Supporting Information for:

Expanding the Coverage of the Metabolome with Nitrogen-Based NMR

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TABLE OF CONTENTS

Chemicals. Deuterium oxide (99.9% D) was obtained from Sigma Aldrich (Milwaukee, WI). 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (98% D) (TMSP) was purchased from Cambridge Isotopes (Andover, MA). Potassium phosphate dibasic salt (anhydrous, 99.1% pure) and monobasic salt (crystal, 99.8% pure) were purchased from Fisher Scientific (Fair Lawn, NJ). All compounds used to obtain reference spectra were obtained from Sigma Aldrich (Milwaukee, WI). ^{15}N-labeled compounds were purchased from Cambridge Isotopes (Andover, MA). ^{13}C labeled compounds were purchased from Sigma Aldrich (Milwaukee, WI).

NMR data collection and processing. One-dimensional (1D) ^{1}H NMR spectra were collected with 64K data points, a spectrum width of 11160 Hz, 128 scans, and 16 dummy scans using an excitation sculpting pulse sequence to remove the solvent peak.[1] The 2D ^{1}H-^{15}N HSQC spectra were collected with 16 scans, 8 dummy scans, and a 2 s relaxation delay. The spectra were collected with 2K data points and a spectrum width of 11160 Hz in the direct dimension, and 256 data points and a spectrum width of 10645 Hz in the indirect dimension. The 2D ^{1}H-^{13}C HSQC spectra were collected with 16 scans, 16 dummy scans, and a 2 s relaxation delay. The spectra were collected with 2K data points and a spectrum width of 11160 Hz in the direct dimension, and 128 data points and a spectrum width of 29052 Hz in the indirect dimension. The NMR data was Fourier transformed and phased using NMRpipe.[2] Further processing was performed in NMRviewJ (Version 8.0) where spectra were manually analyzed to obtain lists of chemical shifts and peak intensities.[3]

The 2D ^{1}H-^{15}N HSQC reference spectra for nitrogen-containing metabolites were collected with

non-uniform sampling (25% sampling sparsity)[4] at 277.15 K with 32 scans, 16 dummy scans, and a 2 s relaxation delay. The 2D ^{1}H-^{15}N HSQC spectra were collected with 2K data points and a spectral width of 11160 Hz in the direct dimension, and 256 data points and a spectral width of 10645 Hz in the indirect dimension. Spectral widths were adjusted as needed for individual metabolites. The NMR data were Fourier transformed, phased and reconstructed using Topspin 3.5. Further NMR processing was performed in NMRviewJ (Version 8.0) where spectra were manually analyzed to obtain lists of chemical shifts and peak intensities.[3] The 2D ^{1}H-^{15}N HSQC reference spectra were used to annotate the NMR spectra obtained for the *E. coli* and *S. aureus* metabolome extracts.

Table S1: Chemical Shifts for Nitrogen-Containing Metabolites from 2D ^{1}H-^{15}N HSQC Spectra

No.	Metabolite	Chemical Shifts (ppm)
1	4'-deoxypyridoxine	96.3 (10.91)
2	5'-ATP	89.2 (8.59), 89.1 (8.57)
3	5-azacytidine	72.9 (6.4), 82.4 (7.54), 82.5 (7.54), 109.2 (7.78), 105.5 (7.86), 105.2 (7.66), 106.3 (7.40)
4	5'-CMP	103.19 (8.96), 103.19 (8.09)
5	B-alanine	31.4 7.32
6	choline	136.2 (2.91)
7	creatine phosphate	101.5 (6.44)
8	cytosine	99.2 (8.12)
9	D-galactosamine HCl	31.2 (7.90)
10	D-gluconic acid	118.7 (3.38)
11	D-glucosamine 6- phosphate	34.0 (7.87), 34.0 (7.92)
12	D-glucosamine HCl	34.0 (7.83)
13	GABA	32.6 (7.33)
14	L-alanine	36.0 (7.60)
15	L-arginine	128.6 (7.78,8.08)
16	L-asparagine	111.8(7.44), 111.4(6.69), 39.4 (7.68)
17	L-aspartic acid	38.3 (7.78)
18	L-citruline	86.9 (6.17), 39.5 (7.56)
19	L-cysteine	38.4 (7.67), 20.64 (6.84)
20	L-glutamic acid	40.1 (7.69)
21	L-glutamine	112.2 (7.39), 112.2 (6.69), 40.2 (7.65), 20.2 (6.82)
22	L-glycine	42.4 (7.49)
23	L-histidine	129.5 (7.88)
24	L-isoleucine	36.7 (7.41)
25	L-leucine	39.6 (7.51)
26	L-lysine	61.2 (8.03)
27	L-methionine	39.7 (7.6)
28	L-ornithine	32.2 (7.38), 40.1 (7.62)

29	L-phenylalanine	38.8 (7.44), 50.7 (6.96)
30	L-proline	54.9 (7.69), 54.9 (8.57)
31	L-serine	35.67 (3.60)
32	L-threonine	37.8 (7.58)
33	L-tryptophan	35.8 (7.40), 42.5 (7.50)
34	L-tyrosine	38.4 (7.50), 130.0 (9.95)
35	L-valine	30.0 (7.45)
36	NAD	110.1 (7.53,8.46)
37	nicotinic acid	15.3 (7.76)
38	pantothenic acid	121.9 (7.84), 31.4 (7.31), 121.9 (3.11)
39	pyridoxine	18.3 (7.2), 164.5 (2.00), 177.7 (2.00)
40	sialic acid	124.1 (7.91)
41	thiamine HCl	107.0 (8.21,8.23), 226.6 (9.33), 234.8 (7.71)
42	thymine	131.9 (10.40), 157.5 (10.78)
43	trans-4-hydroxy-L-proline	52.0 (9.15,7.85)
44	UMP	215.0 (7.73), 185.2 (7.75)
45	uracil	134.4 (10.63)
46	urea	76.9 (5.91)
47	ureidosuccinic acid	88.8 (6.59), 96.0 (7.73)
48	uric acid	144.8 (7.24)
49	UTP	185.2 (7.75)
50	vitamin B12	114.0 (7.72), 121.7 (7.95), 121.4 (8.05), 119.5 (7.60), 113.2 (7.54), 112.7 (7.43), 113.3 (6.81), 114.1 (6.84), 119.5 (6.92), 121.4 (6.91), 113.4 (6.66), 110.3 (6.21), 110.4 (6.15)

Table S2: Nitrogen-containing metabolites with low aqueous solubility

No.	Metabolite
1	adenine
2	5'-ADP
3	allopurinol
4	5'-AMP
5	2'-dAMP
6	IMP
7	2'-dGMP
8	1,3-dimethyl uric acid
9	1,7-dimethylxanthine
10	cyclic GMP
11	5'-GTP
12	oxypurinol
13	pyridoxal-5'-phosphate
14	TMP
15	UTP

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