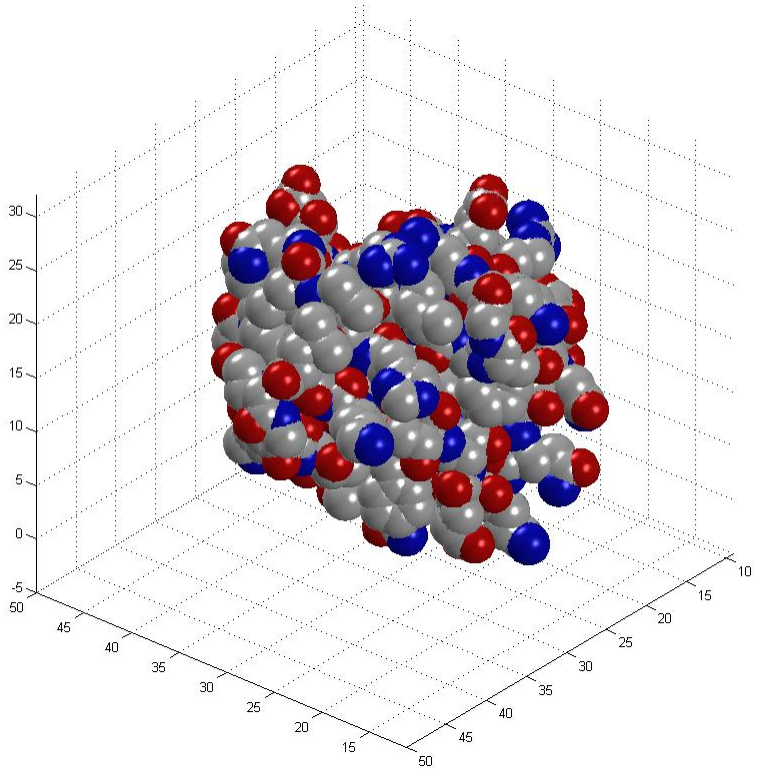
$$< X^m >= \frac{1}{N}\sum_{j=1}^{N} X_j^m$$

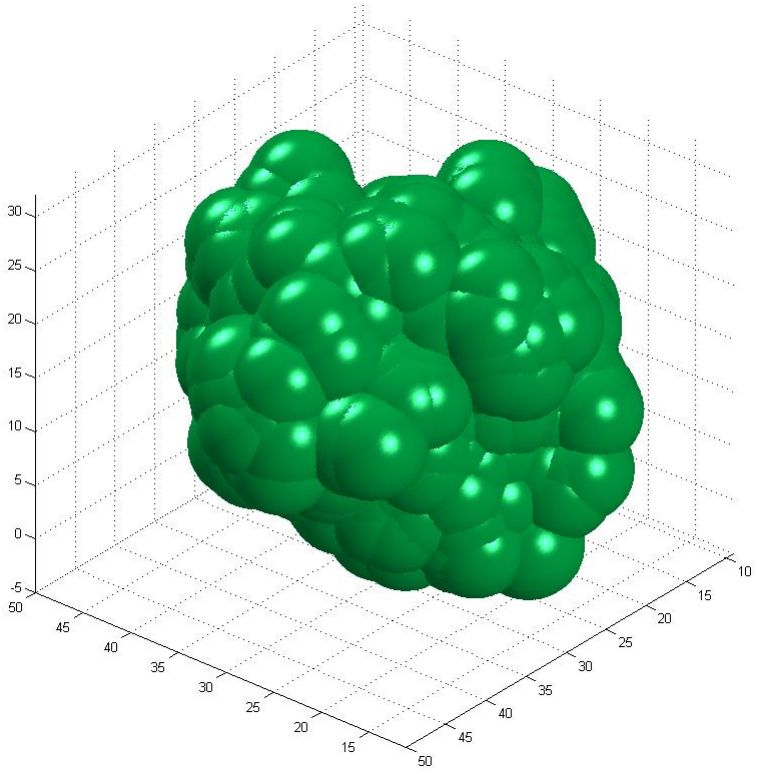
$$\mathbf{Cov}\,\mathbf{S}^n = E^n \mathbf{S}^n$$

$$a_n = \sqrt{3E^n}$$

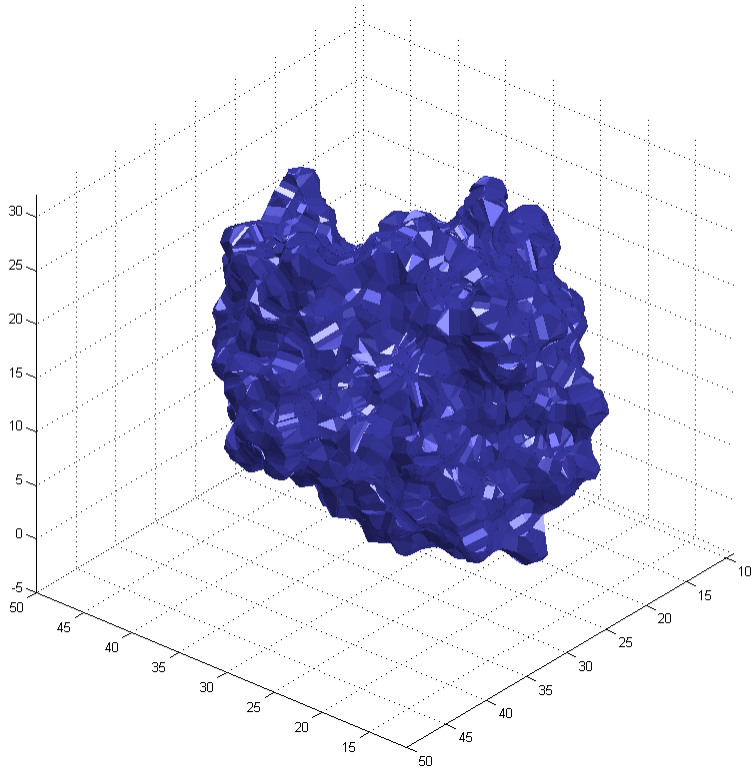


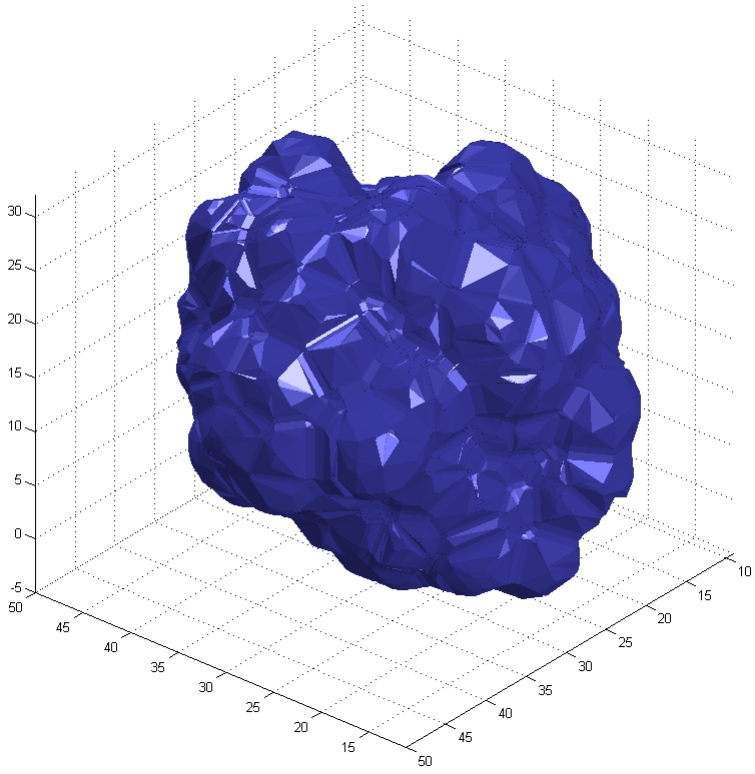
Hydration shell



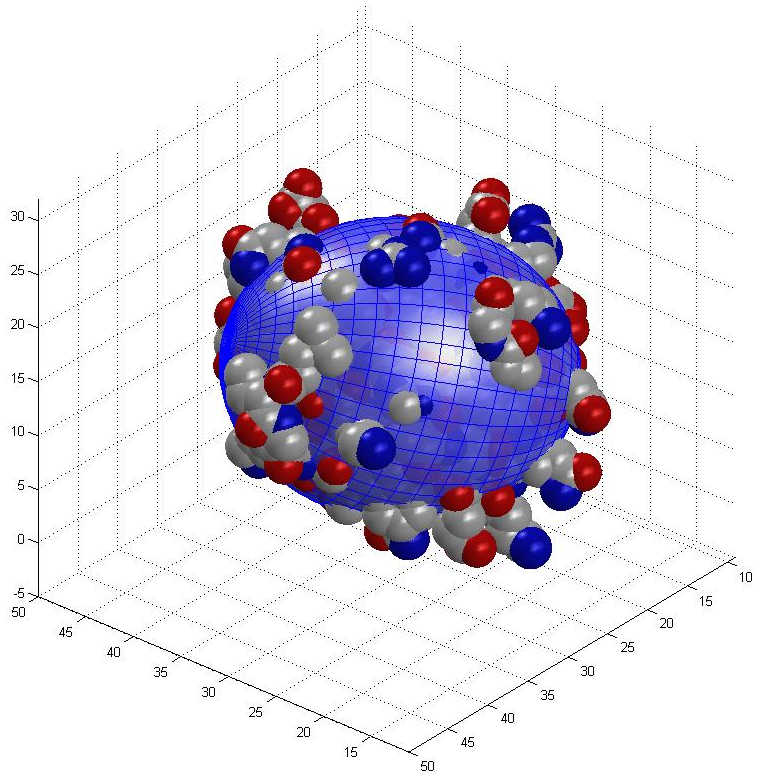


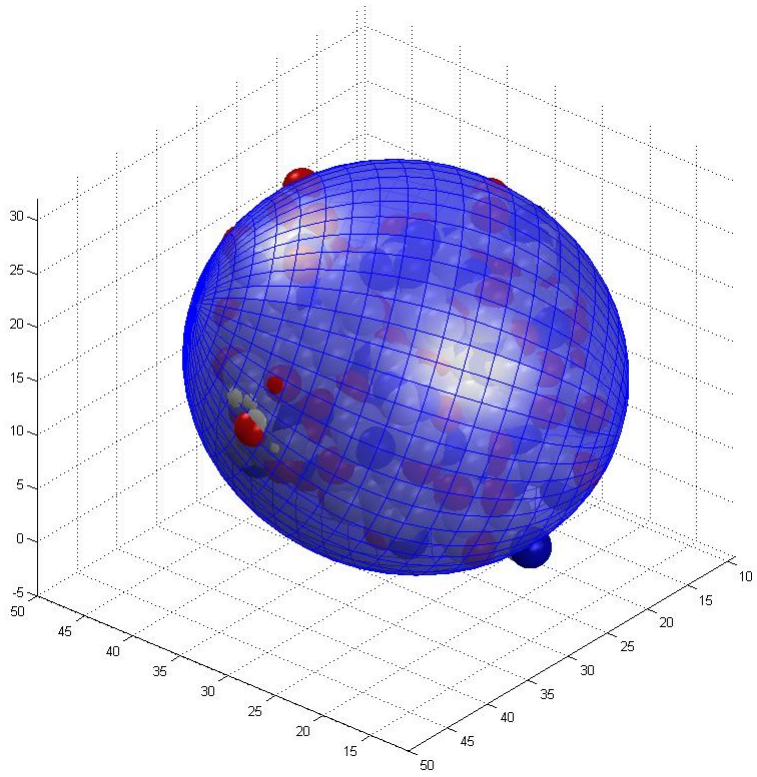
Hydration shell



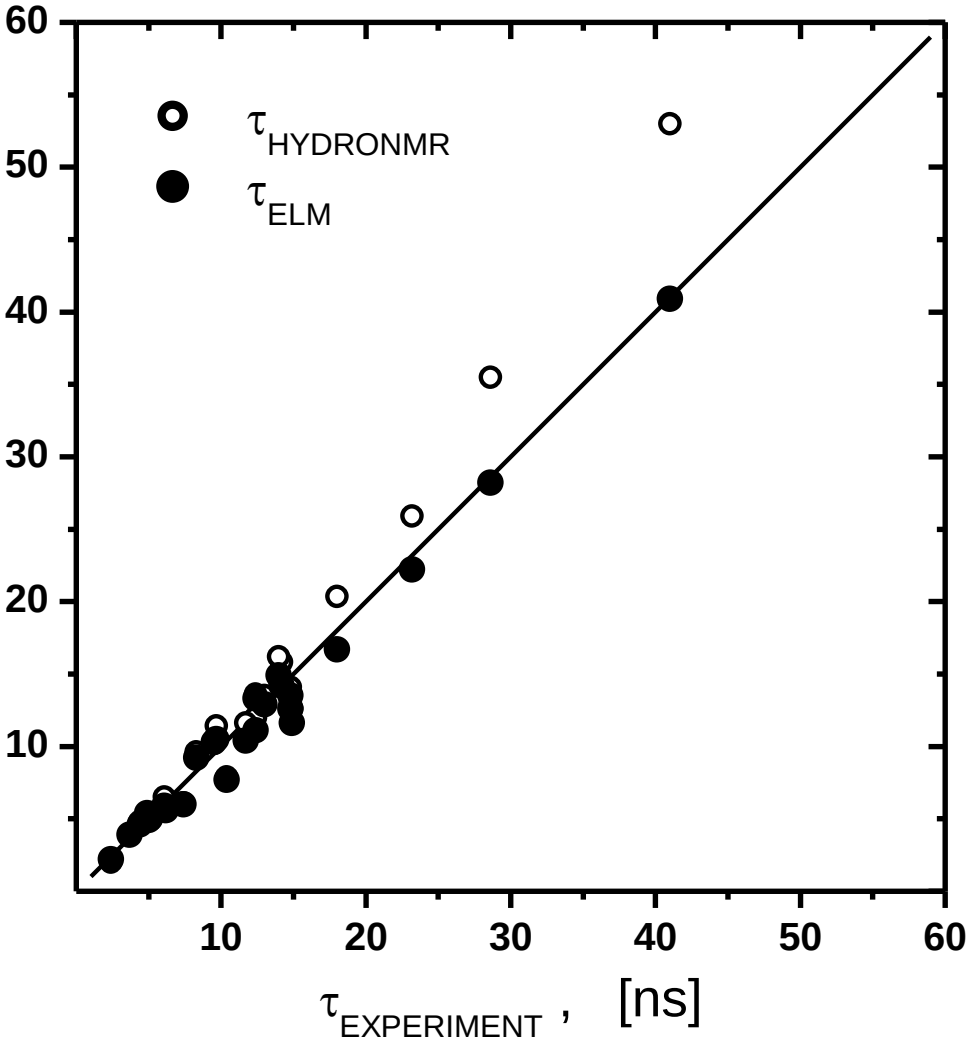


Hydration shell
Equivalent ellipsoid is approximately twice bigger



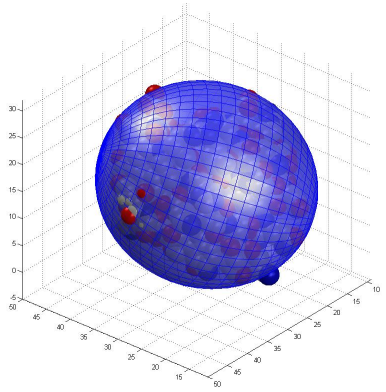


Comparison with the experimental data



A Very General Concept

- During the course of structure elucidation build an equivalent ellipsoid for every snapshot of protein structure conformation



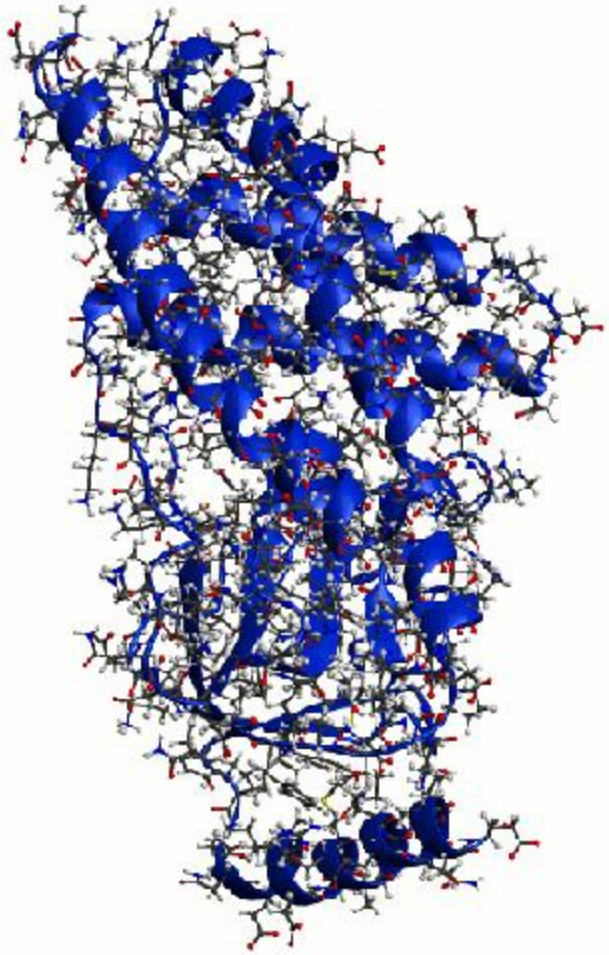
- Then calculate parameters of protein diffusion tensor using the equivalent ellipsoid shape

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

- Compare calculated diffusion tensor parameters with those which were derived from the experimental data and establish a pseudo energy term proportional to the sum of square differences between components of calculated and experimental diffusion tensors

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

Refinement of a protein structure with Xplor-NIH using overall shape restraints from diffusion tensor



N terminal domain from Enzyme I (EIN)

Standard Xplor-NIH simulated annealing protocol started from 3000K down to 25 K
With 12.5 K steps

Experimental restraints:

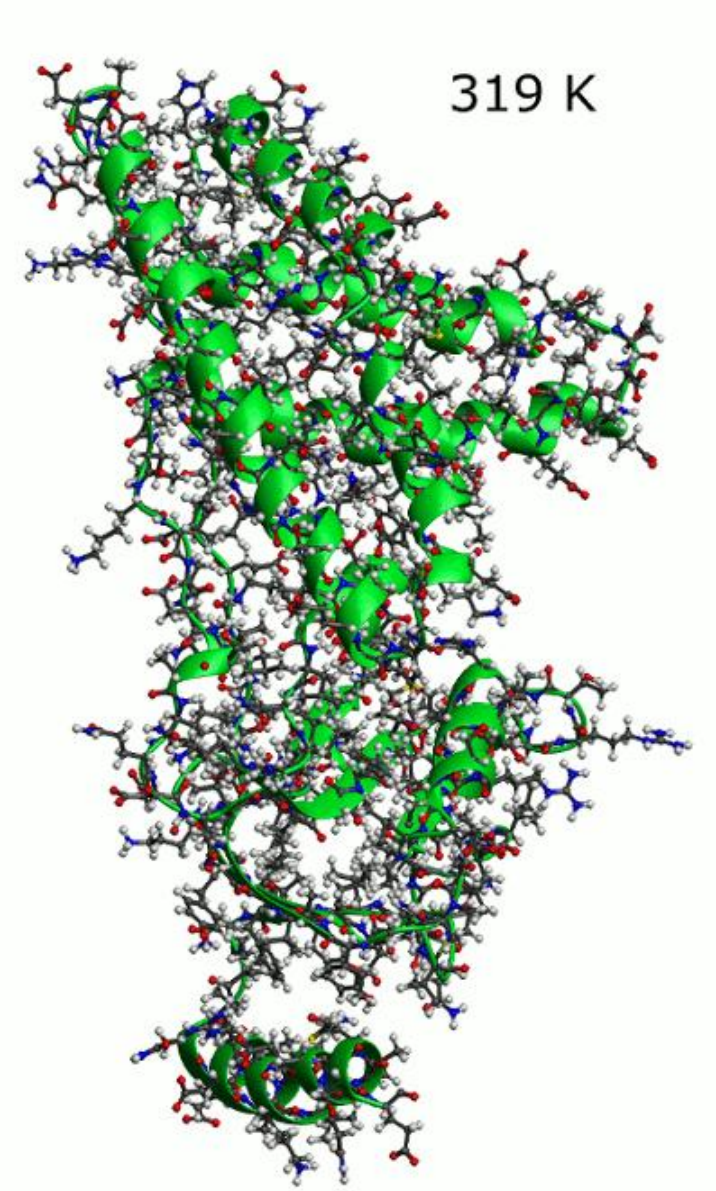
distance restraints derived from NOE

and

Components of Rotation Diffusion Tensor

10 lowest energy structures: Blue with diffusion tensor restraints
Green without diffusion tensor restraints

Effect of temperature settings for diffusion tensor term



Uncertainties in

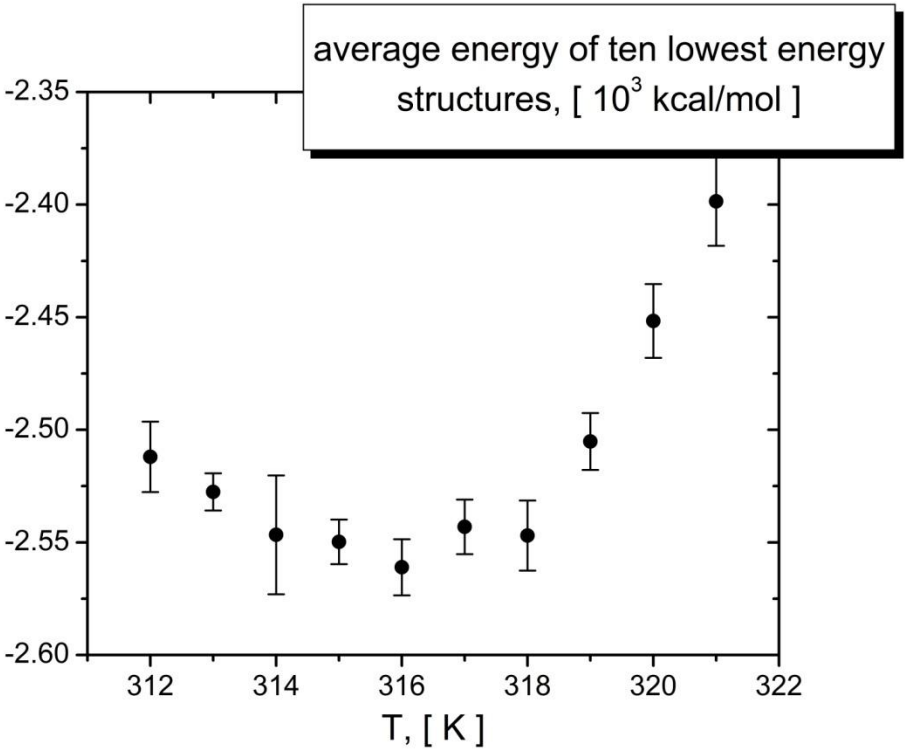
- Thickness of hydration layer
- Sample viscosity
- Sample temperature

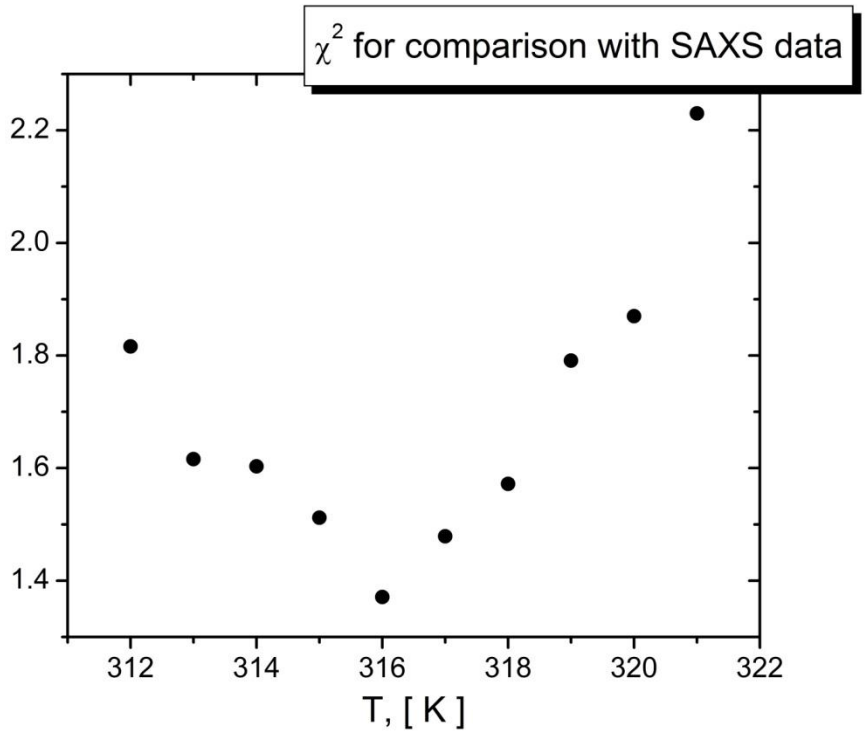
Could be compensated by adjustment of setup for

“Experimental” temperature

Effect of temperature settings for diffusion tensor term

Experimental temperature: 313 K
Temperature of the minimum: 316 K

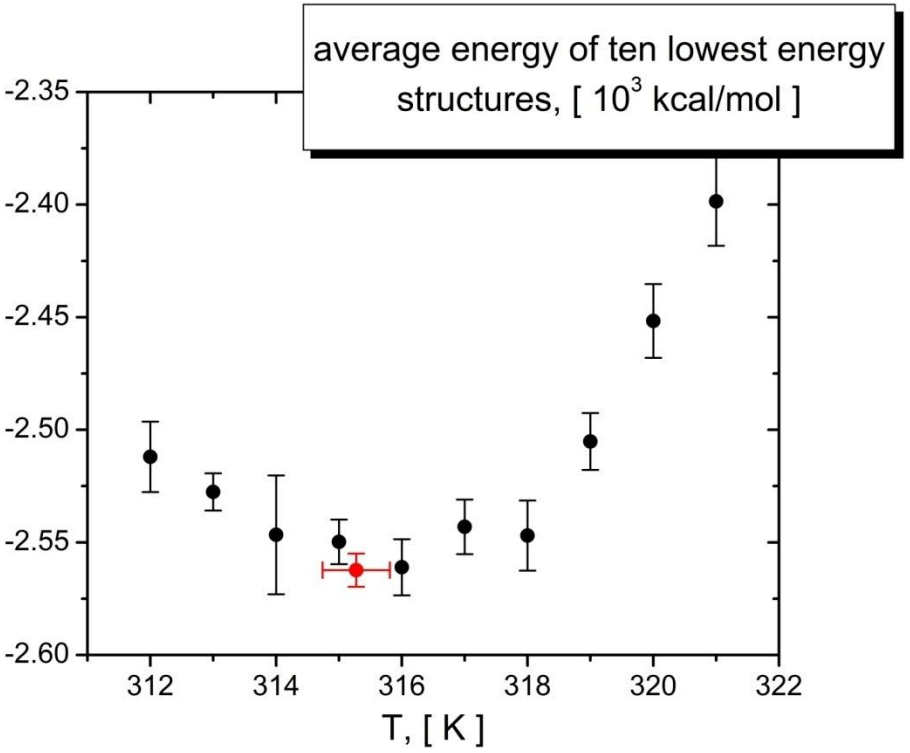


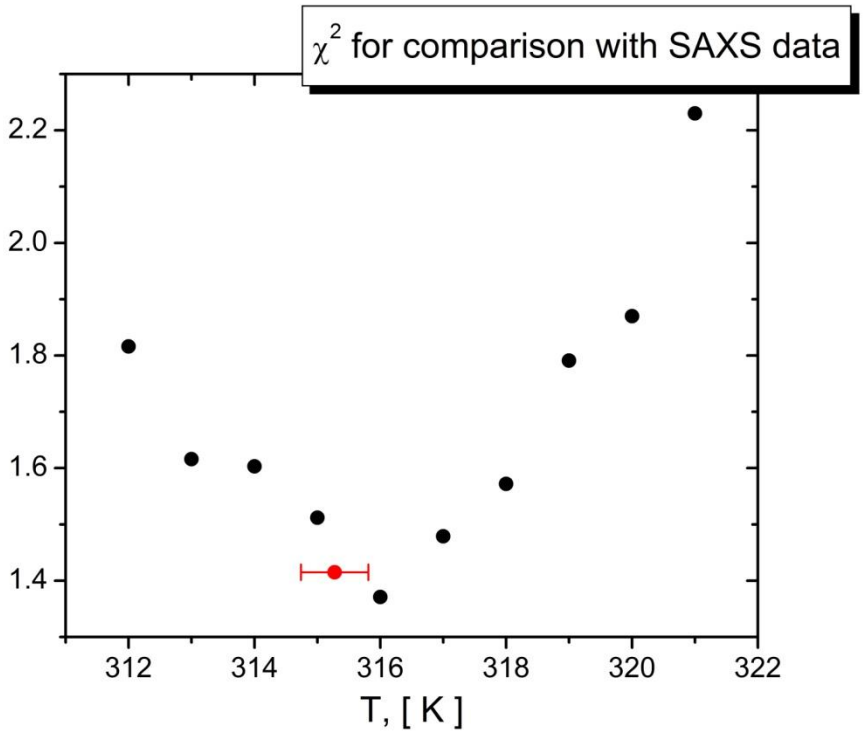


Uncertainties in
thickness of hydration layer, sample temperature, and sample viscosity

Effect of temperature settings for diffusion tensor term

Experimental temperature: 313 K Optimized Temperature: 315.3 ± 0.5
Temperature of the minimum: 316 K

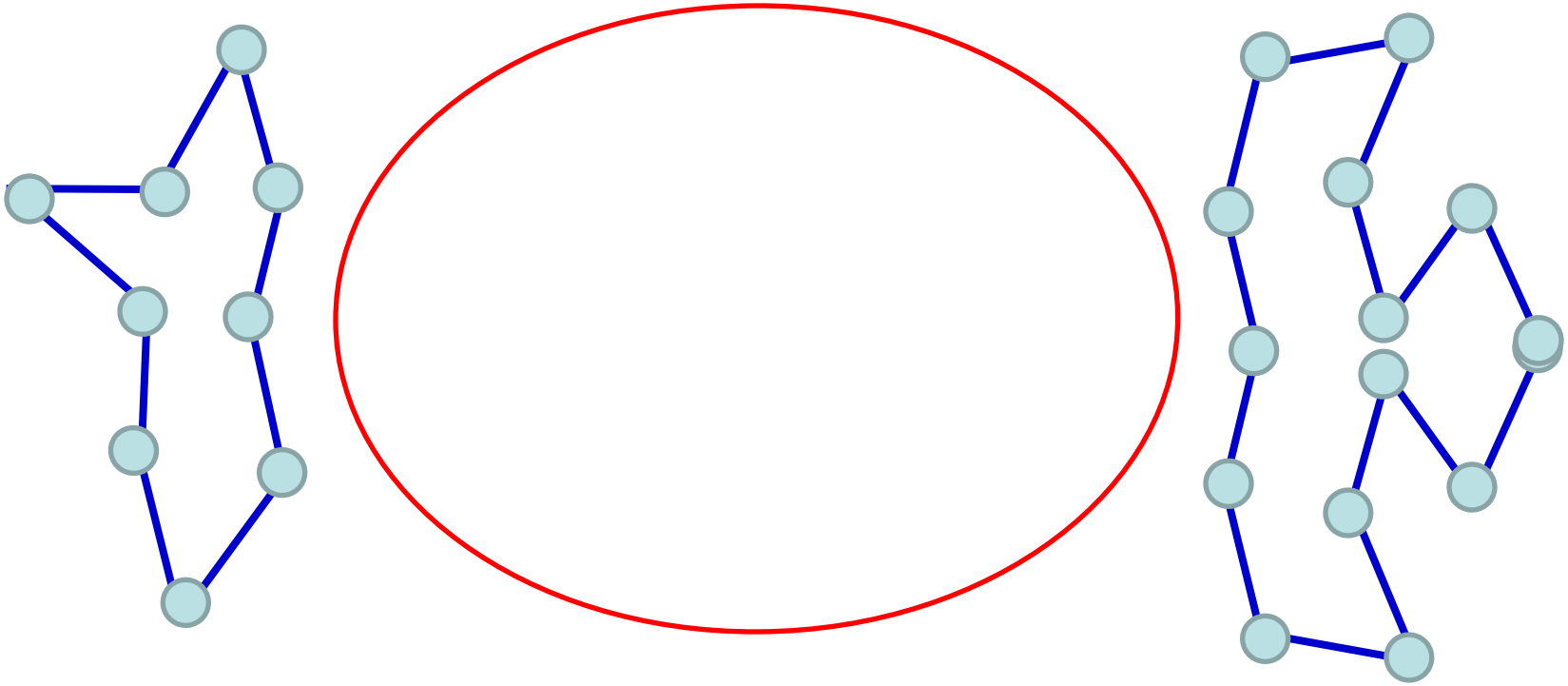




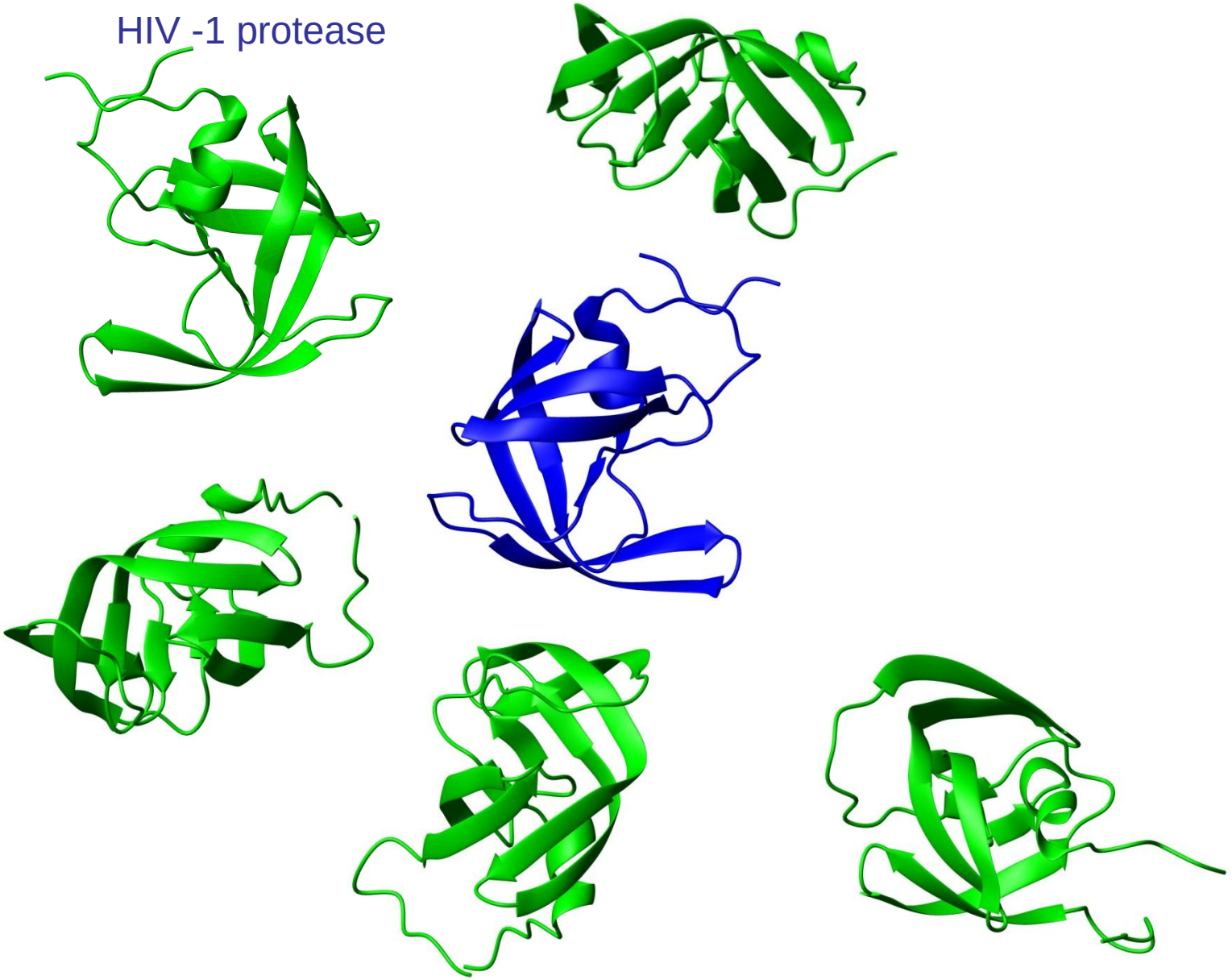
Uncertainties in
thickness of hydration layer, sample temperature, and sample viscosity

Assembling structures of multi domain proteins using the overall shape restraints provided by the diffusion tensor

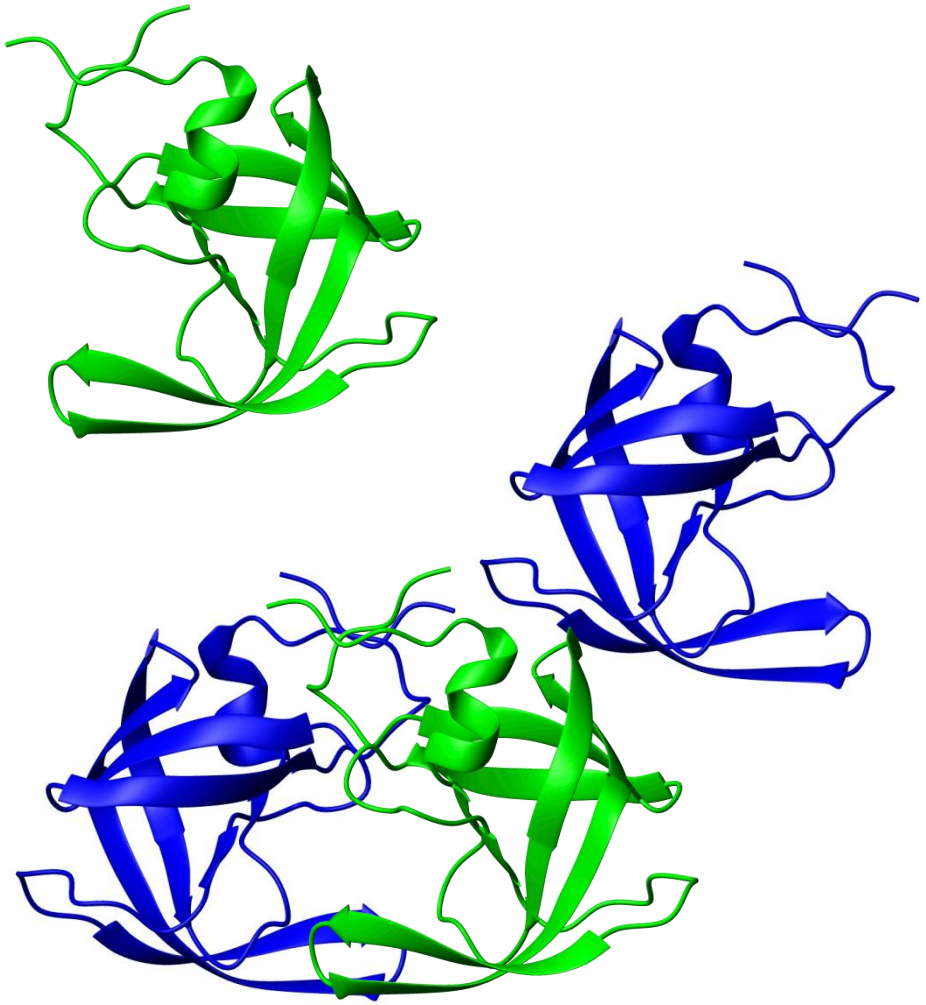
Global restraints on Overall shape



Assembling structure of a symmetric protein homo dimer



Assembling structure of a symmetric protein homo dimer



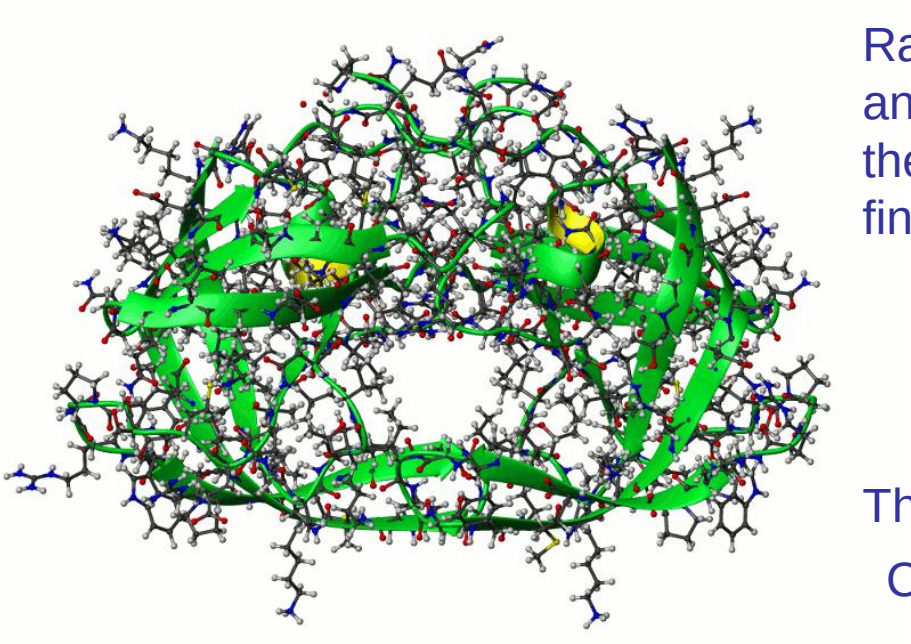
Generic docking protocol

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



10 lowest energy structures

Randomization of domain positions
and Rigid body dynamics repeated 10 times;
then the lowest energy structure submitted to
final simulated annealing part of the protocol

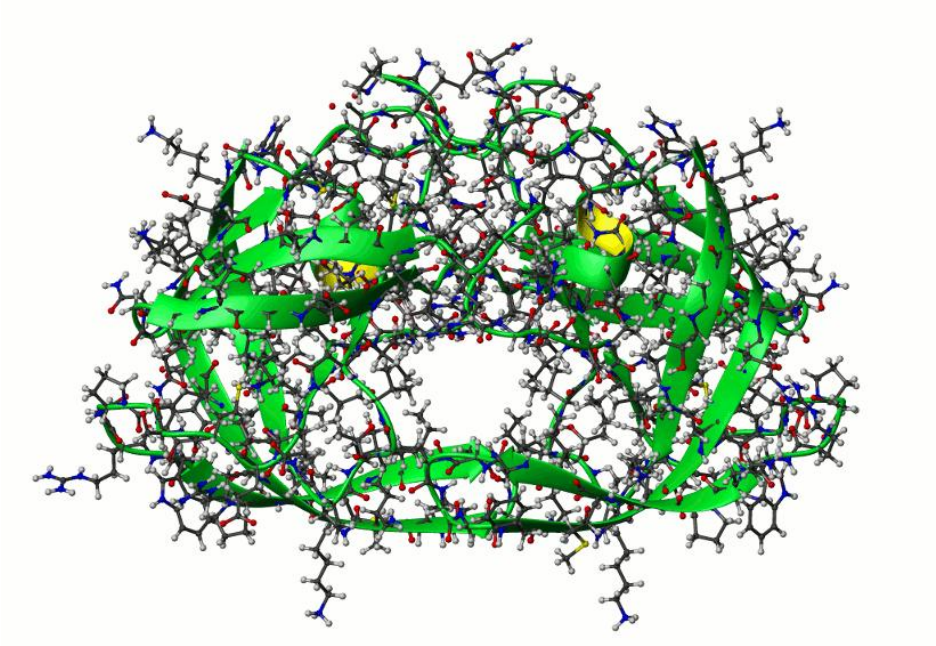
512 structures calculated.

The only experimental restrains are

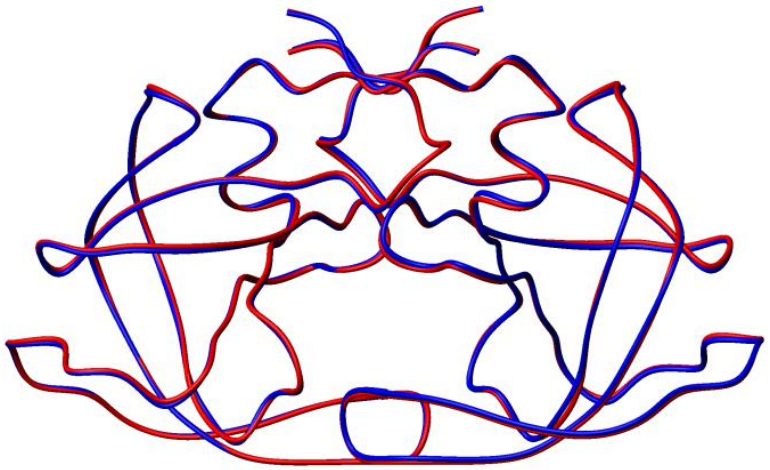
Components of Rotation Diffusion Tensor

Assembling structure of a symmetric protein homo dimer

HIV -1 protease



10 lowest energy structures

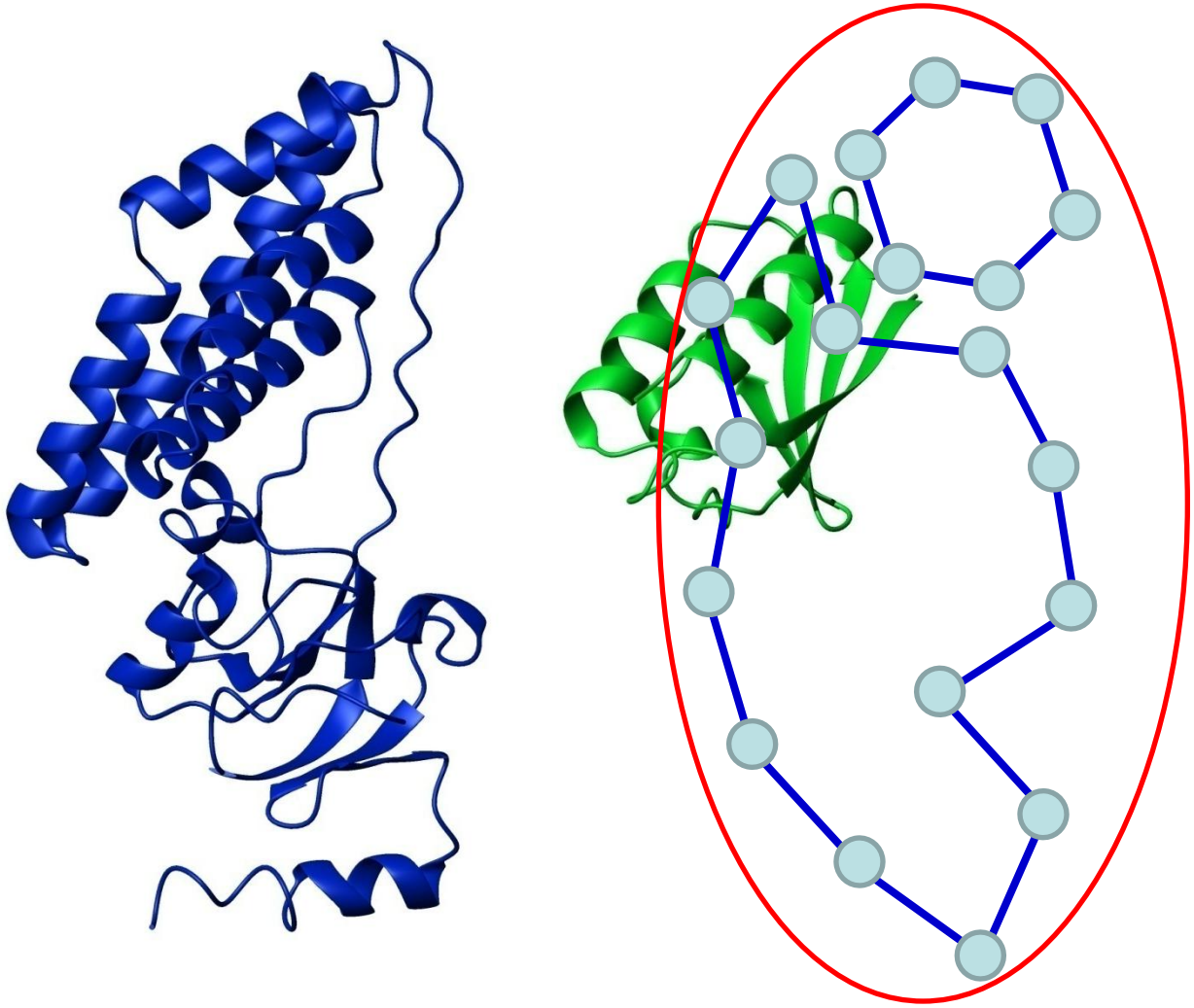


Averaged over 10 lowest energy structures (blue) versus reference (red)

Cα RMSD 0.35 ±0.09 [Å]

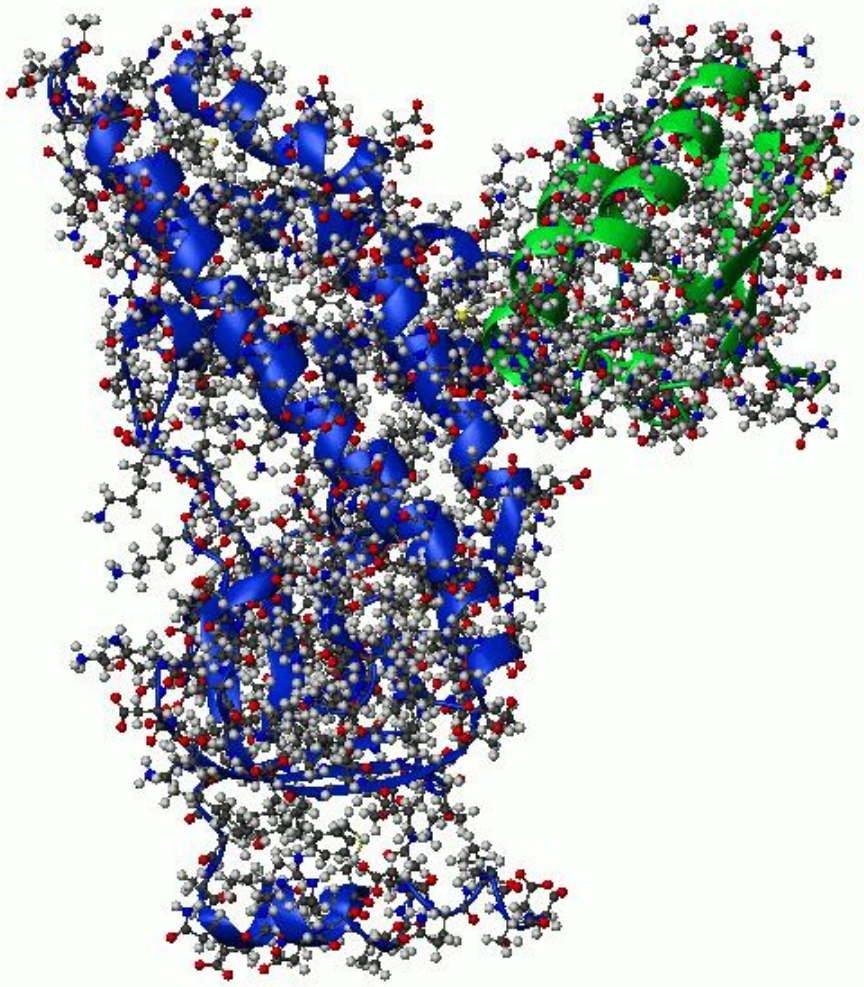
Application to an asymmetric complex

EIN – HPr complex

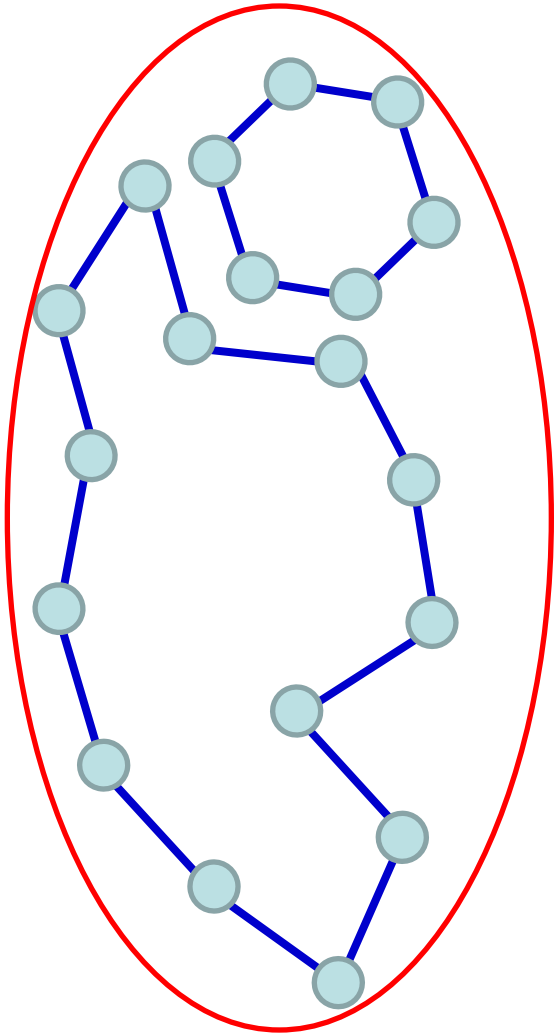


Application to an asymmetric complex

EIN – HPr complex

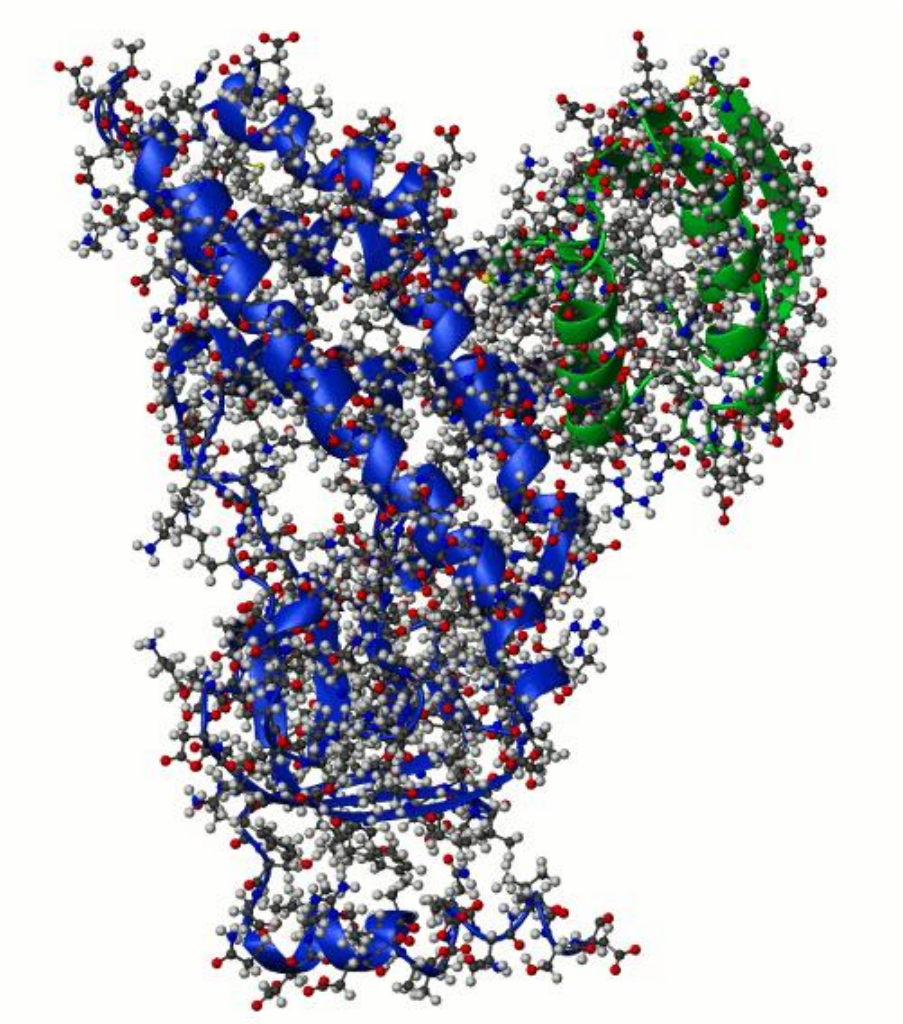


10 lowest energy structures

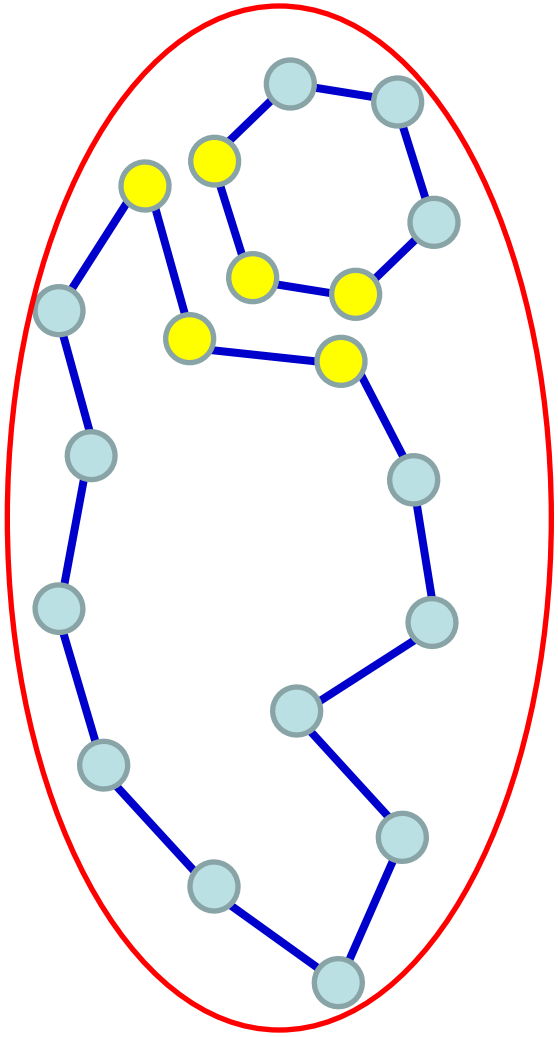


Application to an asymmetric complex

EIN – HPr complex

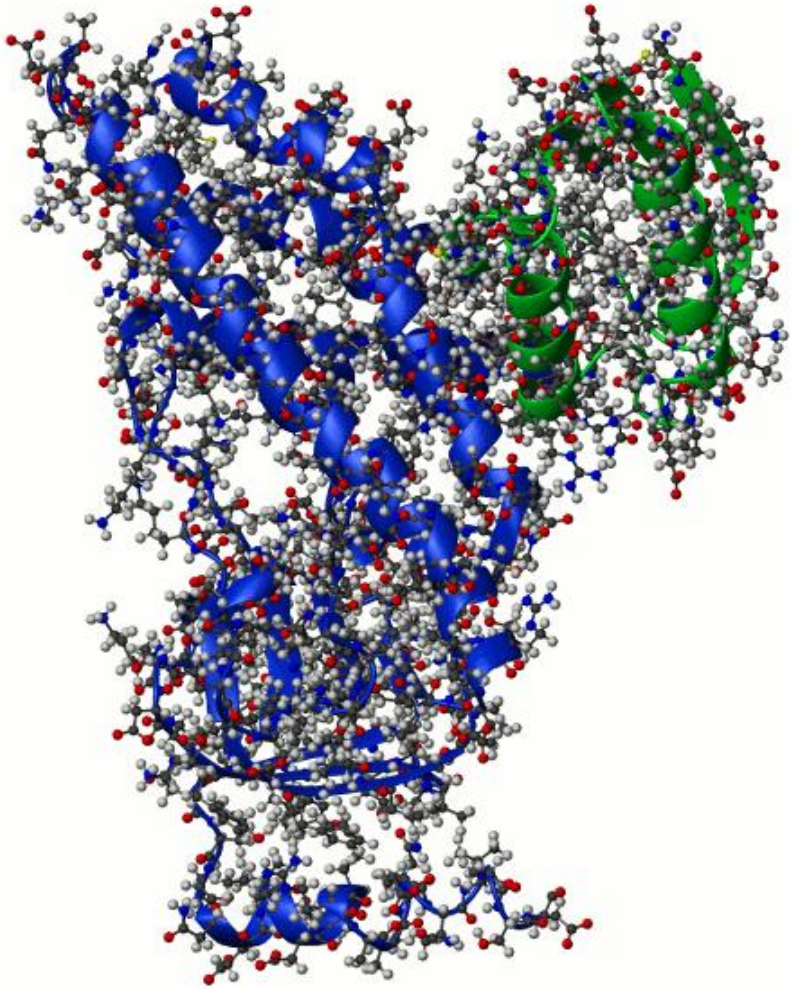


10 lowest energy structures



Application to an asymmetric complex

EIN – HPr complex



10 lowest energy structures

Randomization of domain positions and Rigid body dynamics repeated 10 times; then the lowest energy structure submitted to final simulated annealing part of the protocol

512 structures calculated.

Experimental restrains were

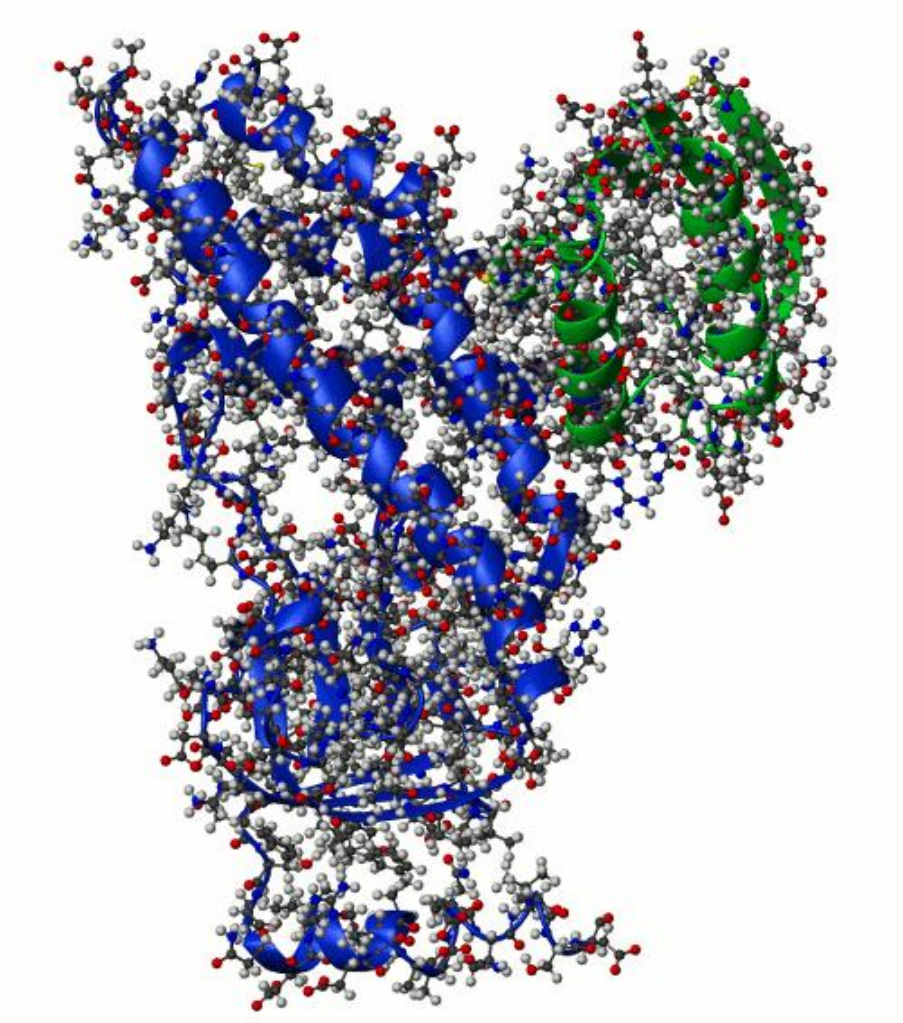
Components of Rotation Diffusion Tensor

and

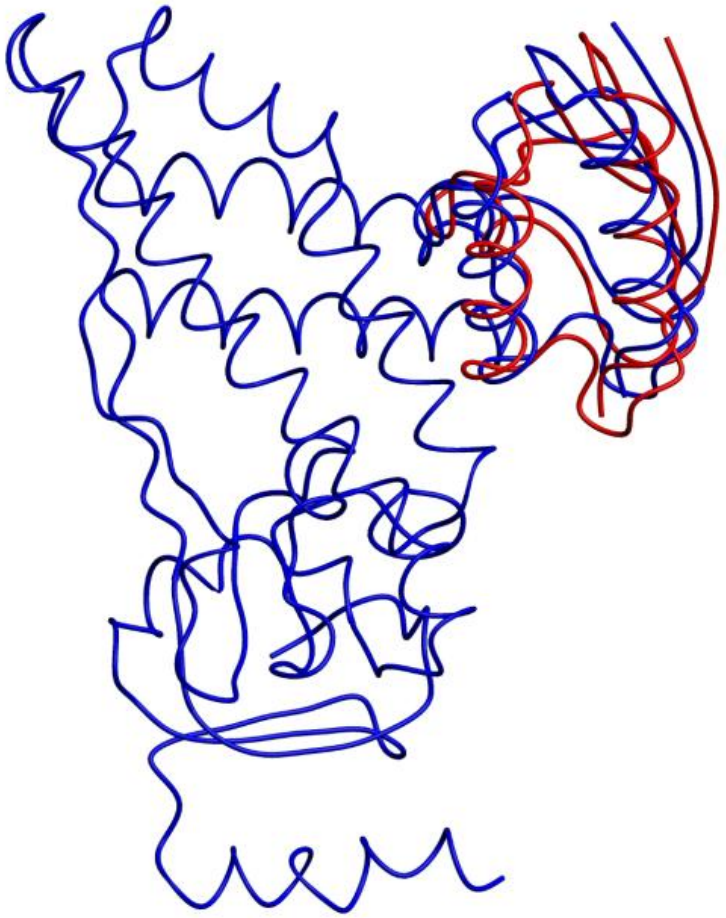
Highly ambiguous distance restraints from chemical shift perturbation mapping

Application to an asymmetric complex

EIN – HPr complex



10 lowest energy structures



Cα RMSD 1.20 ±0.03 [Å]

CONCLUSIONS

Accuracy of the structures obtained using shape restraints derived from protein rotation tensor is comparable to the accuracy of standard structure determination protocols.

When refining structures of globular proteins, these restraints, in combination with other information, could help to solve the problem of poor packing density of NMR protein structures.

When assembling protein complexes, the relaxation data even for one domain of the complex are enough to drive accurate domain assembly.

In some cases these restraints can be the only experimental information necessary to obtain correct domain assembly.

ACKNOWLEDGMENTS

Co-authors of the paper: *JACS* **131** (2009) p.9522	Jeong-Yong Suh, Alexander Grishaev, G. Marius Clore, Charles D. Schwieters
For the help with the NMR data on EIN	Daniel Garrett
For the help with Xplor-NIH code	John Kuszewski
For funding	National Research Council

ACKNOWLEDGMENTS

For all

DCB

for warm, friendly and cooperative working environment !

Current project

Orientation information from residue specific NMR
Relaxation rates