Supporting Information for

Guidelines for the Use of Deuterium Oxide (D_2O) in ^1H NMR Metabolomics

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Experimental Section

Superposition of creatinine CH_3 signal with metformin in 2D HSQC and ^1H spectra

A 2D-HSQC NMR experiment (hsqcetgpsisp2.2) was acquired using a pooled urine sample (QC, based on n=227 samples) from a cohort including patients with various systemic diseases (Gil et al., 2018)[1]. Parameters were used as follows: 4096 x 840 data points were collected using 512 scans per increment, an acquisition time of 0.25 s, and 16 dummy scans. The spectra width was set to 12 and 230 ppm in the 1H and 13C dimension. In addition, 7 selected samples with visible overlap of creatinine and metformin were selected from the dataset to illustrate the overlap of creatinine-CH_3 and metformin.

T_1 measurements for CH_2 and CHD creatinine peaks

The determination of T_1 was executed using a pooled urine sample via an inversion recovery experiment. The standard experiment (t1ir) containing the excitation sequence was complemented by addition of a solvent suppression array[2] (t1iresgp). Delays were defined to be 0.05, 0.1, 0.2, 0.3, 0.5, 0.8, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 sec. Following parameters were used: ns = 72 and ds = 4 per increment, sw = 16 ppm, aq = 1 sec.
Spectra were imported into Matlab software (R2011b; Mathworks). Integrals were calculated using trapezoidal numerical integration. T_1 relaxation times were calculated via polynomial fitting of peak areas over relaxation delays (τ) and determination of zero-crossing points.

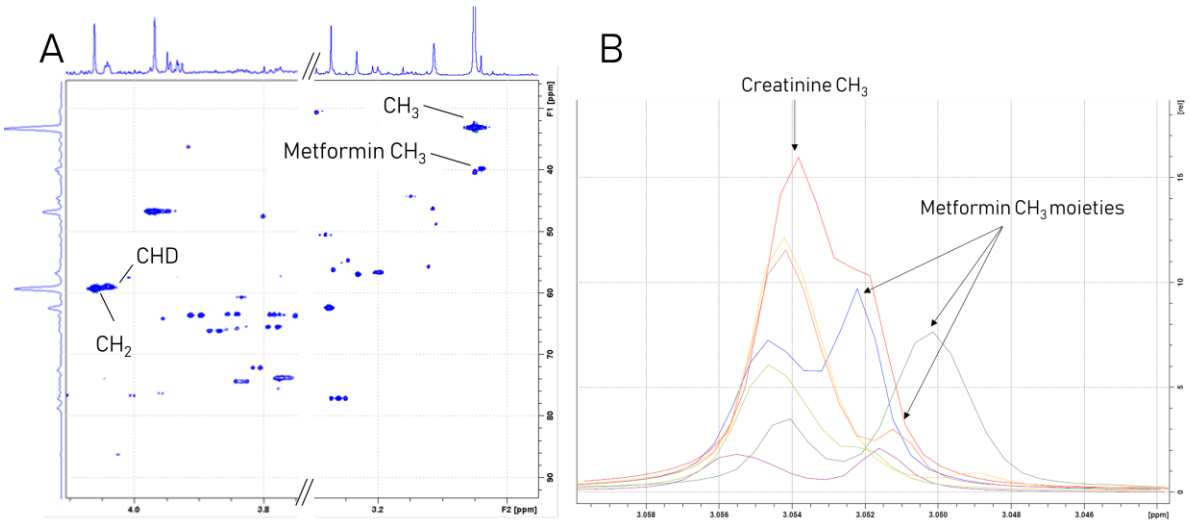

Figure S1: (A) 2D-HSQC from a QC sample highlighting the metformin-overlap of the creatinine-CH_3, while the creatinine-CH_2 and creatinine-CHD show little to no overlap with other signals. (B) Overlap of selected urine samples from a chronic kidney diseases (CKD) study containing metformin with annotation of creatinine CH_3 and metformin CH_3 moieties.

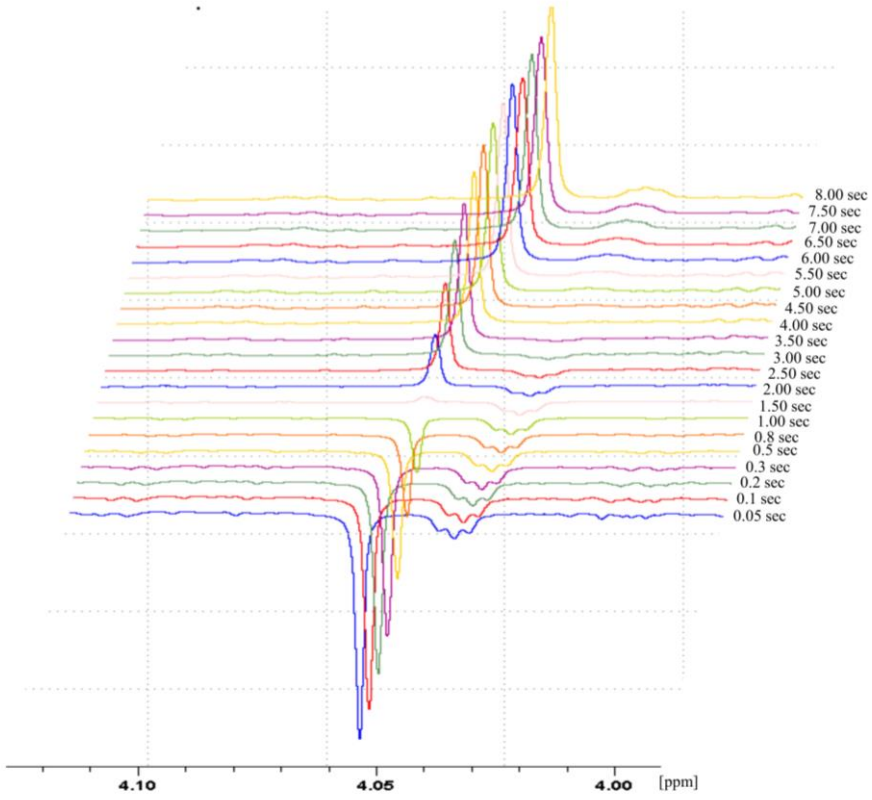

Figure S2: Stacked plot for inversion recovery experiment for urine sample with τ ranging from 0.05 sec to 8.5 sec, enlarged in CH_2/CHD peak area; zero crossing of CH_2 protons at ~ 1.5 sec; CHD protons between 3.5 sec – 4.5 sec

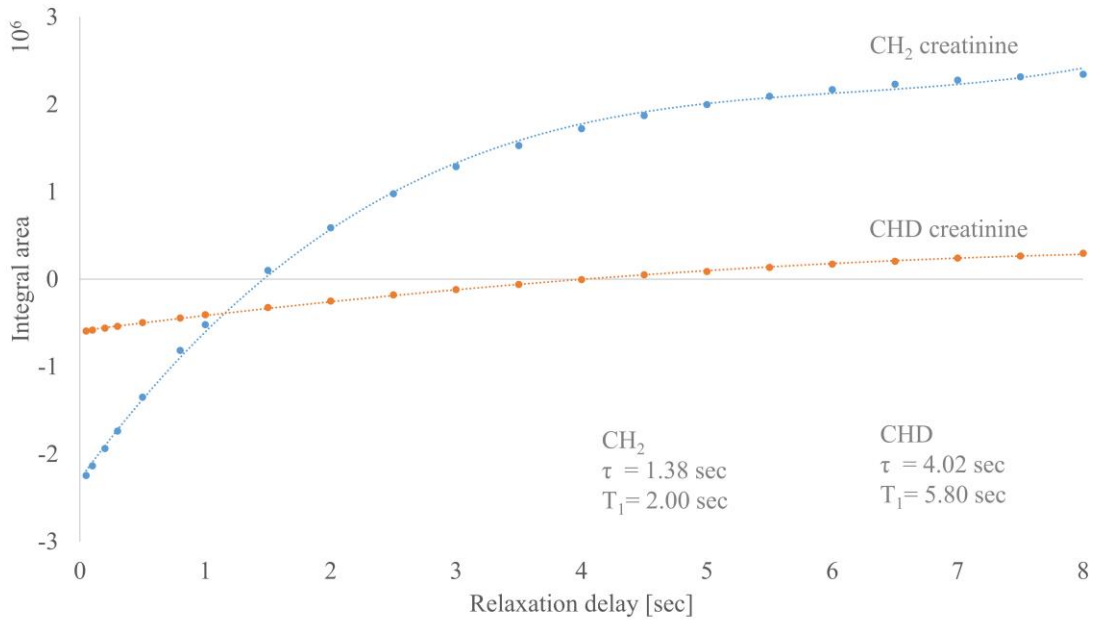

Figure S3: Integral areas over varying relaxation delays (τ) for CH_2 and CHD creatinine peaks

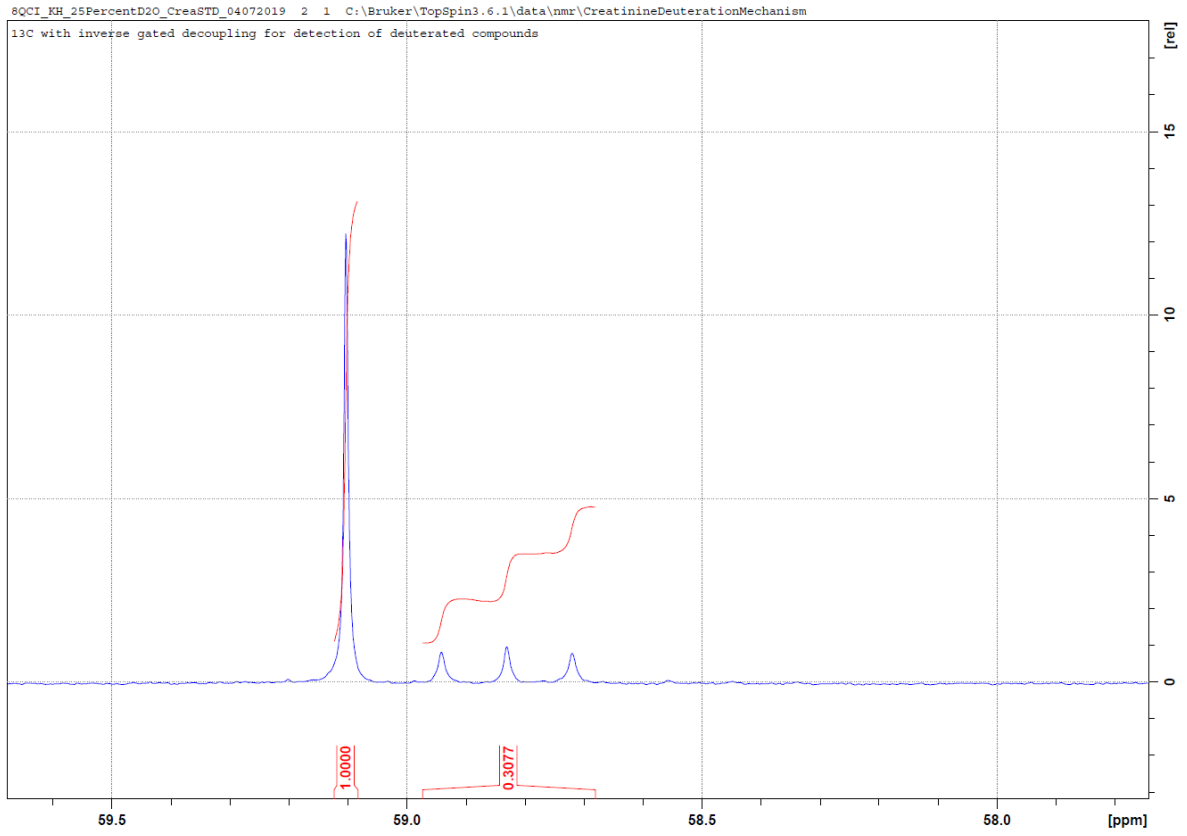

Figure S4: IG ^{13}C for estimation of CD_2 occurrence under realistic measurement conditions: A urine sample containing 25% D_2O was analyzed after a dwell time of 24 h. The singlet of creatinine-CH_2 and the triplet of CHD are clearly visible, the quintet of creatinine-CD_2 is below S/N. Peak area integration was performed in TopSpin 3.6.1.

REFERENCES

(1) Gil, Ryan B., et al. "Increased urinary osmolyte excretion indicates chronic kidney disease severity and progression rate." *Nephrology Dialysis Transplantation* 33.12 (2018): 2156-2164.

(2) Hwang, T.-L. & Shaka, A.J. "Water suppression that works. Excitation sculpting using arbitrary wave-forms and pulsed-field gradients." *Journal of Magnetic Resonance, Series A* 112.2 (1995): 275-279.