Supporting Information for:

Increasing the exchange time-scale that can be probed by CPMG relaxation dispersion NMR

Pramodh Vallurupalli, Guillaume Bouvignies and Lewis E. Kay*

Robustness of $\Delta \tilde{\delta}_{ex}$ and $\varpi_{sQ} - \varpi_{MQ}$ values with respect to temperature miscalibration: Key to the methodology presented is the ability to measure accurate ground state chemical shifts in HSQC and HMQC data sets that are obtained at a number of different static magnetic fields. Because different instruments are used it is essential that the experimental parameters be as similar as possible. We have investigated how small differences in temperature can affect measured values of $\Delta \tilde{\delta}_{ex}$ and $\varpi_{sQ} - \varpi_{MQ}$ using the I44A,V67A FF domain exchanging system as a test case. Values of $\Delta \tilde{\delta}_{\scriptscriptstyle ex}$ are calculated from shift differences measured in HSQC data sets obtained at a pair of magnetic fields so that temperature miscalibration between instruments would be expected, at least in some cases, to have significant implications. In contrast $\varpi_{sQ} - \varpi_{MQ}$ is obtained as a difference between chemical shifts measured from HSQC and HMQC data sets that are recorded at the same magnetic field (same instrument). Thus, the effect of temperature 'subtracts out' to first order, so that even when values of $\varpi_{sQ} - \varpi_{MQ}$ measured at different static magnetic fields are used in the data analysis small temperature miscalibrations are not pathological.



Figure S1: $\Delta \tilde{\delta}_{ex}$ values are more sensitive to temperature miscalibration than $\overline{\omega}_{SQ} - \overline{\omega}_{MQ}$ (A) Linear correlation of $\Delta \tilde{\delta}_{ex}$ values measured on a sample of the I44A,V67A FF domain. Shift differences were obtained from spectra recorded at 11.7 and 18.8T where the temperature is set to 36°C for both measurements, Y-axis, and from spectra recorded with a 0.2°C temperature difference, corresponding to 36°C (18.8T) and 35.8°C (11.7T), X-axis. (B) Comparison of $\overline{\omega}_{SQ} - \overline{\omega}_{MQ}$ values measured on the I44A,V67A FF domain at 36°C (Y-axis) and at 35.8°C (X-axis).