

Supplementary Table and Figure Legends:

Table S1. Percentage of carboxyl group modification for Her4 kinase domain protein. All 37 modified residues detected in LC-MS/MS were listed. Residue numbers of glutamic acid (E) and aspartic acid (D) were based on full length Her4 protein. Her4 residue numbering is based on the mature, full length protein minus its signal peptide.

Figure S1. SDS PAGE of modified Her4 kinase domain protein. Only one band, corresponding to the Her4 monomer, was seen in each sample. No cross-linked Her4 dimer was detected.

Figure S2. MS/MS spectra of Her4 phosphorylation sites. (A) pTyr-708. (B) pThr-732. (C) pSer-828. (D) pTyr-850. (E) pTyr-959. (F) pSer-972. Peaks corresponding to ammonia loss are labeled with (#), and those corresponding to water loss are labeled with (*).

Figure S3. Coupling between the activation loop and α C-helix. The Her4 kinase domain dimer structure is shown as in figure 3B. The donor monomer is in green and the acceptor monomer is in blue. The activation loop of the acceptor monomer is shown as red spheres and the α C-helix of the acceptor monomer is shown as magenta spheres. This figure demonstrates the close packing between the α C-helix and the N-terminal portion of the activation loop. The opposite surface of the α C-helix packs up against the donor monomer.

Figure S4. Titration experiment results of Glu-690 and Glu-730. The Her4 kinase domain protein solution concentration was listed on X axis. The black dots are modification extents on different protein concentrations.

Figure S5. Asp-847 titration curve modeling by different Her4 dimer models. Her4 dimer on nickel liposome with parasitic binding site model is given by the black curve. Simple Her4 dimer model is shown in green and Her4 dimer on Ni liposome model is shown by the red curve.

Table S1. Carboxyl group modification extent for Her4 kinase domain.

Residue	% of Modification								
	Monomer in Liposome			Monomer in Buffer			Dimer in Ni-Liposome		
E690	41.25%	±	7.13%	42.01%	±	6.69%	26.82%	±	4.15%
E692	5.52%	±	1.25%	4.90%	±	0.63%	3.40%	±	0.69%
E715	8.16%	±	0.41%	9.36%	±	0.50%	7.54%	±	0.25%
E717	9.03%	±	0.93%	11.73%	±	1.15%	10.82%	±	0.95%
E730	15.76%	±	1.50%	19.19%	±	2.83%	16.64%	±	1.22%
E739	4.00%	±	0.44%	3.17%	±	0.32%	3.56%	±	0.14%
D742	0.27%	±	0.04%	0.26%	±	0.03%	0.23%	±	0.03%
E743	1.15%	±	0.09%	0.99%	±	0.13%	1.05%	±	0.07%
D751	10.39%	±	1.67%	8.45%	±	0.09%	8.65%	±	0.75%
E810	0.71%	±	0.02%	0.63%	±	0.07%	0.74%	±	0.04%
E811	0.08%	±	0.02%	0.10%	±	0.03%	0.17%	±	0.06%
D836	6.79%	±	0.35%	6.77%	±	0.16%	6.76%	±	0.17%
E844	0.19%	±	0.03%	0.13%	±	0.02%	0.23%	±	0.04%
D846	5.88%	±	0.37%	5.81%	±	0.82%	5.46%	±	0.28%
E847	7.96%	±	0.58%	7.60%	±	0.76%	7.51%	±	0.57%
E849	1.04%	±	0.03%	1.12%	±	0.11%	1.00%	±	0.07%
D853	23.96%	±	3.63%	22.20%	±	2.58%	16.66%	±	3.13%
E865	3.97%	±	0.21%	4.94%	±	0.51%	4.34%	±	0.09%
D877	2.00%	±	0.43%	1.77%	±	0.12%	1.85%	±	0.13%
E887	1.25%	±	0.40%	0.19%	±	0.13%	0.04%	±	0.01%
D897	14.56%	±	3.34%	18.53%	±	6.85%	27.91%	±	3.05%
E903	0.27%	±	0.07%	0.22%	±	0.05%	0.52%	±	0.10%
D906	0.09%	±	0.01%	0.05%	±	0.01%	0.11%	±	0.02%
E909	2.47%	±	0.24%	2.00%	±	0.09%	2.41%	±	0.23%
E912	7.63%	±	2.73%	6.04%	±	1.71%	3.53%	±	0.55%
D923	0.08%	±	0.03%	0.17%	±	0.01%	0.19%	±	0.04%
D935	24.69%	±	9.76%	31.94%	±	3.94%	30.05%	±	4.93%
D937	11.70%	±	6.10%	10.79%	±	3.00%	17.36%	±	2.29%
E944	3.95%	±	0.03%	4.15%	±	0.44%	4.03%	±	0.05%
E948	0.24%	±	0.04%	0.15%	±	0.02%	0.17%	±	0.03%
D965	0.19%	±	0.01%	0.14%	±	0.02%	0.19%	±	0.01%
D966	1.00%	±	0.07%	1.02%	±	0.03%	1.28%	±	0.08%
D975	3.11%	±	0.05%	3.01%	±	0.03%	3.06%	±	0.08%
D984	10.84%	±	0.79%	15.67%	±	4.76%	15.87%	±	4.08%
E989	1.09%	±	0.11%	1.00%	±	0.31%	0.88%	±	0.37%
D990	2.30%	±	0.77%	2.46%	±	0.39%	2.70%	±	1.12%
D993	7.90%	±	2.21%	11.51%	±	0.90%	9.51%	±	0.04%

Figure S2a.

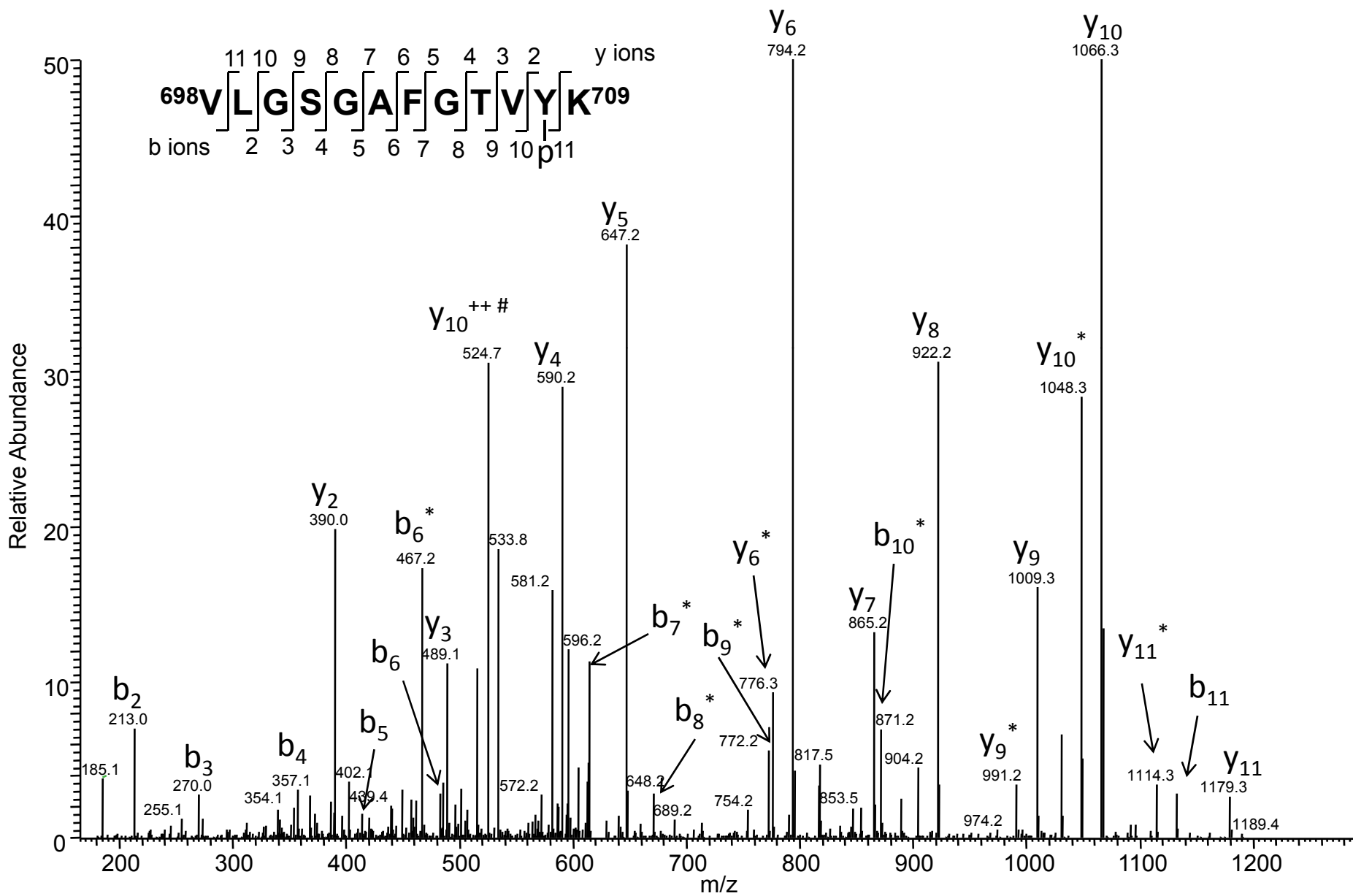


Figure S2b.

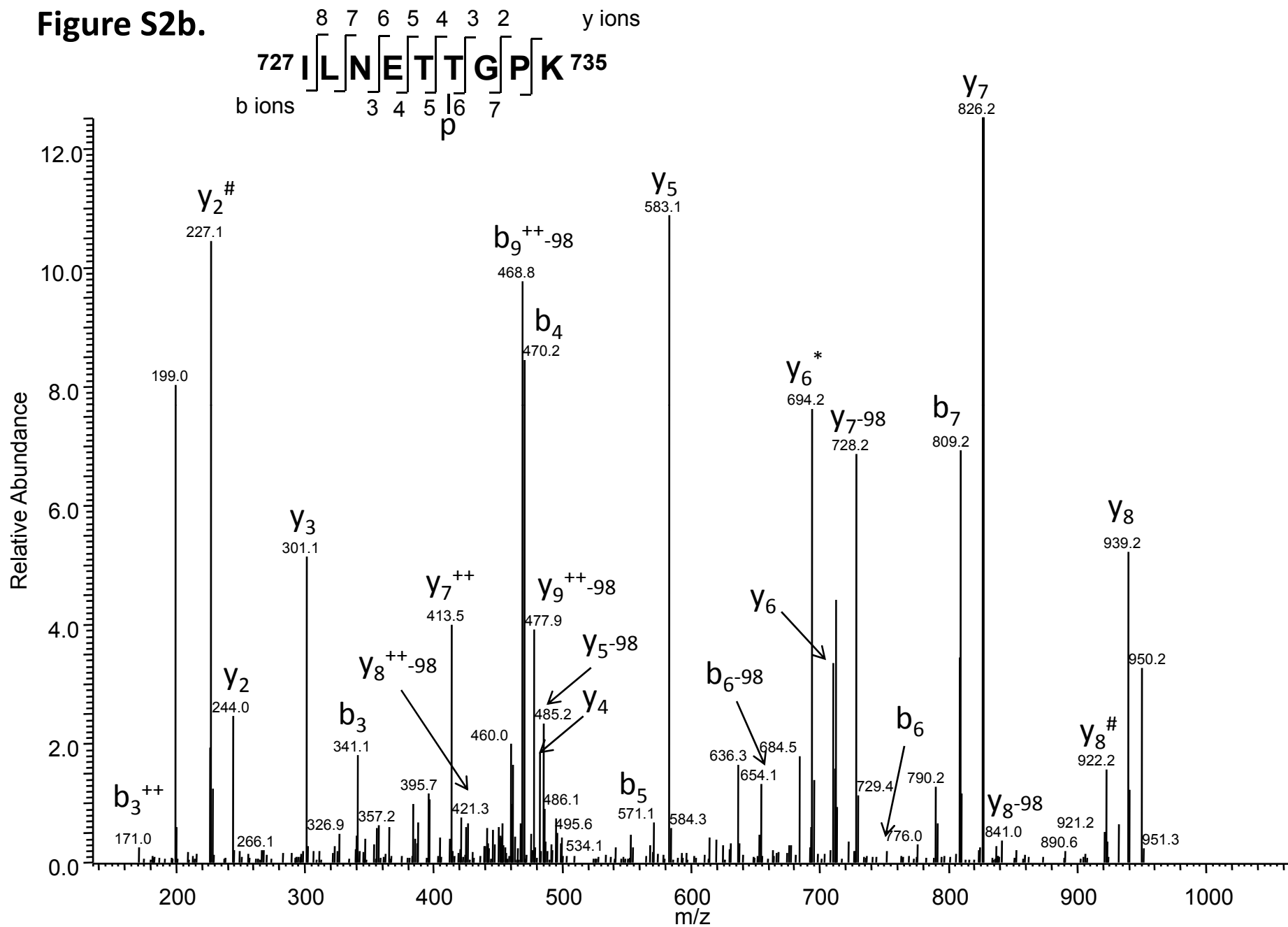


Figure S2c.

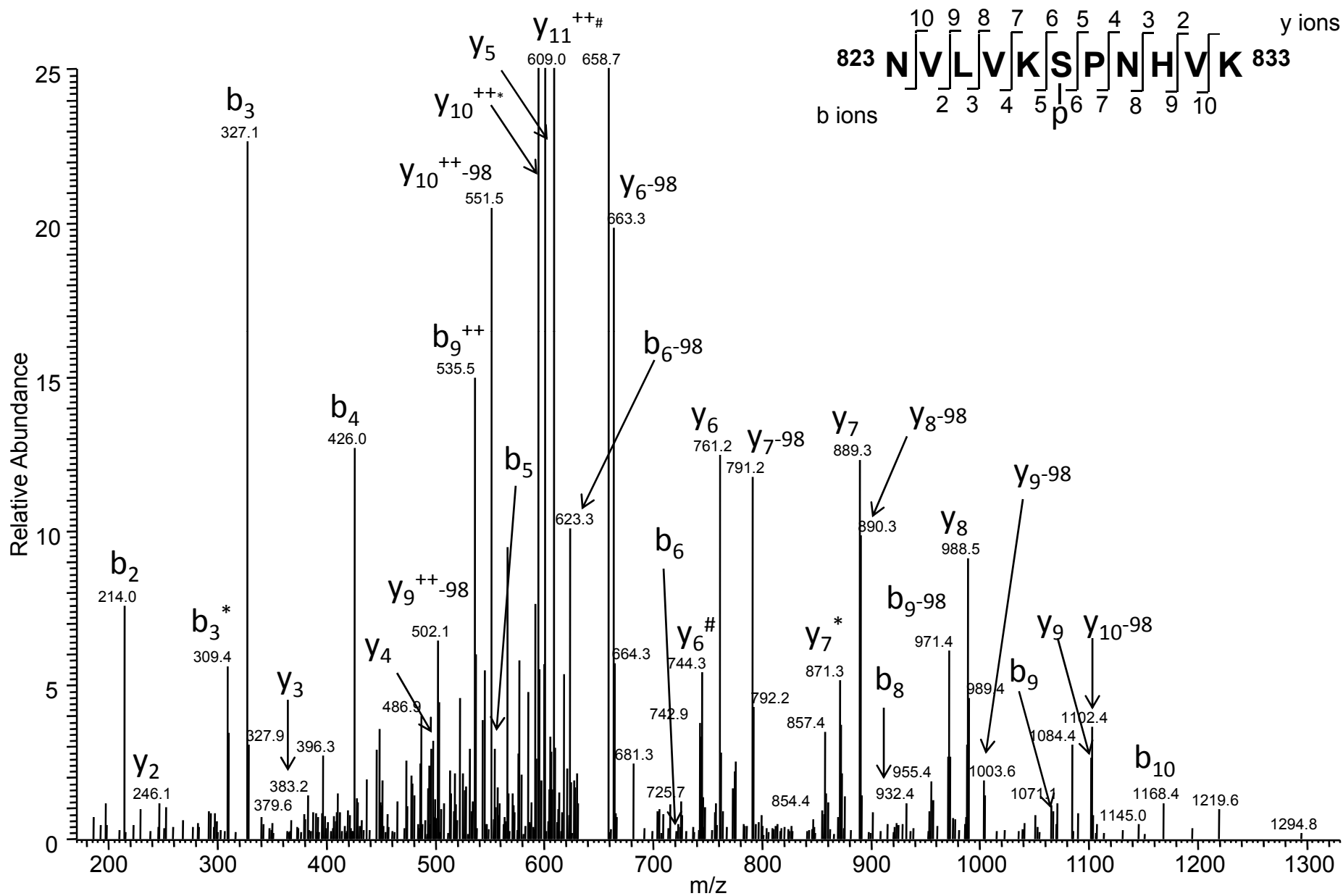


Figure S2e.

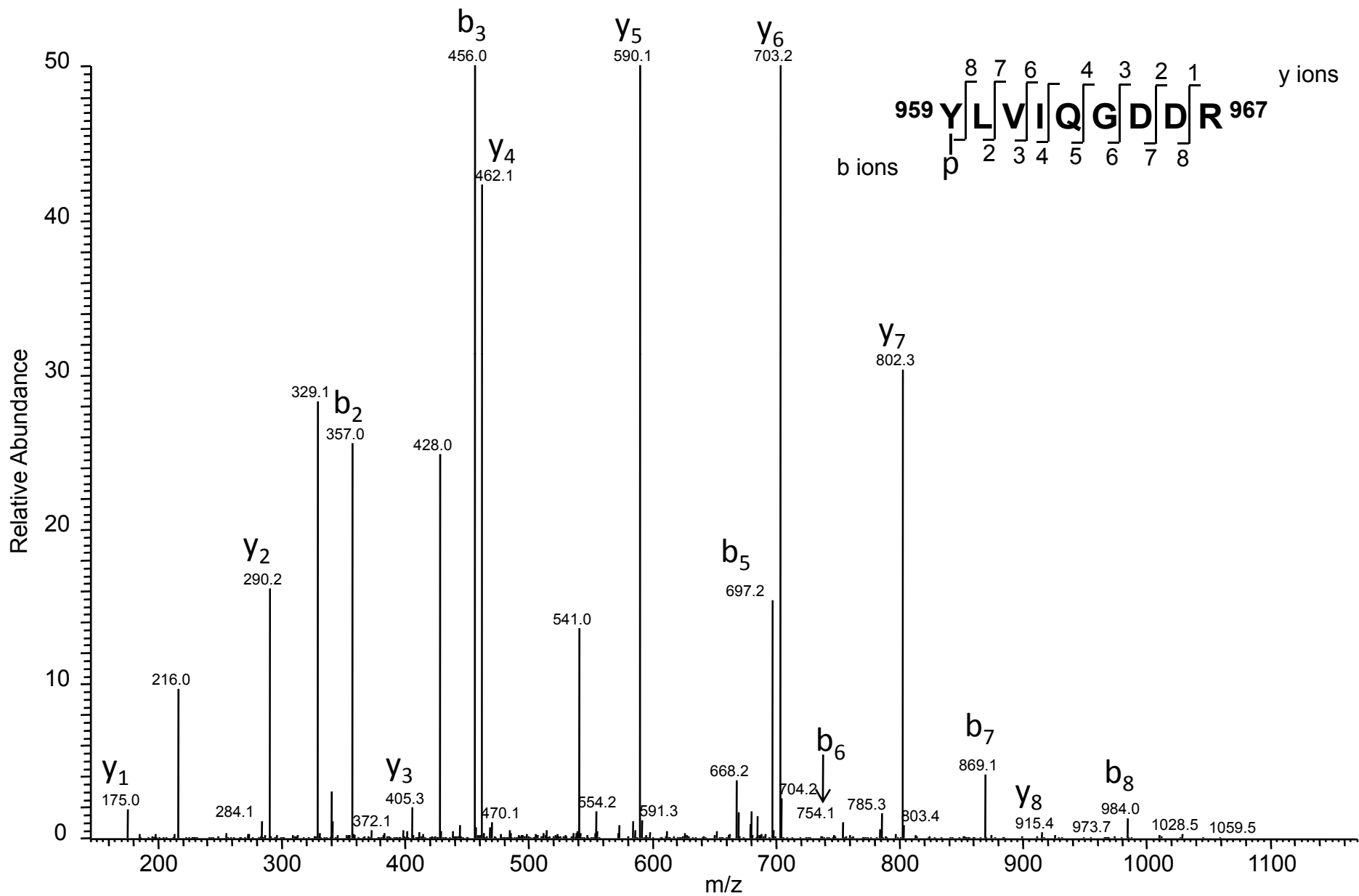


Figure S2f.

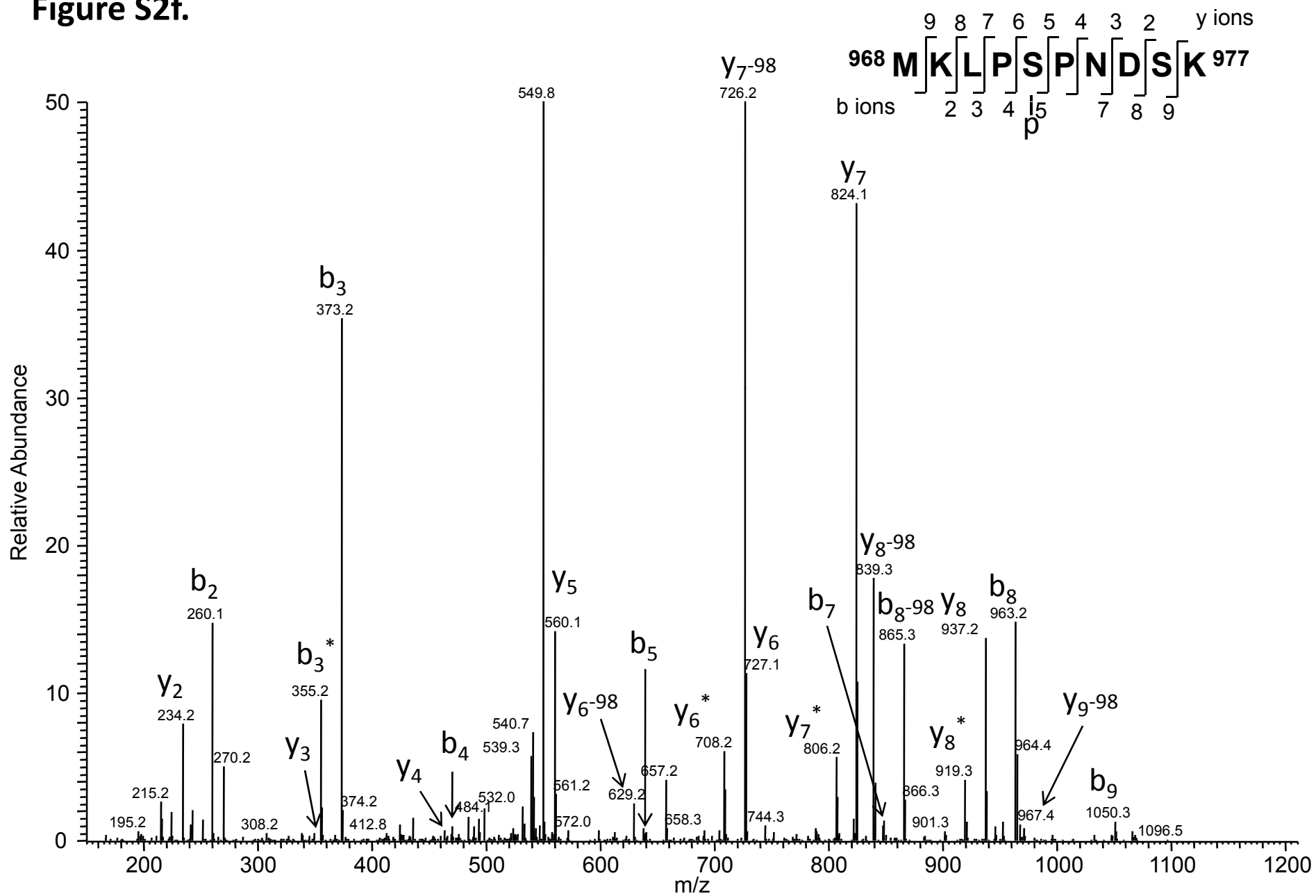


Figure S3.

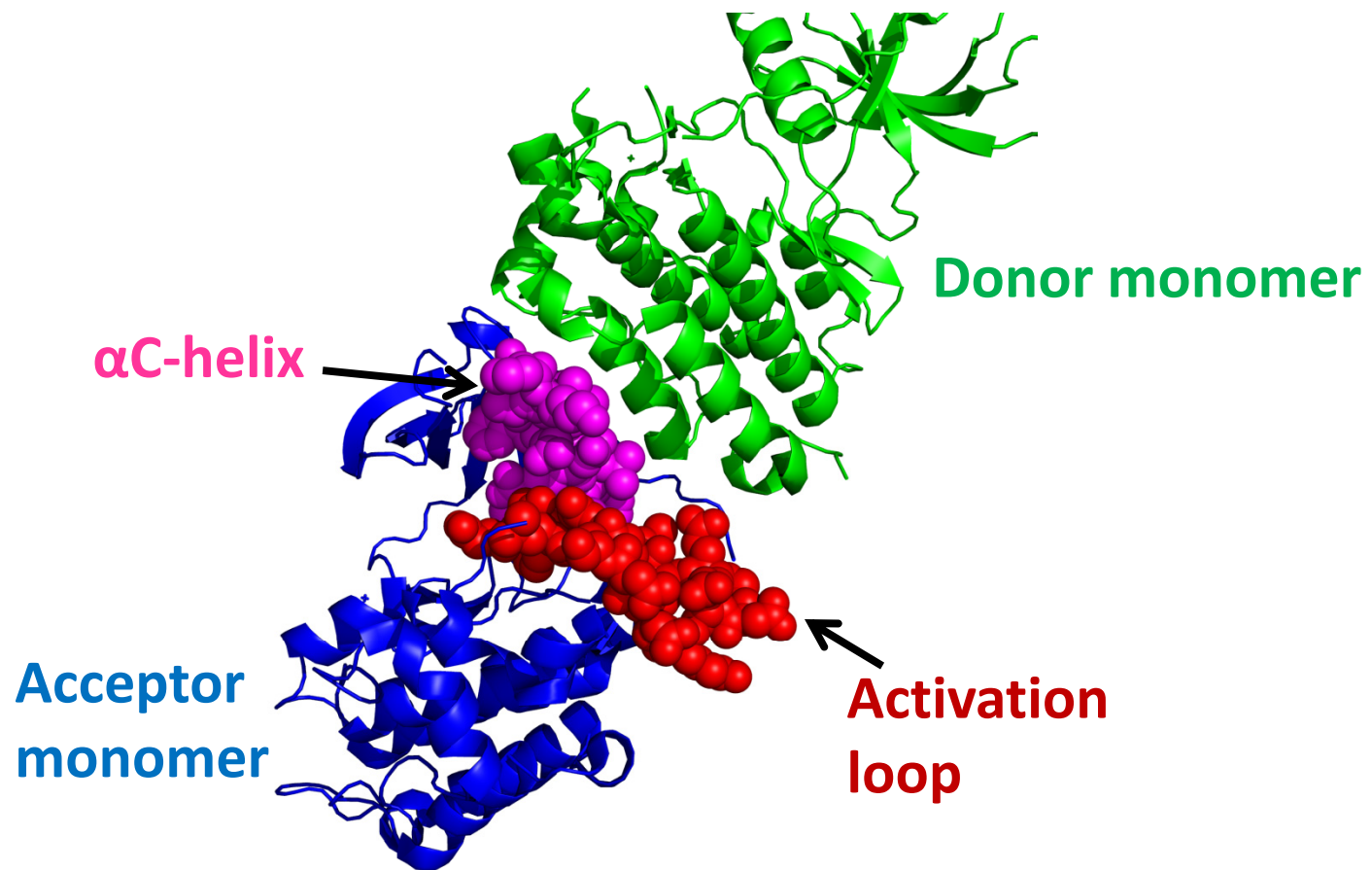


Figure S4.

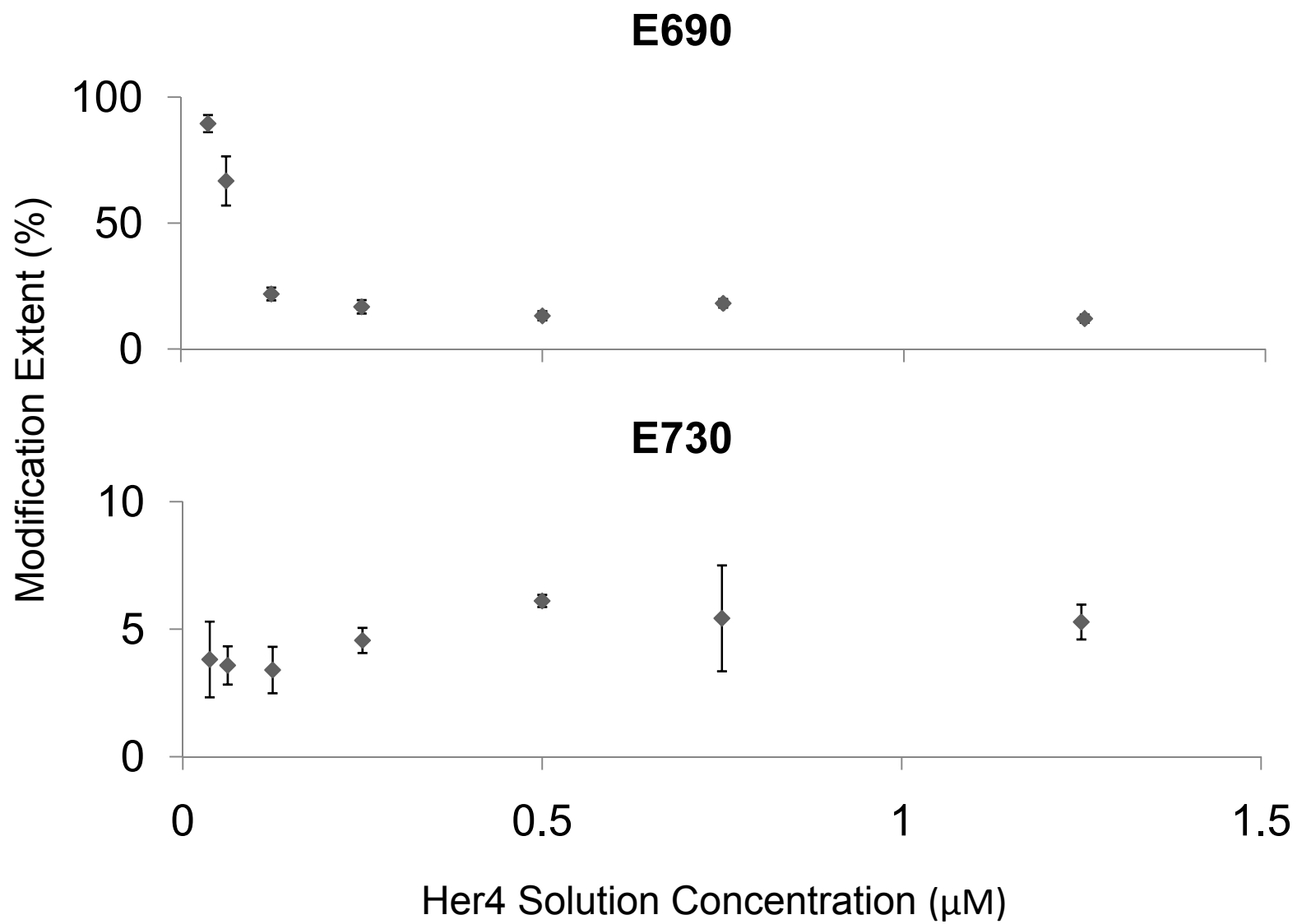


Figure S5.

