

Supporting Information

Transition State Compressibility and Activation Volume of Transient Protein Conformational Fluctuations

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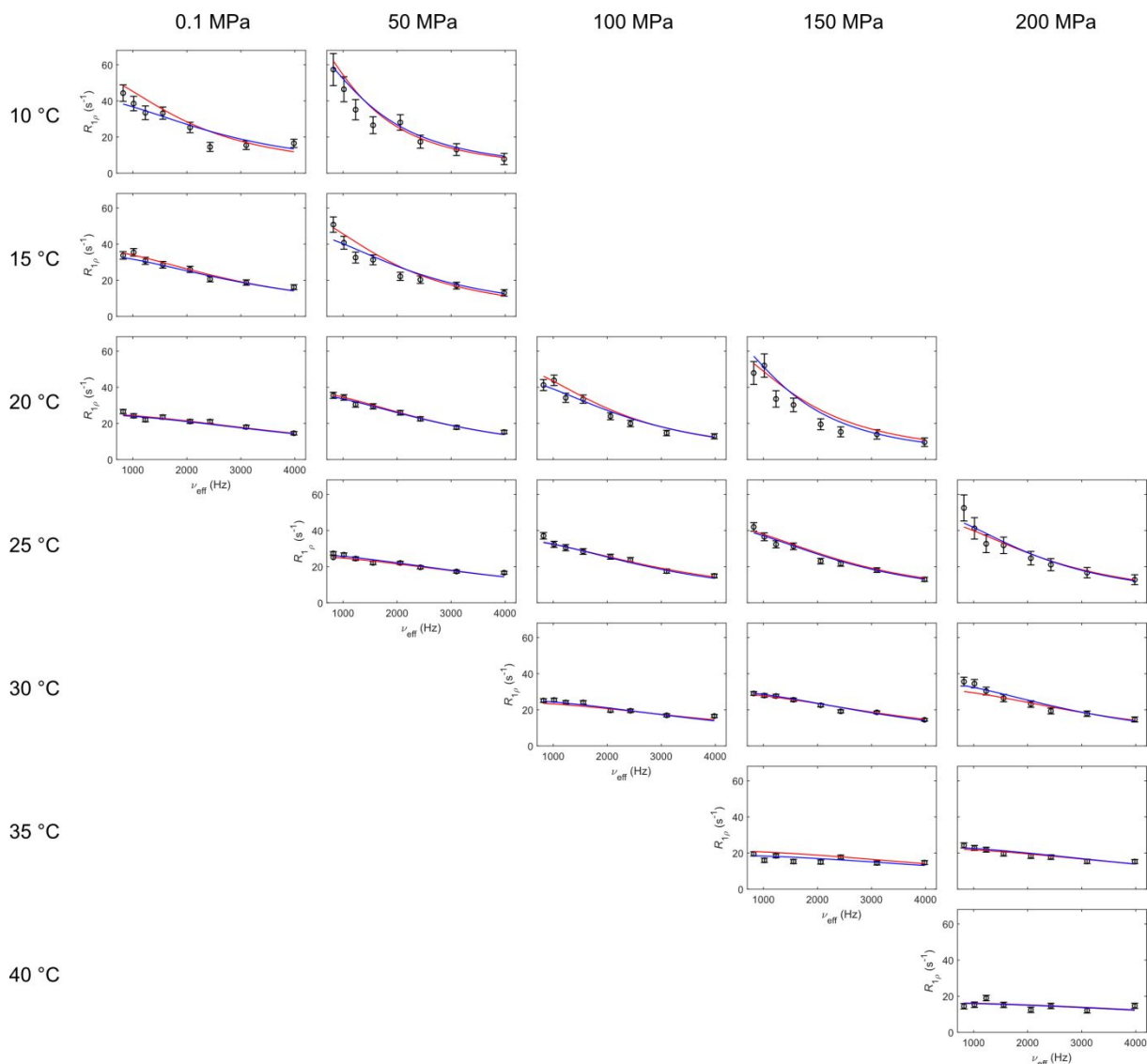


Figure S1: ^{13}C aromatic $R_{1\rho}$ relaxation dispersion profiles for F52 ϵ recorded on-resonant ($\theta > 85^\circ$) on a 5 mM sample of GB1 at pH 7.0 and a static magnetic field strength of 14.1 T. The relaxation dispersions were fitted using a fixed population $p_1 = p_2 = 0.5$ and $\Delta\delta$ fixed at the value measured from HSQC spectra under slow-exchange conditions. Blue lines depict global fits of all temperatures at each pressure, fitted with the restrictions: $k_{\text{flip}}(T_{\text{high}}) > k_{\text{flip}}(T_{\text{low}})$, and $R_{2,0}(T_{\text{high}}) \leq R_{2,0}(T_{\text{low}})$. Derived ring flip rate constants (k_{flip}) are given in Table 1. Red lines depict the global fit of all relaxation dispersions directly to the model given by Eq. (6), with the restriction: $R_{2,0}(T_{\text{high}}) \leq R_{2,0}(T_{\text{low}})$ for sets of equal pressure.

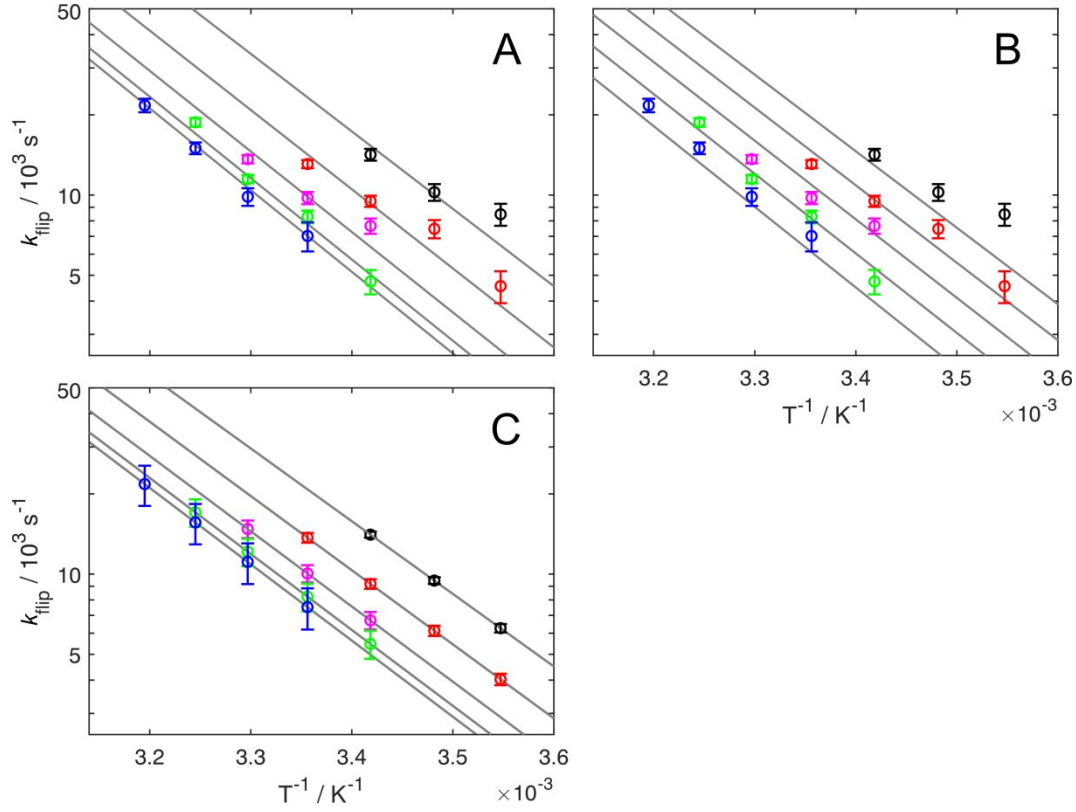


Figure S2: Temperature and pressure dependence of flip rates. k_{flip} is plotted as a function of $1/T$ for F52 at pressures of 0.1 MPa (black), 50 MPa (red), 100 MPa (magenta), 150 MPa (green) and 200 MPa (blue). The solid lines represent the fitted model including the activation parameters $\Delta^\ddagger G_0$, $\Delta^\ddagger S_0$, and $\Delta^\ddagger V_0$ at the reference point of 0.1 MPa and 293 K, as well as $\Delta^\ddagger \kappa'$, according to Eq. (6). (A) Flip rates derived from $R_{1\rho}$ relaxation dispersions, based on simultaneous fits of all data (all temperatures) at a given pressure using the restrictions $k_{\text{flip}}(T_{\text{high}}) > k_{\text{flip}}(T_{\text{low}})$, and $R_{2,0}(T_{\text{high}}) \leq R_{2,0}(T_{\text{low}})$. (B) Flip rates as in A, fitted with the restriction $\Delta^\ddagger \kappa' = 0$. (C) Global fitting of $\Delta^\ddagger G_0$, $\Delta^\ddagger S_0$, $\Delta^\ddagger V_0$, and $\Delta^\ddagger \kappa'$ directly to all temperature and pressure dependent relaxation dispersion data. In C, flip rates are subsequently calculated from the fitted thermodynamic parameters, and error bars are derived by error propagation; for this reason, error bars are larger for data points further from the reference point.

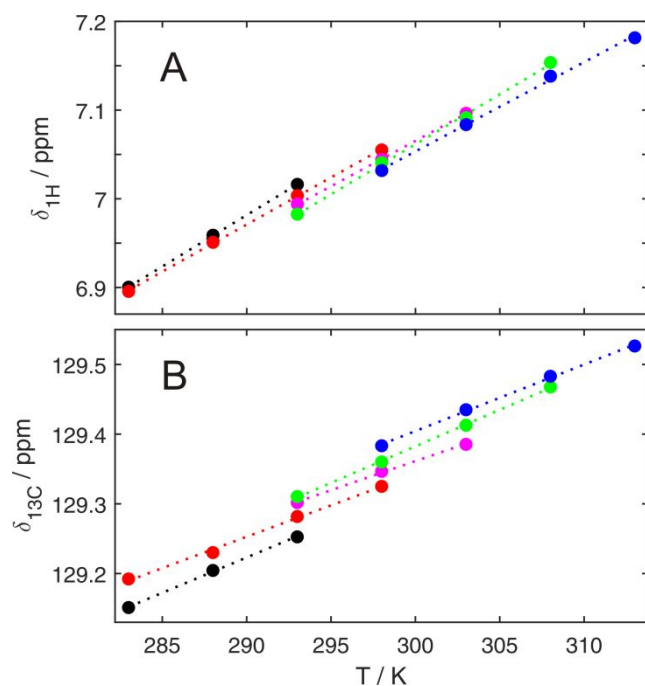


Figure S3: Temperature and pressure dependence of chemical shifts of the averaged signal of F52 ϵ . $\delta_{1\text{H}}$ (A) and $\delta_{13\text{C}}$ (B) are plotted as a function of T at pressures of 0.1 MPa (black), 50 MPa (red), 100 MPa (magenta), 150 MPa (green) and 200 MPa (blue). Dotted lines depict linear fits of $\delta(T)$ at the respective pressures. Slopes of the linear fits range from 0.010 to 0.011 ppm/K for ^1H and from 0.008 to 0.010 ppm/K for ^{13}C . At temperatures of 293 and 298 K, shift changes with pressure are less than or equal to 0.22 ppm/GPa for ^1H and 0.39 ppm/GPa for ^{13}C , respectively.