Supporting Information

Pro-CrossLink— A Software Tool for Protein Crosslinking and Mass Spectrometry

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Figure S-1. SDS-PAGE analysis of the crosslinking reaction of CYP2E1 and b_5 using EDC. Proteins were visualized by staining with Coomassie Blue for 2h and destaining for 12h. *Lane1*, molecular weight markers; *Lane 2*, results of 1:1 incubation of CYP2E1 and b_5 with EDC; *Lane 3*, the same as *Lane 2* without EDC.

Figure S-2. Total ion chromatograms of QTOF-ESI MS runs of ^{16}O-labeled (A) and ^{18}O-labeled (B) tryptic peptide mixtures.

Figure S-3. Isotopic distribution of the crosslinked peptide pair (b_5(E48-R68)-CYP2E1(Y423-K434)) ion, $[M + 4H]^{4+}$ = 907.43 in QTOF-ESI MS runs. Crosslinked complex was digested in ^{16}O- (A) and ^{18}O-water (B). The mass shift of 1.5 for the quadruply charged ion indicates the incorporation of three ^{18}O atoms.

Table S-1. MS/MS assignments generated by *AssignXLink.*

Table S-2. Fragment ions labeled in Figure 3. (*AssignXLink* assigned fragment ions that are caused exclusively by amide bond cleavages.)

Figure S-1

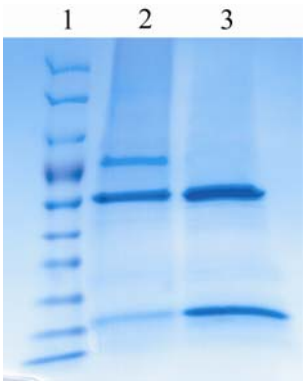

Figure S-2

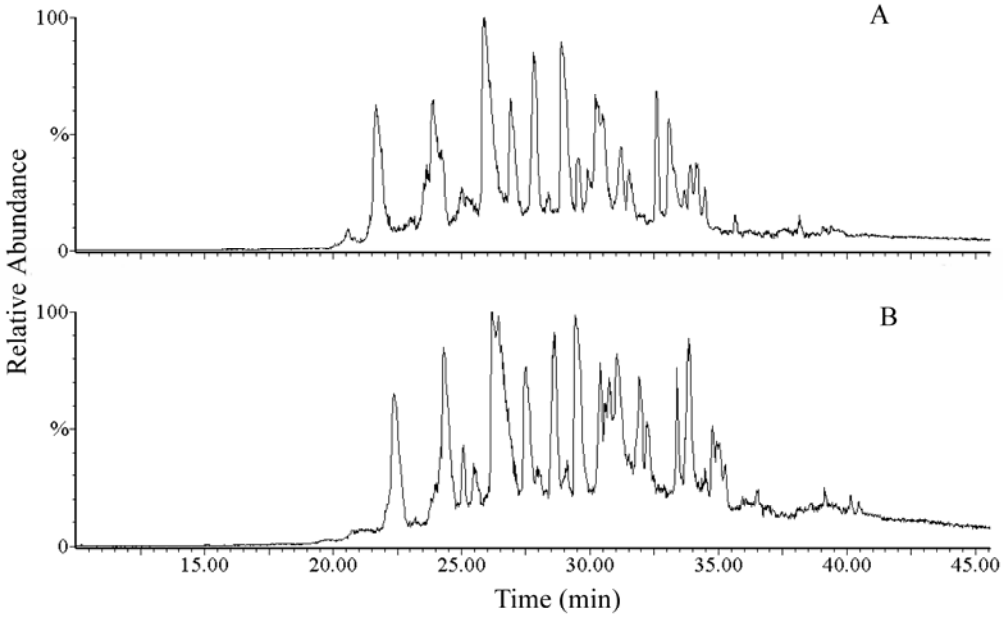

Figure S-3

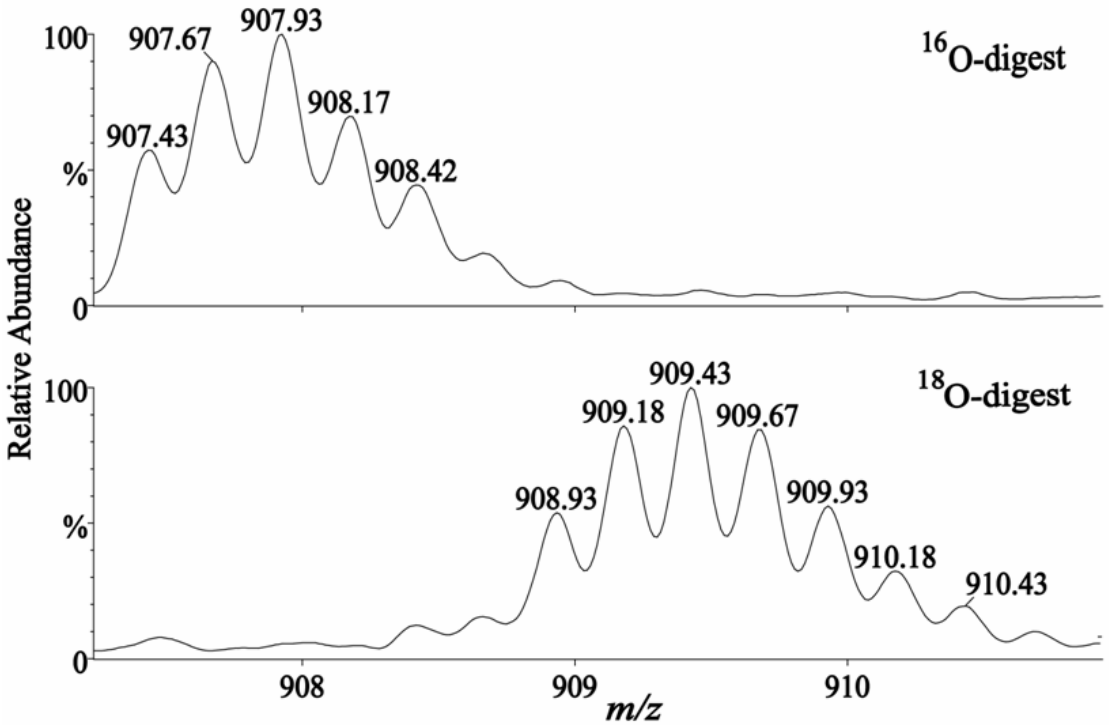

Table S-1. MS/MS assignments generated by *AssignXLink.*

Labeling Number	Measured (m/z)	Calculated (m/z)	Error (ppm)	Assignment
1	498.1947	498.1948	-0.3	b-int(P1)(15-19)(+1)
2	498.1947	498.1948	-0.3	c-int(P1)(15-19)(-NH3)(+1)
3	501.1985	501.1985	-0.1	b4(P2)(-CO)(+1)
4	501.1985	501.1985	-0.1	a4(P2)(+1)
5	549.2642	549.2633	1.6	y5(P1)(+1)
6	549.2642	549.2673	-5.7	a-int(P1)(10-14)(-CO)(+1)
7	549.2642	549.2673	-5.7	a-int(P1)(8-12)(-CO2)(+1)
8	597.2644	597.2633	1.9	b-int(P1)(12-17)(-CO)(+1)
9	597.2644	597.2633	1.9	a-int(P1)(12-17)(+1)
10	597.2644	597.2633	1.9	b-int(P1)(13-18)(+1)
11	597.2644	597.2633	1.9	c-int(P1)(13-18)(-NH3)(+1)
12	597.2644	597.2633	1.9	c-int(P1)(14-19)(-NH3)(+1)
13	597.2644	597.2633	1.9	b-int(P1)(14-19)(+1)
14	618.3269	618.3251	2.9	y6(P2)(-H2O)(+1)
15	618.3269	618.3251	2.9	x6(P2)(-CO2)(+1)

16	636.3372	636.3357	2.3	y6(P2)(+1)
17	648.2709	648.2670	6.0	a5(P2)(+1)
18	648.2709	648.2670	6.0	b5(P2)(-CO)(+1)
19	676.2629	676.2619	1.5	c5(P2)(-NH3)(+1)
20	676.2629	676.2619	1.5	b5(P2)(+1)
21	686.3241	686.3222	2.8	y6(P1)(+1)
22	694.2806	694.2796	1.4	b-int(P1)(13-19)(-H2O)(+1)
23	712.2932	712.2902	4.2	c-int(P1)(13-19)(-NH3)(+1)
24	712.2932	712.2902	4.2	b-int(P1)(13-19)(+1)
25	743.3461	743.3436	3.4	y7(P1)(+1)
26	743.3461	743.3476	-2.0	a-int(P1)(10-16)(-CO)(+1)
27	789.3465	789.3419	5.8	a-int(P1)(8-14)(-H2O)(+1)
28	789.3465	789.3531	-8.5	c-int(P1)(11-17)(+1)
29	823.3247	823.3222	3.0	b-int(P1)(12-19)(-H2O)(+1)
30	842.4153	842.4120	3.8	y8(P1)(+1)
31	957.4433	957.4390	4.6	y9(P1)(+1)
32	957.4433	957.4430	0.4	a-int(P1)(8-16)(-CO2)(+1)
33	1102.4475	1102.4441	3.1	b-int(P1)(10-19)(+1)
34	1102.4475	1102.4441	3.1	c-int(P1)(10-19)(-NH3)(+1)
35	1233.5573	1233.5500	5.9	y11(P1)(+1)
36	1347.5998	1347.5929	5.1	y12(P1)(+1)
37	1403.5788	1403.5715	5.2	c-int(P1)(8-20)(-NH3)(+1)
38	1403.5788	1403.5715	5.2	b-int(P1)(8-20)(+1)
39	1403.5788	1403.5715	5.2	c-int(P1)(7-19)(-NH3)(+1)
40	1403.5788	1403.5715	5.2	b-int(P1)(7-19)(+1)
41	1733.8083	1733.8094	-0.7	c10(P1)x7(P2)(-CO)(+1)
42	1733.8083	1733.8135	-3.0	b9(P1)y8(P2)(-H2O)(+1)
43	1733.8083	1733.8135	-3.0	b9(P1)x8(P2)(-CO2)(+1)
44	824.8672	824.8641	3.8	y15(P1)(+2)
45	875.4144	875.4081	7.2	a9(P1)x8(P2)(+2)
46	875.4144	875.4081	7.2	b9(P1)x8(P2)(-CO)(+2)
47	875.4144	875.4157	-1.5	c8(P1)c8(P2)(-CO2)(+2)
48	939.4034	939.4030	0.4	b11(P1)b6(P2)(-CO)(+2)
49	939.4034	939.4030	0.4	b11(P1)a6(P2)(+2)
50	939.4034	939.4030	0.4	a11(P1)c6(P2)(-NH3)(+2)
51	939.4034	939.4030	0.4	c11(P1)a6(P2)(-NH3)(+2)
52	939.4034	939.4030	0.4	a11(P1)b6(P2)(+2)
53	965.9186	965.9107	8.2	a9(P1)b9(P2)(-NH3)(+2)
54	965.9186	965.9107	8.2	b9(P1)a9(P2)(-NH3)(+2)
55	965.9186	965.9107	8.2	b8(P1)b10(P2)(-NH3)(+2)
56	980.9455	980.9398	5.9	b6(P1)(-H2O)(+2)
57	980.9455	980.9398	5.9	b8(P1)b11(P2)(-CO2)(+2)

58	980.9455	980.9440	1.5	b12(P1)z7(P2)(-H2O)(+2)
59	989.9509	989.9450	5.9	c6(P1)(-NH3)(+2)
60	989.9509	989.9450	5.9	b6(P1)(+2)
61	989.9509	989.9493	1.6	c12(P1)z7(P2)(-NH3)(+2)
62	989.9509	989.9493	1.6	b12(P1)z7(P2)(+2)
63	989.9509	989.9569	-6.1	b11(P1)z8(P2)(-H2O)(+2)
64	1030.4394	1030.4320	7.2	b9(P1)b10(P2)(-NH3)(+2)
65	1131.5078	1131.5035	3.9	a14(P1)a7(P2)(-CO)(+2)
66	1131.5078	1131.5035	3.9	b9(P1)(-H2O)(+2)
67	1197.5329	1197.5302	2.2	a15(P1)x8(P2)(-NH3)(+2)
68	1197.5329	1197.5302	2.2	b10(P1)(+2)
69	1197.5329	1197.5302	2.2	c10(P1)(-NH3)(+2)
70	1271.0659	1271.0644	1.2	c14(P1)a9(P2)(-CO)(+2)
71	1271.0659	1271.0644	1.2	a14(P1)c9(P2)(-CO)(+2)
72	1271.0659	1271.0644	1.2	c11(P1)(-NH3)(+2)
73	1271.0659	1271.0644	1.2	b11(P1)(+2)
74	1353.6074	1353.5999	5.5	b19(P1)y7(P2)(+2)
75	1353.6074	1353.5999	5.5	c19(P1)y7(P2)(-NH3)(+2)
76	1353.6074	1353.6019	4.1	c15(P1)x10(P2)(-CO)(+2)
77	1353.6074	1353.6082	-0.6	a15(P1)z11(P2)(-CO)(+2)
78	1364.5617	1364.5577	3.0	b19(P1)b6(P2)(-H2O)(+2)
79	1384.1064	1384.1058	0.4	a16(P1)z10(P2)(-NH3)(+2)
80	1384.1064	1384.1058	0.4	b17(P1)z9(P2)(-NH3)(+2)
81	1393.1062	1393.0992	5.0	b13(P1)(+2)
82	1393.1062	1393.0992	5.0	c13(P1)(-NH3)(+2)
83	1393.1062	1393.1028	2.4	b20(P1)x7(P2)(-H2O)(+2)
84	1393.1062	1393.1104	-3.0	x19(P1)a7(P2)(-CO2)(+2)
85	1393.1062	1393.1104	-3.0	y19(P1)a7(P2)(-H2O)(+2)
86	1442.6419	1442.6390	2.0	z18(P1)z9(P2)(-NH3)(+2)
87	1442.6419	1442.6390	2.0	b18(P1)y9(P2)(-NH3)(+2)
88	1442.6419	1442.6390	2.0	z16(P1)z10(P2)(-H2O)(+2)
89	1442.6419	1442.6446	-1.9	y17(P1)x9(P2)(-H2O)(+2)
90	1442.6419	1442.6446	-1.9	x17(P1)y9(P2)(-H2O)(+2)
91	1442.6419	1442.6446	-1.9	x17(P1)x9(P2)(-CO2)(+2)
92	1443.1437	1443.1366	4.9	a16(P1)x11(P2)(-CO)(+2)
93	1443.1437	1443.1366	4.9	a17(P1)x10(P2)(-CO)(+2)
94	1443.1437	1443.1366	4.9	x17(P1)y9(P2)(-NH3)(+2)
95	1443.1437	1443.1366	4.9	y17(P1)x9(P2)(-NH3)(+2)
96	1443.1437	1443.1429	0.6	b18(P1)z9(P2)(+2)
97	1443.1437	1443.1429	0.6	c18(P1)z9(P2)(-NH3)(+2)
98	1443.1437	1443.1492	-3.8	a15(P1)(-CO)(+2)
99	1471.1543	1471.1497	3.1	z17(P1)z10(P2)(-H2O)(+2)

100	1471.1543	1471.1553	-0.7	y18(P1)x9(P2)(-H2O)(+2)
101	1471.1543	1471.1553	-0.7	x18(P1)x9(P2)(-CO2)(+2)
102	1471.1543	1471.1553	-0.7	x18(P1)y9(P2)(-H2O)(+2)
103	984.4564	984.4493	7.3	x20(P1)z8(P2)(-CO)(+3)
104	984.4564	984.4493	7.3	z20(P1)x8(P2)(-CO)(+3)
105	984.4564	984.4521	4.4	y7(P2)(+3)
106	984.4564	984.4572	-0.8	x20(P1)y8(P2)(-CO2)(+3)
107	984.4564	984.4572	-0.8	y20(P1)x8(P2)(-CO2)(+3)
108	984.4564	984.4572	-0.8	y20(P1)y8(P2)(-H2O)(+3)
109	991.4550	991.4498	5.2	z19(P1)z9(P2)(+3)
110	991.4550	991.4498	5.2	z18(P1)z10(P2)(-CO2)(+3)
111	991.4550	991.4498	5.2	b18(P1)y10(P2)(-CO2)(+3)
112	991.4550	991.4498	5.2	b19(P1)y9(P2)(-CO2)(+3)
113	991.7609	991.7573	3.6	b6(P2)(-H2O)(+3)
114	991.7609	991.7587	2.2	b17(P1)a11(P2)(-H2O)(+3)
115	991.7609	991.7587	2.2	a17(P1)b11(P2)(-H2O)(+3)
116	997.7663	997.7608	5.4	b6(P2)(+3)
117	997.7663	997.7608	5.4	c6(P2)(-NH3)(+3)
118	997.7663	997.7622	4.1	b17(P1)a11(P2)(+3)
119	997.7663	997.7622	4.1	a17(P1)c11(P2)(-NH3)(+3)
120	997.7663	997.7622	4.1	a17(P1)b11(P2)(+3)
121	997.7663	997.7622	4.1	b17(P1)b11(P2)(-CO)(+3)
122	997.7663	997.7622	4.1	c17(P1)a11(P2)(-NH3)(+3)
123	1000.1125	1000.1096	2.9	z18(P1)z10(P2)(-H2O)(+3)
124	1000.1125	1000.1096	2.9	b19(P1)y9(P2)(-H2O)(+3)
125	1000.1125	1000.1096	2.9	b18(P1)x10(P2)(-CO2)(+3)
126	1000.1125	1000.1096	2.9	b19(P1)x9(P2)(-CO2)(+3)
127	1000.1125	1000.1096	2.9	b18(P1)y10(P2)(-H2O)(+3)
128	1000.1125	1000.1096	2.9	y16(P1)c10(P2)(+3)
129	1006.7863	1006.7850	1.3	z17(P1)z11(P2)(-CO)(+3)
130	1006.7863	1006.7859	0.4	z20(P1)a8(P2)(-CO2)(+3)
131	1006.7863	1006.7929	-6.6	y17(P1)z11(P2)(-CO2)(+3)
132	1006.7863	1006.7929	-6.6	z17(P1)y11(P2)(-CO2)(+3)
133	1026.7900	1026.7850	4.9	c16(P1)(-NH3)(+3)
134	1026.7900	1026.7850	4.9	b16(P1)(+3)
135	1026.7900	1026.7850	4.9	a19(P1)a10(P2)(-CO2)(+3)
136	1026.7900	1026.7887	1.2	y20(P1)a8(P2)(+3)
137	1026.7900	1026.7887	1.2	y20(P1)b8(P2)(-CO)(+3)
138	1026.7900	1026.7929	-2.9	z19(P1)a10(P2)(-CO2)(+3)
139	1026.7900	1026.7958	-5.6	y17(P1)y11(P2)(+3)
140	1033.4810	1033.4749	5.9	y8(P2)(+3)
141	1044.4615	1044.4554	5.8	b19(P1)y10(P2)(+3)

142	1044.4615	1044.4554	5.8	c19(P1)y10(P2)(-NH3)(+3)
143	1044.4615	1044.4563	4.9	z20(P1)a9(P2)(-H2O)(+3)
144	1109.8160	1109.8128	2.9	x20(P1)z11(P2)(-NH3)(+3)
145	1109.8160	1109.8128	2.9	z20(P1)x11(P2)(-NH3)(+3)
146	1121.8215	1121.8170	4.0	b19(P1)(-H2O)(+3)
147	1127.8236	1127.8205	2.8	c19(P1)(-NH3)(+3)
148	1127.8236	1127.8205	2.8	b19(P1)(+3)
149	815.8582	815.8548	4.1	z20(P1)b10(P2)(-H2O)(+4)
150	816.1273	816.1240	4.0	y9(P2)(+4)
151	844.8842	844.8807	4.1	y10(P2)(+4)

Table S-2. Fragment ions labeled in Figure 3. (*AssignXLink* assigned fragment ions that are caused exclusively by amide bond cleavages.)

Labeling Number	Measured (*m/z*)	Calculated (*m/z*)	Error (ppm)	Assignment
1	549.2642	549.2633	1.6	$[y_{5\alpha}]^{+1}$
2	618.3269	618.3251	2.9	$[y_{6\beta} - H_2O]^{+1}$
3	636.3372	636.3357	2.3	$[y_{6\beta}]^{+1}$
4	648.2709	648.2670	6.1	$[b_{5\beta} - CO]^{+1}$
5	676.2629	676.2619	1.5	$[b_{5\beta}]^{+1}$
6	686.3241	686.3222	2.8	$[y_{6\alpha}]^{+1}$
7	694.2806	694.2796	1.4	$[b\text{-int}(\alpha13\text{-}\alpha19) - H_2O]^{+1}$
8	743.3461	743.3436	3.4	$[y_{7\alpha}]^{+1}$
9	816.1273	816.1240	4.0	$[y_{9\beta}]^{+4}$
10	823.3247	823.3222	3.0	$[b\text{-int}(\alpha12\text{-}\alpha19) - H_2O]^{+1}$
11	824.8672	824.8641	3.8	$[y_{15\alpha}]^{+2}$
12	842.4153	842.4120	3.8	$[y_{8\alpha}]^{+1}$
13	844.8842	844.8807	4.1	$[y_{10\beta}]^{+4}$
14	939.4036	939.4030	0.6	$[b_{11\alpha}b_{6\beta} - CO]^{+2}$
15	957.4433	957.4390	4.6	$[y_{9\alpha}]^{+1}$
16	965.9186	965.9107	8.2	$[b_{8\alpha}b_{10\beta} - NH_3]^{+2}$
17	980.9455	980.9398	5.9	$[b_{6\alpha} - H_2O]^{+2}$
17	980.9455	980.9398	5.8	$[b_{8\alpha}b_{11\beta} - CO_2]^{+2}$
18	984.4564	984.4521	4.4	$[y_{7\beta}]^{+3}$
18	984.4564	984.4572	-0.8	$[y_{20\alpha}y_{8\beta} - H_2O]^{+3}$
19	989.9509	989.9450	5.9	$[b_{6\alpha}]^{+2}$

20	991.7609	991.7573	3.6	$[b_{6\beta} - H_2O]^{+3}$
21	997.7662	997.7608	5.4	$[b_{6\beta}]^{+3}$
21	997.7662	997.7622	4.0	$[b_{17\alpha}b_{11\beta} - CO]^{+3}$
22	1000.1125	1000.1096	2.9	$[b_{18\alpha}y_{10\beta} - H_2O]^{+3}$
22	1000.1125	1000.1096	2.9	$[b_{19\alpha}y_{9\beta} - H_2O]^{+3}$
23	1026.7900	1026.7850	4.9	$[b_{16\alpha}]^{+3}$
23	1026.7900	1026.7887	1.2	$[y_{20\alpha}b_{8\beta} - CO]^{+3}$
23	1026.7900	1026.7958	-5.6	$[y_{17\alpha}y_{11\beta}]^{+3}$
24	1030.4394	1030.4320	7.2	$[b_{9\alpha}b_{10\beta} - NH_3]^{+2}$
25	1033.4810	1033.4749	5.9	$[y_{8\beta}]^{+3}$
26	1044.4615	1044.4554	5.8	$[b_{19\alpha}y_{10\beta}]^{+3}$
27	1102.4474	1102.4441	3.0	$[b\text{-int}(\alpha10\text{-}\alpha19)]^{+1}$
28	1121.8215	1121.8170	4.0	$[b_{19\alpha} - H_2O]^{+3}$
29	1127.8236	1127.8205	2.8	$[b_{19\alpha}]^{+3}$
30	1131.5078	1131.5035	3.9	$[b_{9\alpha} - H_2O]^{+2}$
31	1197.5329	1197.5302	2.2	$[b_{10\alpha}]^{+2}$
32	1233.5573	1233.5500	5.9	$[y_{11\alpha}]^{+1}$
33	1271.0659	1271.0644	1.2	$[b_{11\alpha}]^{+2}$
34	1347.5998	1347.5929	5.1	$[y_{12\alpha}]^{+1}$
35	1353.6074	1353.5999	5.5	$[b_{19\alpha}y_{7\beta}]^{+2}$
36	1364.5617	1364.5577	3.0	$[b_{19\alpha}b_{6\beta} - H_2O]^{+2}$
37	1393.1062	1393.0992	5.0	$[b_{13\alpha}]^{+2}$
38	1403.5788	1403.5715	5.2	$[b\text{-int}(\alpha7\text{-}\alpha19)]^{+1}$
38	1403.5788	1403.5715	5.2	$[b\text{-int}(\alpha8\text{-}\alpha20)]^{+1}$
39	1442.6419	1442.6390	2.0	$[b_{18\alpha}y_{9\beta} - NH_3]^{+2}$

EXPERIMENTAL PROCEDURES

QTOF-MS Analysis. Peptide digests were analyzed by on-line nano-LC/ESI MS/MS using a QTOF mass spectrometer equipped with a CapLC system (Waters, Milford, MA). The stream select module was configured with a 5 mm x 300 µm i.d. trap column packed with 5 µm C_4 particles (LC Packings, San Francisco, CA) connected by a ZU1XC metallic union (Valco, Houston, TX) to a 20 cm x 75 µm i.d.

nanoscale analytical column packed in-house with 5 μm Jupiter C18 particles (Phenomenex, Torrance, CA) using the method described by Kennedy *et al.*[1] Peptide samples were injected onto the trap column at 10 μL/min, cleaned-up and back-flushed to the analytical column at 0.5 μL/min using gradient elution. Binary gradients of 5-60% solvent B were generated over 30 min, followed by 60% B for 5 min and 60-90% B for 5min (solvent A = 3.3% acetonitrile, 1.7% 2-propanol and 0.1% TFA; solvent B = 63.3% acetonitrile, 31.7% 2-propanol and 0.1% TFA). QTOF parameters were set as follows: electrospray potential, 3.6 kV; cone voltage, 35 V; extraction cone, 0 V; and source temperature, 100 ^{o}C. The instrument was operated at a mass resolving power of 6,000. For MS/MS, the scan time was set to 2 sec, the precursor isolation width set to 4 Da, and the collision energy set to 25-45 eV according to the *m/z* of the precursor and the charge state.

IT-FT-ICR-MS Analysis. Peptide digests were analyzed by electrospray ionization in the positive ion mode on a hybrid ion trap-fourier transform ion cyclotron resonance mass spectrometer (Thermo Electron Corp., San Jose, CA). Nanoflow HPLC was performed using a similar approach to that described by Yi *et al.*[2] The electrospray voltage was applied via liquid junction using a platinum wire inserted into a micro-tee union (Upchurch Scientific, Oak Harbor, WA). Ion source conditions were as follows: ESI voltage, 1.4 kV; capillary temperature, 200 ^{o}C; capillary voltage, 44 V; and tube lens voltage, 180 V. All other voltages were optimized using a tuning solution composed of caffeine (Sigma, St. Louis, MO), MRFA (Bachem, King of Prussia, PA) and Ultramark 1621 (Lancaster Synthesis,

Windham, NH). Injection waveforms for the LTQ-FT ion trap and ICR cell were kept on for all acquisitions. ICR resolution was set to 50,000. ICR ion populations in the ICR cell were held at 1e6 and 5e5 for MS and MS/MS, respectively. For MS/MS, the precursor isolation width was set to 10 Da and the collision energy set to 55%.

References:

(1) Kennedy, R. T.; Jorgenson, J. W. *Anal. Chem.* **1989**, 61, 436-41.

(2) Yi, E. C.; Lee, H.; Aebersold, R.; Goodlett, D. R. *Rapid Commun. Mass Spectrom.* **2003**, 17, 2093-8.