Supporting Information

Very fast two-dimensional NMR spectroscopy for real-time investigation of dynamic events in proteins on the time scale of seconds

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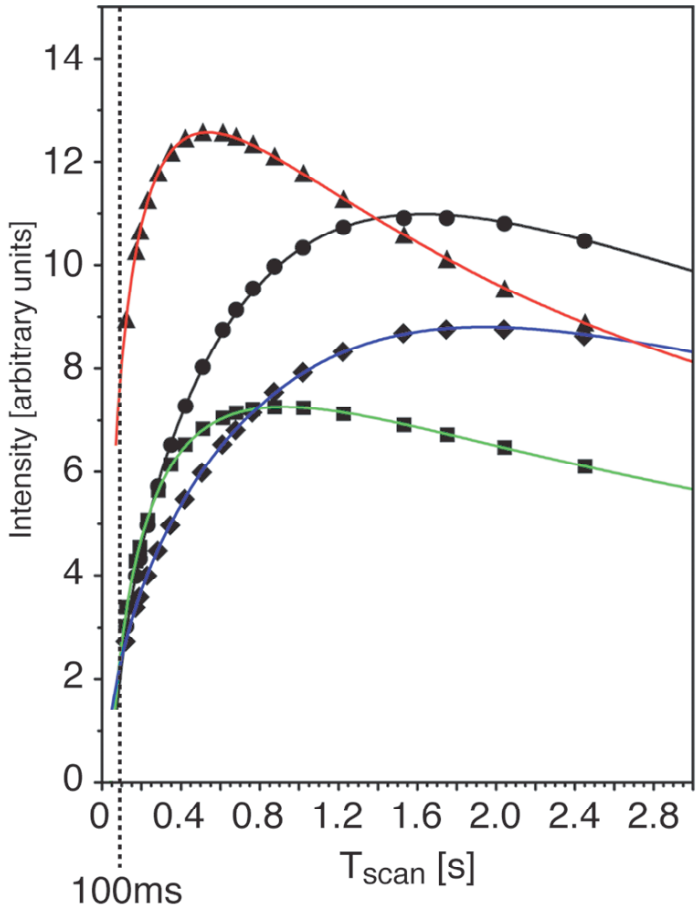

Figure S1: Sensitivity comparison of different ^{1}H-^{15}N correlation experiments: FHMQC with flip angle 120° (squares), FHSQC (diamonds), sensitivity-enhanced (se) water-flipback (wfb) HSQC (circles), and the new SOFAST-HMQC with flip angle 120° (triangles). The signal-to-noise ratio obtained for constant overall experimental time in a series of 1D ^{1}H-^{15}N correlation spectra, recorded on a 2mM sample of ubiquitin (pH 4.7) at 800MHz, is plotted versus the repetition time T_{scan} (comprising the duration of the pulse sequence, the acquisition time and the recycle delay). 1D spectra were recorded for the different experiments by setting t_1=0 using the pulse sequences provided in the Varian Proteinpack for FHSQC and se-wfb-HSQC. The FHMQC sequence was set up according to Ross et al. (*J. Biomol. NMR* 1997, 10, 389). No ^{15}N decoupling was applied during detection in order to avoid heating the probe. The excitation flip angle α in the FHMQC and SOFAST HMQC experiments was set to 120°. Intensities were obtained by integration of the 1D spectra in the amide proton range (7.1 to 9.4 ppm). In addition, to account for the intrinsic sensitivity gain obtained by phase-modulated quadrature detection, the se-wfb-HSQC and FHMQC data were scaled by a factor of $\sqrt{2}$. This comparison does not take into account differences in line width along the ^{15}N dimension between the various experiments. These differences, however, will only little affect the sensitivity comparison as long as t_1^{max} is kept small.

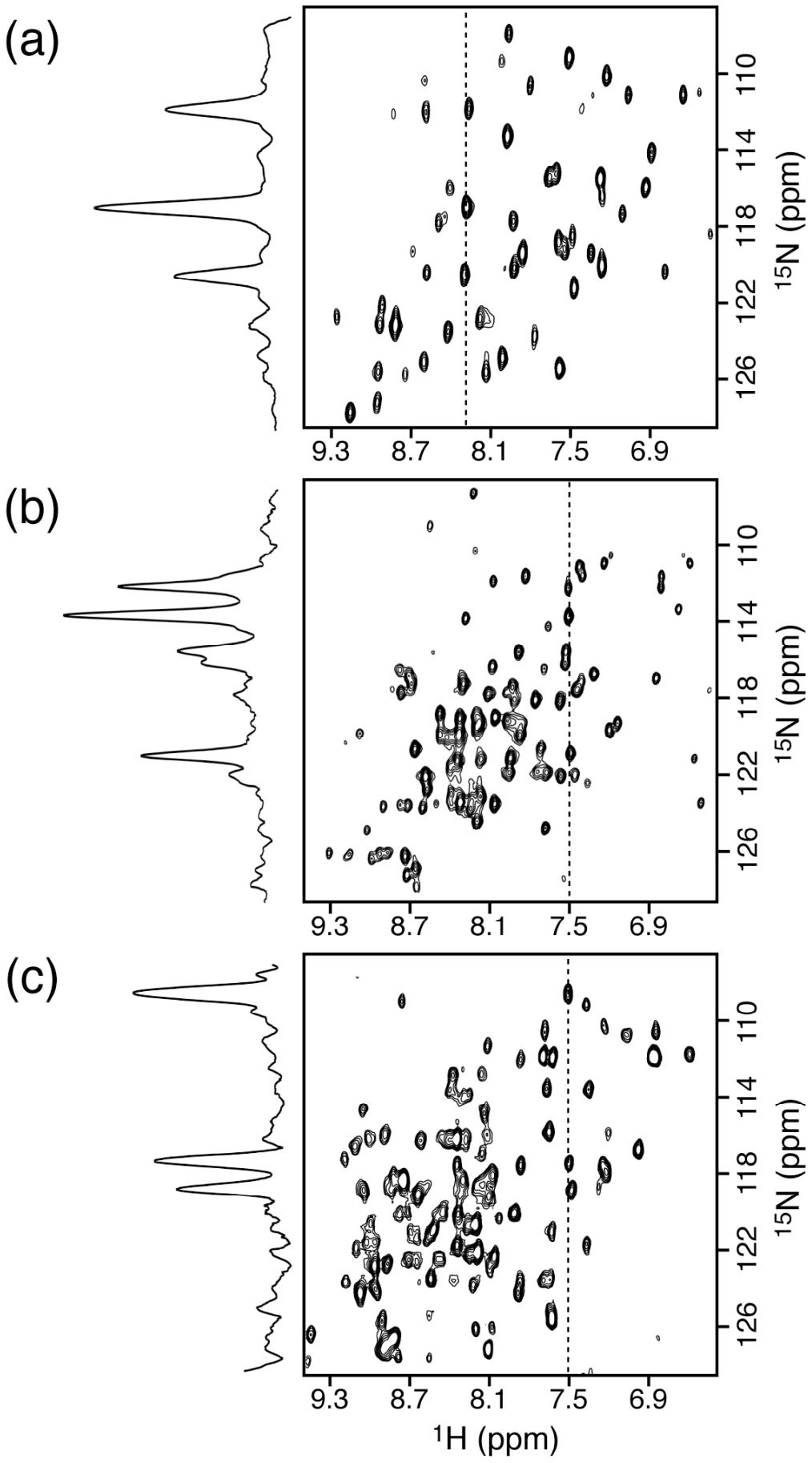
(a)
(b)
(c)
9.3
8.7
8.1
7.5
6.9
110
114
118
122
126
^{15}N (ppm)
^{1}H (ppm)

Figure S2:

^{1}H-^{15}N correlation spectra (central part) recorded using the SOFAST-HMQC sequence on different protein samples : (a) MerAa, protein fragment of the mercuric reductase MerA from *R. metallidurans* (8.2kDa, 1.5mM, pH 7.5, 25°C); (b) SiR-FP18, flavodoxin-like domain of the *E. coli* sulfite reductase (18kDa, 1.8mM, pH 7.0, 25°C); (c) BRP-Blm complex, *S. hindustanus* bleomycine resistance protein (homodimer) in a 1:1 complex with Zn(II)-ligated bleomycine (30.4kDa, 1mM, pH 6.5, 40°C). The band selective ^{1}H pulses were centered at 8.0ppm covering a band width of 4.0ppm. ^{15}N decoupling during signal detection was realized using WURST-2 at an average field strength of $\gamma B_1/2\pi$=550Hz. Gradient strengths and durations were set to 15G/cm and 1ms for G_1, and 20G/cm and 100μs for G_2. All experiments were performed on a Varian INOVA spectrometer operating at 800MHz ^{1}H frequency using the following acquisition parameters : (a) α=120°, n=48, t_1^{max}=14ms, t_2^{max}=50ms, and t_{rec}=1ms, (b) α=120°, n=100, t_1^{max}=28ms, t_2^{max}=40ms, and t_{rec}=20ms, (c) α=120°, n=120, t_1^{max}=17ms, t_2^{max}=40ms, and t_{rec}=50ms. A longer recycle delay was used for proteins with higher molecular weight (increased tumbling correlation time) to experimentally optimize the signal to noise ratio for a given acquisition time. We are currently investigating in detail the influence of molecular weight on the optimal choice of the various acquisition parameters. The results of this study will be presented elsewhere. All spectra were recorded with 4 additional dummy scans to reach a steady state, resulting in total acquisition times of (a) **3s** for MerAa, (b) **8s** for SiR-FP18, and (c) **14s** for BRP-Blm. For data processing the raw data were multiplied with a squared cosine function prior to Fourier transformation along both dimensions. Additional base-line correction in the ^{1}H dimension was applied before Fourier transformation along the ^{15}N dimension. The data were zero-filled to final matrices of 1024*1024 data points. The 1D traces are extracted along the ^{15}N dimension at the ^{1}H frequency indicated by a dashed line in the corresponding 2D spectra.