SUPPORTING INFORMATION

A Chemoselective ^{15}N Tag for Sensitive and High Resolution NMR Profiling of the Carboxyl-Containing Metabolome

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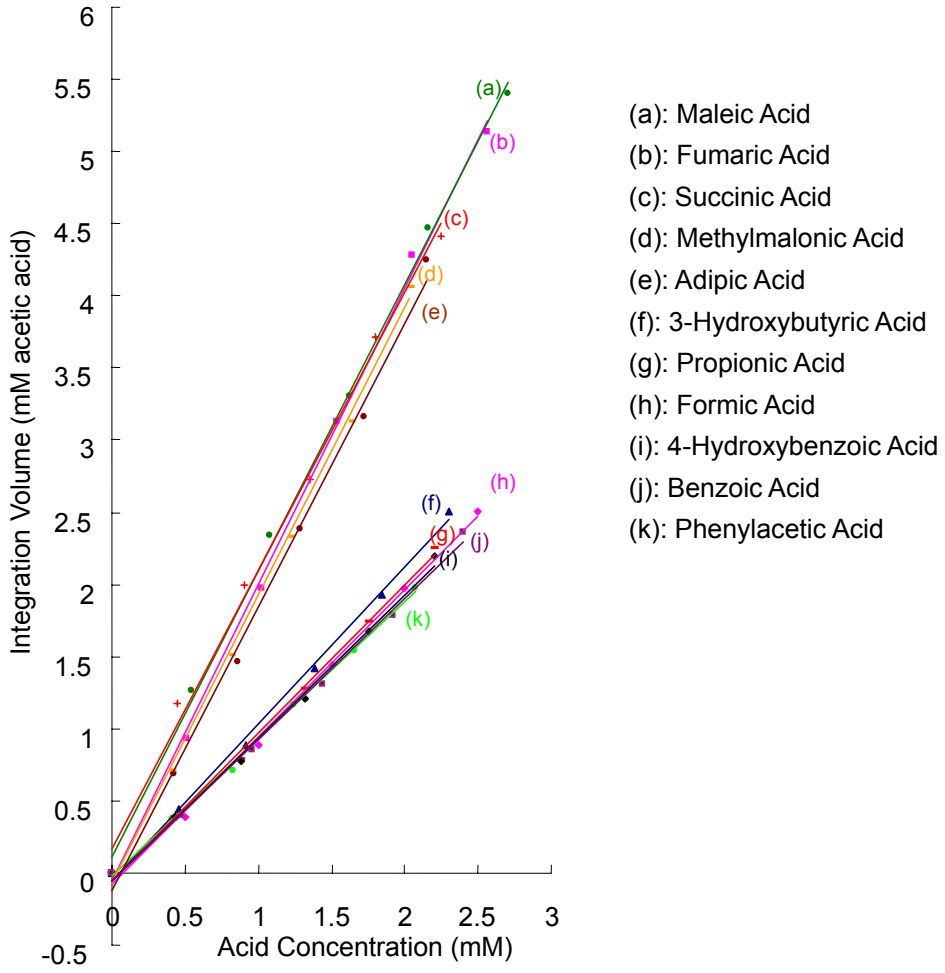

Figure S1. Integrated peak volumes of 11 metabolite standards in 6 mixtures from the 2D ^{1}H-^{15}N HSQC NMR spectra after ^{15}N-ethanolamine tagging plotted against their original concentrations. The integrated signal volume of 1 mM tagged acetic acid (internal standard) was used as the unit for the vertical axis. The original concentrations of metabolite standards in the six samples were determined by 1D ^{1}H NMR before conducting the ^{15}N-ethanolamine tagging reactions. Best-fit lines were generated by linear least square regression. The ^{15}N-ethanolamine reagent contained ~1% formic acid, and thus background subtraction was done on the integrated signals of tagged formic acid in all samples. All 12 compounds (including acetic acid) were quantitatively converted into ^{15}N-amides. All the metabolite signals exhibited good linearities with coefficients of regression (R^2) greater than 0.99. The molar integrated signals (slopes of best-fit lines) for metabolites with two identical carboxyl groups were observed to be twice as large as for those with one carboxyl group because those metabolite molecules were double-tagged.

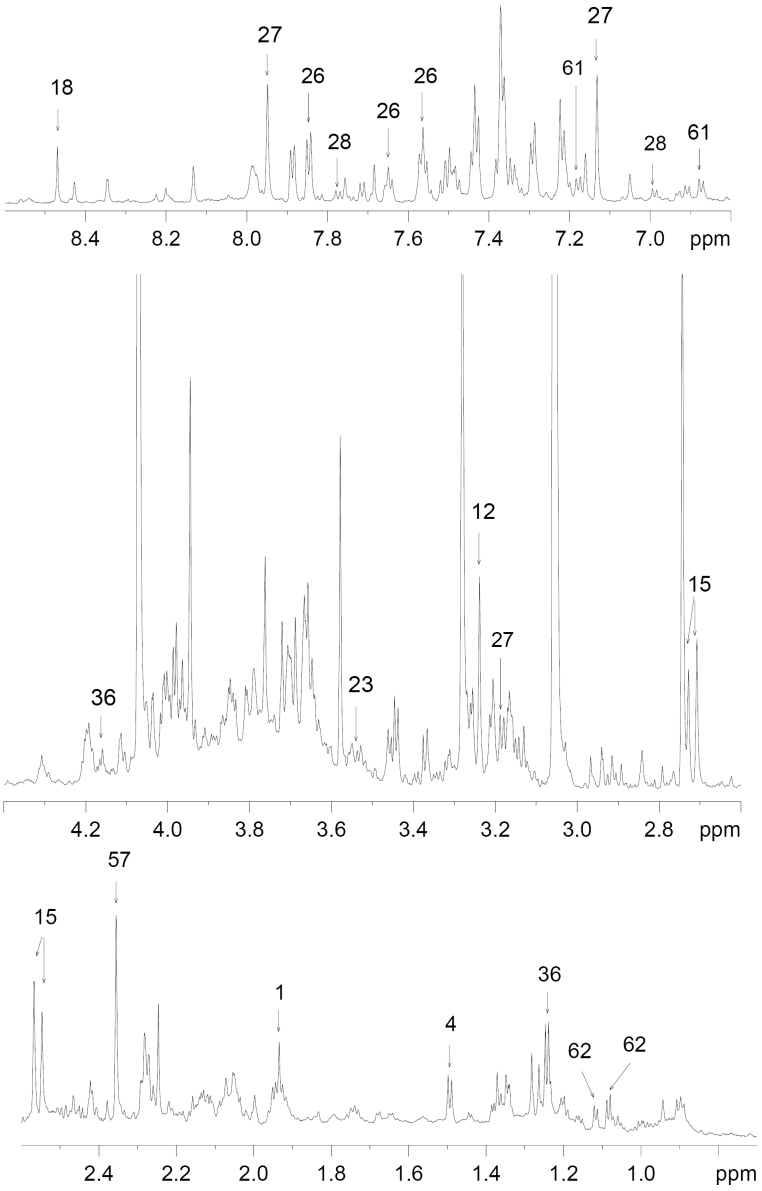

Figure S2. 1D ^{1}H NMR spectrum of a human urine sample measured prior to ^{15}N-ethanolamine tagging. As labeled, signals from only 13 carboxyl-containing metabolites are identifiable in the spectrum. Most of the signals are not quantifiable due to overlap. The labels correspond to the metabolite label numbers shown in Table 1 of the main text.

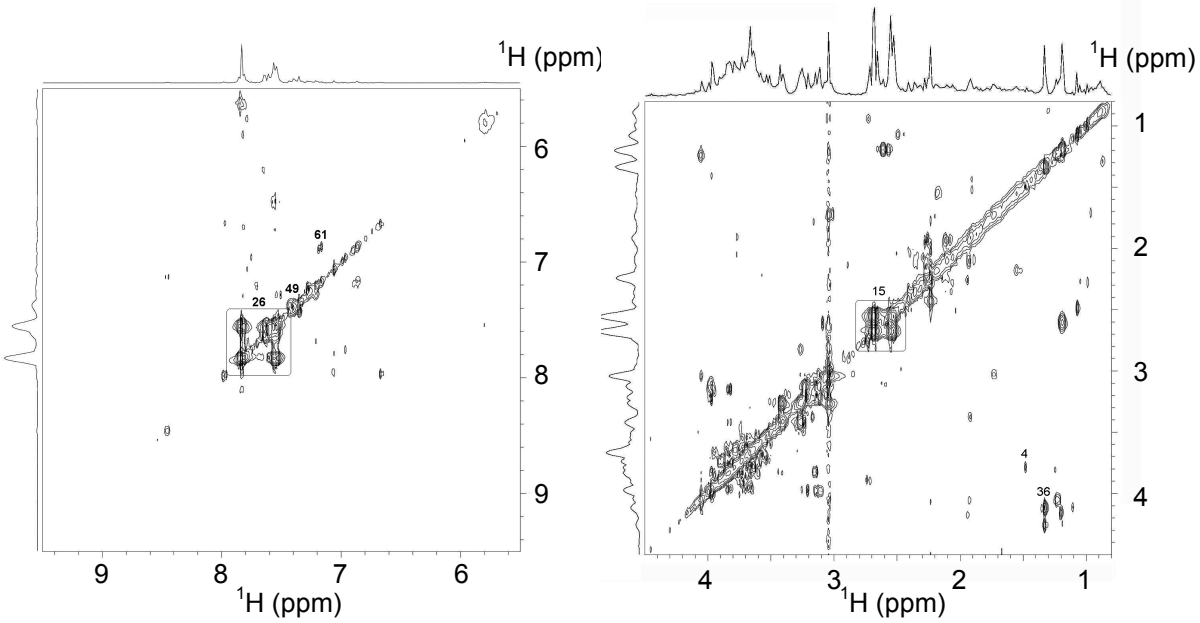

Figure S3. DQF-COSY spectrum of the human urine sample used for ^{15}N-ethanolamine tagging and 2D NMR detection. The spectrum was acquired on a Bruker DRX-500 spectrometer at 298 K This 2D homonuclear experiment does not significantly reduce the signal overlap and spectral complexity in the ^{1}H dimension and thus it is difficult to detect significantly more metabolites than in the 1D spectrum.

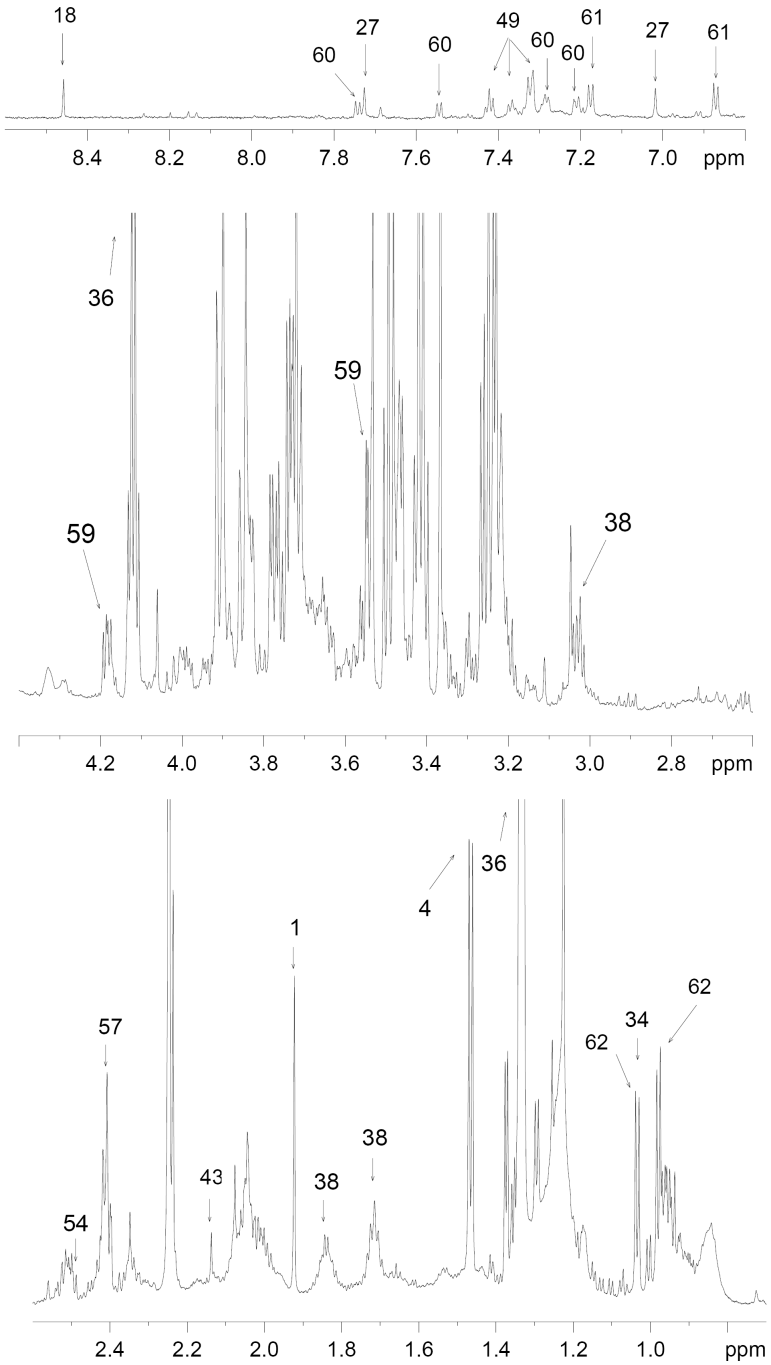

Figure S4. 1D ^{1}H NMR spectrum of a human serum sample measured prior to ^{15}N-ethanolamine tagging. As labeled, signals from only 15 carboxyl-containing metabolites are identifiable from the spectrum. Most of the signals are not quantifiable due to overlap. The labels correspond to the metabolite numbers shown in Table 1.

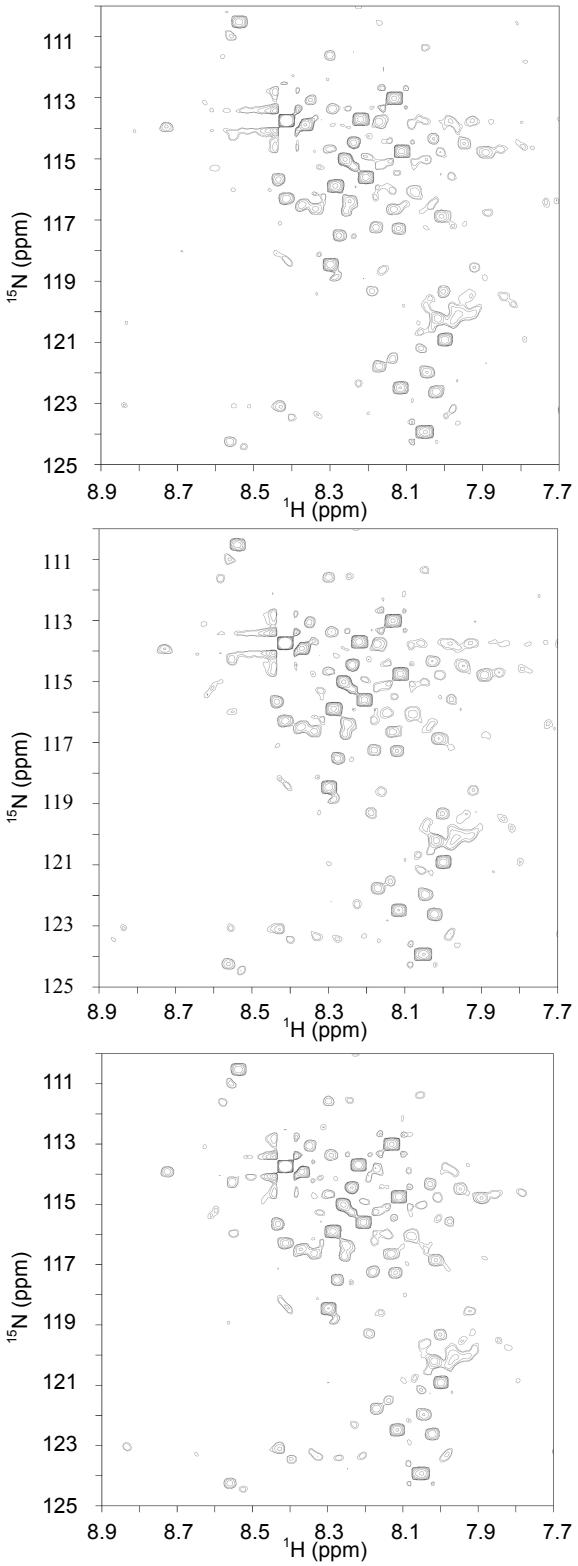

Figure S5. 2D HSQC NMR spectra of human serum obtained after tagging with ^{15}N-ethanolamine performed in triplicate reactions. One-to-one matching of the metabolite peaks is observed in the spectra. Each spectrum was acquired on a Bruker DRX-500 spectrometer at 298 K within 30 min.

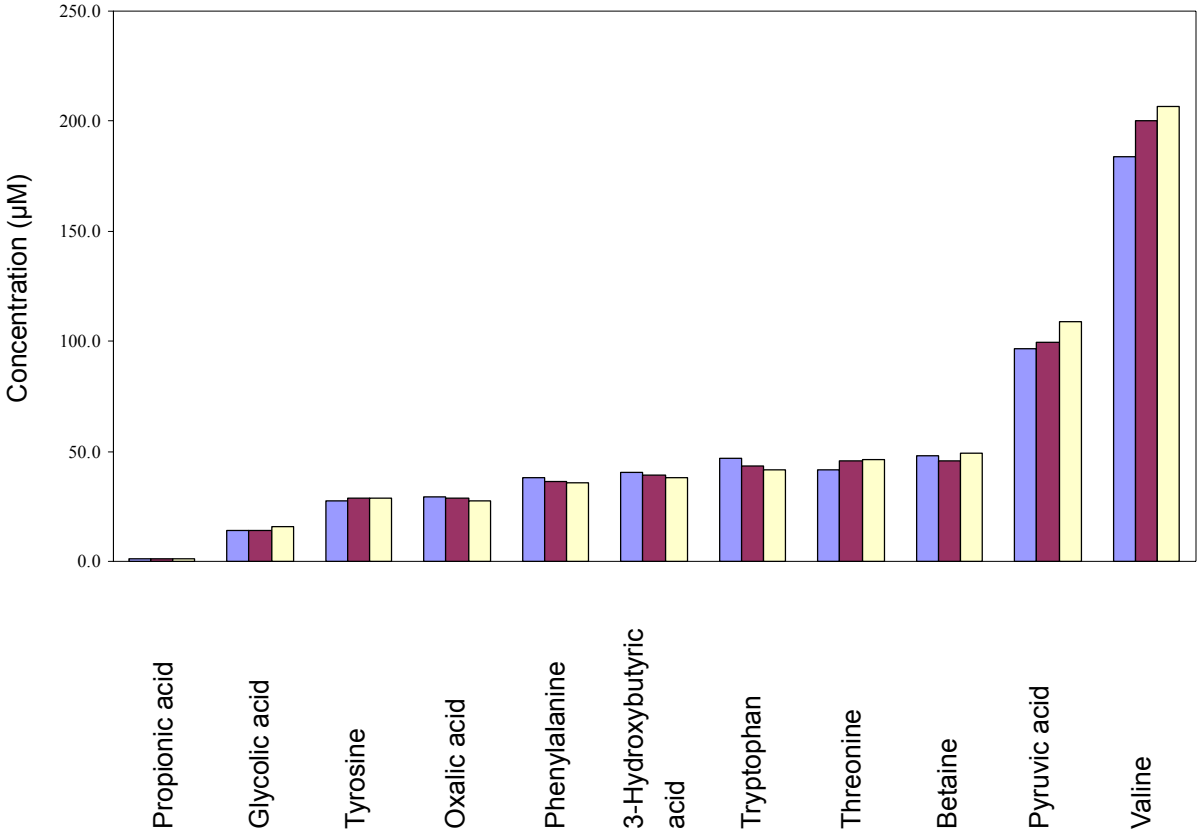

Figure S6. The concentrations of 9 representative carboxyl-containing metabolites in the triplicate reaction and analysis of a split serum sample measured from their 2D signal integration volumes. Metabolites from the same split sample are represented by the same color.

Table S1. Signals of carboxyl-containing metabolites that can be identified from the 1D ^{1}H NMR spectrum of the human urine sample used in this report before ^{15}N tagging. Abbreviations: s: singlet; d: doublet; q: quartet; O: the signal overlap other signals; I: the signal is identifiable; Q: the signal is quantifiable.

Label	Metabolite	^{1}H (ppm)	Multiplicity	Shape
1	Acetic Acid	1.94	s	O, I
4	L-Alanine	1.49	d	I, Q
12	Betaine	3.24	s	O, I
15	Citric Acid	2.58	d	I, Q
		2.71	d	O, I
18	Formic Acid	8.47	s	I, Q
23	Glycine	3.54	s	O, I
26	Hippuric Acid	7.56	t	I, Q
		7.64	t	I, Q
		7.84	d	I, Q
27	L-Histidine	3.18	ABX	O, I
		7.13	s	I, Q
		7.95	s	O, I
28	4-Hydroxybenzoic Acid	6.99	d	I, Q
		7.78	d	O, I
36	L-Lactic Acid	1.34	d	O, I
		4.12	q	O, I
57	Succinic Acid	2.37	s	O, I
61	L-Tyrosine	6.68	d	I, Q
		7.18	d	O, I
62	L-Valine	1.08	d	O, I
		1.13	d	I, Q

Table S2. Signals of carboxyl-containing metabolites that can be identified from the 1D ^{1}H NMR spectrum of the human serum sample used in this report before ^{15}N tagging. Abbreviations: s: singlet; d: doublet; q: quartet; O: the signal overlap other signals; I: the signal is identifiable; Q: the signal is quantifiable.

Label	Metabolite	^{1}H (ppm)	Multiplicity	Shape
1	Acetic Acid	1.94	s	I, Q
4	Alanine	1.48	d	I, Q
18	Formic Acid	8.47	s	I, Q
27	L-Histidine	7.02	s	I, Q
		7.73	s	O, I
34	Isoleucine	1.00	d	O, I
36	L-Lactic Acid	1.34	d	O, I
		4.12	q	I, Q
38	L-Lysine	1.71	m	I, Q
		1.84	m	I, Q
		3.03	t	O, I
43	L-Methionine	2.14	m	I, Q
49	L-Phenylalanine	7.32	d	O, I
		7.36	m	O, I
		7.42	m	I, Q
54	Pyruvic Acid	2.47	s	O, I
57	Succinic Acid	2.41	s	O, I
59	L-Threonine	3.55	d	O, I
		4.18	m	O, I
60	Tryptophan	7.21	dt	O, I
		7.28	dt	O, I
		7.54	d	I, Q
		7.74	d	O, I
61	L-Tyrosine	6.68	d	I, Q
		7.18	d	I, Q
62	L-Valine	1.08	d	O, I
		1.13	d	I, Q

Supplementary Notes

^{15}N Tagging Efficiencies

Most normal carboxyl-containing compounds (having no strong electron-donating or withdrawing functional groups at α-carbons) are converted quantitatively into ^{15}N-amides or with yields greater than 95% using an excess amount of ^{15}N-ethanolamine. It was observed that metabolites with special substitution groups on the α-carbon of the carboxyl showed reduced tagging efficiencies. The tagging efficiencies for α-amino acids were ~30%. Lactic acid which has an α-hydroxyl group showed two signals at (8.40 ppm, 113.69 ppm) and (8.12 ppm, 112.96 ppm) with 21% and 13% tagging efficiencies respectively. Oxalic acid has two conjugated carboxyl groups and showed two signals at (8.40 ppm, 116.22 ppm) and (8.73 ppm, 113.89 ppm) with 80% and 20% tagging efficiencies, respectively. The tagging of citric acid showed three signals at (8.02 ppm, 122.59 ppm), (8.16 ppm, 121.70 ppm) and (8.17 ppm, 113.75 ppm) with tagging efficiencies of 95%, 67%, and 45%. Assignment of these three signals is still underway.

Supplementary Methods

Serum Deproteination

Serum samples were mixed with methanol in a 1:2 (v / v) ratio, vortexed and then incubated at -20 °C for 20 min. The precipitated proteins were removed after centrifugation at 13,200 g for 10 min. The supernatant was dried in vacuum and re-dispersed in water.

Evaluation of Detection Linearity

An evaluation of the linearity of the isotope tagging coupled HSQC detection was made by analyzing 12 standard metabolites including 7 compounds each containing one carboxyl group (acetic, formic, 3-hydroxybutyric, phenylacetic, benzoic, 4-hydroxybenzoic and propionic acids) and 5 compounds each containing two identical carboxyl groups (fumaric, adipic, maleic, succinic, and methylmalonic acids) in a calibration series of 6 mixtures. The integration volumes of the 2D peaks from 11 metabolites were measured relative to the internal standard (acetic acid with a fixed concentration in all 6 mixtures) and plotted against the corresponding concentrations measured by 1D ^{1}H NMR before tagging.

Stock solutions of a mixture of 11 metabolite standards (~5 mM each), 4 mM acetic acid (used as the reference) and 1:9 (v / v) ^{15}N-ethanolamine-H_2O were first prepared. Six calibration samples were then prepared from the stock solutions of the acid mixture and acetic acid, keeping the acetic acid concentration fixed at 2 mM. The other 11 acids were diluted by 0 X, 2 X, 2.5 X, 3.3 X, 5 X and 10 X in concentration. The samples were then reacted with 30 μL ^{15}N-ethanolamine solution and 21 mg DMT-MM. The concentrations

of the metabolite standards in the calibration samples were determined using integrated signals from their 1D ^{1}H NMR spectra and then comparing with those obtained after ^{15}N tagging.