Supporting Information

NOAH- (^{15}N/^{13}C)-CEST NMR Supersequence for Dynamics Studies of Biomolecules

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Material and Methods

The NOAH-(^{15}N/^{13}C)-CEST supersequence was first tested on a uniformly ^{15}N- and ^{13}C-labeled sample of ubiquitin before it was applied to the uniformly ^{15}N- and ^{13}C-labeled Im7 sample. All NMR experiments were performed on an 850 MHz Bruker Ascend magnet equipped with an Avance III HD console and a triple resonance inverse cryoprobe. There were four experiments performed for each sample: conventional ^{15}N-CEST and ^{13}C-CEST experiments, a ^{13}C-CEST experiment modified to mimic the ^{13}C-CEST block in the supersequence, and the combined ^{15}N-CEST and ^{13}C-CEST supersequence. To mimic the ^{13}C-CEST used in the supersequence, a conventional ^{13}C-CEST experiment was modified to use ^{13}C-magnetization as starting magnetization of the sequence. Furthermore, an extra heat compensation block was added, which was of identical duration with the same decoupling elements applied as the one used in the ^{15}N-CEST in the supersequence. All experiments were recorded with 2k x 128 complex points along the F3 and F2 dimensions, respectively, together with 106 and 101 CEST offset saturation frequencies in F1 for ubiquitin and Im7, respectively. Four transients per FID were obtained with an acquisition time of 75 milliseconds and 180 dummy scans. The sweep widths of proton, nitrogen, and carbon dimensions were 16, 35, and 35 ppm, respectively, with carrier frequencies set at 4.7 ppm for ^{1}H, 118.0 ppm for ^{15}N, 55 ppm for ^{13}C in ^{15}N-CEST and 17 ppm for ^{13}C in ^{13}C-CEST. For the Im7 sample, two experiments were acquired to calibrate the saturation field (B_1) of the ^{15}N-CEST and ^{13}C-CEST. The B_1 field strength $\gamma B_1/2\pi$ for the ubiquitin sample was set to 30 and 40 Hz for ^{15}N-CEST and ^{13}C-CEST, respectively, while for the Im7 sample, the B_1 field strength was set to 25 and 40 Hz for ^{15}N-CEST and ^{13}C-CEST, respectively. The recovery delay d1 used for all sequences was 2 seconds.

All spectra were initially processed in Topspin 3.6.2. For the supersequence experiment, the Bruker AU program “split” was used to separately obtain the resulting spectra from each type of CEST experiment and an AU program written in-house was used to fix some parameters of the ^{13}C-CEST block after data splitting. Further processing was done using NMRPipe with scripts written in-house for the processing of pseudo-3D experiments. Peak fitting was done in NMRPipe and the resulting CEST profiles were obtained and fitted using the ChemEx software (http://www.github.com/gbouvignies/chemex) for a 2-site exchange process in the case of Im7. For the signal-to-noise ratio calculation, the noise level was determined using median average deviation in a region of the spectra free of resonances (consisting of ~35k points) using MATLAB Version R2021b (The Mathworks, Inc, Natick, MA).