Supporting information

Fast and accurate fitting of relaxation dispersion data using the flexible software package GLOVE

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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 4.7.2 and Intel C++ compiler version 13.1.1 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 χ^2 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 χ^2 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.

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} ($= R_2^{\mathrm{eff}}(1/\tau_{\mathrm{CP}} = 50) - R_2^{\mathrm{eff}}(1/\tau_{\mathrm{CP}} = 100000)$), and the population of the minor state in a two-state exchange model, p_{B} ($= \left(1-\sqrt{1-4p_{\mathrm{A}}p_{\mathrm{B}}}\right)/2$), 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 $1/\tau_{\mathrm{CP}}$, temperature, magnetic field, index numbers for the temperature and the magnetic field. In the cases of `CALC` and `PLOT`, $1/\tau_{\mathrm{CP}}$ is not necessary for their calculations, thus `*` is used instead of a specific $1/\tau_{\mathrm{CP}}$ value. For temperature, it was shown that chemical shift difference $\Delta\omega$ does not change at different temperatures (Palmer AG et al. 2001). Therefore, relaxation dispersion data collected at multiple temperatures can be fitted globally with $\Delta\omega$ 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 ^{15}N magnetic fields of 60.830911 and 76.010013 MHz, respectively. GLOVE calculates chemical shift differences $\Delta\omega$ 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, experimental conditions, index numbers, the minimum and maximum $1/\tau_{CP}$ 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. The experimental conditions and index numbers are the same as those used for `CALC`.

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. 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 (line 1 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 (line 2-6 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 $\exp(nx)$, $\exp(-nx)$, $\exp(n^2x^2)$, $\exp(-n^2x^2)$, 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_2^0 is little dependent upon other parameters, and is not very sensitive to fitting models. In practice, the slowest R_2^{eff} 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
```

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 fitting methods (lines 4-9), the total data points (line 12), the total degree of freedom (line 13), the global reduced χ^2 value at each step of the fitting method (line 14-19), and the total time spent for the fit (line 20). Note that the fitting method `INIT` is always inserted in the beginning of the fitting methods by GLOVE. This method calculates the global reduced χ^2 value using the lower limits of the parameters or user-defined initial values.

The result section reports the data set name (line 22), fitted parameter values with standard deviations in parentheses (lines 23-27), optionally calculated parameter values (lines 28-30), and the local reduced χ^2 value with the degree of freedom (line 31) for each data set.

Fitting models

The Luz and Meiboom equation

For the condition of fast exchange ($k_{\mathrm{ex}}/2 >> \Delta\omega$), the Luz and Meiboom equation (Luz Z and Meiboom S 1963), called CPMG_`MEIBOOM` in GLOVE, can be used:

$$R_2^{\mathrm{eff}} = R_2^0 + \frac{p_{\mathrm{A}} p_{\mathrm{B}} \Delta\omega^2}{k_{\mathrm{ex}}} \left[1 - \frac{2}{k_{\mathrm{ex}} \tau_{\mathrm{CP}}} \tanh\left(\frac{k_{\mathrm{ex}} \tau_{\mathrm{CP}}}{2} \right) \right] \quad \text{(S1)},$$

where $p_{\mathrm{A}}p_{\mathrm{B}}\Delta\omega^2$ is a single fitting parameter, often represented as Φ_{ex}. In GLOVE, the parameters k_{ex}, $p_{\mathrm{A}}p_{\mathrm{B}}\Delta\omega^2$, and $R_2^0$are 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 CPMG_`TORCHIA` in GLOVE, can be used:

$$R_2^{\mathrm{eff}} = R_2^0 + \frac{p_{\mathrm{A}} p_{\mathrm{B}} \Delta\omega^2 k_{\mathrm{ex}}}{k_{\mathrm{ex}}^2 + \sqrt{p_{\mathrm{A}}^2 \Delta\omega^4 + 144/\tau_{\mathrm{CP}}^4}} \quad \text{(S2)}.$$

In GLOVE, the parameters $\Delta\omega$, k_{ex}, $p_{\mathrm{A}}p_{\mathrm{B}}$, and R_2^0 are defined as `DW`, `KEX`, `PAPB`, and `R20`, respectively.

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 (Grey MJ et al. 2003). Since it is difficult to know the exchange regime of the system of interest *a priori*, an R_2^{eff} rate in a three state exchange is usually calculated numerically by matrix operations (Korzhnev DM et al. 2004):

$$\begin{aligned} R_2^{\mathrm{eff}}(1/\tau_{\mathrm{CP}}) &= -\frac{1}{T_{\mathrm{CPMG}}} \ln\left(\frac{I(1/\tau_{\mathrm{CP}})}{I_0} \right) \\ \mathbf{I}(1/\tau_{\mathrm{CP}}) &= \left[\exp\left(\mathbf{A}\frac{\tau_{\mathrm{CP}}}{2} \right) \exp\left(\mathbf{A}^* \frac{\tau_{\mathrm{CP}}}{2} \right) \exp\left(\mathbf{A}^* \frac{\tau_{\mathrm{CP}}}{2} \right) \exp\left(\mathbf{A}\frac{\tau_{\mathrm{CP}}}{2} \right) \right]^n \mathbf{I}(0) \end{aligned} \quad \text{(S3)}.$$

The calculated R_2^{eff} rate using this matrix manipulation has no approximation for all exchange regimes, but the calculation is time consuming. To calculate R_2^{eff} faster in GLOVE, an R_2^{eff} rate is calculated using the equation shown below. This equation called CPMG_`MATRIX3` in GLOVE was derived according to the same procedure to derive the Carver and Richards equation (Carver and Richards 1972):

$$R_2^{\mathrm{eff}} = R_2^0 - \frac{1}{\tau_{\mathrm{CP}}} \ln\left(\lambda_1\right) \tag{S4}$$

Under the experimentally accessible condition, R_2^{eff} is dominated by the largest eigenvalue λ_1 of the matrix.

$$\begin{pmatrix} R\left[\exp\left(-\mathbf{A}\frac{\tau_{\mathrm{cp}}}{2}\right)\exp\left(-\mathbf{A}^*\frac{\tau_{\mathrm{cp}}}{2}\right)\right] & I\left[\exp\left(-\mathbf{A}\frac{\tau_{\mathrm{cp}}}{2}\right)\exp\left(-\mathbf{A}^*\frac{\tau_{\mathrm{cp}}}{2}\right)\right] \\ I\left[\exp\left(-\mathbf{A}\frac{\tau_{\mathrm{cp}}}{2}\right)\exp\left(-\mathbf{A}^*\frac{\tau_{\mathrm{cp}}}{2}\right)\right] & -R\left[\exp\left(-\mathbf{A}\frac{\tau_{\mathrm{cp}}}{2}\right)\exp\left(-\mathbf{A}^*\frac{\tau_{\mathrm{cp}}}{2}\right)\right] \end{pmatrix} \tag{S5}$$

where $R[\ ]$ and $I[\ ]$ are functions to extract the real or imaginary elements, respectively, of the complex matrix. As the matrix $\mathbf{A}$ is a 3-by-3 evolution matrix as given below ($\mathbf{A}^*$ is its complex conjugate), the matrix size shown as (S5) 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, $\mathbf{A}$ is represented as:

$$\mathbf{A} = \begin{pmatrix} k_{\mathrm{AB}} + k_{\mathrm{AC}} & -k_{\mathrm{BA}} & -k_{\mathrm{CA}} \\ -k_{\mathrm{AB}} & k_{\mathrm{BA}} + k_{\mathrm{BC}} - i\Delta\omega_{\mathrm{AB}} & -k_{\mathrm{CB}} \\ -k_{\mathrm{AC}} & -k_{\mathrm{BC}} & k_{\mathrm{CB}} + k_{\mathrm{CA}} - i\Delta\omega_{\mathrm{AC}} \end{pmatrix} \tag{S6}$$

Note that the equation to derive eigenvalues becomes a sixth degree equation, but is a cubic equation of λ^2, therefore, it can be solved using Cardano's formula. In GLOVE, the parameters $\Delta\omega_{\mathrm{AB}}$, $\Delta\omega_{\mathrm{AC}}$, k_{AB}, k_{BA}, k_{BC}, k_{CA}, k_{AC}, and R_2^0 are defined as `DWAB`, `DWAC`, `KAB`, `KBA`, `KBC`, `KCA`, `KAC`, and `R20`, respectively. k_{CB} is calculated according to the microscopic reversibility: $k_{\mathrm{CB}} = (k_{\mathrm{AB}} \times k_{\mathrm{BC}} \times k_{\mathrm{CA}})/(k_{\mathrm{BA}} \times k_{\mathrm{AC}})$.

Two-state binding models

Since the binding rate is dependent upon concentrations of two interacting molecules ($\mathrm{A} + \mathrm{B} \underset{k_{\mathrm{off}}}{\overset{[\mathrm{B}]k_{\mathrm{on}}}{\rightleftarrows}} \mathrm{AB}$), a global analysis of relaxation dispersions measured with multiple concentration ratio samples provides binding kinetics k_{on} and k_{off}. The model called CPMG_`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. CPMG_`BIND2C` utilizes the Richards and Carver equation with $k_{\mathrm{ex}} = [\mathrm{B}]k_{\mathrm{on}} + k_{\mathrm{off}}$. The concentration of free B, [B], is calculated according to:

$$[\mathrm{B}] = \frac{1}{2}\left\{-K_{\mathrm{D}} - [\mathrm{A}]_0 + a[\mathrm{B}]_0 + \sqrt{\left(K_{\mathrm{D}} + [\mathrm{A}]_0 - a[\mathrm{B}]_0\right)^2 + 4a[\mathrm{B}]_0 K_{\mathrm{D}}}\right\} \quad \text{(S7)},$$

where a is the concentration ratio of B between samples, for example, 0.95, 1, 1.05, 1.1. $[\mathrm{A}]_0$ and $[\mathrm{B}]_0$ represent the total concentrations of A and B, respectively, and the dissociation constant K_{D} is determined as $K_{\mathrm{D}} = k_{\mathrm{off}}/k_{\mathrm{on}}$. In GLOVE, $\Delta\omega$, k_{on}, k_{off}, R_2^0, $[\mathrm{A}]_0$ and $[\mathrm{B}]_0$ are defined as `DW`, `KON`, `KOFF`, `R20`, `A0`, and `B0`, respectively. $[\mathrm{B}]_0$ 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 CPMG_`BIND2`, in which the fitting parameters are $\Delta\omega$, Bk_{on}, k_{off}, and R_2^0, defined as `DW`, `BKON`, `KOFF`, and `R20`, respectively, in GLOVE. Bk_{on} is a single fitting parameter in units of s^{-1}, substituted for $[\mathrm{B}]k_{\mathrm{on}}$. This fitting parameter is determined for each concentration ratio sample. The $\Delta\omega$, k_{off}, and R_2^0 values derived using this model can be good initial values in CPMG_`BIND2C`.

Three-state binding models

GLOVE has two distinct three-state binding models (CPMG_`BIND3C_1F2B` and CPMG_`BIND3C_2F1B`) utilizing the same matrix manipulation as CPMG_`MATRIX3` described above. CPMG_`BIND3C_1F2B` is comprised of one free and two bound states whereas CPMG_`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. CPMG_`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 CPMG_`MATRIX3` become as:

$$\mathbf{A} = \begin{pmatrix} [\mathrm{B}](k_{\mathrm{on1}} + k_{\mathrm{on2}}) & -k_{\mathrm{off1}} & -k_{\mathrm{off2}} \\ -[\mathrm{B}]k_{\mathrm{on1}} & k_{\mathrm{off1}} + k_{12} - i\Delta\omega_{\mathrm{FB1}} & -k_{21} \\ -[\mathrm{B}]k_{\mathrm{on2}} & -k_{12} & k_{\mathrm{off2}} + k_{21} - i\Delta\omega_{\mathrm{FB2}} \end{pmatrix} \quad \text{(S8)},$$

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. $\Delta\omega_{\mathrm{FB1}}$ and $\Delta\omega_{\mathrm{FB2}}$ 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 S7, but in this case, the dissociation constant K_{D} is calculated according to:

$$K_{\mathrm{D}} = \frac{k_{\mathrm{off1}}k_{\mathrm{off2}} + k_{\mathrm{off1}}k_{21} + k_{\mathrm{off2}}k_{12}}{k_{\mathrm{on1}}\left(k_{\mathrm{off2}} + k_{12} + k_{21}\right) + k_{\mathrm{on2}}\left(k_{\mathrm{off1}} + k_{12} + k_{21}\right)} \quad \text{(S9).}$$

In GLOVE, the parameters $\Delta\omega_{\mathrm{FB1}}$, $\Delta\omega_{\mathrm{FB2}}$, k_{on1}, k_{off1}, k_{on2}, k_{off2}, k_{12}, k_{21}, R_2^0, $[\mathrm{A}]_0$ and $[\mathrm{B}]_0$ are defined as `DWFB1`, `DWFB2`, `KON1`, `KOFF1`, `KON2`, `KOFF2`, `K12`, `K21`, `R20`, `A0`, and `B0`, respectively. $[\mathrm{B}]_0$ 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:

$$\mathrm{A} + \mathrm{B} \underset{k_{\mathrm{off1}}}{\overset{[\mathrm{B}]k_{\mathrm{on1}}}{\rightleftarrows}} \mathrm{AB}^* \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} \mathrm{AB} \quad \text{(S10),}$$

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, CPMG_`BIND3C_1F2B` becomes the model in which a ligand binds to a receptor with two different bound conformations:

$$\mathrm{A}^*\mathrm{B} \underset{[\mathrm{B}]k_{\mathrm{on2}}}{\overset{k_{\mathrm{off2}}}{\rightleftarrows}} \mathrm{A} + \mathrm{B} \underset{k_{\mathrm{off1}}}{\overset{[\mathrm{B}]k_{\mathrm{on1}}}{\rightleftarrows}} \mathrm{AB} \quad \text{(S11),}$$

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

On the other hand, the model CPMG_`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 CPMG_`MATRIX3` become as:

$$\mathbf{A} = \begin{pmatrix} k_{12} + [\mathrm{B}]k_{\mathrm{on1}} & -k_{21} & -k_{\mathrm{off1}} \\ -k_{12} & k_{21} + [\mathrm{B}]k_{\mathrm{on2}} - i\Delta\omega_{\mathrm{F1F2}} & -k_{\mathrm{off2}} \\ -[\mathrm{B}]k_{\mathrm{on1}} & -[\mathrm{B}]k_{\mathrm{on2}} & k_{\mathrm{off1}} + k_{\mathrm{off2}} - i\Delta\omega_{\mathrm{F1B}} \end{pmatrix} \quad \text{(S12),}$$

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. $\Delta\omega_{\mathrm{F1F2}}$ and $\Delta\omega_{\mathrm{F1B}}$ 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:

$$
\begin{aligned}
&k_{\mathrm{on1}}k_{\mathrm{on2}}[\mathrm{B}]^3+\left[\left([\mathrm{A}]_0-[\mathrm{B}]_0\right)k_{\mathrm{on1}}k_{\mathrm{on2}}+k_{\mathrm{on1}}\left(k_{\mathrm{off2}}+k_{21}\right)+k_{\mathrm{on2}}\left(k_{\mathrm{off1}}+k_{12}\right)\right][\mathrm{B}]^2\\
&\quad+\left[\left([\mathrm{A}]_0-[\mathrm{B}]_0\right)\left(k_{\mathrm{on1}}k_{21}+k_{\mathrm{on2}}k_{12}\right)-[\mathrm{B}]_0\left(k_{\mathrm{on1}}k_{\mathrm{off2}}+k_{\mathrm{on2}}k_{\mathrm{off1}}\right)+\left(k_{\mathrm{off1}}+k_{\mathrm{off2}}\right)\left(k_{12}+k_{21}\right)\right][\mathrm{B}]\\
&\quad-[\mathrm{B}]_0(k_{\mathrm{off1}}+k_{\mathrm{off2}})(k_{12}+k_{21})=0
\end{aligned}
\tag{S13}
$$

In GLOVE, the parameters $\Delta\omega_{\mathrm{F1F2}}$, $\Delta\omega_{\mathrm{F1B}}$, k_{on1}, k_{off1}, k_{on2}, k_{off2}, k_{12}, k_{21}, $R_2^{\;0}$, $[\mathrm{A}]_0$ and $[\mathrm{B}]_0$ are defined as `DWF1F2`, `DWF1B`, `KON1`, `KOFF1`, `KON2`, `KOFF2`, `K12`, `K21`, `R20`, `A0`, and `B0`, respectively. $[\mathrm{B}]_0$ 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:

$$\mathrm{A}^*+\mathrm{B}\xrightleftharpoons[k_{12}]{k_{21}}\mathrm{A}+\mathrm{B}\xrightleftharpoons[k_{\mathrm{off2}}]{[\mathrm{B}]k_{\mathrm{on1}}}\mathrm{AB}$$

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.

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 $\Delta\omega$ 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, `CPMG_BIND2C`, `CPMG_BIND3C_1F2B`, and `CPMG_BIND3C_2F1B`, the option `-ic` is used instead of `-i` to specify the concentration ratio, corresponding to a in equation S7. Other valid options can be found by executing `cpmg2glove` 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'`.

References

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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
```

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 METHOD INIT
 5 METHOD RANDOM 5
 6 METHOD MCMIN 5 0.10
 7 METHOD MCMIN 5 0.01
 8 METHOD MCMIN 5 0.00
 9 METHOD ONEEX
10
11 # Fitting results
12 # Total data points = 2090
13 # Total degrees of freedom (DoF) = 1923
14 # INIT         X2/DoF = 88.7611
15 # RANDOM       X2/DoF = 3.91054
16 # MCMIN        X2/DoF = 1.45054
17 # MCMIN        X2/DoF = 1.45054
18 # MCMIN        X2/DoF = 1.45047
19 # ONEEX        X2/DoF = 1.45047
20 # Fit duration        0h0m30s
21
22 SET    5-HN
23        DW        784.628 (0.15154)
24        KEX_0     600.292 (4.89726)
25        PAPB_0    0.034356 (0.000180275)
26        R20_0_0   7.75981 (22.0383)
27        R20_0_1   8.96904 (12.0833)
28            REX_60.83MHz_298.0K    12.8993
29            REX_76.01MHz_298.0K    15.1195
30            PB_298.0K              0.0356251
31        X2/DoF    1.3024 (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 $1/\tau_{\mathrm{CP}}$ 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 $1/\tau_{\mathrm{CP}}$ 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.

Table S1. Validation of the fitting protocol using synthetic data including 5% random noise

fitting parameter	input	output[c]	input	output[c]	input	output[c]	input	output[c]	input	output[c]
k_{ex}[a]	500	503	1000	1002	1500	1513	2000	2015	2500	2497
p_{B}[a]	0.02000	0.01987	0.02000	0.02002	0.02000	0.01995	0.02000	0.01993	0.02000	0.02005
$\Delta\omega$[b]	500	492	500	502	500	491	500	501	500	497
	1000	1003	1000	993	1000	1002	1000	1005	1000	1000
	1500	1501	1500	1491	1500	1507	1500	1505	1500	1496
	2000	2016	2000	1990	2000	1992	2000	2005	2000	1992
	2500	2510	2500	2510	2500	2498	2500	2508	2500	2489

[a] Global parameter.

[b] Local parameter.

[c] The best-fit parameter values using the combined method RANDOM+MCMIN+ONEEX.