**GLOVE manual**

Kenji Sugase, Tsuyoshi Konuma, Jonathan C. Lansing, Peter E. Wright

In what follows, characters written in Courier New represent computational words used in the command lines, or GLOVE related files.

**Installation**

*Requirements*

• UNIX system (Linux, Intel-based Mac OSX, and Cygwin were tested)

• C++ compiler (g++ version 4 and Intel C++ compilers were tested)

• gfortran (http://gcc.gnu.org/fortran/)

• lapack (http://www.netlib.org/lapack/)

• blas (http://www.netlib.org/blas/)

• perl (http://www.perl.org/)

• grace (http://plasma-gate.weizmann.ac.il/Grace/)

Most of the programs and libraries required for GLOVE can be obtained using yum for Fedora Linux, apt-get for ubuntu Linux, or port for Mac OSX.

*Environment variables*

The GLOVEDIR environment variable should point to the GLOVE directory. In the following example, the GLOVE directory, glove2, is located under user’s home directory. For csh and tcsh, execute source ~/.cshrc after adding the following lines to the .cshrc file,

setenv GLOVEDIR ~/glove2

set path=($path $GLOVEDIR/bin)

For bash, execute source ~/.bashrc after adding the following lines to the .bashrc file.

export GLOVEDIR=~/glove2

export PATH=$PATH:$GLOVEDIR/bin

*Compilation*

The GLOVE executable binary is installed in $GLOVEDIR/bin by executing the following commands:

cd $GLOVEDIR

make install

**Usage of GLOVE**

A typical usage of GLOVE and the description of valid options are shown below:

glove –i glove.in –o glove.out –v –xmgr

–i This option specifies the GLOVE input file, glove.in. Any file name can be used as long as the operating system allows it.

–o This option specifies the GLOVE output file, glove.out. Any file name can be used as long as the operating system allows it.

–v The reduced χ2 value during the fit is reported on the monitor in real time.

–xmgr Graphical plots in the Xmgr format are created.

–h Valid options are shown.

–d This option specifies glove.in as the GLOVE input file and glove.out as the GLOVE output file, thus the options –i and –o are not necessary.

–vv The reduced χ2 value during the fit are reported on the monitor in real time, and the fitting result is stored after each fitting method is finished.

–noerr Experimental errors are set to 1.0.

**GLOVE input file**

A glove input file is composed of two sections: a header section (Supplementary Fig. 1), and a data set section (Supplementary Fig. 2). In what follows, we explain them in detail along with Supplementary Figs 1 and 2. Examples of input files are given in the examples directory that accompanies the executable.

*Fitting Model (line 2 in Supplementary Fig. 1)*

The keyword FUNCTION specifies the fitting method (equation) to be used in the fit. CPMG\_RICHARDS represents the Carver and Richards equation (Carver and Richards 1972) described in Theory and Methods. Other fitting methods implemented in GLOVE are explained in detail below.

*Fitting Method (lines 4-8 in Supplementary Fig. 1)*

The keyword METHOD specifies the fitting method(s) to be used in the fit. GLOVE has five fitting methods: ONE, ONEEX, GRID, RANDOM, and MCMIN. Here, we describe their usages in a GLOVE input file.

ONE, ONEEX and GRID require no optional parameter whereas RANDOM and MCMIN require the number of iterations. MCMIN further requires the scaling factor that defines the amplitude of random values to be added to the current best-fit parameter values. In the example shown in Supplementary Fig. 1, the fit starts with RANDOM with the number of iterations of 5, followed by three sequential MCMIN runs with the number of iterations of 5 and the scales of 0.1, 0.01, and 0.001, and the fit is finalized with ONEEX.

*Error Estimation (lines 10-11 in Supplementary Fig. 1)*

Standard deviations of fitting parameters are calculated using the covariance matrix method (Press et al. 2007) by default. Optionally, the jackknife and Monte Carlo methods (Press et al. 2007; Mosteller and Tukey 1968) can be used by specifying keywords JACK and MONTE, respectively. JACK requires no optional parameter whereas MONTE requires the number of calculations (100 in the case of Supplementary Fig. 1). Additional output files regarding their calculation results are generated. JACK and MONTE are conducted after a regular fit, and start from the best-fit parameter values determined by the fit as the initial values. Since the same fitting method(s) as the preceding fit are used for JACK and MONTE as well, the calculations often take extremely a long time, especially in the case of complicated models such as a three-state exchange model. Alternatively, GLOVE has simpler and faster versions of JACK and MONTE designated as JACK1 and MONTE1, respectively. They use only the method ONEEX, which is a single point minimization from the initial values. Since each fitting parameter does not deviate much form its initial value (best-fit parameter value) during JACK and MONTE calculations, ONEEX is usually sufficient for the calculation of standard deviations, and of course, it calculates much faster than does the full set of the fitting methods.

*Optional Calculation (lines 13-15 in Supplementary Fig. 1)*

GLOVE can optionally calculate parameters REX and PB, corresponding to an excess contribution caused by chemical exchange to an effective *R*2 rate, *R*ex (), and the population of the minor state in a two-state exchange model, *p*B (), respectively. The keyword CALC specifies what is to be calculated (REX or PB), followed by a label for the calculation, for example REX\_60.83MHz\_298.0K in Supplementary Fig. 1. This label is used in the GLOVE output file to show what was optionally calculated. The keyword CALC further requires experimental conditions and index numbers in the same manner as PLOT and DATA lines, shown as \* 298.0 1.00000 00, corresponding to 1/τCP, temperature, magnetic field, index numbers for the temperature and the magnetic field. In the cases of CALC and PLOT, 1/τCP is not necessary for their calculations, thus \* is used instead of a specific 1/τCP value. For temperature, it was shown that chemical shift difference Δ*ω* does not change at different temperatures (Palmer AG et al. 2001). Therefore, relaxation dispersion data collected at multiple temperatures can be fitted globally with Δ*ω* being treated as a global parameter. Since the model CPMG\_RICHARDS (the Carver and Richards equation) does not involve temperature for the calculation of the effective *R*2 rate, the temperature value is used to discriminate the experimental condition. For the magnetic field, GLOVE uses the relative magnetic field instead of the absolute magnetic fields. In Supplementary Fig.1, the relative magnetic fields of 1.0000 and 1.24953 correspond to 15N magnetic fields of 60.830911 and 76.010013 MHz, respectively. GLOVE calculates chemical shift differences Δ*ω* as DW multiplied by the relative magnetic field, and the fitted DW values are reported for the relative magnetic field of 1.0000, for example, 60.830911 MHz in this case. The two-digit index numbers 00 and 01 indicate that the first relaxation dispersion data were collected at 298.0 K (the left index number) and 60.83 MHz (the right index number), and the second ones were at 298.0 K and 76.01 MHz. Since both data were collected at the same temperature, they use the same left index number of 0. However, the magnetic fields are different, thus they use different right index numbers. For PB, the population of each state does not change with the magnetic field; therefore, it is calculated only for the relative magnetic field of 1.0000.

*Plot Instruction (lines 16-17 in Supplementary Fig. 1)*

The keyword PLOT specifies the relaxation dispersion profiles to be plotted on the Xmgr file as fitting results. PLOT is followed by a graph legend (60.83MHz\_298.0K), experimental conditions (\* 298.0 1.00000), index numbers (00), the minimum and maximum 1/τCP values for which theoretical effective *R*2 rates are to be calculated, and the number of data points for the theoretical effective *R*2 rates (50.0 3000.0 119). The experimental conditions and index numbers are explained in *Optional Calculation*.

*XMGR Option (lines 20-27 in Supplementary Fig. 1)*

The statement starting with @ specifies the properties of an Xmgr plot. In Supplementary Fig. 1, the minimum and maximum values of X (horizontal) and Y (longitudinal) axes, the major and minor tick spacing of X and Y axes are specified. The full lists of the Xmgr options are found on Grace user’s guide (http://plasma-gate.weizmann.ac.il/Grace/doc/UsersGuide.html).

*Global Parameter (lines 29-30 in Supplementary Fig. 1)*

Global parameters must be declared before the data set section. A global parameter consists of a keyword GPAR, a label, lower and upper limits of a fitting parameter, and a grid size. The label is an identifier, and thus is not necessary to be the same as the fitting parameter name. In the data set section, the label of the global parameter substitutes for the lower and upper limits and the grid size of the fitting parameter that is treated as a global parameter, for example, shown on lines 3 and 4 in Supplementary Fig. 2. If they are treated as local parameters, they are specified as:

PAR KEX\_0 5 4000 3

PAR PAPB\_0 0.005 0.09 3

For the data sets (residues) that are involved in the same cluster as the data set of SET 5-HN, their fitting parameters KEX\_0 and PAPB\_0 are also modified in the same manner. If there are more than two clusters (more than two regions in a protein change their conformations at different rates), corresponding global parameter sets for KEX\_0 and PAPB\_0 must be declared in the header section.

The keyword GPAR is the global parameter version of PAR, which is a regular local parameter. As described in Theory and Methods, the fitting parameters specified as PAR can go beyond the lower and upper limits during minimization. This is true for GPAR as well. To restrict a parameter within the user-defined range, the GREST parameter class, which is the global parameter version of REST, should be used instead of GPAR.

*Data Set Name (lines 1, 45 in Supplementary Fig. 2)*

The keyword SET specifies the data set name, for which the residue name or residue number is usually used.

*Fitting Parameters (lines 2-6, 46-50 in Supplementary Fig. 2)*

GLOVE has three different local parameters: PAR, REST, and FIXED. PAR is a regular local parameter, and is used like as:

PAR DW 100 2500 3

PAR KEX\_0 50 4000 3

PAR PAPB\_0 0.005 0.09 3

PAR R20\_0\_0 7.58

PAR R20\_0\_1 8.74

These are the same as what are shown in Supplementary Fig. 2 except that no global parameters are set. DW represents the chemical shift difference in units of rad∙s-1, followed by the lower and upper limits, and the grid size. These values can differ among data sets. The upper limit and grid size can be omitted. If the grid size is omitted, it is automatically set to 2. If both the upper limit and grid size are omitted (the grid size must be omitted if the upper limit is to be omitted), the grid size is set to 1. By default, the parameter range is divided by the grid size with an equal distance; therefore, the parameter space is evaluated with no bias. Optionally, the parameter range can be divided by the distance calculated according to exp(*nx*), exp(-*nx*), exp(*n*2*x2*), exp(-*n*2*x2*), 10*nx*, or 10-*nx*. *n* (n > 0) represents the *n* th grid point and *x* = (parameter range)/(grid size). These dividing methods are specified by adding e, E, g, G, t, or T, respectively, right after the grid size without inserting a space. This option is useful for examining parameter values near the lower or upper limit more intensively than the opposite side without increasing the number of the grid size.

For the exchange rate, KEX, and the sum of the populations, PAPB, they have an index number (\_0) to discriminate the temperature at which the relaxation dispersions were measured. If another relaxation dispersion data were collected at a different temperature, the exchange rate and the populations can differ from KEX\_0 and PAPB\_0, thus KEX\_1 and PAPB\_1 should be added to the fitting parameter set. In practice, the program cpmg2glove automatically add fitting parameters and corresponding index numbers to the GLOVE input file if experimental conditions are correctly provided. For the intrinsic relaxation rate, R20, there are two index numbers (\_0\_0 or \_0\_1) to discriminate the temperature and the magnetic field, corresponding to the first and second index numbers, respectively, since the intrinsic relaxation rate depends upon both of them. Note that the upper limit and grid size for R20 are omitted because *R*20 is little dependent upon other parameters, and is not very sensitive to fitting models. In practice, the slowest *R*2eff rate in a relaxation dispersion profile is a good estimate as an initial value.

An initial value can optionally be specified after the grid size (neither the upper limit nor grid size should be omitted), for example,

PAR KEX\_0 50 4000 3 250

In the case of the PAR class parameters, the optimized values can exceed beyond the user-defined upper and lower limits during fitting, but cannot exceed the hard-coded parameter limits that are written in the GLOVE source code, for instance, 1-12000 for KEX.

REST represents a restricted parameter class. It is similar to PAR, but each parameter value cannot exceed the user-defined lower or upper limit. The upper limit cannot be omitted in the case of REST, and the initial value must be specified within the upper and the lower limits, for example,

REST KEX\_0 50 4000 3 250

FIXED represents a fixed parameter class. The parameter name is followed by a single value, which is not optimized in the fit, for example,

FIXED KEX\_0 250

*Data (lines 7-45, 51-88 in Supplementary Fig. 2)*

The keyword DATA specifies the input data to be fitted such as 100.00 298.0 1.00000 00 20.42862 0.35563, corresponding to 1/τCP, temperature, magnetic field, index numbers for the temperature and the magnetic field, effective *R*2 rate, and experimental error. The experimental conditions and index numbers are explained in *Optional Calculation*.

**Glove output file**

A glove output file is composed of two sections: a header section and a result section (Supplementary Fig. 3). In what follows, we explain them along with Supplementary Fig 3. The header section reports the program version (line 1), the fitting model used in the fit (line 2), the fitting parameters for the model (line 3), the seed numbers of the random generator used for RANDOM and MCMIN (line 4), the fitting methods (lines 5-10), the total data points (line 13), the total degree of freedom (line 14), the global reduced χ2 value at each step of the fitting method (line 15-20), and the total time spent for the fit (line 21). Note that the fitting method INIT is always inserted in the beginning of the fitting methods by GLOVE. This method calculates the global reduced χ2 value using the lower limits of the parameters or user-defined initial values.

The result section reports the data set name (line 23), fitted parameter values with standard deviations in parentheses (lines 24-28), optionally calculated parameter values (lines 29-31), and the local reduced χ2 value with the degree of freedom (line 32) for each data set.

**Fitting models**

The Carver and Richards equation

The Carver and Richards equation (Carver and Richards 1972), called Richards in GLOVE, calculates well-approximated *R*2eff values for all exchange regimes of a two-state exchange model () under the experimentally accessible condition. The original equation is represented as

 (1),

where Δ*ω* represents the chemical shift difference between the two states in units of rad·s-1, *R*2A0 and *R*2B0 represents intrinsic transverse relaxation rates of the states A and B, respectively. Although the intrinsic transverse relaxation rates of the two states can be different, they are usually assumed to be the same, i.e., *R*20 = *R*2A0 = *R*2B0. The assumption has little effect upon the analysis of the exchange when the exchange rate is much faster than the difference between *R*2A0 and *R*2B0 (*k*ex >> |*R*2A0 − *R*2B0|). In addition to the assumption on *R*20, GLOVE adopts *k*ex (sum of the forward and backward rates, *k*AB + *k*BA) and *p*A*p*B (product of the two populations, *p*A × *p*B) instead of *k*AB and *k*BA to reduce the parameter spaces around the commutable *k*AB and *k*BA, enhancing the computational efficiency and stability. The population of state B, designated as the lower-populated state, is calculated according to the formula . The exact equation used in GLOVE is represented as:

 (2).

The Luz and Meiboom equation

For the condition of fast exchange (*k*ex/2 >> Δ*ω*), the Luz and Meiboom equation (Luz Z and Meiboom S 1963), called MEIBOOM in GLOVE, can be used:

 (3),

where *p*A*p*BΔ*ω*2 is a single fitting parameter, often represented as *Φ*ex. In GLOVE, the parameters *k*ex, *p*A*p*BΔ*ω*2, and *R*20are defined as KEX, PDW, and R20, respectively.

The Ishima and Torchia equation

If the population is highly skewed to the major state, the Ishima and Torchia equation (Ishima R and Torchia DA 1999), called TORCHIA in GLOVE, can be used:

 (4).

In GLOVE, the parameters Δ*ω*, *k*ex, *p*A*p*B, and *R*20 are defined as DW, KEX, PAPB, and R20, respectively.

Constant line

The model called CONST in GLOVE is used whether *R*ex exists or not. This model has a single fitting parameter *R*20. i.e. *R*2eff = *R*20.

Basic three-state exchange model

An analytical equation was derived by Grey *et al* for a three-state exchange model, but is only applicable to fast exchange. Since it is difficult to know the exchange regime of the system of interest *a priori*, an *R*2eff rate in a three state exchange is usually calculated numerically by matrix operations (Korzhnev DM et al. 2004):

 (5).

The calculated *R*2eff rate using this matrix manipulation has no approximation for all exchange regimes, but the calculation is time consuming. To calculate *R*2eff faster in GLOVE, an *R*2eff rate is calculated using the equation shown below. This equation called MATRIX3 in GLOVE was derived according to the same procedure to derive the Carver and Richards equation:

 (6).

Under the experimentally accessible condition, *R*2eff is dominated by the largest eigenvalue *λ*1 of the matrix.

 (7),

where *R*[ ] and *I*[ ] are functions to extract the real or imaginary elements, respectively, of the complex matrix. As the matrix **A** is a 3-by-3 evolution matrix as given below (**A**\* is its complex conjugate), the matrix size shown as Equation (7) is 6-by-6 for the three-state exchange model. If the kinetic rate constants are much faster than differences in intrinsic relaxation rate between the states, **A** is represented as:

 (8).

Note that the equation to derive eigenvalues becomes a sixth degree equation, but is a cubic equation of λ2, therefore, it can be solved using Cardano’s formula. In GLOVE, the parameters Δ*ω*AB, Δ*ω*AC, *k*AB, *k*BA, *k*BC, *k*CA, *k*AC, and *R*20 are defined as DWAB, DWAC, KAB, KBA, KBC, KCA, KAC, and R20, respectively. *k*CB is calculated according to the microscopic reversibility: *k*CB = (*k*AB×*k*BC×*k*CA)/(*k*BA×*k*AC).

Two-state binding models

Since the binding rate is dependent upon concentrations of two interacting molecules (), a global analysis of relaxation dispersions measured with multiple concentration ratio samples provides binding kinetics *k*on and *k*off. The model called BIND2C in GLOVE is designed for the experiments in which the concentration of the NMR observable molecule is fixed for all samples whereas the concentration of the target molecule is varied, for example, 1:0.95, 1:1, 1:1.05, and 1:1.1. BIND2C utilizes the Richards and Carver equation with *k*ex = [B]*k*on + *k*off. The concentration of free B, [B], is calculated according to:

 (9),

where *a* is the concentration ratio of B between samples, for example, 0.95, 1, 1.05, 1.1. [A]0 and [B]0 represent the total concentrations of A and B, respectively, and the dissociation constant *K*D is determined as *K*D = *k*off/*k*on. In GLOVE, Δ*ω*, *k*on, *k*off, *R*20, [A]0 and [B]0 are defined as DW, KON, KOFF, R20, A0, and B0, respectively. [B]0 is determined for the sample with the concentration ratio of 1.

In practice, this model is time-consuming to explore the best-fit parameter set, therefore, at the initial stage of the analysis, it is preferable to start with an easier model called BIND2, in which the fitting parameters are Δ*ω*, B*k*on, *k*off, and *R*20, defined as DW, BKON, KOFF, and R20, respectively, in GLOVE. B*k*on is a single fitting parameter in units of s-1, substituted for [B]*k*on. This fitting parameter is determined for each concentration ratio sample. The Δ*ω*, *k*off, and *R*20 values derived using this model can be good initial values in BIND2C.

Three-state binding models

GLOVE has two distinct three-state binding models (BIND3C\_1F2B and BIND3C\_2F1B) utilizing the same matrix manipulation as MATRIX3 described above. BIND3C\_1F2B is comprised of one free and two bound states whereas BIND3C\_2F1B has two free and one bound states. Other three-state binding models can be constructed from either of the two models by fixing some parameters to zero. These two models are designed for the experiments in which the concentration of the NMR observable molecule is fixed for all samples whereas the concentration of the target molecule is varied. BIND3C\_1F2B can deal with the binding event in which a ligand binds to two binding sites on the target receptor with different affinities, or bound conformations, and the two bound conformations interconvert with each other. The matrix **A** in MATRIX3 become as:

 (10),

where *k*on1 and *k*off1 (*k*on2 and *k*off2) represent the association and dissociation rate constants for the bound1 (bound2) state, respectively, and *k*12 and *k*21 are the rate constants for the chemical (conformational) exchange from the bound1 to the bound2 state, and from the bound2 to the bound1 state, respectively. Δ*ω*FB1 and Δ*ω*FB2 represent the chemical shift differences between the free and bound1 states and between the free and bound2 states, respectively. The kinetic rates from the free to two bound states are concentration dependent, and [B] is calculated using Equation (9), but in this case, the dissociation constant *K*D is calculated according to:

 (11).

In GLOVE, the parameters Δ*ω*FB1, Δ*ω*FB2, *k*on1, *k*off1, *k*on2, *k*off2, *k*12, *k*21, *R*20, [A]0 and [B]0 are defined as DWFB1, DWFB2, KON1, KOFF1, KON2, KOFF2, K12, K21, R20, A0, and B0, respectively. [B]0 is determined for the sample with the concentration ratio of 1. If *k*on2 and *k*off2 are fixed to 0 (*k*on1 and *k*off1 can alternatively be fixed to 0), the model becomes the induced fit model, in which there is a conformational change after binding:

 (12),

where AB\* and AB are the intermediate and the bound states, respectively. In this case, the rate constants *k*12 and *k*21 correspond to the folding and unfolding rate constants, respectively. If *k*12 and *k*21 are fixed to 0, BIND3C\_1F2B becomes the model in which a ligand binds to a receptor with two different bound conformations:

 (13),

where AB and A\*B are the bound1 and the bound2 states, respectively.

On the other hand, the model BIND3C\_2F1B can deal with the binding event in which a ligand binds to a single binding site on the target receptor from two different free states, and the two free states interconvert with each other. The matrix **A** in MATRIX3 become as:

 (14),

where *k*12 and *k*21 are rate constant for the chemical (conformational) exchange from the free1 to the free2 state, and from the free2 to the free1 state. Δ*ω*F1F2 and Δ*ω*F1B represent the chemical shift differences between the free1 and free2 states and between the free1 and bound states, respectively. [B] is obtained by solving the following cubic equation:

 (15).

In GLOVE, the parameters Δ*ω*F1F2, Δ*ω*F1B, *k*on1, *k*off1, *k*on2, *k*off2, *k*12, *k*21, *R*20, [A]0 and [B]0 are defined as DWF1F2, DWF1B, KON1, KOFF1, KON2, KOFF2, K12, K21, R20, A0, and B0, respectively. [B]0 is determined for the sample with the concentration ratio of 1. If *k*on1 and *k*off1 are fixed to 0 (*k*on2 and *k*off2 can alternatively be fixed to 0), the model becomes the conformational selection model, in which there is a conformational change from the inactive form to the active form before binding:

 (16),

where A\* and A are the inactive and active conformations, respectively. The rate constants *k*21 and *k*12 correspond to the folding and unfolding rate constants, respectively.

The Carver and Richards equation for extraction of dipolar couplings for a two state model

GLOVE is able to fit data to coupling data to allow the determination of RDCs for low populated intermediates using CPMG experiments. The model CPMG\_RDC fits KEX, PAPB, R20, DW and DJ to three sets of dispersion data (0:RC, 1:TROSY, 2:anti-TROSY). The RDCs and signs can be then determined from the DJ as detailed by Vallurupalli et al. (2007)

The Carver and Richards equation for multiple probes and temperatures

GLOVE is able to fit data to multiple probes (for example if 15N and 13C carbonyl dispersion data are recorded) using a single kex and pb. The model CPMG\_RICHARDS\_PROBES fits KEX, PAPB, R20, DW, where a different DW value is fit for each different probe. Different temperatures are treated in a similar way, however PB and KEX are able to vary between temperatures.

R1rho models

GLOVE is able to fit data to four analytical R1rho models. The first model is R1RHO\_LONDON and fits PHI, KEX and R20 to the equation from Davis et al. (1994). The rest of the models are R1RHO\_PALMER02, R1RHO\_PALMER05, R1RHO\_KAY, and GLOVE fits DW, KEX, PB, R1, and R20 to Eqn 21 of Trott et. al 2002, Eqn 19 of Miloushev et al. 2005, and Eqns 1 and 3 of Korzhnev et al. 2005, respectively.

**Utility programs contained in the GLOVE software package**

*pkfit*

The program pkfit extracts chemical shifts and peak intensities from a series of relaxation dispersion spectra in the NMRView or NMRpipe format. It searches the highest peak position within a peak box defined in an NMRView xpk file, and obtains a sum of intensities at N × N grid points centered on the peak top (N is an integer and usually N = 3). Noise amplitudes are also extracted from noise floors specified in the same NMRView peak list, in which noises are assigned as 1.noise, 2.noise, …, and N.noise (N ≥ 10). pkfit can be executed without signal assignment except for noise peaks as long as peaks of interest are correctly picked. Unassigned signals are represented as N.tmp (N represents the peak number) in GLOVE. A typical usage of pkfit and the description of valid options are shown below:

pkfit –i pkfit.in –o pkfit.out –v

–i This option specifies the pkfit input file, pkfit.in. Any file name can be used as long as the operating system allows it.

–o This option specifies the pkfit output file, pkfit.out. Any file name can be used as long as the operating system allows it.

–v The process of pkfit is shown on the monitor.

–abs All intensities except noise are obtained as absolute values.

Supplementary Figs 4 and 5 show examples of pkfit input and output files, respectively. It should be noted that pkfit determines the peak top positions accurately; therefore, it can also be used for determining the sign of Δ*ω* by comparing the chemical shifts between HSQC and HMQC spectra or between HSQC spectra collected at different magnetic fields.

*chop*

The program chop compiles peak intensities of each residue in a row from a pkfit output file, in which they are reported by the residue. The file created by pkfit, designated as an Int file, is subsequently used as an input file of the program cpmg2glove. A typical usage of chop and the description of valid options are shown below:

chop –i pkfit.out –int

–i This option specifies the pkfit output file, pkfit.out. Any file name can be used as long as the operating system allows it.

–int Intensities are extracted.

Other than intensities, chop can extract peak numbers, peak names, chemical shifts in the horizontal dimension, and chemical shifts in the vertical dimension by specifying –num, -label, -pos1, and -pos2, respectively, instead of –int. The name of the created Int file is Int\_XXXX. XXXX is the magnetic field at which relaxation dispersion data were collected.

*pkfiti*

pkfiti is a shell script to execute pkfit and chop sequentially. The valid options for pkfiti are the same as those for pkfit.

*initval*

The program initval extracts the fitted parameter values from a GLOVE output file, and adds them to a new GLOVE input file as initial values. A typical usage of initval and the description of valid options are shown below:

initval –i glove.in –o glove.out > glove2.in

–i This option specifies the GLOVE input file, glove.in. Any file name can be used as long as the operating system allows it.

–o This option specifies the GLOVE output file, glove.out. Any file name can be used as long as the operating system allows it.

–ov The old GLOVE input file is overridden by the new GLOVE input file. Thus the option ‘> glove2.in’ is not necessary.

–h Valid options are shown.

–d This option specifies glove.in as the GLOVE input file and glove.out as the GLOVE output file instead of using the options –i and –o.

*mfit*

The program mfit runs glove multiple times with updating the GLOVE input file using initval. A typical usage of mfit is shown below:

mfit –i glove.in –o glove.out –n 10

The option –n specifies the number of repeats, and other options are the same as those for glove.

*cpmg2glove*

The program cpmg2glove produces a GLOVE input file from Int files created by chop or pkfiti. A typical usage of cpmg2glove and the description of valid options are shown below.

cpmg2glove –t RICHARDS –i Int\_76.01 298 > glove.in

–t This option specifies the fitting model.

–i This option specifies the Int file, Int\_76.01, and the temperature at which relaxation dispersions were collected. Any file name can be used as long as the operating system allows it.

For the models, BIND2C, BIND3C\_1F2B, and BIND3C\_2F1B, the option –ic is used instead of –i to specify the concentration ratio, corresponding to *a* in Equation (9). Other valid options can be found by executing cpmg2glove with no arguments.

*r1rho2glove*

The program r1rho2glove produces a GLOVE input file from Int files created by chop or pkfiti. A typical usage of r1rho2glove and the description of valid options are shown below.

cpmg2glove –t PALMER05 W –i Int\_76.01 298 > glove.in

–t This option specifies the fitting model and X-axis.

The option X-axis is w1 for on-resonance R1rho dispersion data and W for off-resonance R1rho dispersion data

–i This option specifies the Int file, Int\_76.01, and the temperature at which relaxation dispersions were collected. Any file name can be used as long as the operating system allows it.

Other valid options can be found by executing r1rho2glove with no arguments.

*mplot*

The program mplot merges graphical plots of relaxation dispersion profiles created separately for each residue by GLOVE after the fit with the reduced size of the plots. mplot is typically used to merge all plots into a single PDF file by executing:

mplot –PDF

Other than the PDF format, mplot can produce PS, EPS, MIF, SVG, PNM, JPEG, PNG, and CGM files. Other valid options can be found by executing ‘mplot –h’.

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1 ################# Fitting Model #################

2 FUNCTION CPMG\_RICHARDS

3 ################# Fitting Method #################

4 METHOD RANDOM 5

5 METHOD MCMIN 5 0.1

6 METHOD MCMIN 5 0.01

7 METHOD MCMIN 5 0.001

8 METHOD ONEEX

9 ################# Error Estimation #################

10 JACK

11 MONTE 100

12 ################# Optional Calculation #################

13 CALC REX REX\_60.83MHz\_298.0K \* 298.0 1.00000 00

14 CALC REX REX\_76.01MHz\_298.0K \* 298.0 1.24953 01

15 CALC PB PB\_298.0K \* 298.0 1.00000 00

16 ################# Plot Instruction #################

17 PLOT 60.83MHz\_298.0K \* 298.0 1.00000 00 50.0 3000.0 119

18 PLOT 76.01MHz\_298.0K \* 298.0 1.24953 01 50.0 3000.0 119

19 ################# XMGR Option #################

20 @ WORLD XMIN 0

21 @ WORLD XMAX 3000

22 @ XAXIS TICK MAJOR 500

23 @ XAXIS TICK MINOR 250

24 @ WORLD YMIN 0

25 @ WORLD YMAX 45

26 @ YAXIS TICK MAJOR 10

27 @ YAXIS TICK MINOR 5

28 ################# Global Parameter #################

29 GPAR KEX 5 4000 3

30 GPAR PAPB 0.005 0.09 3

**Supplementary Figure 1 A header section of a GLOVE input file.**

A line starting with # is a comment. Some comment lines inserted by the program cpmg2glove are omitted for clarity. The line numbers shown in light blue were added to guide the line(s) explained in the text, thus they are not necessary in an actual input file.

1 SET 5-HN

2 PAR DW 100 2500 3

3 PAR KEX\_0 KEX

4 PAR PAPB\_0 PAPB

5 PAR R20\_0\_0 7.58

6 PAR R20\_0\_1 8.74

7 DATA 100.00 298.0 1.00000 00 20.42862 0.35563

8 DATA 200.00 298.0 1.00000 00 18.20412 0.32535

9 DATA 300.00 298.0 1.00000 00 14.74989 0.28337

10 DATA 300.00 298.0 1.00000 00 15.04105 0.28669

11 DATA 400.00 298.0 1.00000 00 12.73597 0.26144

12 DATA 500.00 298.0 1.00000 00 11.21044 0.24596

13 DATA 600.00 298.0 1.00000 00 10.00580 0.23439

14 DATA 700.00 298.0 1.00000 00 9.58696 0.23049

15 DATA 800.00 298.0 1.00000 00 9.10373 0.22608

16 DATA 1000.00 298.0 1.00000 00 8.79323 0.22329

17 DATA 1000.00 298.0 1.00000 00 8.59268 0.22151

18 DATA 1200.00 298.0 1.00000 00 8.42577 0.22003

19 DATA 1400.00 298.0 1.00000 00 8.31555 0.21907

20 DATA 1600.00 298.0 1.00000 00 8.07264 0.21695

21 DATA 1800.00 298.0 1.00000 00 7.93837 0.21579

22 DATA 2000.00 298.0 1.00000 00 8.17706 0.21786

23 DATA 2000.00 298.0 1.00000 00 8.33593 0.21924

24 DATA 2500.00 298.0 1.00000 00 8.02470 0.21653

25 DATA 3000.00 298.0 1.00000 00 7.57790 0.21270

26 DATA 100.00 298.0 1.24953 01 23.56888 0.56658

27 DATA 200.00 298.0 1.24953 01 21.51731 0.52194

28 DATA 300.00 298.0 1.24953 01 18.56210 0.46375

29 DATA 300.00 298.0 1.24953 01 18.06511 0.45462

30 DATA 400.00 298.0 1.24953 01 15.64430 0.41267

31 DATA 500.00 298.0 1.24953 01 13.69360 0.38169

32 DATA 600.00 298.0 1.24953 01 13.30667 0.37583

33 DATA 700.00 298.0 1.24953 01 12.38000 0.36215

34 DATA 800.00 298.0 1.24953 01 10.96410 0.34221

35 DATA 1000.00 298.0 1.24953 01 10.28702 0.33307

36 DATA 1000.00 298.0 1.24953 01 10.84764 0.34062

37 DATA 1200.00 298.0 1.24953 01 10.41255 0.33474

38 DATA 1400.00 298.0 1.24953 01 10.52699 0.33628

39 DATA 1600.00 298.0 1.24953 01 9.24555 0.31948

40 DATA 1800.00 298.0 1.24953 01 9.22325 0.31919

41 DATA 2000.00 298.0 1.24953 01 9.23549 0.31935

42 DATA 2000.00 298.0 1.24953 01 9.69786 0.32531

43 DATA 2500.00 298.0 1.24953 01 9.92347 0.32826

44 DATA 3000.00 298.0 1.24953 01 8.73760 0.31305

45 SET 6-HN

46 PAR DW 100 2500 3

47 PAR KEX\_0 KEX\_0

48 PAR PAPB\_0 PAPB\_0

49 PAR R20\_0\_0 14.09

50 PAR R20\_0\_1 14.89

51 DATA 100.00 298.0 1.00000 00 25.43456 0.60688

52 DATA 200.00 298.0 1.00000 00 22.26203 0.53455

53 DATA 300.00 298.0 1.00000 00 19.55237 0.47965

54 DATA 300.00 298.0 1.00000 00 18.62029 0.46209

55 DATA 400.00 298.0 1.00000 00 18.14712 0.45343

56 DATA 500.00 298.0 1.00000 00 16.68735 0.42771

57 DATA 600.00 298.0 1.00000 00 16.35414 0.42205

58 DATA 700.00 298.0 1.00000 00 16.11809 0.41808

59 DATA 800.00 298.0 1.00000 00 15.36257 0.40564

60 DATA 1000.00 298.0 1.00000 00 14.85221 0.39744

61 DATA 1000.00 298.0 1.00000 00 15.23160 0.40352

62 DATA 1200.00 298.0 1.00000 00 14.81200 0.39680

63 DATA 1400.00 298.0 1.00000 00 15.17059 0.40253

64 DATA 1600.00 298.0 1.00000 00 14.46560 0.39134

65 DATA 1800.00 298.0 1.00000 00 14.67624 0.39465

66 DATA 2000.00 298.0 1.00000 00 14.53529 0.39243

67 DATA 2000.00 298.0 1.00000 00 14.96568 0.39925

68 DATA 2500.00 298.0 1.00000 00 14.62914 0.39391

69 DATA 3000.00 298.0 1.00000 00 14.09184 0.38553

70 DATA 100.00 298.0 1.24953 01 28.37915 0.70986

71 DATA 200.00 298.0 1.24953 01 26.50886 0.65869

72 DATA 300.00 298.0 1.24953 01 22.80643 0.56802

73 DATA 300.00 298.0 1.24953 01 21.81220 0.54588

74 DATA 400.00 298.0 1.24953 01 19.99902 0.50769

75 DATA 500.00 298.0 1.24953 01 18.72142 0.48239

76 DATA 600.00 298.0 1.24953 01 18.33500 0.47499

77 DATA 700.00 298.0 1.24953 01 18.01723 0.46900

78 DATA 800.00 298.0 1.24953 01 16.23357 0.43670

79 DATA 1000.00 298.0 1.24953 01 16.48358 0.44109

80 DATA 1000.00 298.0 1.24953 01 16.18115 0.43579

81 DATA 1200.00 298.0 1.24953 01 16.42672 0.44009

82 DATA 1400.00 298.0 1.24953 01 17.08885 0.45190

83 DATA 1600.00 298.0 1.24953 01 15.48813 0.42387

84 DATA 1800.00 298.0 1.24953 01 14.89289 0.41390

85 DATA 2000.00 298.0 1.24953 01 15.58354 0.42549

86 DATA 2000.00 298.0 1.24953 01 16.24244 0.43686

87 DATA 2500.00 298.0 1.24953 01 16.86562 0.44788

88 DATA 3000.00 298.0 1.24953 01 15.59247 0.42564

**Supplementary Figure 2 A data set section of a GLOVE input file.**

The line numbers shown in light blue were added to guide the line(s) explained in the text, thus they are not necessary in an actual input file.

1 # GLOVE VERSION 2.3.5

2 MODEL CPMG\_Richards

3 EQN f(dw, kex, papb, R20)

4 SEED 288019632111244372

5 METHOD INIT

6 METHOD RANDOM 5

7 METHOD MCMIN 5 0.10

8 METHOD MCMIN 5 0.01

9 METHOD MCMIN 5 0.00

10 METHOD ONEEX

11

12 # Fitting results

13 # Total data points = 2090

14 # Total degrees of freedom (DoF) = 1923

15 # INIT X2/DoF = 88.7611

16 # RANDOM X2/DoF = 3.91054

17 # MCMIN X2/DoF = 1.45054

18 # MCMIN X2/DoF = 1.45054

19 # MCMIN X2/DoF = 1.45047

20 # ONEEX X2/DoF = 1.45047

21 # Fit duration 0h0m30s

22

23 SET 5-HN

24 DW 784.628 (0.15154)

25 KEX\_0 600.292 (4.89726)

26 PAPB\_0 0.034356 (0.000180275)

27 R20\_0\_0 7.75981 (22.0383)

28 R20\_0\_1 8.96904 (12.0833)

29 REX\_60.83MHz\_298.0K 12.8993

30 REX\_76.01MHz\_298.0K 15.1195

31 PB\_298.0K 0.0356251

32 X2/DoF 1.3024 (DoF = 33)

33

34 SET 6-HN

35 DW 642.567 (0.102615)

36 KEX\_0 600.292 (4.89726)

37 PAPB\_0 0.034356 (0.000180274)

38 R20\_0\_0 14.4686 (13.898)

39 R20\_0\_1 15.4686 (9.28874)

40 REX\_60.83MHz\_298.0K 10.7068

41 REX\_76.01MHz\_298.0K 13.1432

42 PB\_298.0K 0.0356251

43 X2/DoF 1.39968 (DoF = 33)

**Supplementary Figure 3 An example of a GLOVE output file.**

The line numbers shown in light blue were added to guide the line(s) explained in the text, thus they are not shown in an actual output file.

# pkfit input file

FIELD 76.010013

BOXSIZE 1

PEAKLIST test.xpk

TOLERANCE 10 10

SPECTRUM 750r2\_1.ft2 0 0

SPECTRUM 750r2\_2.ft2 0 0

SPECTRUM 750r2\_3.ft2 0.04 100

SPECTRUM 750r2\_4.ft2 0.04 200

SPECTRUM 750r2\_5.ft2 0.04 300

SPECTRUM 750r2\_6.ft2 0.04 300

SPECTRUM 750r2\_7.ft2 0.04 400

SPECTRUM 750r2\_8.ft2 0.04 500

SPECTRUM 750r2\_9.ft2 0.04 600

SPECTRUM 750r2\_10.ft2 0.04 700

SPECTRUM 750r2\_11.ft2 0.04 800

SPECTRUM 750r2\_12.ft2 0.04 1000

SPECTRUM 750r2\_13.ft2 0.04 1000

SPECTRUM 750r2\_14.ft2 0.04 1200

SPECTRUM 750r2\_15.ft2 0.04 1400

SPECTRUM 750r2\_16.ft2 0.04 1600

SPECTRUM 750r2\_17.ft2 0.04 1800

SPECTRUM 750r2\_18.ft2 0.04 2000

SPECTRUM 750r2\_19.ft2 0.04 2000

**Supplementary Figure 4 An example of a pkfit input file.**

A line starting with # is a comment. FIELD is the magnetic field at which relaxation dispersion were collected. If relaxation dispersions were collected at multiple magnetic fields, the pkfit input file must be prepared for each of them. BOXSIZE determines the box size from which peak intensities are obtained. BOXSIZE of 1 corresponds to a 3 × 3 box, 2 for 5 × 5, and 3 for 7 × 7. PEAKLIST specifies the NMRView xpk file. TOLERANCE specifies the ranges in which differences in the peak top position of each signal among a series of spectra are permitted. The ranges are specified for the horizontal and vertical dimensions of the 2D NMR spectra in units of Hz. SPECTRUM specifies a relaxation dispersion spectrum with *T*CPMG and 1/τCP values.

FIELD(MHz): 76.010013

SPECTRUM 1: 750r2\_1.ft2

PARAMETERS: 0 0

# Num Label Pos1 Pos2 Int

0 36.HN 10.1077 120.187 2.82754e+06

1 29.HN 9.93679 115.562 1.90427e+06

2 23.HN 9.63594 119.687 -1.92504e+06

3 19.HN 9.465 124.687 2.09456e+06

4 42.HN 9.30774 115.562 1.91047e+06

5 27.HN 9.23253 116.812 2.37208e+06

6 21.HN 9.19151 120.187 2.01295e+06

7 26.HN 9.14364 122.937 2.193e+06

8 28.HN 9.12313 126.687 2.35233e+06

9 46.HN 8.94536 122.937 2.82009e+06

10 44.HN 8.87015 117.312 3.06093e+06

: : : : :

56 1.noise 10.0872 117.562 -32073

57 2.noise 10.0188 124.062 -27747.7

58 3.noise 8.64451 118.937 -20059.1

59 4.noise 8.624 115.687 -51750.8

60 5.noise 6.81891 116.937 -30862.3

61 6.noise 7.05822 122.937 39349.8

62 7.noise 9.75218 127.187 34877.6

63 8.noise 8.72656 125.562 29051

64 9.noise 7.54368 125.937 -24599.5

65 10.noise 6.91463 128.312 -51846.8

**Supplementary Figure 5 An example of a pkfit output file.**

A line starting with # is a comment. FIELD is the magnetic field at which relaxation dispersion are collected. SPECTRUM shows the spectrum number and its name. PARAMETERS report *T*CPMG and 1/τCP values specified by the line staring SPECTRUM in the pkfit input file (Supplementary Fig. 4). The rest of the part reports the peak numbers, peak names, chemical shifts of the horizontal and vertical dimensions, and intensities for each spectrum.

