Supplementary Information for

Probing transient excited states of the bacterial cell division regulator MinE by relaxation dispersion NMR spectroscopy

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SI Materials and Methods

Protein Expression and purification. Plasmids containing the gene encoding for the full length Neisseria gonorrhoeae ngMinE(E46A/R10Q) mutant (referred to here as wt*, and used as the starting point for all other constructs) and the various derivative constructs (Δ10 and Δ30 comprising 10 and 30 residue N-terminal deletions, respectively; full length I24N; and Δ10/I24N) were cloned into the pet-11a vector (Novagen, EMD Millipore) as C-terminal His-tagged fusion proteins and expressed in E. coli BL21(DE3) cells. All perdeuterated proteins were expressed according to our previously published procedure (1) in a modified M9/D2O medium containing 18 g/L deuterated glucose (D-glucose-1,2,3,4,5,6,6-d7) and 5g/L 15NH4Cl. 250 ml culture cells in 2.8 L baffled Fernbuch flasks were grown to an OD600 ~10 at 37 °C and then induced at 25 °C for 20 h with 0.5 mM isopropyl β-d-1-thiogalactopyranoside (IPTG). Protonated [13C/15N]-labeled proteins were expressed in modified M9 medium (1) containing 9 g/L U-15C6-glucose and 3 g/L 15NH4Cl. 250 ml cultures were grown in 2.8 L (or larger) baffled Fernbuch flasks to an OD600 of between 2 and 3 at 37 °C. The temperature was then reduced to 30 °C and the cultures grown up to an OD600 between 5 and 6, prior to induction at 19 °C with 0.5 mM IPTG overnight. Cells were harvested by centrifugation and stored as a cell paste at -80 °C.

The cell paste from 250 ml cultures was suspended in 200 ml buffer containing 50 mM Tris-HCl (pH 7.5) and 200 mM NaCl, and carefully homogenized. The cell suspension was then passed twice through the Avestin Emusiflex system at a pressure between 15,000-20,000 psi. The cell pellet was removed by centrifugation for 1 h at 11,000 × g. The supernatant was loaded onto a 10 ml Ni-column pre-equilibrated with 50 mM Tris-HCl (pH 7.5), 200 mM NaCl and 0.1 mM benzamidine hydrochloride. Prior to elution of the protein fractions with an imidazole gradient (40 mM to 1 M in 100 mL), the column was washed thoroughly with about 200 mL buffer containing 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.1 mM benzamidine and 40 mM imidazole until the UV absorbance of the eluent remained unchanged. The fractions containing the ngMinE proteins were pooled together and further purified on a gel filtration column (superdex75, GE healthcare). The gel filtration column was pre-equilibrated 50 mM Tris-HCl (pH 6.5), 0.5 mM ethylenediamine-tetraacetic acid (EDTA), 0.1 mM benzamidine and 300 mM NaCl, and eluted with the same buffer. Fractions contain the MinE proteins were pooled together, concentrated using a centrifriprep (Millipore) with a molecular weight cut-off of 3 kDa. The concentrated protein solutions were dialyzed against 25 mM potassium phosphate (pH 6.5), 0.5 mM EDTA and 0.1 mM benzamidine chloride. For storage, the protein solutions with glycerol added to a final concentration of 20 % v/v were rapidly frozen in liquid nitrogen and stored at -80 °C.

E. coli His-GFP-ecMinD and Alexa647-ecMinE-his were prepared as previously described (2). Alexa647-ngMinE(E46A/R10Q/L88C)-his and his-GFP-ngMinD were expressed, purified and dye-labeled essentially as described previously (2).

Total Internal Reflection Fluorescence Microscopy (TIRF). Details of TIRF experiments used to monitor the Alexa647-MinE/GFP-MinD patterning on a supported lipid bilayer (SLB) in a flow cell was as described previously (2). Briefly, the SLB used to coat the flow cell comprised 67% DOPC (1, 2-dioleoyl-sn-glycerol-3 phosphocholine; catalog no. 850375, Avanti) and 33 % DOPG (1,2-dioleoyl-sn-glycerol-3 phospho-rac-1-glycerol; catalog no. 840475, Avanti). 1 μM GFP-MinD and 2 μM Alexa647-MinE (typically diluted 9-fold with unlabeled protein) were premixed in a buffer containing 25 mM Tris-HCl (pH 7.4), 200 mM KCl, 2 mM dithiothreitol (DTT), 5 mM MgCl2, 2.5 mM ATP, 0.5 mg/ml ascorbate, 10 μg/mL pyruvate kinase (Sigma) and 5 mM phosphoenol pyruvate. The reaction mixture was loaded into a 1 ml syringe connected to the flow cell inlet and injected into the 3 μL flowcell (~25 μm x 4 mm x 30 mm) at a flow rate of 1 μl/min for 10 min. Sample flow was stopped for 30 min before movie acquisition. The prism-type TIRF microscopy setup based on an Eclipse TE2000E (Nikon) stand and a PlanApo 10x (NA = 0.45) objective lens was as described (2).

NMR sample preparation. The buffer for NMR samples comprised 25 mM potassium phosphate (pH 6.5), 0.5 mM EDTA and 0.1 mM benzamidine chloride in 95% (v/v) H2O/5% (v/v) D2O. Samples for RDC measurements also contained 200 mM NaCl, 11mg/ml phage pf1 and 90 % (v/v) H2O/10% (v/v) D2O. Triple resonance backbone assignment experiments (3) were collected on 0.5 mM to 1.0 mM (in monomer
units) uniformly $^{13}$C/$^{15}$N-labeled samples. All $^{15}$N-CPMG relaxation dispersion and RDC experiments were collected on $^2$H/$^{15}$N-labeled protein samples. $^{15}$N CPMG relaxation dispersion experiments were collected on 1 mM (in monomer units) samples for wt*, $\Delta 30$ and $\Delta 10/\Delta 24N$ constructs, and at 2 mM (in monomer units) for the $\Delta 10$ constructs. In addition CPMG data were also collected on 0.5 and 2 mM (in monomer units) samples for $\Delta 10$ and $\Delta 30$, respectively. $^1$D$_{na}$ RDC data, measured as the difference in $^1$H$_{na}$ scalar couplings in aligned (11 mg/ml fd phage; (4)) and isotropic (water) media, were collected on 1.0 mM protein samples using the $^1$H-$^{15}$N HSQC IPAP scheme (5); the RDC data were recorded at a spectrometer frequency of 800 MHz for wt*, $\Delta 10$ and $\Delta 30$ constructs, and at 600 MHz for the $\Delta 10/\Delta 24N$ construct. Samples of the wt*/$\Delta 10$ heterodimer were prepared using a denaturation/renaturation process. wt* and $\Delta 10$ constructs were mixed in different and defined ratios, and guanidinium chloride added to a final concentration of 6 M. For heterodimer samples containing $^{15}$N-labeled wt*, unlabeled $\Delta 10$ was added in a 10-fold molar-excess, and vice versa for samples containing $^{15}$N-labeled $\Delta 10$ and unlabeled wt*. The denatured wt*/$\Delta 10$ protein mixture was then dialyzed into 25 mM potassium phosphate (pH 6.5), 0.5 mM EDTA and 0.1 mM benzamidine hydrochloride, and concentrated to a final total protein concentration between 0.5 and 2.0 mM.

NMR experiments. All heteronuclear NMR experiments were recorded at 25 °C on Bruker 500, 600 and 800 MHz spectrometers equipped with z-gradient triple resonance cryoprobes. Spectra were processed using NMRPipe (6) and analyzed using the in-house software package, XIPP, a new version of PIPP (7). $^1$H, $^13$C and $^{15}$N backbone resonance assignments were obtained from analysis of 3D CBCA(CO)NH, HNCACB and HNCO through-bond heteronuclear scalar correlation experiments; H\(\alpha\) and H\(\beta\) protons were assigned from 3D HBHA(CBCACO)NH experiments (3).

$^{15}$N Relaxation dispersion measurements. $^{15}$N-CPMG relaxation dispersion experiments were recorded at 800 MHz and either 500 or 600 MHz using a pulse scheme with amide proton decoupling to measure the rates of in-phase $^{15}$N coherences (8) and a constant time period of 40 ms. The CPMG field strengths ($\nu = 1/2\tau_{CP}$ where $\tau_{CP}$ is the time between two consecutive 180° pulses of the CPMG pulse train) employed were 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 and 1000 Hz. Constant wave (CW) $^1$H saturation with an 11 kHz radiofrequency (RF) field was applied during the entire CPMG period. For each sample, a total of 22 spectra were collected including a reference spectrum without the 40 ms delay and a duplicate spectrum at $\nu = 150$Hz. For data collected on 500 and 600 MHz spectrometers, 88 $t_1$ complex points in the indirect ($^{15}$N) dimension were collected with 32 scans per free induction decay (fid) for a total data collection time of $\sim$2.5 hrs. per spectrum. For data collected on the 800 MHz spectrometer, 128 $t_1$ complex points in the indirect ($^{15}$N) dimension were collected with 16 scans per fid for a total collection time of $\sim$2.2 hrs. per spectrum. In all experiments, the delay between scans was set to 1.7 s.

To investigate any possible concentration dependence of the exchange parameters, CPMG relaxation dispersion experiments for $\Delta 10$ and $\Delta 30$ constructs were carried out on 0.5 and 2 mM, and 1 and 2 mM samples, respectively. As the relaxation dispersion curves were found to be independent of concentration (Fig. S3), quantitative analysis and fitting focused on the data collected at a protein concentration of 1 mM except for the $\Delta 10$ construct where a 2 mM sample was employed.

The $^{15}$N-CPMG relaxation dispersion curves were fit to two-state and three-state (linear and branched) exchange models by solving the appropriate McConnell equations (9) optimizing the relevant rate constants as global parameters, and the transverse relaxation rate and differences in chemical shifts for each residue. The standard deviation of the optimized parameters was obtained from the variance-covariance matrix; but it should be noted that these values systematically underestimate the real uncertainties. The choice of appropriate model was made on the basis of goodness-of-fit and examination of systematic errors in the fits (see Figs. S6-S8). It should be noted that the calculated reduced $\chi^2$ values are based on uncertainties in $R_{2,eff}$ values obtained from duplicate measurements at one $v_{CPMG}$ value for each construct; these uncertainties, however, are smaller than the point to point variation from a smooth curve that is directly apparent by looking at the $v_{CPMG}$ series, and therefore the $\chi^2$ values should only be interpreted in relative rather than absolute terms. The best model for the wt*, $\Delta 30$ and $\Delta 10/\Delta 24N$ was a linear three-state exchange system $\textbf{A} \leftrightarrow \textbf{B} \leftrightarrow \textbf{C}$ where $\textbf{A}$ is the major species. For the $\Delta 10$ data, a two-state exchange model, $\textbf{A} \leftrightarrow \textbf{B}$ where $\textbf{A}$ is the major species, was sufficient to represent the data.
For the fits to the three state schemes, the data were fit simultaneously to a two-member three-state exchanging system where the two members share the kinetic rate constants and the residue-specific transverse relaxation rate constants; however, in the first member, the A major state is chosen as the chemical shift reference (i.e. $\omega_A = 0$), while in the second member, the B minor state is chosen as the chemical shift reference (i.e. $\omega_B = 0$). In this manner $\Delta \omega_{AB}$ (shared between the two members) and $\Delta \omega_{BC}$ (for the second member) are optimized, while $\Delta \omega_{AC}$ for the first member is given by $\Delta \omega_{AB} + \Delta \omega_{BC}$. This procedure ensures self-consistency of the three sets of chemical shift differences and preserves the relative sign information of the $\Delta \omega$ values.

$^{15}$N-CEST experiments. $^{15}$N-CEST (chemical exchange saturation transfer) experiments (10) on the $\Delta 30$ ngMinE construct were recorded as described previously (11) at a spectrometer frequency of 900 MHz in an interleaved manner with the exchange time $T_{CEST}$ set to 200 ms and the CW saturation field strength to 25 Hz. $^{15}$N CW saturation was applied at 25 Hz increments from -1250 to +1250 Hz (with the $^{15}$N carrier set to 119.5 ppm). The $^{15}$N-CEST profiles are shown in Fig. S9.

CS-Rosetta structure modeling. Structures of the wt*, $\Delta 10$, $\Delta 30$ and $\Delta 10/\Delta 24$ ngMinE constructs were modeled using the CS-ROSETTA fold-and-docking protocol (12, 13) making use of backbone ($^{15}$N, $^1$H$_N$, Cα, Cβ and C') chemical shifts and $^1$D$_{NH}$ RDCs measured in f1 phage alignment medium. Residues at the N- and C-termini, judged to be disordered on the basis of secondary backbone chemical shifts (using TALOS-N) and near-zero RDC value, were excluded from the calculations. Thus residues 1-81, 16-81, 38-81 and 38-81 were used for wt*, $\Delta 10$, $\Delta 30$ and $\Delta 10/\Delta 24$, respectively. The backbone chemical shift data indicate that the secondary structures of wt* and $\Delta 10$ are identical, except that the latter is missing the first helix (residues 3-8), and likewise the secondary structures of $\Delta 30$ and $\Delta 10/\Delta 24$ are the same (see Fig. 2, main text). The chemical shift and RDC data were therefore combined as follows: the wt* and $\Delta 10$ calculations were carried out with their respective chemical shifts but with the RDCs from both constructs; similarly the $\Delta 30$ and $\Delta 10/\Delta 24$ calculations were carried out with their respective chemical shifts but with the RDCs for both constructs combined. This facilitates convergence, as this procedure is in effect equivalent to using two different alignment media with different alignment tensors. The values of the axial component of the alignment tensors $D_a$ and the rhombicity $\eta$ are 1.4 Hz/0.33, 12.5 Hz/0.26, 5.1 Hz/0.13 and 5.6 Hz/0.43 for wt*, $\Delta 10$, $\Delta 30$ and $\Delta 10/\Delta 24$, respectively. 40,000, 20,000, 10,000 and 10,000 structures were generated for wt*, $\Delta 10$ and $\Delta 30$ and $\Delta 10/\Delta 24$, respectively, and the 10 lowest energy structures in each instance were selected for further analysis.

It should be noted that the high resolution three-dimensional protein coordinate database employed in the CS-ROSETTA calculations does not include the previously published NMR structures of full length ngMinE (six-stranded resting state; 2KXO (14)) and $\Delta 30$-ecMinE (four-stranded form; 1EVO (15)) and the 4.3 Å resolution crystal structure of $\Delta 24$-ecMinE (four-stranded form) complexed to MinD (PDB ID 3R9J (16)). The registers of the $\beta$-sheets, both intramolecular and intermolecular at the dimer interface, in the CS-ROSETTA structures of both the six-stranded (wt* and $\Delta 10$) and four-stranded ($\Delta 30$ and $\Delta 10/\Delta 24$) forms of ngMinE are fully consistent with these previously published structures. The sequence identity between $\Delta 30$ ngMinE and $\Delta 30$ ecMinE is 47%, and ecMinE has a single residue insertion between residues 71 and 72 in the turn connecting strands $\beta 2$ and $\beta 3$. The backbone rmsds for residues 37-71/37’-71’ and 72-80/72’-80’ (using the $\Delta 30$ ngMinE numbering scheme, and deleting the single residue insertion in the E. coli variants) between the lowest energy CS-ROSETTA structure of ngMinE $\Delta 30$ and the E. coli 1EVO and 3R9J structures are 2.3 and 1.9 Å, respectively. Given the agreement with the existing published structures there was no need, in this particular instance, to further confirm the $\beta$-sheet registers by recording 3D-$^{15}$N NOESY spectra for the four ngMinE constructs.

Sedimentation velocity analytical ultracentrifugation. Sedimentation velocity experiments were carried out at 50,000 rpm and 20 °C on a Beckman Coulter ProteomeLab XI-I analytical ultracentrifuge following standard protocols (14). The $\Delta 10$ ngMinE construct was $^2$H/$^{15}$N-labeled; the $\Delta 30$ ngMinE construct was $^2$H/$^{15}$N/selective $^{13}$C-methyl protonated (Val $\gamma$, Leu $\delta$ and Ile $\delta$) labeled. Both $\Delta 10$ and $\Delta 30$ constructs were studied at different loading concentrations in 25 mM potassium phosphate (pH 6.5), 0.5 mM EDTA and 0.1 mM benzamidine. Samples, prepared by dilution of stock solutions, were loaded in 2-channel centerpiece cells, and data collected using absorbance (280 nm) and interference (655 nm, when
available) optical detection systems. Sedimentation data were time-corrected (15) and analyzed in SEDFIT 15.01c (16) in terms of a continuous c(s) distribution of Lamm equation solutions with a maximum entropy regularization confidence level of 0.68. Excellent data fits were observed with r.m.s.d. values of 0.0033 – 0.0055 absorbance units and 0.0021 - 0.0065 fringes. Solution densities and viscosities were determined based on composition in SEDNTERP (17). Protein partial specific volumes were calculated based on the amino acid composition in SEDNTERP, and corrected for isotopic substitution. Sedimentation coefficients were corrected to $s_{20,w}$ values at standard conditions.
Figure S1. $^{15}$N-$R_2$ transverse relaxation rates as a function of residue for the wt*, $\Delta 10$, $\Delta 10/I24N$ and $\Delta 30$ constructs. The disordered region at the N-terminus of each construct is indicated by the transparent blue bar. The data were collected using the same sequence as that used for the in-phase $^{15}$N CPMG relaxation dispersion experiments at a 1 kHz CPMG field ($v_{\text{CPMG}}$). The data for wt*, $\Delta 10$ and $\Delta 30$ constructs were recorded at a spectrometer field of 500 MHz; the data for the $\Delta 10/I24N$ construct were recorded at a spectrometer frequency of 600 MHz. All data were recorded at 25°C. The estimated uncertainties in $R_2$ values obtained from duplicate measurements at one intermediate $v_{\text{CPMG}}$ value (see SI Methods) are indicated by the error bars (= 1 S.D.); these values, however, are significantly smaller than the true uncertainties as they are less than the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves (see Figs. 5 and 6 in the main text, and Figs. S3, S5-S8 and S10-S13 in the SI). The $R_2$ data for residues 60, 71 and 74 for the $\Delta 10/I24N$ and $\Delta 30$ constructs are indicated by an asterisks as their values are larger than expected compared to those of adjacent residues, indicating that exchange line broadening for these three residues is not fully suppressed at a CPMG field of 1 kHz.
Figure S2. Correlation between experimental backbone amide RDCs ($^{1}$D$_{NH}$) and those calculated from the CS-Rosetta derived model structures based on backbone chemical shifts and RDCs. The reported RDC $R$-factor is given by $\sqrt{\langle(D_{\text{obs}} - D_{\text{calc}})^2\rangle}/(2\langle D_{\text{obs}}^2 \rangle)^{1/2}$, where $D_{\text{obs}}$ and $D_{\text{calc}}$ are the observed and calculated $^{1}$D$_{NH}$ RDCs, respectively (18). The experimental RDCs were recorded at 25°C.
Figure S3. Concentration dependence of $^{15}$N CPMG relaxation dispersion curves for the Δ10 and Δ30 ngMinE constructs. (A) Δ10 at 2 (blue) and 0.5 (red) mM. (B) Δ30 at 2 (blue) and 1 (red) mM. The CPMG relaxation dispersion curves at the two concentrations have been superimposed by displacement along the $R_{2,\text{eff}}$ axis, since the curves are uniformly shifted upwards at higher concentrations owing to higher viscosity. The data were recorded at 800 MHz and 25 °C.
Figure S4. Characterization of the multimeric state of the Δ10 and Δ30 ngMinE constructs by sedimentation velocity analytical ultracentrifugation. Interference $c(s)$ distributions at different loading concentrations (as indicated in the figure) for Δ10 (top) and Δ30 (bottom). The data at the highest concentration (455 µM for Δ10, 890 µM for Δ30) were collected in 3 mm pathlength cells; data at all other concentrations were collected in 12 mm pathlength cells. The Δ10 and Δ30 data over the concentration range studied (4.6 to 455 µM and 5 to 890 µM, respectively) are indicative of a single species at 2.24 S and 1.93 S, respectively, corresponding to molar masses of 21.4 and 16.3 kDa. These data indicate the presence of only a dimer species for both constructs and provide no evidence for any monomer within the limit of detection. The sedimentation data for the Δ10 construct at 4.6 µM were also modeled using the Lamm equation in terms of discrete monomers and dimers to estimate the maximum amount of monomer present. Best-fits were obtained with a dimer only model. However, the presence of a maximum of 1.3% monomer was statistically indistinguishable based on the observed rmsd and a 95% confidence interval. A maximum $K_{dimer}$ limit of ~1.6 nM is determined, indicating that at a loading concentration of 1 mM there will be at most ~0.09% of the monomer.
Figure S5. $^{15}$N-CPMG relaxation dispersion curves for residues 60, 71 and 74 of the Δ30 and Δ10/I24N ngMinE constructs. The experimental data (circles) were recorded at 800 (blue) and 500 (red) MHz for Δ30 and 800 (blue) and 600 (red) MHz for Δ10/I24N. The temperature was 25°C. For all three residues chemical exchange line broadening is not fully suppressed at a CPMG field of 1 kHz, indicating the existence of a faster process. While these data can be fit globally together with the CPMG data for the other residues to a 3-state linear exchange model, the values of $\Delta\omega_{AC}$ appear to be unusually (and in two cases unphysically) large: for residues 60, 71 and 74 of Δ30, the optimized values of $\Delta\omega_{AC}$ are 5.2, 10.6 and 8.9 ppm, respectively; for Δ10/I24N, the optimized values of $\Delta\omega_{AC}$ are 7.5, 11.7 and 13.0 ppm, respectively; these should be compared to secondary shifts (folded protein minus predicted random coil shifts) of 5.2, 6.3 and 0.2 ppm, respectively. That being said, inclusion of the CPMG data for residues 60, 71 and 74 in the global fits does not result in any statistically significant changes in the populations of the three states or in the values of the rate constants. This suggests that the larger than expected values of $\Delta\omega_{AC}$ for residues 60, 71 and 74 obtained from the fits is likely due to incorporation of additional chemical shifts changes into the $\Delta\omega_{AC}$ values attributable to a faster exchanging process that is not suppressed at a 1 kHz CPMG field. In addition, these fits also underestimate the $\Delta\omega_{AB}$ values of D60 and V74 for the slow A→B transition ($k_{ex} \sim 180$ s$^{-1}$; see Fig. S7) as judged by the $^{15}$N CEST data recorded on the Δ30 construct. Thus, the $\Delta\omega_{AB}$ values for D60 and V74 from the CPMG fits shown in this figure (left panels) are 0.04 and 0.83 ppm, respectively, compared to ~0.9 and ~ 2 ppm, respectively, from $^{15}$N-CEST. For these reasons the data for D60, G71 and V74 were excluded from the global analysis of the $^{15}$N CPMG data for Δ30 and Δ10/I24N reported in the paper (Fig. 6, main text, and Figs. S7, S8, S12 and S13).
Figure S6. Comparison of global fits to the $^{15}$N CPMG relaxation dispersion data for selected residues of wt* ngMinE for 2-state, 3-state branched and 3-state linear models. The circles represent the experimental data, and the lines are the best fits. Systematic errors in the fits are clearly apparent for the 2-state and 3-state branched models. The reduced $\chi^2$ values are indicated and should be interpreted in relative terms only, as the uncertainties in $R_{2,\text{eff}}$ values obtained from duplicate measurements at one $v_{\text{CPMG}}$ value (see Fig. S1 and Fig. S10 legend) are smaller than the point to point variation than the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves. Note that a two-fold increase in the estimated measurement error results in a four-fold decrease in the $\chi^2$ value.
Figure S7. Comparison of global fits to the $^{15}$N CPMG relaxation dispersion data for selected residues of the D30 ngMinE construct for 2-state, 3-state branched and 3-state linear models. The circles represent the experimental data, and the lines are the best fits. Systematic errors in the fits are clearly apparent for the 2-state model; for the 3-state branched model, smaller systematic errors in the fits are present (e.g. for residue 39 at 800 MHz and residue 57 at 500 MHz). The reduced $\chi^2$ values are indicated and should be interpreted in relative terms only, as the uncertainties in $R_{2,eff}$ values obtained from duplicate measurements at one VCPMG value (Fig. S1 and Fig. S12 legend) are smaller than than the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves. Note that a two-fold increase in the estimated measurement error results in a four-fold decrease in the $\chi^2$ value.
Figure S8. Comparison of global fits to the $^{15}$N CPMG relaxation dispersion data for selected residues of the D10/I24N ngMinE construct for 2-state, 3-state branched and 3-state linear models. The circles represent the experimental data, and the lines are the best fits. Systematic errors in the fits are clearly apparent for the 2-state model; for the 3-state branched model a few systematic errors in the fits are present (e.g. for residue 39 and 57 at 800 MHz) that are larger than for the 3-state linear model. The reduced $\chi^2$ values are indicated and should be interpreted in relative terms only, as the uncertainties in $R_{2,\text{eff}}$ values obtained from duplicate measurements at one $v_{\text{CPMG}}$ value (Fig. S1 and Fig. S13 legend) are smaller than the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves. Note that a two-fold increase in the estimated measurement error results in a four-fold decrease in the $\chi^2$ value.
Figure S9. $^{15}$N-CEST profiles recorded at 900 MHz for the Δ30 ngMinE construct. Shown in red are the $\Delta \omega_{AB}$ values obtained from global analysis of the $^{15}$N CPMG relaxation dispersion data which are fully consistent with the CEST profiles. Note that only the A↔B transition is slow enough ($k_{ex} \sim 180 \text{ s}^{-1}$; see Fig. S7) to be probed by CEST.
**Figure S10.** $^{15}$N-CPMG curves for residues 14-16 (located in the loop connecting helix α1 to strand β1) of $^{15}$N-labeled wt* ngMinE in the context of the wt* homodimer (blue) and the wt*/Δ10 heterodimer (red). A sizeable reduction in the magnitude of the dispersions is apparent for these three residues in the context of the heterodimer. The heterodimer was prepared, as described in the SI Methods, by mixing 91 μM (in monomer units) $^{15}$N-labeled wt* with 910 μM (in monomer units) unlabeled Δ10. Under these conditions the concentrations of wt*/Δ10 heterodimer and wt*/wt* homodimer are ~83 and ~8 μM (in monomer units), respectively. The data were recorded at a spectrometer frequency of 500 MHz and 25 °C. Owing to the low concentration of heterodimer, the S/N ratio is too low to permit quantitative analysis. The corresponding experiments in which $^{15}$N-labeled Δ10 was mixed with a 10-fold excess of unlabeled wt* did not reveal any significant differences in dispersions for Δ10 in the context of the homodimer and heterodimer.
Figure S11 (Part 1). $^{15}$N-CPMG dispersion profiles for wt* ngMinE with experimental data shown as circles and global best-fits using the three-state linear model as lines. The data were collected at 800 (blue) and 500 (red) MHz, and 25°C. The average error and error range for the $R_{2,\text{eff}}$ values, estimated from a single $V_{\text{CPMG}}$ value duplicate, are 0.21±0.10 and 0.10-0.70 s$^{-1}$, respectively, at 800 MHz, and 0.64±0.30 and 0.31-0.95 s$^{-1}$, respectively, at 500 MHz. These errors, which were used in the calculation of $\chi^2$, are likely underestimates as is readily seen from the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves.
Figure S11 (Part 2). $^{15}$N-CPMG dispersion profiles for wt* ngMinE with experimental data shown as circles and global best-fits using the three-state linear model as lines. The data were collected at 800 (blue) and 500 (red) MHz, and 25°C. The average error and error range for the $R_{2,\text{eff}}$ values, estimated from a single $\nu_{\text{CPMG}}$ value duplicate, are 0.21±0.10 and 0.10-0.70 s$^{-1}$, respectively, at 800 MHz, and 0.64±0.30 and 0.31-0.95 s$^{-1}$, respectively, at 500 MHz. These errors, which were used in the calculation of $\chi^2$, are likely underestimates as is readily seen from the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves.
Figure S12 (Part 1). $^{15}$N-CPMG dispersion profiles for the D10 ngMinE construct with experimental data shown as circles and global best-fits using the two-state model as lines. The data were collected at 800 (blue) and 500 (red) MHz, and 25°C. The average error and error range for the $R_{2,\text{eff}}$ values, estimated from a single $\nu_{\text{CPMG}}$ value duplicate, are 0.33±0.12 and 0.15-0.80 s$^{-1}$, respectively, at 800 MHz, and 0.27±0.15 and 0.11-1.10 s$^{-1}$, respectively, at 500 MHz. These errors, which were used in the calculation of $\chi^2$, are likely underestimates as is readily seen from the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves. The reduced $\chi^2$ is 2.3.
Figure S12 (Part 2). $^{15}$N-CPMG dispersion profiles for the Δ10 ngMinE construct with experimental data shown as circles and global best-fits using the two-state model as lines. The data were collected at 800 (blue) and 500 (red) MHz, and 25°C. The average error and error range for the $R_{2,\text{eff}}$ values, estimated from a single $v_{\text{CPMG}}$ value duplicate, are 0.33±0.12 and 0.15-0.80 $s^{-1}$, respectively, at 800 MHz, and 0.27±0.15 and 0.11-1.10 $s^{-1}$, respectively, at 500 MHz. These errors, which were used in the calculation of $\chi^2$, are likely underestimates as is readily seen from the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves. The reduced $\chi^2$ is 2.3.
Figure S13 (Part 1). $^{15}$N-CPMG dispersion profiles for the Δ30 ngMinE construct with experimental data shown as circles and global best-fits using the two-state model as lines. The data were collected at 800 (blue) and 500 (red) MHz, and 25°C. The average error and error range for the $R_{2,\text{eff}}$ values, estimated from a single $v_{\text{CPMG}}$ value duplicate, are 0.13±0.06 and 0.11-0.36 s$^{-1}$, respectively, at 800 MHz, and 0.19±0.11 and 0.10-0.61 s$^{-1}$, respectively, at 500 MHz. These errors, which were used in the calculation of $\chi^2$, are likely underestimates as is readily seen from the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves.
Figure S13 (Part 2). $^{15}$N-CPMG dispersion profiles for the Δ30 ngMinE construct with experimental data shown as circles and global best-fits using the two-state model as lines. The data were collected at 800 (blue) and 500 (red) MHz, and 25°C. The average error and error range for the $R_{2,\text{eff}}$ values, estimated from a single $v_{\text{CPMG}}$ value duplicate, are 0.13±0.06 and 0.11-0.36 s$^{-1}$, respectively, at 800 MHz, and 0.19±0.11 and 0.10-0.61 s$^{-1}$, respectively, at 500 MHz. These errors, which were used in the calculation of $\chi^2$, are likely underestimates as is readily seen from the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves.
Figure S14 (Part 1). $^{15}$N-CPMG dispersion profiles for the Δ10/I24N ngMinE construct with experimental data shown as circles and global best-fits using the two-state model as lines. The data were collected at 800 (blue) and 600 (red) MHz, and 25°C. The average error and error range for the $R_{2,\text{eff}}$ values, estimated from a single $v_{\text{CPMG}}$ value duplicate, are 0.31±0.27 and 0.10-0.90 s$^{-1}$, respectively, at 800 MHz, and 0.19±0.12 and 0.10-0.81 s$^{-1}$, respectively, at 600 MHz. These errors, which were used in the calculation of $\chi^2$, are likely underestimates as is readily seen from the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves.
Figure S14 (Part 2). $^{15}$N-CPMG dispersion profiles for the Δ10/I24N ngMinE construct with experimental data shown as circles and global best-fits using the two-state model as lines. The data were collected at 800 (blue) and 600 (red) MHz, and 25°C. The average error and error range for the $R_{2,\text{eff}}$ values, estimated from a single $v_{\text{CPMG}}$ value duplicate, are 0.31±0.27 and 0.10-0.90 s$^{-1}$, respectively, at 800 MHz, and 0.19±0.12 and 0.10-0.81 s$^{-1}$, respectively, at 600 MHz. These errors, which were used in the calculation of $\chi^2$, are likely underestimates as is readily seen from the point to point variation of the experimental CPMG relaxation dispersion curves from smooth curves.
Supplementary References