Electronic Supplementary Material (ESI) for Molecular Omics.

Supplementary Information

CPMG based TOCSY: Efficient Toolbox for Metabolomics

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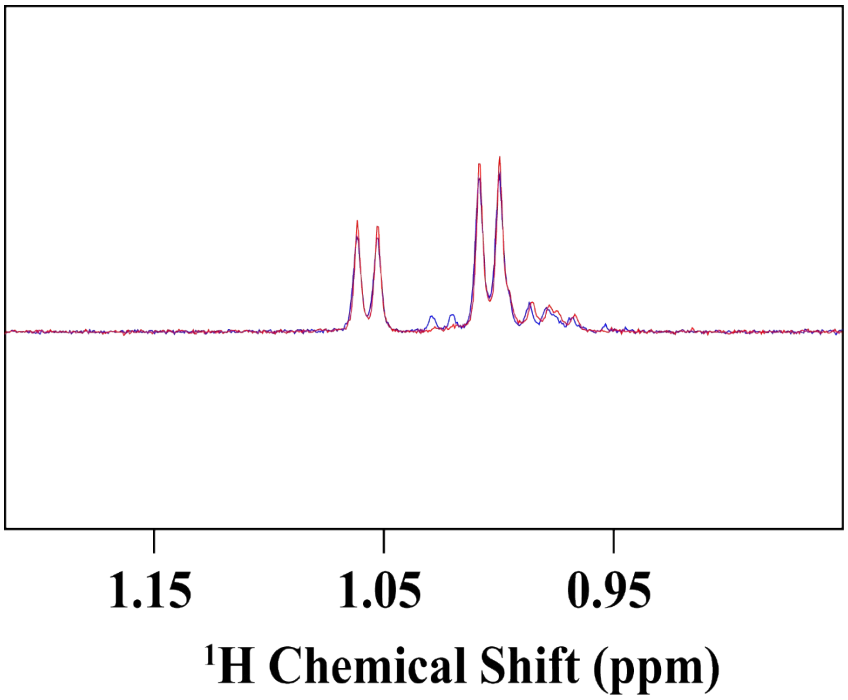

Figure S1: Overlay of selective ^{1}H TOCSY using Reburp-shaped 180° inversion pulse with a duration of 80ms (blue) and 300ms (red) with the same offset. 300ms was more effective for selective excitations.

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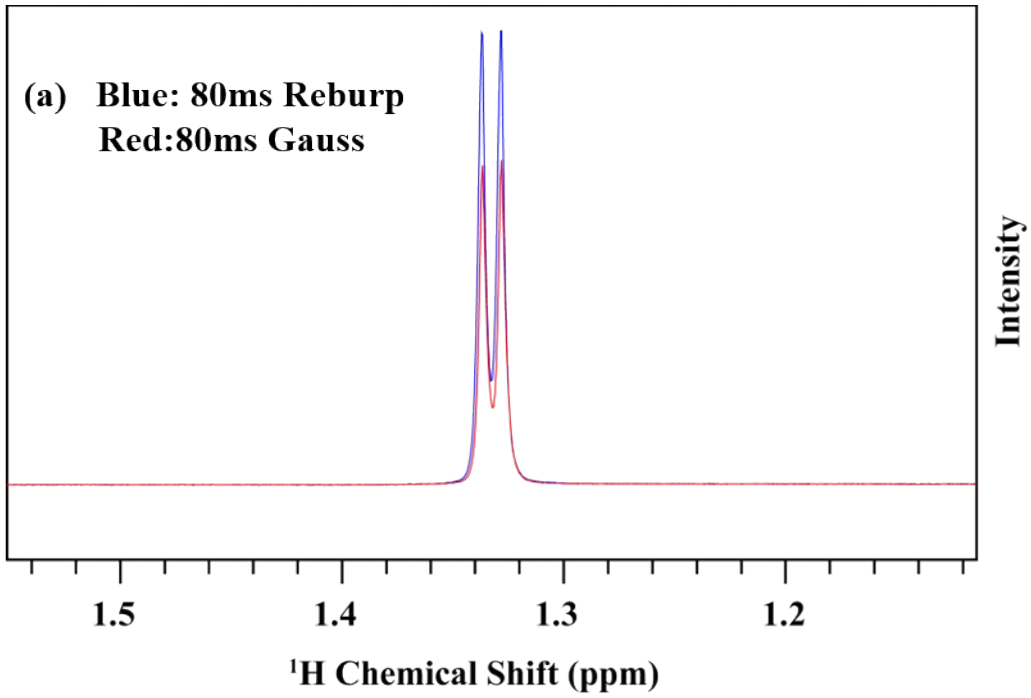

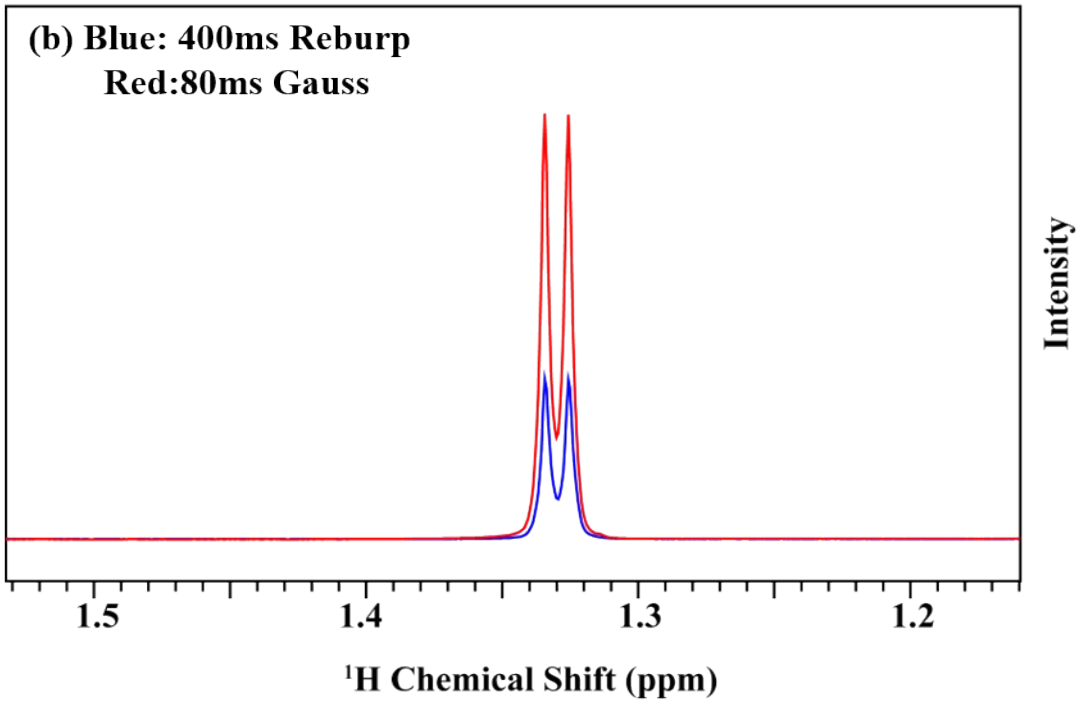

Figure S2: (a) Overlay of 1D CPMG-TOCSY spectra highlighting the peak of lactate at 1.33 ppm. The blue denotes the spectra using Reburp as refocusing pulse and red indicates usage of Gauss_180 as refocusing pulse. In each case, the duration was kept 80ms. The integral drops more than 30% (1:0.69) upon using the Gauss upon keeping the duration of the pulse same (80ms). However The bandwidth of 80ms Reburp (78 Hz) is more than 80ms Gauss_180 (15 Hz). The bandwidth of 400 ms Reburp (15 Hz) is equivalent to 80ms Gauss_180 (15 Hz). (b) represents the overlay of 1D CPMG-TOCSY spectra highlighting the peak of lactate at 1.33 ppm using 400ms Reburp as refocusing pulse (blue)

and 80 ms Gauss_180 as refocusing pulse (red). In each case, the bandwidth was kept same 80ms. The integral drops more than 60% (1:0.38) upon using the Reburp keeping the bandwidth same (15 Hz). The Reburp was chosen for its better off-resonance effect.

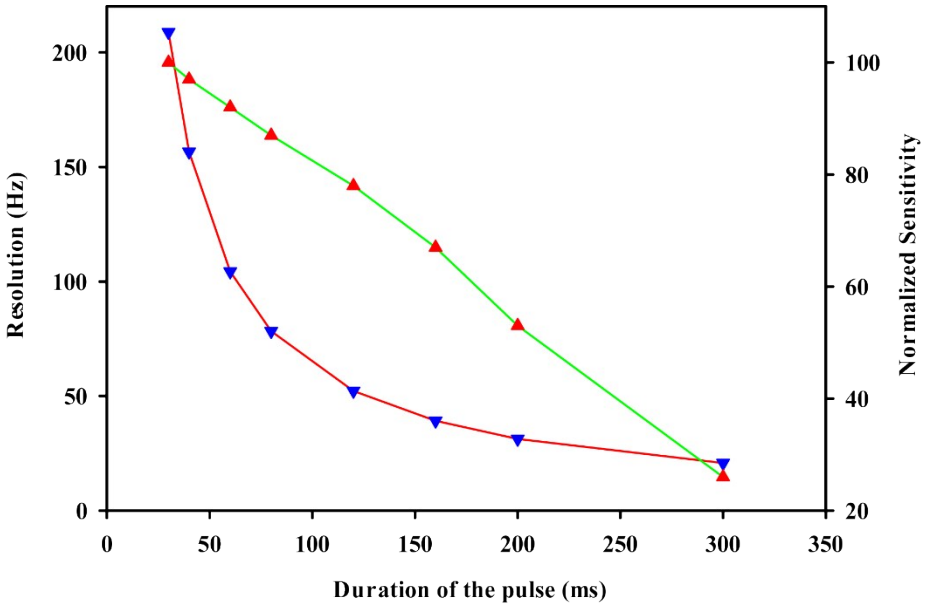

Fig S3: The excitation bandwidths of the Reburp pulses (denoted as resolution and shown as a blue down triangle) are plotted against the duration of Reburp pulses (ms). For the sensitivity analysis, an isolated doublet peak of lactate resonance at 1.33 ppm was integrated and normalized to 100 for the lowest duration pulse of 30ms. The normalized integral value is denoted as sensitivity and plotted as red up-triangle and is plotted as a function of the applied pulse duration. The corresponding sensitivity axis is shown on the right side of the graph.

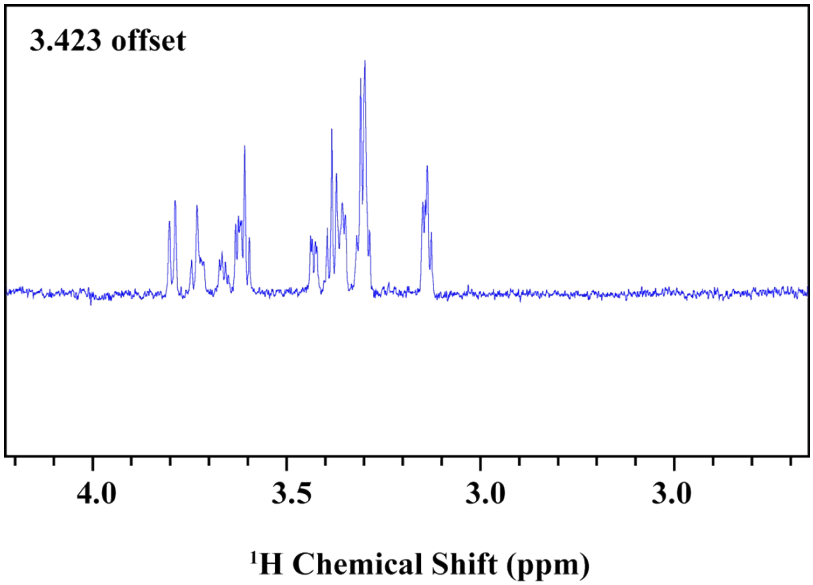

Fig S4: 1D selective ^{1}H CPMG TOCSY spectra of glucose with an offset of 3.42 ppm. The data is recorded with a 300ms Reburp pulse, 16 scans, and 5 seconds recycle delay. The total measurement time was 2min 16 seconds.

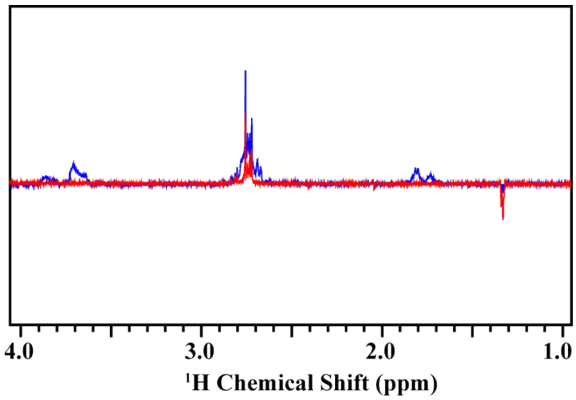

Fig S5: Overlay of the 1D selective ^{1}H CPMG TOCSY spectra with offset at 2.75ppm, which is embedded in broad lipid resonance, with (red) and without (blue) CPMG. Clearly, with CPMG, much cleaner spectra can be achieved.

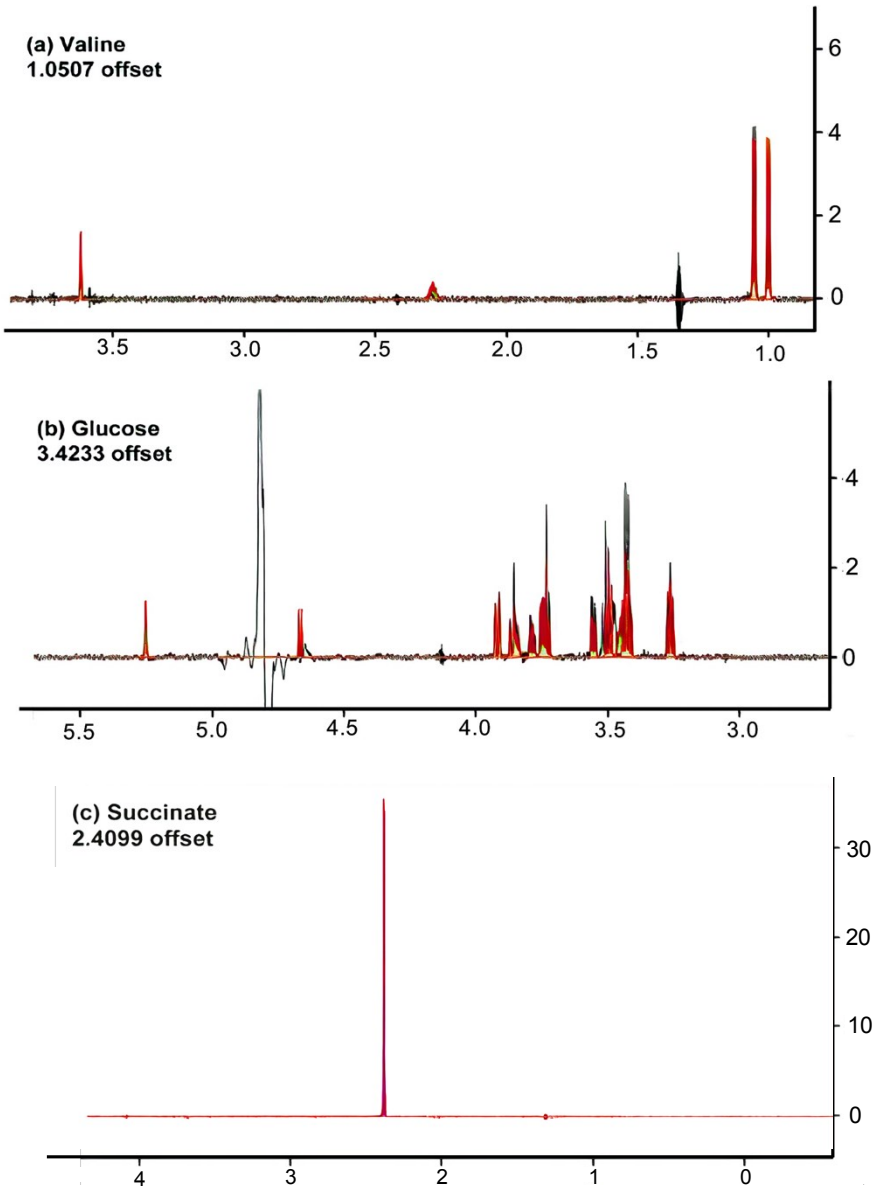

Fig S6: Overlay of the 1D selective ^{1}H CPMG TOCSY spectra with different offset (black) frequencies and the peak pattern from the inbuilt library of Chenomx (red). Offset frequencies are denoted within each panel. A clear match of the experimentally observed signal and the peak pattern of the inbuilt library was observed for valine (a), glucose (b), and succinate (c).

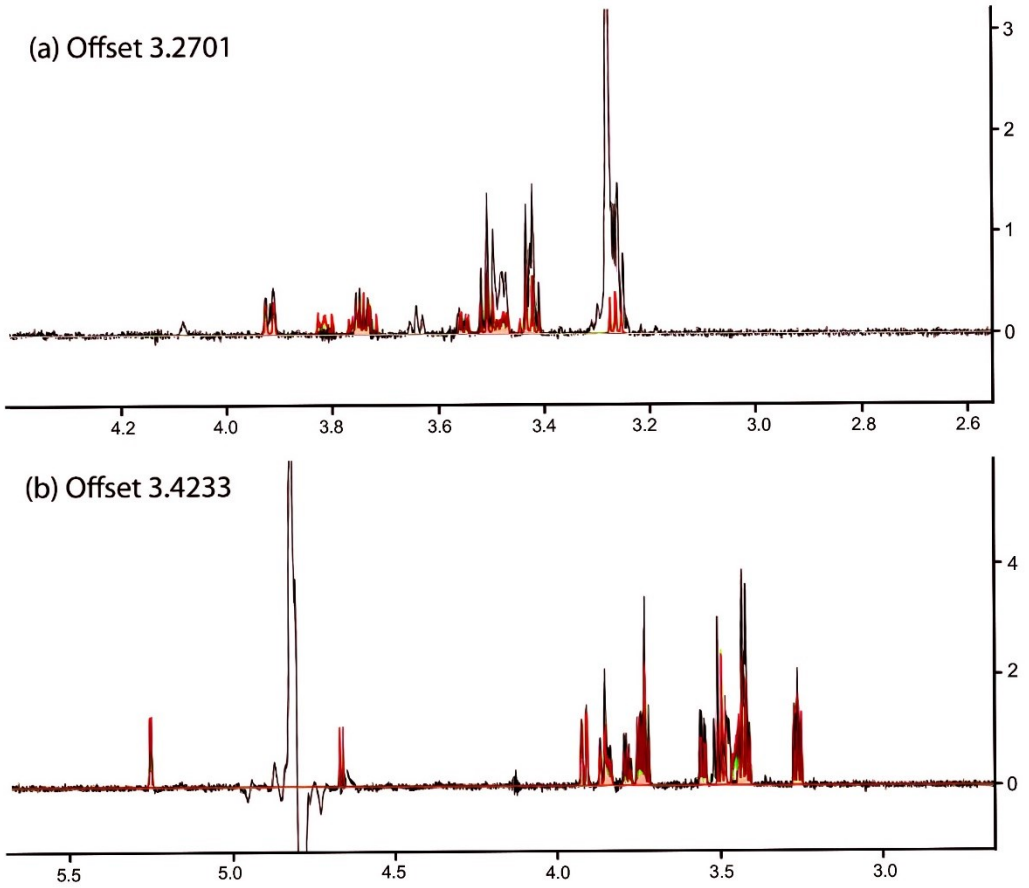

Fig S7: Overlay of the 1D selective ^{1}H CPMG TOCSY spectra with different offset (black) frequencies and the peak pattern from the inbuilt library of Chenomx (red). Offset frequencies are denoted within each panel. When the offset was chosen at 3.2701 ppm (panel a), assuming this is only one of the glucose peaks, the resultant spectra do not match the library's standard glucose peak patterns upon shifting the offset to 3.4233 ppm. A clear match of the experimentally observed signal and the peak pattern of the inbuilt library was observed for glucose.

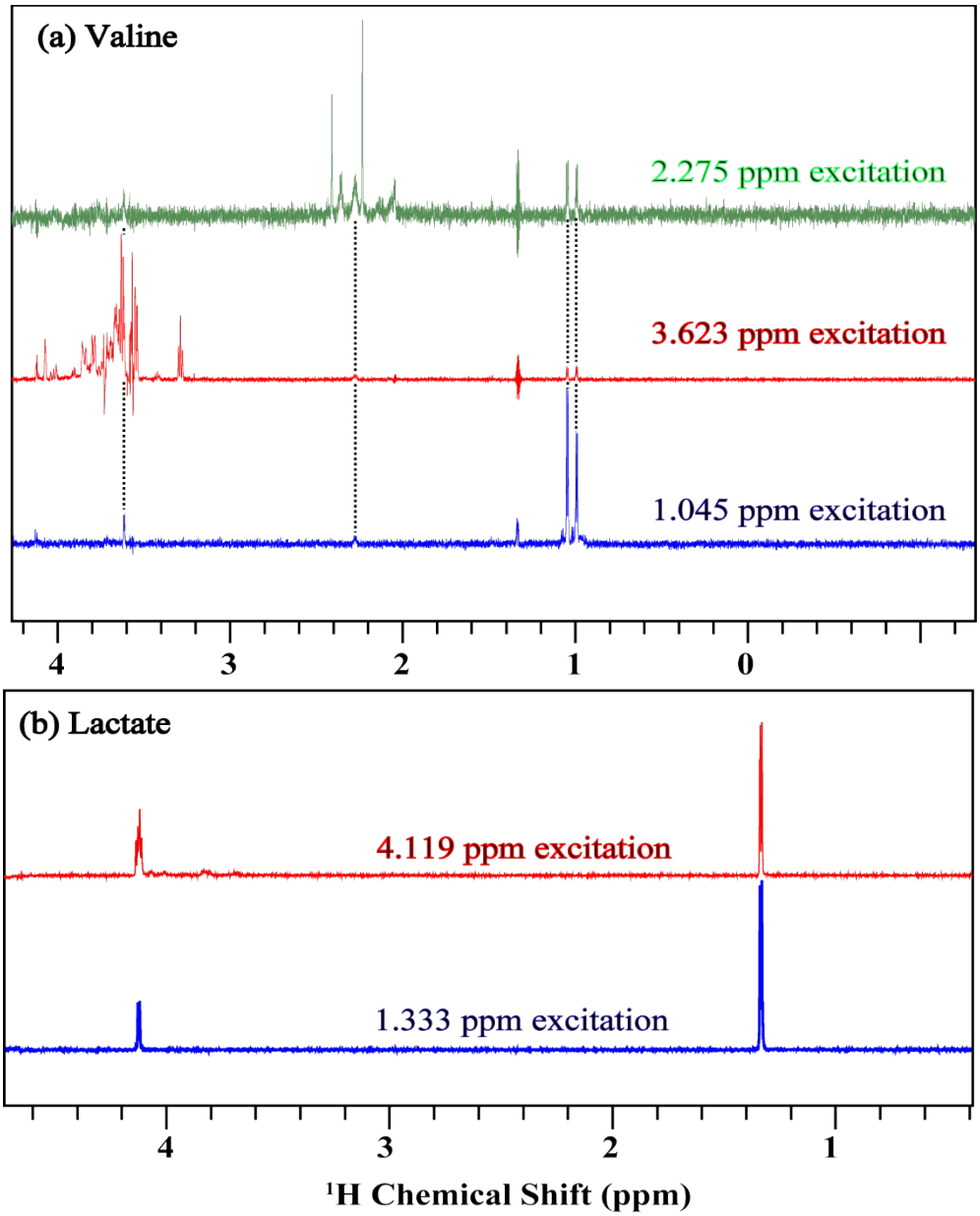

Fig S8: The 1D selective ^{1}H CPMG TOCSY spectra with different offset frequencies for valine (a) and lactate (b). For valine, we find that shifting the offset to the 3.623 or 2.275 excites multiple peaks. The TOCSY pattern is preserved and the chemical shifts can be confirmed as highlighted by dotted black lines in (a). For the isolated resonances of lactate, shifting of offset between 4.119 and 1.333 ppm yields identical spectra (b).

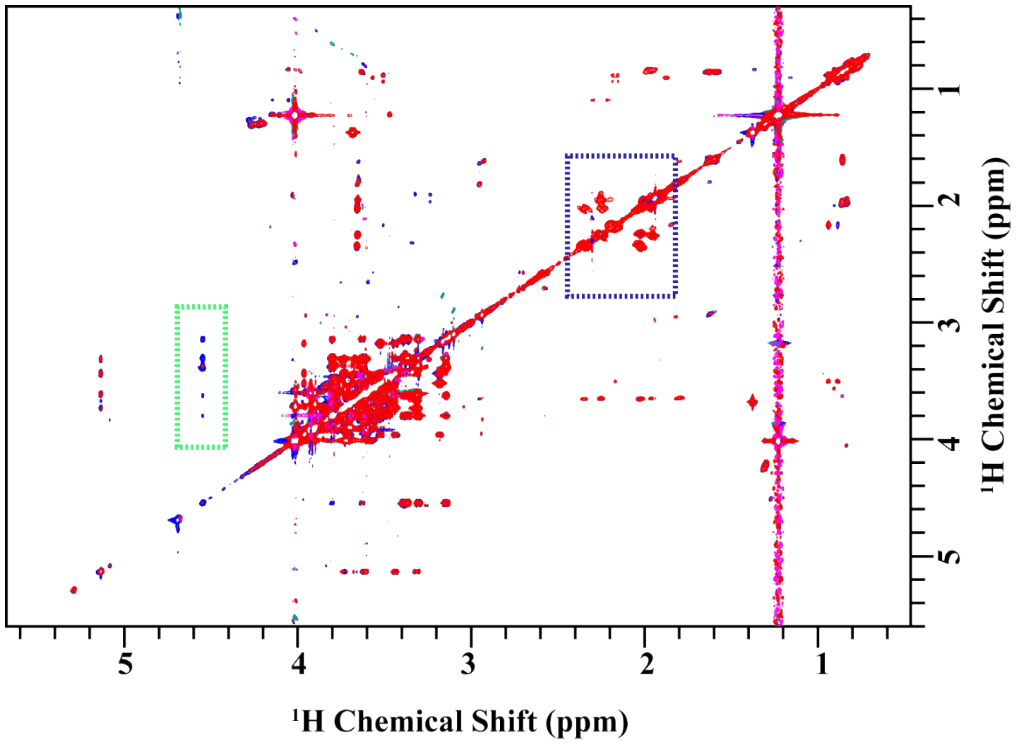

Figure S9: Overlay of the 2D ^{1}H-^{1}H TOCSY spectra acquired with 3-9-19 water suppression (blue) and with excitation sculpting (red). Near the vicinity of the water signal at 4.5 ppm, 3-9-19 performed better, as highlighted by the green box. Some peaks around 2.2 ppm showed better sensitivity upon using excitation sculpting, as underlined by the purple box.

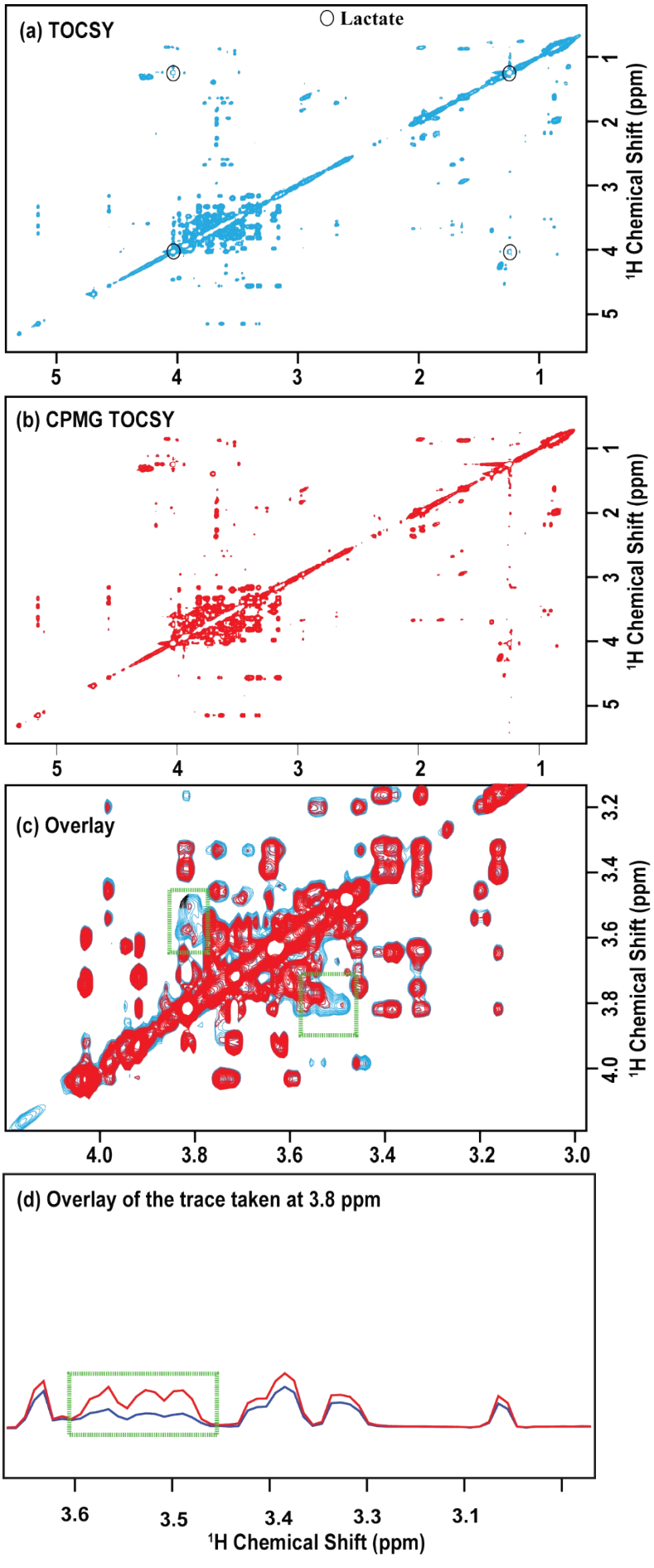
(a) TOCSY
Lactate
(b) CPMG TOCSY
(c) Overlay
(d) Overlay of the trace taken at 3.8 ppm
¹H Chemical Shift (ppm)

Figure S10: (a) The 2D ^{1}H-^{1}H TOCSY spectra (blue) and (b) the 2D ^{1}H-^{1}H CPMG TOCSY spectra (red) of bovine serum. (c) represents the overlay of (a) and (b). In (a), the lactate resonance assignment is highlighted. The highlighted regions marked with green rectangles indicate the cleaning of the spectra. To illustrate it further, we have taken trace along 3.8ppm from (a) and (b) and have shown the overlay of the traces in (d). The broad signals coming from the macromolecules or from the metabolites bound to macromolecules are reduced, as highlighted by the green rectangle in the same region.

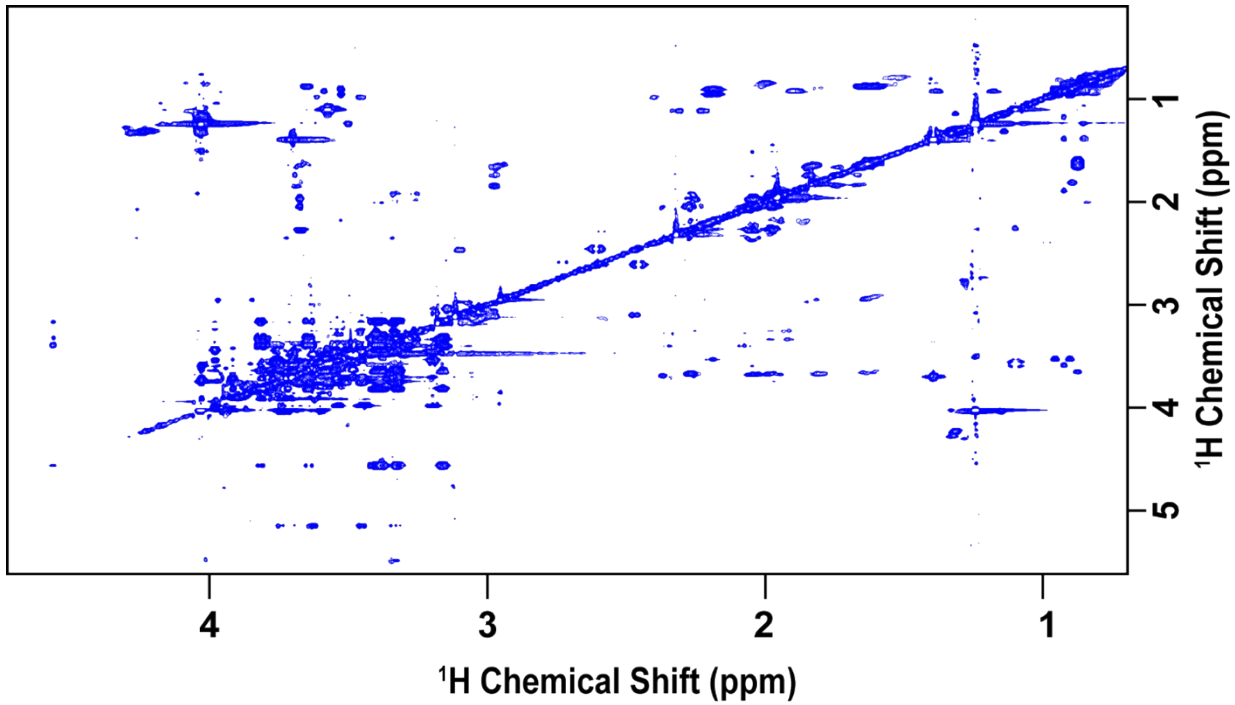

Figure S11: The 2D ^{1}H-^{1}H CPMG TOCSY spectra of bovine serum acquired with 50% non-uniform sampling. The spectra were processed with IST reconstruction.

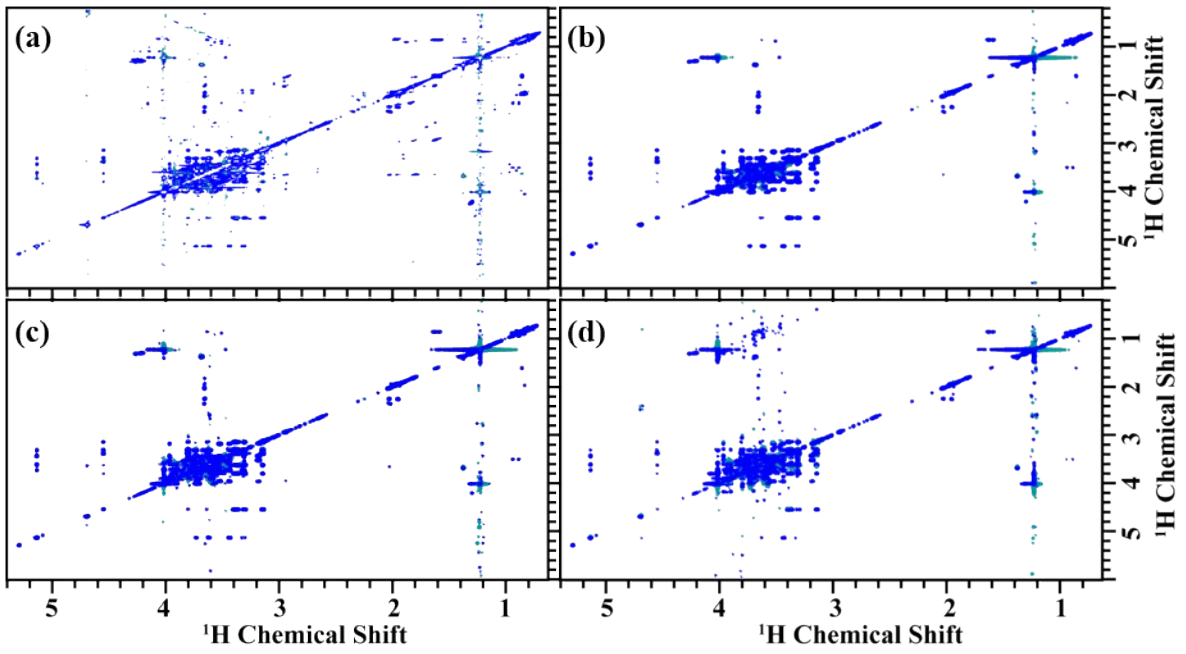

Figure S12: The 2D ^{1}H-^{1}H CPMG TOCSY spectra of bovine serum acquired with linear (100%) acquisition (a), 50% non-uniform sampling (b), 25% non-uniform sampling (c) and 12.5% non-uniform sampling (d). The spectra were processed with IST reconstruction.

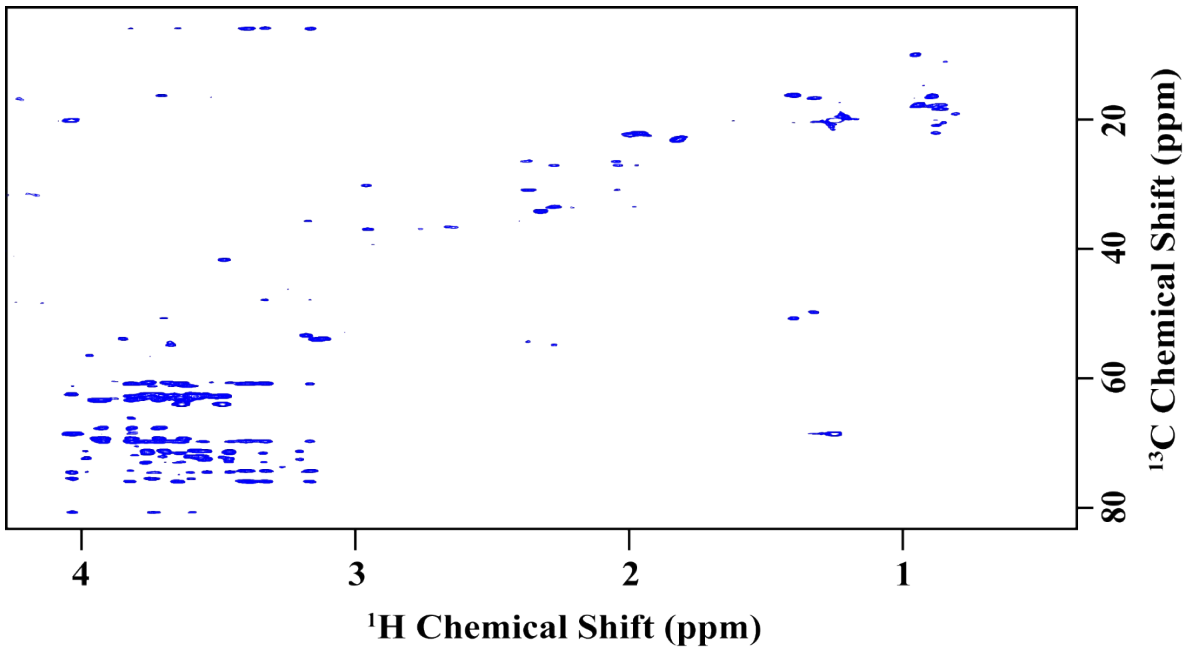

Figure S13: The 2D CPMG-HSQC TOCSY spectra (T_2 delay = 120ms) of bovine serum. The pulse program is depicted in Fig 1(c).