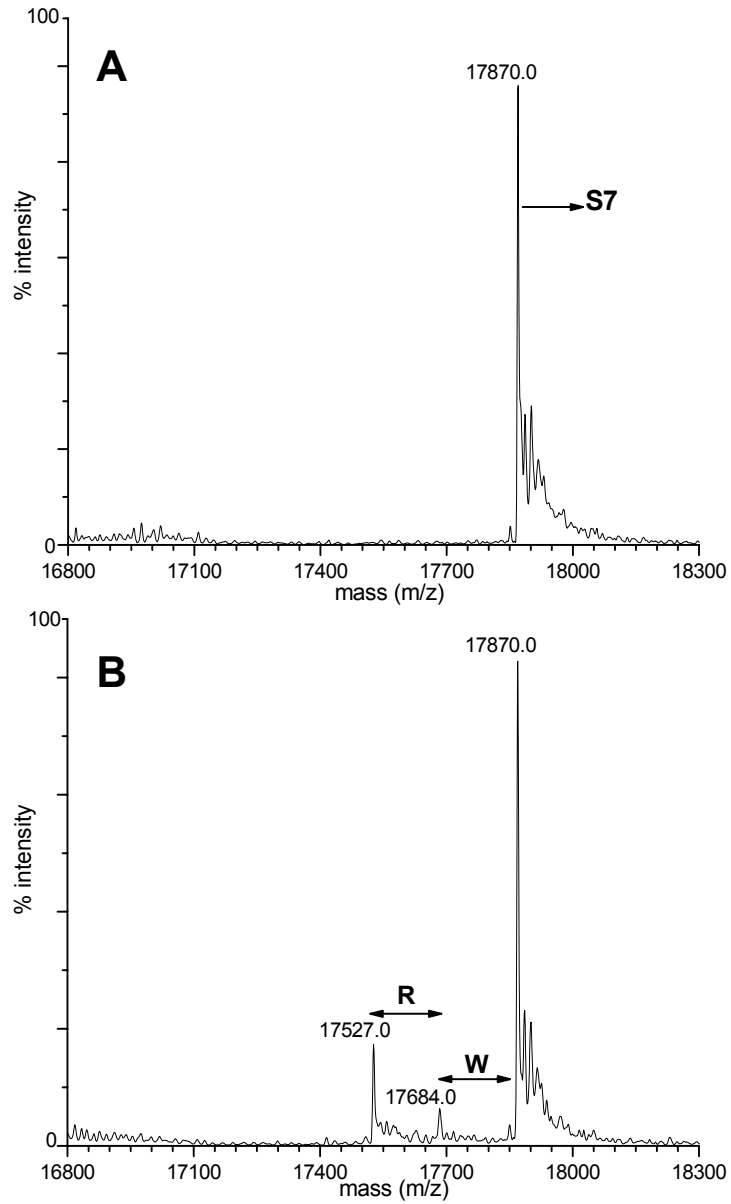
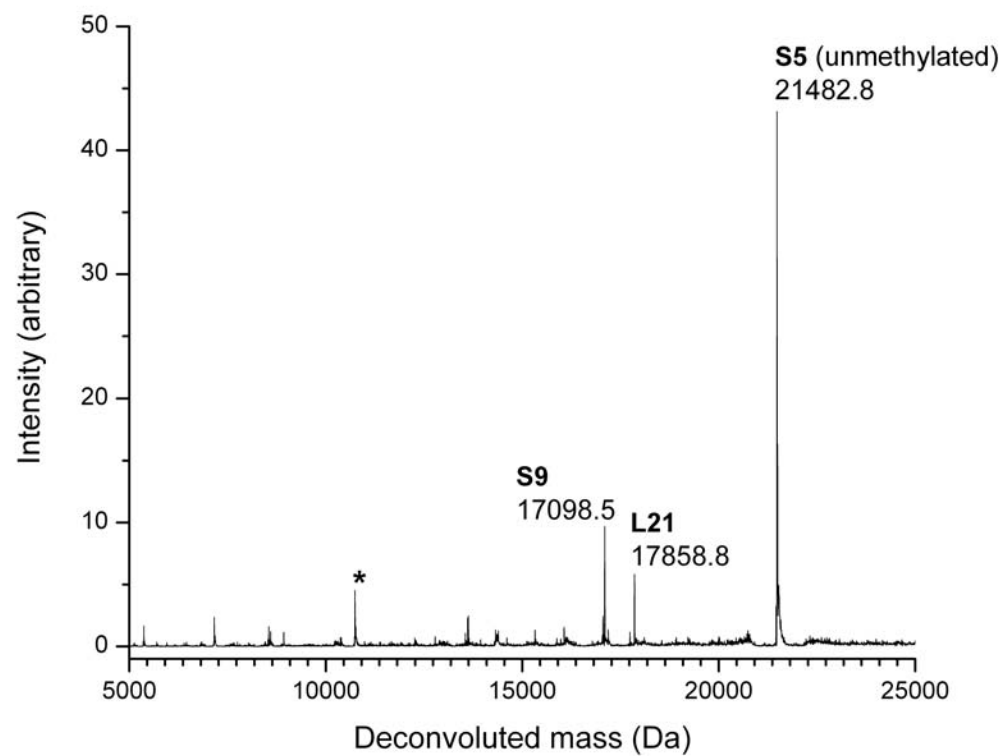


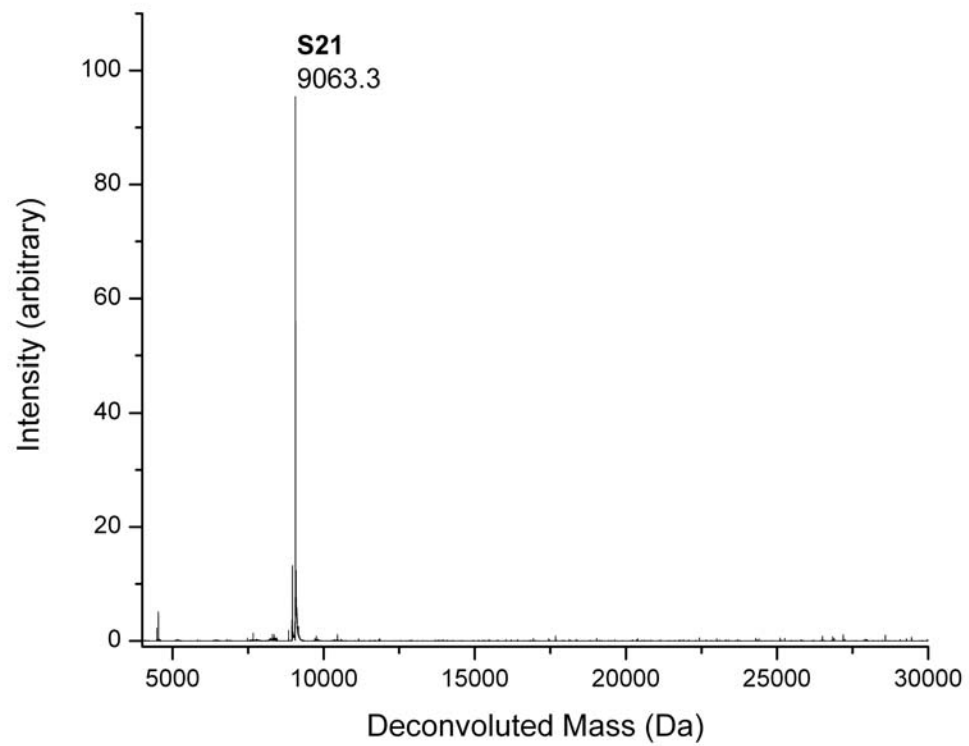
**Supplemental Figure 1:** Fragmentation spectra generated from the ions in Figure 8. The high intensity fragment ions are consistent with enhanced cleavage adjacent to the aspartate residues and glutamate residue. Fragments whose m/z are consistent with the methylation of K88 are marked with an asterisk.



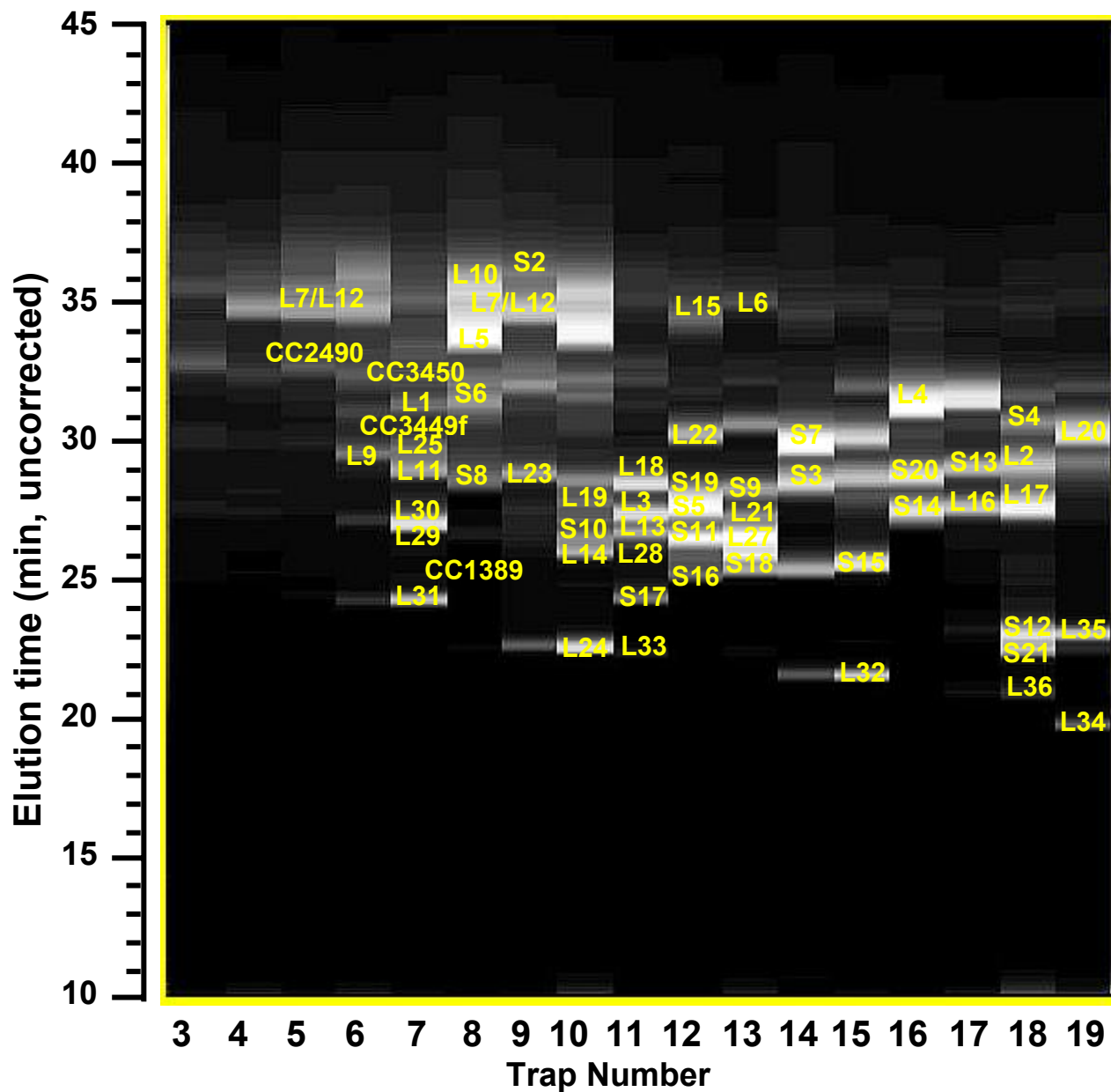
**Supplemental Figure 2:** Deconvoluted mass spectra of C-Terminal sequence analysis of S7 using CPY/CPP. **(A)** Digestion time = 0 seconds. **(B)** Digestion time = 15 seconds.



**Supplemental Figure 3:** A deconvoluted spectrum from the Trap 13 TIC, showing a weak signal for L21. The ribosomes used for these experiments were from *Caulobacter crescentus* cells grown to mid-log phase rather than stationary phase, resulting in some differences in the post-translational modifications from those reported in Table 1. The peak marked with an asterisk represents an artifact of the deconvolution algorithm.



**Supplemental Figure 4:** Deconvoluted mass spectrum showing the intense, recurring mass corresponding to S21 with an eleven amino acid truncation at the N-terminus.



**Supplemental Figure 5:** Twenty serial reversed phase trap chromatograms with protein identifications. Labels indicate the positions of maximum whole protein signal intensity, except in the 25 minute region of Traps10-13 and the 28 minute region of Traps16-18, where some labels have been displaced for the sake of visibility. This figure was generated by exporting the intensity vs. time data from each trap's TIC, normalizing each chromatogram to the maximum intensity in its data set then plotting the data as a contour plot. Brightness of each band represents normalized TIC intensity within each chromatogram on a linear scale.

**Supplemental Table 1:** IEC dimension gradient. Mobile phase A: 20 mM acetic acid, 6 M urea. Mobile phase B: same as A with 500 mM NaCl

Time	Flow rate ( $\mu\text{L}/\text{min}$ )	%A	%B
0.00	150	100	0
20.0	300	100	0
25.0	300	100	0
45.0	300	90	10
95.0	300	65	35
96.0	300	30	70
102.0	300	0	100
103.0	300	100	0
110.0	300	100	0

**Supplemental Table 2.** Regular RPLC gradient. Flow rate 50  $\mu\text{L}/\text{min}$  at the pump, split to 7  $\mu\text{L}/\text{min}$  for ESI-MS. Mobile phases as described in main text.

Time (min)	%A	%B
0	100	0
7	100	0
7.1	80	20
37.0	30	70
40.0	10	90
44.0	10	90
44.1	100	0
53.0	100	0

**Supplemental Table 3.** Longer RPLC Gradient. Flow rate 50  $\mu$ L/min. Mobile phases as described in text.

Time (min)	%A	%B
0	100	0
7	100	0
7.1	80	20
75.0	30	70
75.1	10	90
80	10	90
80.1	100	0
90	100	0

**Supplemental Table 4.** Carboxypeptidase-Y and -P digest analysis gradient. Flow rate was 50  $\mu$ L/min. Mobile phase A: 0.3% (v/v) formic acid in water. Mobile phase B: 0.3% (v/v) formic acid in acetonitrile.

Time (min)	%A	%B
0	95	5
5	95	5
5.1	80	20
95	55	45
95.1	35	65
100	5	95
105	95	5
110	95	5
120	95	5

**Supplemental Table 5.** Large scale 1-D RPLC gradient for isolation of L11 and L21 for CPY/CPP analysis. Mobile phase A: 0.1% trifluoroacetic acid in water. Mobile phase B: 0.1% trifluoroacetic acid in acetonitrile. Flow rate was 1 mL/min throughout.

Time (min)	%A	%B
0	95	5
5	95	5
5.1	80	20
20	70	30
45	65	35
70	60	40
110	50	50
120	5	95
130	95	5

**Supplemental Table 6.** RPLC gradient used for Capillary LC/ESI-MS experiments. Flow rate: 100  $\mu$ L/min at the pump, split to 5  $\mu$ L/min before sample valve. Mobile phases are described in the main text.

Time (min)	%A	%B
0	95	5
10	95	5
40	60	40
41	20	80
46	20	80
47	95	5
60	95	5