# Supporting Information

for the manuscript

**Backbone Assignment of Perdeuterated Proteins Using Long-Range H/C-Dipolar Transfers**

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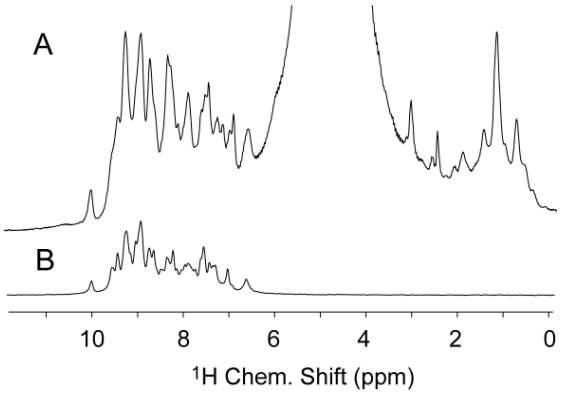
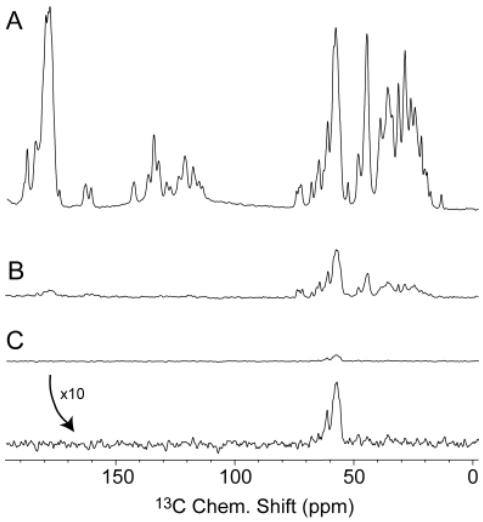
*Transfer efficiencies:*



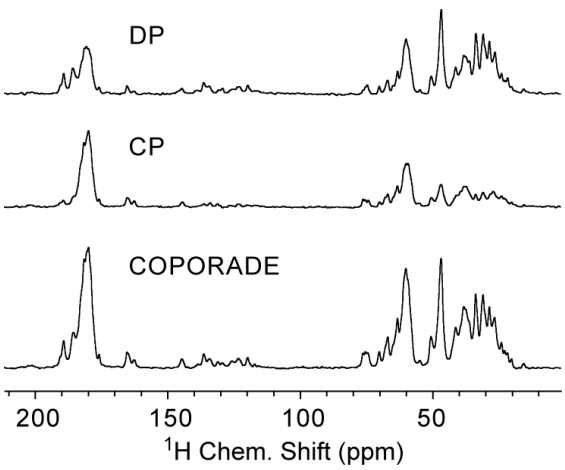

**Supporting Figure 1:** Comparison between (A) direct excitation and (B) an experiment in which proton magnetization is transferred to $^{15}$N and back (pulse scheme A in Supporting Information of (Linser et al. 2011b)). Signal decreases by 2.96fold (to 33.8%), meaning a decrease to 58% per transfer. Spectra were recorded using 128 scans each and apodized employing 10 Hz exponential linebroadening.




**Supporting Figure 2:** Comparison between (B) direct H/C CP transfer and (C) HNC double CP (DCP). The last strip represents the HNC spectrum scaled up 10-fold. A) shows the direct excitation spectrum for comparison, roughly 4x higher in signal intensity than the H/C CP spectrum due to the reduced protonation of the sample (25% in amides, and around 3% in each site on aliphatic carbons). The DCP spectrum including two transfers is 7.3-fold weaker than the transfer from $^{1}$H to $^{13}$C directly.

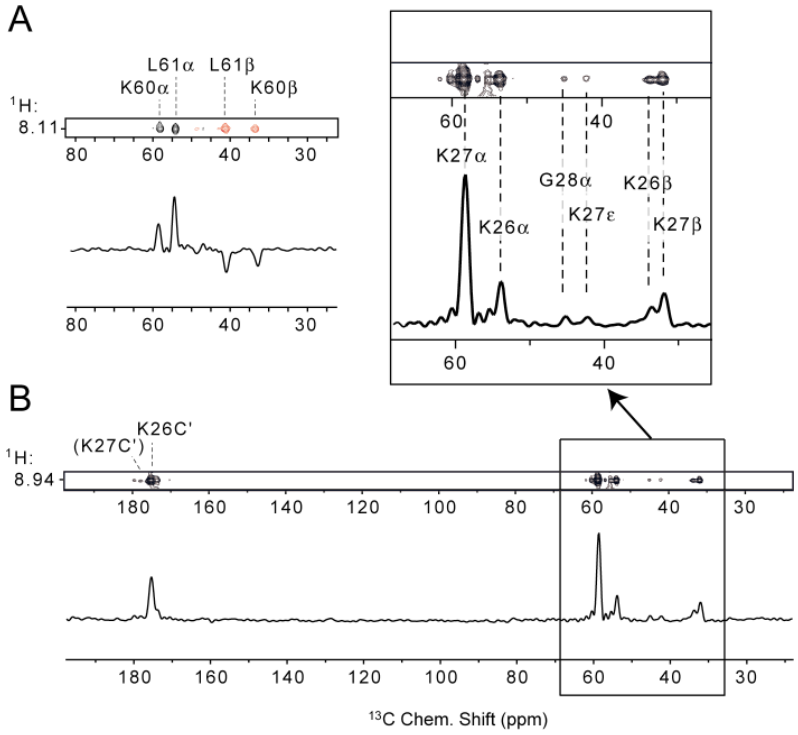
Spectra were recorded with 128 scans apart from the DCP spectrum (512 scans, but scaled down accordingly) with 1 s recycle delay (apart from 5.4 s for $^{13}$C direct excitation) and apodized using 100 Hz exponential linebroadening.

*Excitation:*



**Supporting Figure 3:** $^{13}C$ excitation profiles of COPORADE excitation at 500ms recycle delay. Only a quarter of directly excited $^{13}C$ spins will give rise to a signal in experiments with subsequent magnetization transfer to partially back-substituted amide protons as employed in this work.

*Distribution of peak intensities:*




**Supporting Figure 4:** Visualization of the distribution of signal intensities obtained in an $hC^{x}hNH$ experiment (B) as compared with an HNCACB experiment (A). The spectra were recorded with comparable parameters, using 3 and 2.5 d of spectrometer time for A) and B), respectively. Strips shown were taken at $^{15}N$ chemical shifts of 125.9 and 122.2 ppm in A) and B), respectively, and were chosen to reflect a representative intensity distributions. Compared to interresidual CO, intra- and interresidual $C^{\alpha}$, and intraresidual $C^{\beta}$ resonances, intraresidual CO and interresidual $C^{\beta}$ peaks bear a relatively weak signal to noise in many cases. Additionally,

other correlation signals turn up occasionally with a very weak s/n, like G28α and K27ε in this strip.

The 3D strips of an hC$^{x}$hNH experiment show a relatively high variation of ratios between inter- and intraresidual peaks, which can be explained by structure-dependent H$^{N}$-C$^{x}$ distances rather than distance-independent scalar interactions. Although there are exceptions with higher intraresidual CO signals for example, a peak in the H/N plane splits up in the $^{13}$C dimension by a representative ratio of intensities of approximately 43%:14%:10%:3%:26%:3%, referring to peaks from intra- and intermolecular C$^{\alpha}$, intra- and intermolecular C$^{\beta}$, and intra- and intermolecular CO, respectively. Based on the intensity obtained in the first 1D and 2D slices of 3D experiments, an intensity comparison was pursued regarding the hC$^{x}$hNH experiment proposed here and out-and-back HNCO, HNCA, and HNCACB experiments with a dipolar H/N but scalar N/C transfer. Comparing first 1D spectra of 64 scans each, all recorded on the SH3 domain, we find a factor of 2.7 in signal to noise of the hC$^{x}$hNH experiment over both, the HNCO and the HNCA. The HNCACB sensitivity can only be assessed via HC 2D planes due to evolution of both, positive and negative terms. In comparison to an HNCA, we find an intensity loss of 57 % averaged over representative groups of C$^{\alpha}$ peaks in the HNCACB. In both experiments, all interresidual correlations are weaker in intensity than intraresidual correlations by approximately 30 %, and C$^{\beta}$ peaks in the HNCACB are approximately 2-fold lower than C$^{\alpha}$ peaks, as determined from a 3D HNCACB recorded over 3d. Intraresidual carbonyl correlations as obtained from HNCACO experiments have been described in the literature to be lower than the HNCO correlations by a factor of 4, as assessed for a similar sample preparation. (Linser et al. 2010a)

Consequently, for this preparation (which has comparably long $T_2$ ($^{15}$N)), the hC$^{x}$hNH experiment delivers signal to noise that (in comparison to scalar transfer-based experiments) is higher for intraresidual C$^{\alpha}$ (1.9fold) and C$^{\beta}$ (1.6 fold) and weaker for inter- (70%) and intraresidual CO (32%), interresidual C$^{\alpha}$ (92%), and interresidual C$^{\beta}$ (70%).

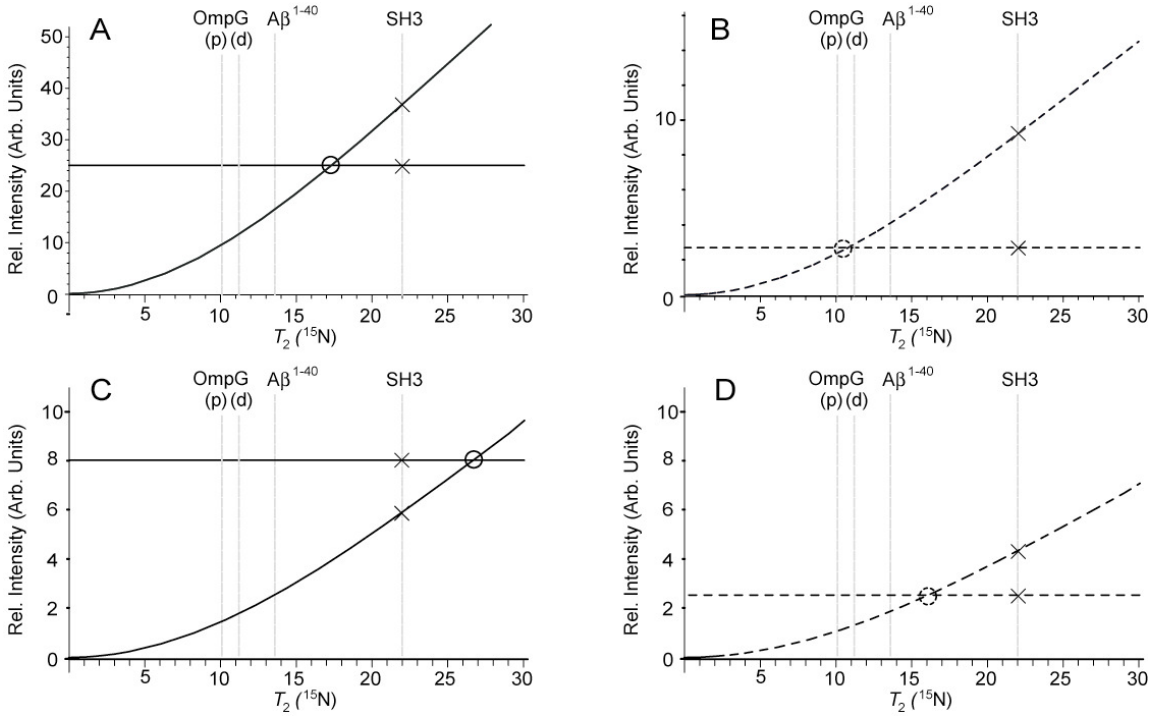



**Figure 5:** Approximation of peak intensities obtainable for carbonyl (interresidual correlation, A, and intraresidual correlations, B) and $C^{\beta}$ resonances (intraresidual correlations, C, and interresidual correlations, D) as observed for the SH3 domain of chicken α-spectrin and samples providing shorter $^{15}N$ relaxation times $T_2$. The relative intensities for long-range dipolar transfer experiments as obtained for this preparation can be assumed to be $T_2$ ($^{15}N$) independent to a first-order approximation and are depicted as horizontal lines. The intensities obtainable for scalar transfer-based experiments are shown by matching a theoretical $T_2$ dependence (see main manuscript) with values obtained for this preparation (at the position of the rightmost grey bar). The theoretical performance for scalar-based experiments on samples with lower $^{15}N$ $T_2$ can be extracted from the curves at the positions marked by the other grey bars. Circles mark the expected $^{15}T_2$ at which a break-even $T_2$ of the scalar and dipolar based experiments occurs. Shorter $T_2$ left from the circles would benefit from experiments based on long-range dipolar H/C transfers. (p) and (d) denotes preparations of OmpG reconstituted in protonated and deuterated lipids, respectively. The relaxation values are taken from (Linser et al. 2011b). The ordinate values are to be read as arbitrary units. *J*-couplings chosen to calculate optimal transfer efficiency during INEPTs were 15 Hz for $^{1}J_{NCO}$ and 9 Hz for transfer with both, $^{1}J_{NC\alpha i}$ and

$^{1}J_{NC\alpha(i-1)}$. INEPT duration optimized experimentally for the SH3 domain was 24 ms for each of the two steps, evolution and reconversion of $N_xC_z$ antiphase magnetization.