

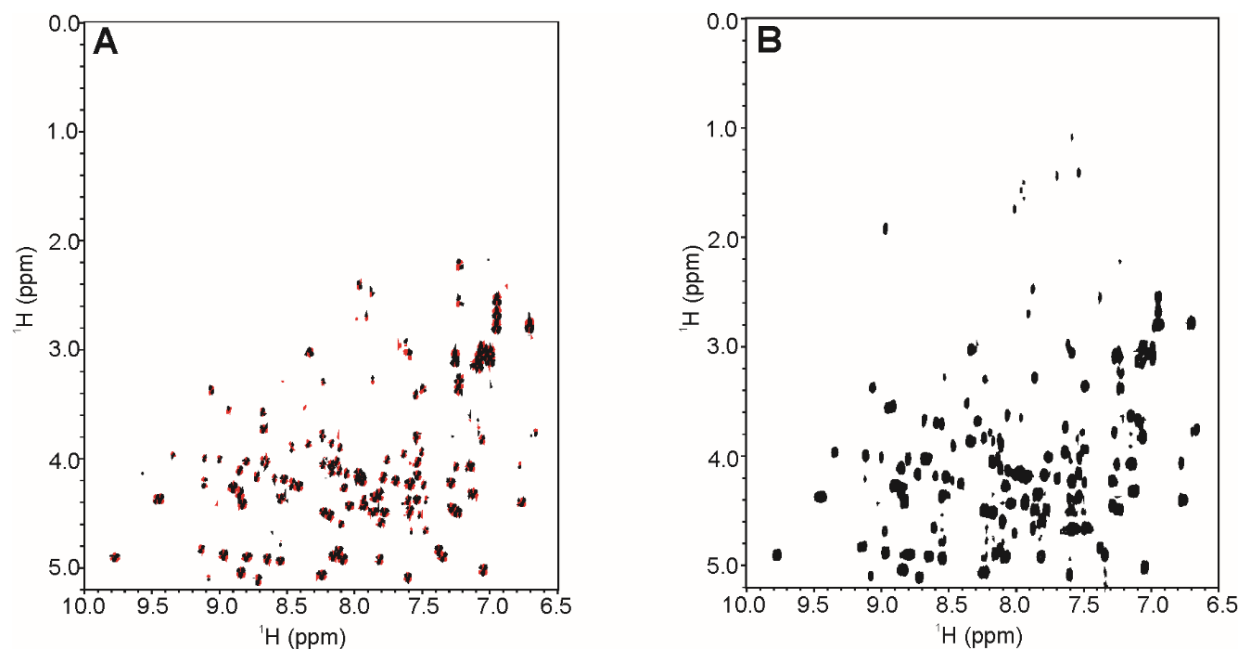
## Appendix A: Supporting Materials for

### 2D $J$ -Correlated Proton NMR Experiments for Structural Fingerprinting of Biotherapeutics

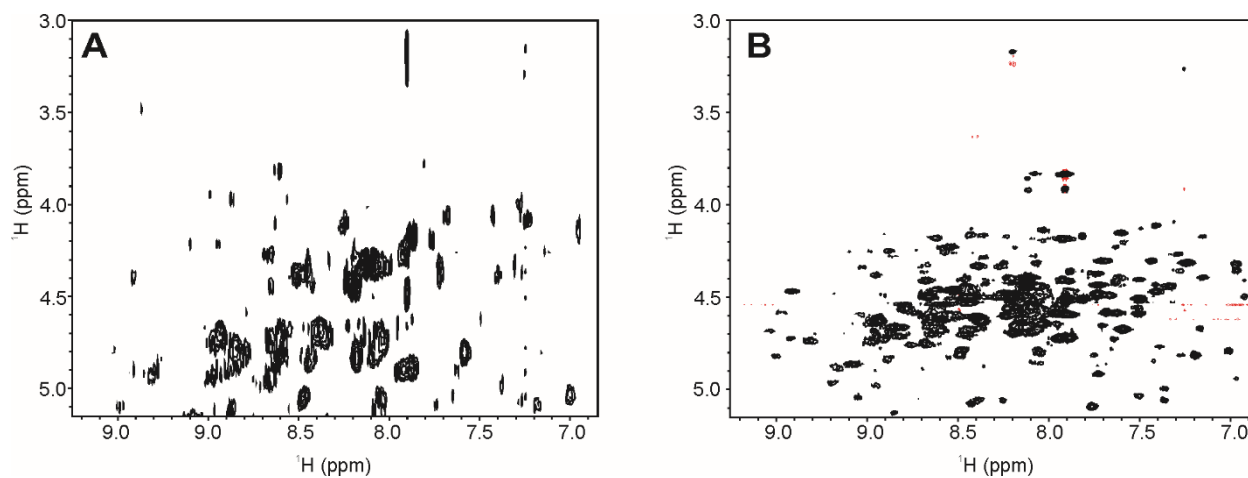
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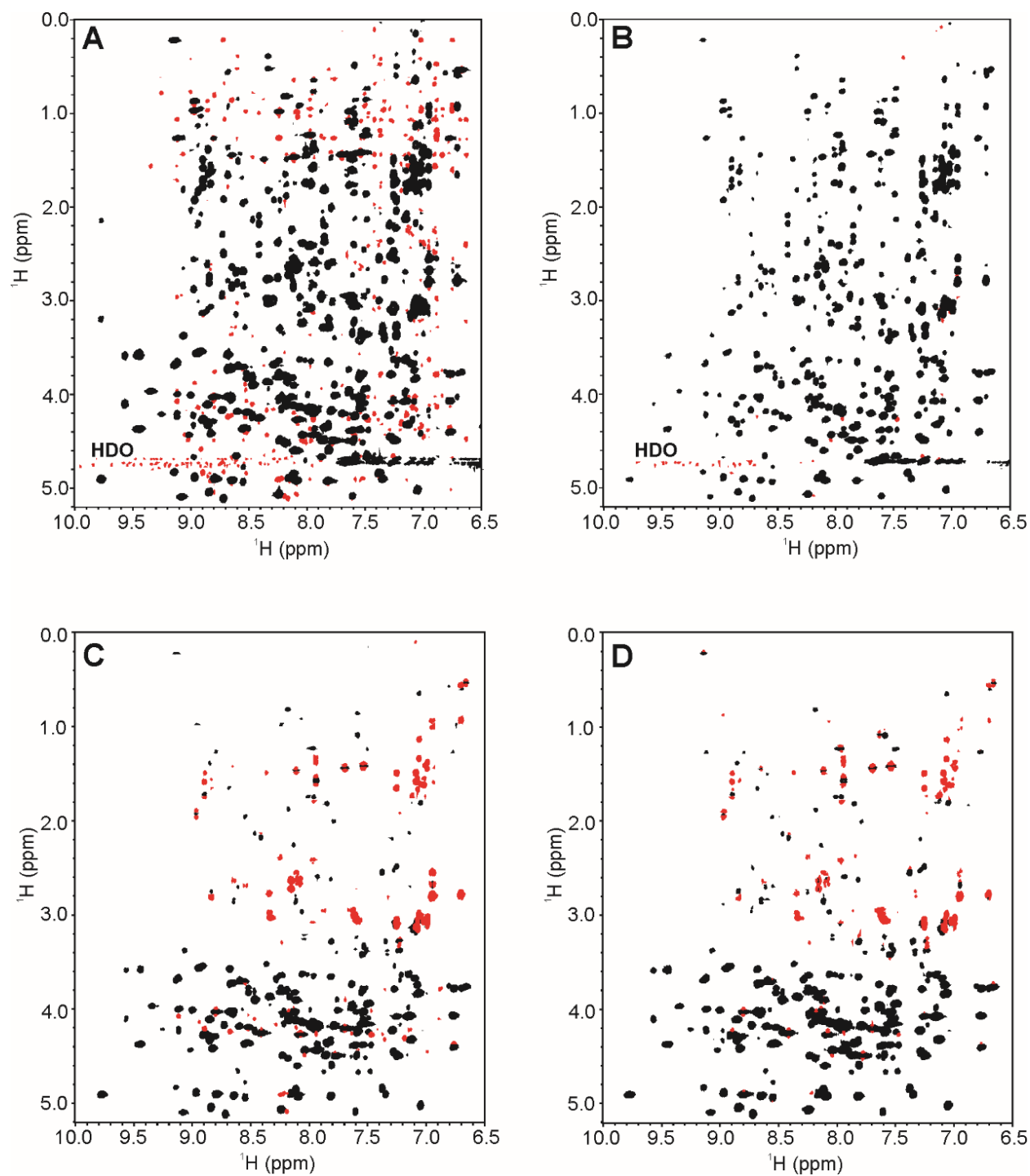
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**Fig. S1.** 2D  $^1\text{H}$  COSY spectral fingerprints of  $^1\text{H}_\text{N}$  -  $^1\text{H}_\text{aliphatic}$  region of HEWL collected at 600 MHz and 25 °C. (A) 2D  $^1\text{H}$  DQF-COSY; (B) 2D IP-COSY. Positive contours are shown in black and negative contours in red. The upfield aliphatic region is included for comparative purposes. While setting 3.0 ppm as the upfield cut-off for the fingerprint region, only a few upfield  $\text{H}_\alpha$  resonances are lost for subsequent analysis. However, as can be seen from the TOCSY spectra (Figure S2A, B), many other side chain  $^1\text{H}$  resonances appear  $\leq 3.0$  ppm. Choosing this cut-off predominantly selects for  $^1\text{H}_\text{N}$ - $^1\text{H}_\alpha$  correlations.



**Fig. S2.** 2D  $^1\text{H}$  homonuclear spectra of  $^1\text{H}_\text{N}$  -  $^1\text{H}_\text{aliphatic}$  region of the NIST-Fab collected at 600 MHz and 50  $^\circ\text{C}$ . (A) 2D  $^1\text{H}$  IP-COSY; (B) 2D  $^1\text{H}$  COIN-TACS using DIPSI-2rc. Positive contours are shown in black and negative contours in red. Negative artifacts in panel B are from residual water, apodization artifacts, or magnetization leakage in the COIN-TACS. The IP-COSY was collected with 32 scans per transient and the COIN-TACS with 16 scans per increment, affording total experimental times of 3 hours 19 minutes and 8 hours 23 minutes, respectively. All other experimental parameters were as described in the Materials and Methods section and Table S2.



**Fig. S3.** 2D  $^1\text{H}$  TOCSY-type spectra of  $^1\text{H}_\text{N}$  -  $^1\text{H}_\text{aliphatic}$  region of lysozyme collected at 600 MHz and 25  $^\circ\text{C}$ . (A) 2D  $^1\text{H}$  TOCSY using DIPSI-2; (B) 2D  $^1\text{H}$  TOCSY using DIPSI-2rc; (C) 2D  $^1\text{H}$  COIN-TACSYP using DIPSI-2; (D) 2D  $^1\text{H}$  COIN-TACSYP using DIPSI-2rc. Positive contours are shown in black and negative contours in red. ROE artifacts in panels A and C are removed by the addition of a relaxation compensation delay in the DIPSI-2rc pulse train (panels B and D). Additional positive and negative artifacts in panels C and D are from magnetization leakage in the COIN-TACSYP.



**Table S1: Relative peak intensities: qualitative comparison of the sensitivity of the 2D  $^1\text{H}$ ,  $^1\text{H}$  J-correlated spectra**

	Relative Peak Height†				
	Slice at 5.05 ppm		Slice at 4.07 ppm		
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Clean TACS	1.00	1.00	1.00	1.00	1.00
TACS	0.63	0.66	0.76	0.77	0.78
TOCS	0.89	1.09	0.75	0.65	0.80
Clean TOCS	0.33	0.35	0.46	0.27	0.82
IP-COSY	0.26	0.26	0.08	0.09	0.08

†Each peak is normalized to the intensity in the clean COIN-TACS experiment. The five peaks are labeled accordingly in Fig. 3.

**Table S2: Selected Experimental parameters and overall experimental times. See Materials and Methods Section for complete details.**

	Peak Type	F1, total points	F2, total points	Mixing Time	Scans	Experimental Time
Clean TACS	$\text{H}_\text{N}-\text{H}_\alpha$	2,690	1,024	85 ms	8	3 h
TACS	$\text{H}_\text{N}-\text{H}_\alpha$	2,690	1,024	83 ms	8	3 h
Clean TOCS	$\text{H}_\text{N}-\text{H}_\text{all}$	2,690	1,024	80 ms	8	3 h
TOCS	$\text{H}_\text{N}-\text{H}_\text{all}$	2,690	1024	80 ms	8	3 h
IP-COSY†	$\text{H}_\text{N}-\text{H}_\alpha$	2,690	208	N/A	8	0.5 h
DQF-COSY	$\text{H}_\text{N}-\text{H}_\alpha$	2,690	1,024	N/A	32	12 h

†Due to the use of a constant time period for  $t_1$  evolution, the maximum number of total points that could be collected was 208.

### Supplementary References

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