Tailoring Relaxation Dispersion Experiments for Fast-Associating Protein Complexes

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Supporting Information

A. Sample preparation

Uniformly ^{15}N-labeled C-terminal activation domain (residues 776-826) of human HIF-1α was expressed as a GB1 fusion protein in BL21-DE3 cells in M9 minimal medium; complete and specific Asn803 hydroxylation was accomplished in *E. coli* by coexpression with human asparaginyl hydroxylase, the factor inhibiting HIF-1 (FIH). Following thrombin cleavage of the GB1 fusion protein, hydroxylated HIF (HIF-OH) was purified to homogeneity by reverse-phase HPLC. Unlabeled TAZ1 domain (residues 345-439) of mouse CBP was prepared as described.[1] The proteins were dissolved separately in NMR buffer [90% H_2O/10% D_2O, 20 mM MES (pH 6.12), 2 mM dithiothreitol (DTT), 2 mM NaN_3] and concentrated. NMR samples of the [^{15}N]-HIF-OH:TAZ1 complex for the R_2 dispersion experiments, in which HIF-OH concentration was kept at 510 μM while the effective TAZ1 concentration was 26.9, 21.5, 16.1, or 10.8 μM, were prepared from a single concentrated solution of each protein to make the concentration ratios accurate. The concentration of HIF-OH was determined from the absorbance at 280 nm, using an extinction coefficient of 1.4 $mM^{-1}\cdot cm^{-1}$. The effective concentrations of TAZ1 were determined from fitting the dispersion data; TAZ1 refolding is technically difficult, and the effective concentrations of correctly folded protein are lower than determined from UV absorbance measurements.

B. NMR measurements

^{1}H-^{15}N HSQC,[2] ^{15}N TOCSY-HSQC and ^{15}N NOESY-HSQC,[3] HNCA, HN(CO)CA, and HNCO,[4] (HCA)CO(CA)NH,[5] HNCACB[6] spectra were acquired at 25 °C on a Bruker DRX600 spectrometer for

chemical shift assignments.

^{15}N R_2 relaxation rates were measured for the four [^{15}N]-HIF-OH:TAZ1 samples on Bruker DRX600 and Avance900 spectrometers at 25 °C using relaxation-compensated constant-time Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences.[7,8] R_2 dispersion spectra were acquired as two-dimensional data sets with a constant relaxation delay of 40, 60 or 80 ms. Some data points (three to seven), including a reference spectrum acquired with the CPMG blocks omitted, were collected in duplicate and were used to estimate the absolute uncertainties and the signal-to-noise ratio of each spectrum.

A ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) titration was performed for 510 μM [^{15}N]-HIF-OH with unlabeled TAZ1 on a Bruker DRX600 spectrometer at 25 °C. The HIF-OH:TAZ1 concentration ratio ranged from 1:0 to 1:1.2 (Fig. S1). Exchange between the free and fully bound states is slow on the chemical shift time scale. However, very small shifts are observed for a subset of HIF-OH cross peaks upon addition of substoichiometric amounts of TAZ1; this exchange process is fast and does not contribute to R_2 relaxation.

Fitting of R_2 dispersion profiles

^{15}N R_2 dispersion profiles of HIF-OH for all four samples at the two spectrometer frequencies (Fig. S3) were fit simultaneously for each residue using the program GLOVE as described previously.[9,10] The association and dissociation rate constants, k_{on} and k_{off}, were treated as global parameters for all residues in the C-terminal helix of HIF-OH. The data fitted well to a two-site exchange model. Fits to a three-site exchange model yielded physically unreasonable parameters and could not reproduce the macroscopic K_D measured by ITC.

An analytical equation derived by Carver and Richards was used:[11]

$$R_2^{\text{eff}} = R_2^0 + \frac{1}{2}\left\{[\text{TAZ1}]k_{\text{on}} + k_{\text{off}} - \frac{1}{\tau_{\text{cp}}}\cosh^{-1}\left[D_+\cosh(\eta_+) - D_-\cos(\eta_-)\right]\right\}$$

$$D_\pm = \frac{1}{2}\left[\pm 1 + \frac{\Psi + 2\Delta\omega_{\text{FB}}^2}{\sqrt{\Psi^2 + \xi^2}}\right]$$

$$\eta_\pm = \tau_{\text{CP}}\sqrt{\frac{1}{2}\left(\pm\Psi + \sqrt{\Psi^2 + \xi^2}\right)}$$

$$\Psi = \left([\text{TAZ1}]k_{\text{on}} + k_{\text{off}}\right)^2 - \Delta\omega_{\text{FB}}^2$$

$$\xi = 2\Delta\omega_{\text{FB}}\left([\text{TAZ1}]k_{\text{on}} - k_{\text{off}}\right),$$

where fitting parameters are described in the main text. The free TAZ1 concentration, [TAZ1], can be calculated from the total concentrations $[\text{TAZ1}]_0$ and $[\text{HIF-OH}]_0$ and K_{D} ($=k_{\text{off}}/k_{\text{on}}$):

$$[\text{TAZ1}] = \frac{1}{2}\left\{-K_{\text{D}} + [\text{TAZ1}]_0 - [\text{HIF-OH}]_0 + \sqrt{\left(K_{\text{D}} - [\text{TAZ1}]_0 + [\text{HIF-OH}]_0\right)^2 + 4[\text{TAZ1}]_0 K_{\text{D}}}\right\}$$

Simulation of R_2 dispersion profiles

R_2 dispersion profiles can be simulated using the same equations as used in the fitting. By varying $[\text{TAZ1}]_0$, the TAZ1 concentration dependence of R_2^{eff} can be obtained, as shown in Figure 2a. For this simulation, $1/\tau_{\text{CP}}$ was fixed to 100 s^{-1}, which corresponds to the first data point when R_2 rates are measured with a constant relaxation delay of 40 ms. On the other hand, by varying $1/\tau_{\text{CP}}$, typical R_2 dispersion profiles as shown in Figure 2b can be simulated.

Simulations were also performed for the pKID/KIX system, using the kinetic parameters derived previously from R_2 dispersion experiments performed with KIX:pKID concentration ratios in the range 0.95-1.10.[10] The simulations confirm that concentration ratios near 1:1 represent the optimal stoichiometry for the pKID/KIX system (Fig. S5). Because the apparent association rate (average $k_{\text{on}}^* = 6.3 \times 10^6$ $M^{-1}\cdot s^{-1}$) is much slower than for binding of HIF-OH to TAZ1 ($k_{\text{on}} = 1.3 \times 10^9$ $M^{-1}\cdot s^{-1}$), exchange is too slow to contribute significantly to R_2 relaxation under conditions of a large excess of pKID. In practical terms, KIX:pKID concentration ratios > 0.33 would be required to give R_{ex} >3, conditions under which the signal intensity of the free pKID resonances has been greatly reduced.

Reference List

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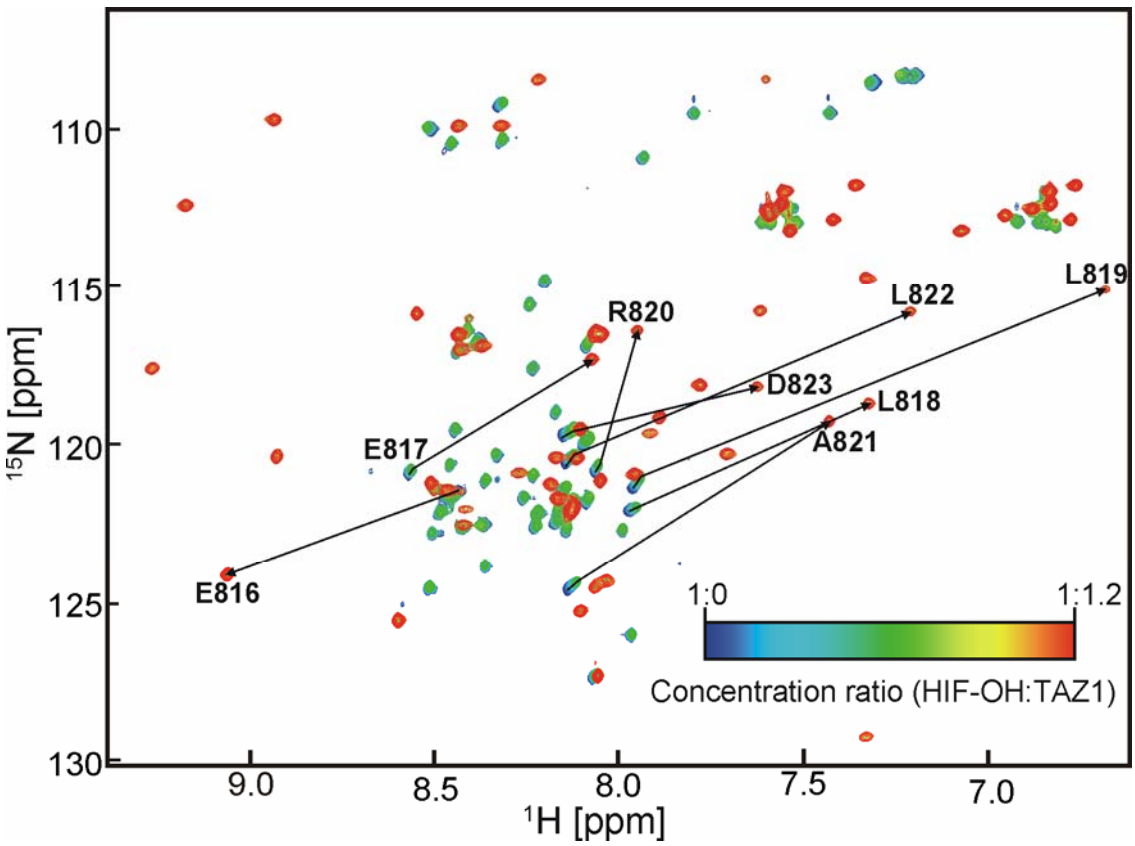
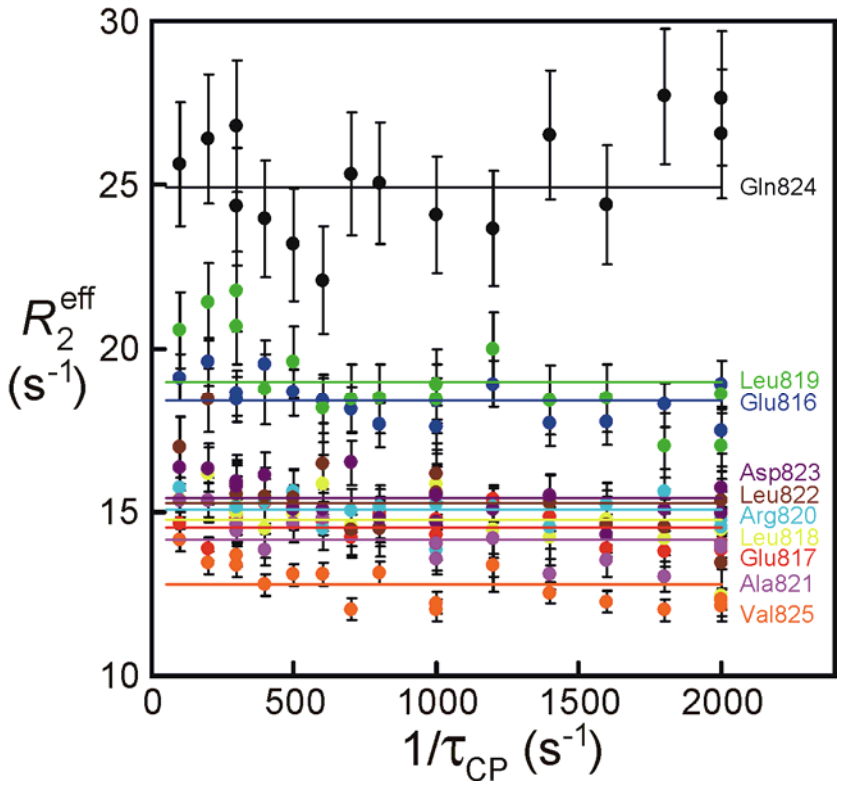


Supplementary Figure S1. ^{1}H-^{15}N HSQC titration of [^{15}N]-HIF-OH with unlabeled TAZ1 over a HIF-OH:TAZ1 concentration ratios ranging from 1:0 to 1:1.2. The cross peaks are color coded from blue (free HIF-OH) through green to red (1:1.2).



Supplementary Figure S2. R_2 dispersion data recorded at 600 MHz for ^{15}N-labeled HIF-OH in the complex with TAZ1 at 1:1 concentration ratio.

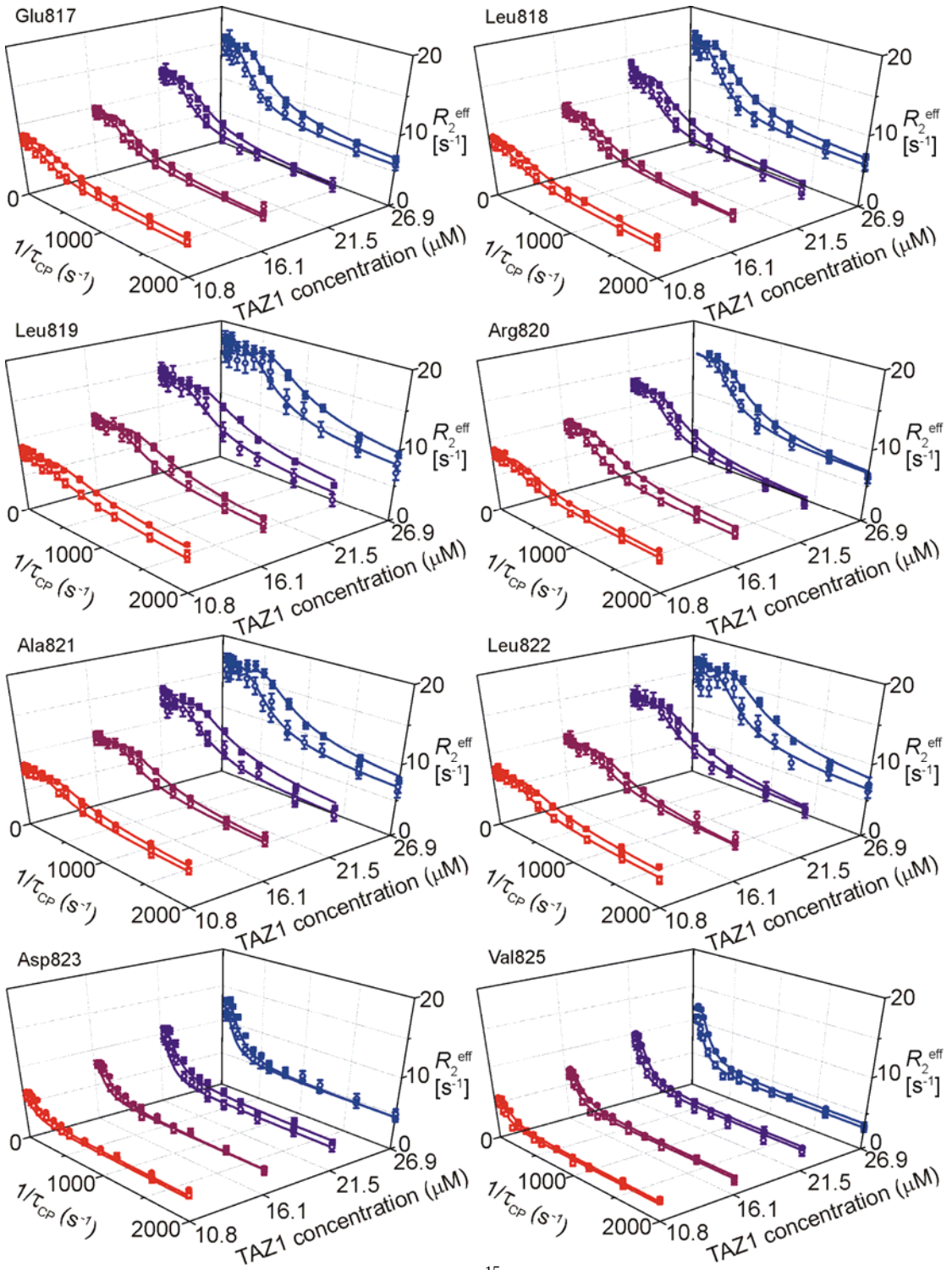


Figure S3. TAZ1 concentration dependence of ^{15}N R_2 dispersion curves recorded at 900 MHz (filled circles) and 600 MHz (open circles). Dispersion curves for 505 μM [^{15}N]-HIF-OH in the presence of 10.8, 16.1, 21.5, and 26.9 μM TAZ1 are shown.

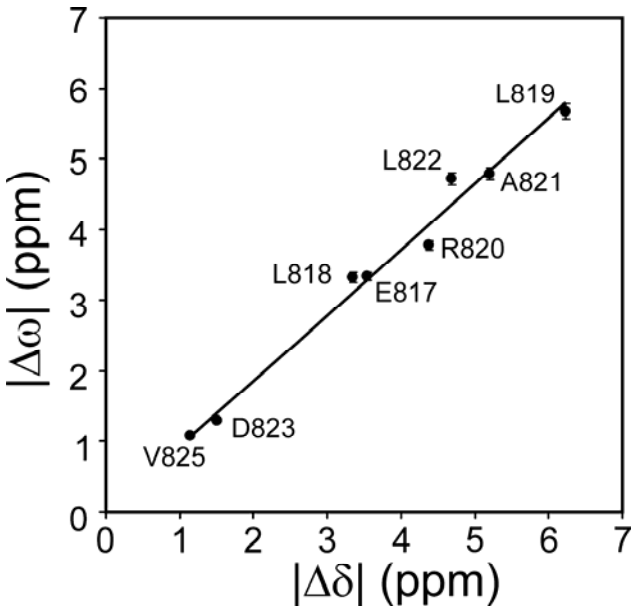


Figure S4. Correlation of ^{15}N chemical shift differences, $\Delta\omega$, determined from the R_2 dispersion measurements with equilibrium chemical shift differences, $\Delta\delta$, between free and TAZ1-bound HIF-OH. The slope is 0.93 (R^2 = 0.98).

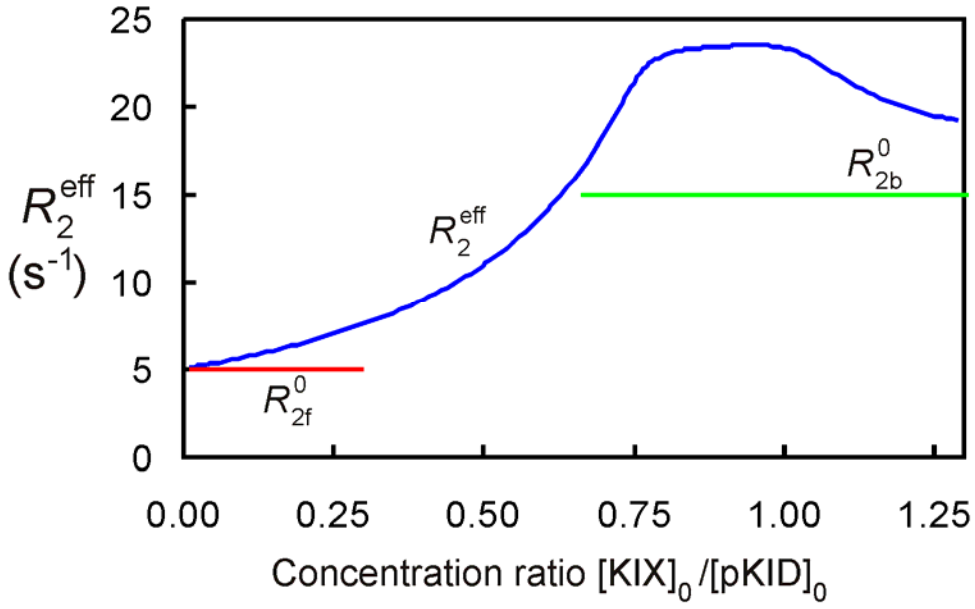


Figure S5. R_2^{eff} rates for pSer133 of pKID simulated using the parameters listed in Table 1 of ref. 10. The R_2^{eff} rates are plotted versus the concentration ratio, $[KIX]_0/[pKID]_0$. The red and green lines indicate R_2^0, where the R_2^0 rates for the free and bound states are 5 and 15 s^{-1}, respectively.