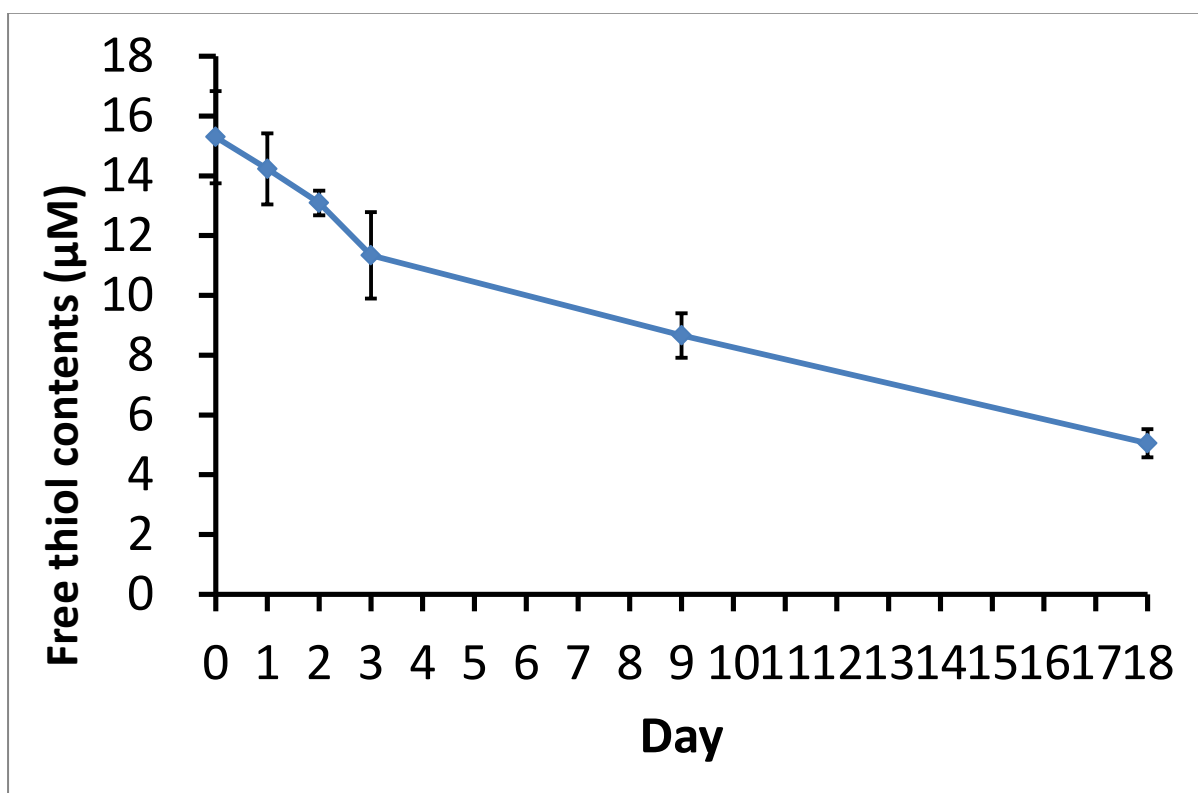


## Supplementary Material

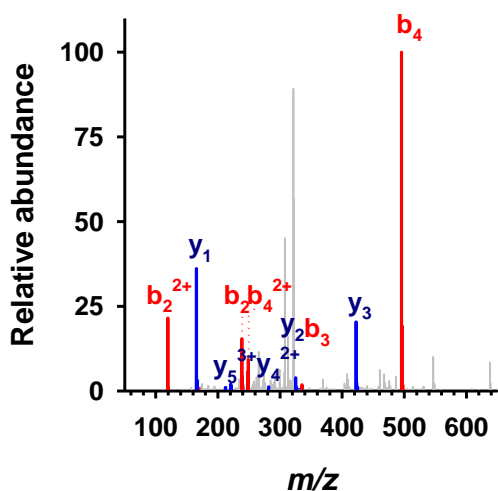
**Supplementary Figure S1. The cysteine residues of CueP are gradually oxidized in the absence of a reducing agent.** The purified CueP was reduced by 10 mM DTT, and then, the DTT was removed using a desalting column. The concentrated CueP sample (5  $\mu\text{M}$ ) was incubated at 277 K for the given amount of time and then subjected to Ellman's assay to measure the redox state. The means of the three independent experiments are displayed with the standard error (error bars).



**Supplementary Figure S2. Tandem mass spectra assigned to b- and y-ions of cysteine- and/or histidine-modified peptides of trypsin/chymotrypsin-digested CueP.** After the purified CueP protein was stored for two weeks in the absence of a reducing agent, the peptide peaks with variable modifications were detected by LC-MS/MS, as described in the main text and Figure 2.

(a) Tandem mass spectra of the modified peptide peaks of  $^{93}\text{THPCF}^{97}$

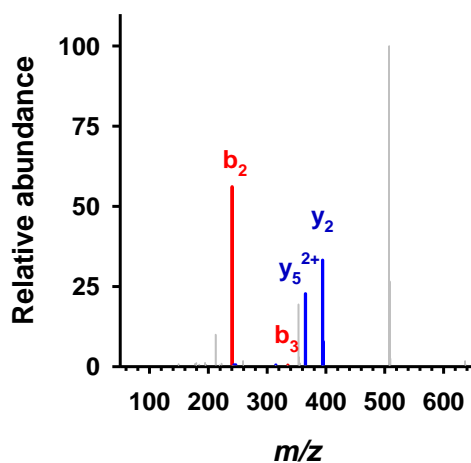
$a_{\text{IAA}}$ : carbamidomethyl cysteine at position 96



#	b	b++	Seq.	y	y++	y+++	#
1			T	661.72	331.36	221.24	5
2	239.25	120.13	H	560.62	280.81		4
3	336.37	168.69	P	423.48	212.24		3
4	496.53	248.77	C <sup>CAM</sup>	326.36	163.68		2
5			F	166.2			1

SEQUEST result:  
Matches = 31,  $S_p$ Score = 381.3

$a_{\text{NEM}}$ : N-ethylmaleimide cysteine at position 96

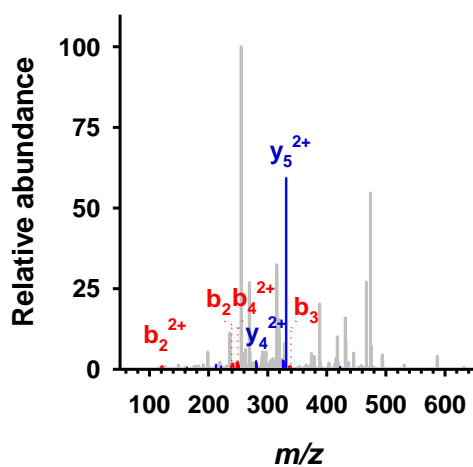


#	b	b++	Seq.	y	y++	y+++	#
1			T	729.83	365.42	243.95	5
2	239.25	120.13	H	628.72	314.87	210.25	4
3	336.37	168.69	P	491.58	246.3		3
4	564.64	282.82	C <sup>NEM</sup>	394.47	197.74		2
5			F	166.2			1

SEQUEST result:  
Matches = 25,  $S_p$ Score = 206.3

(continued Figure S2a)

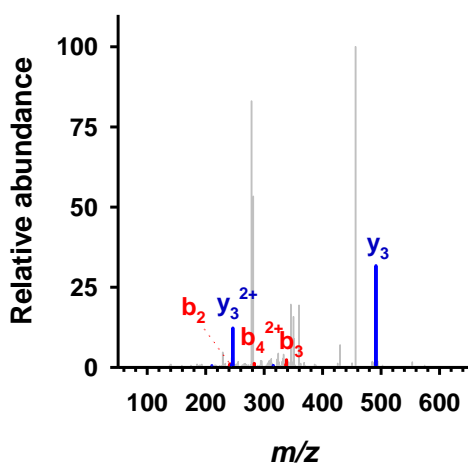
$a_{H,IAA}$ : protonated histidine at position 94 and carbamidomethyl cysteine at position 96



#	b	b++	Seq.	y	y++	y+++	#
1			T	662.73	331.87	221.58	5
2	240.26	120.63	H <sup>H</sup>	561.62	281.32		4
3	337.37	169.19	P	423.48	212.24		3
4	497.54	249.27	C <sup>CAM</sup>	326.36	163.68		2
5			F	166.2			1

SEQUEST result:  
Matches = 31,  $S_p$ Score = 363.4

$a_{H,NEM}$ : protonated histidine at position 94 and N-ethylmaleimide cysteine at position 96

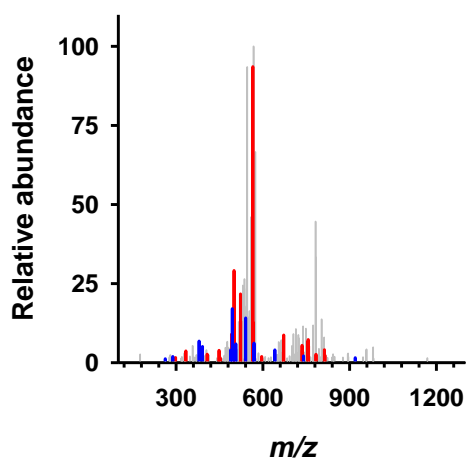


#	b	b++	Seq.	y	y++	y+++	#
1			T	730.84	365.92	244.28	5
2	240.26	120.63	H <sup>H</sup>	629.73	315.37	210.58	4
3	337.37	169.19	P	491.58	246.3		3
4	565.65	283.33	C <sup>NEM</sup>	394.47	197.74		2
5			F	166.2			1

SEQUEST result:  
Matches = 27,  $S_p$ Score = 248.7

(b) Tandem mass spectra of the modified peptide peaks of  $^{98}\text{NHSLSGCGGEMPKNKPF}^{113}$

$b_{\text{IAA}}$ : carbamidomethyl cysteine at position 104

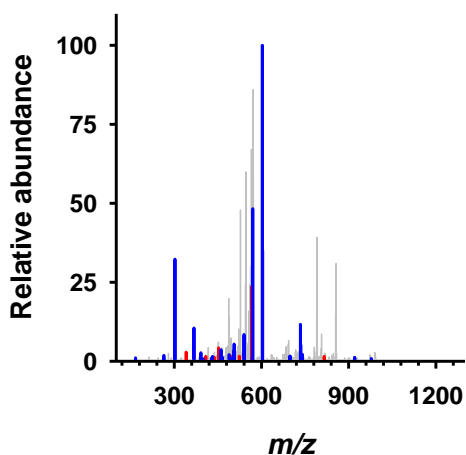


#	b	b++	b+++	Seq.	y	y++	y+++	#
1				N	1732.89	866.95	578.3	16
2	252.25	126.63		H	1618.78	809.9	540.27	15
3	339.33	170.17		S	1481.64	741.33	494.55	14
4	452.49	226.75		L	1394.57	697.79	465.53	13
5	539.56	270.28		S	1281.41	641.21	427.81	12
6	596.61	298.81		G	1194.33	597.67	398.78	11
7	756.78	378.89	252.93	C <sup>CAM</sup>	1137.28	569.14	379.77	10
8	813.83	407.42	271.95	G	977.12	489.06	326.38	9
9	870.88	435.94	290.97	G	920.06	460.54	307.36	8
10	1000	500.5	334	E	863.01	432.01	288.34	7
11	1131.19	566.1	377.74	M	733.9	367.45	245.3	6
12	1228.31	614.66	410.11	P	602.7	301.85	201.57	5
13	1342.41	671.71	448.14	N	505.59	253.3		4
14	1470.58	735.8	490.87	K	391.48	196.25		3
15	1567.7	784.35	523.24	P	263.31	132.16		2
16				F	166.2			1

SEQUEST result:

Matches = 58,  $S_p$ Score = 330.6

$b_{\text{H,IAA}}$ : protonated histidine at position 99 and carbamidomethyl cysteine at position 104

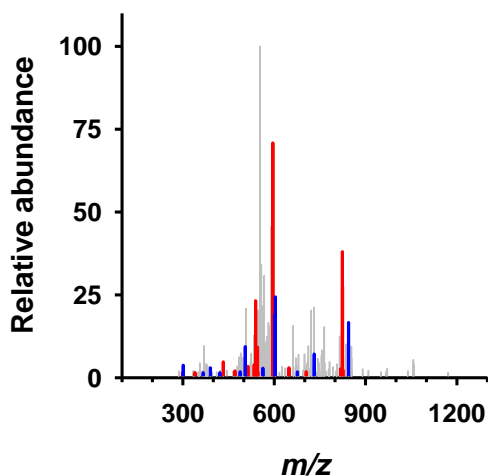


#	b	b++	b+++	Seq.	y	y++	y+++	#
1				N	1733.9	867.45	578.64	16
2	253.26	127.13		H <sup>H</sup>	1619.79	810.4	540.6	15
3	340.34	170.67		S	1481.64	741.33	494.55	14
4	453.49	227.25		L	1394.57	697.79	465.53	13
5	540.57	270.79		S	1281.41	641.21	427.81	12
6	597.62	299.31		G	1194.33	597.67	398.78	11
7	757.79	379.4	253.27	C <sup>CAM</sup>	1137.28	569.14	379.77	10
8	814.84	407.92	272.28	G	977.12	489.06	326.38	9
9	871.89	436.45	291.3	G	920.06	460.54	307.36	8
10	1001	501.01	334.34	E	863.01	432.01	288.34	7
11	1132.2	566.6	378.07	M	733.9	367.45	245.3	6
12	1229.32	615.16	410.44	P	602.7	301.85	201.57	5
13	1343.42	672.21	448.48	N	505.59	253.3		4
14	1471.59	736.3	491.2	K	391.48	196.25		3
15	1568.71	784.86	523.57	P	263.31	132.16		2
16				F	166.2			1

SEQUEST result:

Matches = 62,  $S_p$ Score = 429.7

$b_{\text{NEM}}$ : N-ethylmaleimide cysteine at position 104



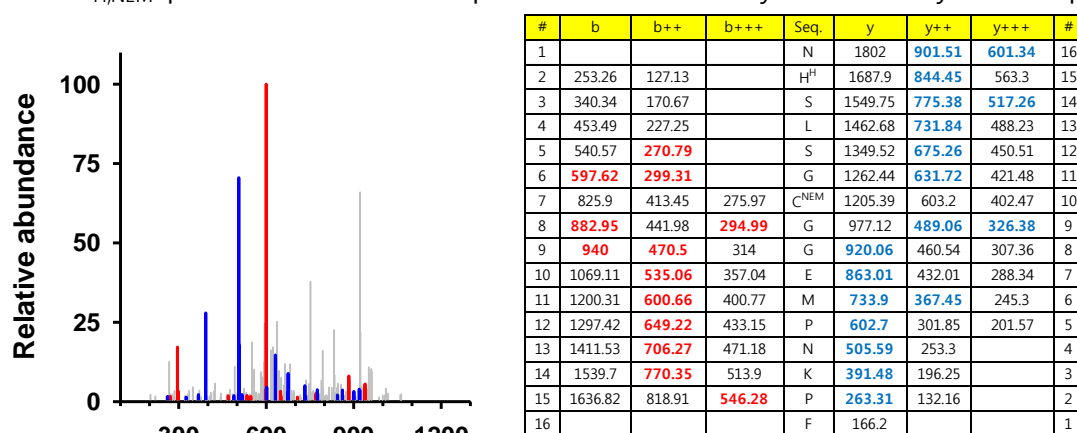
#	b	b++	b+++	Seq.	y	y++	y+++	#
1				N	1801	901	601	16
2	252.25	126.63		H	1686.89	843.95	562.97	15
3	339.33	170.17		S	1549.75	775.38	517.26	14
4	452.49	226.75		L	1462.68	731.84	488.23	13
5	539.56	270.28		S	1349.52	675.26	450.51	12
6	596.61	298.81		G	1262.44	631.72	421.48	11
7	824.89	412.95	275.63	C <sup>NEM</sup>	1205.39	603.2	402.47	10
8	881.94	441.47	294.65	G	977.12	489.06	326.38	9
9	938.99	470	313.67	G	920.06	460.54	307.36	8
10	1068.1	534.56	356.71	E	863.01	432.01	288.34	7
11	1199.3	600.15	400.44	M	733.9	367.45	245.3	6
12	1296.42	648.71	432.81	P	602.7	301.85	201.57	5
13	1410.52	705.76	470.84	N	505.59	253.3		4
14	1538.69	769.85	513.57	K	391.48	196.25		3
15	1635.81	818.41	545.94	P	263.31	132.16		2
16				F	166.2			1

SEQUEST result:

Matches = 57,  $S_p$ Score = 304.3

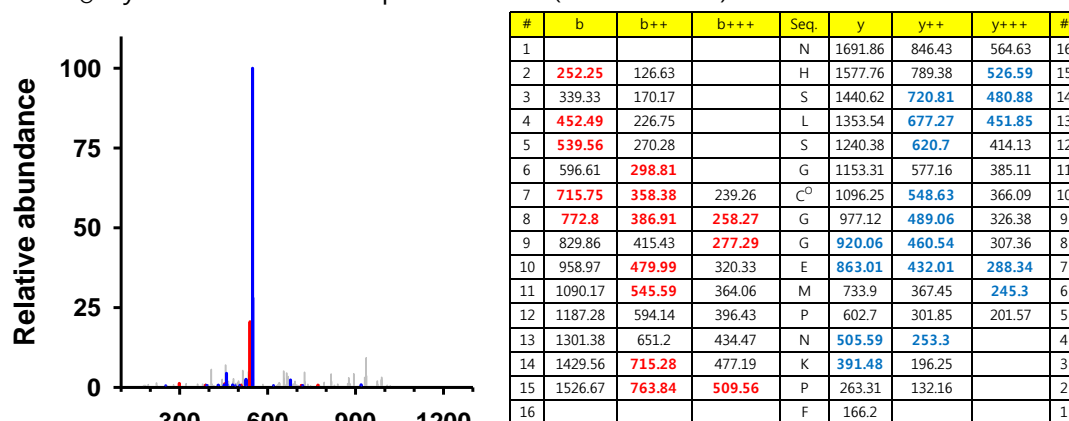
(continued Figure S2b)

$b_{H,NEM}$ : protonated histidine at position 99 and N-ethylmaleimide cysteine at position 104



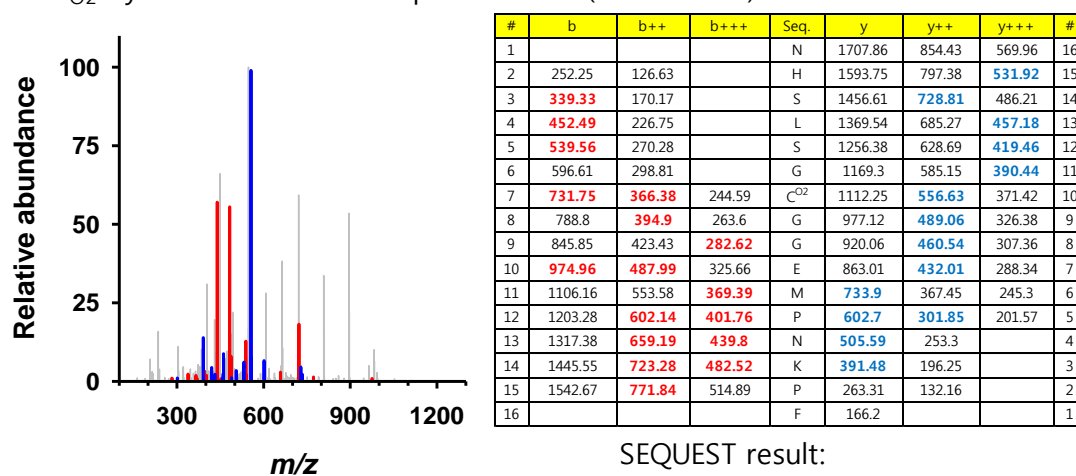
SEQUEST result:  
Matches = 54,  $S_p$ Score = 333.1

$b_O$ : cysteine oxidation at position 104 (sulfenic acid)



SEQUEST result:  
Matches = 61,  $S_p$ Score = 365.9

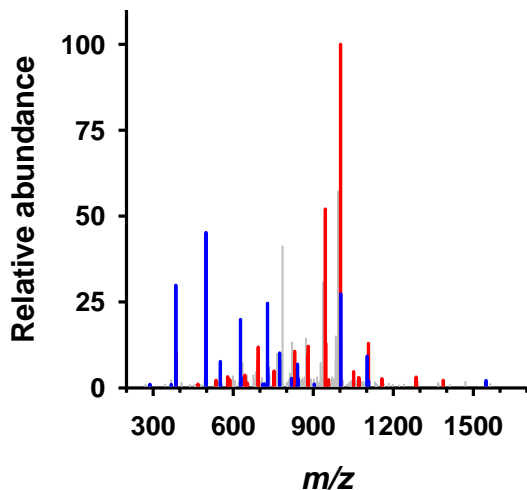
$b_{O_2}$ : cysteine dioxidation at position 104 (sulfinic acid)



SEQUEST result:  
Matches = 55,  $S_p$ Score = 290

(c) Tandem mass spectra for the modified peptide peaks of  $^{158}\text{TASHAIATSDDSQTCLTELPLR}^{179}$

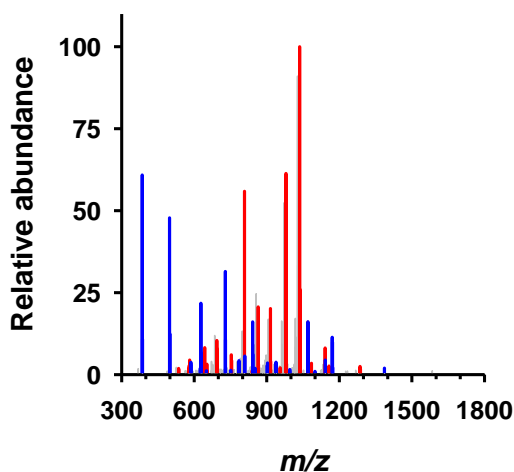
$\text{C}_{\text{IAA}}$ : carbamidomethyl cysteine at position 172



#	b	b++	b+++	Seq.	y	y++	y+++	#
1				T	2388.56	1194.78	796.86	22
2	173.19			A	2287.45	1144.23	763.16	21
3	260.27	130.64		S	2216.38	<b>1108.69</b>	739.46	20
4	397.41	199.21		H	2129.3	1065.15	<b>710.44</b>	19
5	<b>468.48</b>	234.75		A	1992.16	996.58	664.72	18
6	<b>581.64</b>	291.32		I	1921.08	961.04	641.03	17
7	<b>652.72</b>	326.86	218.24	A	1807.92	<b>904.47</b>	603.31	16
8	<b>753.82</b>	377.42	251.95	T	1736.85	868.93	<b>579.62</b>	15
9	<b>840.9</b>	420.95	280.97	S	1635.74	<b>818.37</b>	545.92	14
10	<b>955.99</b>	478.5	319.33	D	<b>1548.66</b>	<b>774.84</b>	516.89	13
11	<b>1071.08</b>	<b>536.04</b>	357.7	D	1433.58	<b>717.29</b>	478.53	12
12	<b>1158.15</b>	<b>579.58</b>	<b>386.72</b>	S	1318.49	659.75	440.17	11
13	<b>1286.28</b>	<b>643.65</b>	429.43	Q	1231.41	616.21	411.14	10
14	<b>1387.39</b>	<b>694.2</b>	463.13	T	<b>1103.28</b>	<b>552.15</b>	<b>368.43</b>	9
15	<b>1547.55</b>	<b>774.28</b>	516.52	$\text{C}^{\text{CAM}}$	<b>1002.18</b>	501.59	334.73	8
16	1660.71	<b>830.86</b>	554.24	L	<b>842.01</b>	421.51	281.34	7
17	1761.81	<b>881.41</b>	<b>587.94</b>	T	<b>728.86</b>	364.93	243.62	6
18	1890.93	<b>945.97</b>	630.98	E	<b>627.75</b>	314.38	209.92	5
19	2004.09	<b>1002.55</b>	668.7	L	<b>498.64</b>	249.82		4
20	2101.2	<b>1051.1</b>	701.07	P	<b>385.48</b>	193.24		3
21	2214.36	<b>1107.68</b>	738.79	L	<b>288.37</b>	144.69		2
22				R	175.21			1

SEQUEST result:  
Matches = 90,  $S_p$ Score = 961.6

$\text{C}_{\text{NEM}}$ : N-ethylmaleimide cysteine at position 172



#	b	b++	b+++	Seq.	y	y++	y+++	#
1				T	2456.67	1228.84	819.56	22
2	173.19			A	2355.56	1178.29	<b>785.86</b>	21
3	260.27	130.64		S	2284.48	<b>1142.8</b>	762.17	20
4	397.41	199.21		H	2197.41	<b>1099.2</b>	733.14	19
5	468.48	234.75		A	2060.27	1030.64	687.43	18
6	<b>581.64</b>	291.32		I	1989.19	<b>995.1</b>	663.73	17
7	<b>652.72</b>	326.86	218.24	A	1876.03	<b>938.52</b>	<b>626.02</b>	16
8	<b>753.82</b>	377.42	251.95	T	1804.95	<b>902.98</b>	602.32	15
9	<b>840.9</b>	420.95	280.97	S	1703.85	<b>852.43</b>	568.62	14
10	<b>955.99</b>	478.5	319.33	D	1616.77	<b>808.89</b>	539.6	13
11	<b>1071.08</b>	<b>536.04</b>	357.7	D	1501.69	<b>751.35</b>	501.23	12
12	<b>1158.15</b>	<b>579.58</b>	<b>386.72</b>	S	<b>1386.6</b>	<b>693.8</b>	462.87	11
13	<b>1286.28</b>	<b>643.65</b>	429.43	Q	1299.52	<b>650.26</b>	433.85	10
14	<b>1387.39</b>	<b>694.2</b>	463.13	T	<b>1171.39</b>	<b>586.2</b>	391.14	9
15	1615.66	<b>808.33</b>	539.23	$\text{C}^{\text{CAM}}$	<b>1070.29</b>	<b>535.65</b>	357.43	8
16	1728.82	<b>864.91</b>	<b>576.94</b>	L	<b>842.01</b>	421.51	281.34	7
17	1829.92	<b>915.46</b>	610.65	T	<b>728.86</b>	364.93	243.62	6
18	1959.04	<b>980.02</b>	<b>653.68</b>	E	<b>627.75</b>	314.38	209.92	5
19	2072.19	<b>1036.6</b>	691.4	L	<b>498.64</b>	249.82		4
20	2169.31	<b>1085.16</b>	723.77	P	<b>385.48</b>	193.24		3
21	2282.47	<b>1141.74</b>	761.49	L	288.37	144.69		2
22				R	175.21			1

SEQUEST result:  
Matches = 103,  $S_p$ Score = 1335.4

**Supplementary Figure S3. The electron density map surrounding Cys104.**

The 2Fo-Fc map contoured at 1.0 $\sigma$  is displayed in gray.

