Supporting Information

$^{13}CHD_2$ methyl group probes of ms timescale exchange in proteins by ^{1}H relaxation dispersion: An application to proteasome gating residue dynamics

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Sample Preparation. A U-[^{2}H], Ile-[$^{13}CHD_2$ δ1], Leu,Val-[$^{13}CHD_2$, $^{13}CHD_2$] labeled Abp1p SH3 domain sample was prepared by protein over-expression in *E. coli*. (BL21(DE3)). The growth was carried out in D_2O and M9 media with ^{12}C,^{2}H glucose and ^{15}N ammonium chloride as carbon and nitrogen sources, respectively, and with the precursors α-ketobutyrate [$^{13}CHD_2CD_2COCO_2Na$] and α-isovalerate [$(^{13}CHD_2)_2CDCOCO_2Na$] (Sigma-Aldrich) added one hour prior to induction of protein over-expression to obtain the appropriately labeled methyl groups[1,2]. Details of protein expression and purification have been presented elsewhere[3]. The final protein concentration was ≈1.5 mM, in a buffer consisting of 50 mM sodium phosphate, 100 mM NaCl, 1mM EDTA, 1mM NaN_3, 100% D_2O, pH=7 (uncorrected). ^{2}H Ark1p peptide[4], which binds the SH3 domain, was expressed and purified as described previously[3]. Approximately 2 mole percent peptide was added to the Abp1p SH3 domain sample. Chemical shift differences between peptide free and bound SH3 domains were obtained by analysis of spectra recorded on apo and holo forms of the SH3 domain.

A sample of U-[^{2}H], Met-[$^{13}CHD_2$]-α_7 was expressed and purified as described previously[5-7]. Methyl groups of Met were labeled with $^{13}CHD_2$ by supplementing the M9 D_2O-

based growth media with 100 mg/L of $^{13}CHD_2$ (otherwise ^1H) labeled Met (Sigma-Aldrich) one hour prior to protein induction. A sample comprising 2.5 mM protein (in monomer of α; 0.36 mM in α_7 particle) was prepared in 100% D_2O, 25 mM potassium phosphate, pH 6.8, 50 mM NaCl, 1 mM EDTA, 0.03% NaN_3 and used for all dispersion experiments.

NMR Spectroscopy and Data Analysis.

(1) Magnetization transfer:

Figure 1 illustrates the pulse scheme for recording methyl ^1H relaxation dispersion profiles using $^{13}CHD_2$ spin-probes. Briefly, the flow of magnetization can be summarized as follows

$$I_z \rightarrow 2I_zC_z \rightarrow 2I_yC_z(CPMG,T_{relax}) \rightarrow 2I_zC_y\cos(\omega_C t_1) - 2I_zC_x\sin(\omega_C t_1) \rightarrow I_{tr}(t_2) \qquad [1]$$

where the effects of relaxation, pulse imperfections and coherence transfer gradients have been neglected. In the above scheme A_i is the $i \in \{x,y,z\}$ component of A magnetization, I=^1H, C=^{13}C and I_{tr} denotes transverse ^1H magnetization. With respect to ^1H,^{13}C the $^{13}CHD_2$ probes are effectively AX spin systems; an enhanced sensitivity scheme has therefore been used whereby both cosine and sine chemical shift modulated t_1 components are transferred to ^1H magnetization for detection[8]. The back-transfer from ^{13}C to ^1H is achieved using a planar-TOCSY mixing scheme of duration $1/J_{CH}$ that has been described in some detail previously[9,10] and where the effective Hamiltonian (including the ^1H and ^{13}C 90° pulses flanking the DIPSI-2x mixing scheme) is given by $H = \frac{\pi J_{CH}}{2}(2I_XC_X + 2I_YC_Y)$.

(2) Abp1p SH3 - Ark1p peptide exchanging system:

A set of 18 constant-time CPMG relaxation dispersion data-sets were recorded with the scheme of Figure 1, T_{relax} = 40 ms, at 25ºC and 5ºC (17 CPMG frequencies between 25 and 1000 Hz for each dispersion curve along with 1 reference, N=0). Data-sets were obtained at a pair of static magnetic field strengths corresponding to ^{1}H resonance frequencies of 600 and 800 MHz. Acquisition times of 0.66(0.86) hours/2D spectrum at 600(800) MHz were used for net measuring times of 12 (15.5) hours for a complete dispersion data-set.

^{13}C,^{1}H dispersion spectra were processed and analyzed with the program NMRPipe[11] and signal intensities were quantified by using the program FuDA (http://pound.med.utoronto.ca/software). Relaxation dispersion data were interpreted within the context of a two-state exchange model $A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B$ and fitted using in-house software (http://pound.med.utoronto.ca/software) following protocols described in detail previously[3,12,13]. In cases where fits of dispersion profiles produced reduced χ^2 values > 2, or where cross-peaks were highly overlapped, the data were excluded from further analysis. Dispersion profiles for all residues were analyzed together to extract global exchange parameters as well as residue specific chemical shift differences between ground and excited states and intrinsic relaxation rates. Values of $k_{ex} = k_{AB} + k_{BA}$ = 206±13 s^{-1} (300±40 s^{-1}) and p_B (minor state population) = 2.7±0.1% (1.3±0.1%) were obtained from independent global fits of data recorded at 25ºC (5ºC). It is noteworthy that for the small k_{ex} values characterizing the SH3-peptide exchanging system considered here, k_{ex} and p_B are correlated. Indeed, essentially identical fits of the dispersion data recorded at 5ºC were obtained by fixing p_B to 2.7%. In this case k_{ex}=108±5 s^{-1}, r^2 = 0.95 and rmsd=12.6 ppb, see Figure 2b.

(3) α_7 exchanging system:

Sets of 41 (26) values of ν_{CPMG} ranging from 25 – 2000 Hz (25 – 1000 Hz) were recorded at 600 MHz (800 MHz), T_{relax} = 40 ms, 50ºC. Each 2D spectrum, corresponding to a single ν_{CPMG} value, was recorded in 0.5 hours (0.65 hours) for a total measuring time of 20.3 hours (17 hours) for the complete dispersion series. Data was processed and analyzed as described above for the abp1p SH3 domain – Ark1p peptide complex with dispersion profiles for M-1(A,B,C), M1(A,B,C) and M6 included in a global analysis. Values of k_{ex} = 1030±30 s^{-1} and p_B = 6.0±0.4% were obtained, with values of $|\Delta\varpi|$ listed in Figure 4 of the text.

An Experimental Approach for Estimating $R_{2,I} - R_{2,A}$

The ^{13}C longitudinal relaxation rate, $R_1(^{13}C)$, in a $^{13}CHD_2$ methyl spin system is given by[14,15]

$$\begin{aligned} R_1\left(^{13}C\right) &= 0.1 d_{CH}^2\left[3J\left(\omega_C\right) + J\left(\omega_H - \omega_C\right) + 6J\left(\omega_H + \omega_C\right)\right] \\ &+0.2 d_{CD}^2\left[3J\left(\omega_C\right) + J\left(\omega_D - \omega_C\right) + 6J\left(\omega_D + \omega_C\right)\right] + c^2 J'\left(\omega_C\right) \end{aligned} \quad [2]$$

where $d_{CH}^2 = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_H^2 \gamma_C^2 \hbar^2}{r_{CH}^6}$, $d_{CD}^2 = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{8\gamma_D^2 \gamma_C^2 \hbar^2}{3 r_{CD}^6}$, $c^2 = \frac{2}{15}\left(\omega_C \Delta\sigma_C\right)^2$, with $\Delta\sigma_C$ the methyl ^{13}C CSA. *J(ω)* and *J'(ω)* are spectral densities defined as[16]

$$\begin{aligned} J(\omega) &= a^2 S_{axis}^2 \frac{\tau_C}{1+\left(\omega\tau_C\right)^2} + \left(1 - a^2 S_{axis}^2\right)\frac{\tau'}{1+\left(\omega\tau'\right)^2} \\ J'(\omega) &= S_{axis}^2 \frac{\tau_C}{1+\left(\omega\tau_C\right)^2} + \left(1 - S_{axis}^2\right)\frac{\tau'}{1+\left(\omega\tau'\right)^2} \end{aligned} \quad [3]$$

where τ_C is the molecular correlation time (isotropic tumbling is assumed), S^2_{axis} is the generalized order parameter of the methyl 3-fold symmetry axis (located along the $C^{\gamma 1}$-$C^{\delta 1}$ bond

in Ile, C^{γ}-$C^{\delta 1}$ or C^{γ}-$C^{\delta 2}$ bonds in Leu, along C^{β}-$C^{\gamma 1}$ or C^{β}-$C^{\gamma 2}$ bonds in Val and along the S-C^{ε} bond in Met), $a=(3\cos^2(\theta)-1)/2$, θ is the angle between the C-H / C-D bond and the methyl 3-fold symmetry axis (a^2=1/9 and $r_{CH}=r_{CD}$=1.135Å) and $\frac{1}{\tau'}=\frac{1}{\tau_e}+\frac{1}{\tau_C}$ where τ_e is the effective correlation time of the ps-ns motions. It is straightforward to show that the relaxation contribution from the intra-methyl ^{1}H-^{13}C dipolar interaction dominates $R_1(^{13}C)$. For example, assuming τ_C = 5 ns, S^2_{axis}=0.5 and τ_e = 15 ps the ^{1}H-^{13}C dipolar interaction is over 5 (45) fold larger than the total ^{2}H-^{13}C (^{13}C CSA) interaction and the ^{1}H-^{13}C dipolar interaction becomes more dominant with increasing τ_C. Thus,

$$R_1\left(^{13}C\right)\approx 0.1d_{CH}^2\left[3J\left(\omega_C\right)+J\left(\omega_H-\omega_C\right)+6J\left(\omega_H+\omega_C\right)\right] \quad [4]$$

and assuming $(\omega_C\tau_C)^2>>1$, $(\omega_C\tau')^2<<1$ it follows that

$$R_1\left(^{13}C\right)\approx 0.1d_{CH}^2\left[A\left(\frac{3}{\omega_C^2}+\frac{1}{(\omega_H-\omega_C)^2}+\frac{6}{(\omega_H+\omega_C)^2}\right)+10B\right]\approx 0.1d_{CH}^2\left(\frac{3.35A}{\omega_C^2}+10B\right) \quad [5]$$

where $A=\frac{S^2_{axis}}{9\tau_C}$ and $B=\left(1-\frac{S^2_{axis}}{9}\right)\tau'$. Thus by recording $R_1(^{13}C)$ rates at a pair of static magnetic fields it is possible to extract A and B. Finally, it can be shown that[17]

$$R_{2,I}-R_{2,A}=0.3d_{CH}^2J(\omega_C) \quad [6]$$

which is readily simplified under the same limiting conditions as above

$$R_{2,I}-R_{2,A}=0.1d_{CH}^2\left(\frac{3A}{\omega_C^2}+3B\right) \quad [7]$$

Thus once residue specific values of A and B are obtained they can be substituted into Eq [7] so that values of $R_{2,I} - R_{2,A}$ can be calculated. Values of $R_1(^{13}C)$ have been measured at magnetic fields of 600 and 800 MHz using a previously developed pulse scheme[15]. Average values of $R_{2,I} - R_{2,A}$ are ~0.3 s^{-1} for the Ile, Leu, Val $^{13}CHD_2$ methyls of the abp1p SH3 domain (very weak dependence on temperature between 5 and 25ºC) and ~ 0.06 s^{-1} for the Met $^{13}CHD_2$ probes of α_7, 50ºC.

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