Supporting Information: Deriving Quantitative Dynamics Information for Proteins and RNAs using ROTDIF with a Graphical User Interface

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A Spectral Density Functions $J(\omega)$

Here we present expressions for the spectral density function $J(\omega)$ in the Lipari-Szabo (model-free) formalism, corresponding to various models for the overall rotational diffusion tensor. The most general is the *fully anisotropic* model, where all three eigenvalues of the rotational diffusion tensor are assumed to be different. In the case when two eigenvalues of the diffusion tensor are assumed to be equal, the fully anisotropic model can be simplified to an *axially-symmetric* model. Finally, in the simplest case, when all three eigenvalues are assumed to be equal, a simple *isotropic* model is used. The axially-symmetric and isotropic models can be directly derived from the fully anisotropic model.

A.1 Fully Anisotropic Diffusion Tensor Spectral Density Function

The rotational diffusion tensor is a symmetric 3 × 3 matrix, and can be decomposed using eigendecomposition, such that

$$\mathbf{D} = \mathbf{V} \begin{bmatrix} D_x & 0 & 0 \\ 0 & D_y & 0 \\ 0 & 0 & D_z \end{bmatrix} \mathbf{V}^T, \tag{S1}$$

where $D_x \leq D_y \leq D_z$ are the eigenvalues of the diffusion tensor, and $\mathbf{V}$ is an orthonormal matrix of the eigenvectors of $\mathbf{D}$.

We start with the most general case of a fully anisotropic diffusion tensor, with all three eigenvalues, D_x, D_y, and D_z, being different. Assuming that the local motion of a PQ bond can be described using the extended model-free formalism[1, 2], the spectral density function can be written as

$$\begin{aligned} J(\omega, \mathbf{v}, \mathbf{D}, \tau_{slow}, \tau_{fast}) = \frac{2}{5} \sum_{k=1}^{5} \Bigg[& S^2 \frac{d_k(\mathbf{D}) a_k(\mathbf{v}, \mathbf{D})}{d_k^2(\mathbf{D}) + \omega^2} + \\ & (S_{fast}^2 - S^2) \frac{e_k(\mathbf{D}, \tau_{slow}) a_k(\mathbf{v}, \mathbf{D})}{e_k^2(\mathbf{D}, \tau_{slow}) + \omega^2} + \\ & (1 - S_{fast}^2) \frac{\hat{e}_k(\mathbf{D}, \tau_{fast}) a_k(\mathbf{v}, \mathbf{D})}{\hat{e}_k^2(\mathbf{D}, \tau_{fast}) + \omega^2} \Bigg], \end{aligned} \tag{S2}$$

where S_{fast}^2 and τ_{fast} are the squared order parameter and the corresponding correlation time for the fast motion, S_{slow}^2 and τ_{slow} are for slow motion (naturally, $\tau_{fast} \ll \tau_{slow}$), and the generalized squared order parameter is defined as $S^2 = S^2_{slow}S^2_{fast}$. When only one local motion is present, usually characterized by the squared order parameter S^2 and the (local) correlation time τ_{loc}, the model-free[1] form of the spectral density function can be obtained from Eq. S2, by setting $S^2_{slow} = 1$, hence $S^2 = S^2_{fast}$, and renaming τ_{fast} as τ_{loc}, or, alternatively, by setting $S^2_{fast} = 1$, hence $S^2 = S^2_{slow}$, and renaming τ_{slow} as τ_{loc}.

In Eq. S2 the components independent of the PQ bond are

$$\begin{aligned}
&d_1(\mathbf{D}) = 4D_x + D_y + D_z,\\
&d_2(\mathbf{D}) = D_x + 4D_y + D_z,\\
&d_3(\mathbf{D}) = D_x + D_y + 4D_z,\\
&d_4(\mathbf{D}) = 6k_3 + 2k_4,\\
&d_5(\mathbf{D}) = 6k_3 - 2k_4,\\
&e_i(\mathbf{D}, \tau_{loc}) = \tau_{slow}/(d_i\tau_{slow} + 1),\\
&\hat{e}_i(\mathbf{D}, \tau_{fast}) = \tau_{fast}/(d_i\tau_{fast} + 1)\\
&k_1 = D_y - D_x,\\
&k_2 = D_z - D_x,\\
&k_3 = (D_x + D_y + D_z)/3,\\
&k_4 = \sqrt{k_1^2 - k_1k_2 + k_2^2},
\end{aligned} \tag{S3}$$

the components that depend on the PQ bonds are

$$\begin{aligned}
&a_1(\mathbf{v}, \mathbf{D}) = 3\bar{v}_2^2\bar{v}_3^2,\\
&a_2(\mathbf{v}, \mathbf{D}) = 3\bar{v}_1^2\bar{v}_3^2,\\
&a_3(\mathbf{v}, \mathbf{D}) = 3\bar{v}_1^2\bar{v}_2^2,\\
&a_4(\mathbf{v}, \mathbf{D}) = p_1 - p_2,\\
&a_5(\mathbf{v}, \mathbf{D}) = p_1 + p_2,\\
&p_1 = \frac{1}{4}[3(\bar{v}_1^4 + \bar{v}_2^4 + \bar{v}_3^4) - 1],\\
&p_2 = \frac{1}{12}[\delta_1(3\bar{v}_1^4 + 2a_1 - 1) + \delta_2(3\bar{v}_2^4 + 2a_2 - 1) + \delta_3(3\bar{v}_3^4 + 2a_3 - 1)],\\
&\bar{\mathbf{v}} = \mathbf{V}^T\mathbf{v},
\end{aligned} \tag{S4}$$

and the shared components are

$$\begin{aligned}
&\delta_1 = (-k_1 - k_2)/k_4,\\
&\delta_2 = (2k_1 - k_2)/k_4,\\
&\delta_3 = (2k_2 - k_1)/k_4,
\end{aligned} \tag{S5}$$

Note that we reformulated how d_4 and d_5 were calculated in Ghose et al. [3] to increase numerical stability.

In the case of a "rigid" bond $S^2_{slow}S^2_{fast} = 1$, and Eq. S2 reduces to

$$J(\omega, \mathbf{v}, \mathbf{D}) = \frac{2}{5}\sum_{k=1}^{5}\frac{d_k(\mathbf{D})a_k(\mathbf{v}, \mathbf{D})}{d_k^2(\mathbf{D}) + \omega^2}. \tag{S6}$$

A.2 Axially-Symmetric Diffusion Tensor Model

If two eigenvalues of $\mathbf{D}_{exp}$ are equal, then an axially-symmetric diffusion tensor model can be used for the computation of $\mathbf{D}_{exp}$. We label the two equal eigenvalues as $D_\perp$, and the unique eigenvalue as $D_\|$. The expression for $J(\omega, \mathbf{v}, \mathbf{D})$ can be simplified greatly from the case of fully anisotropic diffusion model [3, 4].

Without loss of generality, we simplify the fully anisotropic model for the case when $D_\perp = D_x = D_y$ and $D_\| = D_z$. For simplicity, we assume $S^2_{slow}, S^2_{fast} = 1$, however the analysis can also be extended to general order parameters values. In this case the spectral density function for an axially-symmetric diffusion model can be written as:

$$J(\omega, \mathbf{v}, \mathbf{D}) = \frac{2}{5} \sum_{k=1}^{3} \frac{\hat{d}_k(\mathbf{D}) \hat{a}_k(\mathbf{v}, \mathbf{D})}{\hat{d}_k^2(\mathbf{D}) + \omega^2}, \tag{S7}$$

where the components independent of the PQ bonds are

$$\begin{aligned} \hat{d}_1(\mathbf{D}) &= 5D_\perp + D_\|, \\ \hat{d}_2(\mathbf{D}) &= 2D_\perp + 4D_\|, \\ \hat{d}_3(\mathbf{D}) &= 6D_\perp, \end{aligned} \tag{S8}$$

the components dependent on the PQ bonds are

$$\begin{aligned} \hat{a}_1(\mathbf{v}_i, \mathbf{D}) &= 3\bar{v}_3^2(1 - \bar{v}_3^2), \\ \hat{a}_2(\mathbf{v}_i, \mathbf{D}) &= \frac{3}{4}(1 - \bar{v}_3^2)^2, \\ \hat{a}_3(\mathbf{v}_i, \mathbf{D}) &= \frac{1}{4}(3\bar{v}_3^2 - 1)^2, \\ \bar{\mathbf{v}} &= \mathbf{V}^T \mathbf{v}. \end{aligned} \tag{S9}$$

A.3 Isotropic Diffusion Tensor Model

If all three eigenvalues of the diffusion tensor are equal, a simple isotropic diffusion tensor model can be used. We label the eigenvalue as D_c. Note that now $\hat{d}_1 = \hat{d}_2 = \hat{d}_3 = 6D_c$. From Eq. S7 we have:

$$J(\omega, \mathbf{v}, \mathbf{D}) = \frac{2}{5} \frac{\tau_c}{1 + (\omega\tau_c)^2}, \tag{S10}$$

where $\tau_c = 1/(6D_c)$.

B Default CSA Values

The default CSA values used in ROTDIF are given in Table S1.

Table S1: Default Chemical Shift Anisotropy (CSA) values.

Bond Type	Molecule	Nucleus	CSA (ppm)
N-H	Protein	^{15}N	160
N_1-H_1	RNA	^{15}N	130
N_3-H_3	RNA	^{15}N	95
C_α-H_α	Protein	^{13}C	30
C'_1-H'_1	RNA	^{13}C	29
C_2-H_2	RNA	^{13}C	150
C'_2-H'_2	RNA	^{13}C	23.1
C'_3-H'_3	RNA	^{13}C	83
C'_4-H'_4	RNA	^{13}C	79.5
C_5-H_5	RNA	^{13}C	170
C'_5-H'_5	RNA	^{13}C	55.5
C''_5-H''_5	RNA	^{13}C	55.5
C_6-H_6	RNA	^{13}C	180
C_8-H_8	RNA	^{13}C	140

C ROTDIF Algorithms

Below we describe the algorithms for solving all three diffusion tensor models. Note that our algorithms use a nonlinear least-squares function "lsqnonlin($\chi^2(\mathbf{x}), \mathbf{x}_0$)", that we define as a generic convex nonlinear least-squares solver.

Algorithm S1 rotdifIso

```
Input: $\rho^{exp}, \sigma_i$ – defined in Eq. 9.
Output: $\mathbf{D}_{exp}$ – the experimental diffusion tensor.
for all bonds do
    $\tau_c^i \leftarrow$ solution from Eq. 11.
end for
$D_{iso} \leftarrow 1/\left[6\langle\tau_{\mathbf{c}}\rangle\right]$, {where $\langle\tau_{\mathbf{c}}\rangle$ is the mean of all the $\tau_c$ values for all bonds.}
$\mathbf{x}_0 \leftarrow \begin{bmatrix} D_{iso} & 0 & 0 \\ 0 & D_{iso} & 0 \\ 0 & 0 & D_{iso} \end{bmatrix}$
$\mathbf{D}_{exp} \leftarrow$ lsqnonlin($\chi^2(\emptyset, \mathbf{x}), \mathbf{x}_0$) {$\emptyset$ represents the fact that the first parameter $\mathbf{v}$ in $\chi^2$ in Eq. 9
    is not used in the isotropic model.}
return $\mathbf{D}_{exp}$
```

Algorithm S2 rotdifAxi

```
Input: $\rho^{exp}, \sigma_i$ – defined in Eq. 9 and $\mathbf{v}$ – array of the normalized $PQ$ vectors, where $\mathbf{v}_i$ is
    associated with $\rho_i^{exp}$.
Output: $\mathbf{D}_{exp}$, the experimental diffusion tensor.
$\mathbf{D}_{iso} \leftarrow$ rotdifIso($\rho^{exp}$)
$\hat{\mathbf{D}} \leftarrow \mathbf{D}_{iso}$
$\mathbf{D}_{exp} \leftarrow \mathbf{D}_{iso}$
for oblate and prolate case do
  {Switch between the prolate and oblate cases. The first eigenvalue changes from being $D_{\|}$
  to $D_{\perp}$.}
  if prolate case then
    $\hat{D}_x = \hat{D}_y = 0.75 D_{iso}, \hat{D}_z = 1.25 D_{iso}$
  else if oblate case then
    $\hat{D}_x = 0.75 D_{iso}, \hat{D}_y = \hat{D}_z = 1.25 D_{iso}$ {oblate case}
  end if
  for $\alpha = 0, \pi/2$ do
    for $\beta = 0, \pi/2$ do
      $\mathbf{x}_0 \leftarrow \mathbf{R}(\alpha, \beta, 0) \hat{\mathbf{D}} \mathbf{R}^T(\alpha, \beta, 0)$
      $\mathbf{x}^* \leftarrow$ lsqnonlin($\chi^2(\mathbf{v}, \mathbf{x}), \mathbf{x}_0$)
      if $\chi^2(\mathbf{v}, \mathbf{x}^*) < \chi^2(\mathbf{v}, \mathbf{D}_{exp})$ then
        $\mathbf{D}_{exp} \leftarrow \mathbf{x}^*$
      end if
    end for
  end for
end for
return $\mathbf{D}_{exp}$
```

Algorithm S3 rotdifAni

```
Input: $\rho^{exp}, \sigma_i$ – defined in Eq. 9 and $\mathbf{v}$ – array of the normalized $PQ$ vectors, where $\mathbf{v}_i$ is associated with $\rho_i^{exp}$.
Output: $\mathbf{D}_{exp}$, the experimental diffusion tensor.
$\mathbf{D}_{iso} \leftarrow$ rotdifIso($\rho_{exp}$)
$\hat{\mathbf{D}} \leftarrow \mathbf{D}_{iso}$
$\mathbf{D}_{exp} \leftarrow \mathbf{D}_{iso}$
$\hat{D}_{11} \leftarrow .75D_{iso}$, $\hat{D}_{22} \leftarrow D_{iso}$, $\hat{D}_{33} \leftarrow 1.25D_{iso}$ {Move away from the isotropic case.}
for $\alpha = 0, \pi/2$ do
  for $\beta = 0, \pi/2$ do
    for $\gamma = 0, \pi/2$ do
      $\mathbf{x}_0 \leftarrow \mathbf{R}(\alpha, \beta, \gamma)\hat{\mathbf{D}}\mathbf{R}^T(\alpha, \beta, \gamma)$
      $\mathbf{x}^* \leftarrow$ lsqnonlin($\chi^2(\mathbf{v}, \mathbf{x}), \mathbf{x}_0$)
      if $\chi^2(\mathbf{v}, \mathbf{x}^*) < \chi^2(\mathbf{v}, \mathbf{D}_{exp})$ then
        $\mathbf{D}_{exp} \leftarrow \mathbf{x}^*$
      end if
    end for
  end for
end for
return $\mathbf{D}_{exp}$
```

References

[1] Lipari, G.; Szabo, A. *Journal of the American Chemical Society* **1982**, *104*, 4546–4559.

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[3] Ghose, R.; Fushman, D.; Cowburn, D. *Journal of Magnetic Resonance* **2001**, *149*, 204–217.

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