CHEMBIOCHEM

Supporting Information

CHEMBIOCHEM

Supporting Information

for

Hydrogen-Bond Detection, Configuration Assignment, and Rotamer Correction of Side-Chain Amides in Large Proteins by NMR through Protium/Deuterium Isotope Effects

Aizhuo Liu,* Jifeng Wang, Zhenwei Lu, Lishan Yao, Yue Li, and Honggao Yan*

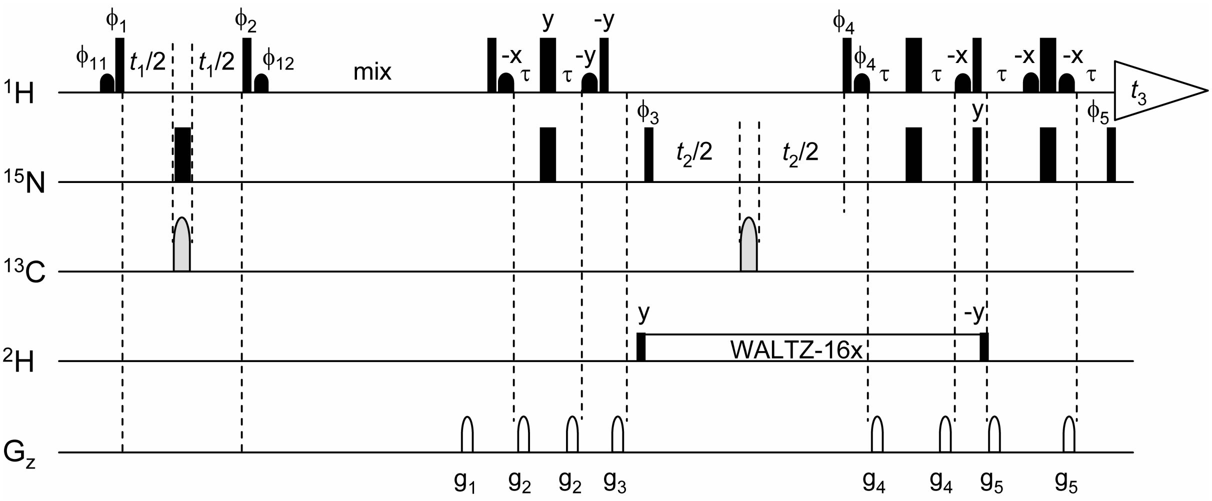
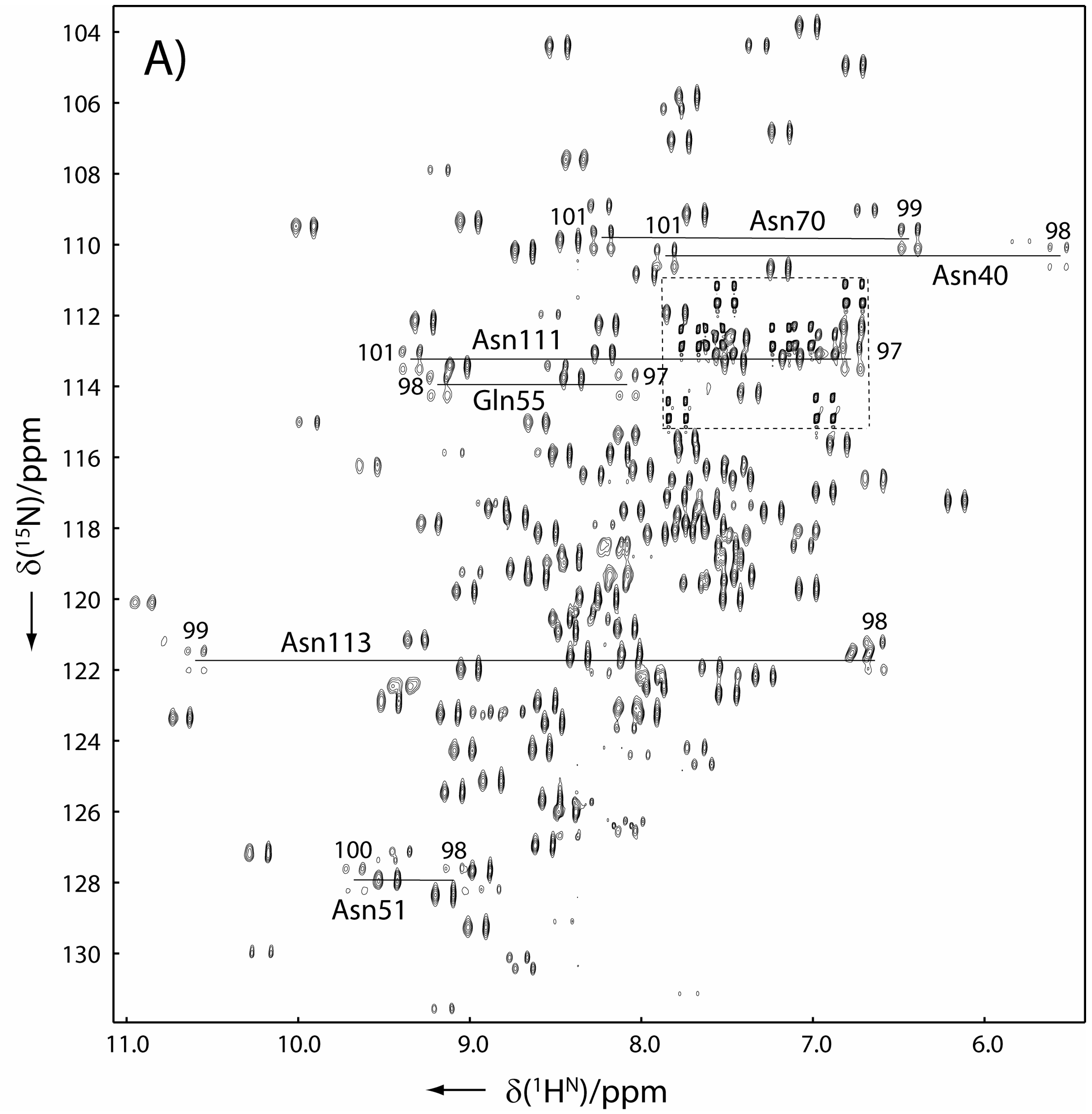


Figure S1. Pulse sequence scheme of 3D NOESY-[^{15}N,^{1}H]-IS-TROSY experiment for the simultaneous NOE observation for both backbone and side chain amides of proteins in the mixture solvent of 50%H_2O/50%D_2O. Unlike existing 3D NOESY-[^{15}N,^{1}H]-TROSY experiments which exclusively detect NOEs with backbone amides, an isotopomer-selective (IS)-TROSY instead of normal TROSY element is used in the new experiment. Narrow and broad black bars indicate 90° pulses and 180° pulses, respectively, in either ^{1}H frequency channel with 24.3 kHz power or ^{15}N channel with 5.0 kHz power. Small black shapes in ^{1}H channel are 90° water-flip-back pulses having a 1.5 ms pulse length with the shape of the central lobe of Sinc function and maximum power level of 283 Hz. The shaped 180° pulse in gray of ^{13}C channel is an adiabatic Chirp pulse with 500 µs width and maximum power of 9.8 kHz. Deuterium decoupling scheme is the WALTZ-16 sequence with phase *x* and flanked with a 90° pulse with phase *y* in front and a 90° pulse with phase –*y* behind at the power level of 1.4 kHz. The offsets in different channels were set at 4.82, 118, 115 and 7.3 ppm for ^{1}H, ^{15}N, ^{13}C and ^{2}H, respectively, with DSS as inner reference at 25 °C. The delay *t* was set to 2.7 ms. Phase cycling scheme was: $f_1 = x$; $f_2 = 4(x), 4(-x)$; $f = (y, -y, -x, x)$; $f = -y$; $f_5 = -x$; $f = -f$; $f = -f$; receiver $f(t_3) = (x, -x, -y, y, -x, x, y, -y)$. Unless specifically indicated, the phase of the pulse was *x*. TPPI for the t_1 dimension was achieved through f_1 with each t_1 increment and for the t_2 dimension by inverting f_3 and receiver $f(t_3)$ with each t_2 increment. For each value of t_2, quadrature detection was achieved by acquiring two FIDs and stored separately with pulse phases $f = (-y, y, -x, x)$; $f = y$; $f = x$ for the second FID. The length of the *z*-axis pulsed field gradients was set to 1 ms with following strengths: g_1 (42 G/cm), g_2 (18 G/cm), g_3 (48 G/cm), g_4 (27 G/cm) and g_5 (30 G/cm) with the sine profile. The gradient g_3 is optional, but necessary for NMR samples in this study in order to further suppress the strong signals from the unlabeled ligand.


A)
104
106
108
110
112
114
116
118
120
122
124
126
128
130
δ(15N)/ppm
11.0
10.0
9.0
8.0
7.0
6.0
δ(1HN)/ppm
101
101
Asn70
99
98
Asn40
101
Asn111
97
98
97
Gln55
99
Asn113
98
100
98
Asn51

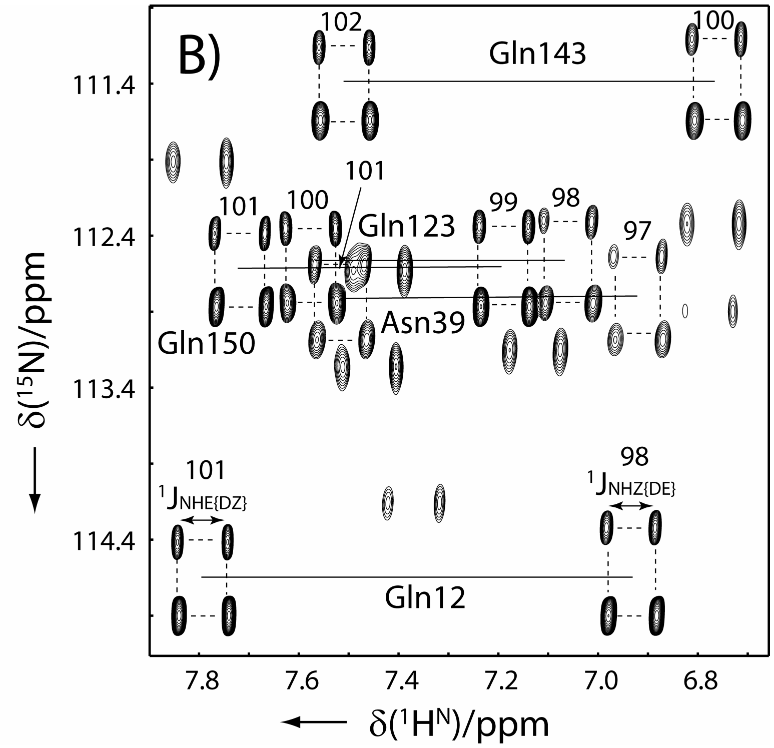


Figure S2. A) ^{15}N-coupled 2D ^{15}N,^{1}H HSQC spectrum of yCD for the measurement of $^{1}J_{NH}$ coupling constants in side-chain amides. 1.5 mM (protomer concentration) u-^{2}H/^{13}C/^{15}N-labeled NMR sample was solved in 75% H_2O/25% D_2O under buffer conditions the same as other preparations. The spectrum was recorded on a Bruker AVANCE 900 MHz NMR spectrometer equipped with a TCI cryoprobe at 25 °C. Each pair of resonances for a side-chain amide is linked by a horizontal line. The protonated doublet in each “quartet” is at ^{15}N downfield and the semideuterated doublet upfield. The one-bond $^{1}J_{NHE\{DZ\}}$ ($^{1}J_{NHZ\{DE\}}$) scalar coupling is measured from the splitting of the semideuterated doublet and shown as ppb in the ^{1}H dimension. The region enclosed with dashed lines is enlarged and shown in B). The spectrum was recorded with 32 scans and a 2.0 s delay time, t_{1max}(^{15}N) = 66 ms and t_{2max}(^{1}H^{N}) = 285 ms, resulting in the experimental time of 4.2 h. Before Fourier transformation, the raw data were zero filled resulting in a 1.0 Hz digital resolution in ^{1}H dimension and 1.5 Hz in ^{15}N diemnsion. Repeated measurements essentially led to the same result.