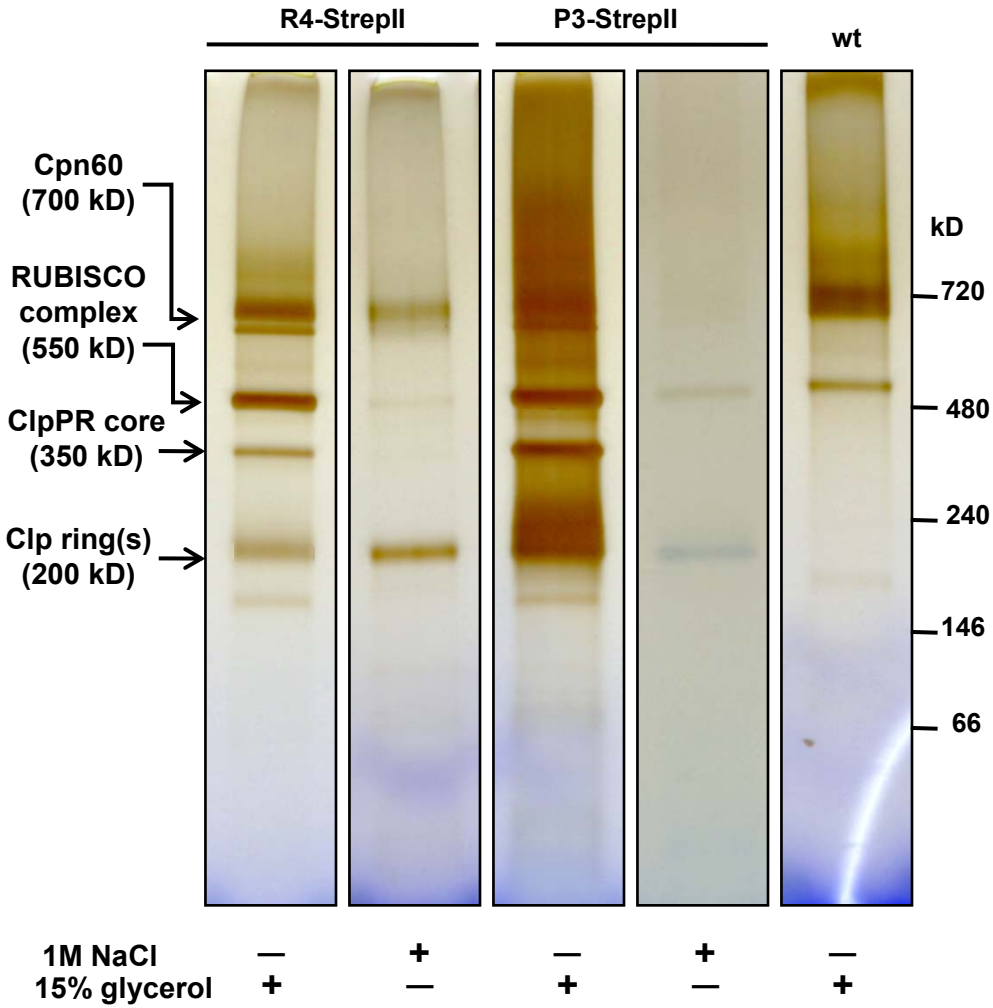
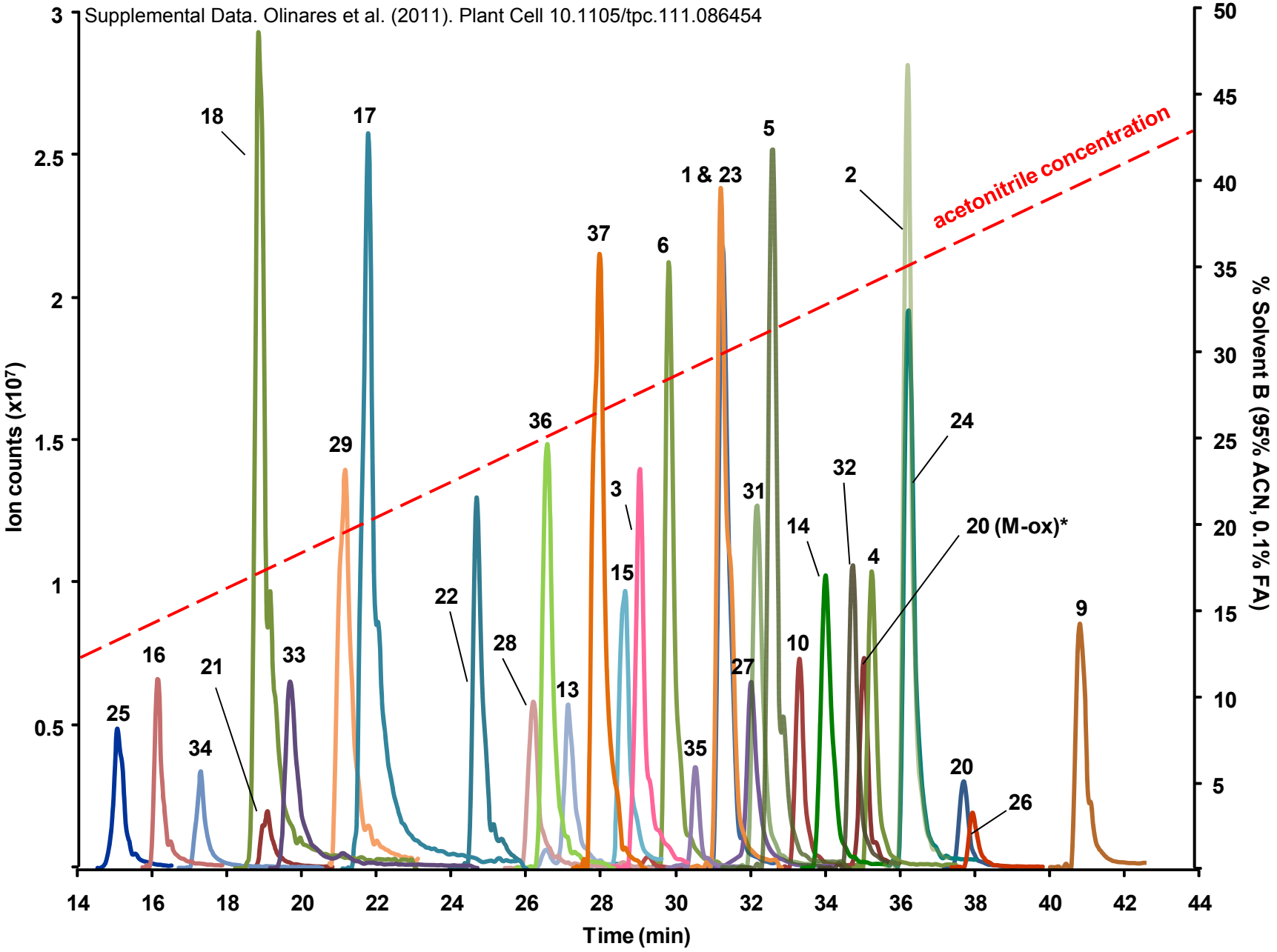
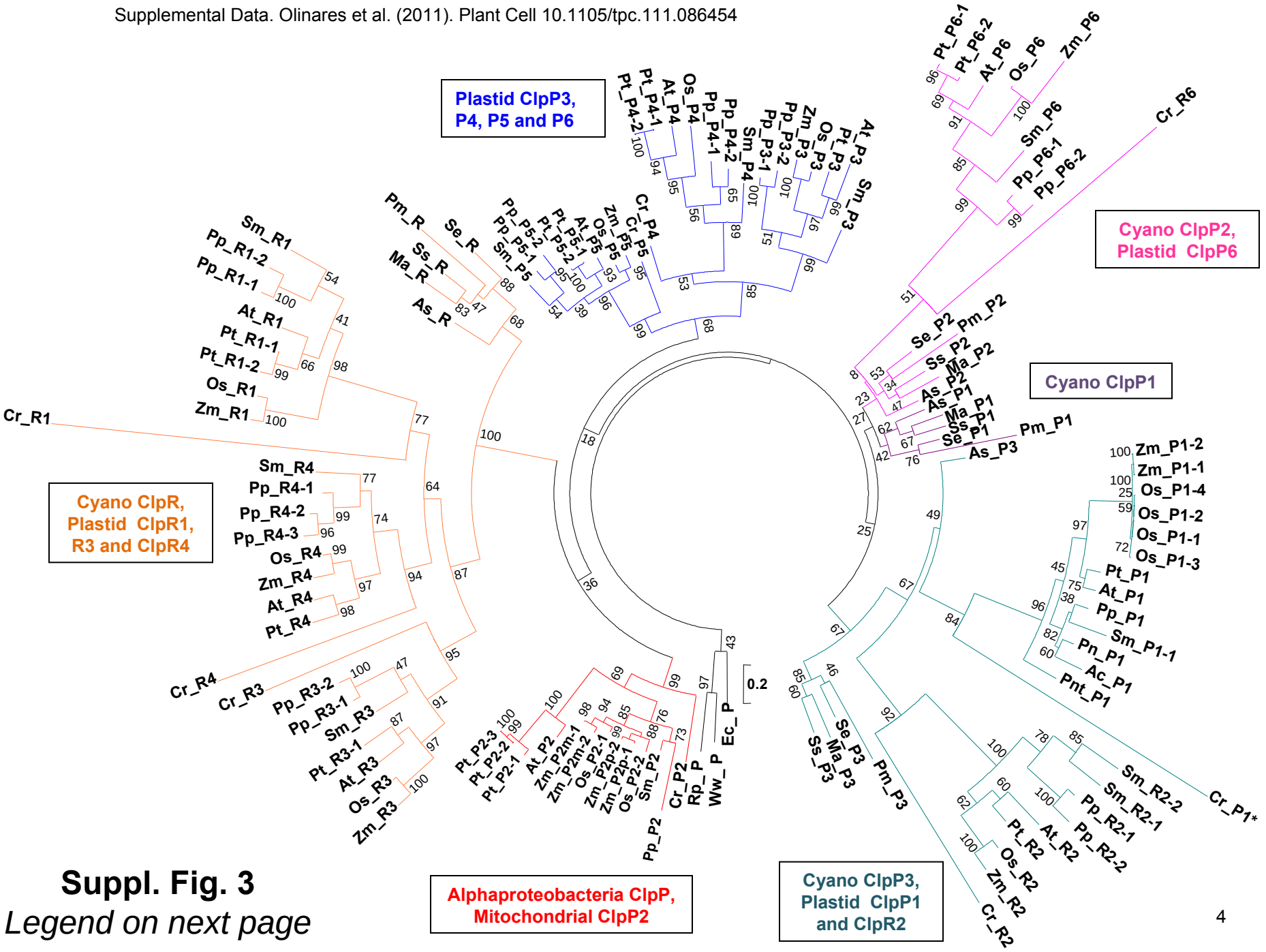


Supplemental Figure 1. Affinity purification and MS-based protein component identification of StrepII-tagged Clp assemblies. Native PAGE analysis of affinity-purified StrepII-tagged Clp assemblies (350 and 200kDa) from total leaf extractions showing the gel lanes at full-length. On-column salt incubation dissociated the individual rings from the core revealing the Strep-tagged Clp ring. Additional bands at 720 and 500 kDa were observed but were also found in mock purifications (with wild-type). MS/MS analyses of these bands indicate the co-purification of the Chaperone 60 complex (700 kDa) and the highly abundant Ribulose-1,5-bisphosphate carboxylase oxygenase (RUBISCO) complex (~550 kDa)



Supplemental Fig. 2 *Legend on next page*

Supplemental Figure 2. Representative extracted ion chromatograms (XICs) of the peptides derived from 100 fmol Clp-QconCAT protein. The numbers designate the position and identity of the peptide (see Supplemental Table 2). 32 out of the 37 Clp-QconCAT peptides can be detected and quantified. All the quantifiable Clp-QconCAT peptides eluted within the first 45 minutes of the run. The % acetonitrile concentration (red dashed line) was estimated based on the mobile phase gradient used with correction for dead volume during the chromatographic run.



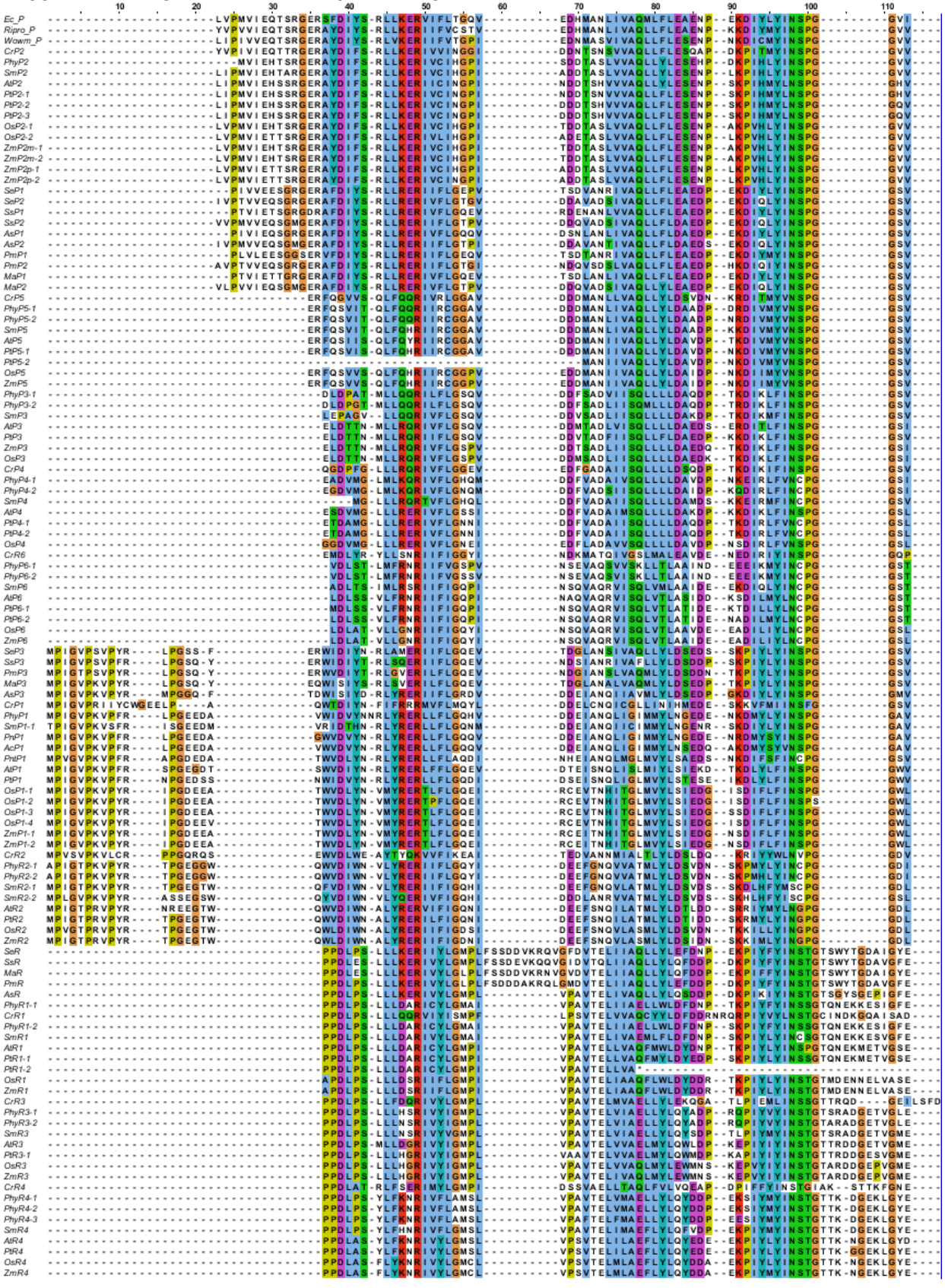
Suppl. Fig. 3

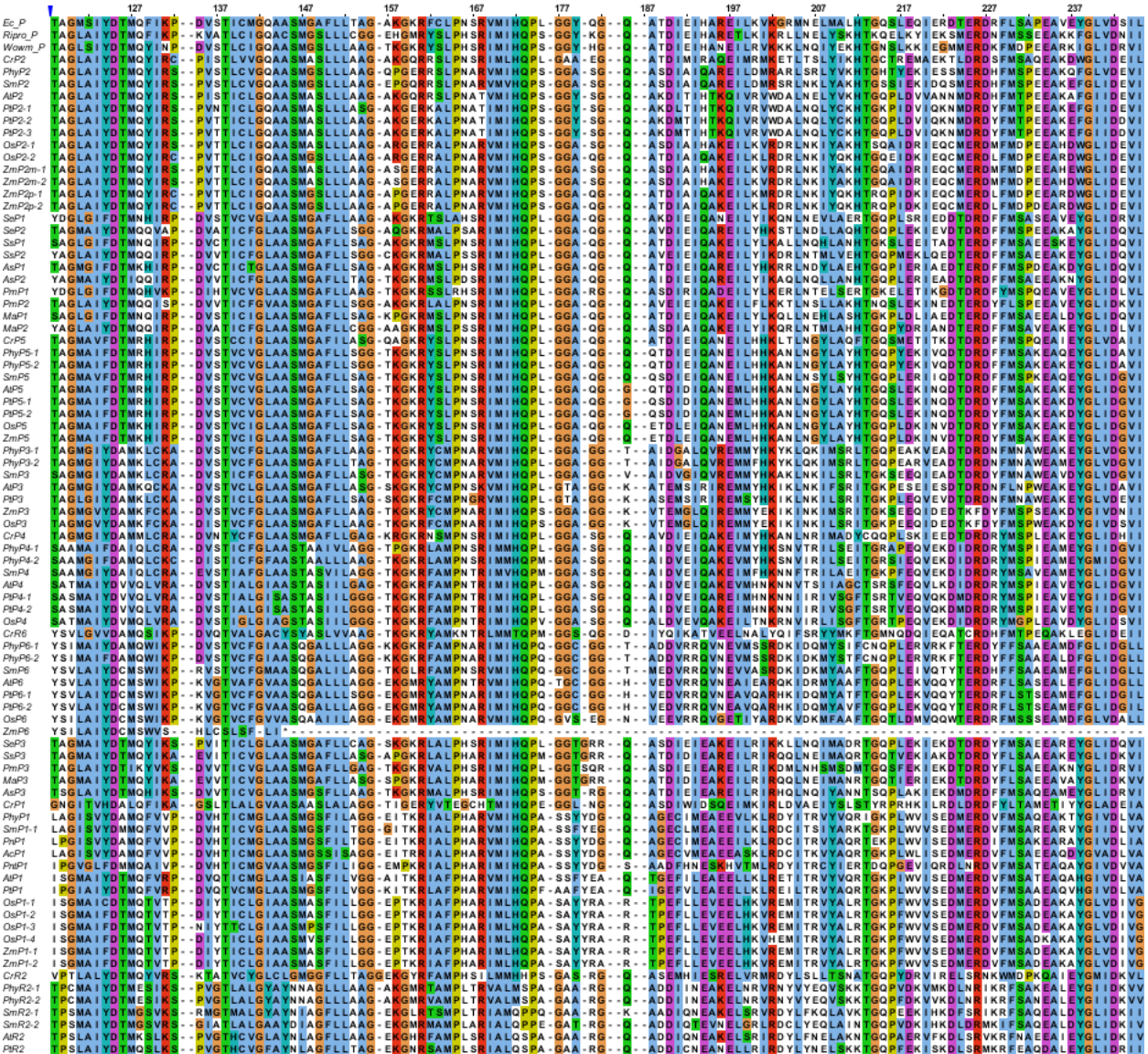
Legend on next page

Supplemental Figure 3. Phylogenetic analysis of the ClpP/R proteins from proteobacteria, photosynthetic bacteria, green algae and plants. The phylogenetic tree inferred for the 118 Clp sequences from various prokaryotes and photosynthetic eukaryotes employing the Maximum Likelihood (ML) approach (Felsenstein, 1981) with the General Time Reversible (GTR) model for nucleotide substitution (Tavar, 1986) and a gamma model of substitution rate heterogeneity (Yang, 1996). Support values are shown from 1,000 non-parametric bootstrap inferences. This includes the Escherichia coli ClpP; the ClpP from alphaproteobacteria presumed to be the ancestor of mitochondrial organelles: *Rickettsia prowazekii* (Rp) and *Wolbachia wmel* (Ww); the cyanobacteria which are the ancestor of plastids: *Anabaena sp. (strain PCC 7120)* (As), *Synechocystis sp. (strain PCC 6803)* (Ss), *Synechococcus elongatus* (strain PCC 7942) (Se), *Microcystis aeruginosa* (Ma) and *Prochlorococcus marinus* MED4 (Pm); the green alga *Chlamydomonas reinhardtii* (Cr) and representative plant species: the bryophyte *Physcomitrella patens* (Pp), the lycopod *Selaginella moellendorfii* (Sm), the dicots *Arabidopsis thaliana* (At) and *Populus trichocarpa* (Pt) as well as dicots *Zea mays* (Zm) and *Oryza sativa* (Os). The plastid ClpP1 homologues for the ferns *Psilotum nodum* (Pn) and *Adiantum capillus renesis* (Ac) and the gymnosperm *Pinus thunbergii* (Pnt) were also collected from recent chloroplast genome sequencing projects for these species. The Cr_P1* is the plastid-encoded ClpP1 in green alga with the large insertion (IS1) sequence removed. Ec_P was used as the outgroup.

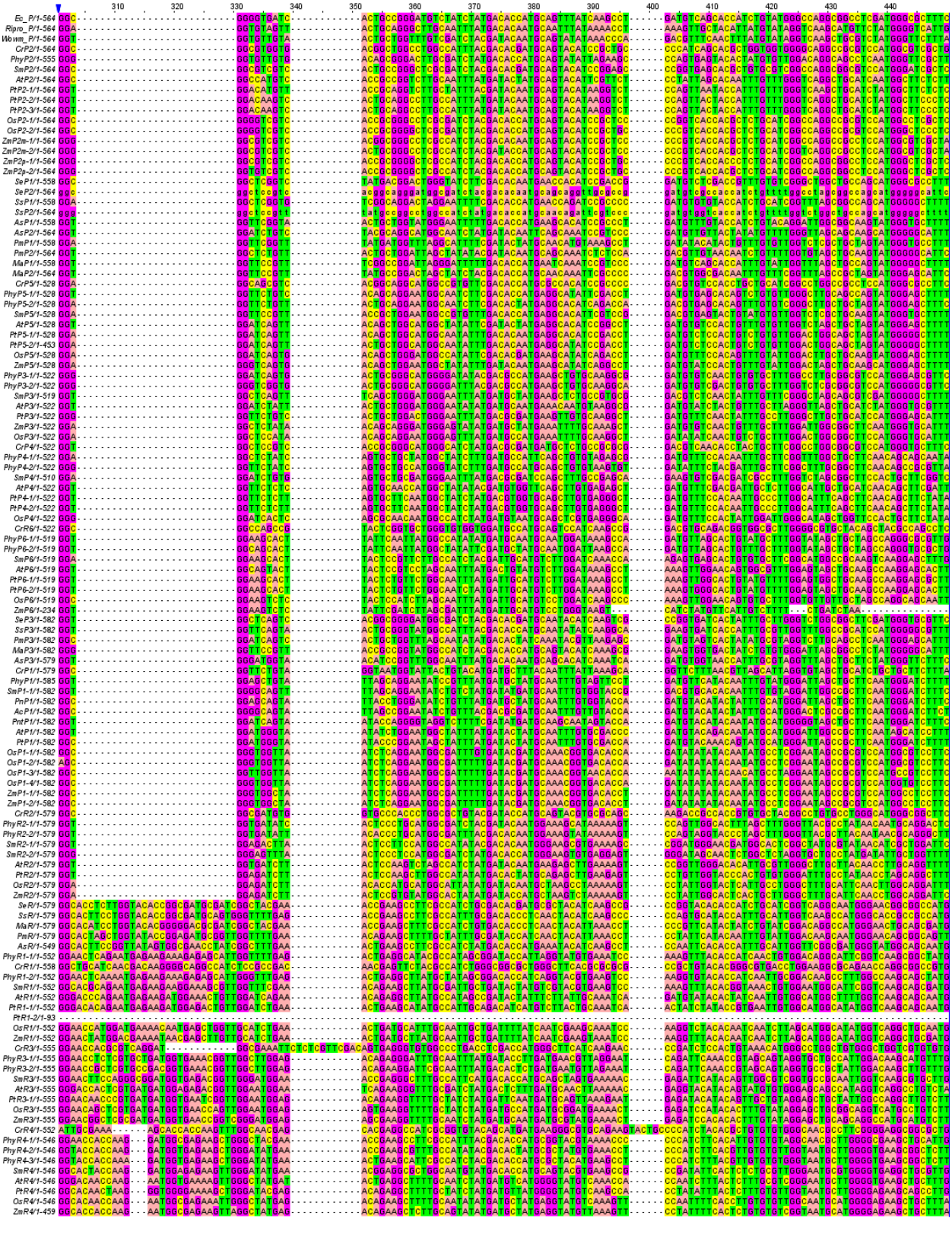
Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol **17**: 368-376.
Tavar, S. (1986). Some probabilistic and statistical problems on the analysis of DNA sequences. In Some mathematical questions in biology-DNA sequence analysis., R.M. Miura, ed (Providence, R.I.), pp. 57-86.
Yang, Z. (1996). Among-site rate variation and its impact on phylogenetic analyses. Trends Ecol. Evol. **11**: 367-372.

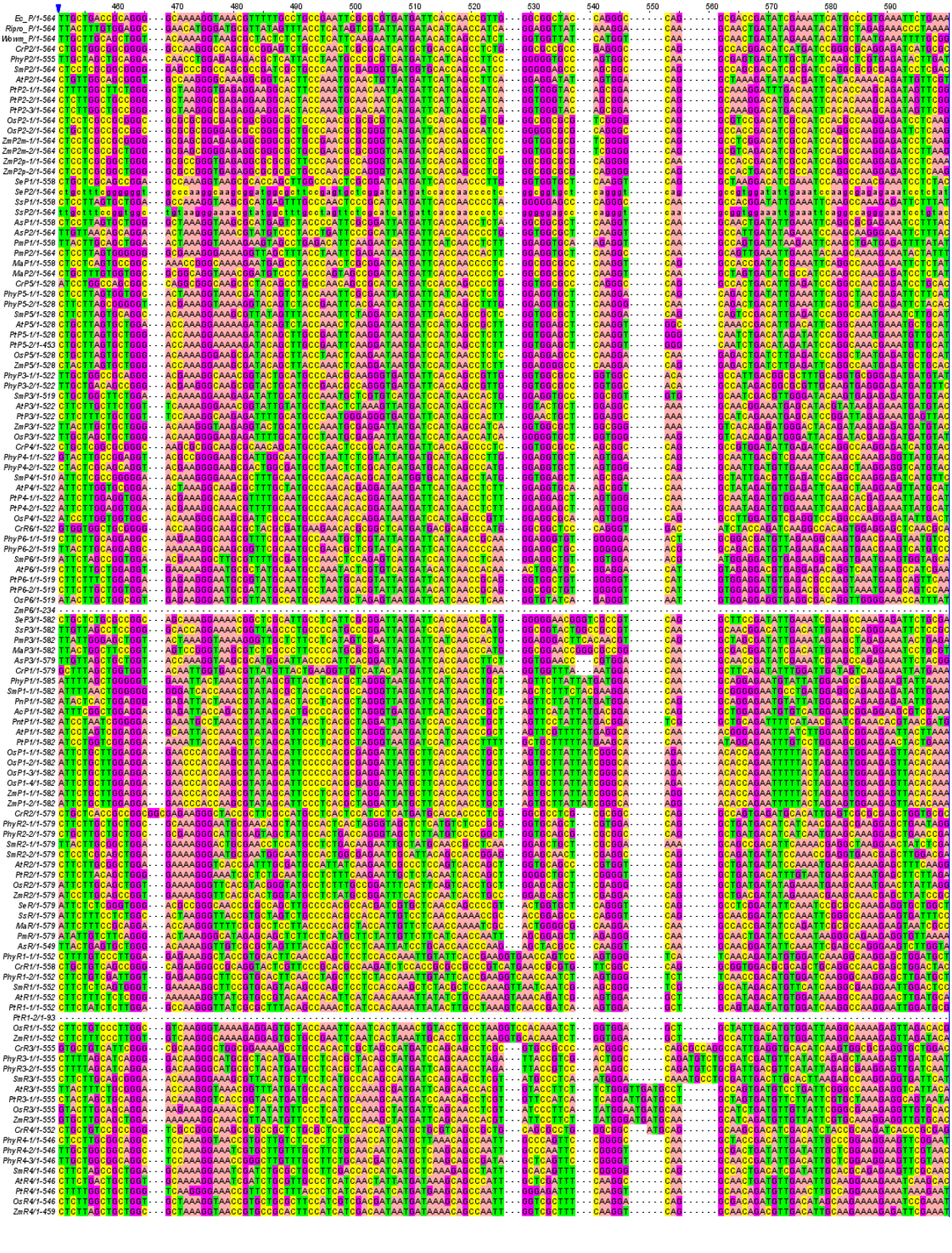
Supplemental Figure 4. Protein sequence alignment:

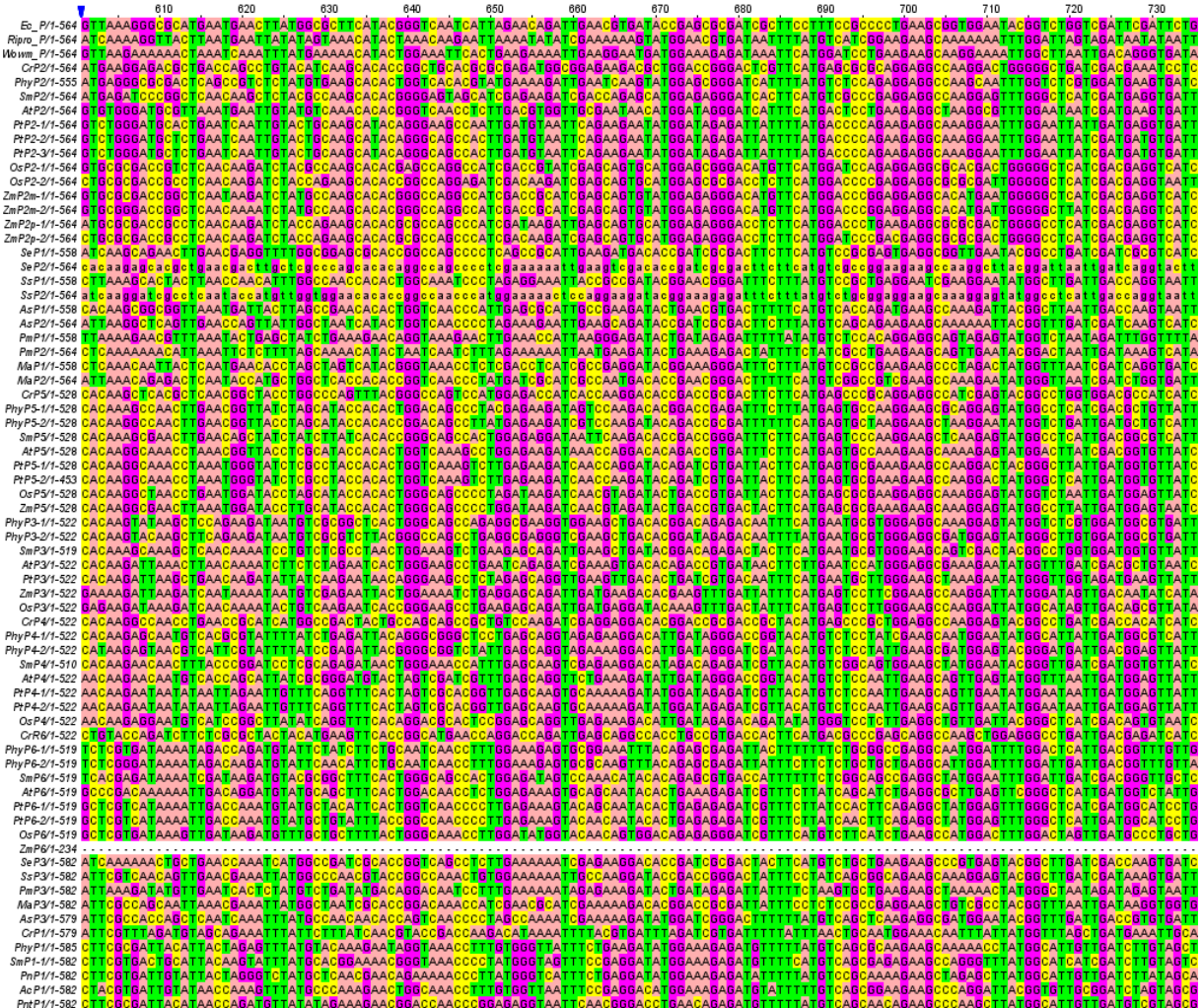




cDNA sequence alignment:







Supplemental Table 1. MS/MS analyses of the affinity-purified Clp assemblies[a]

		R4-StrepII			P3-StrepII			
		no salt		+ salt	no salt			+ salt
Accession	**Clp**	**core (350 kDa)**	**ring(s) (200 kDa)**	**ring (200 kDa)**	**core (350 kDa)**	**ring(s) (200 kDa)[b]**	**ring(s) (180 kDa)[b]**	**ring (200 kDa)**
ATCG00670.1	P1	5	2	6	43	11	40	
AT1G49970.1	R1	8	1	19	19	10	28	
AT1G12410.1	R2	6	4	30	25	20	60	
AT1G09130.1	R3	11	2	33	35	34	39	
AT4G17040.1	R4	10	4	43	57	26	77	
AT1G66670.1	P3	3	1		35	50	70	18
AT5G45390.1	P4	9	6	3*		122	127	72
AT1G02560.1	P5	8	4	2*	66	86	109	41
AT1G11750.1	P6	10	6		43	86	76	40
AT4G25370.1	T1	2			38	36	26	
AT4G12060.1	T2	3			6	2		

[a] MS/MS analyses was performed from peptides extracted from the in-gel trypsin-digested protein bands from native gel separations of the affinity-purified Clp complexes (see Fig.1 for the corresponding gel images). Shown here are the total adjusted spectral counts per protein.

[b] Two bands were observed at slightly different masses and were excised separately for in-gel digestion and MS/MS analysis. Nevertheless, both bands contained all the ClpP/R subunits.

* These are spectral counts from minor contaminating hydrophobic peptides of ClpP4 and ClpP5 that could not be fully removed from the column ("sticky peptides") even after two blank LC-MS runs.

Supplemental Table 2. The peptides comprising Clp-QconCAT and their corresponding properties.

Accession	Peptide	Position[a]	Peptide	Residue order[b]	Charge states	Number of Residues	$[M+2H]^{2+}$	$[M+3H]^{3+}$	Q?[c]
Clp core subunits									
ATCG00670.1	P1-1	19	IAFPHAR	245-251	2,3	7	406.2323, 271.1573	271.1573	N
	P1-2	6	SPGEGDTSWVDIYNR	86-100	2	15	848.3841		Y
AT1G49970.1	R1-1	11	YLQAQAAIDYGIADK	143-157	2	15	820.4199		Y
	R1-2	24	TAPPDLPSLLLDAR	323-336	2	14	739.9143		Y
AT1G12410.1	R2-1	25	IALQSPAGAAR	337-347	2	11	527.8038		Y
	R2-2	4	FNAEEAIEYGLIDK	56-69	2	14	806.3987		Y
AT1G09130.1	R3-1	12	EPIYIYINSTGTTR	158-171	2	14	814.4199		N
	R3-2	26	DILVELLSK	348-356	2	9	515.3132		Y
AT4G17040.1	R4-1	27	GSAHEQPPPDLASYLFK	357-373	2, 3	17	928.9625	619.6441	Y
	R4-2	14	YFSPTEAVEYGIIDK	181-195	2	15	866.4274		Y
AT1G66670.1	P3-1	7	DNTNLPSER	101-109	2	9	523.2491		N
	P3-2	20	LPSFEELDTTNMLLR	252-266	2	15	889.9533		Y
	P3-2 (ox)	20	LPSFEELDTTNM(ox)LLR	252-266	2	15	897.9508		Y
AT5G45390.1	P4-1	8	SFEQVLK	110-116	2	7	425.7371		Y
	P4-2	21	ADVSTIALGIAASTASIILGAGTK	267-290	3	24	1101.579		N
AT1G02560.1	P5-1	22	ANLNGYLAYHTGQSLEK	291-307	2, 3	17	939.9709	626.983	Y
	P5-2	9	FQSIISQLFQYR	117-128	2	12	765.4092		Y
AT1G11750.1	P6-1	23	IIFIGQPINAQVAQR	308-322	2	15	834.4832		Y
	P6-2	10	VISQLVTLASIDDK	129-142	2	14	751.4272		Y
Clp chaperones/adaptors									
AT4G25370.1	T1-1	13	DETLSLLGK	172-180	2	9	488.2715		Y
	T1-2	28	AIAWAIDEK	374-382	2	9	508.7742		Y
AT4G12060.1	T2-1	29	ALDSALDQNLK	383-393	2	11	594.317		Y
	T2-2	15	ILATLGFTDEK	196-206	2	11	604.3321		Y
AT1G68660.1	S-1	30	VILHNDNFNK	394-403	2, 3	10	607.3198	405.2157	N
	S-2	16	GGGVLDKPIIEK	207-218	2, 3	12	613.3612	409.2432	Y
AT5G50920.1	C1-1	31	VPEPTVDETIQILK	404-417	2	14	791.4403		Y
AT5G50920.1,	C1/2-1	17	VLENLGADPSNIR	219-231	2	13	699.3728		Y
AT3G48870.1	C1/2-2	32	GSGFVAVEIPFTPR	418-431	2	14	738.8959		Y
AT5G51070.1	D1-1	18	VVGQDEAVAAISR	232-244	2	13	657.8542		Y
	D1-2	33	VFEAAVEYSR	432-441	2	10	585.7931		Y
Localization markers[d]									
AT1G04410.1	MD-1	1	VLVVANPANTNALILK	8-23	2	16	825.5011		Y
	MD-2	34	LSVPVSDVK	442-450	2	9	472.2766		Y
AT5G20290.1	RPS8-1	3	VLDVVYNASNNELVR	41-55	2	15	852.9494		Y
	RPS8-2	36	SAIVQVDAAPFK	465-476	2	12	623.3455		Y
AT3G04790.1	PRI-2	35	SLGIPLVGLDTHPR	451-464	2, 3	14	737.9225	492.2841	Y
	PRI-1	2	LLSSGELYDIVGIPTSK	24-40	2	17	896.4906		Y
AT5G51820.1	PGM1-1	5	VAEIPDIDLSQVGVTK	70-85	2	16	842.4618		Y
	PGM1-2	37	IYGNTLSISEIK	477-488	2	12	669.3692		Y

[a] Order by which these proteotypic peptide appeared starting from the N-terminus of the Clp-QconCAT construct

[b] Residue numbers of the peptide sequence within the Clp-QconCAT construct.

[c] Can be detected and quantified. Y=yes, N = no.

[d]These proteins serve as reference for organellar location. Malate dehydrogenase (MD) and the 40S ribosomal subunit S8 (RPS8) are both cytosolic whereas ribose 5-phosphate isomerase (PRI) and phosphoglucomutase (PGM) are both plastid-localized.

Supplemental Table 3. Linear instrument response for individual Clp-QconCAT peptides.*

Peptide	Position	slope	y-intercept	Regression coefficient
ClpP1/R ring				
P1-2	6	1.40	-0.05	0.995
R1-1	11	1.09	-0.04	0.971
R1-2	24	1.20	-0.04	0.990
R2-1	25	1.05	0.14	0.999
R2-2	4	1.12	0.04	0.987
R3-2	26	0.76	0.13	0.968
R4-1	27	1.13	-0.06	0.996
R4-2	14	1.32	-0.05	0.991
ClpP3-P6 ring				
P3-2	20	0.89	0.26	0.987
P4-2	8	1.04	0.05	0.999
P5-1	22	1.827	-0.07	0.999
P5-2	9	0.57	0.42	0.956
P6-1	23	1.21	-0.05	0.994
P6-2	10	1.22	-0.33	0.975
Clp chaperones/adaptors				
T1-1	13	1.05	0.04	0.998
T1-2	28	1.10	0.07	0.998
T2-1	29	1.00	0.08	0.998
T2-2	15	0.72	0.19	0.987
S-2	16	0.86	0.12	0.998
C1/2-1	17	1.07	0.07	0.997
C1/2-2	32	0.70	0.30	0.971
C1-1	31	0.92	0.02	0.986
D1-1	18	1.08	0.09	0.999
D1-2	33	1.31	0.004	0.993
Localization markers				
MD-1	1	1.11	-0.09	0.992
MD-2	34	1.03	0.002	0.998
PGM1-1	5	1.09	-0.09	0.988
PGM1-2	37	1.30	-0.06	0.996
PRI-1	2	0.87	0.13	0.981
PRI-2	35	1.14	0.03	0.996
RPS8-1	3	1.26	-0.04	0.992
RPS8-2	36	0.85	0.15	0.989

* For 100 fmol of heavy Clp-Qconcat peptides mixed with varying amounts of their light versions spanning two orders of magnitude (10 fmol to 1 pmol) with L/H ratios of 0.1, 0.2, 0.5, 1, 2, 5 and 10.

Supplemental Table 4. Quantification of Clp subunits and determination of subunit stoichiometry in the Clp core complex and constituent Clp rings.[a]

Clp core complex (350 kDa)

Clp	Pep	Quantity (x 100 fmol)								Stoichiometry						
		R4-1	%CV	R4-2	%CV	P3-1	%CV	P3-2	%CV	R4-1	R4-2	P3-1	P3-2	AVE	SD	%CV
P1	Q6	1.62	1.2	0.59	1.2	3.30	1.4	4.37	0.8	3.35	3.15	2.51	2.72	**2.93**	**0.38**	**13.1**
R1	Q11	0.59	0.9	0.28	0.9	1.31	0.9	1.67	0.1	1.22	1.50	1.00	1.04	**1.19**	**0.23**	**19.0**
R2	Q25	0.74	4.9	0.29	4.9	1.45	1.0	1.82	0.8	1.53	1.54	1.10	1.13	**1.33**	**0.24**	**18.2**
	Q4	0.33	0.5	0.15	0.5	0.90	1.2	1.30	1.1	0.69	0.82	0.69	0.81	**0.75**	**0.07**	**9.5**
R3	Q26	0.47	0.04	0.18	0.04	1.04	1.2	1.74	0.6	0.97	0.97	0.79	1.08	**0.95**	**0.12**	**12.6**
R4	Q27	0.51	1.5	0.17	1.5	0.92	3.2	1.22	0.4	1.05	0.92	0.70	0.76	**0.86**	**0.16**	**18.4**
	Q14	0.46	1.9	0.20	1.9	1.11	0.1	1.45	0.6	0.95	1.08	0.85	0.90	**0.94**	**0.10**	**10.5**
TOTAL[b]						7.85		10.68								

Clp ring with no salt incubations (200 kDa)

Clp	Pep	Quantity (x 100 fmol)												Stoichiometry								
		R4-3	%CV	R4-4	%CV	P3-3	%CV	P3-4	%CV	P3-5	%CV	P3-6	%CV	R4-3	R4-4	P3-3	P3-4	P3-5	P3-6	AVE	SD	%CV
P1	Q6	4.56	3.6	0.86	4.4	1.19	1.6	1.75	1.0	6.45	1.5	6.89	0.4	3.19	3.13	3.34	3.32	3.46	3.53	**3.33**	**0.15**	**4.6**
R1	Q11	1.54	0.8	0.38	0.2	0.56	0.9	0.67	0.4	2.17	0.4	2.28	0.5	1.08	1.40	1.57	1.28	1.16	1.17	**1.28**	**0.18**	**14.3**
R2	Q25	1.84	2.4	0.49	3.6	0.67	0.6	0.81	1.9	2.30	1.9	3.04	1.1	1.29	1.80	1.89	1.54	1.24	1.55	**1.55**	**0.26**	**16.9**
	Q4	0.93	1.2	0.24	3.3	0.27	0.7	0.47	0.9	1.74	15.5	1.79	0.3	0.65	0.89	0.75	0.90	0.93	0.92	**0.84**	**0.11**	**13.4**
R3	Q26	1.35	0.8	0.29	1.0	0.38	1.1	0.51	1.0	1.77	7.0	2.47	0.9	0.94	1.07	1.06	0.97	0.95	1.26	**1.04**	**0.12**	**11.6**
R4	Q27	1.43	0.5	0.23	2.5	0.37	1.3	0.48	3.1	1.69	2.5	1.82	0.7	1.00	0.84	1.03	0.91	0.91	0.93	**0.94**	**0.07**	**7.3**
	Q14	1.43	0.2	0.32	1.9	0.34	1.3	0.57	0.8	2.04	1.4	2.09	1.6	1.00	1.16	0.97	1.09	1.09	1.07	**1.06**	**0.07**	**6.4**

purified Clp ring from on-column salt incubation (200 kDa)

Clp		Quantity (x 100 fmol)					Stoichiometry					
		%CV	R4-6	%CV	R4-7	%CV	R4-6	R4-7	R4-8	AVE	SD	%CV
P1	Q6	1.5	2.74	1.0	1.15	1.2	2.79	3.38	2.68	**2.95**	**0.38**	**12.7**
R1	Q11	0.4	0.96	0.9	0.58	0.6	1.10	1.19	1.35	**1.21**	**0.12**	**10.1**
R2	Q25	6.8	1.03	8.4	0.52	0.2	1.42	1.27	1.21	**1.30**	**0.11**	**8.1**
	Q4	0.6	0.68	0.3	0.39	0.4	0.67	0.84	0.91	**0.81**	**0.12**	**15.3**
R3	Q26	0.9	0.81	0.6	0.58	1.2	0.62	1.00	1.34	**0.99**	**0.36**	**36.9**
R4	Q27	1.5	0.73	1.1	0.38	1.7	0.92	0.90	0.89	**0.90**	**0.02**	**1.7**
	Q14	0.8	0.89	1.7	0.48	0.6	1.08	1.10	1.11	**1.10**	**0.02**	**1.4**

Clp core complex (350 kDa)

Clp	Pep	Quantity (x 100 fmol)								Stoichiometry						
		R4-1	%CV	R4-2	%CV	P3-1	%CV	P3-2	%CV	R4-1	R4-2	P3-1	P3-2	AVE	SD	%CV
P3	Q20	0.47	1.8	0.26	0.5	1.60	2.8	1.84	0.4	0.98	1.38	1.22	1.15	**1.18**	**0.16**	**13.8**
P4	Q8					2.82	1.6	3.53	1.2			2.15	2.20	**2.17**	**0.04**	**1.6**
P5	Q22	1.60	1.0	0.48	1.5	2.51	1.3	3.74	3.4	3.31	2.56	1.91	2.33	**2.53**	**0.59**	**23.2**
	Q9	1.08	0.9	0.50	0.4	4.86	0.9	6.25	0.7	2.24	2.68	3.70	3.90	**3.13**	**0.80**	**25.5**
P6	Q23	0.72	1.8	0.32	1.1	1.50	1.2	1.86	0.8	1.48	1.68	1.14	1.16	**1.37**	**0.26**	**19.3**
	Q10	0.69	0.4	0.24	2.6	1.13	1.9	1.35	0.3	1.42	1.27	0.86	0.84	**1.10**	**0.29**	**26.7**
TOTAL[b]						9.43		11.97								
T1	Q28	0.42	0.4	0.16	2.2	0.96	0.4	1.29	0.4	0.86	0.83	0.73	0.80	**0.81**	**0.06**	**7.1**

Clp ring with no salt incubations (200 kDa)

Clp	Pep	Quantity (x 100 fmol)										Stoichiometry							
		R4-3	%CV	P3-3	%CV	P3-4	%CV	P3-5	%CV	P3-6	%CV	R4-3	P3-3	P3-4	P3-5	P3-6	AVE	SD	%CV
P3	Q20	0.24	13.5	1.70	0.4	2.19	0.3	2.22	14.5	3.56	0.9	0.73	1.16	1.13	0.86	1.02	**0.98**	**0.18**	**18.6**
P4	Q8			3.75	1.9	5.24	0.7	5.68	6.0	9.25	0.9		2.57	2.71	2.20	2.66	**2.53**	**0.23**	**9.1**
P5	Q22	0.86	0.42	2.47	1.7	5.59	0.6	6.63	2.0	9.82	1.2	2.60	1.69	2.89	2.57	2.83	**2.52**	**0.48**	**19.2**
	Q9	0.68	1.71	4.33	1.2	7.54	0.9	7.56	0.5	12.91	0.7	2.04	2.96	3.90	2.93	3.72	**3.11**	**0.74**	**23.9**
P6	Q23	0.35	0.87	1.69	0.3	2.27	0.7	3.01	1.3	3.78	1.2	1.05	1.16	1.17	1.16	1.09	**1.13**	**0.05**	**4.8**
	Q10	0.31	2.33	1.23	0.2	1.60	1.4	2.16	0.5	3.16	2.0	0.95	0.84	0.83	0.84	0.91	**0.87**	**0.05**	**6.2**
TOTAL[b]																			

T1	**Q28**			0.85	0.72	1.31	0.74	0.72	2.35	1.31	0.23		0.58	0.68	0.28	0.38	**0.48**	**0.18**	**38.1**

purified Clp ring from on-column salt incubation (200 kDa)

		Quantity (x 100 fmol)								**Stoichiometry**						
Clp	**Pep**	**P3-7**	**%CV**	**P3-8**	**%CV**	**P3-9**	**%CV**	**P3-10**	**%CV**	**P3-7**	**P3-8**	**P3-9**	**P3-10**	**AVE**	**SD**	**%CV**
P3	**Q20**	0.57	1.8	1.52	c	0.59	1.2	2.14	1.5	0.99	0.95	0.86	1.15	**0.99**	**0.12**	**12.3**
P4	**Q8**	1.37	1.2	3.23	3.4	1.48	1.6	4.92	2.1	2.40	2.02	2.14	2.64	**2.30**	**0.28**	**12.0**
P5	**Q22**	0.85	0.5	3.62	4.4	1.55	1.5	4.76	0.2	1.49	2.26	2.24	2.55	**2.14**	**0.45**	**21.3**
	Q9	1.45	1.1	5.00	1.2	2.11	0.5	7.73	0.8	2.54	3.12	3.05	4.15	**3.21**	**0.67**	**21.0**
P6	**Q23**	0.65	1.3	1.78	1.6	0.76	1.3	2.08	0.4	1.14	1.11	1.10	1.12	**1.12**	**0.02**	**1.8**
	Q10	0.49	1.7	1.43	1.8	0.63	0.3	1.65	1.8	0.86	0.89	0.90	0.88	**0.88**	**0.02**	**2.2**

[a] Based on affinity purification and native gel separation of various Clp assemblies from R4-StrepII (n=8) or P3-StrepII (n=10) transgenic lines . Molar amounts are derived from peak area ratios between the endogenous Clp peptides and 100 fmol spiked Clp-QconCAT peptides). ClpP4 could not be quantified in runs R4-1, R4-2 and R4-5. Stoichiometry was calculated by normalization against the molar amounts of ClpR4 or ClpP6 proteins. Peak areas for peptides identified with multiple charge states (R4-1, Q27 and P5-1, Q22) or modifications (P3-2, Q20 abd P5-1, Q22) were combined prior to calculating the light-to-heavy ratios.

[b] Sum of the molar amounts of the constituent Clp subunits per ring. The molar amounts for proteins quantified with two peptides were averaged. The average ratio between the total moles of ClpP1/R and ClpP3-6 within the Clp core is 1.16 for n=2 (Runs P3-1 and P3-2).

Supplemental Table 5. Accession numbers for protein and cDNA sequences of the Clp proteins from prokaryotes, green algae and plants.

Protein Accessions (Prokaryotic Clps)					
	Proteo-bacteria P	**Cyano P1**	**Cyano P2**	**Cyano P3**	**Cyano R**
E. coli (Ec)	POA6G7				
Rickettsia prowazekii (Rp)	RP520				
Wolbachia wmel (Ww)	WD0319				
Anabaena sp. (strain PCC 7120) (As)		Q8YXH5	Q8YQX8	Q8YP43	Q8YP42
Synechocystis sp. (strain PCC 6803) (Ss)		P54416	Q59993	P74467	P74466
Synechococcus elongatus (strain PCC 7942) (Se)		P54415	O34125	Q9L4P3	Q9L4P4
Microcystis aeruginosa (Ma)		MAE_11870	MAE_62720	MAE_57180	MAE_57190
Prochlorococcus marinus MED4 (Pm)		PMM0742	PMM1656	PMM1314	PMM1313
cDNA Accessions (prokaryotic Clps)					
	Proteo-bacteria P	**Cyano P1**	**Cyano P2**	**Cyano P3**	**Cyano R**
E. coli (Ec)	ENA|AAA23588				
Rickettsia prowazekii (Rp)	RP520				
Wolbachia wmel (Ww)	WD0319				
Anabaena sp. (strain PCC 7120) (As)		gi|17227497: 1470240 -1470854	gi|17227497: 4443275- 4443973	gi|17227497: 5219511-5220104	gi|17227497: 5220184-5220846
Synechocystis sp. (strain PCC 6803) (Ss)		gi|16329170: 3212598-3213194	gi|16329170: 3245421-3246101	gi|16329170: 2206551-2207159	gi|16329170: 2205774-2206451
Synechococcus elongatus (strain PCC 7942) (Se)		ENA|AAC67306	ENA|AAB68677	ENA|AAB68677	ENA|CAB81780
Microcystis aeruginosa (Ma)		MAE_11870	MAE_62720	MAE_57180	MAE_57190
Prochlorococcus marinus MED4 (Pm)		PMM0742	PMM1656	PMM1314	PMM1313
Protein Accessions (Plastid Clps)					
	P1	**P3**	**P4**	**P5**	**P6/R6**
Chlamydomonas reinhardtii (Cr)	P42380		A8IJ60	A8IL21	A8INX1
Physcomitrella patens (PhyP)	Q6YXM7	P3-1: A9RYV9, P3-2: A9T6I1	P4-1: A9TZR6, P4-2: A9RE44	P5-1: A9S5E8, P5-2: A9TE25	P6-1: A9TH43, P6-2: A9SWP4
Selaginella moellendorfii (Sm)	C7B2H1	XP_002960346.1	XP_002981945.1	XP_002963649.1 , XP_002983780.1	XP_002968942.1
Arabidopsis thaliana (At)	ATCG00670.1	AT1G66670.1	AT5G45390.1	AT1G02560.1	AT1G11750.1
Populus trichocarpa (Pt)	A4GYT6	B9H362	P4-1: B9GZW8, P4-2: A9PA38	P5-1: B9GST6, P5-2:B9I9I1	P6-1: A9PDP3, P6-2: B9HRQ3
Zea mays (Zm) ("GRM" removed from Accessions)	ZM2G448161_P01, ZM2G427444_P06	ZM2G001755_P01		ZM2G121456_P01	ZM2G092632_P01
Oryza sativa (Os)	Os08g15270, Os10g21300, Osp1g005990, Os12g10590	0s01g32350.1	Os10g43050.1	Os03g19510.1	Os03g29810.1
Psilotum nodum (Pn)	Q8WHZ7				
Adiantum capillus renesis (Ac)	Q85FJ8				
Pinus thunbergii (Pnt)	P41609				
cDNA accessions (for plastid ClpPs)					
	P1	**P3**	**P4**	**P5**	**P6/R6**
Chlamydomonas reinhardtii (Cr)	ENA|ACJ50097		gi|159465134: 119-1156	gi|159465360: 108-878	gi|159466487: 159-1010
Physcomitrella patens (PhyP)	gi|34501376:11632-11702, 12338-12632, 13074-13307	P3-1: gi|168013185, P3-2: gi|168043510	P4-1: gi|168064315, P4-2: gi|167999543	P5-1: gi|168018158: 395-1306 , P5-2: gi|168048811	P6-1: gi|168050942, P6-2: gi|168036512
Selaginella moellendorfii (Sm)	gi|255961289: c>68286-68216, c67451-66902	XM_002960300.1	XM_002981899.1	XM_002963603.1 XM_002983734.1	XM_002968896.1

Arabidopsis thaliana (At)	ATCG00670.1	gi|145337232: 57-986	gi|145358888	gi|145334998	gi|145335409
Populus trichocarpa (Pt)	ENA|ABO36731	gi|224079412	P4-1: gi|224074499:77-739 , P4-2: EF145046	P5-1: gi|224068557: 123-1025 , P5-2: gi|224128301: 37-543	P6-1: gi|224080314: 72-869 , P6-2: gi|224103436:1-741
Zea mays (Zm) ("GRMZM" removed from Accessions)	P1-1: 2G448161_T01 , P1-2: 2G427444_T06	2G001755_P01	2G001755_T01	2G121456_T03	2G121456_T03
Oryza sativa (Os)	P1-1: 13108.m09245, P1-2: 13110.m07757, P1-3: gi|11466763: 67638-68288 , P1-4: 13112.m01122	13101.m03260	13110.m04073	13103.m02334	13103.m03417
Psilotum nodum (Pn)	ENA|BAB84241				
Adiantum capillus renesis (Ac)	ENA|AAP29415				
Pinus thurnbergii (Pnt)	gi|7524593: 29611-30201				
Protein accessions (plastid ClpR and mitochondrial ClpP2)					
	R1	**R2**	**R3**	**R4**	**mito P2**
Chlamydomonas reinhardtii (Cr)	A8INX1	jgi|Chlre4|183767	A8I547	A8IH07	A8IX06
Physcomitrella patens (PhyP)	P6-1: A9TH43, P6-2: A9SWP4	R1-1:A9TVY1, R1-2: A9SI89	R2-1: A9SWI4, R2-2: A9T3W3	R3-1: A9SG20, R3-2: A9RZ41	R4-1: A9RQL9, R4-2: A9TF07, R4-3: A9SSY7
Selaginella moellendorfii (Sm)	XP_002968942.1	XP_002970082.1	XP_002968834.1	XP_002990376.1	XP_002982594.1
Arabidopsis thaliana (At)	AT1G11750.1	AT1G49970.1	AT1G12410.1	AT1G09130.1	AT4G17040.1
Populus trichocarpa (Pt)	P6-1: A9PDP3, P6-2: B9HRQ3	R1-1: B9HMY7, R1-2: B9NHA6	B9N3L0	A9PFT0	A9PAS6
Zea mays (Zm)	ZM2G092632_P01	ZM2G099529_P01	ZM2G148106_P01	ZM2G030072_P02	AC207652.3_FGP003
Oryza sativa (Os)	Os03g29810.1	Os05g51450.1	Os06g04530.1	Os03g22430.1	Os01g16530.1
cDNA accessions (Plastid ClpR and mitochondrial ClpP2)					
	R1	**R2**	**R3**	**R4**	**mito P2**
Chlamydomonas reinhardtii (Cr)	jgi|Chlre4|183767| estExt_fgenesh2 _kg.C_10275	gi|159467460: 144-992	gi|159464778: 1-1248	gi|159470090: 140-970	gi|159477908
Physcomitrella patens (PhyP)	R1-1: gi|168061570, R1-2: gi|168026988	R2-1: gi|168036369, R2-2: gi|168041626	R3-1: gi|168025571, R3-2: gi|168013354	R4-1: gi|168007319, R4-2: gi|168049468, R4-3: gi|168033938:1-714	gi|168031791
Selaginella moellendorfii (Sm)	XM_002970036.1	XM_002968788.1	XM_002990330.1,	XM_002982548.1	XM_002983734.1
Arabidopsis thaliana (At)	gi|145336568	gi|145335431	gi|145335309	gi|30683910	gi|186524962: 74-799
Populus trichocarpa (Pt)	R1-1:gi|224103734:80-1255, R1-2: gi|224097119	gi|224125071: 126-965	gi|224125177: 74-1099	gi|224126446: 25-957	P2-1: gi|224081362: 28-762 , P2-2: gi|224094112: 142-876 , P2-3: gi|224094112: 142-876
Zea mays (Zm) ("GRMZM" removed from Accessions)	2G099529_T01	2G148106_T01	2G030072_T02	AC207652.3_FGT003	P2m-1: 2G320135_T02, P2m-2: 2G474883_T01, P2p-1: 2G053236_T01, P2p-2: 2G111756_T01
Oryza sativa (Os)	13105.m05548	13106.m00412	13103.m02699	13101.m01848	P2-1: 13104.m04426, P2-2: 13102.m04675

Supplemental Table 6. Primers used in this study

Construct	Primers (5' to 3')
R4cDNAStrepII Step1, for PCR amplification	1: CCATGGAGGTAGCAGCAGCGA 2: AGGATGAGACCAAATGAGTTGTGCC
R4cDNAStrepII Step2, for PCR amplification	1: CACCATGGAGGTAGCAGCAGCGAC 2: TCACTTCTCGAATTGAGGATGAGACCA
R4StrepII in pEARLEYGATE100, for genotyping	1: GGGAATGTCTCTCGTACCTTCAGTT 2: GGCGCTCTATCATAGATGTCGCTATAAACC
R4StrepII in pEARLEYGATE100, for genotyping	1: CCATGGAGGTAGCAGCAGCGA 2: GGCGCTCTATCATAGATGTCGCTATAAACC
R4 gene specific, for genotyping	1; GGGAATGTCTCTCGTACCTTCAGTT 2: ACAACTGGACACTGTTGCATAATGA
P3 cDNA Strep II, for PCR amplification	1: ATGGAGATGAGTTTGCGTCTCGCTTC 2:CTACTTCTCGAATTGAGGATGAGACCATTCAA T GGCGGCATAACCATTCTGTGTC
P3StrepII in pEARLEYGATE100, for genotyping	1: ATGGAGATGAGTTTGCGTCTCGCTTC 2: GGCGCTCTATCATAGATGTCGCTATAAACC
TDNA insertion, for genotyping	1: GGCAATCAGCTGTTGCCCGTCTCACTGGTG 2: ACAACTGGACACTGTTGCATAATGA
ClpQconCAT in pET21, for confirmation	1: TTATGCTAGTTATTGCTCAGCGGTG 2: CCATTTCTCGTATCGCATTTCCAC
ClpQconCAT in pET21, for confirmation	1: TCCGGCGTAGAGGATCGAGATC 2: TGGAAATGCGATACGAGAAATGG

Supplementary Text. Selection of the Clp-QconCAT peptides. Sequence coverage of the subunits of the plastid-localized Clp protease complex from extensive MS analysis of *Arabidopsis* chloroplasts, total leaf extracts and purified Clp complexes. The identified tryptic peptides are show in red and are separated by bars. The predicted chloroplast transit peptide sequence is underlined. Chemically reactive residues (methionine and cysteine) and positions in the sequence that confer instability (e.g., N-terminal Asn, N-terminal Gln, Asn-Gly) or missed cleavages (e.g., Pro after Lys or Arg) within the identified peptides are shown in green. The selected Clp-QconCAT peptides are highlighted in yellow. Additional comments regarding the behavior of the selected Clp peptides during the Clp-QconCAT quantification experiments are also included.

ClpP1

MPIGVPKVPFR|SPGEGDTSWVDIYNR|LYRER|LFFLGQEVDTEISNQLISLMIYLSIEK|DTKD LYLFINSPGGWVISGMAIYDTMQFVR|PDVQTICMGLAASIASFILVGGAITK|R|IAFPHAR|V MIHQPASSFYEAQTGEFILEAEELLK|LR|ETITR|VYVQR|TGKPIWVISEDMER|DVFMSATE AQAHGIVDLVAVQ

For ClpP1, only one peptide (SPGEGDTSWVDIYNR, P1-2) passed the requirements for a good quantifiable peptide. It harbors tryptophan which can be oxidized. However, peak area analyses of the unmodified peptide and its various oxidized forms (e.g., kynurenine, hydroxytryptophan and dihydroxytryptophan (see also (Perdivara et al., 2010)) showed that 98% of this peptide remained unoxidized in our Clp-QconCAT experiments. IAFPHAR (P1-1) was rarely detected in our extensive MS analysis of various *Arabidopsis* large-scale proteome experiments but we still included it in our Clp-QconCAT construct hoping that we would observe it in purified Clp complexes. However, we still did not detect this peptide in our Clp purifications most likely due to its short peptide length and high hydrophilicity. Most of the other peptides are long, methionine-bearing peptides that could be differentially modified and could be very hydrophobic.

ClpR2

MAVSFNTTLHQPSLSPSCSIKLYSGLKPQSASFLASGYQNLNKEFYGRVYKSLQSGTGKASR SRVKMMPIGTPRVPYR|NR|EEGTWQWVDIWNALYR|ER|VIFIGQNIDEEFSNQILATMLYL DTLDDSR|R|IYMYLNGPGGDLTPSLAIYDTMK|SLK|SPVGTHCVGLAYNLAGFLLAAGEK|G HR|FAMPLSR|IALQSPAGAAR|GQADDIQNEAK|ELSR|IR|DYLFNELAK|NTGQPAER|VFK|D LSR|VK|R|FNAEEAIEYGLIDK|IVRPPR|IKEDAPRQDESAGLG

We were able to quantify the two ClpR2 peptide standards which exhibited good instrument responses. However, peptide IALQSPAGAAR (R2-1) yielded a slightly higher stoichiometry value than the other peptide standard (see Table 2) and this might have been due to a potential missed cleavage within the Clp-QconCAT construct (see Methods section for the Clp-QconCAT sequence) yielding slightly lower amounts of the labeled standard and higher sample-to-standard ratios.

ClpR1

MATALVSPLTSQLNHEAVCSKFVLPKSPFMSGSKLFSSNMPCSTVPRRTRRSHCFASAKDM SFDHIPKQFRGDNLKDGVMQNFKNVPQYFYGLNSAQMDMFMTEDSPVRRQAEKVTEESIS SRNNYLNNGGIWSMSGMNAADARRYSMSVQMYRGGGGGGGSERPR|TAPPDLPSLLLDAR| ICYLGMPIVPAVTELLVAQFMWLDYDNPTKPIYLYINSPGTQNEK|METVGSETEAYAIADTI SYCK|SDVYTINCGMAFGQAAMLLSLGK|KGYRAVQPHSSTKLYLPKVNR|SSGAAIDMWIK| AK|ELDANTEYYIELLAK|GTGK|SK|EQINEDIK|R|PK|YLQAQAAIDYGIADK|IADSQDSSEK| R|DYDGTLAQR|AMRPGGGSPAAPAGLR|

The two peptides were both observed in our large-scale experiments. However, the peptide YLQAQAAIDYGIADK (R1-1) exhibited low signal-to-noise ratio even for the stable isotope-labeled version (see Figure 2) and is thereby not amenable for quantification. The rest of the tryptic peptides were not selected since they exhibited weak MS intensity response (e.g., peptides EQINEDIK and IADSQDSSEK) and were occasionally detected, was part of a missed cleavage (peptide DYDGTLAQR) or possessed methionine/cysteine residues.

ClpR3

MASCLQASMNSLLPRSSSFSPHPPLSSNSSGRRNLKTFRYAFRAKASAKIPMPPINPK|DPFLS TLASIAANSPEK|LLNRPVNADVPPYLDIFDSPQLMSSPAQVER|SVAYNEHRPR|TPPPDLPSM LLDGR|IVYIGMPLVPAVTELVVAELMYLQWLDPK|EPIYIYINSTGTTR|DDGETVGMESEG FAIYDSLMQLK|NEVHTVCVGAAIGQACLLLSAGTK|GKRFMMPHAK|AMIQQPRVSSGLMP ASDVLIR|AK|EVITNR|DILVELLSK|HTGNSVETVANVMR|R|PYYMDAPKAK|EFGVIDR|ILW RGQEK|IIADVVPSEEFDK|NAGIKSVV

Similar to ClpR1, the two selected peptides for ClpR3 were both observed in our large-scale experiments. However, the peptide EPIYIYINSTGTTR (R3-1) exhibited low signal-to-noise ratio even for the stable isotope-labeled version (see Figure 2) and is thereby not amenable for quantification. The

other tryptic peptides also exhibited weak MS intensity response (peptide EFGVIDR), was part of a missed cleavage (peptide EVITNR) or have reactive residues and are thus not good for quantification. Peptide IIADVVPSEEFDK could have been chosen but this was occasionally detected from our initial experiments and was thus not selected included then.

ClpR4

MEVAAATATSFTTLRARTSAIIPSSTRNLRSKPRFSSSSSLRASLSNGFLSPYTGGSISS DLCGAKLRAESLNPLNFSSSKPKRGVVTMVIPFSK|GSAHEQPPPDLASYLFK|NRIVYLGM SLVPSVTELILAEFLYLQYEDEEKPIYLYINSTGTTKNGEKLGYDTEAFAIYDVMGYVKP PIFTLCVGNAWGEAALLLTAGAKGNR|SALPSSTIMIK|QPIAR|FQGQATDVEIAR|K|EIKHI KTEMVKLYSKHIGK|SPEQIEADMK|RPK|YFSPTEAVEYGIIDK|VVYNER|GSQDR|GVVSDL K|K|AQLI

Both ClpR4 peptides were reliably quantified.

ClpP3

MEMSLRLASSSTSNPICLLNPGKNLNFPIRNHRIPKTSKPFCVRSSMSLSKPPR|QTLSSNWDV SSFSIDSVAQSPSR|LPSFEELDTTNMLLR|QR|IVFLGSQVDDMTADLVISQLLLLDAEDSER|D ITLFINSPGGSITAGMGIYDAMK|QCK|ADVSTVCLGLAASMGAFLLASGSK|GKRYCMPNSK| VMIHQPLGTAGGK|ATEMSIRIREMMYHKIKLNKIFSR|ITGKPESEIESDTDR|DNFLNPWEA K|EYGLIDAVIDDGKPGLIAPIGDGTPPPK|TK|VWDLWK|VEGTKK|DNTNLPSER|SMTQNG YAAIE

For ClpP3, most identified peptides had methionine or had potential internal missed cleavage sites (KP in their sequences). We resorted to selecting a methionine-bearing peptide (LPFEELDTTNMLLR, P3-2) that exhibited good MS instrument response. We also included DNTNLPSER (P3-1) which was rarely observed. We did not observe peptide DNFLNPWEAK in our initial experiments and was thereby not selected but we had been detecting it in enriched Clp complex purifications.

ClpP4

MGTLSLSSSLKPSLVSSRLNSSSSASSSSFPKPNNLYLKPTKLISPPLRTTSPSPLR|FAN ASIEMSQTQESAIR|GAESDVMGLLLR|ER|IVFLGSSIDDFVADAIMSQLLLLDAK|DPK|KDI K|LFINSPGGSLSATMAIYDVVQLVR|ADVSTIALGIAASTASIILGAGTK|GK|R|FAMPNTR|I MIHQPLGGASGQAIDVEIQAKEVMHNK|NNVTSIIAGCTSR|SFEQVLK|DIDR|DR|YMSPIEA

VEYGLIDGVIDGDSIIPLEPVPDR|VKPR|VNYEEISK|DPMK|FLTPEIPDDEIY

Most detected peptides in ClpP4 have methionine and peptide VNYEEISK was only detected as part of missed cleavages. Only SFEVLK (P4-1) worked. We included ADVSTIALGIAASTASIILGAGTK (P4-2) but it was a very hydrophobic sticky peptide that does not completely elute during an LCMS run (we still detected in subsequent blank runs).

ClpP5

MAHACVSTSASSLRFTAGFVSASPNGSSFDSPKLSLPFEPLRSRKTNKLVSDRKNWKNSTPK
AVYSGNLWTPEIPSPQGVWSIRDDLQVPSSPYFPAYAQGQGPPPMVQER|FQSIISQLFQYR|I
IR|CGGAVDDDMANIIVAQLLYLDAVDPTK|DIVMYVNSPGGSVTAGMAIFDTMR|HIR|PDVS
TVCVGLAASMGAFLLSAGTK|GK|R|YSLPNSR|IMIHQPLGGAQGGQTDIDIQANEMLHHK|A
NLNGYLAYHTGQSLEK|INQDTDR|DFFMSAKEAKEYGLIDGVIMNPLK|ALQPLAAA

Peptide YSLPNSR was only detected in one experiment and peptide INQDTDR was only observed as part of a missed cleavage. The selected peptide FQSIISQLFQYR (P5-2) elutes later in the LC gradient although this does not fully explain why it exhibits a higher stochiometry ratio than peptide P5-1 (ANLNGYLAYHTGQSLEK). Deamidation of Asn, most common in -Asn-Gly-sequences, yields aspartic acid (Asp) and isoaspartic acid (βAsp) and is usually observed in proteomic workflows involving overnight tryptic digestion (Krokhin et al., 2006). Two peaks were observed for the deamidated species (aspartic acid and isoaspartic acid are isomeric) of P5-1 and one species eluted closely with the unmodified P5-1. Previous studies revealed that reversed-phase LC using formic acid as ion-pairing modifier separated native and deamidated peptides in the order: -Asn- /-βAsp-/-Asp- (Krokhin et al., 2006).

ClpP6

MAGLAISPPLGLSFSSRTRNPKPTSFLSHNQRNPIRRIVSALQSPYGDSLK|AGLSSNVSGSPIK|
IDNKAPRFGVIEAK|K|GNPPVMPSVMTPGGPLDLSSVLFR|NR|IIFIGQPINAQVAQR|VISQL
VTLASIDDK|SDILMYLNCPGGSTYSVLAIYDCMSWIKPK|VGTVAFGVAASQGALLLAGGE
K|GMR|YAMPNTR|VMIHQPQTGCGGHVEDVR|R|QVNEAIEAR|QKIDR|MYAAFTGQPLEK|
VQQYTERDRFLSASEALEFGLIDGLLETEY

The two ClpP6 peptides worked well.