**SUPPLEMENTARY INFORMATION**

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

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## 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.

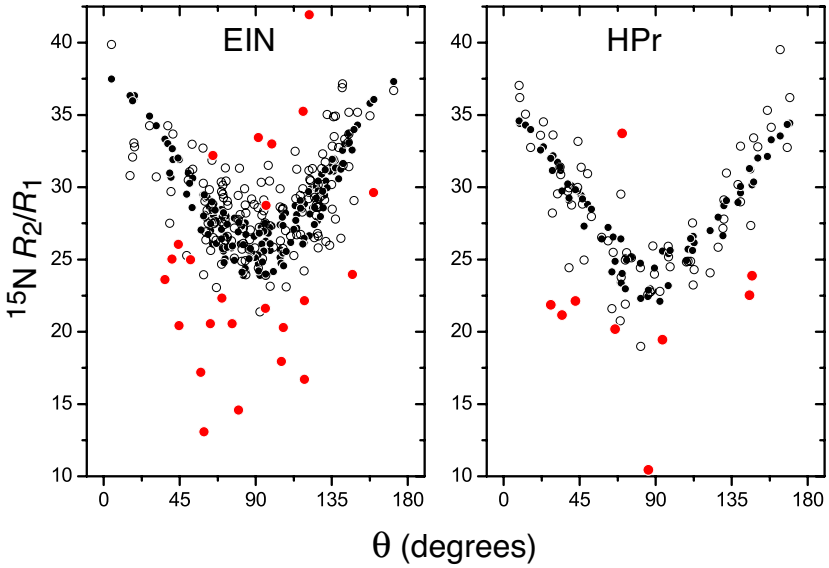


***Figure S1.*** Filtering of experimental $^{15}$N $R_2/R_1$ relaxation data. Black circles are simulated data; open circles are accepted data; and red circles are outliers. θ denotes the angle between an N-H bond vector and the long axis of the fitted diffusion tensor. 13% of the data were removed 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 | $\chi^2$ | 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

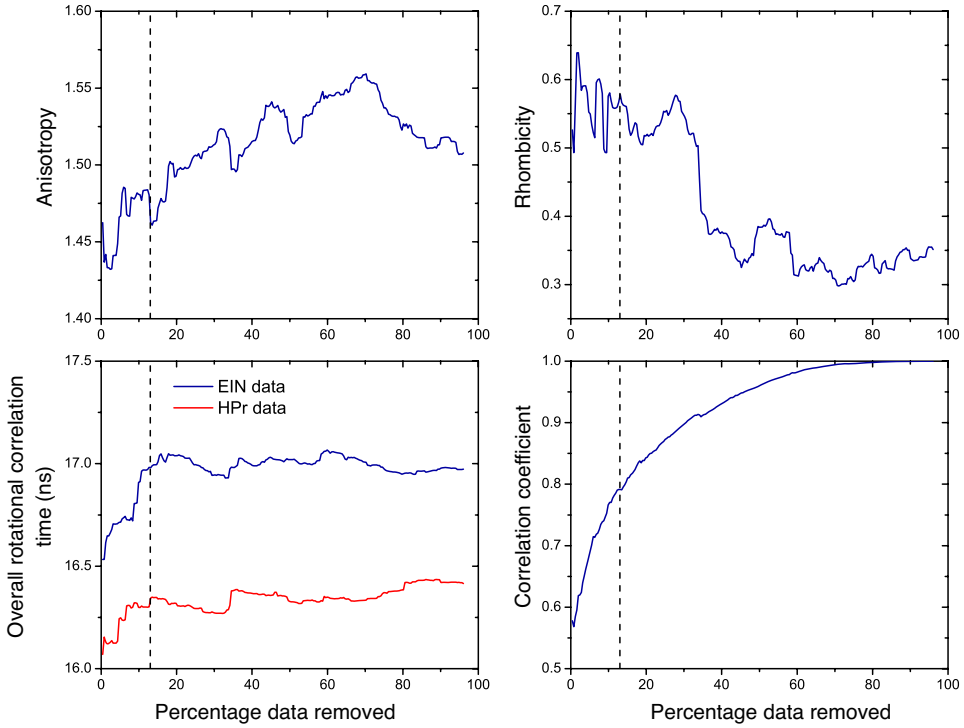


***Figure S2.*** Dependence of the fitted parameters of the diffusion tensor on the percentage of experimental data points removed from the original data set. The panel in the right bottom corner shows the correlation between experimental data points and back calculated data. The dashed line marks the 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 $\omega_N$ and $\omega_H$ 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 $\alpha$, $\beta$, and $\gamma$ 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 $\tau_{loc}$ 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 $\rho$. 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:

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

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:

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

where the function

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

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
```