

# Supporting Information

A simple method for measuring signs of  $^1\text{H}^{\text{N}}$  chemical shift differences between ground and excited protein states

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## Bloch-McConnell Equations

For a two-site exchange process interconverting states  $G$  and  $E$ ,  $G \xrightleftharpoons[k_E]{k_G} E$ , the evolution of spin coherence  $X$  that is not coupled to any other spin can be written as follows

$$\frac{d}{dt} \begin{pmatrix} X_G(t) \\ X_E(t) \end{pmatrix} = \begin{pmatrix} -k_G - R_G + i\omega_G & k_E \\ k_G & -k_E - R_E + i\omega_E \end{pmatrix} \begin{pmatrix} X_G(t) \\ X_E(t) \end{pmatrix} \quad (\text{S1})$$

where  $X_i$  is the ‘amount’ of coherence  $X$  in the exchanging state  $i$ ,  $\omega_i$  is the Larmor frequency ( $\text{rad}\cdot\text{s}^{-1}$ ) of  $X_i$  and  $R_i$  its relaxation rate (McConnell 1958). The evolution frequencies of  $G$  and  $E$ , including contributions from exchange, can be obtained from the imaginary parts of the two eigenvalues of the matrix on the right-hand-side of eq S1. It is thus possible to calculate the shift of the peak corresponding to state  $G$  in spectra recorded at a different static magnetic fields,  $\tilde{\delta}_X$ , so long as the parameters of the exchange process are known.

## Sample Preparation

[U-<sup>2</sup>H, <sup>15</sup>N] enriched Pfl6 I58D was obtained by IPTG-induced protein over-expression in *E. coli* BL21(DE3) in M9 minimal medium with 99.9% D<sub>2</sub>O and [<sup>12</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub>]-glucose, following a previously described protocol (Roessler et al. 2008). The NMR sample was 1.4 mM in protein, 2 mM EDTA, 2 mM NaN<sub>3</sub>, 50 mM sodium phosphate, pH 6.2, 90% H<sub>2</sub>O, 10% D<sub>2</sub>O. A sample of 1.2 mM [U-<sup>2</sup>H, <sup>13</sup>C, <sup>15</sup>N] A39V/N53P/V55L Fyn SH3, 0.2 mM EDTA, 0.05% NaN<sub>3</sub>, 50 mM sodium phosphate, pH 7.0, 90% H<sub>2</sub>O, 10% D<sub>2</sub>O was prepared as described in detail previously (Neudecker et al. 2006). A sample of [U-<sup>2</sup>H, <sup>15</sup>N] enriched WT FF domain was generated as previously described (Korzhnev et al. 2007). The sample contained 1.0 mM [U-<sup>2</sup>H, <sup>15</sup>N] protein, 50 mM sodium acetate, 100 mM NaCl, 0.05% (w/v) NaN<sub>3</sub>, 0.2 mM EDTA (pH 5.7), 90% H<sub>2</sub>O, 10% D<sub>2</sub>O.

## NMR Spectroscopy and Data Analysis

Single quantum  $^1\text{H}^{\text{N}}$  relaxation dispersion profiles (Ishima and Torchia 2003) were recorded at 5 °C for Pfl6 I58D, at 30 °C for the WT FF domain and at 20 °C for the A39V/N53P/V55L Fyn SH3 domain. Data were obtained at static magnetic fields strengths of 11.7 and 18.8 T. Relaxation dispersion profiles,  $R_{2,\text{eff}}(\nu_{\text{CPMG}})$ , were generated from peak intensities,  $I_1(\nu_{\text{CPMG}})$ , in a series of 2D  $^1\text{H}^{\text{N}}$ - $^{15}\text{N}$  correlation maps measured as a function of CPMG frequency,  $\nu_{\text{CPMG}} = 1/(4\tau_{\text{CP}})$ , where  $2\tau_{\text{CP}}$  is the interval between consecutive 180°-refocusing pulses of the CPMG sequence. Effective relaxation rates were calculated as  $R_{2,\text{eff}}(\nu_{\text{CPMG}}) = \ln(I_0/I_1(\nu_{\text{CPMG}}))/T_{\text{CPMG}}$ , where  $I_0$  is the peak intensity in a reference spectrum recorded without the relaxation delay  $T_{\text{CPMG}}$  (Tollinger et al. 2001).

All data sets were processed and analyzed with the NMRPipe program (Delaglio et al. 1995) and signal intensities were quantified using the program FuDA (<http://pound.med.utoronto.ca/software.html>) or MUNIN (Korzhnev et al. 2001; Orekhov et al. 2001). Relaxation dispersion data were analyzed using a two-state exchange model and the best-fit model parameters were extracted as described previously (Hansen et al. 2008) using the program CATIA, which is available from <http://pound.med.utoronto.ca/software.html>. Briefly, the parameters are obtained by minimization of the target function,

$$\chi^2(\xi) = \sum \left( \frac{R_{2,\text{eff}}^{\text{calc}}(\xi) - R_{2,\text{eff}}^{\text{exp}}}{\Delta R_{2,\text{eff}}^{\text{exp}}} \right)^2 \quad (\text{S2})$$

where  $R_{2,\text{eff}}^{\text{exp}}$  and  $\Delta R_{2,\text{eff}}^{\text{exp}}$  are experimental effective relaxation rates and their uncertainties, respectively,  $R_{2,\text{eff}}^{\text{calc}}(\xi)$  are calculated relaxation rates obtained by numerical integration of

the Bloch-McConnell equation (McConnell 1958),  $\xi = \{x_1, \dots, x_n\}$  denotes the set of adjustable model parameters (including  $\Delta\tilde{\omega}$ ) and the summation in eq S2 is over the number of experimental data points.

$[^1\text{H}, ^{15}\text{N}]$ -HSQC spectra were recorded with the following parameters: (1) Pfl6 I58D  $(t_1, t_2) = (103.0, 64.0)$  ms at 11.4 T and  $(t_1, t_2) = (89.5, 64.0)$  ms at 18.8 T, (2) WT FF domain  $(t_1, t_2) = (117.8, 64.0)$  ms at 11.4 T and  $(t_1, t_2) = (99.5, 64.0)$  ms at 18.8 T, (3) A39V/N53P/V55L Fyn SH3 domain  $(t_1, t_2) = (145.2, 64.0)$  ms at 11.4 T and  $(t_1, t_2) = (121.0, 64.0)$  at 18.8 T.  $[^1\text{H}, ^{15}\text{N}]$ -HMQC spectra were recorded on the Pfl6 I58D sample with  $(t_1, t_2)$  values identical to those used for the HSQC spectra. After extensive zero-filling, peak positions were determined by two-dimensional parabolic interpolation (Press et al. 2007). Overlapped peaks for which no maximum could be defined were discarded from the analysis.

## Bootstrap Analysis

Errors on fitted  $|\Delta\omega_{\text{H}}|$  and calculated  $|\Delta\tilde{\delta}_{\text{H}}^{\text{calc}}|$  values were determined by a standard bootstrap analysis (Press et al. 2007):

1) 1000 ‘re-sampled’ data sets of the same size as the original  $^1\text{H}^{\text{N}}$  relaxation dispersion data set were generated by randomly picking  $R_{2,\text{eff}}(v_{\text{CPMG}})$  points from the original data set – the same point could be picked once, several times or not at all.

2) The dispersion profiles  $i \in \{1, \dots, 1000\}$  generated in step (1) were independently fitted to extract  $|\Delta\omega_{\text{H},i}|$  for each residue, assuming previously fitted values for  $p_{\text{E}}$  and  $k_{\text{ex}}$ .

Simultaneously, exchange-induced shifts  $|\Delta\tilde{\delta}_{\text{H},i}^{\text{calc}}|$  were calculated for each profile

$i \in \{1, \dots, 1000\}$  by numerically solving the Bloch-McConnell equations, S1, using the exchange parameters  $(p_E, k_{ex}, |\Delta\omega_{H,i}|)$ .

4) Errors on fitted  $|\Delta\omega_H|$  and calculated  $|\Delta\tilde{\delta}_H^{calc}|$  values were estimated as the standard deviation of  $\{|\Delta\omega_{H,i}|\}_{i \in \{1, \dots, 1000\}}$  and  $\{|\Delta\tilde{\delta}_H^{calc}|\}_{i \in \{1, \dots, 1000\}}$ , respectively, for each residue.

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