Supporting informations for

High-resolution 3D CANCA NMR experiments for complete mainchain assignments using C^{α} direct-detection

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Supplemental Figure 1. C α -C α plane of the CANCA 3D experiment for the 52 kDa protein GST. The

NMR spectrum was recorded on a Bruker (Billerica, MA) Avance 500 spectrometer equipped with a

triple-resonance carbon-detection optimized cryogenic probe (TXO). The spectrum was recorded in 5 days at 25°C for 1mM sample of [ul-²H, ¹⁵N, ¹³C] GST dimer (2mM in monomer concentration) in D₂O buffer containing 10 mM sodium phosphate (pH 6.8), 100 mM NaCl. The spectral width for carbon is 2753 Hz (centered at 55 ppm). 512 and 100 complexed data points were uniformly recorded for direct and indirect dimensions, respectively. The recycling delay was set to 4.5 sec.



Supplemental Figure 2. Comparison of signal intensities in the CANCA and CANCO experiments.

(A) 1D trace of CANCA (left) and CANCO (right) experiments for 6 kDa and 73 kDa conditions. 4 mM uniformly ²H¹⁵N¹³C-labeled GB1 sample was used for this comparison. Residue numbers are indicated for the resonances that are used for calculating average intensities. (B) Normalized signal

intensity of CANCA and CANCO experiments simulating various molecular weights. Each point corresponds to the average intensity for selected resonances (see A). All points are normalized against the first point of the CANCO experiment (308 K condition: 6 kDa). The broken line indicates the noise level of the spectra used in this comparison.