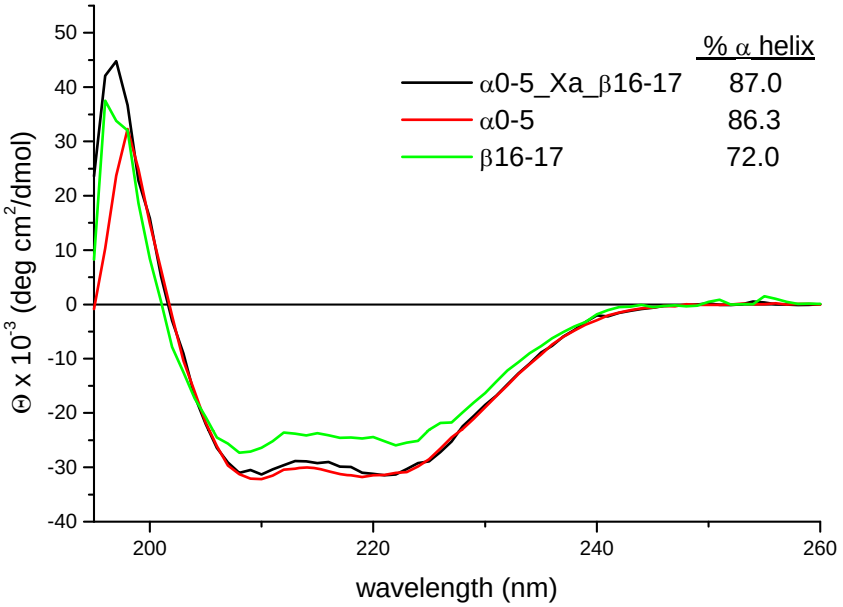
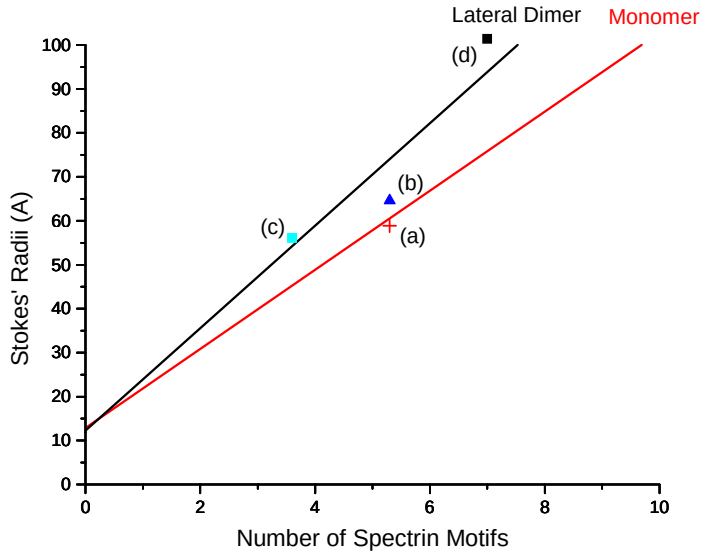
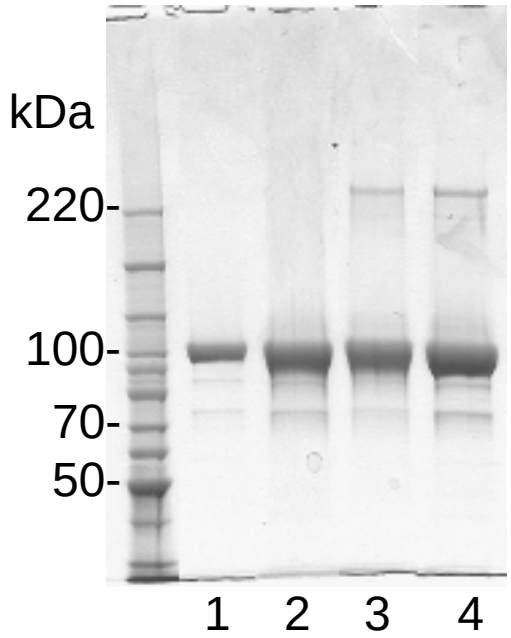


Supplemental Figure 1. Circular dichroism results for the mini-spectrin tetramer. *A* Circular dichroism results for the mini-spectrin dimer, α0-5 and β16-17 in 10 mM MOPS, 130 mM NaCl, 1 mM TCEP pH 7.4 at 25 ^{o}C are shown.

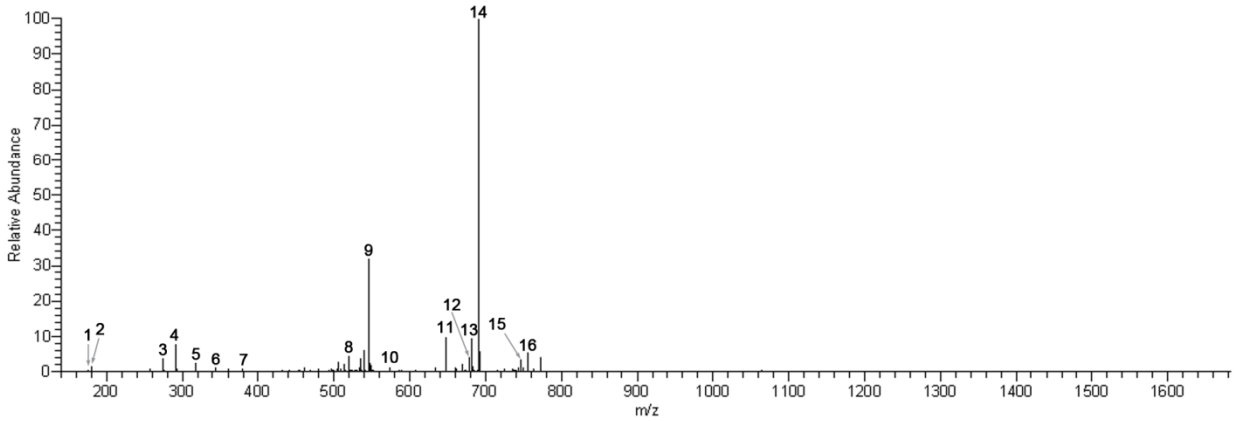


Supplemental Figure 2. Relationship between Stokes' radii and the number of spectrin repeats. Monomeric spectrin recombinants with different numbers of repeats were used to create a standard curve for number of repeats versus Stokes' radii (red line). Several two-stranded dimeric spectrin recombinant protein complexes were used to create a similar standard curve for two-stranded, laterally associated spectrin complexes (black line). The locations of the various mini-spectrin species are plotted for comparison based upon the number of two-strand or single-strand (monomer) repeats in the model: *(a)* α0-5, *(b)* open dimer, *(c)* closed dimer, *(d)* tetramer.



Supplemental Figure 3. SDS-PAGE gel of cross-linked mini-spectrin dimer. Mini-spectrin dimer was cross-linked using EDC and sulfo-NHS at a concentration of 10 mM and 5 mM, respectively. The reaction mixtures were incubated at 4 ^{o}C, and 100 μl aliquots were removed after 1h, 2h, and 4h. *Lane 1*, non-cross-linked control. *Lane 2*, 1 h reaction. *Lane 3*, 2 h reaction. *Lane 4*, 4 h reaction.

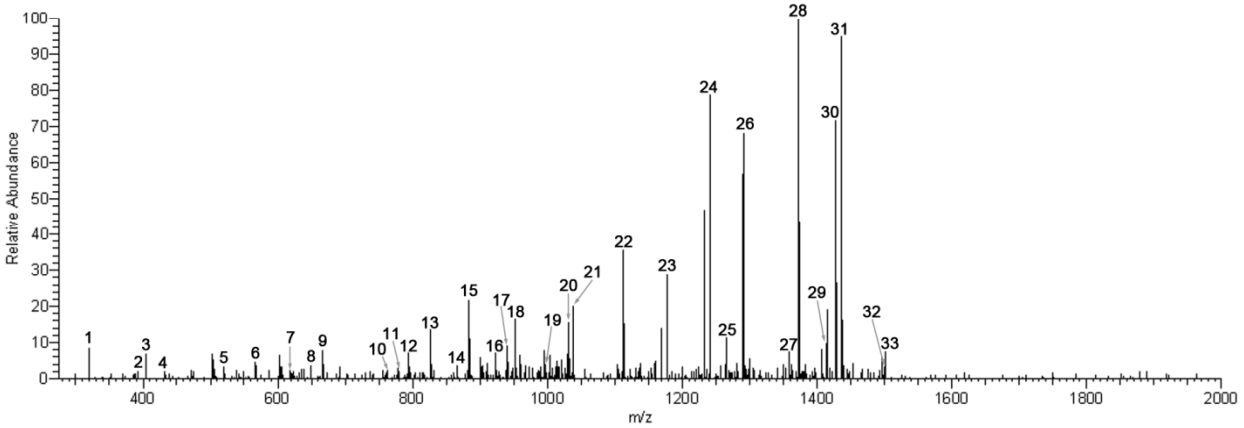
Supplemental Figure 4. MS/MS spectra of intra-molecular cross-linked peptides supporting an open dimer conformation: DAD*D*LGK and YQSFKR*ER with precursor m/z 557.9376 and a charge state of +3. See Supplemental Table 1 for the MS/MS assignments. Most ion assignments were from GPMAW and Fuzzy Ions.



Supplemental Table 1 MS/MS assignments for cross-linked peptide : DAD*D*LGK and YQSFKR*ER. The MS/MS spectra is shown in Supplemental Fig 4.

No.	Peaks	Assignments	No.	Peaks	Assignments
1	175.22	$y1_A$	9	546.50	MH_3^{+3}-$2NH_3$
2	181.09	$(b3_A)^{+2}$-H_2O	10	573.72	$(y3_A)^{+2}$
3	274.99	$b2_A$-NH_3	11	647.63	$(y4_AB_{int})^{+2}$
4	292.11	$b2_A$	12	678.48	$(A_{int}b4_B)^{+2}$
5	317.36	$y3_B$	13	682.12	$(y5_AB_{int})^{+2}$-H_2O
6	361.09	$b3_A$-H_2O	14	691.05	$(y5_AB_{int})^{+2}$
7	379.19	$b3_A$	15	749.76	$(b6_AB_{int})^{+2}$
8	519.81	$(A_{int}y6_B)^{+3}$	16	755.13	$(y6_AB_{int})^{+2}$

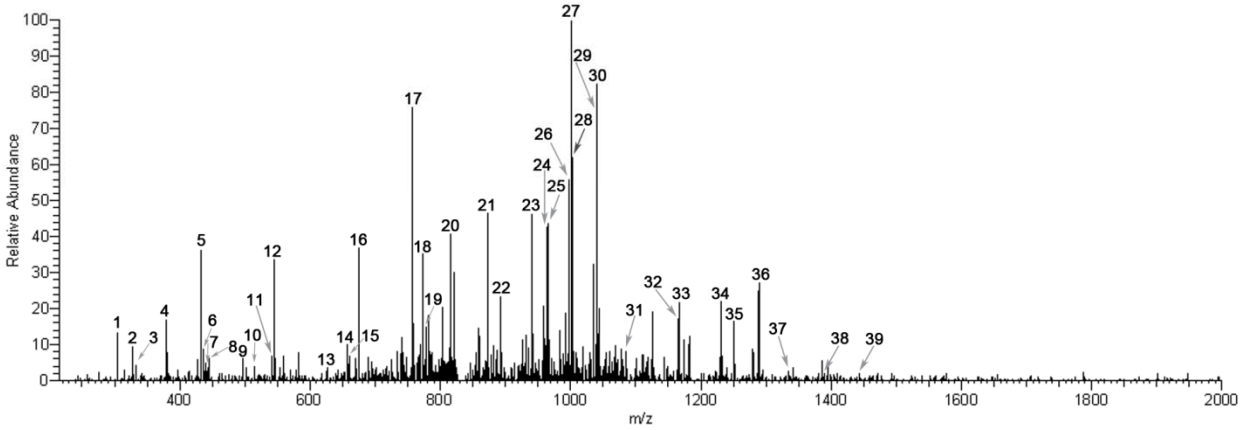
Supplemental Figure 5. MS/MS spectra of intra-molecular cross-linked peptides supporting an open dimer conformation: FQQYVQEC@ADILE*WIGDK and G*SMEQFPK with a precursor mass of 1049.4920 and a charge state of +3. See Supplemental Table 2 for the MS/MS assignments. Most ion assignments were made from GPMAW and Fuzzy Ions.



Supplemental Table 2 MS/MS assignments for cross-linked peptide: FQQYVQEC@ADILE*WIGDK and G*SMEQFPK . The MS/MS spectra is shown in Supplemental Fig 5.

No.	Peaks	Assignments	No.	Peaks	Assignments
1	319.16	$y3_A$	18	951.95	$(b16_AB_{int})^{+3}$-$2NH_3$
2	391.29	$y3_B$	19	997.75	$(y9_AB_{int})^{+2}$
3	404.15	$b3_A$	20	1032.43	$(y10_AB_{int})^{+2}$
4	432.34	$y4_A$	21	1038.57	MH_3^{+3}-$2NH_3$
5	520.14	$y4_B$	22	1112.59	$(y11_AB_{int})^{+2}$
6	566.91	$b4_A$	23	1177.09	$(y12_AB_{int})^{+2}$
7	618.39	$y5_A$	24	1241.14	$(y13_AB_{int})^{+2}$
8	648.39	$y5_B$	25	1265.46	$(b13_AB_{int})^{+2}$
9	666.24	$b5_A$	26	1291.29	$(y14_AB_{int})^{+2}$
10	761.91	$(A_{int}b1_B)^{+3}$	27	1358.28	$(b14_AB_{int})^{+2}$
11	779.33	$y6_B$	28	1372.42	$(y15_AB_{int})^{+2}$
12	794.45	$b6_A$	29	1414.73	$(b15_AB_{int})^{+2}$
13	826.56	$(y6_AB_{int})^{+2}$	30	1427.95	$(y16_AB_{int})^{+2}$-H_2O
14	866.40	$y7_B$	31	1437.00	$(y16_AB_{int})^{+2}$
15	883.18	$(y7_AB_{int})^{+2}$	32	1499.76	$(y17_AB_{int})^{+2}$
16	923.33	$b7_A$	33	1501.00	$(b17_AB_{int})^{+2}/(A_{int}b7_B)^{+2}$
17	939.50	$(y8_AB_{int})^{+2}$			

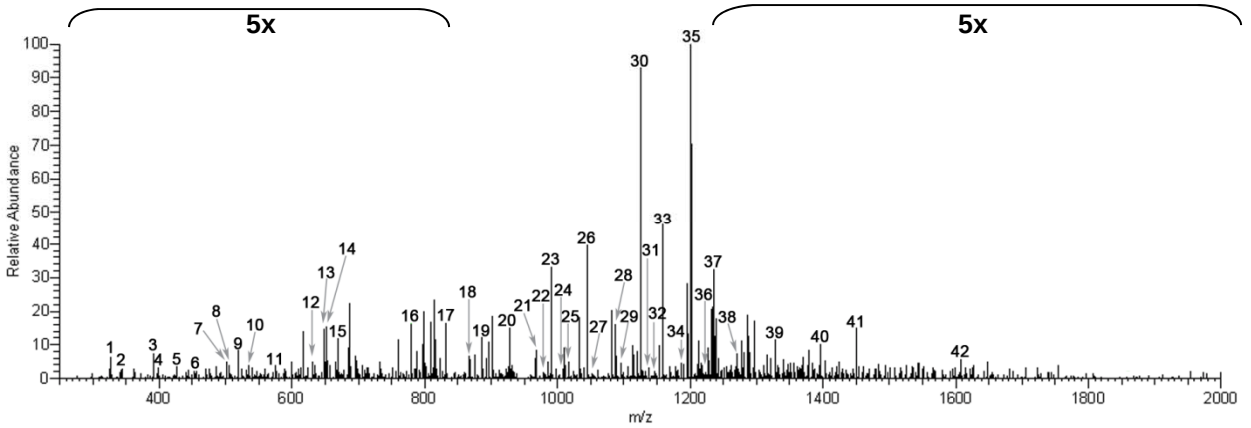
Supplemental Figure 6. MS/MS spectra of intra-molecular cross-linked peptides supporting an open dimer conformation: VNILTDK*SYEDPTNIQGK and VLETAE*EIQER with a precursor mass of 833.9279 and a charge state of +4. See Supplemental Table 3 for the MS/MS assignments. Most ion assignments were made from GPMAW and Fuzzy Ions.



Supplemental Table 3 MS/MS assignments for cross-linked peptide VNILTDK*SYEDPTNIQGK and VLETAE*EIQER . The MS/MS spectra is shown in Supplemental Fig 6.

No.	Peaks	Assignments	No.	Peaks	Assignments
1	304.28	$y2_B$	21	872.33	$y8_A$
2	327.28	$b3_A$	22	893.30	$(y12_A B_{int})^{+3}$
3	332.19	$y3_A$	23	940.67	$(A_{int} y6_B)^{+3}$
4	379.26	$(y7_A)^{+2}$	24	964.44	$(A_{int} y7_B)^{+3}$
5	432.19	$b3_B/y3_B$	25	965.46	$(y14_A B_{int})^{+3}$
6	436.77	$(y8_A)^{+2}$	26	998.20	$(A_{int} y8_B)^{+3}$
7	440.01	$b4_A$	27	1001.46	$y9_A/(b15_A B_{int})^{+3}$
8	445.25	$y4_A$	28	1002.65	$(y15_A B_{int})^{+3}$
9	496.19	$b5_B$-H_2O	29	1040.75	$(A_{int} y9_B)^{+3}$
10	514.30	$b5_B$	30	1041.36	$(b7_A B_{int})^{+2}$
11	541.23	$b5_A$	31	1085.75	$(b8_A B_{int})^{+2}$
12	545.38	$y4_B$	32	1164.53	$y10_A$
13	626.63	$y11_A$	33	1166.47	$(b9_A B_{int})^{+2}$
14	656.30	$b6_A$	34	1231.28	$(b10_A B_{int})^{+2}$
15	660.43	$y6_A$	35	1251.47	$y11_A$
16	674.35	$y5_B$	36	1289.06	$(b11_A B_{int})^{+2}$
17	757.30	$y7_A$	37	1337.74	$(b12_A B_{int})^{+2}$
18	772.11	$(A_{int} y9_B)^{+4}$-$2H_2O$	38	1387.75	$(b13_A B_{int})^{+2}$
19	778.01	$(b9_A B_{int})^{+3}$	39	1444.92	$(b14_A B_{int})^{+2}$
20	815.25	$(b10_A B_{int})^{+3}$-NH_3			

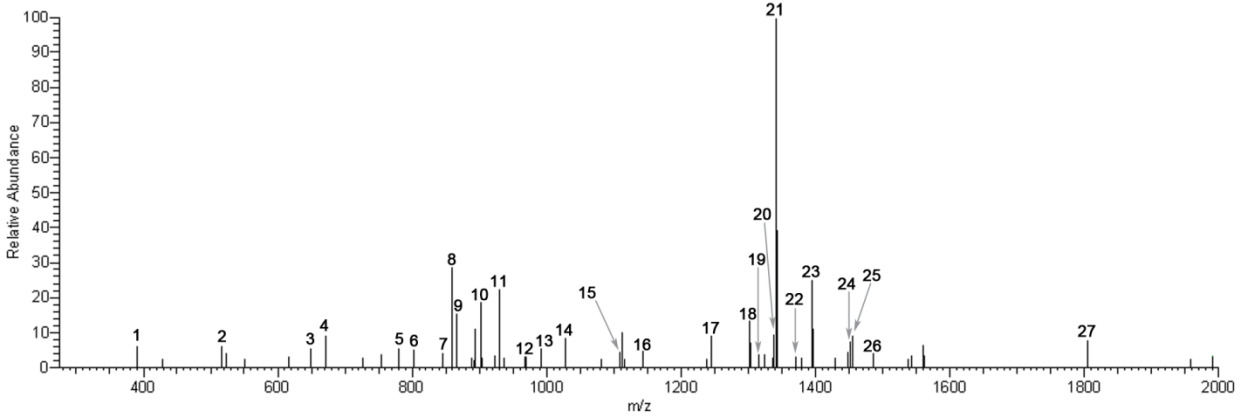
Supplemental Figure 7. MS/MS spectra of intra-molecular cross-linked peptides supporting an open dimer conformation: G*SMEQFPK and VVEVNQYANEC@AEE*NHPDLPLIQSK with a precursor mass of 950.9517 and a charge state of +4. See Supplemental Table 4for the MS/MS assignments. Most ion assignments were made from GPMAW and Fuzzy Ions.



Supplemental Table 4. MS/MS assignments for cross-linked peptide G*SMEQFPK and VVEVNQYANEC@AEE*NHPDLPLIQSK . The MS/MS spectra is shown in Supplemental Fig 7.

No.	Peaks	Assignments	No.	Peaks	Assignments
1	327.92	$b3_A$	22	979.39	$(A_{int}b1_B)^{+3}$
2	343.66	$(y6_A)^{+2}$	23	990.72	$(y18_AB_{int})^{+3}$
3	391.39	$(y7_A)^{+2}$-NH_3/$y3_B$	24	1008.46	$(A_{int}b2_B)^{+3}$
4	399.06	$(y7_A)^{+2}$	25	1017.28	$b9_A$
5	427.26	$b1_A$	26	1045.10	$(y19_AB_{int})^{+3}$
6	457.20	$(y8_A)^{+2}$	27	1052.83	$(A_{int}b3_B)^{+3}$
7	502.35	$(b13_A)^{+2}$	28	1087.69	$(y20_AB_{int})^{+3}$
8	506.06	$y9_A$	29	1095.23	$(A_{int}b4_B)^{+3}$
9	519.45	$y4_B$	30	1126.19	$(y21_AB_{int})^{+3}$
10	541.14	$b5_A$	31	1137.68	$(A_{int}b5_B)^{+3}$
11	574.68	$y10_A$	32	1148.22	$(y12_AB_{int})^{+2}$
12	631.34	$(y11_A)^{+2}$	33	1159.36	$(y22_AB_{int})^{+3}$
13	648.52	$y5_B$	34	1186.69	$(A_{int}b6_B)^{+3}$
14	652.40	$b6_A$-NH_3	35	1201.81	$(y23_AB_{int})^{+3}$
15	669.37	$b6_A$	36	1219.09	$(A_{int}b7_B)^{+3}$
16	779.42	$y6_B$	37	1236.66	$(b14_AB_{int})^{+2}$-$4NH_3$
17	832.56	$(y14_AB_{int})^{+3}$/$b7_A$	38	1271.15	$(b14_AB_{int})^{+2}$
18	866.37	$y7_B$	39	1327.76	$(b15_AB_{int})^{+2}$
19	886.23	$(y15_AB_{int})^{+3}$	40	1396.63	$(b16_AB_{int})^{+2}$
20	929.04	$(y16_AB_{int})^{+3}$	41	1450.68	$(A_{int}b1_B)^{+2}$-$2H_2O$
21	967.64	$(y17_AB_{int})^{+3}$	42	1607.40	$(b20_AB_{int})^{+2}$

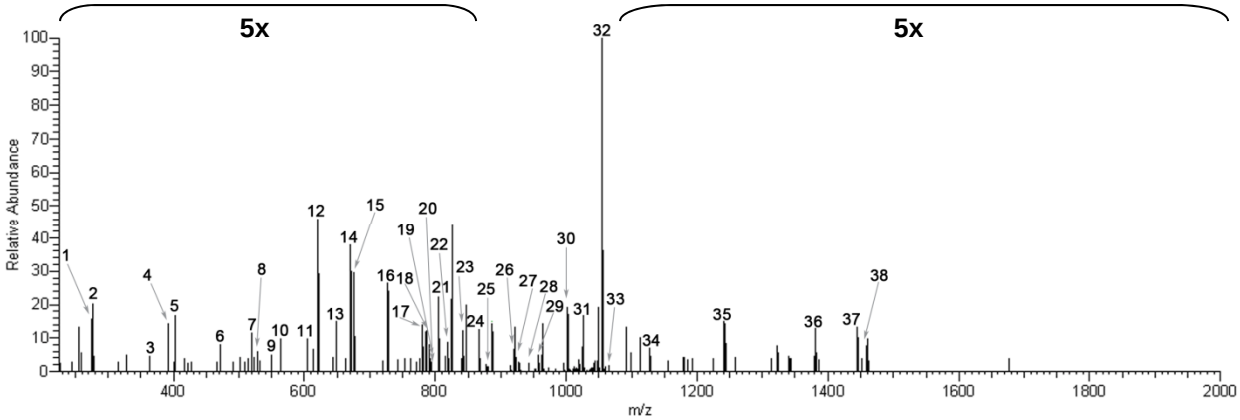
Supplemental Figure 8. MS/MS spectra of intra-molecular cross-linked peptides supporting a closed dimer conformation: G*SMEQFPK and TAAINAD*E*LPTDVAGGEVLLDR with a precursor mass of 1048.8553 and a charge state of +3. See Supplemental Table 5 for the MS/MS assignments. Most ion assignments were made from GPMAW and Fuzzy Ions.



Supplemental Table 5. MS/MS assignments for cross-linked peptide G*SMEQFPK and TAAINAD*E*LPTDVAGGEVLLDR. The MS/MS spectra is shown in Supplemental Fig 8.

No.	Peaks	Assignments	No.	Peaks	Assignments
1	391.24	$y3_B$	15	1108.53	$(b13_A B_{int})^{+2}$
2	516.32	$y4_A$	16	1144.05	$(b14_A B_{int})^{+2}$
3	648.34	$y5_B$	17	1244.64	$y12_A$
4	671.36	$(y13_A)^{+2}$	18	1302.13	$(y16_A B_{int})^{+2}$
5	779.38	$y6_B$	19	1315.13	$(b18_A B_{int})^{+2}$
6	801.44	$y7_A$	20	1337.66	$(y17_A B_{int})^{+2}$
7	845.90	$(b8_A B_{int})^{+2}$	21	1341.71	$y13_A$
8	858.47	$y8_A$	22	1371.66	$(b19_A B_{int})^{+2}$
9	866.41	$y7_B$	23	1394.68	$(y18_A B_{int})^{+2}$
10	902.44	$(b9_A B_{int})^{+2}$	24	1451.21	$(A_{int} b6_B)^{+2}$
11	929.51	$y9_A$	25	1454.78	$y14_A$
12	967.82	$(y19_A B_{int})^{+3}$	26	1486.77	$(y20_A B_{int})^{+2}$
13	991.47	$(y20_A B_{int})^{+3}$	27	1803.87	$b9_A B_{int}$
14	1028.58	$y10_A$			

Supplemental Figure 9. MS/MS Spectra of intra-molecular cross-linked peptides supporting a closed dimer conformation: G*SM#EQFPK and FQSADE*TGQDLVNANHEASDEVR with a precursor mass of 1151.5157/863.8886 and a charge state of +3/4. See Supplemental Table 6 for the MS/MS assignments. Most ion assignments were made from GPMAW and Fuzzy Ions.



Supplemental Table 6. MS/MS assignments for cross-linked peptide G*SM#EQFPK and FQSADE*TGQDLVNANHEASDEVR . The MS/MS spectra is shown in Supplemental Fig 9.

No.	Peaks	Assignments	No.	Peaks	Assignments
1	274.19	$y2_A$	20	791.87	$(b6_A B_{int})^{+2}$
2	276.14	$b2_A$	21	805.37	$y7_A$
3	363.17	$b3_A$	22	823.38	$(A_{int} b7_B)^{+4}$
4	391.24	$y3_B$	23	842.37	$(b7_A B_{int})^{+2}$
5	403.23	$y3_A$	24	866.42	$y7_B$
6	471.72	$(y8_B)^{+2}$	25	877.41	$(y16_A)^{+2}$
7	519.30	$y4_B$	26	921.10	$(b17_A B_{int})^{+3}$
8	528.74	$(y9_A)^{+2}$	27	927.93	$(y17_A)^{+2}$
9	549.24	$b5_A$	28	942.43	$y8_A$
10	564.26	$(y10_A)^{+2}$	29	963.45	$(y18_A B_{int})^{+3}$
11	605.29	$y5_A$	30	1002.12	$(y19_A B_{int})^{+3}$
12	621.28	$(y11_A)^{+2}$	31	1025.47	$(y20_A B_{int})^{+3}$
13	648.34	$y5_B$	32	1055.15	$(y21_A B_{int})^{+3}$
14	670.81	$(y12_A)^{+2}$	33	1065.11	$(A_{int} b6_B)^{+3}$
15	676.33	$y6_A$	34	1127.50	$y10_A$
16	727.36	$(y13_A)^{+2}$	35	1241.55	$y11_A$
17	779.39	$y6_B$	36	1380.60	$(b17_A B_{int})^{+2}$
18	784.87	$(y14_A)^{+2}$	37	1445.13	$(y18_A)^{+2}$
19	791.61	$(b21_A B_{int})^{+4}$	38	1459.64	$(A_{int} b4_B)^{+2}$