SUPPLEMENTARY INFORMATION

Direct Use of ^{15}N Relaxation Rates as Experimental Restraints on Molecular Shape and Orientation for Docking of Protein-Protein Complexes

Yaroslav Ryabov,[1] G. Marius Clore,[2,] and Charles D. Schwieters[1,]**

[1]Division of Computational Bioscience, Building 12A, Center for Information Technology, National Institutes of Health, Bethesda, Maryland 20892-5624, and [2]Laboratory of Chemical Physics, Building 5, National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0520

1. Docking protocol

Step 1: Randomization of positions and orientations of one protein relative to the other: the position and orientation of EIN is randomized within a cube 45x45x45 Å around the center of gravity of HPr.
Step 2: Initial rigid body gradient minimization with experimental restraints.
Step 3: Initial rigid body gradient minimization with experimental restraints and quartic van der Waals repulsion term.
Steps 1-3 are repeated 50 times for every run of the protocol, and the structure with the lowest energy is refined in the following two steps.
Step 4: Simulated annealing.
Step 5: Final gradient minimization in torsion angle space.

Simulated annealing:
Starting temperature: 1000 K.
Final temperature: 10 K.
Temperature steps: 10 K.
Duration of simulations at every temperature: 1 ps or 600 steps, whichever happens first.

Altogether 512 structures are calculated.

Potential terms used in docking protocol.

Potential term (units of force constant)	Description	Force constant		
		initial minimization	simulated annealing	final minimization
`ANGL` ($kcal.mol^{-1}.rad^{-2}$)	bond angle	0	ramped from 200 to 500	500
`BOND` ($kcal.mol^{-1}.Å^{-2}$)	bond length	0	1000	1000
`relaxRatioPot` (`kcal.mol`$^{-1}$)	Ratios of ^{15}N R_2/R_1 relaxation rates	0.5	ramped from 0.5 to 5	5
`DistSymmPot`[a] ($kcal.mol^{-1}.Å^{-2}$)	Distance symmetry restraints to maintain C_2 symmetry	5	5	5
`PosDiffPot`[a] ($kcal.mol^{-1}.Å^{-2}$)	Restraint dimer subunits to be identical	1000	1000	1000
`IMPR` ($kcal.mol^{-1}.rad^{-2}$)	Improper torsion angles	0	ramped from 50 to 500	500
`NOEpot`[b] ($kcal.mol^{-1}.Å^{-2}$)	Highly ambiguous distance restraints from chemical shift perturbation mapping	0.3	ramped from 0.3 to 60	60
`RAMA` ($kcal.mol^{-1}$)	Multidimensional torsion angle database of mean force	0	ramped from 0.002 to 1	1
`residueAffPot`[c]	Low-resolution contact potential	0	ramped from 1 to 50	50
`VDW` ($kcal.mol^{-1}.Å^{-4}$)	Quartic van der Waals repulsion	0.01 Cα atoms only	ramped from 0.01 to 4 all atoms	4

[a] Used only for HIV dimer.
[b] Used only for EIN/HPr complex.
[c]The pairwise interaction strengths M_{ij} between amino acid residue types in the hydrophobic contact potential used in Xplor-NIH as described in ref. 12 (main text) are directly proportional to those in the Miyazawa & Jernigan contact potential (*Proteins*, **1999**, *34*, 49-68).

In additional calculations incorporating backbone amide RDCs, the RDC force constant was set to 0.1 kcal.mol^{-1}.Hz^{-2} during the minimization stage, ramped from 0.1 to 1.0 kcal.mol^{-1}.Hz^{-2} during simulated annealing, and kept at 1.0 kcal.mol^{-1}.Hz^{-2} in the final minimization stage.

2. Filtering of the experimental ^{15}N R_2/R_1 relaxation data

To exclude outliers in the experimental ^{15}N R_2/R_1 relaxation data (taken from Ryabov *et al. J. Am. Chem. Soc.* **2009**, *131*, 9522) arising either from coordinate uncertainties, measurement uncertainties, or significant local motions, we developed the following iterative filtering procedure that makes use of a simplex algorithm to optimize the parameters of the diffusion tensors for EIN and HPr independently while constraining the anisotropy and rhombicity of the tensors to be the same. Initially the complete set of relaxation data is used to estimate the parameters of the diffusion tensors. Then the data point with the largest relative deviation from the back-calculated value of the R_2/R_1 ratio is marked as an "outlier" and removed from the experimental data set. This relative deviation is computed as the ratio of the difference between observed and calculated R_2/R_1 over the mean value for the same pair of observed and calculated R_2/R_1 ratios. The fitting is repeated with the reduced experimental data set and the next outlier determined using updated values of the fitted parameters. This procedure is repeated until a given threshold is reached. In our study we stopped the filtering procedure after removing 13% of the data points, which, for a Gaussian distribution, corresponds to retaining all data for which the deviation between observed and calculated R_2/R_1 values is less than 1.5σ.

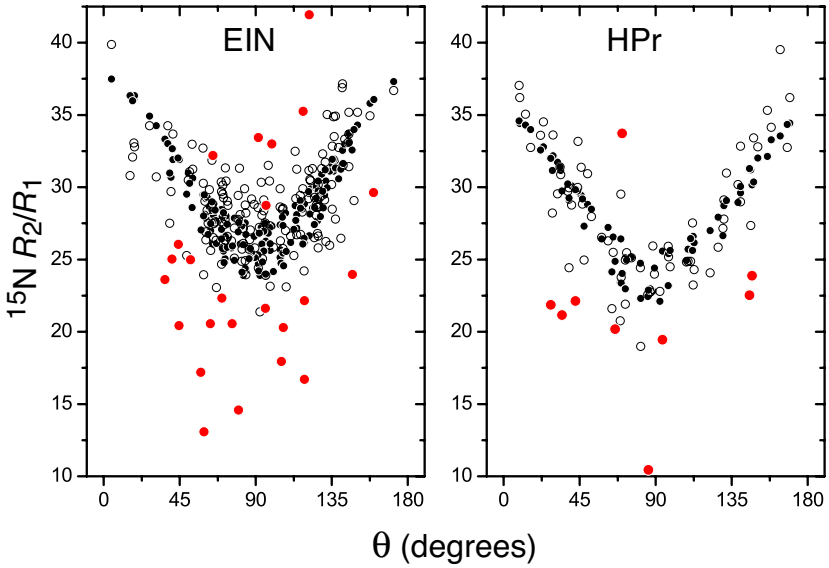

Figure S1. Filtering of experimental ^{15}N R_2/R_1 relaxation data. Black circles are simulated data; open circles are accepted data; and red circles are outliers. θ denotes the angle between an N-H bond vector and the long axis of the fitted diffusion tensor. 13% of the data were removed in the filtering procedure, which, assuming a Gaussian distribution, corresponds to retaining all data for which the deviation between the observed and calculated R_2/R_1 values falls within 1.5 σ.

Table S1. Fitted parameters of the diffusion tensors for EIN and HPr data obtained from the filtered data set.

	Anisotropy	Rhombicity	τ [ns]	α	β	γ	χ^2	rms
EIN	1.46±0.05	0.57±0.14	16.99±0.13	5±10°	160±4°	37±11°	23.96	2.26
HPr			16.35±0.11	137±3°	62±4°	67±7°		

Explicit lists of residues included in the E_{relax} energy term:

EIN data (153 data points)
4, 6, 8, 11-16, 18-19, 26-29, 31-32, 36-37, 39-42, 44-47, 50, 52-53, 56-67, 72-75, 78, 80-81, 83, 85-87, 89, 91-97, 99-103, 105, 107, 109-110, 113-118, 120-124, 128, 132, 134, 136-143, 145, 149, 154-158, 160-163, 166-168, 170-171, 173, 175-183, 185, 189-190, 192-198, 201-207, 209, 212-215, 217, 219, 221-224, 226, 228-230, 232, 235-236

HPr data (66 data points)
303-310, 312, 314-315, 317, 319-322, 324-328, 330-337, 340, 342-347, 350-352, 356-382

Explicit lists of residues excluded from the E_{relax} energy term:

EIN data (24 data points)
5, 7, 10, 20, 23-25, 30, 70-71, 84, 98, 119, 146-148, 150-152, 210, 220, 241, 243-244

HPr data (9 data points)
313, 316, 339, 348, 354-355, 383-385

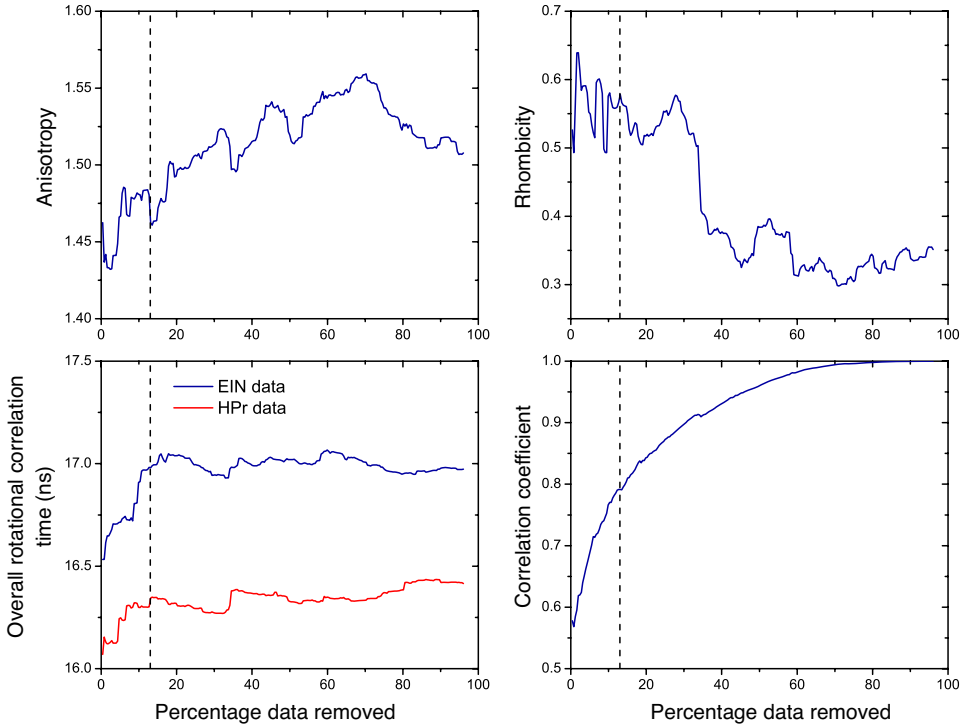

Figure S2. Dependence of the fitted parameters of the diffusion tensor on the percentage of experimental data points removed from the original data set. The panel in the right bottom corner shows the correlation between experimental data points and back calculated data. The dashed line marks the 13% threshold, which corresponds to retaining data for which the relative deviation between observed and calculated values falls within 1.5σ.

3. Calculation of the ratio of relaxation rates

The values of longitudinal (R_1) and transverse (R_2) relaxation rates can be calculated as follows:

$$R_1 = (d^2 + c^2)\big(6J(\omega_H - \omega_N) + J(\omega_H + \omega_N) + 3J(\omega_N)\big) \quad \text{(S1)}$$

$$R_2 = \frac{1}{2}(d^2 + c^2)\big(R_1/(d^2 + c^2) + 6J(\omega_H) + 4J(0)\big) \quad \text{(S2)}$$

where ω_N and ω_H are the Larmor frequencies for precession of ^{15}N and ^{1}H nuclei spins, d^2 is the strength of the ^{1}H-^{15}N dipolar coupling, and c^2 describes the effect of the anisotropy of the ^{15}N chemical shift. The spectral density function, $J(\omega)$, is given by the cosine Fourier transform of the correlation function $C(t)$:

$$J(\omega) = 2\int_0^\infty \cos(\omega t)\, C(t) dt \quad \text{(S3)}$$

Thus, evaluation of the correlation function $C(t)$ is central to the calculation of the R_1 and R_2 relaxation rates. This function describes correlations between the initial orientation of a vector associated with particular N-H bond and that bond's orientation at some subsequent time t.

Since the interaction between the ^{1}H and ^{15}N nuclei can be described as the interaction between two quantum spins, it is convenient to represent the correlation function $C(t)$ using the basis of eigen functions of the symmetric quantum rotator - Wigner rotation matrices of second rank, $D^{(2)}_{q,0}$, in the form:

$$C(t) = \langle D^{(2)*}_{q,0}(\Omega^0_{L\to I}) D^{(2)}_{q,0}(\Omega^t_{L\to I})\rangle \quad \text{(S4)}$$

where the angular brackets denote ensemble averaging, the asterisk denotes complex conjugation, and $\Omega^0_{L\to I}$ and $\Omega^t_{L\to I}$ denote the rotational transformation from the laboratory reference frame, L, to the reference frame associated with instantaneous orientations, I, of the N-H bond at times 0 and t, respectively.

Explicit expressions for the components of the Wigner rotation matrices can be written in simple form using the y-convention for Euler angles to describe the rotation transformations described above: the angle $0 \le \alpha < 2\pi$ describes clockwise rotation about the z axis of the initial reference frame; the angle $0 \le \beta \le \pi$ describes clockwise rotation about the y axis obtained as a result of the first rotation; and the angle $0 \le \gamma < 2\pi$ describes the rotation about the z axis obtained as a result of the second rotation.

For this convention the angular dependence of the Wigner rotation matrices on the angles α, β, and γ can be factorized in the form

$$D^{(2)}_{m,n}(\Omega) = \exp(-im\alpha)\, d^{(2)}_{m,n}(\beta) \exp(-in\gamma) \quad \text{(S5)}$$

where *i* denotes the imaginary unit, the indices *m* and *n* take the values -2,-1,0,1,2 and $d^{(2)}_{m,n}(\beta)$ are the components of the second rank reduced Wigner rotation matrix:

$$
\begin{aligned}
d^{(2)}_{2,2}(\beta) &= \tfrac{1}{4}(1+\cos(\beta))^2 \\
d^{(2)}_{2,1}(\beta) &= -\tfrac{\sin(\beta)}{2}(1+\cos(\beta)) \\
d^{(2)}_{2,0}(\beta) &= \sqrt{\tfrac{3}{8}}\sin^2(\beta) \\
d^{(2)}_{2,-1}(\beta) &= -\tfrac{\sin(\beta)}{2}(1-\cos(\beta)) \\
d^{(2)}_{2,-2}(\beta) &= \tfrac{1}{4}(1-\cos(\beta))^2 \\
d^{(2)}_{1,1}(\beta) &= \tfrac{1}{2}(2\cos^2(\beta)+\cos(\beta)-1) \\
d^{(2)}_{1,0}(\beta) &= -\sqrt{\tfrac{3}{2}}\sin(\beta)\cos(\beta) \\
d^{(2)}_{1,-1}(\beta) &= -\tfrac{1}{2}(2\cos^2(\beta)-\cos(\beta)-1) \\
d^{(2)}_{0,0}(\beta) &= \tfrac{1}{2}(3\cos^2(\beta)-1)
\end{aligned}
\tag{S6}
$$

The other 16 elements of $d^{(2)}_{m,n}(\beta)$ are given by the symmetry relationships:

$$
d^{(2)}_{m,n}(\beta) = d^{(2)}_{-n,-m}(\beta) = (-1)^{m-n} d^{(2)}_{n,m}(\beta) \tag{S7}
$$

Let us again consider correlation function $C(t)$. It is natural to introduce together with the laboratory reference frame, *L*, and the reference frame of instantaneous N-H bond vector orientation, *I*, the reference frame associated with the average orientation of the N-H vector within a molecular reference frame of the entire protein, *A*, the molecular reference frame itself, *M*, and a reference frame associated with the principal axis of the diffusion tensor describing tumbling of whole protein, *D*. In this case the correlation function under consideration can be written as follows:

$$
\begin{aligned}
C(t) = \sum_{m=-2}^{2}\sum_{n=-2}^{2}\sum_{k=-2}^{2}\sum_{l=-2}^{2}\sum_{s=-2}^{2}\sum_{h=-2}^{2} \langle & D^{(2)*}_{q,m}(\Omega^0_{L\to D}) D^{(2)}_{q,n}(\Omega^t_{L\to D}) D^{(2)*}_{m,k}(\Omega^0_{D\to M}) D^{(2)}_{n,l}(\Omega^t_{D\to M}) \\
& D^{(2)*}_{k,s}(\Omega^0_{M\to A}) D^{(2)}_{l,h}(\Omega^t_{M\to A}) D^{(2)*}_{s,0}(\Omega^0_{A\to I}) D^{(2)}_{h,0}(\Omega^t_{A\to I})\rangle
\end{aligned}
\tag{S8}
$$

where $\Omega_{L\to D}$ describes the transformation from the laboratory reference frame, *L*, to the reference frame of the overall rotation diffusion tensor, *D*; $\Omega_{D\to M}$ describes the transformation from the reference frame of the overall rotation diffusion tensor, *D*, to the molecular reference frame of the protein, *M*; $\Omega_{M\to A}$ describes the transformation from molecular reference frame, *M*, to the average orientation of the N-H bond vector of a particular amino acid residue, *A*; and, finally, $\Omega_{A\to I}$ describe the transformation from the average orientation of the N-H bond vector of an amino acid residue, *A*, to its instantaneous orientation, *I*.

In this work we assume that the structure of the protein, and hence its overall rotation diffusion tensor, are constant. Hence, the time dependence of $\Omega_{D\to M}$ and $\Omega_{M\to A}$ can be ignored which, in this case, simply define the orientations of the principal axis frame of the protein rotational diffusion tensor and the orientation of the N-H bond vector with respect to the protein molecular frame. Thus, the only motions remaining are the overall diffusion tumbling of the whole protein encoded in the transformation $\Omega_{L\to D}$ and the fast libration of the N-H bond around its average orientation encoded in the transformation $\Omega_{A\to I}$. These types of motion occur on substantially different time scales: nanoseconds for overall tumbling and picoseconds for libration. Therefore, averaging of the correlation function with respect to these two motional modes can be treated independently which simplifies the equation for $C(t)$ to:

$$C(t) = \sum_{m=-2}^{2}\sum_{n=-2}^{2}\sum_{k=-2}^{2}\sum_{l=-2}^{2}\sum_{s=-2}^{2}\sum_{h=-2}^{2} \langle D^{(2)*}_{q,m}(\Omega^0_{L\to D}) D^{(2)}_{q,n}(\Omega^t_{L\to D})\rangle\, D^{(2)*}_{m,k}(\Omega_{D\to M}) D^{(2)}_{n,l}(\Omega_{D\to M})$$
$$D^{(2)*}_{k,s}(\Omega_{M\to A}) D^{(2)}_{l,h}(\Omega_{M\to A})\, \langle D^{(2)*}_{s,0}(\Omega^0_{A\to I}) D^{(2)}_{h,0}(\Omega^t_{A\to I})\rangle \qquad \text{(S9)}$$

Bond libration is usually treated within the framework of the "Model Free" approach (Lipary & Szabo *J. Am. Chem. Soc.* **1982**, *104*, 4546) which assumes that

$$\langle D^{(2)*}_{s,0}(\Omega^0_{A\to I}) D^{(2)}_{h,0}(\Omega^t_{A\to I})\rangle = \delta_{s,0}\delta_{h,0}(S^2 + (1-S^2)\exp(-t/\tau_{loc})) \qquad \text{(S10)}$$

where $\delta_{s,0}$ is Kronecker delta symbol, $0 \le S^2 \le 1$ is the order parameter, and τ_{loc} is the characteristic time scale of local N-H bond motion.

The overall diffusion tumbling of a rigid body was treated by Favro (*Phys. Rev.* **1960**, *119*, 53). Applied to the case of interest this theory leads to the expression

$$\langle D^{(2)*}_{q,m}(\Omega^0_{L\to D}) D^{(2)}_{q,n}(\Omega^t_{L\to D})\rangle = \frac{1}{5}\sum_{r=-2}^{2} \exp(-E_r t)\, a^*_{r,m} a_{r,n} \qquad \text{(S11)}$$

where E_r denotes the eigenvalues of the differential operator of the anisotropic three-dimensional rotational diffusion, and $a_{r,n}$ are decomposition coefficients of the eigenvectors of this operator on the complete set of basis vectors of the symmetric quantum rotator - Wigner rotation matrices $D^{(2)}_{m,n}(\Omega)$.

E_r and $a_{r,n}$ depend only on eigenvalues of the protein diffusion tensor, D_x, D_y, and D_z, while the orientation of the diffusion tensor relative to the protein molecular frame is described by $\Omega_{D\to M}$. Thus, the general form of the correlation function is as follows:

$$C(t) = \frac{1}{5}\sum_{r=-2}^{2}\sum_{m=-2}^{2}\sum_{n=-2}^{2}\sum_{k=-2}^{2}\sum_{l=-2}^{2} \exp(-E_r t)\, a^*_{r,m} a_{r,n}\, D^{(2)*}_{m,k}(\Omega_{D\to M}) D^{(2)}_{n,l}(\Omega_{D\to M})$$
$$D^{(2)*}_{k,0}(\Omega_{M\to A}) D^{(2)}_{l,0}(\Omega_{M\to A})(S^2 + (1-S^2)\exp(-t/\tau_{loc})) \qquad \text{(S12)}$$

where E_r is given by the expressions:

$$\begin{aligned} E_2 &= 6D_s + 2\Delta \\ E_{-2} &= 3(D_z + D_s) \\ E_1 &= 3(D_x + D_s) \\ E_{-1} &= 3(D_y + D_s) \\ E_0 &= 6D_s - 2\Delta \end{aligned} \tag{S13}$$

with

$$D_s = \tfrac{1}{3}(D_x + D_y + D_z) \tag{S14}$$

$$\Delta = \begin{cases} +\sqrt{(D_y - D_x)^2 + (D_z - D_x)(D_z - D_y)} \\ -\sqrt{(D_y - D_x)^2 + (D_z - D_x)(D_z - D_y)} \end{cases}, \; only\ for\ D_z < D_x = D_y \tag{S15}$$

and $a_{r,n}$ are given by the matrix:

		n				
r		2	1	0	-1	-2
	2	$\frac{w}{N\sqrt{2}}$	0	$\frac{u}{N}$	0	$\frac{w}{N\sqrt{2}}$
	1	0	$\frac{1}{\sqrt{2}}$	0	$\frac{1}{\sqrt{2}}$	0
	0	$-\frac{u}{N\sqrt{2}}$	0	$\frac{w}{N}$	0	$-\frac{u}{N\sqrt{2}}$
	-1	0	$\frac{1}{\sqrt{2}}$	0	$\frac{1}{\sqrt{2}}$	0
	-2	$\frac{1}{\sqrt{2}}$	0	0	0	$\frac{1}{\sqrt{2}}$

with

$$u = \sqrt{3}(D_x - D_x)$$
$$N = 2\sqrt{|w\Delta|}$$

$$w = \begin{cases} 2\,D_z - D_x - D_y + 2\Delta \\ -2\,D_z + D_x + D_y - 2\Delta \end{cases}, \; only\ for\ D_z < D_x = D_y$$

For the current implementation of the potential term which uses the ratio of relaxation rates $\rho = R_2/R_1$, the impact of local mobility is canceled in the final equations for ρ. Thus, for our current purposes we can neglect the impact of local mobility by assuming $S^2 = 1$. In this case after Fourier transformation one obtains the following expression for the spectral density $J(\omega)$ that is used in the structure calculations:

$$J(\omega) = \frac{2}{5}\sum_{r=-2}^{2}\sum_{m=-2}^{2}\sum_{n=-2}^{2}\sum_{k=-2}^{2}\sum_{l=-2}^{2}\frac{E_r}{E_r^2+\omega^2}a_{r,m}^{*}a_{r,n}\,D_{m,k}^{(2)*}(\Omega_{D\to M})D_{n,l}^{(2)}(\Omega_{D\to M})D_{k,0}^{(2)*}(\Omega_{M\to A})D_{l,0}^{(2)}(\Omega_{M\to A}) \quad \text{(S16)}$$

In Eq. [S16], E_r and $a_{r,n}$ depend only on the eigenvalues of the protein diffusion tensor D_x, D_y, and D_z; $\Omega_{D\to M}$ specifies the orientation of the rotation diffusion tensor; and $\Omega_{M\to A}$ specifies the orientation of the particular N-H bond vector relative to protein molecular frame. Using the properties of Wigner rotation matrices, Eq. [S16] can be rewritten in shorter form as:

$$J(\omega) = \frac{2}{5}\sum_{r=-2}^{2}\frac{E_r}{E_r^2+\omega^2}F_r(\{D_x,D_y,D_z\},\Omega_{D\to A}) \quad \text{(S17)}$$

where the function

$$F_r(\{D_x,D_y,D_z\},\Omega_{D\to A}) = \sum_{m=-2}^{2}\sum_{n=-2}^{2}a_{r,m}^{*}a_{r,n}\,D_{m,0}^{(2)*}(\Omega_{D\to A})D_{n,0}^{(2)}(\Omega_{D\to A}) \quad \text{(S18)}$$

depends only on the eigenvalues of the protein rotation diffusion tensor, $\{D_x, D_y, D_z\}$, and on the orientation of a particular N-H bond vector relative to the principal axis frame of the tensor, $\Omega_{D\to A}$.

4. HIV-1 protease ^{15}N relaxation data

The experimental ^{15}N R_1 and R_2 relaxation data were taken from from Tjandra et al. *J. Biomol. NMR* **1996**, *8*, 273. The list of residues included in the E_{relax} energy term (which excludes residues with significant local motions, as described by Tjandra et al.) is as follows: 10-15, 18-22, 24, 32, 33, 43, 45, 46, 48, 49, 52, 53, 55, 58, 60, 61, 63, 66, 70, 71, 74, 77, 84, 85, 87-93, 96. Altogether 45 ^{15}N R_2/R_1 ratios per HIV-1 protease subunit were employed in the calculations.

5. Docking protocol used in this study: file dock_rrp.py (in eginput/relaxRatio directory of Xplor_NIH 2.25 release)

```
# ----- sample of docking script with diffusion temperature optimization ----- #
# ---- which uses NMR relaxation data and chemical shift pertrubation data --- #
# ------as structural restraints implemented in Xplor-NIH 2.25 --------------- #

# this checks for typos on the command-line. User-customized arguments can
# also be specified.
#
(opts,args) = xplor.parseArguments(["quick"])

quick=False
for opt in opts:
    if opt[0]=="quick":  #specify -quick to just test that the script runs
        quick=True
        pass
    pass

outFilename = "SCRIPT_STRUCTURE.sa"
numberOfStructures = 2 if quick else 512
numberOfLoops      = 2 if quick else 50
numberOf_cool_Loops      = 1


# protocol module has many high-level helper functions. #
import protocol

# explicitly set random seed #
protocol.initRandomSeed(7654)

command = xplor.command

protocol.initParams("protein")

# read in the EIN domain coordinates #
protocol.loadPDB("randomized_sch_EIN.pdb")

# read in the HPr domain coordinates #
protocol.loadPDB("randomized_sch_HPr.pdb")

xplor.simulation.deleteAtoms("not known")

# subtract EIN center of gravity coordinates #
# from original EIN coordinates #
xplor.command("vector do (x=x+6.6) (resid 1:250)")
xplor.command("vector do (y=y-2.3) (resid 1:250)")
xplor.command("vector do (z=z-138.5) (resid 1:250)")

# subtract HPr center of gravity coordinates #
# from original HPr coordinates #
xplor.command("vector do (x=x-13) (resid 301:385)")
xplor.command("vector do (y=y-7.9) (resid 301:385)")
xplor.command("vector do (z=z-8.8) (resid 301:385)")

# create PotLists which contains lists of potential terms. #

from potList import PotList

potList = PotList()
score = PotList()
potList_0 = PotList()
potList_1 = PotList()
potList_2 = PotList()


from simulationTools import MultRamp, StaticRamp, InitialParams

rampedParams=[]
highTempParams=[]

from xplorPot import XplorPot

# set up the therms which will use EIN and HPr relaxation data#
from diffPotTools         import readInRelaxData, make_ratio
```

```
from relaxRatioPotTools   import create_relaxRatioPot

relax_data_EIN = readInRelaxData(['ein_r1r2_7pts.tbl'],
              pattern=['resid','R1','R1_err','R2','R2_err','skip','skip'])

for item in relax_data_EIN:
     make_ratio(item)
     pass

icls_EIN="not ((resid 5) or (resid 7) or (resid 10) or (resid 20)\
               or (resid 23:25) or (resid 30) or (resid 70:71)\
               or (resid 84) or (resid 98) or (resid 119)\
               or (resid 146:148) or (resid 150:152) or (resid 210)\
               or (resid 220) or (resid 241) or (resid 243:244))"

r_ratio_EIN=create_relaxRatioPot('rrp_EIN', data_in = relax_data_EIN,
     inc_sel = icls_EIN, freq = 600.141, temperature = 313, addAtoms=True)

potList_0.append(r_ratio_EIN)
score.append(r_ratio_EIN)
potList.append(r_ratio_EIN)
potList_1.append(r_ratio_EIN)
potList_2.append(r_ratio_EIN)

r_ratio_EIN.setScale(0.5)
r_ratio_EIN.setRangeTmpFit(10)

rampedParams.append( MultRamp(0.5,5, "r_ratio_EIN.setScale( VALUE )") )


relax_data_HPr = readInRelaxData(['hpr_r1r2_7pts.tbl'],
              pattern=['resid','R1','R1_err','R2','R2_err','skip','skip'])

for item in relax_data_HPr:
     make_ratio(item)
     pass

icls_HPr="not ((resid 313) or (resid 316) or (resid 339)\
                or (resid 348) or (resid 354:355)\
                or (resid 383:385))"

r_ratio_HPr=create_relaxRatioPot('rrp_HPr', data_in = relax_data_HPr,
                inc_sel = icls_HPr, freq = 600.141, temperature = 313,
                addAtoms=True, link_to=r_ratio_EIN)

potList_0.append(r_ratio_HPr)
score.append(r_ratio_HPr)
potList.append(r_ratio_HPr)
potList_1.append(r_ratio_HPr)
potList_2.append(r_ratio_HPr)

r_ratio_HPr.setScale(0.5)
r_ratio_HPr.setRangeTmpFit(10)

rampedParams.append( MultRamp(0.5,5, "r_ratio_HPr.setScale( VALUE )") )

# conract shifts
noe=PotList('noe')
potList_0.append(noe)
score.append(noe)
potList.append(noe)
potList_1.append(noe)
potList_2.append(noe)

from noePotTools import create_NOEPot
for (name,scale,file) in [('all',10,"shifts_noe_newx_EINHPr.tbl")]:
    pot = create_NOEPot(name,file)
    pot.setScale(scale)
    noe.append(pot)
rampedParams.append( MultRamp(0.3,60, "noe.setScale( VALUE )") )

# set up Miyazawa contact potential
from residueAffPotTools import create_ResidueAffPot
ra = create_ResidueAffPot('hydphob', interdomainContacts=True,
                          potentialName="Miyazawa")
ra.setAveType("center")
```

```
ra.setMoveTol(0.5)
ra.setCutoffLong(20)
ra.setScale(10)

potList_1.append(ra)
potList_2.append(ra)

rampedParams.append( MultRamp(1,50,"ra.setScale(VALUE)") )

# IVM setup
#   the IVM is used for performing dynamics and minimization in torsion-angle
#   space, and in Cartesian space.
#
from ivm import IVM
from ivm import PublicIVM
dyn_fix = IVM()           # ivm for rigid body dynamics
dyn_free_sch = IVM()

# reset ivm topology for torsion-angle dynamics
dyn_fix.reset()
dyn_free_sch.reset()

protocol.torsionTopology(dyn_fix)
protocol.torsionTopology(dyn_free_sch)

# ivms used for initial rigid body gradient minimization #
dyn_fix.group( 'resid 1:249' )
dyn_fix.group( 'resid 301:385' )

# ivms used for simulated annealing which let side chains to be flexible  #
dyn_free_sch.group( 'resid 1:249 and \
                     (name CA or name C or name N or name O or name HN)' )
dyn_free_sch.group( 'resid 301:385 and \
                     (name CA or name C or name N or name O or name HN)' )

# Give atoms uniform weights, except for the anisotropy axis
from atomAction import SetProperty
import varTensorTools
AtomSel("not resname ANI").apply( SetProperty("mass",100.) )
AtomSel("all            ").apply( SetProperty("fric",10.) )


# compare atomic Cartesian rmsd with a reference structure
#  backbone and heavy atom RMSDs will be printed in the output
#  structure files

from posDiffPotTools import create_PosDiffPot

refRMSD = create_PosDiffPot("refRMSD",
          selection="(name CA and resid 3:249) or (name CA and resid 301:385)",
          selection2="(name CA and resid 3:249) or (name CA and resid 301:385)",
          pdbFile='reference_docking_structure.pdb',
          cmpSel=" (name CA and resid 3:249) or (name CA and resid 301:385) ")

# setup parameters for atom-atom repulsive term. (van der Waals-like term)
#
potList_1.append( XplorPot('VDW') )
potList_2.append( XplorPot('VDW') )
score.append( XplorPot('VDW') )

rampedParams.append( StaticRamp("protocol.initNBond()") )
rampedParams.append( MultRamp(0.9,0.8,
                              "command('param nbonds repel VALUE end end')") )
rampedParams.append( MultRamp(.01,4,
                              "command('param nbonds rcon VALUE end end')") )
# nonbonded interaction only between CA atoms
highTempParams.append( StaticRamp("""protocol.initNBond(cutnb=100,
                                                        tolerance=45,
                                                        repel=1.2,
                                                        rcon=0.01,
                                                        onlyCA=1)""") )

#Rama torsion angle database
#
protocol.initRamaDatabase()
potList_2.append( XplorPot('RAMA') )
```

```
rampedParams.append( MultRamp(.002,1,"potList_2['RAMA'].setScale(VALUE)") )


potList_2.append( XplorPot("BOND") )
potList_2.append( XplorPot("ANGL") )
potList_2['ANGL'].setThreshold( 5 )
rampedParams.append( MultRamp(0.4,1,"potList_2['ANGL'].setScale(VALUE)") )
potList_2.append( XplorPot("IMPR") )
potList_2['IMPR'].setThreshold( 5 )
rampedParams.append( MultRamp(0.1,1,"potList_2['IMPR'].setScale(VALUE)") )



# object which performs simulated annealing
#
from simulationTools import AnnealIVM
init_t  = 1000 # 3000.     # Need high temp and slow annealing to converge
cool = AnnealIVM(initTemp =init_t,
                 finalTemp=10,
                 tempStep =100 if quick else 10,
                 ivm=dyn_free_sch,
                 rampedParams = rampedParams)


from atomAction import randomizeDomainPos

import random

def calcOneStructure(loopInfo):
    """ this function calculates a single structure, performs analysis on the
    structure, and then writes out a pdb file, with remarks.
    """

    initial_tmp_pos = xplor.simulation.atomPosArr()
    tmp_pos_swap = xplor.simulation.atomPosArr()

    tmp_energy = 1e9      # big number
    k=0                   # set up initial minimaizing loop

    while k < numberOfLoops:

      xplor.simulation.setAtomPosArr(initial_tmp_pos)

      randomizeDomainPos( 'resid 1:249', deltaPos=45 )


      # initialize parameters for high temp dynamics. #
      InitialParams( rampedParams )
      InitialParams( highTempParams )

      protocol.initMinimize(dyn_fix,potList=potList_0,
                            printInterval=50)
      dyn_fix.run()

      dyn_fix.run()

      protocol.initMinimize(dyn_fix,potList=potList,
                            printInterval=50)
      dyn_fix.run()

      dyn_fix.run()

      protocol.initMinimize(dyn_fix,potList=score,
                            printInterval=50)
      dyn_fix.run()

      dyn_fix.run()

      dyn_fix.run()

      print potList.calcEnergy(), score['VDW'].calcEnergy()

      tmp_energy_swap = score.calcEnergy()

      print tmp_energy_swap
```

```
      if tmp_energy_swap < tmp_energy :
            tmp_pos_swap = xplor.simulation.atomPosArr()
            tmp_energy = tmp_energy_swap

      k=k+1
      print k

   xplor.simulation.setAtomPosArr(tmp_pos_swap)

   tmp_energy = potList.calcEnergy() # debugging line
   print tmp_energy                  # debugging line



   k2=0                  # set up initial cooling loop
   while k2 < numberOf_cool_Loops:

      InitialParams( rampedParams )

      # initialize integrator for simulated annealing
      protocol.initDynamics(dyn_free_sch,
                            potList=potList_2,
                            numSteps=3 if quick else 600,
                            finalTime=1 ,
                            printInterval=100)

      # perform simulated annealing
      cool.run()

      k2=k2+1
      print k2


   # final torsion angle minimization
   protocol.initMinimize(dyn_free_sch,potList=potList_2,
                         printInterval=50)
   dyn_free_sch.run()

   #do analysis and write structure
   loopInfo.writeStructure(potList_2)
   pass


from simulationTools import StructureLoop, FinalParams
StructureLoop(numStructures = numberOfStructures ,
              pdbTemplate=outFilename,
              structLoopAction=calcOneStructure,
              genViolationStats=1,
              averagePotList=potList_2,
              averageSortPots=[noe, r_ratio_HPr, r_ratio_EIN, potList_2['ANGL'],
                               potList_2['BOND'], potList_2['IMPR'],
                               potList_2['RAMA'], potList_2['VDW'], ra],
              averageCrossTerms=refRMSD,
              averageTopFraction=0.0195,
              averageContext=FinalParams(rampedParams),
              averageFilename="SCRIPT_ave.pdb",    #generate regularized ave structure
              averageFitSel="name CA",
              averageCompSel="not resname ANI and not hydro" ).run()
```

6. R_2/R_1 data filtering script: file filter_data.py (in eginput/relaxRatio directory of Xplor_NIH 2.25 release)

```
# -- sample script which demonstrates how to filter NMR NH relaxation data --- #
# ----------------- used in docking protocol dock_rrp.py --------------------- #


# protocol module with many high-level helper functions.
import protocol

# explicitly set random seed
#
protocol.initRandomSeed(7654)

protocol.initParams("protein")

# read in EIN domain coordinates
#
protocol.loadPDB("randomized_sch_EIN.pdb")

# read in HPr domain coordinates
#
protocol.loadPDB("randomized_sch_HPr.pdb")

xplor.simulation.deleteAtoms("not known")

from diffPotTools         import readInRelaxData, mergeRelaxData
from relaxRatioPotTools   import filterRelaxData

# read in data for EIN domain
#
relax_data_EIN = readInRelaxData(['ein_r1r2_7pts.tbl'],
              pattern=['resid','R1','R1_err','R2','R2_err','skip','skip'])

# read in data for EIN domain
#
relax_data_HPr = readInRelaxData(['hpr_r1r2_7pts.tbl'],
              pattern=['resid','R1','R1_err','R2','R2_err','skip','skip'])

# combine two sets of relaxation data in one list
#
relax_data=mergeRelaxData(relax_data_EIN+relax_data_HPr)

# run filtering routine
# for input and output description refer to relaxRatioPotTools.py
#
exc_sel=filterRelaxData(data_in=relax_data, Fr=[600.141,600.141],
                        domain_sel=['resid 1:250','resid 300:400'] )

print '\n',"Selection of excluded residues:",'\n',exc_sel,'\n'
```

7. NMR relaxation data (in eginput/relaxRatio directory of Xplor_NIH 2.25 release)

(data from Ryabov, Y.; Suh, J.- Y.; Grishaev, A.; Clore, G. M.; Schwieters, C. D. *J. Am. Chem. Soc.* 2009, *131*, 9522-9531)

EIN NMR relaxation data: file ein_r1r2_7pts.tbl

(Note the R1rho data are not required in the input table)

#residue	R1	error	R2	error	R1rho	error
4	0.735	0.004	21.410	0.878	19.852	0.812
5	0.769	0.010	32.262	4.084	26.289	3.309
6	0.831	0.017	20.856	0.492	18.474	0.433
7	0.747	0.006	24.658	1.125	20.537	0.931
8	0.741	0.004	22.154	0.583	21.481	0.565
10	0.704	0.007	22.654	0.985	19.219	0.831
11	0.789	0.005	19.258	0.158	19.230	0.157
12	0.824	0.008	20.655	0.485	18.445	0.431
13	0.763	0.007	20.063	0.218	19.775	0.215
14	0.806	0.006	19.282	1.164	17.747	1.067
15	0.793	0.001	19.758	0.097	19.677	0.097
16	0.740	0.006	24.198	0.273	23.518	0.265
18	0.814	0.002	21.580	0.503	18.986	0.440
19	0.788	0.006	21.884	0.274	18.678	0.233
20	0.821	0.019	14.123	0.206	13.148	0.191
23	0.876	0.022	17.893	0.129	17.311	0.124
24	0.843	0.004	17.336	0.110	16.410	0.104
25	0.825	0.008	23.695	2.445	19.547	2.001
26	0.728	0.003	20.020	0.790	18.164	0.714
27	0.806	0.002	22.021	0.422	19.197	0.366
28	0.677	0.010	22.810	0.184	21.545	0.174
29	0.795	0.008	23.594	0.128	22.805	0.124
30	0.901	0.012	19.495	0.247	18.741	0.237
31	0.765	0.005	21.130	0.119	21.065	0.119
32	0.698	0.003	23.019	0.324	22.810	0.321
36	0.695	0.006	25.638	0.321	25.208	0.315
37	0.758	0.005	23.078	0.345	23.012	0.344
39	0.773	0.009	23.330	0.374	22.716	0.363
40	0.740	0.006	24.910	0.188	24.216	0.183
41	0.780	0.010	22.323	0.309	22.240	0.308
42	0.740	0.006	24.300	0.282	23.687	0.274
44	0.731	0.008	25.718	0.539	25.304	0.530
45	0.766	0.006	23.110	0.423	23.104	0.423
46	0.705	0.008	26.191	0.510	25.058	0.487
47	0.737	0.007	25.749	0.348	24.746	0.334
50	0.729	0.007	25.453	0.321	24.860	0.313
52	0.792	0.005	24.667	0.316	23.812	0.305
53	0.714	0.007	24.901	0.461	24.882	0.461
56	0.745	0.011	23.291	0.140	23.286	0.140
57	0.735	0.009	24.485	0.365	23.285	0.347
58	0.746	0.006	24.977	0.331	24.569	0.325
59	0.803	0.008	23.076	0.334	22.925	0.332
60	0.784	0.008	24.528	0.153	23.541	0.146
61	0.789	0.004	24.790	0.339	23.895	0.326
62	0.816	0.013	21.719	0.916	19.089	0.801
63	0.848	0.015	22.032	0.171	21.633	0.168
64	0.781	0.015	22.239	0.321	21.977	0.317
65	0.806	0.009	21.752	0.207	21.745	0.207
66	0.759	0.006	17.495	0.494	16.523	0.466
67	0.819	0.024	21.579	0.213	21.414	0.211
70	0.849	0.012	21.220	0.252	21.074	0.250
71	0.840	0.004	19.814	0.278	19.761	0.277
72	0.748	0.008	24.727	0.225	24.637	0.224
73	0.769	0.007	23.291	0.139	23.291	0.139
74	0.784	0.007	23.903	0.281	23.415	0.275
75	0.735	0.010	24.092	1.397	21.425	1.237
78	0.710	0.006	24.319	0.252	24.315	0.252
80	0.722	0.013	24.731	0.318	24.730	0.318
81	0.755	0.007	23.606	0.430	23.231	0.423
83	0.802	0.018	23.081	0.289	20.171	0.251
84	0.868	0.007	21.731	0.188	21.169	0.183
85	0.767	0.007	22.880	0.186	22.048	0.179
86	0.841	0.007	20.922	0.124	20.918	0.124
87	0.761	0.006	23.831	0.431	23.802	0.431

89	0.784	0.009	22.025	0.269	21.840	0.267
91	0.812	0.008	24.658	0.189	24.228	0.186
92	0.747	0.006	23.222	0.385	23.199	0.384
93	0.775	0.010	22.337	0.633	22.065	0.625
94	0.764	0.008	23.930	0.350	23.847	0.349
95	0.768	0.002	21.822	0.194	21.762	0.193
96	0.748	0.007	21.439	0.339	21.376	0.338
97	0.737	0.008	23.069	0.422	23.050	0.422
98	0.684	0.009	24.117	0.216	24.116	0.216
99	0.728	0.003	21.310	1.106	18.940	0.978
100	0.798	0.005	22.407	0.374	21.894	0.365
101	0.770	0.004	23.357	0.749	21.872	0.700
102	0.803	0.012	24.007	0.464	23.111	0.446
103	0.804	0.008	23.421	0.182	22.945	0.179
105	0.793	0.006	24.130	0.303	24.101	0.303
107	0.801	0.006	22.899	0.253	22.810	0.252
109	0.817	0.007	23.323	0.195	23.195	0.194
110	0.801	0.005	21.401	1.150	19.487	1.043
113	0.776	0.013	22.680	0.338	22.034	0.328
114	0.823	0.011	23.577	0.228	22.386	0.217
115	0.809	0.006	21.135	0.174	21.123	0.174
116	0.779	0.012	22.035	0.225	22.024	0.225
117	0.810	0.008	21.711	0.143	21.698	0.143
118	0.893	0.003	19.088	0.163	19.012	0.162
119	0.968	0.027	16.187	0.129	16.153	0.128
120	0.737	0.006	23.230	0.255	23.072	0.254
121	0.757	0.010	21.755	1.000	19.555	0.896
122	0.881	0.019	20.384	0.372	20.174	0.368
123	0.838	0.007	21.627	0.503	21.349	0.496
124	0.842	0.010	22.170	0.352	22.033	0.349
128	0.773	0.010	23.298	0.162	23.131	0.161
132	0.805	0.007	22.840	0.390	22.234	0.379
134	0.817	0.006	20.152	1.023	18.545	0.938
136	0.803	0.006	22.654	0.300	22.468	0.297
137	0.789	0.008	23.036	0.408	22.309	0.394
138	0.817	0.007	21.986	0.213	21.863	0.212
139	0.768	0.005	23.370	0.322	23.355	0.322
140	0.756	0.013	22.253	0.507	22.157	0.505
141	0.752	0.007	22.188	0.410	21.461	0.396
142	0.799	0.005	21.045	0.290	21.018	0.290
143	0.721	0.013	23.420	0.586	21.911	0.547
145	0.775	0.011	20.368	0.102	20.115	0.101
146	0.917	0.019	16.445	0.470	14.997	0.426
147	0.921	0.014	18.692	0.324	16.085	0.276
148	0.842	0.021	18.789	0.544	17.131	0.494
149	0.801	0.016	22.268	0.632	20.367	0.576
150	0.798	0.004	23.646	0.516	23.620	0.515
151	0.871	0.016	20.874	0.509	19.545	0.475
152	0.765	0.005	16.944	0.079	16.843	0.079
154	0.726	0.015	18.736	0.109	18.529	0.108
155	0.681	0.004	18.736	0.147	18.724	0.147
156	0.753	0.004	22.146	0.268	22.106	0.267
157	0.781	0.004	21.463	0.079	20.971	0.077
158	0.821	0.007	22.799	0.413	19.869	0.358
160	0.782	0.005	22.705	0.413	20.826	0.378
161	0.787	0.006	20.856	0.467	19.385	0.433
162	0.774	0.005	19.936	0.439	18.936	0.416
163	0.753	0.003	18.017	0.167	17.911	0.166
166	0.868	0.029	21.017	0.273	20.532	0.267
167	0.854	0.005	22.086	0.495	21.622	0.484
168	0.789	0.006	23.593	0.489	22.411	0.464
170	0.888	0.010	20.527	0.338	19.968	0.329
171	0.827	0.003	20.903	0.164	20.903	0.164
173	0.764	0.010	22.213	0.282	20.619	0.261
175	0.695	0.008	25.499	0.595	25.478	0.594
176	0.764	0.008	20.414	0.309	20.411	0.309
177	0.783	0.006	22.279	0.268	21.380	0.257
178	0.757	0.013	20.028	1.641	15.659	1.269
179	0.795	0.005	22.953	0.359	22.897	0.358
180	0.792	0.003	21.086	0.196	20.886	0.194
181	0.789	0.006	23.427	0.271	22.767	0.263
182	0.780	0.007	23.535	0.212	22.206	0.200
183	0.823	0.011	22.567	0.263	21.905	0.255
185	0.698	0.021	20.480	0.566	17.533	0.482
189	0.730	0.017	24.168	1.189	23.453	1.152
190	0.818	0.013	23.421	0.176	23.268	0.175

192	0.722	0.005	25.398	0.276	24.911	0.271
193	0.826	0.008	23.167	0.112	22.776	0.110
194	0.802	0.003	24.496	0.303	23.799	0.295
195	0.758	0.006	23.880	0.330	23.860	0.330
196	0.745	0.008	23.639	0.233	23.632	0.233
197	0.827	0.006	21.704	0.348	21.380	0.343
198	0.730	0.009	23.924	0.417	23.920	0.417
201	0.749	0.004	18.933	0.408	18.849	0.406
202	0.821	0.008	21.673	0.281	20.959	0.271
203	0.801	0.004	23.246	0.389	23.239	0.389
204	0.719	0.006	22.088	1.327	19.720	1.180
205	0.819	0.014	22.249	1.730	19.933	1.543
206	0.773	0.009	23.809	0.434	23.765	0.434
207	0.683	0.004	21.911	0.352	21.523	0.345
209	0.812	0.009	23.792	0.460	23.446	0.453
210	0.842	0.006	21.944	0.456	21.802	0.453
212	0.622	0.004	21.787	0.248	21.672	0.247
213	0.658	0.004	26.243	0.345	24.732	0.325
214	0.756	0.002	22.075	0.106	22.024	0.106
215	0.764	0.005	21.882	0.262	21.881	0.262
217	0.736	0.003	23.448	0.249	22.543	0.239
219	0.766	0.008	20.364	0.310	20.280	0.309
220	0.805	0.008	26.935	2.045	22.322	1.684
221	0.792	0.006	21.564	0.197	20.273	0.184
222	0.781	0.003	21.172	0.401	18.560	0.350
223	0.806	0.016	22.246	0.569	22.208	0.568
224	0.753	0.013	19.358	0.409	19.358	0.409
226	0.813	0.005	20.823	0.241	20.771	0.240
228	0.793	0.008	21.436	0.469	19.389	0.422
229	0.780	0.006	20.048	0.238	19.190	0.228
230	0.735	0.004	23.760	0.237	22.350	0.223
232	0.812	0.007	19.399	0.515	18.201	0.482
235	0.807	0.005	22.207	0.241	21.662	0.234
236	0.825	0.007	21.297	0.321	20.976	0.316
241	0.953	0.007	19.596	0.175	19.136	0.171
243	1.070	0.026	15.586	0.102	15.356	0.100
244	1.056	0.020	13.819	0.183	13.745	0.182

HPr NMR relaxation data: file hpr_r1r2_7pts.tbl

#residue	R1	error	R2	error	R1rho	error
303	0.722	0.002	22.333	0.333	21.315	0.318
304	0.727	0.002	21.174	0.303	20.943	0.300
305	0.720	0.003	20.130	0.113	19.393	0.108
306	0.811	0.004	20.144	0.058	19.678	0.057
307	0.768	0.005	19.183	0.146	18.573	0.141
308	0.832	0.003	18.775	0.745	17.014	0.672
309	0.901	0.013	19.453	0.156	19.182	0.153
310	0.763	0.003	25.314	0.603	21.364	0.506
312	0.905	0.023	17.196	0.339	17.092	0.337
313	0.919	0.008	17.876	1.048	16.081	0.937
314	0.904	0.005	18.761	0.157	18.018	0.151
315	0.861	0.019	21.666	0.147	21.208	0.143
316	0.836	0.023	19.966	0.423	19.717	0.417
317	0.842	0.018	23.037	0.281	22.886	0.279
319	0.773	0.005	30.549	0.356	29.848	0.348
320	0.715	0.002	24.412	0.120	24.219	0.119
321	0.766	0.003	22.206	0.080	22.190	0.080
322	0.734	0.005	24.518	0.338	23.974	0.331
324	0.730	0.004	23.942	0.064	23.859	0.064
325	0.757	0.004	22.852	0.153	22.834	0.152
326	0.711	0.003	25.108	0.652	23.506	0.609
327	0.672	0.004	24.332	0.405	23.888	0.397
328	0.798	0.005	19.204	0.879	16.971	0.772
330	0.762	0.004	20.223	0.085	20.128	0.084
331	0.828	0.002	19.855	0.258	19.633	0.255
332	0.677	0.002	24.504	0.233	23.910	0.227
333	0.738	0.002	20.506	0.286	17.887	0.248
334	0.721	0.003	22.629	0.568	21.149	0.530
335	0.841	0.002	19.535	0.469	17.768	0.424
336	0.790	0.002	19.841	0.093	19.258	0.090
337	0.792	0.003	19.405	0.155	19.283	0.154
339	0.762	0.016	25.714	3.365	21.427	2.787
340	0.798	0.001	19.840	0.246	19.087	0.236
342	0.784	0.003	19.981	0.252	18.987	0.239

```
 343   0.766 0.005  18.616 0.321  18.505 0.319
 344   0.694 0.005  25.710 0.844  22.973 0.752
 345   0.746 0.004  18.634 0.911  17.159 0.836
 346   0.801 0.001  20.399 0.356  20.180 0.352
 347   0.786 0.003  20.387 0.356  17.413 0.302
 348   0.832 0.007  16.793 0.127  16.780 0.127
 350   0.881 0.004  20.042 0.096  19.287 0.092
 351   0.846 0.006  20.683 0.238  20.265 0.233
 352   0.815 0.004  19.916 0.918  17.904 0.822
 354   0.813 0.024  17.212 0.965  15.779 0.881
 355   0.855 0.013  19.271 0.389  18.128 0.364
 356   0.805 0.005  19.132 0.284  18.521 0.275
 357   0.725 0.014  23.746 0.148  23.049 0.144
 358   0.863 0.003  18.900 0.186  18.874 0.186
 359   0.776 0.004  21.069 0.141  21.022 0.141
 360   0.814 0.003  18.547 0.525  16.492 0.464
 361   0.780 0.005  20.206 0.251  18.465 0.229
 362   0.752 0.003  20.704 0.121  20.630 0.121
 363   0.771 0.004  22.750 0.493  19.339 0.417
 364   0.734 0.004  22.741 0.296  22.272 0.290
 365   0.756 0.003  21.794 0.366  20.568 0.345
 366   0.689 0.001  22.625 0.187  22.470 0.185
 367   0.781 0.002  20.526 0.122  20.465 0.121
 368   0.783 0.008  19.467 0.266  19.379 0.265
 369   0.806 0.006  20.845 0.334  20.689 0.331
 370   0.766 0.005  22.634 0.235  22.633 0.235
 371   0.754 0.002  22.587 0.164  22.412 0.163
 372   0.731 0.002  21.021 0.278  20.922 0.277
 373   0.734 0.005  24.670 0.135  24.267 0.133
 374   0.689 0.003  24.151 0.241  24.111 0.241
 375   0.745 0.004  22.998 0.317  22.995 0.317
 376   0.772 0.002  23.144 0.285  23.098 0.284
 377   0.720 0.004  24.870 0.268  24.768 0.267
 378   0.736 0.004  24.720 0.252  24.531 0.250
 379   0.772 0.008  22.946 0.275  22.617 0.271
 380   0.741 0.004  23.271 0.151  23.225 0.150
 381   0.715 0.003  23.414 0.172  23.403 0.172
 382   0.788 0.006  22.239 0.233  22.113 0.232
 383   0.822 0.004  18.183 0.129  18.178 0.129
 384   0.805 0.004  17.593 0.145  17.155 0.141
 385   1.055 0.003  11.018 0.303  10.183 0.278
```

8. Ambiguous pseudo NOE distance restrains derived from chemical shift perturbation mapping: file shifts_noe_newx_EINHPr.tbl (in eginput/relaxRatio directory of Xplor_NIH 2.25 release)

(from Clore, G.M.;Schwieters, C.D. *J. Am. Chem. Soc.* 2003, *15*, 2902-2912).

```
!!hpr to e1

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 312 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 313 and (hydro or name o* or name n*))
    4.0 2.8 1.0
```

```
assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 314 and (hydro or name o* or name n*))
    4.0 2.8 1.0
assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 315 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 316 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 317 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 321 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 324 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 343 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 349 and (hydro or name o* or name n*))
    4.0 2.8 1.0
```

```
assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 351 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 352 and (hydro or name o* or name n*))
    4.0 2.8 1.0


assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 353 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 354 and (hydro or name o* or name n*))
    4.0 2.8 1.0

assign
    ((hydro or name o* or name n*)
    and ( resid 68:69 or resid 72 or resid 79
        or resid 82 or resid 83 or resid 84:85
          or resid 110:111 or resid 115 or resid 120 or resid 123
        or resid 126 ))
    (resid 355 and (hydro or name o* or name n*))
    4.0 2.8 1.0


!!e1 to hpr

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 68 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 69 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 72 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 79 and (hydro or name o* or name n*))
   4.0 2.8 1.0
```

```
assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 82 and (hydro or name o* or name n*))
   4.0 2.8 1.0


assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 83 and (hydro or name o* or name n*))
   4.0 2.8 1.0


assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 84 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 85 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 110 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 111 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 115 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 120 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 123 and (hydro or name o* or name n*))
   4.0 2.8 1.0

assign
   ((hydro or name o* or name n*)
   and (resid 312:317 or resid 321 or resid 324
       or resid 343 or resid 349 or resid 351:355))
   (resid 126 and (hydro or name o* or name n*))
   4.0 2.8 1.0
```