Supporting Information

Fawzi et al. 10.1073/pnas.0909370107

SI Text

Sample Preparation. Uniformly labeled *E. coli* [^{2}H, ^{15}N]-EIN (1–249) and histidine-containing phosphocarrier protein (HPr) at natural isotopic abundance were expressed in *E. coli* cells [BL21 Star (DE3); Invitrogen] grown in M9 minimal media (supplemented with 1 g/L of ^{2}H, ^{15}N-ISOGRO (Sigma Aldrich) and Luria broth, respectively, at 37 °C until an OD ≈0.6–0.8. Cells were induced with 1 mM isopropyl β-D-1-thiogalactopyranoside at 37 °C for approximately 8 h and then harvested by centrifugation. For both Enzyme I (EIN) and HPr, cells were resuspended in 20 mM Tris (pH 8.0), 0.3 mM EDTA, and 2 mM β-mercaptoethanol and lysed by sonication. The lysate was then recentrifuged, filtered, and loaded onto a DEAE anionic-exchange column (HiPrep 16/10 DEAE FF; GE Healthcare). Protein was eluted with a salt gradient of 0 mM to 1 M NaCl, concentrated with an Amicon ultracentrifugal concentrator (3-kDa cutoff; Millipore Corporation), and loaded onto a gel-filtration column (HiLoad 26/60 Superdex 75; GE Healthcare). Relevant fractions were pooled and buffer exchanged into 0 mM NaCl before loading onto a Mono-Q anionic-exchange column (GE Healthcare). Protein was eluted with buffers treated with Chelex 100 (Sigma-Aldrich) to remove all contaminating divalent paramagnetic ions. HPr cysteine mutants were kept in 2 mM β-mercaptoethanol throughout the purification to prevent cysteine cross-linking.

Paramagnetic Labeling of HPr. Immediately prior to conjugation with the EDTA-Mn^{2+} (or EDTA-Ca^{2+}) tag, the HPr protein was passed through a desalting column (HiPrep 26/10 Desalting; GE Healthcare) to remove residual reducing agent. The protein was collected into a solution containing 10 mg of N-[S-(2-Pyridylthio)cysteaminyl]ethylenediamine-N,N,N',N'-tetraacetic acid (#P996250; Toronto Research Chemicals, Inc.) and incubated at room temperature for 3 h. Unconjugated HPr was separated from the tagged protein by Mono-Q anionic-exchange chromatography.

To remove contaminating divalent ions, HPr was incubated overnight at room temperature with 50 mM EDTA. The excess EDTA was removed by exchanging with a high salt buffer (500 mM NaCl) and the Amicon ultracentrifugal concentrator. Mn^{2+} (Sigma Aldrich M-1787) or Ca^{2+} (Sigma Aldrich C-5080) was then added at a protein to metal ratio of approximately 1∶5. Excess metal cation was removed by exchange with the high salt buffer, followed by exchange into the NMR buffer with 20 mM Tris-HCl, pH 7.4, and 150 mM NaCl. HPr protein was then concentrated to 5 mM for titration into EIN.

NMR Experiments for ^{1}H-Γ_2 Measurements. ^1H_N-R_2 rates for U-[^{2}H, ^{15}N]-EIN in the presence of HPr (paramagnetic or diamagnetic) were obtained from a two time point measurement (12 μs and 27 ms, $\Delta T = 27$ ms) recorded in an interleaved manner using a transverse relaxation optimized (TROSY) version of the ^{1}H-^{15}N heteronuclear single quantum correlation based pulse scheme described previously in figure 1 of Iwahara et al. (1), where the relaxation delay is incorporated in the first insensitive nuclei enhanced by polarization transfer step. The ^1H_N-Γ_2 rates are given by the difference in ^1H_N-R_2 rates between the paramagnetic and diamagnetic samples (at identical HPr concentrations), as described in ref. 2. The errors in the ^1H_N-Γ_2 are calculated as described in ref. 2. Data were acquired with $128(t_1) \times 898(t_2)$ complex points along the indirect ^{15}N (32 ppm sweep width) and direct ^{1}H (13 ppm sweep width) dimensions, respectively. To achieve the high signal-to-noise ratio required for precise paramagnetic relaxation enhancement measurements, at least 32 scans were collected per t_1 increment. Data were processed via standard methods using a shifted cosine-bell window function, zero filling, and baseline correction for both ^{1}H and ^{15}N dimensions. Data were processed with NMRPipe (2) and analyzed with NMRDraw (3) and XIPP, an updated version of PIPP (4).

1. Iwahara J, Tang C, Clore GM (2007) Practical aspects of ^{1}H transverse paramagnetic relaxation enhancement measurements on macromolecules. *J Magn Reson* 184:185–195.
2. Iwahara J, Schwieters CD, Clore GM (2004) Ensemble approach for NMR structure refinement against ^{1}H paramagnetic relaxation enhancement data arising from a flexible paramagnetic group attached to a macromolecule. *J Am Chem Soc* 126:5879–5896.
3. Delaglio F, et al. (1995) NMRpipe—A multidimensional spectral processing system based on unix pipes. *J Biomol NMR* 6:277–293.
4. Garrett DS, Powers R, Gronenborn AM, Clore GM (1991) A common sense approach to peak picking in two-, three- and four-dimensional spectra using automatic computer analysis of contour diagrams. *J Magn Reson* 95:214–220.