

Table S1. Mass spectrometry analyses of purified cytochrome *bc*₁: Comprehensive list of detected peptides relevant to F144, H217, wild type, mutations and related epitope tags. Digestions, nano-LC-MS/MS conditions and data analysis procedures are described in Materials and Methods. Sequest analysis results were filtered using standard values for X_{corr} (1.5, 2.0, 2.5 for m/z of +1,+2 and +3, respectively) and $\Delta C_N(> 0.1)$. Methionine modifications are: oxidation (*, +16), homoserine formation (@, -30) and homoserine lactone formation (#, -48).

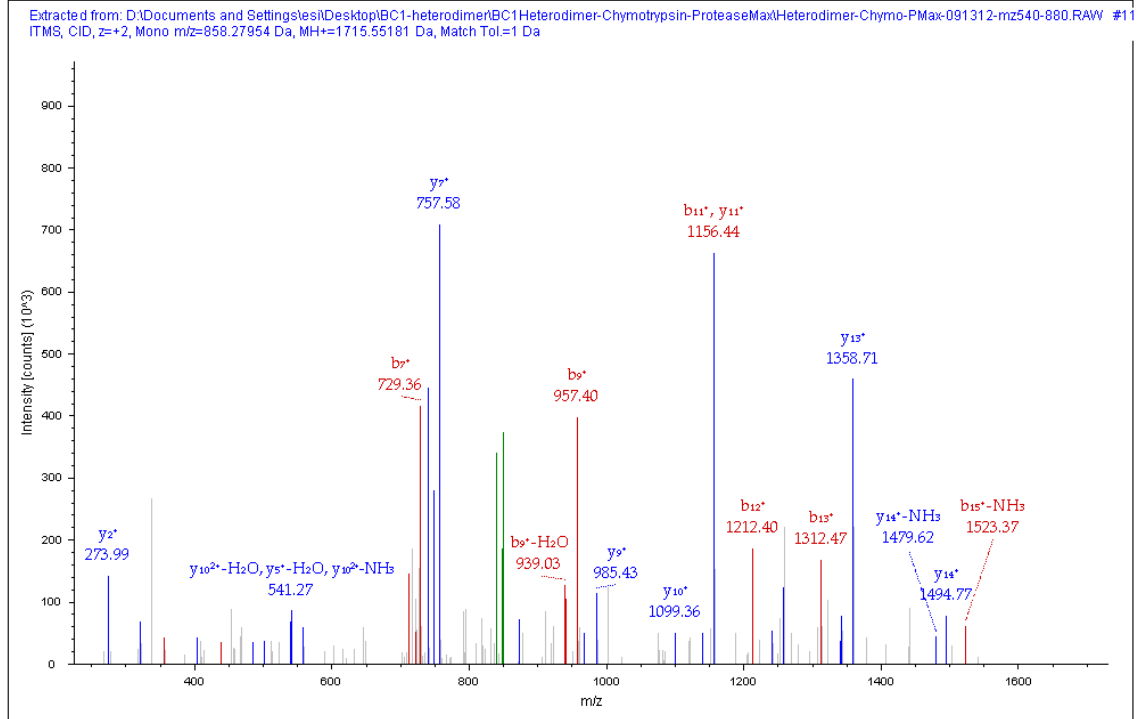
Purified cytochrome <i>bc</i> ₁	Protease / treatment	Cytochrome <i>b</i> peptide	Peptide unique to
• Native homodimer (No tag control)	• Chymotrypsin / Aqueous	W.AFH <u>TT</u> GNNNPTGVEVR.R W.AFH <u>TT</u> GNNNPTGVEV.R W.AFH <u>TT</u> GNNNPTGVE.V W.AFH <u>TT</u> GNNNPTGV.E	H217 (wild type)
	• CNBr cleavage	M.VIYLLM*M*GTAFM@.G M.VIYLLM*M#GTAFM@.G M.VIYLLM@M*GTAFM@.G	F144 (wild type)
• Native-S homodimer (tag control)	• Chymotrypsin / 60% Methanol	F.NSHYGNPAEW.S	Strep tag
• H217L-F homodimer (control)	• CNBr cleavage	M.VIYLLM*M*GTAFM@.G M.VIYLLM#M#GTAFM@.G M.VIYLLM*M@GTAFM@.G	F144 (wild type)
	• Chymotrypsin / Aqueous	<u>L</u> .TTGNNNPTGVEVR.R	H217L (mutant)
• F144R-S homodimer (control)	• CNBr cleavage	M.VIYLLM*M*GTARM@.G M.VIYLLM*M*GTARM#.G M.VIYLLM#M*GTARM@.G M.GTARM*GYVLPWGQM@.S M.M*GTARM*GYVLPWGQM@.S M.GTARM#GYVLPWGQM@.S	F144R (mutant)

• F144R-S + H217L-F heterodimer	• Chymotrypsin / 60% Methanol	W.AFH <u>TTG</u> NNNPTGVEVR.R	H217 (wild type)
		Y.KAPREITWIVGMVIYLLM*MGTAR.M	F144R (mutant)
		Y.LLMM*GTARMGY.V	
		Y.LLMM*GTARM*GY.V	
	• Chymotrypsin / Aqueous	Y.GNPAEWSHPQFEK.-	Strep tag
		F.NSHYGNPAEW.S	
		A.SIEEDFN <u>SHYGNPAEW</u> .S	
		A.SIEEDFN <u>SHYGNPAEDY</u> .K	Flag tag
		A.SIEEDFN <u>SHYGNPAEDYKDDDDK</u> .-	
• CNBr cleavage	W.AFH <u>TTG</u> NNNPTGVEVR.R	H217 (wild type)	
	W.AFH <u>TTG</u> NNNPTGVEV.R		
	F. <u>L</u> TTGNNNPTGVEVR.R	H217L (mutant)	
	<u>L</u> .TTGNNNPTGVEVR.R		
	M.VIYLLM*M*GTA <u>F</u> M@.G	F144 (wild type)	
	M.VIYLLM*M@GTA <u>F</u> M#.G		
	M.VIYLLM*M*GTA <u>R</u> M@.G	F144R (mutant)	
	M.VIYLLM#MGT <u>A</u> RM#.G		

Sequence: **W.AFHHTGNNNPTGVEVR.R**, Charge: +2, Monoisotopic m/z: 85
 MH+: 1714.31694 Da, RT: 26.59 min

Identified with: SEQUEST (v1.20); XCorr: 4.13, Ions matched by search: 1
 Fragment match tolerance used for search: 1 Da

Fragments used for search: b; b-H₂O; b-NH₃; y; y-H₂O; y-NH₃

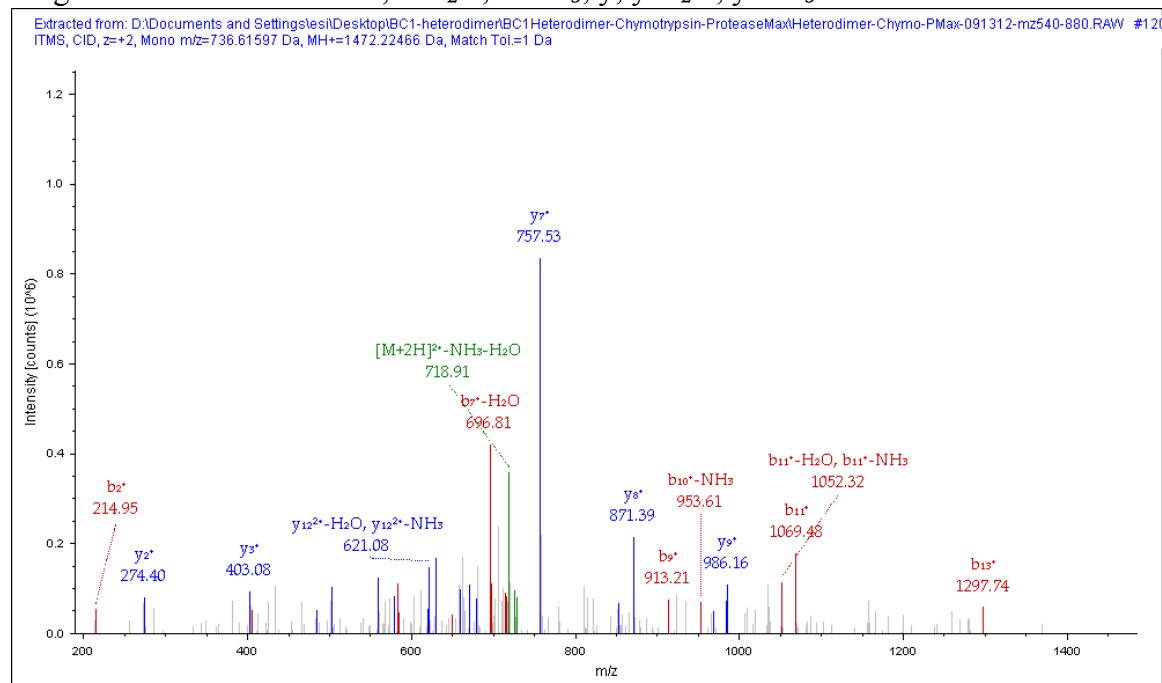


#1	b ⁺	b ²⁺	Seq.	y ⁺	y ²⁺	#2
1	72.04440	36.52584	A			16
2	219.11282	110.06005	F	1642.79327	821.90027	15
3	356.17173	178.58950	H	1495.72485	748.36606	14
4	457.21941	229.11334	T	1358.66594	679.83661	13
5	558.26709	279.63718	T	1257.61826	629.31277	12
6	615.28856	308.14792	G	1156.57058	578.78893	11
7	729.33149	365.16938	N	1099.54911	550.27819	10
8	843.37442	422.19085	N	985.50618	493.25673	9
9	957.41735	479.21231	N	871.46325	436.23526	8
10	1054.47012	527.73870	P	757.42032	379.21380	7
11	1155.51780	578.26254	T	660.36755	330.68741	6
12	1212.53927	606.77327	G	559.31987	280.16357	5
13	1311.60769	656.30748	V	502.29840	251.65284	4
14	1440.65029	720.82878	E	403.22998	202.11863	3
15	1539.71871	770.36299	V	274.18738	137.59733	2
16			R	175.11896	88.06312	1

Sequence: F.LTTGNNNPTGVEVR.R, Charge: +2, Monoisotopic m/z: 736.61597 Da
 MH+: 1472.22466 Da, RT: 26.86 min

Identified with: SEQUEST (v1.20); XCorr: 3.60, Ions matched by search engine: 16/26
 Fragment match tolerance used for search: 1 Da

Fragments used for search: b; b-H₂O; b-NH₃; y; y-H₂O; y-NH₃

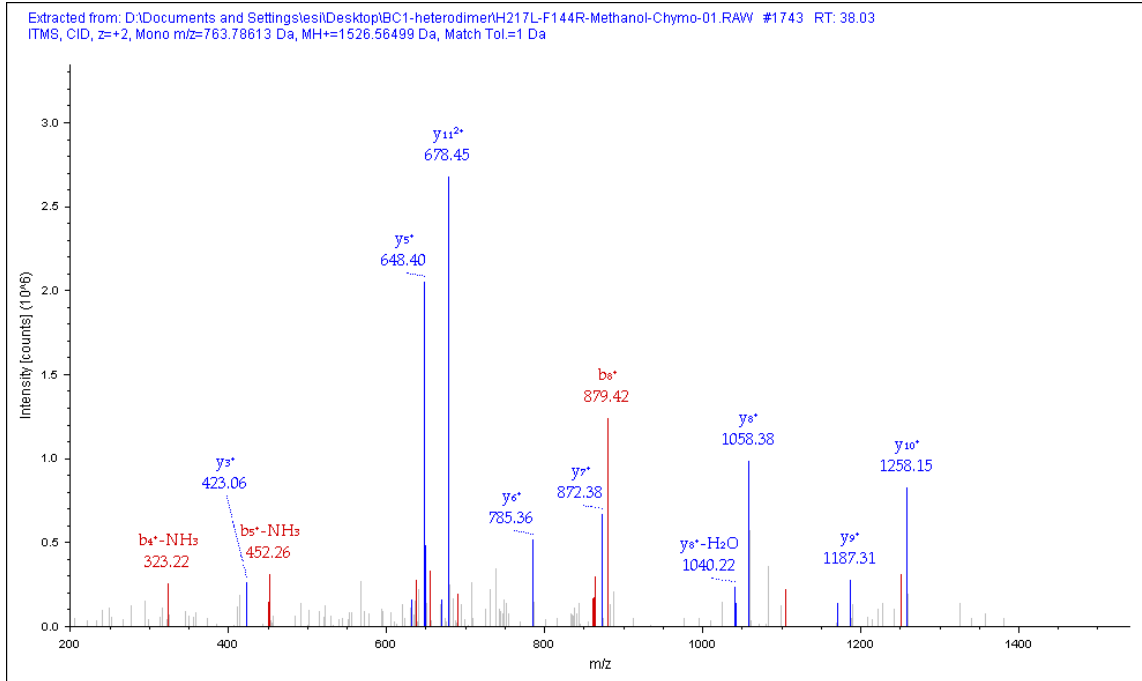


#1	b ⁺	b ²⁺	Seq.	y ⁺	y ²⁺	#2
1	114.09135	57.54931	L			14
2	215.13903	108.07315	T	1358.66594	679.83661	13
3	316.18671	158.59699	T	1257.61826	629.31277	12
4	373.20818	187.10773	G	1156.57058	578.78893	11
5	487.25111	244.12919	N	1099.54911	550.27819	10
6	601.29404	301.15066	N	985.50618	493.25673	9
7	715.33697	358.17212	N	871.46325	436.23526	8
8	812.38974	406.69851	P	757.42032	379.21380	7
9	913.43742	457.22235	T	660.36755	330.68741	6
10	970.45889	485.73308	G	559.31987	280.16357	5
11	1069.52731	535.26729	V	502.29840	251.65284	4
12	1198.56991	599.78859	E	403.22998	202.11863	3
13	1297.63833	649.32280	V	274.18738	137.59733	2
14			R	175.11896	88.06312	1

Sequence: **Y.GNPAEWSHPQFEK.-**, Charge: +2, Monoisotopic m/z: 763.78613 Da
 MH+: 1526.56499 Da, RT: 38.03 min

Identified with: SEQUEST (v1.20); XCorr: 2.90, Ions matched by search engine: 14/24
 Fragment match tolerance used for search: 1 Da

Fragments used for search: b; b-H₂O; b-NH₃; y; y-H₂O; y-NH₃

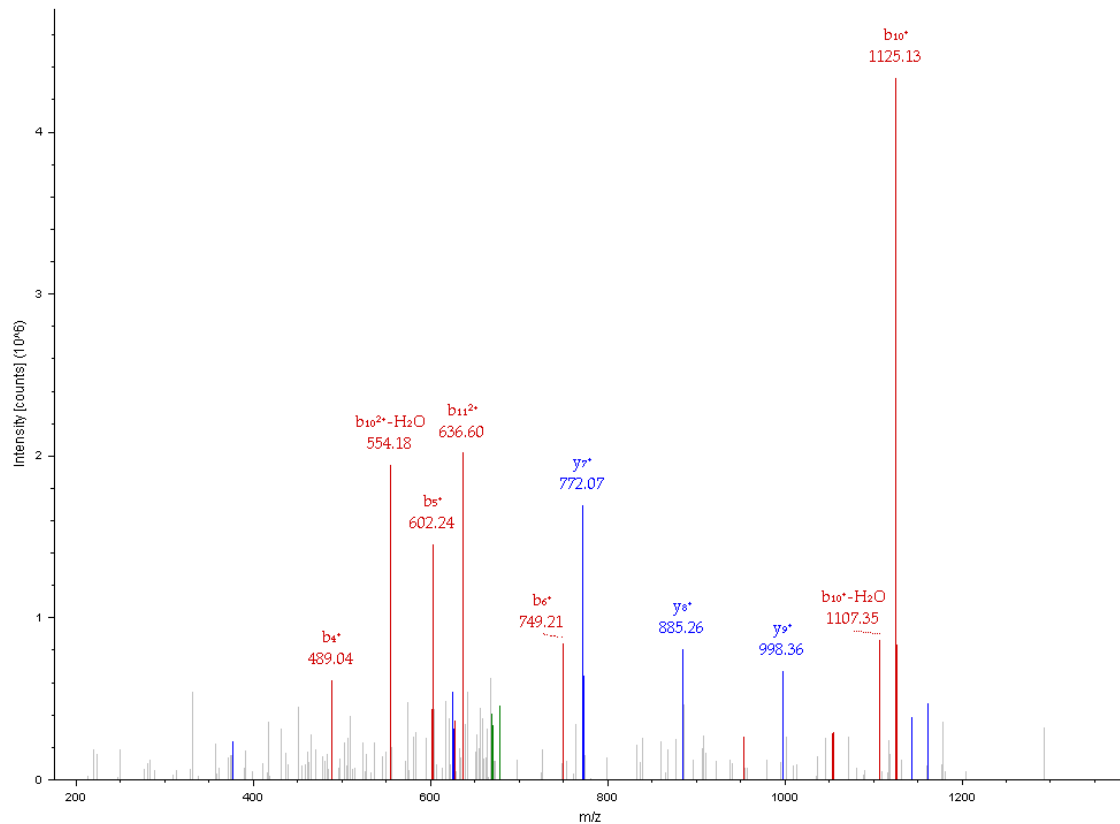


#1	b ⁺	b ²⁺	Seq.	y ⁺	y ²⁺	#2
1	58.02875	29.51801	G			13
2	172.07168	86.53948	N	1469.68086	735.34407	12
3	269.12445	135.06586	P	1355.63793	678.32260	11
4	340.16157	170.58442	A	1258.58516	629.79622	10
5	469.20417	235.10572	E	1187.54804	594.27766	9
6	655.28349	328.14538	W	1058.50544	529.75636	8
7	742.31552	371.66140	S	872.42612	436.71670	7
8	879.37443	440.19085	H	785.39409	393.20068	6
9	976.42720	488.71724	P	648.33518	324.67123	5
10	1104.48578	552.74653	Q	551.28241	276.14484	4
11	1251.55420	626.28074	F	423.22383	212.11555	3
12	1380.59680	690.80204	E	276.15541	138.58134	2
13			K	147.11281	74.06004	1

Sequence: M.VIYLLMMGTAFM.G, M6-Oxidation (15.99492 Da), M7-Oxidation (15.99492 Da)
M12-Met-HomoserineLactone (-48.00337 Da), Charge: +2, Monoisotopic m/z: 687.46436 Da
MH+: 1373.92143 Da, RT: 78.02 min

Identified with: SEQUEST (v1.20); XCorr: 2.70, Ions matched by search engine: 13/22
Fragment match tolerance used for search: 1 Da

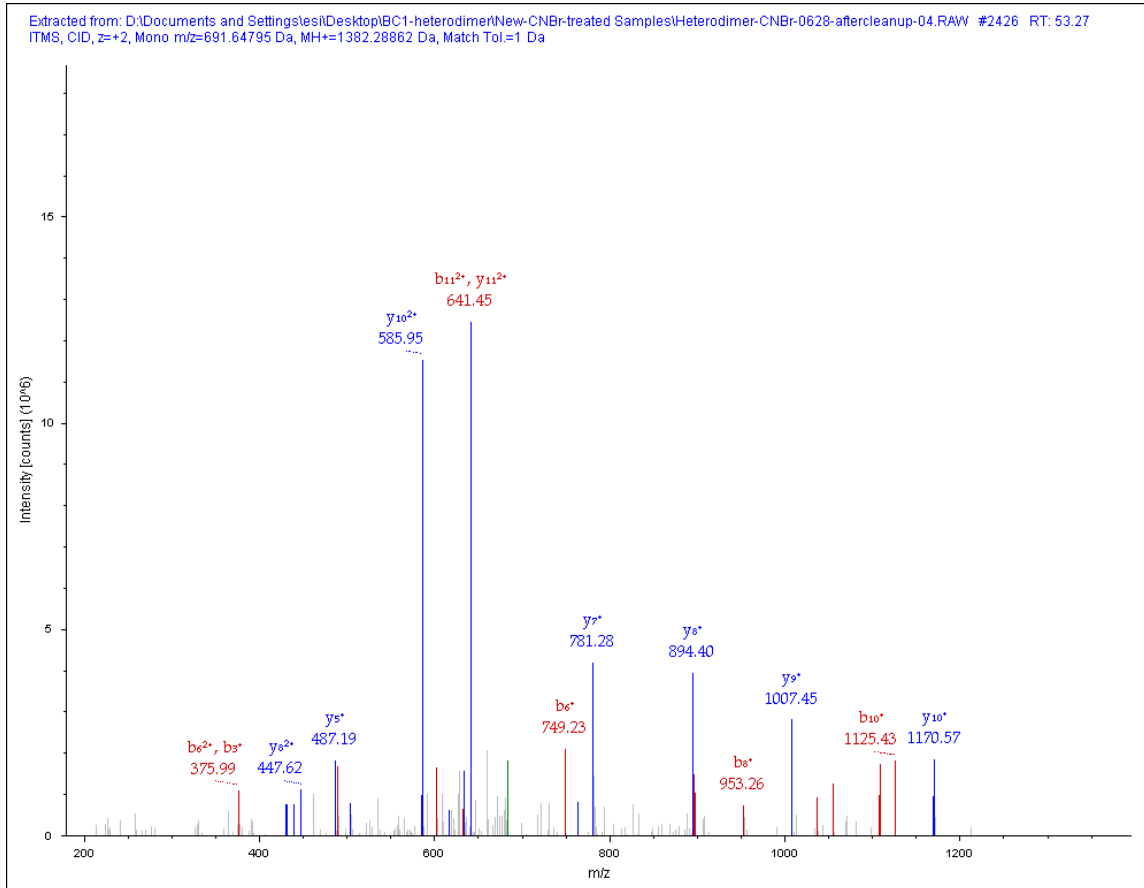
Extracted from: D:\Documents and Settings\es\Desktop\BC1-heterodimer\New-CNBr-treated Samples\Heterodimer-CNBr-062512-02.RAW #3490 RT: 78.09
ITMS, CID, z=+2, Mono m/z=687.38086 Da, MH+=1373.75444 Da, Match Tol.=1 Da



#1	b ⁺	b ²⁺	Seq.	y ⁺	y ²⁺	#2
1	100.07570	50.54149	V			12
2	213.15977	107.08352	I	1274.61602	637.81165	11
3	376.22309	188.61518	Y	1161.53195	581.26961	10
4	489.30716	245.15722	L	998.46863	499.73795	9
5	602.39123	301.69925	L	885.38456	443.19592	8
6	749.42664	375.21696	M-Oxidation	772.30049	386.65388	7
7	896.46206	448.73467	M-Oxidation	625.26508	313.13618	6
8	953.48353	477.24540	G	478.22966	239.61847	5
9	1054.53121	527.76924	T	421.20819	211.10773	4
10	1125.56833	563.28780	A	320.16051	160.58389	3
11	1272.63675	636.82201	F	249.12339	125.06533	2
12			M-Met-HS...	102.05497	51.53112	1

Sequence: M.VIYLLMMGTARM.G, M6-Oxidation (15.99492 Da), M7-Oxidation (15.99492 Da)
M12-Met-HomoserineLactone (-48.00337 Da), Charge: +2, Monoisotopic m/z: 691.64795 Da
MH+: 1382.28862 Da, RT: 53.27 min

Identified with: SEQUEST (v1.20); XCorr: 4.07, Ions matched by search engine: 18/22
Fragment match tolerance used for search: 1 Da

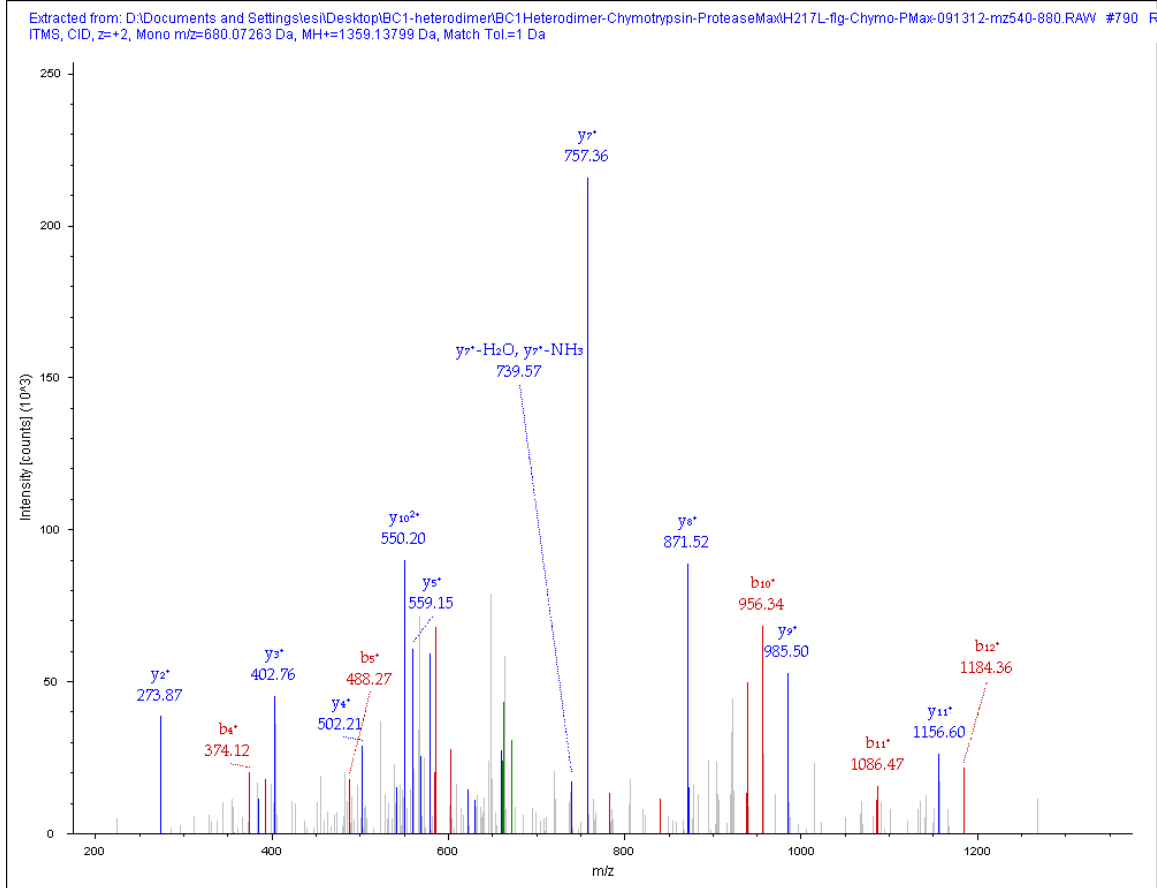


#1	b ⁺	b ²⁺	Seq.	y ⁺	y ²⁺	#2
1	100.07570	50.54149	V			12
2	213.15977	107.08352	I	1283.64872	642.32800	11
3	376.22309	188.61518	Y	1170.56465	585.78596	10
4	489.30716	245.15722	L	1007.50133	504.25430	9
5	602.39123	301.69925	L	894.41726	447.71227	8
6	749.42664	375.21696	M-Oxidation	781.33319	391.17023	7
7	896.46206	448.73467	M-Oxidation	634.29778	317.65253	6
8	953.48353	477.24540	G	487.26236	244.13482	5
9	1054.53121	527.76924	T	430.24089	215.62408	4
10	1125.56833	563.28780	A	329.19321	165.10024	3
11	1281.66945	641.33836	R	258.15609	129.58168	2
12			M-Met-HS...	102.05497	51.53112	1

Sequence: L.TTGNNTPTGVEVR.R, Charge: +2, Monoisotopic m/z: 680.07263 Da
 MH+: 1359.13799 Da, RT: 18.37 min

Identified with: SEQUEST (v1.20); XCorr: 3.44, Ions matched by search engine: 15/24
 Fragment match tolerance used for search: 1 Da

Fragments used for search: b; b-H₂O; b-NH₃; y; y-H₂O; y-NH₃



#1	b ⁺	b ²⁺	Seq.	y ⁺	y ²⁺	#2
1	102.05496	51.53112	T			13
2	203.10264	102.05496	T	1257.61826	629.31277	12
3	260.12411	130.56569	G	1156.57058	578.78893	11
4	374.16704	187.58716	N	1099.54911	550.27819	10
5	488.20997	244.60862	N	985.50618	493.25673	9
6	602.25290	301.63009	N	871.46325	436.23526	8
7	699.30567	350.15647	P	757.42032	379.21380	7
8	800.35335	400.68031	T	660.36755	330.68741	6
9	857.37482	429.19105	G	559.31987	280.16357	5
10	956.44324	478.72526	V	502.29840	251.65284	4
11	1085.48584	543.24656	E	403.22998	202.11863	3
12	1184.55426	592.78077	V	274.18738	137.59733	2
13			R	175.11896	88.06312	1

Figure S1. Light-induced, time-resolved cytochrome *b* reduction and cytochrome *c* re-reduction kinetics of an *R. capsulatus* RecA⁻ strain producing heterodimeric (cyt *b*-S: F144R + cyt *b*-F: H217L) cytochrome *bc*₁ variant. See Materials and Methods, Figure 5 legend, and the text for description and interpretation of these data.

The traces shown here obtained using a RecA⁻ background are qualitatively similar to those shown in Figure 5 obtained using a RecA⁺ background except that the signal/noise ratio in the former case is poor, due to lower yield of chromatophores.

