Calculation of transverse paramagnetic relaxation enhancement rates

Transverse intermolecular paramagnetic relaxation enhancement (PRE) rates are calculated from the ensemble of simulation structures by averaging over the distances between the amide hydrogen atoms of EIN, IIA$^{\text{Mtl}}$, or IIA$^{\text{Man}}$ and the paramagnetic Mn^{2+} ion linked to HPr via a (cysteaminyl-EDTA)-Cys adduct. To account for the flexibility of the Mn^{2+} labels, for a given structure j a single Gaussian distribution of Mn^{2+} is assumed around the center $\mathbf{r}_j$ of a three-conformer ensemble representation for the EDTA-Mn^{2+} groups (1, 2):

$$P_{\text{Mn},j}(\mathbf{r}) = \frac{1}{(2\pi\sigma^2)^{3/2}} e^{-|\mathbf{r}-\mathbf{r}_j|^2/2\sigma^2}, \qquad [1]$$

where σ is the width of the Gaussian distribution. Note that the three-conformer ensemble representation for the EDTA-Mn^{2+} groups was derived from the intramolecular PREs that measure the distance between the residues of HPr and the Mn^{2+} labels (1, 2). The PRE for a residue i, $\Gamma_2^{\text{calc}}(i)$, is then calculated as follows;

$$\Gamma_2^{\text{calc}}(i) = \frac{1}{N_{\text{bound}}} \sum_{j=1}^{N_{\text{bound}}} C \left\langle \frac{1}{r^6} \right\rangle_{ij}, \qquad [2]$$

where N_{bound} is the total number of bound structures, r is the distance between the amide nitrogen of residue i and Mn^{2+}, and $\langle \cdots \rangle_{ij}$ denotes the average over the Gaussian distribution of Mn^{2+} from a structure j. The constant C is given by (2)

$$\begin{aligned} C &= \frac{1}{15} \left(\frac{\mu_0}{4\pi}\right)^2 \gamma_I^2 g^2 \mu_B^2 s(s+1)\tau_c \left\{ 4 + \frac{3}{1+(w_I\tau_c)^2} \right\}, \\ &\simeq 5.72 \times 10^9 \ \text{Å}^6/\text{s}, \end{aligned} \qquad [3]$$

where s is the spin quantum number for an unpaired electron of Mn^{2+}, g the electron g-factor, γ_I the proton gyromagnetic ratio, μ_0 the permeability of a vacuum, μ_B the magnetic moment of the free electron, $w_I/2\pi$ the Larmor frequency of the proton, and τ_c the correlation time.

To estimate the $\langle r^{-6} \rangle$ average of the distance r between the amide proton and the paramagnetic label, we use the Mn^{2+} distribution of Eq. 1. For a structure j and residue i, the average is then given by

$$\begin{aligned} \left\langle \frac{1}{r^6} \right\rangle_{ij} &= \int \frac{1}{|\mathbf{r}_i - \mathbf{r}|^6} P_{\text{Mn},j}(\mathbf{r}) d\mathbf{r} \\ &= \frac{1}{(2\pi\sigma^2)^{3/2}} \int \frac{1}{|\mathbf{r}_i - \mathbf{r}|^6} e^{-|\mathbf{r}-\mathbf{r}_j|^2/2\sigma^2} d\mathbf{r}, \end{aligned} \qquad [4]$$

ignoring the small difference between the alpha-carbon position $\mathbf{r}_i$ of residue i and the amide-hydrogen position. After expanding the integrand in powers of $r_{ij} \equiv |\mathbf{r}_i - \mathbf{r}_j|$ and integrating over the angle, we obtain

$$\begin{aligned} \left\langle \frac{1}{r^6} \right\rangle_{ij} &= \frac{1}{r_{ij}^6} \left[1 + \frac{4\pi}{(2\pi\sigma^2)^{3/2}} \left\{ \frac{1}{r_{ij}^2} \int_0^\infty r^4 e^{-r^2/2\sigma^2} dr \right. \right. \\ &\left. \left. + \frac{1}{r_{ij}^4} \int_0^\infty r^6 e^{-r^2/2\sigma^2} dr \right\} + O\left(\frac{1}{r_{ij}^6}\right) \right]. \end{aligned} \qquad [5]$$

After radial integration one obtains

$$\left\langle \frac{1}{r^6} \right\rangle_{ij} = \frac{1}{r_{ij}^6} \left[1 + \frac{15\sigma^2}{r_{ij}^2} + \frac{210\sigma^4}{r_{ij}^4} + O\left(\frac{1}{r_{ij}^6}\right) \right]. \qquad [6]$$

The width of the Gaussian distribution, σ, is taken as

$$\sigma = \begin{cases} 0 & \text{if } r_{ij} < 8\text{Å}, \\ \frac{5}{7}(r_{ij} - 8) & \text{if } 8\text{Å} \le r_{ij} < 15\text{Å}, \\ 5 & \text{if } r_{ij} \ge 15\text{Å}. \end{cases} \qquad [7]$$

Simulation of the IIA$^{\text{Mtl}}$-HPr complex

The simulation results for the IIA$^{\text{Mtl}}$-HPr complex are presented in Supporting Information (SI) Figs. S1 and S3. The simulated equilibrium binding affinity ($K_D \simeq 15\mu\text{M}$) is close to the experimental value ($\sim 30\mu\text{M}$) (3). However, the DRMS values show that the majority ($\sim 80\%$) of the simulated structures exhibit large DRMS deviations (~ 10 Å). In these structures, HPr is bound to IIA$^{\text{Mtl}}$ at the same binding interface as in the stereospecific complex but with a different relative orientation. The large population in this cluster, compared to the clusters of native-like structures with DRMS <5 Å, may result from the neglect of detailed atomistic interactions in the coarse-grained potential energy function. In the dominant cluster, the superimposed Mn^{2+} coordinates are very close to the residues of IIA$^{\text{Mtl}}$ so that the resulting PRE rates exhibit large amplitudes deviating significantly from the experimental values. However, removing these structures by using a larger cutoff distance of 15 Å between the center of the Mn^{2+} ions and IIA$^{\text{Mtl}}$ residues yields qualitatively good agreement between the simulated and experimental PRE rates (see SI Fig. S3A). Further, structure-refinement via Monte Carlo energy minimization with the remaining structures ($\sim 20\%$) and reweighting the resulting clusters yields the same quality of agreement between the reweighted PREs and experimental values as for the other two complexes (EIN-HPr and IIA$^{\text{Man}}$-HPr, main text). The clusters with a combined population of less than 10% account for the non-specific contributions in the PRE profile (see SI Fig. S3B). The structures of the two non-specific complexes are shown in SI Fig. S3C in blue. Note that in the encounter complexes probed by the E5C-EDTA-Mn^{2+} label, HPr is bound at the same interface of IIA$^{\text{Mtl}}$ as in the stereospecific complex (green) with a different orientation.

References

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