

## **First proteomic study of S-glutathionylation in cyanobacteria**

**Solenne Chardonnet, Samer Sakr, Corinne Cassier-Chauvat, Pierre Le Maréchal, Franck Chauvat, Stéphane D. Lemaire and Paulette Decottignies**

### **ASSOCIATED CONTENT**

#### **Supporting Information**

**Supplemental Table 1: Sequence of the PCR primers used in this study**

<b>Name</b>	<b>Sequence</b>
<b>Cloning into pET21b for over-expression of sll1908 (SerA PGDH) in <i>E.coli</i></b>	
<i>serA</i> - <b>NdeI</b> -Fw	CCGGAATTCCATATGGCTAAAGTTTGTAGTTTCTGACTCC-
<i>serA</i> - <b>XhoI</b> -rev	CCCCGCTCAGGAGCTTAACGGTGTAGGCATCC
<b>Cloning into pET14 for over-expression of sll1621 (PrxII AhpC) in <i>E.coli</i></b>	
prx- <b>NdeI</b> -Fw	CCGGAATTCCATATGACCCCCGAACGAGTTCCCAGTG
prx- <b>XhoI</b> -rev	CCCCGCTCGAGTTAGCCGACAAAAGCTTTAACGGGC

**Supplemental Table 2: Proteins glutathionylated by BioGSSG in *Synechocystis* sp PCC6803.**

AC : accession number in UniProtKB data bank. NUP : number of unique peptides. E-values correspond to the peptide scores given by the X!Tandem software. Protein E-values < 0.01 were significant in the X!Tandem database. %SC: percent of sequence coverage. MW: theoretical molecular weights. Cys, total number of cysteines in the full-length sequence. T, proteins previously identified as putative Trx targets in *Synechocystis*.<sup>57-60</sup>

G, proteins previously identified as putative Grx targets in *Synechocystis*.<sup>35</sup> Site, putative site of glutathionylation identified (this work, Table 1)

AC	orf	Description	Gene	NUP	log(E value)	Spectra	% SC	MW	Cys	T	G	Site
<b>CARBON METABOLISM</b>												
<b>Calvin cycle</b>												
P37101	slI1525	Phosphoribulokinase	Prk	12	-64.12	16	46	38	4	X <sup>58,59</sup>	X	X
P54205	slr0009	Ribulose bisphosphate carboxylase large chain	RbcL	13	-43.51	36	26	52	11	X <sup>57,59</sup>	X	X
P54206	slr0012	Ribulose bisphosphate carboxylase small chain	RbcS	3	-15.88	39	37	13	3		X	X
P72797	slr1793	Transaldolase	TalB	13	-44.59	42	31	43	3			
P73282	slI1070	Transketolase	TktA	6	-31.29	6	13	72	6	X <sup>58</sup>		
P73922	slr2094	D-fructose 1,6-bisphosphatase class 2/sedoheptulose 1,7-bisphosphatase	Fpbl	7	-30.88	29	25	37	9		X	X
P74061	slI0807	Ribulose-phosphate 3-epimerase	Rpe	2	-12.99	3	15	25	3			
P74421	slr0394	Phosphoglycerate kinase	PgK	13	-61.95	20	40	42	5	X <sup>58</sup>	X	X
P80505	slI1342	Glyceraldehyde-3-phosphate dehydrogenase 2	Gap2	11	-53.09	19	46	36	5	X <sup>58,59</sup>	X	X
P73654	ssl3364	CP12 polypeptide	CP12	2	-5.50	2	26	8	4			
<b>Pentose Phosphate pathway</b>												
P52208	slI0329	6-phosphogluconate dehydrogenase, decarboxylating	Gnd	11	-55.32	28	28	53	4			X
P73411	slr1843	Glucose-6-phosphate 1-dehydrogenase	Zwf	10	-34.51	12	24	58	4			X
P74618	slI1479	6-phosphogluconolactonase	DevB	2	-5.60	2	7	26	1			
<b>Other sugar metabolism</b>												
P49433	slr0884	Glyceraldehyde-3-phosphate dehydrogenase 1	Gap1	7	-28.17	13	23	36	6		X	X
P52983	slr1349	Glucose-6-phosphate isomerase	Pgi	9	-45.57	12	24	58	1			
Q55863	slI0587	Pyruvate kinase 1	Pyk1	2	-8.71	2	7	53	2			X
P73534	slI1275	Pyruvate kinase 2	Pyk2	5	-15.24	5	10	63	5			
P73684	slI1709	Glucose dehydrogenase	Gdh	2	-10.86	3	15	27	1			
P74308	slr0942	Aldehyde reductase	ADH	3	-16.84	3	12	36	3			
P74643	slI0726	Phosphoglucomutase	Pgm	7	-32.43	16	16	61	1	X <sup>57</sup>		X
P77972	slr0752	Enolase	Eno	11	-53.28	19	35	46	1			
Q55664	slI0018	Fructose-bisphosphate aldolase class 2	CbbA	11	-43.19	76	33	39	3	X <sup>58</sup>		
Q55855	slI0593	Glucokinase	Glk	4	-17.71	4	16	38	4			
<b>Energetic metabolism (including tricarboxylic acids cycle)</b>												
P72661	slr0721	Malic enzyme	Me	4	-26.06	4	14	49	6			
P73162	slI1299	Acetate kinase	AckA	2	-9.09	2	6	45	6			
P73405	slI1721	Pyruvate dehydrogenase E1 beta subunit	PdhB	6	-25.10	6	26	36	3	X <sup>59</sup>		
P73723	slI1625	Succinate dehydrogenase iron-sulphur protein subunit	SdhB	2	-3.36	2	6	37	13			
P74299	slI0920	Phosphoenolpyruvate carboxylase	Ppc	2	-10.02	2	3	119	7			
P74490	slr1934	Pyruvate dehydrogenase E1 component, $\alpha$ SU	PdhA	9	-41.75	40	30	38	5	X <sup>59</sup>		
P74510	slI1841	Dihydrolipoamide acetyltransferase component (E2) of pyruvate dehydrogenase complex	PdhC	4	-18.16	5	14	45	1	X <sup>58,59</sup>		
P74582	slr0665	Aconitate hydratase 2	AcnB	24	-99.93	28	36	93	14			
P80046	slr1289	Isocitrate dehydrogenase [NADP]	Icd	6	-18.24	7	17	52	5			
Q55383	slI0891	Malate dehydrogenase	CitH/Ldh	4	-16.55	4	13	34	5			
Q55674	slr0018	Fumarate hydratase class II	FumC	2	-11.84	2	6	50	7			X
Q59977	slI0401	Citrate synthase	GltA	4	-16.45	5	11	45	3			
<b>Polysaccharide metabolism</b>												
P52415	slr1176	Glucose-1-phosphate adenyltransferase	GlgC	10	-55.93	27	31	49	6	X <sup>57</sup>		X
P72586	slI1212	GDP-D-mannose dehydratase	rffB	9	-37.91	10	27	41	2	X <sup>57</sup>	X	
P72903	slr1067	UDP-glucose-4-epimerase	GalE	2	-9.16	2	6	37	7			
P73511	slI1356	Glycogen phosphorylase	GlgP	11	-51.03	12	17	97	9			
P73546	slr1367	Phosphorylase	GlgpP2	10	-48.39	10	15	98	6	X <sup>58</sup>		
P73608	slr1857	Glycogen operon protein	GlgX	2	-5.43	2	3	80	10			
P74690	slr0453	Probable phosphoketolase	XfP	8	-28.26	8	12	91	11			
<b>Metabolism of porphyrins, chlorophyll and isoprenoids</b>												
P51634	slr1030	Magnesium-chelatase subunit ChlI	ChlI	5	-23.08	7	17	39	4			
P54224	slr0536	Uroporphyrinogen decarboxylase	HemE	5	-25.54	7	20	39	1			
P72683	slr0739	Geranylgeranyl pyrophosphate synthase	CrtE	3	-16.39	4	14	32	4			
P72848	slI1185	Coproporphyrinogen-III oxidase, aerobic	HemF	2	-3.83	4	7	39	5			
P73495	slI1127	Naphthoate synthase	MenB	3	-13.51	4	13	30	6			
P73660	slr1887	Porphobilinogen deaminase	HemC	5	-26.79	12	21	35	4			X
P73672	slr2136	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	GcpE	10	-36.47	12	26	44	6			
P74323	slr0951	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	IspD	2	-5.78	2	15	25	5			

P77969	sll1994	Delta-aminolevulinic acid dehydratase	HemB	12	-50.70	16	38	36	4	X <sup>57</sup>	
Q55663	sll0019	1-deoxy-D-xylulose 5-phosphate reductoisomerase	Dxr	7	-24.51	8	20	42	5		X
Q55665	sll0017	Glutamate-1-semialdehyde 2,1-aminomutase	HemL	8	-44.53	48	26	46	5		X
Q55809	slr0089	Delta(24)-sterol C-methyltransferase	Erg6	2	-6.65	2	6	36	7		
<b>Cofactor metabolism</b>											
P73849	slr1718	2-phosphosulfolactate phosphatase	ComB	5	-25.68	10	23	26	5		X
P73955	sll1415	Inorganic polyphosphate/ATP-NAD kinase 2	PpnK	3	-11.08	3	11	34	7		
P74045	slr0812	Type III pantothenate kinase		2	-6.35	2	13	28	4		
P74301	slr0936	Nicotinate-nucleotide pyrophosphorylase	NadC	3	-14.17	3	12	32	1		
Q55382	sll0892	Aspartate 1-decarboxylase	PanD	3	-13.77	3	28	16	5		
Q55710	slr0633	Bifunctional protein thiO	ThiG	3	-10.87	4	6	70	7		
Q55894	slr0118	Phosphomethylpyrimidine synthase	ThiC	6	-25.98	6	17	51	7		
Q55982	sll0660	4-hydroxythreonine-4-phosphate dehydrogenase	PdxA	4	-25.88	4	20	37	4		X
<b>Lipid metabolism</b>											
P73033	slr1755	Glycerol-3-phosphate dehydrogenase [NAD(P)+]	GspA	2	-7.08	3	8	33	2		X
P73128	slr1020	Sulfolipid biosynthesis protein	SqdB	9	-41.65	27	25	43	5		X
P73162	sll1299	Acetate kinase	AckA	2	-9.09	2	6	45	6		
P73283	sll1069	3-oxoacyl-[acyl-carrier-protein] synthase 2	FabF	2	-6.05	2	5	44	9		
P74286	sll1557	Succinyl-CoA synthetase	SucD	2	-4.60	2	9	32	3		
Q55404	sll0542	Acetyl-coenzyme A synthetase	AcS	4	-18.14	4	8	73	8		
Q55867	slr0624	UDP-N-acetylglucosamine 2-epimerase	NfrC	3	-10.14	3	9	41	4		
Q57310	slr0015	Lipid-A-disaccharide synthase	LpxB	3	-13.41	3	10	43	2		
<b>Nucleotide metabolism</b>											
P49057	slr0213	GMP synthase [glutamine-hydrolyzing]	GuaA	3	-11.03	3	7	61	7		X
P72644	sll1056	Phosphoribosylformylglycinamide synthase 2	PurL2	7	-27.73	15	12	82	12		X
Q55843	slr0520	Phosphoribosylformylglycinamide synthase 1	PurL1	2	-11.43	3	11	24	4		
P72659	sll1043	Polyribonucleotide nucleotidyltransferase	Pnp	3	-8.18	3	6	78	3		
P72753	sll1035	Uracil phosphoribosyltransferase	Upp	16	-52.20	21	69	24	1		X
P72931	slr1090	GTPase	ObgE	2	-4.45	2	8	39	5		
P72940	slr0676	Probable adenylyl-sulfate kinase	CysC	7	-28.42	13	51	20	2		X
P73290	sll1823	Adenylosuccinate synthetase	purA	8	-31.31	12	21	49	10		
P73440	sll1459	Putative 5'-nucleotidase alr3139	SurE	2	-8.87	2	10	25	4		
P73471	slr1226	Phosphoribosylaminoimidazole-succinocarboxamide synthase	PurC	7	-34.97	9	35	30	5		
P73761	sll0838	Orotidine 5'-phosphate decarboxylase	PurF	2	-10.54	2	13	25	4		
P73935	sll1430	Adenine phosphoribosyltransferase	Apt	3	-20.79	4	28	19	3		
P74208	sll1443	CTP synthase	PyrG	7	-39.06	8	16	61	9		X
P74384	sll0421	Adenylosuccinate lyase	PurB	8	-32.20	29	19	48	5		
P74457	sll0144	Uridylate kinase	PurK	8	-38.37	9	38	28	3		X
P74724	sll0578	N5-carboxyaminoimidazole ribonucleotide synthase	PyrH	3	-12.02	3	10	43	6		
P74741	slr0597	Bifunctional purine biosynthesis protein	PurH	3	-12.85	12	8	54	6		
Q55336	slr0861	Phosphoribosylglycinamide formyltransferase 2	PurT	6	-28.96	8	19	42	1		
Q55422	slr0838	Phosphoribosylformylglycinamide cyclo-ligase	PurM	5	-25.19	7	18	36	5		X
Q55503	sll0900	ATP phosphoribosyltransferase	HisG	2	-10.68	2	14	23	2		
Q55593	slr0379	Thymidylate kinase	Tmk	3	-11.91	3	20	24	4		
Q55641	sll0320	Ribonuclease D	Rnd	5	-23.40	14	26	24	8		
<b>AMINOACIDS AND NITROGEN METABOLISM</b>											
P20170	slr0738	Probable anthranilate synthase component 1	TrpE	3	-8.33	3	5	57	6		X
P29107	sll1363	Ketol-acid reductoisomerase	IlvC	11	-50.30	74	37	36	2		
P48576	slr0186	2-isopropylmalate synthase	LeuA	11	-44.71	13	34	57	3		X
P52986	sll0455	Homoserine dehydrogenase	thrA	6	-32.96	6	18	45	6		
P54261	sll0171	Aminomethyltransferase	GcvT	3	-8.20	3	11	41	1		
P54384	sll1470	3-isopropylmalate dehydratase large subunit	LeuC	4	-24.07	9	11	50	7		
P54691	slr0032	Probable branched-chain-amino-acid aminotransferase	IlvE	11	-41.14	20	42	34	3		
P54899	sll0080	N-acetyl-gamma-glutamyl-phosphate reductase	ArgC	8	-45.87	12	36	38	7		
P54902	sll0373	Probable gamma-glutamyl phosphate reductase	ProA	4	-23.93	7	13	46	9		X
P55037	sll1502	Ferredoxin-dependent glutamate synthase 1	GltB	7	-21.51	7	5	169	19	X <sup>57</sup>	
P55038	sll1499	Ferredoxin-dependent glutamate synthase 2	GltS	4	-10.93	4	3	169	20		X
P55175	sll0601	UPF0012 hydrolase		5	-31.85	6	29	30	3		
P72642	sll1058	Dihydrodipicolinate reductase	DapB	6	-16.42	8	23	29	3		X
P72662	sll0712	Cysteine synthase	CysM	8	-37.10	13	29	35	5		X
P72720	sll0220	Glucosamine-fructose-6-phosphate aminotransferase	GlmS	3	-13.66	3	6	70	5		
P72762	sll1027	NADH-glutamate synthase small subunit	GltD	5	-31.93	8	13	54	13		X
P72775	sll1682	Alanine dehydrogenase	Ald	2	-11.03	2	6	38	1		
P72808	sll1662	Chorismate mutase /prephenate dehydratase	PheA	2	-6.96	4	10	32	4		
P72864	sll0934	Carboxysome formation protein	CcmA	6	-15.93	7	17	38	3		X

P73043	slI1641	Glutamate decarboxylase	Gad	3	-11.82	3	8	53	7				
P73058	slr1848	Histidinol dehydrogenase 1	HisD	13	-49.71	14	36	46	3				
P73133	slr1022	Acetylornithine aminotransferase	ArgD	4	-14.67	4	10	46	7				
P73257	slr1133	Argininosuccinate lyase slr1133	ArgH	9	-47.56	13	23	51	3	X <sup>58</sup>			
P73326	slr1898	Acetylglutamate kinase	ArgB	3	-15.86	5	14	31	4				
P73410	slr1842	Cysteine synthase	CysK	3	-16.19	3	14	33	2				X
P73562	slI0873	Carboxynorspermidine decarboxylase	NapC	6	-23.13	8	19	43	7				
P73646	slI1760	Homoserine kinase	thrB	3	-16.63	5	16	32	4				
P73807	slI1958	Histidinol-phosphate aminotransferase	HisC	4	-17.58	10	14	39	5				X
P73821	slI1908	D-3-phosphoglycerate dehydrogenase	SerA	10	-43.20	13	27	59	3				
P73833	slr2002	Cyanophycin synthetase	CphA	5	-18.62	6	7	95	6				
P73906	slr2081	Arogenate dehydrogenase	TyrA	2	-11.43	2	12	30	5				
P73913	slr2088	Acetohydroxy acid synthase	IlvG	3	-8.34	3	5	68	10				
P73918	slI1981	Acetolactate synthase	ilvB	6	-26.95	15	12	60	10				
P73997	slr2130	3-dehydroquinate synthase	AroB	4	-22.23	5	20	39	2				
P74008	slI1234	Adenosylhomocysteinase	AhcY	5	-17.70	6	15	46	6				X
P74106	slI1893	Imidazole glycerol phosphate synthase subunit hisF	HisF	10	-33.88	12	44	28	4				
P74122	slI1883	Arginine biosynthesis bifunctional protein ArgJ	ArgJ	2	-6.88	2	5	43	6				
P74207	slI1444	3-isopropylmalate dehydratase small subunit	leuD	3	-14.60	5	19	22	5				
P74497	slr1938	Methylthioribose-1-phosphate isomerase	MtnA	3	-15.28	3	9	38	4				X
P74569	slr0657	Aspartokinase	LysC	6	-25.19	6	14	63	7				X
P74667	slr1665	Diaminopimelate epimerase	DapF	3	-11.55	3	15	30	9				
P74689	slr0452	Dihydroxy-acid dehydratase	IlvD	6	-21.11	7	14	59	7				X
P77960	slr0966	Tryptophan synthase alpha chain	TrpA	6	-18.90	6	31	28	3				X
P77961	slr1756	Glutamine synthetase	GlnA	12	-54.17	47	33	53	7				
P77962	slI1931	Serine hydroxymethyltransferase	GlyA	6	-22.76	11	20	46	5				X
P77973	slr0585	Argininosuccinate synthase	ArgG	13	-65.13	27	41	44	3	X <sup>57</sup>	X	X	
Q55128	slI0402	Aspartate aminotransferase	AspC	4	-21.85	5	15	42	5				X
Q55141	slI0065	Acetolactate synthase small subunit	ilvN	3	-14.95	5	21	19	1	X <sup>59</sup>			X
Q55366	slr0898	Ferredoxin--nitrite reductase	NirA	8	-28.64	16	17	56	9				
Q55484	slI0504	Diaminopimelate decarboxylase	LysA	2	-13.15	5	6	51	10				
Q55497	slI0902	Ornithine carbamoyltransferase	ArgF	4	-19.54	4	17	34	4				
Q55512	slr0549	Aspartate-semialdehyde dehydrogenase	Asd	3	-12.42	4	10	31	4				
Q55513	slr0550	Dihydrodipicolinate synthase	DapA	6	-20.70	11	23	32	5				X
Q55679	slI0006	Aspartate aminotransferase	AspC	7	-33.39	12	27	44	2				
Q55756	slI0370	Carbamoyl-phosphate synthase large chain	carB	15	-65.43	21	18	119	12				X
Q55786	slr0212	Methionine synthase	MetH	9	-26.07	9	7	132	17				
Q55828	slI0480	LL-diaminopimelate aminotransferase	AspB	12	-63.25	36	33	45	6				X
<b>SULFUR METABOLISM</b>													
P72622	slI1394	Peptide methionine sulfoxide reductase MsrA 1	MsrA	2	-11.88	2	12	24	4				
P72794	slr1791	Phosphoadenosine phosphosulfate reductase	CysH	2	-9.19	3	8	28	1				X
P72871	slI0927	S-adenosylmethionine synthase	MetK	2	-13.66	3	5	46	5				
P73493	slr1238	Glutathione synthetase	GshB	3	-16.68	8	11	35	3				X
P74241	slr1165	Sulfate adenylyltransferase	SopT	13	-58.05	24	39	44	4	X <sup>57</sup>			X
P74344	slI1536	Molybdopterin biosynthesis MoeB protein GN=moeB	MoeB	6	-29.45	7	20	43	6		X	X	
<b>LIGHT HARVESTING/PHOTOPROTECTION</b>													
P19050	slr1280	NAD(P)H-quinone oxidoreductase subunit K 1	NdhK1	6	-19.20	11	21	27	4				
P19125	slr1281	NAD(P)H-quinone oxidoreductase subunit J	NdhJ1	5	-15.16	5	34	21	3				
P26290	slI1316	Cytochrome b6-f complex iron-sulfur subunit 2	PetC1	2	-6.25	3	16	19	4				
P26525	slI0520	NAD(P)H-quinone oxidoreductase subunit I	NdhI	3	-13.13	3	19	22	9				
P27724	slr0261	NAD(P)H-quinone oxidoreductase subunit H	NdhH	4	-15.14	5	15	45	5				
P32422	ssl0563	Photosystem I iron-sulfur center	PsaC2	3	-17.71	4	51	9	9				X
P72870	slI0928	Allophycocyanin subunit alpha-B	ApcD	6	-16.94	6	32	18	1				
P73092	slr2049	Chromophore lyase CpcS/CpeS 2	CpeS	3	-5.76	3	14	22	4				
P73093	slr2051	Phycobilisome rod-core linker polypeptide	CpcG	10	-30.90	11	39	27	1				X
P74102	slr1963	Orange carotenoid-binding protein	OCP	4	-20.20	5	20	35	3				X
P74338	slr1623	NAD(P)H-quinone oxidoreductase subunit M	NdhM	2	-6.53	3	14	14	1				
Q01950	ssr3383	Phycobilisome 7.8 kDa linker polypeptide, allophycocyanin-associated, core	ApcC	2	-4.72	4	36	8	1				X
Q55013	slI0258	Cytochrome c-550	psbV	4	-19.81	5	29	18	2				
Q55124	slI0404	Glycolate oxidase subunit	GlcD	2	-9.58	2	5	53	11				
Q55544	slr0335	Phycobiliprotein ApcE	ApcE	15	-47.56	16	22	100	3	X <sup>59</sup>	X	X	
Q59987	slr0506	Light-dependent protochlorophyllide reductase	Por	2	-8.55	2	8	36	6				
<b>CELL WALL BIOSYNTHESIS</b>													
P73632	slr1874	D-alanine--D-alanine ligase	DdlA	5	-22.99	5	18	39	6				
P74706	slr1179	Anhydro-N-acetylmuramic acid kinase	AnmK	7	-38.50	7	25	42	8				X



TRANSPORT/STORAGE											
P73452	slI1450	Nitrate transport protein NrtA	NrtA	2	-7.49	3	5	49	4		X
P73704	slI1694	General secretion pathway protein G	PilA1	3	-9.54	5	17	18	2		X
P73287	slr1890	Bacterioferritin	BfrB	2	-8.31	2	16	20	1		
P74060	slr0821	Putative sulfur carrier protein		2	-7.66	2	32	12	3		
P74390	slr0447	Negative aliphatic amidase regulator (urea transporter)	UrtA	2	-14.05	2	6	48	4		X
Q55460	slr0040	Bicarbonate-binding protein CmpA	CmpA	5	-23.37	7	16	49	1		
Q55463	slr0537	Bicarbonate transport ATP-binding protein		4	-23.36	4	19	31	7		
SIGNAL TRANSDUCTION											
P73008	slr1044	Methyl-accepting chemotaxis protein	PilJ	2	-6.28	2	3	93	2		
P73175	slI1292	CheY subfamily	Rre11	2	-7.91	4	27	14	3		X
P74314	slr0947	OmpR subfamily	RpaB	7	-38.44	12	35	26	1		
MISCELLANEOUS											
P73264	slr1140	3-amino-5-hydroxybenzoic acid synthase		4	-14.09	5	13	41	5		
P73720	slr1734	Putative OxPP cycle protein	OpcA	6	-22.16	7	19	52	11		X
P73806	slI1959	Extragenic suppressor	SuhB	2	-5.31	2	9	29	5		
P73828	slI1905	Hybrid sensory kinase	Hik19	2	-9.88	2	2	111	13		
P73990	slr2123	D-isomer specific 2-hydroxyacid dehydrogenase family		3	-23.57	3	14	34	7		
P73991	slr2124	Short-chain alcohol dehydrogenase family		2	-8.28	3	10	26	3		
P74036	slr0809	dTDP-glucose 4-6-dehydratase	RfbB	2	-3.91	2	5	37	4		
P74158	slI1383	Inositol-1-monophosphatase	SuhB	3	-14.31	3	13	31	6		
P74668	slr1666	Pleiotropic regulatory protein	DegT	7	-44.76	8	28	42	5		
P74729	slI0576	HrEpiB putative sugar-nucleotide epimerase		8	-31.17	16	34	35	7	X <sup>57</sup>	X
Q55034	slr0746	Glucosylglycerol-phosphate phosphatase	StpA	2	-6.70	2	5	46	3		
Q55419	slr0835	MoxR protein	MoxR	5	-25.28	6	21	33	2		
Q55536	slr0329	Xylose repressor	XylR	2	-7.09	2	8	35	4		
Q55626	slI0753	Bifunctional protein FoLD	FoLD	3	-16.06	5	14	31	4		
Q55760	slr0427	CinA-like protein (putative competence-damage protein)		2	-5.42	2	6	45	5		X
HYPOTHETICAL PROTEINS (proposed function)											
P72585	slI1213	Hypothetical protein (GDP-fucose synthetase)	WcaG	8	-33.19	9	28	35	6		
P72653	slI1049	Hypothetical protein (transglutaminase-like domain)		2	-6.83	2	5	64	10		X
P72700	slr0244	Hypothetical protein (univ stress protein Usp1)	Usp1	8	-32.42	9	33	31	4	X <sup>59</sup>	X
P72731	slr1417	Uncharacterized protein (ycf57)		2	-7.03	2	13	13	5		
P72742	slr1098	Hypothetical protein (PBS lyase HEAT domain protein repeat-containing protein)		3	-13.29	4	15	28	5		
P72779	slI1680	Hypothetical protein (methionine-R-sulfoxide reductase)		2	-3.28	2	13	20	5		
P72895	slr1617	Hypothetical protein (UDP-glucose-4- epimerase)		3	-10.25	3	10	45	7		X
P72896	slr1618	Hypothetical protein		2	-10.89	2	15	29	7		
P72937	slr0670	Hypothetical protein (univ stress protein Usp2)	Usp2	5	-28.41	9	28	33	4	X <sup>59</sup>	
P73040	slr1763	Hypothetical protein (probable methyl transferase)		4	-19.67	5	11	45	5		
P73066	slr2032	Ycf23 protein		6	-32.92	34	28	25	2		
P73111	slI1835	Hypothetical protein (periplasmic protein )		2	-9.70	2	9	29	2		
P73120	slI1830	Hypothetical protein		3	-14.75	3	13	40	1		
P73155	slr1039	Hypothetical protein (probable methyl transferase)		4	-15.41	5	19	26	4		
P73168	slr1385	Hypothetical protein		2	-9.40	2	8	36	3		
P73217	slI1570	Hypothetical protein (Transmembrane fragment)		2	-15.24	2	13	27	2		
P73272	slr1143	Hypothetical protein		5	-18.30	5	18	39	3		
P73321	slr1894	Uncharacterized protein		2	-8.15	3	17	18	2		
P73328	slr1900	Hypothetical protein		3	-11.06	3	12	27	3		X
P73342	slr1194	Hypothetical protein		5	-24.68	5	34	25	5		X
P73370	slr2070	Hypothetical protein		3	-9.45	3	12	32	4		
P73389	slr1829	Hypothetical protein (putative poly(3-hydroxyalkanoate) synthase component)		2	-5.19	2	7	38	2		
P73393	slI1734	Hypothetical protein (protein involved in low CO <sub>2</sub> -inducible, high affinity CO <sub>2</sub> uptake)	CupA	5	-21.04	9	13	50	3		
P73409	slr1841	Hypothetical protein (probable porin, major outer membrane protein)		3	-14.83	3	6	68	2		
P73422	slr1537	Hypothetical protein		7	-31.66	12	31	32	2		
P73478	ssr2047	Hypothetical protein		2	-5.82	2	23	11	1		
P73488	slI1130	Hypothetical protein		3	-12.43	4	31	13	1		X

P73591	slI1308	Hypothetical protein (oxidoreductase, short chain dehydrogenase)		4	-18.27	4	19	26	2				
P73594	slr1409	Uncharacterized WD repeat-containing protein		2	-7.66	2	7	36	1				
P73598	slI1305	Hypothetical protein (probable hydrolase)		3	-11.02	5	12	33	4				
P73601	slI1784	Hypothetical protein (periplasmic protein)		5	-18.72	12	18	30	3				X
P73602	slI1783	Uncharacterized protein		2	-7.99	2	18	17	1				
P73603	slr1852	Hypothetical protein		2	-10.56	12	13	22	4				
P73604	slr1853	Hypothetical protein (carboxymuconolactone decarboxylase)		3	-16.77	6	28	12	2				
P73605	slr1854	Hypothetical protein		3	-12.76	4	16	22	4				
P73606	slr1855	Hypothetical protein		3	-6.82	3	4	70	3	X <sup>58</sup>			
P73611	slr1861	Hypothetical protein (probable sigma regulatory factor)		2	-10.00	2	18	16	2				X
P73620	slI1774	Hypothetical protein		3	-9.94	6	13	30	1				
P73626	slI1771	Hypothetical protein (protein serine-threonine phosphatase)		7	-45.33	12	39	28	4				X
P73647	slr1880	Hypothetical protein		2	-9.17	2	8	43	7				
P73680	slI2002	Hypothetical protein		6	-26.28	6	22	33	3				
P73759	slr0865	Hypothetical protein (putative methyltransferase)		2	-7.60	2	8	38	4				
P73765	slr0869	Hypothetical protein		13	-53.34	18	19	92	10				
P73799	slr1259	Hypothetical protein (hydroxyacylglutathione hydrolase like )	GloX	2	-13.56	2	13	26	5				
P73818	slI1950	Hypothetical protein		2	-4.18	2	9	27	2				
P73838	slr1712	Hypothetical protein		2	-6.94	3	9	31	1				
P73846	slr1717	Uncharacterized protein (ExsB family protein)		3	-12.25	3	10	30	3				
P73886	slI0245	Hypothetical protein (probable GTP binding protein)		4	-17.83	5	13	39	3	X <sup>59</sup>			
P74002	slr1322	Uncharacterized protein (putative modulator of DNA		7	-35.69	7	20	53	8				
P74033	slr0806	Hypothetical protein (FAD linked oxidase-like)		2	-5.61	2	6	48	4				
P74073	slI1258	Hypothetical protein (dCTP deaminase)		3	-13.69	3	20	21	4				
P74079	slr1342	Hypothetical protein (type III effector Hrp-dependent outers)		11	-52.63	12	31	48	4				
P74113	slr1970	Hypothetical protein		2	-11.14	2	18	19	1				
P74219	slr1533	Hypothetical protein		2	-12.65	3	24	13	5				
P74341	slI1537	Hypothetical protein (probable glycosyl transferase)		3	-11.39	3	30	16	2				
P74428	slr0398	Hypothetical protein		2	-6.30	2	8	18	4				
P74453	slI0148	Hypothetical protein		2	-10.17	2	4	78	7				
P74560	slr0651	Hypothetical protein		2	-5.35	2	14	14	3				
P74659	slr1658	Hypothetical protein		2	-10.59	3	15	23	2				
P74682	slI0446	Hypothetical protein		2	-6.55	2	2	121	9				
P74720	slI1106	Hypothetical protein		4	-20.77	13	31	18	1	X <sup>59</sup>			X
P74722	slr0586	Hypothetical protein		3	-12.52	4	13	30	3				
P74778	ssl1533	Hypothetical protein		2	-13.00	4	38	8	1				
Q55131	slr0049	Hypothetical protein		5	-20.20	6	16	44	8				X
Q55146	slI0064	Hypothetical protein (periplasmic protein, putative polar amino acid transport system substrate-binding protein)		2	-10.99	3	11	30	2				
Q55157	slr0065	Hypothetical protein		2	-10.11	2	15	24	3				X
Q55167	slI0461	Hypothetical protein (gamma-glutamyl phosphate reductase)	ProA	3	-14.20	3	8	46	11				
Q55374	slr0907	Hypothetical protein		3	-11.79	3	3	115	13				
Q55376	slr0912	Hypothetical protein		2	-9.83	4	11	29	4				
Q55390	slI0553	Hypothetical protein		5	-21.60	6	16	38	2				
Q55412	slr0583	Hypothetical protein (GDP-fucose synthetase)		4	-12.26	4	14	35	7				
Q55437	slI0048	Hypothetical protein		2	-9.30	2	6	35	7				
Q55458	slr0039	Uncharacterized protein		4	-14.02	12	12	38	4				
Q55480	slI0504	Uncharacterized protein (putative sugar kinase)		2	-6.84	3	8	35	10				
Q55514	slr0552	Hypothetical protein		8	-28.47	11	46	27	2				X
Q55517	slI0529	Hypothetical protein		6	-30.26	7	12	78	7				
Q55629	slr0782	Uncharacterized protein (putative flavin-containing monoamine oxidase/ L-amino acid dehydrogenase activity)		2	-4.95	2	4	51	7				
Q55667	slr0006	Hypothetical protein		6	-21.73	10	27	23	4				
Q55712	slr0635	Hypothetical protein		3	-11.89	3	11	35	1				X
Q55734	slI0395	Hypothetical protein ( phosphoglycerate mutase)		3	-14.97	3	18	24	2				X
Q55780	slr0208	Hypothetical protein		3	-6.63	3	14	30	1				
Q55874	slI0103	Uncharacterized protein		3	-9.44	3	7	46	2				
Q55992	slI0740	Hypothetical protein (DUF820)		2	-9.55	2	11	27	5				
Q57456	slr0351	ORF416		2	-10.32	2	8	47	5				

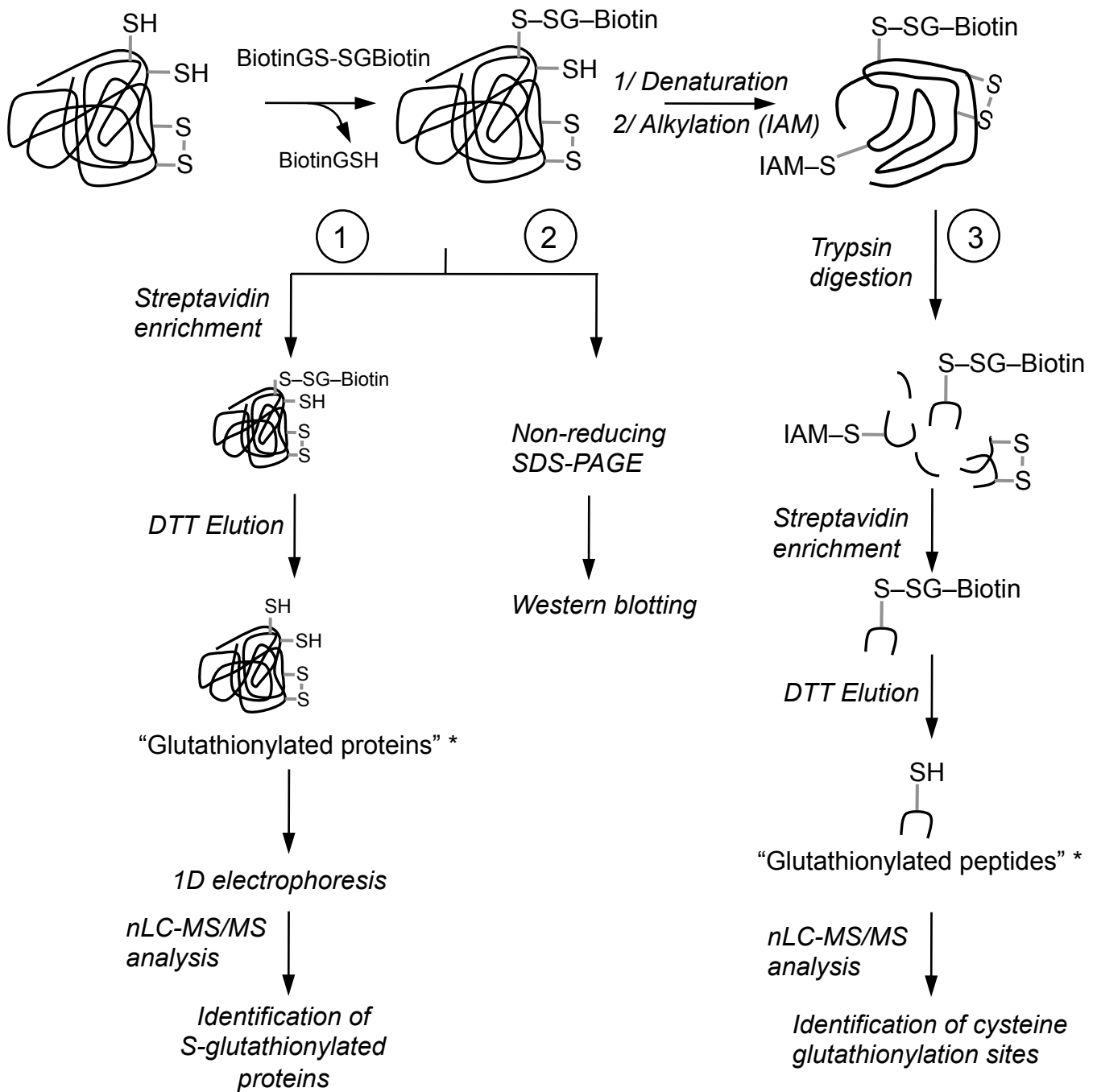


Encoded by plasmid													
Q6YRS8	sll6017	Hypothetical protein	3	-9.94	6	13	29	1					
Q6ZE80	sll8012	Putative uncharacterized protein	3	-12.17	3	26	16	1					
Q6ZED6	sll7062	Putative uncharacterized protein	2	-8.46	2	7	42	5					
Q6ZEG5	sll7033	Hypothetical protein	2	-11.84	3	28	13	4					
Q6ZEK6	slr5124	Hypothetical protein	2	-4.96	2	11	31	1					
Q6ZEL1	slr5119	Hypothetical protein	2	-4.96	2	12	29	1					
Q6ZEL2	slr5118	Hypothetical protein	2	-4.96	2	12	31	1					
Q6ZEP8	slr5082	Hypothetical protein	3	-9.94	6	12	31	1					
Q6ZES1	sll5059	Two-component response regulator	4	-13.43	7	15	33	4					X
Q6ZEU6	sll5034	Hypothetical protein	2	-9.52	2	14	23	3					

**Supplemental Table 3: Characteristics of the 34 new putative targets glutathionylated by BioGSSG in *Synechocystis* sp PCC6803 (identified by their glutathionylation site).**

**AC:** accession number in UniProtKB data bank. **MW:** theoretical molecular weight. **Cys:** total number of cysteines in the full-length sequence.

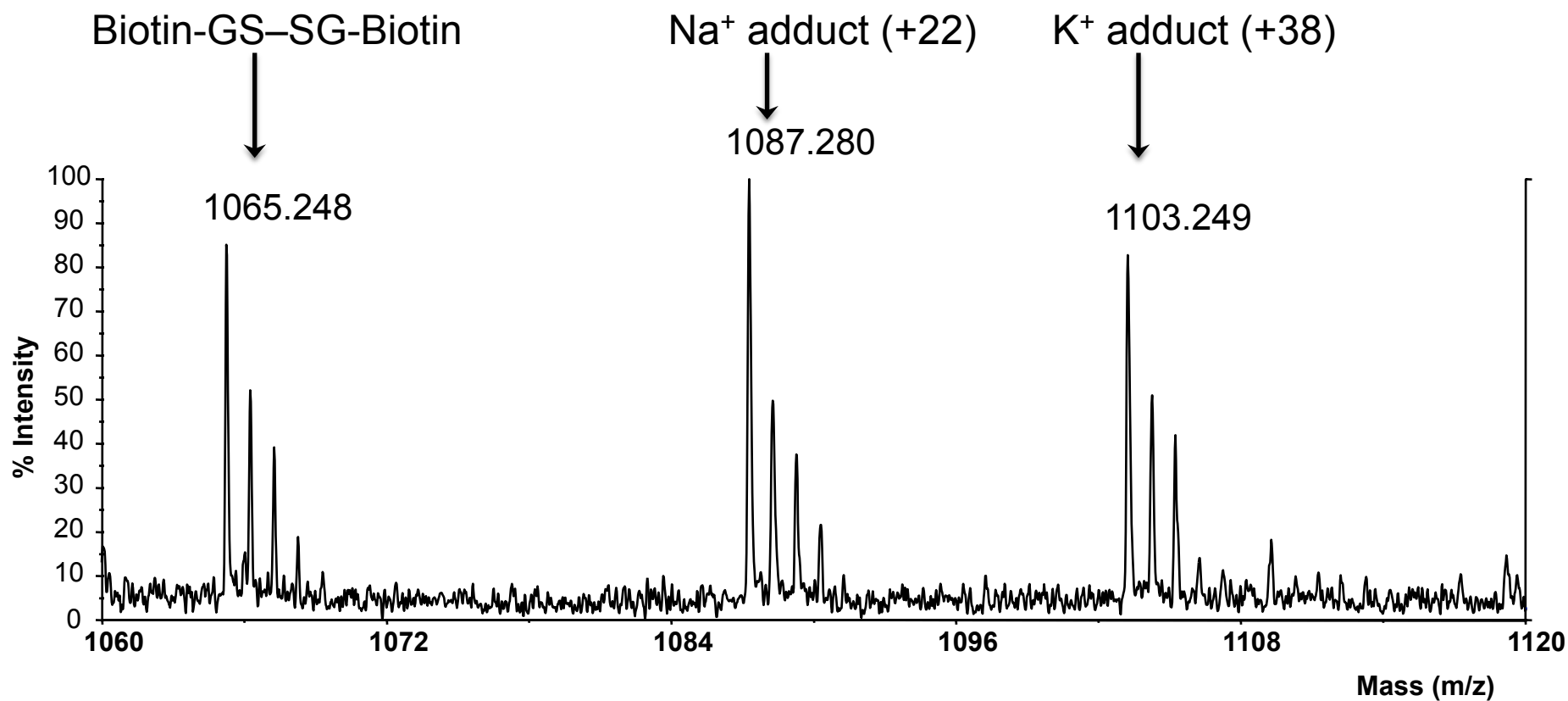
AC n°	Orf	Protein name	MW	Cys
P73951	<i>slr1511</i>	3-oxoacyl-[acyl-carrier-protein] synthase 3	35,166	4
P73293	<i>sll1822</i>	30S ribosomal protein S9	15,086	1
P72663	<i>sll0711</i>	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	34,081	4
P73637	<i>slr1877</i>	5-oxo-1,2,5-tricarboxylic-3-penten acid decarboxylase	30,050	2
P73825	<i>sll1923</i>	Acetyl coenzyme A acetyltransferase (Thiolase)	43,281	4
P72991	<i>slr1604</i>	ATP-dependent zinc metalloprotease FtsH 3	67,250	1
P73456	<i>sll1633</i>	Cell division protein ftsZ	44,774	3
P74138	<i>slr1982</i>	CheY subfamily	13,659	1
P74172	<i>sll1370</i>	GDP-mannose pyrophosphorylase	40,464	3
P73824	<i>slr1992</i>	Glutathione peroxidase	16,646	2
P74024	<i>sll1221</i>	Hydrogenase subunit	57,774	15
P73853	<i>slr1722</i>	Inosine monoPhosphate dehydrogenase subunit	40,235	7
P74298	<i>sll0921</i>	NarL subfamily	24,166	5
P74558	<i>ssl2667</i>	NifU protein	8,374	2
P19569	<i>slr0737</i>	Photosystem I reaction center subunit II	15,644	1
P72712	<i>ssr0390</i>	Photosystem I reaction center subunit PsaK 1	8,644	1
P74342	<i>slr1626</i>	Probable dihydroneopterin aldolase	13,146	1
P73492	<i>ssr2061</i>	Probable glutaredoxin	9,735	4
P74430	<i>slr0400</i>	Probable inorganic polyphosphate/ATP-NAD kinase	33,398	5
Q55233	<i>slr1719</i>	Protein drgA	23,703	3
P53383	<i>slr0067</i>	Protein mrp homolog	37,131	3
Q55794	<i>sll0086</i>	Putative arsenical pump-driving ATPase	44,626	2
P74373	<i>sll1521</i>	Putative diflavin flavoprotein A 3	65,093	12
P52761	<i>slr0709</i>	RutC family protein	13,834	3
P72756	<i>sll1033</i>	Sll1033 protein	73,712	14
P72629	<i>slr1116</i>	Slr1116 protein	20,103	1
P72891	<i>slr1613</i>	Slr1613 protein	22,345	1
P73700	<i>slr1815</i>	Slr1815 protein	20,798	3
P73587	<i>ssl2595</i>	Ssl2595 protein	10,356	1
P74639	<i>ssr1256</i>	Ssr1256 protein	10,046	3
P74193	<i>sll1172</i>	Threonine synthase	40,414	9
Q59994	<i>slr0783</i>	Triosephosphate isomerase	26,159	3
P73887	<i>sll0244</i>	UDP-glucose 4-epimerase	37,130	3
P74786	<i>ssl0294</i>	UPF0426 protein	7,833	1



\* Proteins and peptides initially glutathionylated with BioGSSG

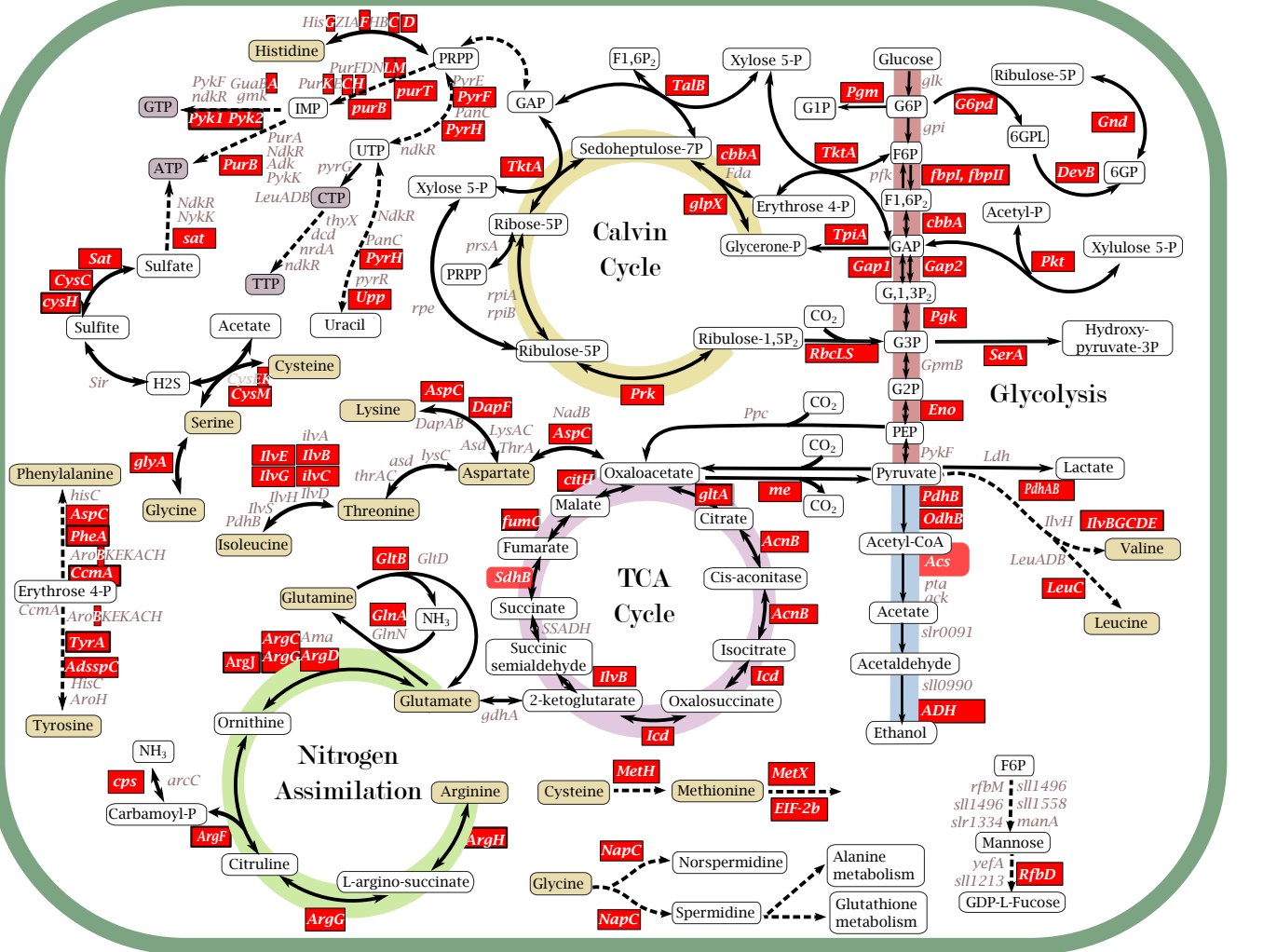
**Supplemental Figure 1: Schematic diagram of the procedure employed in this study.**

Procedure 1: work flow for identification of putative glutathionylated targets. Procedure 2: control of glutathionylation by western blot using anti-biotin antibody. Procedure 3: work flow for identification of cysteine S-glutathionylation sites on proteins.

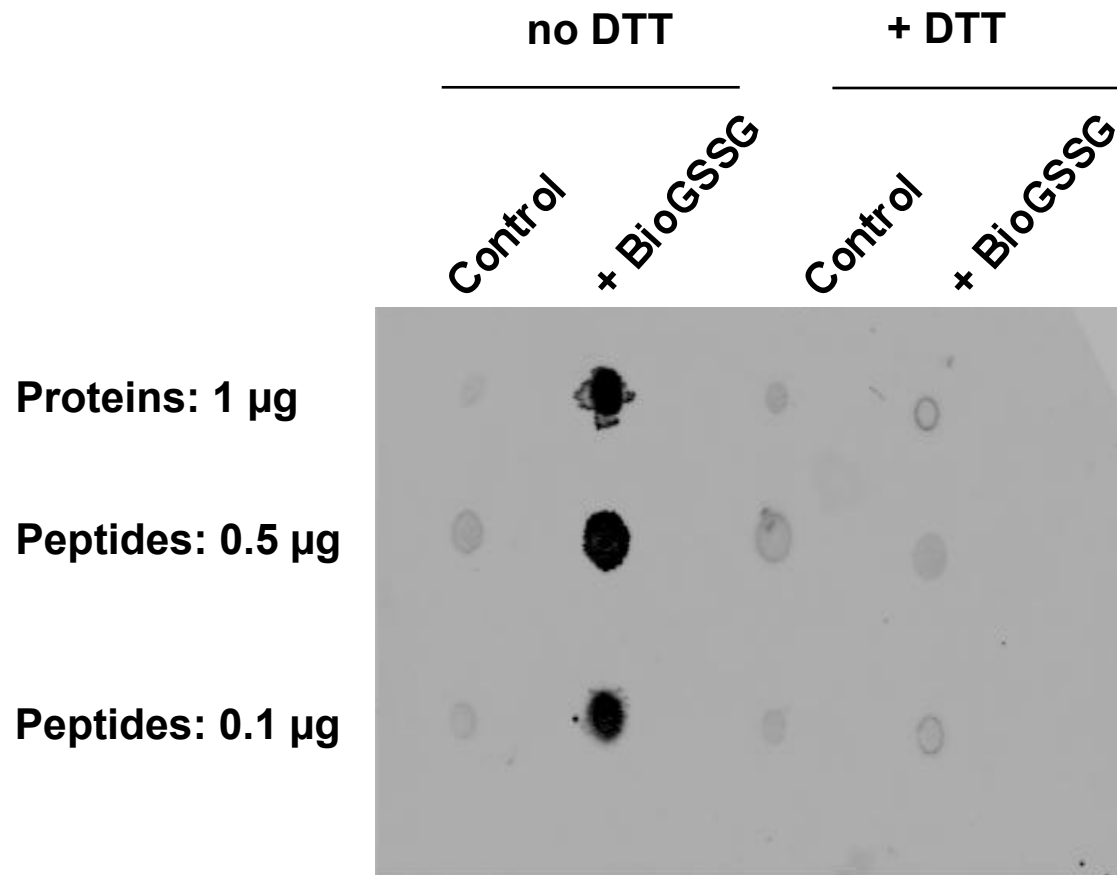


**Supplemental Figure 2: MALDI-TOF analysis of N,N'-biotinyl glutathione disulfide (BioGSSG).**

After synthesis from EZ link Sulfo-NHS-Biotin and GSSG, the resulting mixture was loaded on C18 reverse-phase column and analyzed by HPLC (Alliance Systems, Waters, UK, equipped with a diode array detector). Two peaks were obtained and analyzed by mass spectrometry. The major peak (more than 80%) with a mass of 1065.25 Da (theoretical mass = 1065.4 Da) corresponded to N,N'-biotinyl glutathione disulfide (BioGSSG), the expected compound formed by the addition of one biotin moiety on each amino group of  $\gamma$ -Glu residues. The presence of Na<sup>+</sup> adduct and K<sup>+</sup> adduct was also observed. The minor peak (less than 20%) corresponded to N-biotinyl glutathione disulfide, i.e. one biotin moiety on one of the two amino groups of  $\gamma$ -Glu residues (data not shown).



Supplemental Figure 3: Scheme of central metabolism in *Synechocystis*. Proteins identified in our study as glutathionylatable targets are boxed in red.



**Supplemental Figure 4: Control of the effectiveness of peptide S-glutathionylation.**

After reaction with BioGSSG, proteins were digested using immobilized trypsin as described in the Experimental Section. Resulting peptides (0.1 and 0.5  $\mu$ g) before streptavidin-agarose column were loaded on Whatman paper and glutathionylation was revealed using anti-biotin antibodies as described. Glutathionylation of intact proteins (1  $\mu$ g) before SDS-PAGE was also checked by the same procedure. Control samples correspond to the same extracts without BioGSSG treatment. The reversibility of the reaction was assessed by treatment with 20 mM DTT for 30 min.