Supplemental Material

Activity-based protein profiling of ammonia monooxygenase in *Nitrosomonas europaea*.

Kristen Bennett[a], Natalie C. Sadler[b], Aaron T. Wright[b], Chris Yeager[c], and Michael R. Hyman[a#]

[a]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, USA

[b]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA

[c]Biosciences Division, Los Alamos National Laboratory, Los Alamos, NM, USA

Address correspondence to Michael Hyman, mrhyman@ncsu.edu

METHODS

CuAAC reaction condition optimization. To optimize the CuAAC reaction conditions used in this study incubations were conducted using either freshly prepared whole cells (Figs S1A, 2A, 3A, and 4A) or previously frozen, lysed cells of *N. europaea* (Figs S1B, 2B, 3B, and 4B). In all cases the cells used had previously been incubated for 1 h in the presence of NH_4Cl (10 mM) and 17OD (1 μmol) to fully (≤99%) inactivate AMO activity, as measured by NH_4^+-dependent NO_2^--generating activity. After exposure to 17OD, the cells were harvested by centrifugation, washed and in buffer (50 mM sodium phosphate + 2 mM $MgCl_2$, pH 7.0). The cells were either used immediately (whole cells) or were stored at $^-20^oC$ as a cell suspension (frozen, lysed cells).

In a typical CuAAC reaction, whole or frozen, lysed cells (150 μg total protein) in buffer were added to plastic microfuge tubes (500 μL) then mixed with AlexaFluor 647-azide added from a stock solution (0.6 mM) in DMSO. The reactions were initiated by the addition of $CuSO_4$ and sodium ascorbate, both added from freshly prepared aqueous stock solutions. In some reactions tris-(3-hydroxypropyltriazolyl-methyl)amine (THPTA) was also added from a concentrated aqueous stock solution. In all cases, distilled water was added as required to obtain a final reaction volume of 75 μL. Unless otherwise stated, the CuAAC reactions were conducted for 90 min at room temperature in darkness. The reactions were stopped by the addition of excess 3-butyn-1-ol (13 mM) added from an aqueous stock solution (1M). Samples from the CuAAC reactions were then solubilized at room temperature by adding an equivalent volume of 2X SDS-PAGE sample buffer (75 μL) that contained 0.125M Tris (pH 6.8), 4% SDS, 20% glycerol, 10% β-mercaptoethanol and 0.002% bromophenol blue. The solubilized cell samples were then centrifuged (10,000 x *g*,

2 min) to remove insoluble materials. The resulting supernatant was stored in the dark at -20°C prior to analysis by SDS-PAGE. All SDS-PAGE analyses were conducted using pre-cast 12% discontinuous SDS-polyacrylamide gels and a Bio-Rad Mini-Protean Tetra System (Bio-Rad Laboratories, Hercules, CA). The gels were electrophoresed at room temperature for 30 min at a fixed current of 25 - 35 mA. To visualize fluorescently labeled polypeptides, the unfixed gel was immediately scanned with an excitation wavelength of 650 nm and a detection wavelength of 668 nm using an Odyssey 9120 IR scanner (LI-COR Biosciences, Lincoln, NE). An infrared NIR marker protein ladder (Thermo Scientific, Waltham, MA) was used to estimate the molecular mass of fluorescently labeled polypeptides.

In-gel trypsin digestion and mass spectrometry. Portions of SDS-PAGE gels that contained the 28 kDa AlexaFluor 647-labeled polypeptide were excised from the gel and in-gel trypsin digestion was performed using a ProGest™ Protein Digestion Station (Digilab Inc, Marlborough, MA) using sequencing grade trypsin (Promega Corp., Madison WI) according to the manufacturers instructions. Extracted peptide solutions were lyophilized and reconstituted in 50% methanol/0.1% formic acid. The resulting peptide fragments were subject to MS and MS/MS analyses using a 4800 MALDI TOF/TOF mass spectrometer (AB Sciex, Framingham, MA). The mass spectrometer was operated in a reflector positive ion mode. A saturated solution of α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile/0.1% trifluoroacetic acid/5mM ammonium citrate was used as the matrix solution. MS spectra were collected for a mass range of 700 – 4000 Da and 1250 total shots/spectrum. MS/MS spectra were obtained with a collision energy of 2 kV and total shots/spectrum of 1350 or less. The peptides with signal-to-noise ratio >40 were selected

for MS/ MS analysis and a maximum of 45 MS/MS spectra was allowed per spot. All spectra were searched against all entries in the NCBInr database and Uniprot sprot databases, using ProteinPilot™ Software (AB Sciex, Framingham, MA) and Mascot (MatrixScience, Boston, MA) search engine. Variable modifications included: Oxidation (M). Mass tolerance was 80 ppm for precursor ions and 0.6 Da for fragment ions; two missed cleavages were allowed

On-bead trypsin digestion and mass spectrometry. The protein content of the crude membrane fraction from 17OD-treated cells and untreated control cells of *N. europaea* was determined by the BCA assay (1) after solubilization in 0.4% SDS. Protein concentrations were normalized to 863 µg per reaction prior to CuAAC-catalyzed conjugation with biotin azide (36 µM in DMSO), tris(2-carboxyethyl) phosphine (TCEP) (2.5 mM), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) (250 µM; prepared in 4:1 *tert*-butanol:DMSO), and $CuSO_4$ (0.50 mM) and incubated at room temperature for 90 min. Samples were processed for streptavidin affinity purification, on-bead trypsin digestion and subsequent LC-MS analysis as previously described (2), except the samples were not reduced and alkylated prior to on-bead trypsin digestion.

We employed tag-free quantitative accurate mass and time (AMT) tag proteomics as described previously (2, 3), with the following modifications. Tryptic peptide fragments from enriched proteins were separated by LC on in-house manufactured reverse phase resin columns, and analyzed on a ThermoFisher Orbitrap MS. Data was acquired for 100 min, beginning 65 min after sample injection into the LC. Spectra were collected from 400–2000 *m/z* at a resolution of 100k, followed by data-dependent ion trap generation of MS/MS spectra of the six most abundant ions using a collision energy of 35%. A dynamic

exclusion time of 30s was used to discriminate against previously analyzed ions. Generated MS/MS spectra were searched using the mass spectrometric generating function (MSGF+) algorithm against the publicly available *N. europaea* translated genome sequence (RefSeq: NC_004757). Identified peptides of at least six amino acids in length having MSGF scores $\leq 1E^{-10}$, which corresponds to an estimated false discovery rate (FDR) of <1% at the peptide level (4), were used to generate an accurate mass and time (AMT) tag database (5). Using the AMT tag approach, measured arbitrary abundance values for a particular peptide were determined by integrating the area under each LC–MS peak for the detected LC-MS feature matching to a given peptide. Matched features from each MS dataset were then filtered on an FDR of ≤5% (5,6). Peptide and protein abundances were obtained as previously described (4). To mitigate false protein identifications by probe labeling, we employed the following statistical confidence metrics to all datasets: (a) only peptides unique in identifying a single protein were used; (b) proteins represented by <2 unique peptides were removed; (c) if peptides for a protein were not measured in at least 2 probe labeled replicates they were removed from further analysis; and (d) the peptides identified for a given protein must have covered at ≥10% of the protein sequence. Additionally, we required ≥5 spectral counts per protein and that the AMT tag protein abundance in the probe labeled datasets was ≥2.0-fold higher than the no probe controls. Protein abundances were generated using a rollup algorithm as defined previously (2, 3).

The log2 values reported for the proteins listed in Table 1 and S1 represent the averaging of log2 abundances of peptides as determined by mass spectrometry. The values are defined as the area under the ion peak curves for each peptide measured by MS. Because the protein values shown in Table S1 represent an averaging of all measured

peptides, these Log2 values can also be considered as relative protein abundances. As for fold changes, we evaluated the abundance of specific protein species in the 17OD-treated versus untreated control samples. As seen in Table S1, the targets of the 17OD probe in the 17OD-treated samples are significantly more abundant than in the untreated control samples. The "fold-change" data more clearly defines the magnitude this difference, as compared to Log2 values which can appear to reflect only small differences between the two sample types.

TABLE S1

Complete quantitative mass spectral analysis of peptide fragments detected from on-bead digestion of crude membrane fractions of N. europaea cells pretreated with (Plus) and without (Minus) 17OD

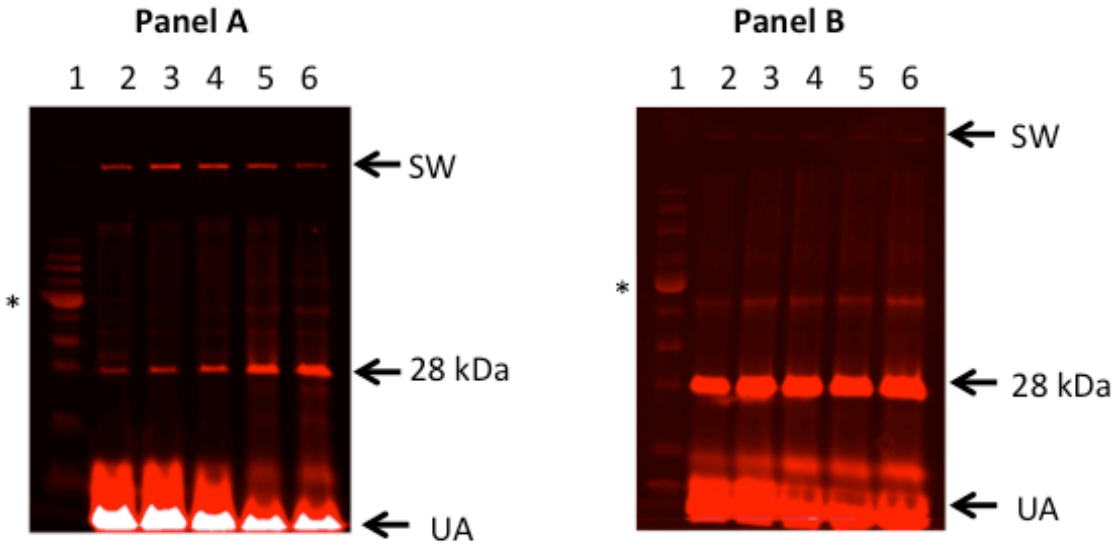
Locus Tag (a)	Percent Coverage (b)	Peptide Count (c)	Protein Description (d)	Plus 17OD sample 1(e)	Plus 17OD sample 2	Plus 17OD sample 3	Minus 17OD sample 1	Minus 17OD sample 2	Mean Plus 17OD samples	Mean Minus 17OD samples	Fold Difference (f)
ALW85_RS04940 ALW85_RS10750	21.38	20	AmoA: ammonia monooxygenase, subunit A **(g)**	31.19	30.05	30.02	24.92	25.80	30.42	25.36	33.46
ALW85_RS04935 ALW85_RS10745	68.10	65	AmoB: ammonia monooxygenase, subunit B **(h)**	30.38	29.29	29.57	22.70	22.87	29.75	22.79	124.37
ALW85_RS04945 ALW85_RS10755	35.06	30	AmoC: ammonia monooxygenase subunit C **(i)**	29.06	28.70	28.63	21.14	21.76	28.80	21.45	163.05
ALW85_RS05300	42.50	28	CoxB: cytochrome C oxidase polypeptide II precursor	27.82	26.79	26.91	20.71	21.41	27.17	21.06	69.27
ALW85_RS10005	45.67	54	CbbL: ribulose bisphosphate carboxylase, large chain	27.26	26.63	26.35	20.35	20.72	26.75	20.53	74.16
ALW85_RS09655	67.73	36	hypothetical protein	26.59	26.04	25.70	18.54	19.70	26.11	19.12	127.19
ALW85_RS05030 ALW85_RS10645 ALW85_RS12195	35.79	40	Hao: hydroxylamine dehydrogenase **(j)**	26.53	26.53	26.04	20.31	20.18	26.37	20.24	69.78
ALW85_RS03575	54.60	18	CoxB: possible cytochrome-c oxidase chain II	26.47	25.30	25.32	18.14	19.46	25.70	18.80	119.20
ALW85_RS10075	23.07	22	inorganic H+ pyrophosphatase	26.06	25.09	25.09	19.57	20.09	25.41	19.83	47.86
ALW85_RS09995	65.54	28	CbbQ: nitric oxide reductase NorQ protein	25.70	25.39	25.08	19.57	19.27	25.39	19.42	62.71
ALW85_RS13365	66.34	40	general diffusion Gram-negative porins	25.64	25.34	24.72	20.28	20.79	25.23	20.54	25.88
ALW85_RS04925 ALW85_RS10735	34.24	7	possible (AF047705) unknown [Nitrosococcus oceani] **(k)**	25.60	25.75	25.35	19.26	19.41	25.57	19.33	75.38
ALW85_RS04225	50.75	21	Rieske iron-sulfur protein 2Fe-2S subunit	25.46	25.02	24.90	18.96	18.61	25.13	18.79	81.20
ALW85_RS03415	66.45	16	AccB1: biotin carboxyl carrier protein of acetyl-CoA carboxylase	25.15	25.21	25.54	21.02	22.27	25.30	21.64	12.62
ALW85_RS04235	43.59	17	PetC: cytochrome c1	24.58	23.90	23.95	19.04	18.74	24.14	18.89	38.17
ALW85_RS09670	49.49	28	hypothetical protein	24.57	24.22	24.26	18.59	19.01	24.35	18.80	47.01
ALW85_RS02050	39.41	37	60 Kda inner membrane protein	24.54	24.16	24.10	17.66	18.23	24.27	17.94	80.28
ALW85_RS05020 ALW85_RS10635 ALW85_RS12185	39.57	22	CycA: cytochrome c-554 precursor **(l)**	24.45	25.05	24.45	19.04	19.15	24.65	19.09	46.99
ALW85_RS06890	58.58	29	IlvC probable ketol-acid reductoisomerase oxidoreductase	24.43	24.43	24.52	19.46	19.43	24.46	19.45	32.30
ALW85_RS10000	53.39	13	CbbS: ribulose bisphosphate carboxylase, small chain	24.37	24.40	24.24	17.62	18.45	24.34	18.03	79.06
ALW85_RS01050	66.24	14	AtpF: ATP synthase B/B' CF(0)	24.22	23.80	23.67	17.86	18.46	23.89	18.16	53.09
ALW85_RS01135	72.52	13	Pal: bacterial outer membrane protein	24.17	24.39	24.29	19.24	20.29	24.29	19.76	23.02
ALW85_RS02790	39.50	45	HflB: ATP-dependent zinc metallopeptidase	24.17	24.07	24.00	18.14	18.55	24.08	18.35	53.18
ALW85_RS06685	76.03	40	band 7 protein	24.11	24.07	23.72	17.50	18.20	23.97	17.85	69.32
ALW85_RS02160	76.74	22	RpsC: ribosomal protein S3	24.09	23.44	23.67	17.07	17.19	23.73	17.13	96.96
ALW85_RS00135	36.07	10	cytochrome c, class I	23.98	23.96	23.81	19.09	18.95	23.92	19.02	29.76
ALW85_RS13385	36.96	23	conserved hypothetical protein	23.96	23.95	23.83	17.68	18.13	23.91	17.91	64.25
ALW85_RS06690	64.14	33	band 7 protein	23.90	24.32	24.02	17.96	18.77	24.08	18.36	52.53
ALW85_RS05295	16.95	19	CoxA: cytochrome c oxidase, subunit I	23.87	23.18	22.77	16.16	16.17	23.27	16.16	138.17
ALW85_RS02210	70.06	17	RpsE: ribosomal protein S5	23.85	23.69	23.78	18.57	18.72	23.78	18.64	35.05
ALW85_RS05015	18.03	5	CycX3: putative tetraheme c-cytochrome	23.78	23.40	23.25	15.96	16.30	23.48	16.13	163.32
ALW85_RS12180	18.03	5	CycX1: putative tetraheme c-cytochrome	23.78	23.40	23.25	15.96	16.30	23.48	16.13	163.32
ALW85_RS01915	37.90	12	hypothetical protein	23.63	23.44	23.50	17.65	17.65	23.52	17.65	58.69
ALW85_RS00155	71.30	64	GroEL: TCP-1/cpn60 chaparonin family	23.63	23.71	23.67	17.91	18.37	23.67	18.14	46.36
ALW85_RS02190	63.13	17	ribosomal protein L5	23.63	23.27	23.43	17.64	17.18	23.44	17.41	65.46
ALW85_RS01060	57.31	42	AtpA: FoF1-type ATP synthase alpha subunit	23.59	23.74	23.54	18.27	18.11	23.62	18.19	43.34
ALW85_RS11520	80.95	69	PpiD: PpiC-type peptidyl-prolyl cis-trans isomerase	23.59	23.93	24.02	16.85	17.26	23.85	17.05	110.69
ALW85_RS03555	25.68	10	YrhG: formate and nitrite transporters	23.57	22.81	23.20	15.84	16.72	23.19	16.28	120.54
ALW85_RS04505	25.71	20	PntB: NAD(P) transhydrogenase beta subunit	23.56	23.25	23.27	17.59	17.50	23.36	17.54	56.40
ALW85_RS07500	47.60	19	GlyA: serine hydroxymethyltransferase	23.53	22.79	22.84	17.85	17.78	23.06	17.81	37.86
ALW85_RS10725	24.76	18	cytochrome c, class I	23.53	23.07	23.12	16.87	17.44	23.24	17.15	67.77
ALW85_RS08940	80.48	31	RpsB: ribosomal protein S2	23.47	23.17	23.33	18.14	18.01	23.32	18.08	37.97
ALW85_RS02150	48.92	21	ribosomal protein L2	23.39	23.18	23.51	18.60	18.23	23.36	18.42	30.71
ALW85_RS12890	67.43	17	conserved hypothetical protein	23.33	23.23	22.78	16.24	16.68	23.12	16.46	100.74
ALW85_RS03825	46.01	36	TonB-dependent receptor protein	23.30	22.82	22.73	17.62	17.51	22.95	17.56	41.88
ALW85_RS04010	21.23	21	CcmF: cytochrome c-type biogenesis protein	23.24	22.78	22.73	16.44	16.46	22.91	16.45	88.32
ALW85_RS05925	45.20	38	SecD/SecF/SecDF: export membrane proteins	23.09	22.92	23.06	17.08	17.07	23.02	17.08	61.72
ALW85_RS13290	49.35	16	bacterial outer membrane protein	23.08	23.41	22.89	18.73	19.25	23.13	18.99	17.64
ALW85_RS00875	28.85	40	MexB: multidrug resistance protein	23.06	22.90	23.03	16.72	16.77	22.99	16.74	76.11
ALW85_RS09990	52.39	41	CbbO: von Willebrand factor type A domain	23.02	22.54	22.68	17.17	16.65	22.75	16.91	57.10
ALW85_RS02240	47.60	11	RpsD: ribosomal protein S4:S4 domain	23.00	22.41	22.65	17.41	17.25	22.68	17.33	40.87
ALW85_RS08085	42.09	29	acyl-CoA dehydrogenase	22.98	22.65	22.75	17.03	16.77	22.79	16.90	59.34
ALW85_RS11920	64.72	25	conserved hypothetical protein	22.83	22.87	22.95	17.47	17.40	22.88	17.43	43.68
ALW85_RS03335	38.73	21	TonB-dependent receptor protein	22.83	22.57	22.20	16.99	16.88	22.53	16.94	48.30
ALW85_RS09400	50.78	20	chain length determinant protein	22.81	22.80	22.78	16.57	16.43	22.80	16.50	78.52
ALW85_RS02165	50.73	11	RplP: ribosomal protein L16	22.81	22.78	22.73	17.15	16.29	22.77	16.72	66.40
ALW85_RS03640	40.37	11	possible transmembrane protein	22.78	23.87	23.27	17.13	18.10	23.31	17.62	51.67
ALW85_RS02125 ALW85_RS10695	49.24	18	Tuf2: GTPases-translation elongation factors **(m)**	22.78	22.06	21.99	16.70	16.15	22.28	16.42	57.80
ALW85_RS01070	71.30	40	AtpD: FoF1-type ATP synthase beta subunit	22.67	22.69	22.47	17.33	17.21	22.61	17.27	40.50
ALW85_RS04020	50.30	14	CcmH: putative cytochrome C-type biogenesis protein	22.60	22.61	22.48	16.50	15.87	22.56	16.18	83.22
ALW85_RS03605	54.58	35	possible transmembrane protein	22.53	22.70	22.62	16.82	17.45	22.62	17.13	44.71
ALW85_RS01030	58.22	10	RpsF: ribosomal protein S6	22.52	22.59	22.68	18.26	17.84	22.60	18.05	23.38
ALW85_RS10790	38.81	16	MreB: heat shock protein hsp70	22.49	22.02	22.28	16.45	16.35	22.26	16.40	58.04

ALW85_RS02135	48.84	12	RplC: ribosomal protein L3	22.48	22.53	22.62	16.72	16.63	22.54	16.67	58.55
ALW85_RS00190	38.03	17	Aminotransferase class-V	22.33	21.78	21.46	17.30	15.78	21.86	16.54	40.00
ALW85_RS04740	45.23	33	FtsH: cell division protein	22.31	22.63	22.82	16.00	16.13	22.59	16.06	91.80
ALW85_RS00880	53.89	20	AcrA,MtcA,Lir HlyD family secretion protein	22.29	22.63	22.34	16.55	16.73	22.42	16.64	54.91
ALW85_RS05920	39.87	8	domain of unknown function DUF219	22.29	22.84	22.69	15.83	16.97	22.60	16.40	73.57
ALW85_RS12450	41.02	22	DsbD: thioredoxin:cytochrome c biogenesis protein	22.15	22.30	22.12	15.72	15.95	22.19	15.84	81.69
ALW85_RS04230	15.42	10	cytochrome b/b6	22.05	21.27	21.10	14.75	14.61	21.47	14.68	110.89
ALW85_RS01865	41.73	12	ExbB1: MotA/TolQ/ExbB proton channel family	22.03	22.11	21.86	15.64	15.18	22.00	15.41	96.31
ALW85_RS04310	37.01	26	TPR repeat	22.02	22.61	22.54	16.96	16.89	22.39	16.92	44.23
ALW85_RS04025	49.89	24	CycH: TPR repeat	21.97	22.45	22.26	16.06	16.06	22.23	16.06	71.93
ALW85_RS07865	45.87	15	hypothetical protein	21.92	22.49	22.51	16.52	16.42	22.31	16.47	57.13
ALW85_RS09930	50.00	7	hypothetical protein	21.87	23.40	22.44	18.25	18.38	22.57	18.32	19.02
ALW85_RS04220	55.45	49	SecA protein:SEC-C motif	21.81	21.99	21.91	16.73	16.87	21.90	16.80	34.31
ALW85_RS10655	43.92	56	RpoB: RNA polymerases beta subunit	21.73	21.42	21.71	16.84	15.93	21.62	16.39	37.57
ALW85_RS06645	43.45	32	hypothetical protein	21.66	22.07	21.98	16.42	16.13	21.91	16.27	49.66
ALW85_RS00820	29.10	29	probable transmembrane multidrug-efflux system	21.64	21.65	21.65	15.71	15.85	21.65	15.78	58.17
ALW85_RS07555	62.33	54	TPR repeat	21.58	21.91	21.54	15.77	15.94	21.68	15.86	56.62
ALW85_RS08090	37.42	21	AMP-dependent synthetase and ligase	21.56	21.87	21.75	15.40	14.77	21.73	15.09	99.76
ALW85_RS05025	57.45	18	hypothetical protein	21.42	21.06	21.16	15.33	15.20	21.21	15.27	61.64
ALW85_RS10640	57.45	18	hypothetical protein	21.42	21.06	21.16	15.33	15.20	21.21	15.27	61.64
ALW85_RS12190	57.45	18	hypothetical protein	21.42	21.06	21.16	15.33	15.20	21.21	15.27	61.64
ALW85_RS07515	31.00	24	MetE: methionine synthase, vitamin-B12 independent	21.42	20.41	20.53	14.81	15.39	20.79	15.10	51.44
ALW85_RS04345	41.63	23	DNA mismatch repair protein MutS family	21.41	21.28	21.34	15.50	15.47	21.34	15.49	57.96
ALW85_RS07520	52.72	18	ArgG: argininosuccinate synthase	21.38	20.86	20.90	15.43	15.81	21.05	15.62	42.99
ALW85_RS01065	56.12	17	AtpG: ATP synthase gamma subunit	21.29	21.32	21.00	15.55	16.00	21.20	15.78	43.07
ALW85_RS09285	36.98	19	carboxy-terminal processing protease	21.22	22.21	21.67	15.51	15.19	21.70	15.35	81.72
ALW85_RS00815	48.71	17	MexE: HlyD family secretion protein	21.16	21.41	21.27	15.60	15.29	21.28	15.44	57.09
ALW85_RS10650	43.88	56	RpoC: RNA polymerase, alpha subunit	21.12	21.17	21.01	16.23	15.55	21.10	15.89	37.06
ALW85_RS09235	30.94	15	NuoD NADH-ubiquinone oxidoreductase	21.12	21.95	21.91	16.22	15.92	21.66	16.07	48.32
ALW85_RS02245	60.12	16	RpoA: bacterial RNA polymerase, alpha chain	21.11	21.12	21.28	15.83	15.33	21.17	15.58	48.16
ALW85_RS00885	36.82	17	OprM: outer membrane efflux protein	21.08	21.24	20.80	15.37	15.44	21.04	15.40	49.69
ALW85_RS08905	41.54	18	membrane-associated Zn-dependent proteases 1	21.07	20.36	20.54	14.35	14.33	20.66	14.34	79.71
ALW85_RS00525	45.59	23	GuaB: inosine-5'-monophosphate dehydrogenase	20.93	21.28	21.15	15.15	15.38	21.12	15.27	57.77
ALW85_RS00775	21.75	12	conserved hypothetical protein	20.89	20.98	20.87	14.28	14.93	20.91	14.61	79.10
ALW85_RS05930	23.87	14	SecF: protein-export membrane protein	20.87	20.83	21.24	16.13	15.36	20.98	15.74	37.70
ALW85_RS01980	26.89	25	acriflavin resistance protein:Heavy metal efflux pump CzcA	20.84	20.78	20.75	14.56	14.60	20.79	14.58	73.75
ALW85_RS03970	40.05	30	putative translation initiation factor protein	20.55	20.38	20.35	14.75	14.17	20.43	14.46	62.55
ALW85_RS07625	27.78	20	ribonucleases G and E	20.41	19.82	20.28	14.32	14.03	20.17	14.17	63.90
ALW85_RS03095	36.91	28	phage integrase:Chain length determinant protein	20.33	21.24	21.08	14.65	14.93	20.88	14.79	68.23
ALW85_RS06315	41.22	11	short-chain dehydrogenase/reductase (SDR) superfamily	20.26	20.33	20.61	13.26	12.95	20.40	13.11	157.07
ALW85_RS10605	17.12	17	AcrD4: Acriflavin resistance protein	20.16	20.18	20.10	13.80	13.80	20.15	13.80	81.51
ALW85_RS12085	31.82	20	MrcA: penicillin-binding 1 transmembrane protein	19.93	19.96	19.96	ND	ND	19.95	NA	NA
ALW85_RS10700	43.10	23	FusA1: translation elongation and release factors	19.78	19.60	19.66	13.21	13.06	19.68	13.13	93.60
ALW85_RS00895	41.81	26	Pnp: polyribonucleotide nucleotidyltransferase protein	19.78	20.37	20.43	14.02	14.36	20.19	14.19	64.28
ALW85_RS06500	23.03	15	Pilj: bacterial chemotaxis sensory transducer	19.52	19.67	19.76	14.92	14.41	19.65	14.67	31.54
ALW85_RS00790	53.74	11	conserved hypothetical protein	19.07	19.32	18.89	16.29	17.22	19.09	16.75	5.05
ALW85_RS06495	23.06	26	two-component hybrid sensor and regulator	18.88	17.99	18.50	13.82	13.69	18.46	13.75	26.11
ALW85_RS10140	41.30	20	DnaK: heat shock protein hsp70	18.80	20.62	20.57	13.68	15.21	20.00	14.44	46.90
ALW85_RS10205	34.97	13	RpsA: ribosomal protein S1	18.55	19.52	19.69	15.59	15.34	19.25	15.46	13.81

(a) Locus tag assigned in NCBI Gene after 4th February, 2015
(b) Defined as the number of amino acids from unique peptides experimentally observed for a protein divided by the total number of amino acids in the protein sequence
(c) Number of unique peptides observed by LC-MS analyses
(d) Annotated protein descriptors
(e) All AMT data presented are in Log2 format for three biological replicates treated with (plus) 17OD and two biological replicates treated without (minus) 17OD
(f) Data presented are the fold difference in Log 2 protein abundance between 17OD pretreated and untreated samples
(g) Peptide amino acid sequences did not enable differentiation between ALW85_RS04940 and ALW85_RS10750
(h) Peptide amino acid sequences did not enable differentiation between ALW85_RS04935 and ALW85_RS10745
(i) Peptide amino acid sequences did not enable differentiation between ALW85_RS04945, and ALW85_RS10755
(j) Peptide amino acid sequences did not enable differentiation between ALW85_RS05030, ALW85_RS10645, and ALW85_RS12195
(k) Peptide amino acid sequences did not enable differentiation between ALW85_RS04925, and ALW85_RS10735
(l) Peptide amino acid sequences did not enable differentiation between ALW85_RS05020, ALW85_RS10635, and ALW85_RS12185
(m) Peptide amino acid sequences did not enable differentiation between ALW85_RS02125, and ALW85_RS10695
ND = Not detected
NA = not applicable

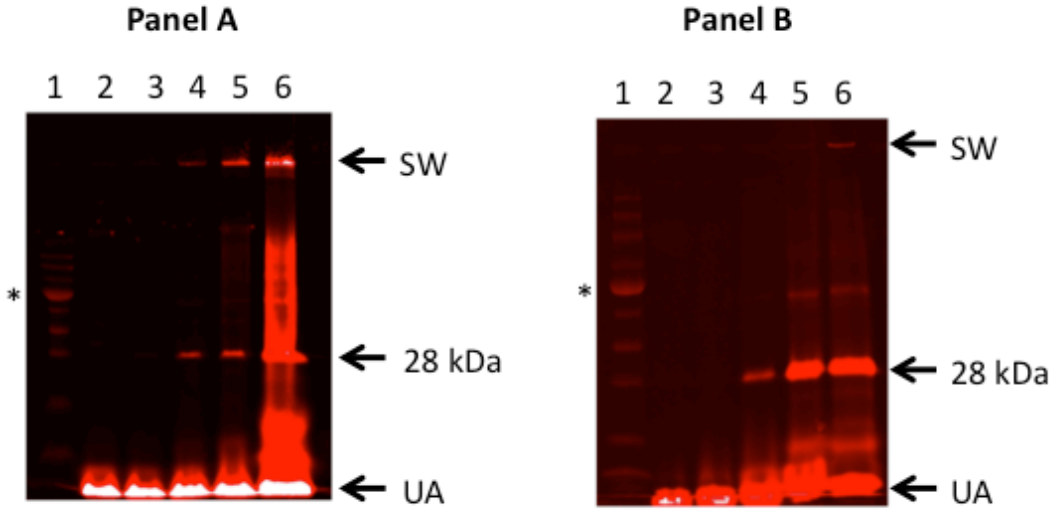
FIGURES

Figure S1A & B. Effect of incubation time on CuAAC reaction-dependent fluorescent labeling of the 28 kDa polypeptide in *N. europaea*.



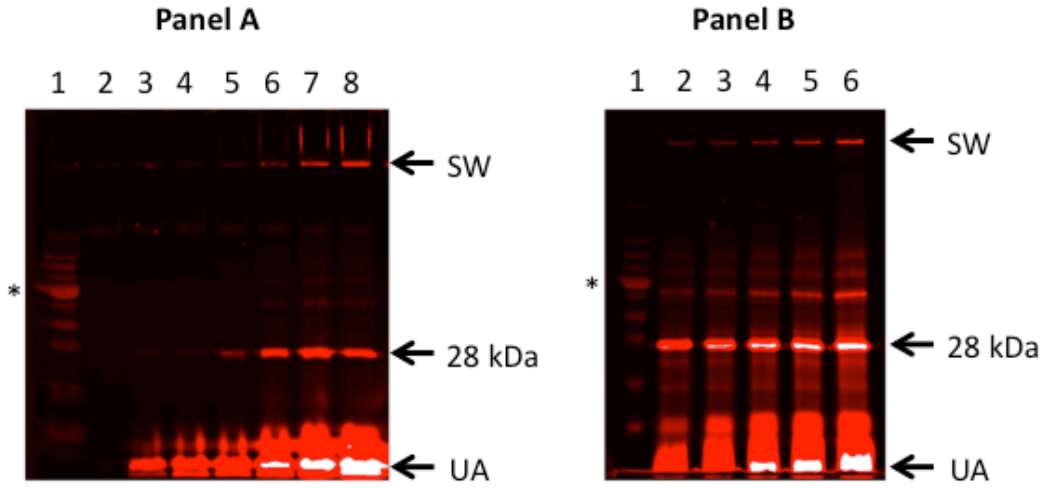
Samples (150 µg total protein) of 17OD-pretreated whole cells (**Panel A**) or frozen, lysed cells (**Panel B**) were incubated in CuAAC reactions containing AlexaFluor 647 azide (8 µM for whole cells, 4 µM for frozen, lysed cells), sodium ascorbate (11 mM), and $CuSO_4$ (2 mM), as described in the Methods section. After the CuAAC reaction, samples (25 µg total protein) were solubilized in 2X SDS-PAGE sample buffer and analyzed by SDS-PAGE and IR scanning, as described above in the Supplemental Material Methods section. Both Panels show the resulting fluorescent labeling observed for (Lane 1) NIR markers and cells incubated in the reaction mixture for (Lane 2) 15 min, (Lane 3) 30 min, (Lane 4) 60 min, (Lane 5) 90 min or (Lane 6) 120 min. Abbreviations: SW = bottom of sample well; UA = unreacted AlexaFluor 647-azide at gel dye front; * = 55 kDa marker protein.

Figure S2A & B. Effect of sodium ascorbate concentration on CuAAC reaction-dependent fluorescent labeling of the 28 kDa polypeptide in *N. europaea*.



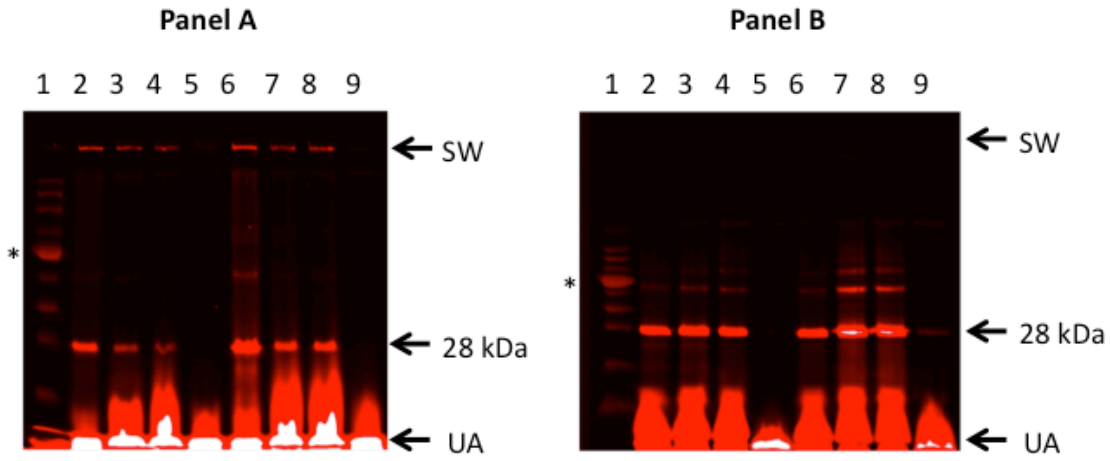
Samples (150 µg total protein) of 17OD-pretreated whole cells (**Panel A**) or frozen, lysed cells (**Panel B**) were incubated for 60 min in CuAAC reactions containing AlexaFluor 647-azide (40µM for whole cells, 4 µM for frozen, lysed cells), sodium ascorbate (11 µM -111 mM), and $CuSO_4$ (2 mM), as described above in the Methods section. After the CuAAC reaction, samples (25 µg total protein) were solubilized in 2X SDS-PAGE sample buffer and analyzed by SDS-PAGE and IR scanning, as described in the Supplemental Material Methods section. Both Panels shows the resulting fluorescent labeling observed for (Lane 1) NIR markers and cells incubated with the following concentrations of sodium ascorbate: (Lane 2) 11µM, (Lane 3) 111 µM, (Lane 4) 1 mM, (Lane 5) 11 mM, (Lane 6) 111 mM. Abbreviations: SW = bottom of sample well; UA = unreacted AlexaFluor 647-azide at gel dye front; * = 55 kDa marker protein.

Figure S3A & B. Effect of AlexaFluor 647-azide concentration on CuAAC reaction-dependent fluorescent labeling of the 28 kDa polypeptide in *N. europaea*.



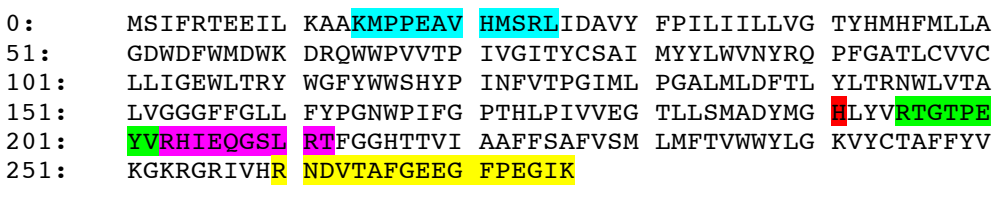
Samples (150 µg total protein) of 17OD-pretreated whole cells (**Panel A**) or frozen, lysed cells (**Panel B**) were incubated for 60 min in CuAAC reactions containing AlexaFluor 647-azide (0 - 80 µM), sodium ascorbate (11 mM), and $CuSO_4$ (2 mM). After the CuAAC reaction, samples (25 µg total protein) were solubilized in 2X SDS-PAGE sample buffer and analyzed by SDS-PAGE and IR scanning, as described in the Supplemental Material Methods section. **Panel A** shows the resulting fluorescent labeling observed for (Lane 1) NIR markers, and whole cells incubated with the following concentrations of AlexaFluor 647-azide: (Lane 2) none, (Lane 3) 2 µM, (Lane 4) 4 µM, (Lane 5) 8 µM, (Lane 6) 20 µM, (Lane 7) 40 µM or, (Lane 8) 80 µM AlexaFluor 647-azide. **Panel B** shows the resulting fluorescent labeling observed for (Lane 1) NIR markers, and frozen, lysed cells incubated with the following concentrations of AlexaFluor 647-azide: (Lane 2) 4 µM, (Lane 3) 8 µM, (Lane 4) 12 µM, (Lane 5) 16 µM, (Lane 6) 20 µM. Abbreviations: SW = bottom of sample well; UA = unreacted AlexaFluor 647-azide at gel dye front; * = 55 kDa marker protein.

Figure S4A & B. Effect of $CuSO_4$ concentration and Cu^{2+}:THPTA molar ratio on CuAAC reaction-dependent fluorescent labeling of the 28 kDa polypeptide in *N. europaea*.



Samples (150 µg total protein) of 17OD-pretreated whole cells (**Panel A**) or frozen, lysed cells (**Panel B**) were incubated for 60 min in CuAAC reactions containing AlexaFluor 647-azide (40µM for whole cells, 4µM for frozen, lysed cells), sodium ascorbate (11 mM), $CuSO_4$ (1-2mM) and THPTA (0-10 mM). After the CuAAC reaction, samples (25 µg total protein) were solubilized in 2X SDS-PAGE sample buffer and were analyzed by SDS-PAGE and IR scanning, as described in the Supplemental Material Methods section. Both Panels show the resulting fluorescent labeling observed for (Lane 1) NIR markers, and cells incubated with: (Lane 2) 1mM $CuSO_4$ alone, (Lane 3) 1mM $CuSO_4$ plus 0.5 mM THPTA, (Lane 4) 1mM $CuSO_4$ plus 1 mM THPTA, (Lane 5) 1mM $CuSO_4$ plus 5 mM THPTA, (Lane 6) 2mM $CuSO_4$ alone, (Lane 7) 2mM $CuSO_4$ plus 1 mM THPTA, (Lane 8) 2mM $CuSO_4$ plus 2 mM THPTA, and (Lane 9) 2mM $CuSO_4$ plus 10 mM THPTA. Abbreviations: SW = bottom of sample well; UA = unreacted AlexaFluor 647-azide at gel dye front; * = 55 kDa marker protein.

Figure S5: Polypeptides from AmoA detected following in-gel digestion and mass spectral analysis of fluorescent 28 kDa polypeptide.



The Figure shows the amino acid sequence of AmoA from *N. europaea* (ALW85_RS04940). The blue, yellow, green and purple highlighted sequences were detected by in gel tryptic digestion and subsequent MALDI-TOF/TOF analysis of the excised portion of an SDS-PAGE gel that contained the 28 kDa fluorescent polypeptide detected after treatment of cells with 17OD and CuAAC conjugation with AlexaFluor 647-azide. Amino acid H191 (highlighted in red) has been identified as an amino acid that binds ^{14}C when whole cells of *N. europaea* are exposed to $^{14}C_2H_2$ (8).

REFERENCES

1. **Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Proenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC**. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150: 76-85.
2. **Sadler NC, Melnicki MR, Serres MH, Merkley ED, Chrisler WB, Hill EA, Romine MF, Kim S, Zink EM, Datta S, Smith RD, Beliaev AS, Konopka A, Wright AT.** 2014. Live

cell chemical profiling of temporal redox dynamics in a photoautotrophic cyanobacterium. ACS Chem Biol. 9: 291-300.

3. **Ansong C, Ortega C, Payne SH, Haft DH, Chauvigne-Hines LM, Lewis MP, Ollodart AR, Purvine SO, Shukla AK, Fortuin S, Smith RD, Adkins JN, Grundner C, Wright AT.** 2013. Identification of widespread adenosine nucleotide binding in *Mycobacterium tuberculosis*. Chem. Biol. 20: 123-133.
4. **Kim S, Gupta N, Pevzner PA. 2008**. Spectral probabilities and generating functions of tandem mass spectra: A strike againsts decoy databases. J. Proteome Res. 7: 3354-3363.
5. **Pasa-Tolic´ L, Masselon C, Barry RC, Shen Y, Smith RD.** 2004. Proteomic analyses using an accurate mass and time tag strategy. Biotechniques 37: 621-624.
6. **Stanley JR, Adkins JN, Slysz GW, Monroe ME, Purvine SO, Karpievitch YV, Anderson GA, Smith RD, Dabney AR.** 2011. A statistical method for assessing peptide identification confidence in accurate mass and time tag proteomics. Anal. Chem. 83: 6135-6140.
7. **Polpitiya AD, Qian W-J, Jaitly N, Petyuk VA, Adkins JN, Camp DG, Anderson GA, Smith RD.** 2008. DAnTE: A statistical tool for quantitative analysis of –omics data. Bioinformatics 24: 1556-1558.
8. **Gilch S, Vogel M, Lorenz MW, Meyer O, Schmidt I.** 2009. Interaction of the mechanism-based inactivator acetylene with ammonia monooxygenase of *Nitrosomonas europaea*. Microbiology 155: 279-284.