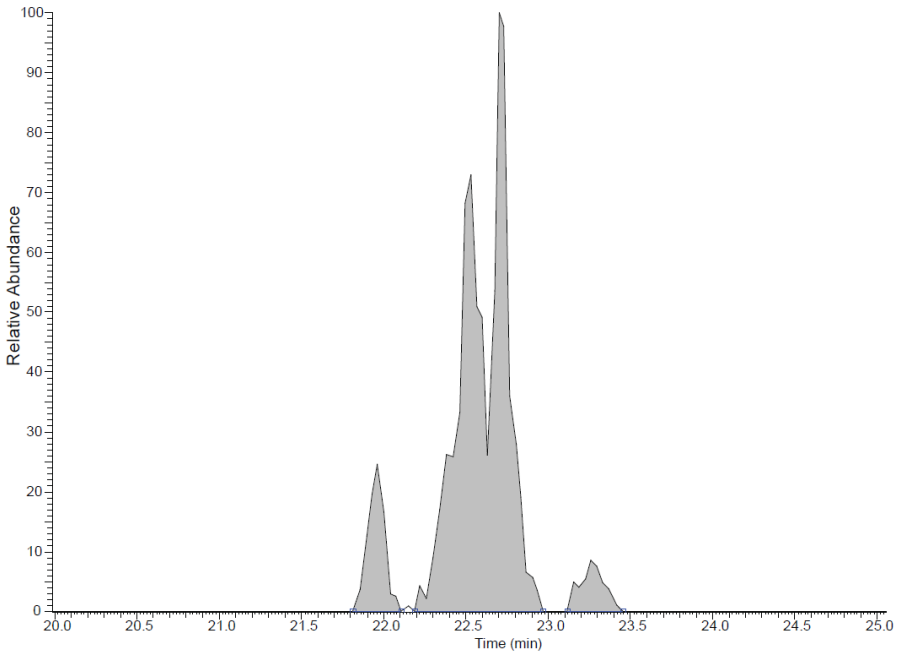
Supplemental Data

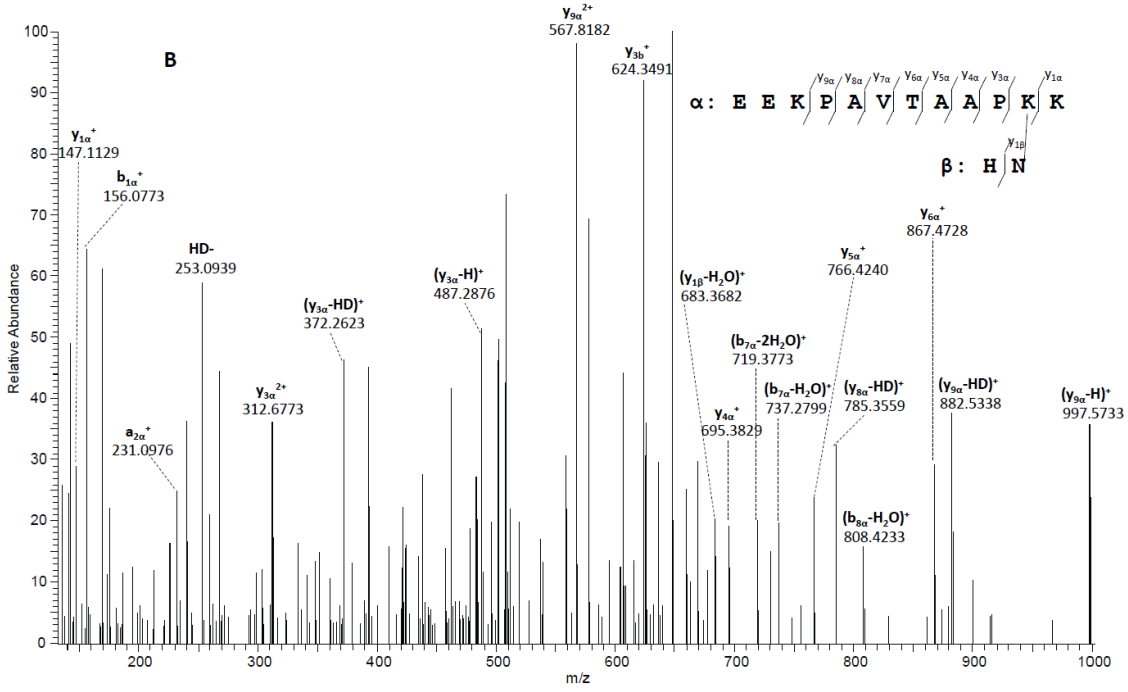
Supplemental Table 1: Potential Asn crosslinked peptides identified in human lenses

Peptide A	Peptide B	[MH]+exp	[MH]+cal	error in ppm
βB1 (Asn 162) or βB2(Asp 170): G**N***	Filensin (Lys 451): E**K***VR	703.3737	703.3733	0.58
βB1 (Asn 162) or βB2 (Asp 170): G**N***	Filensin (Lys 447): **K***VK	546.3252	546.3246	1
βB1 (Asn 162) or βB2 (Asp 170): G**N***	αB (Lys 121): **K***YR	638.3259	638.3257	0.36

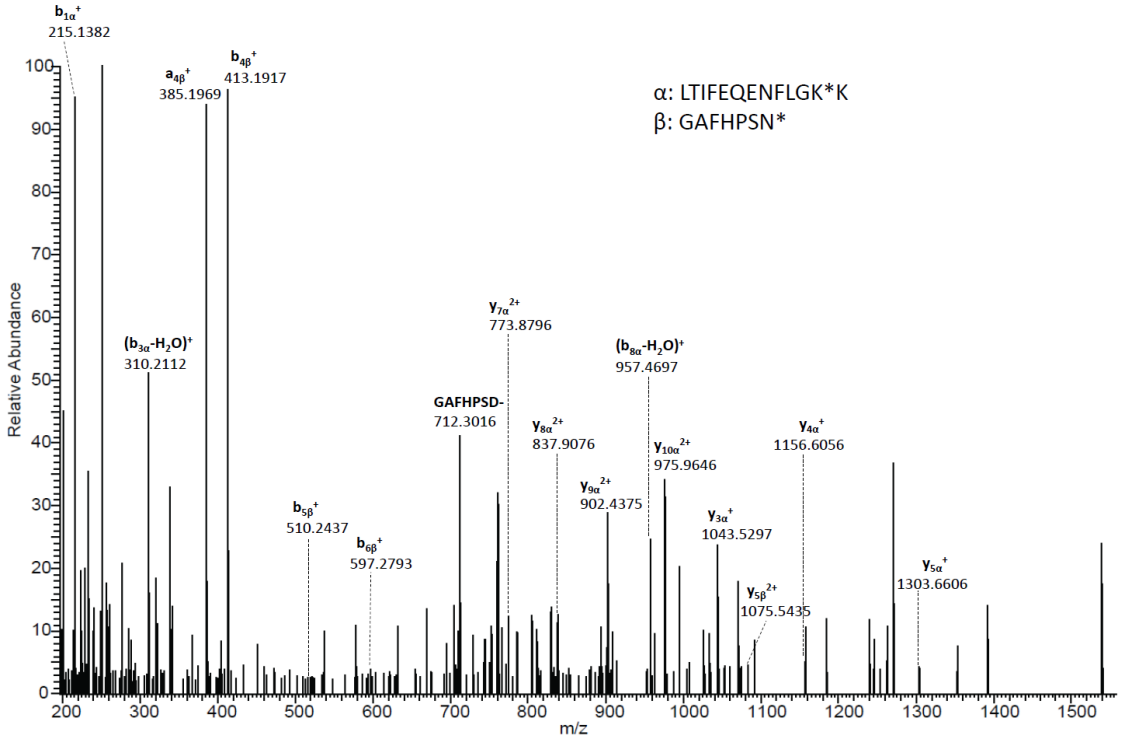
* Asterisks indicate the residues that are involved in crosslinking. All masses listed are monoisotopic masses.



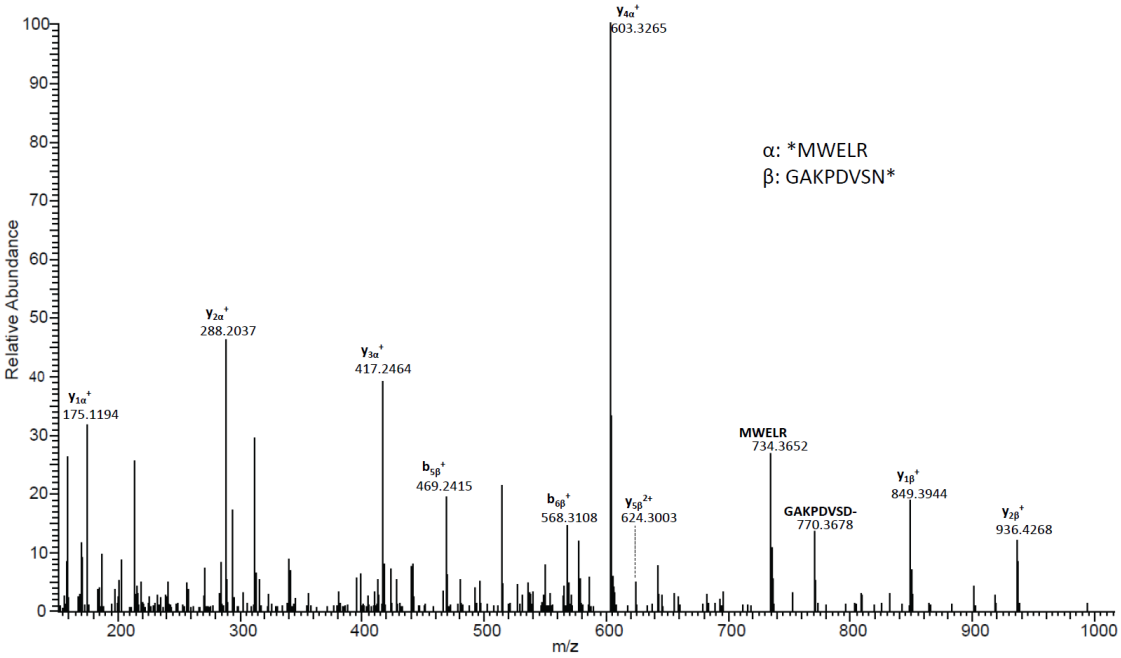
Supplemental Figure 1) Selected Ion Chromatogram for AQP0 227-233 crosslinked to AQP0 239-246, m/z 534.6213±10ppm, Charge 3+



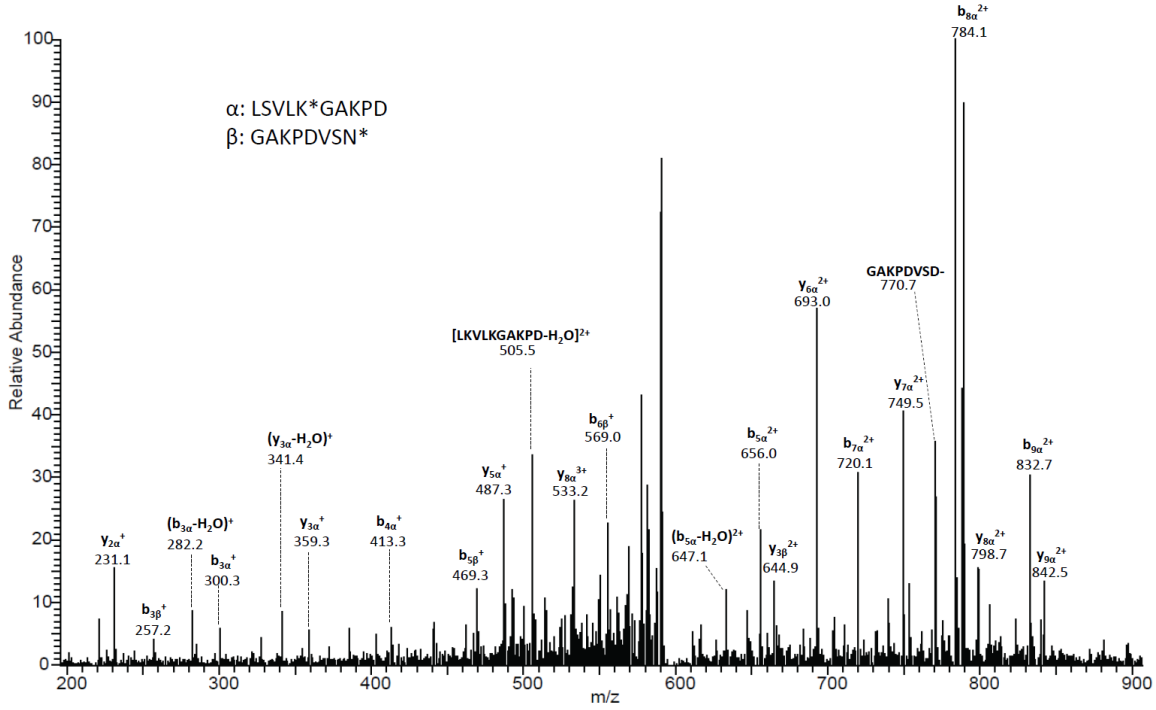
Supplemental Figure 2) MS/MS spectrum of a crosslinked peptide detected in the tryptic digest of human lens proteins. The crosslink involves Asn 101 from αA crystallin and Lys 174 from αB crystallin.



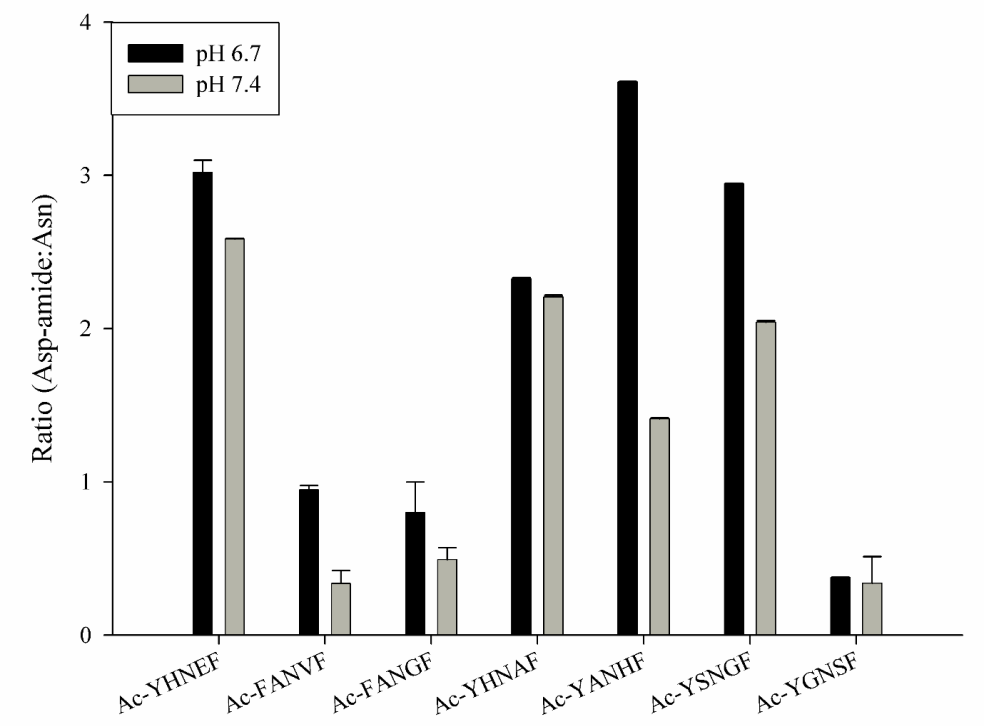
Supplemental Figure 3) MS/MS spectrum of a crosslinked peptide detected in the tryptic digest of human lens proteins. The crosslink involves Asn 205 from βB2 crystallin and Lys 118 from βA4 crystallin.



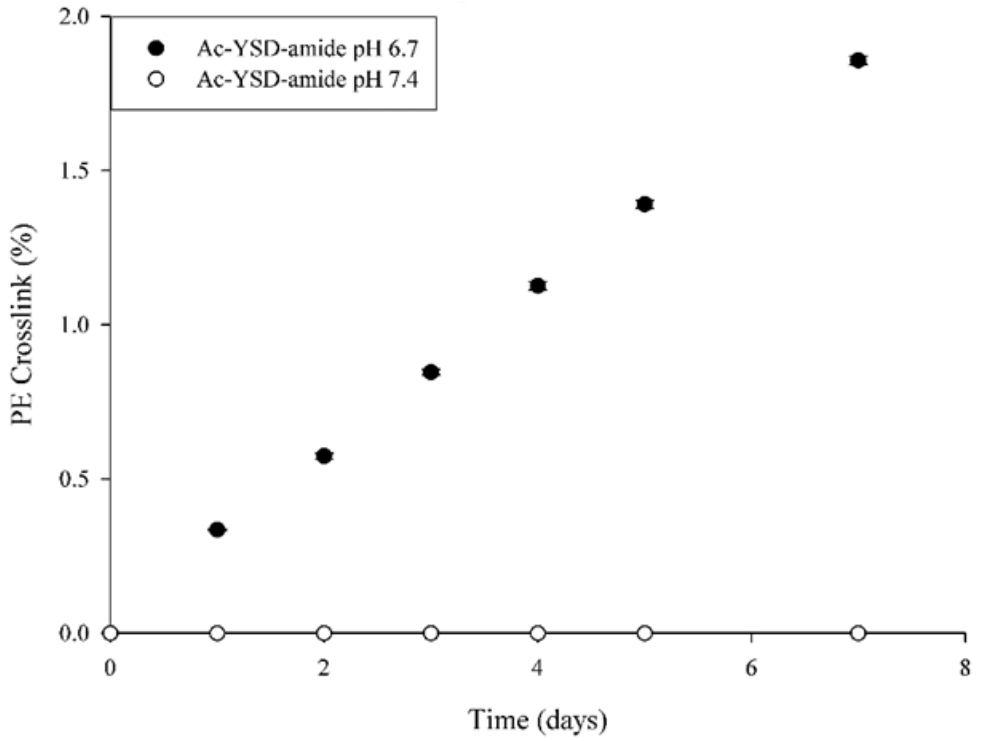
Supplemental Figure 4) MS/MS spectrum of a crosslinked peptide detected in the tryptic digest of human lens proteins. The crosslink involves Asn 246 from AQP0 and the N-terminus of Met 1 from AQP0.



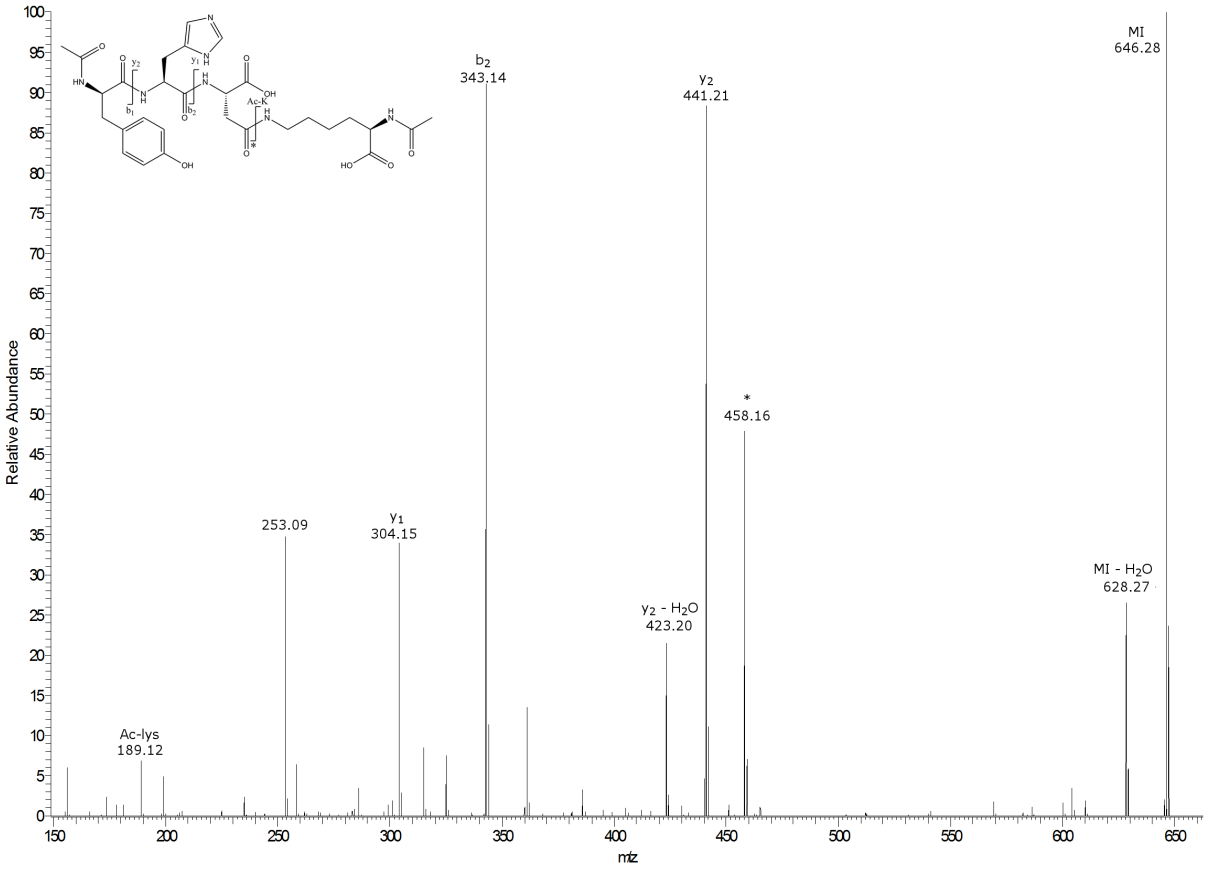
Supplemental Figure 5) MS/MS spectrum of a crosslinked peptide detected in the tryptic digest of human lens proteins. The crosslink involves Asn 246 from AQP0 and Lys 238 from AQP0.



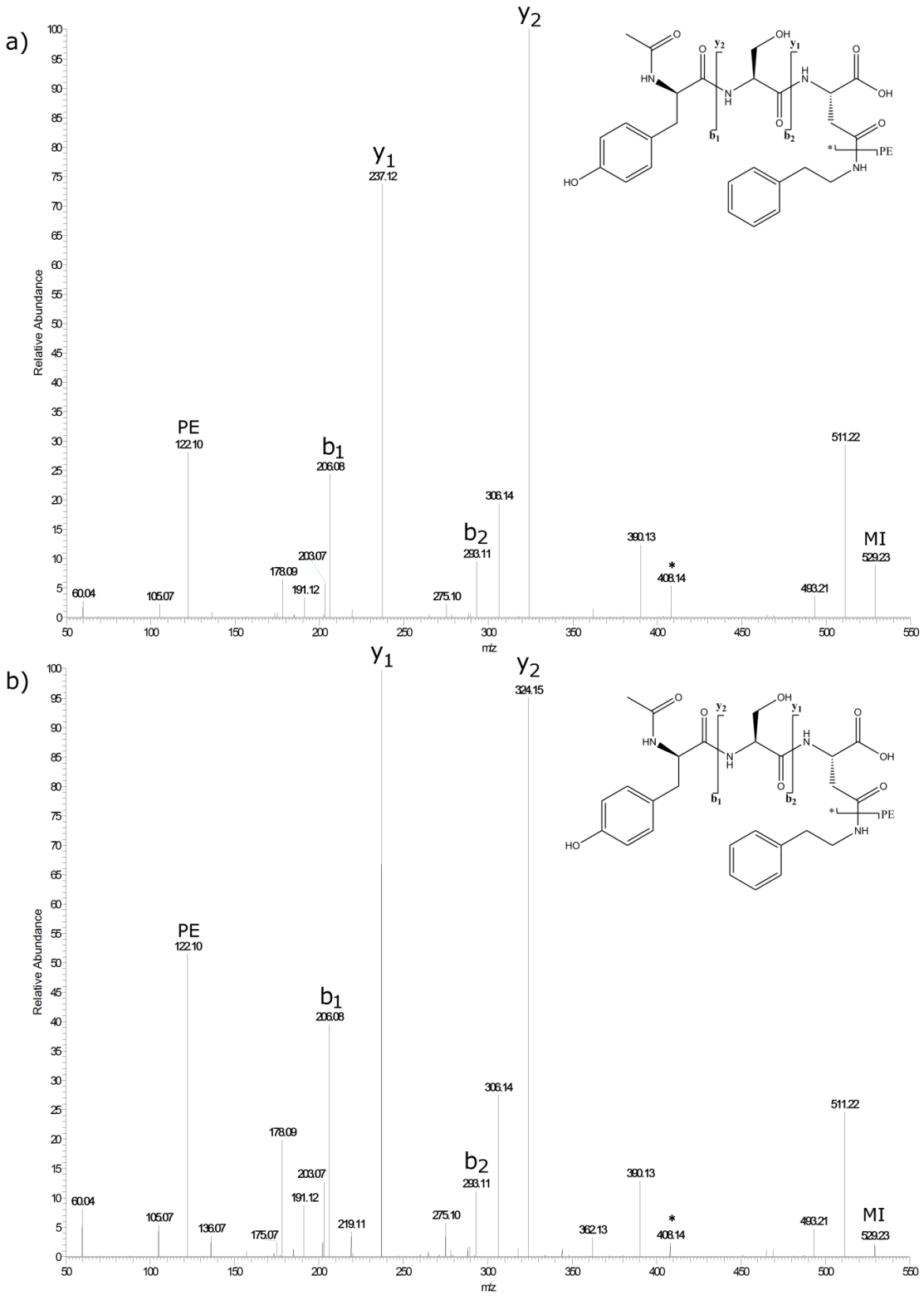
Supplemental Figure 6) Ratio of C-terminal Asp-amide to Asn formed from original peptide at pH 6.7 and 7.4. Peptides incubated for 4 days at 60°C. Values are n=3 +/- SD.



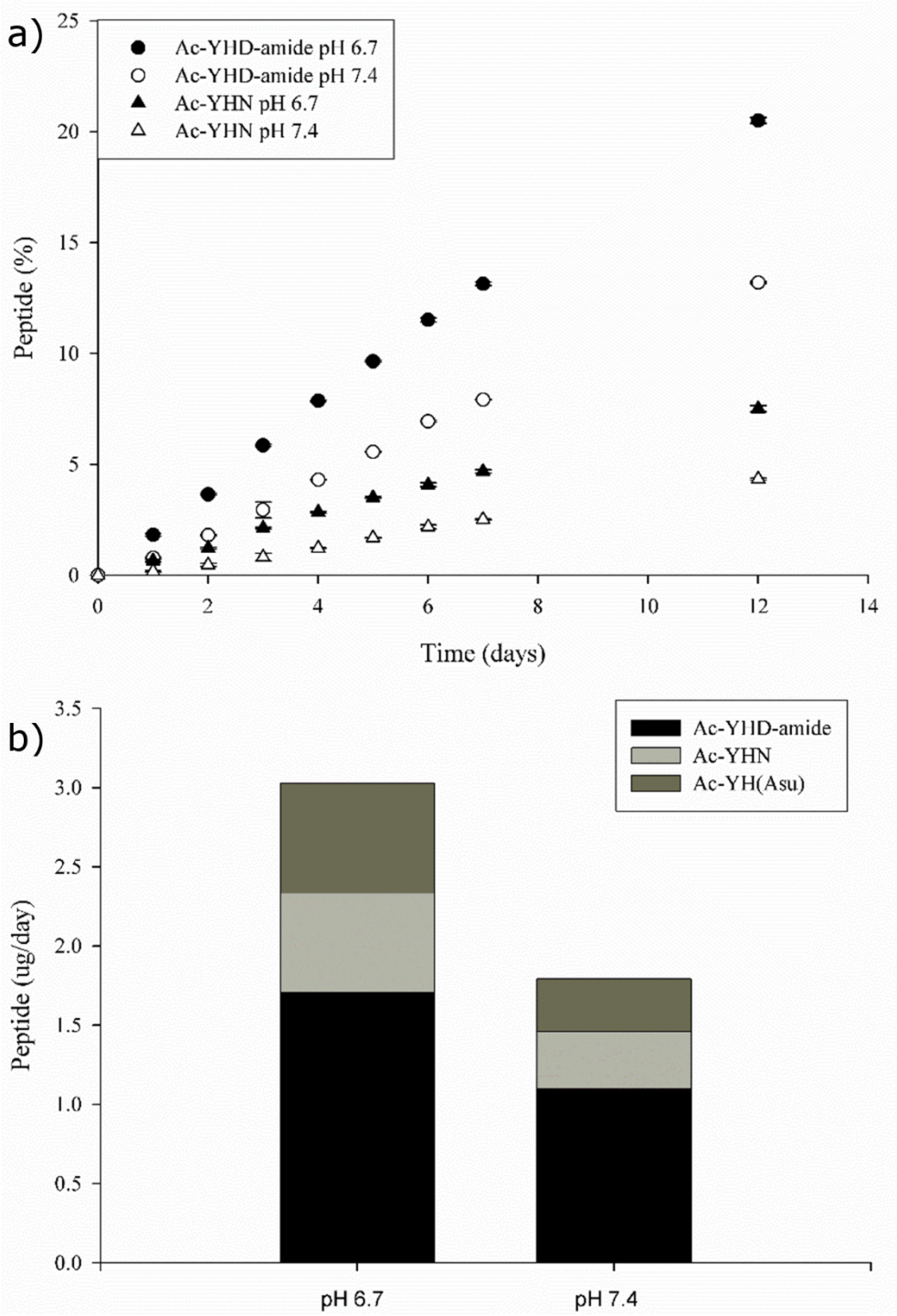
Supplemental Figure 7) Ac-YSD-amide readily forms a crosslink with PE. Crosslink formation following incubation at 60 °C of a) Ac-YHD-amide and Ac-YHN b) Ac-YSD-amide with PE at pH 6.7 and 7.4. Ac-YSN did not form a detectable PE crosslink under these conditions.



Supplemental Figure 8). Ac-YHD crosslink with Ac-K. MS/MS spectrum of the Ac-YHD Ac-K crosslink from an incubation of Ac-YHD-amide with 5 molar excess of Ac-K at pH 6.7 for 3 days at 60°C. * = loss of Ac-K, MI = molecular ion.

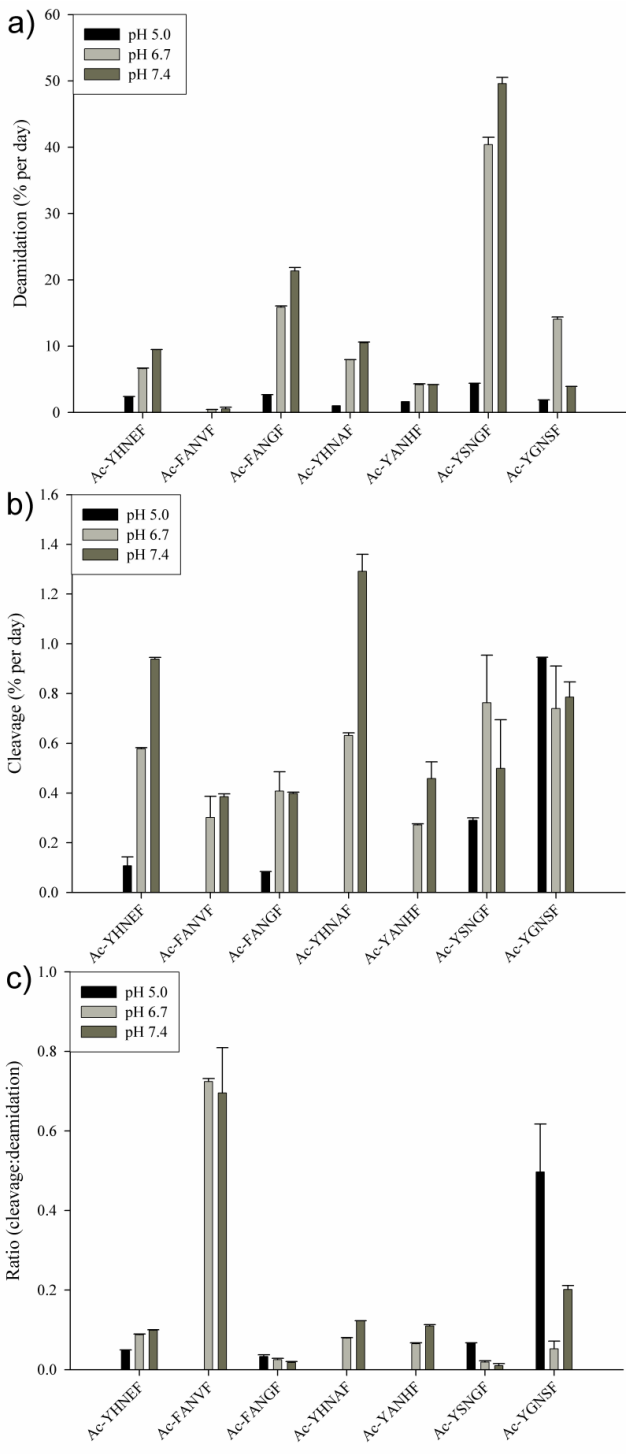


Supplemental Figure 9). Ac-YSD crosslink with PE forms from Ac-YSD-amide via an anhydride. a) MS/MS spectrum of the Ac-YSD PE crosslink isolated by HPLC from incubation of Ac-YSD-amide with PE at pH 6.7 for 3 days at 60°C. b) MS/MS spectrum of the Ac-YSD PE crosslink formed by reacting Ac-YH succinic anhydride with PE. * = loss of PE, MI = molecular ion.



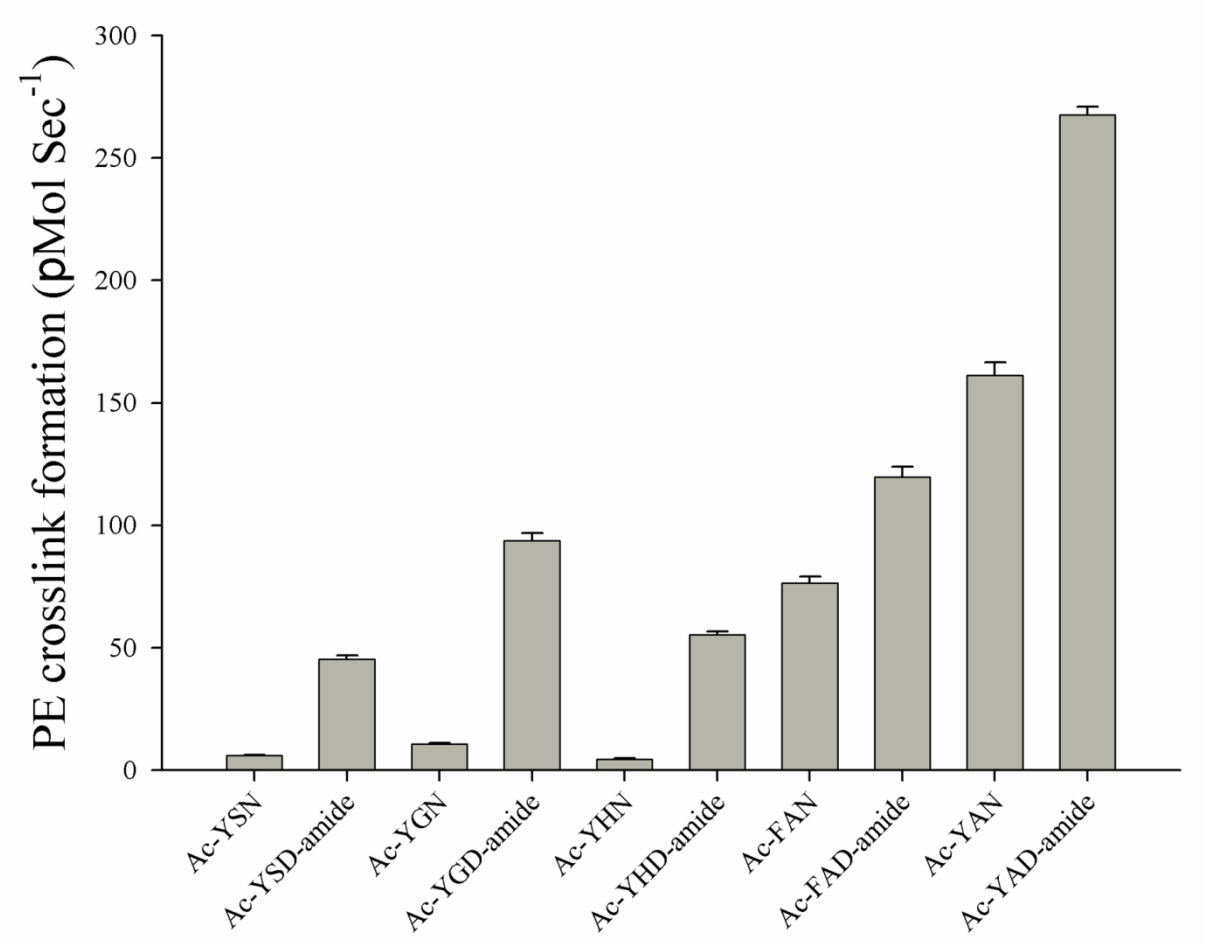
Supplemental Figure 10) Decomposition of Ac-YHNPF at pH 6.7 and 7.4. a) Time course of Ac-YHNPF breakdown products Ac-YHD-amide and Ac-YHN. Ac-YHNPF was incubated at 60°C in 100mM phosphate buffer, pH 6.7 and pH 7.4. Peaks corresponding to Ac-YHD-amide and Ac-YHN were identified by co-elution with synthetic peptide standards and MS/MS. Peak areas were determined at 280nm by comparison to that of the original peptide.

b) Comparison of three Asn breakdown product observed at pH 6.7 and 7.4 as a function of the rate of formation.



Supplemental Figure 11) The effect of amino acid sequence on Asn deamidation and cleavage at Asn.

a) Deamidation of peptides at pH 5.0, 6.7 and 7.4. Deamidation rate was calculated as for Fig 3, b) Cleavage of the same peptides at pH 5.0, 6.7 and 7.4. c) The ratio of cleavage to deamidation for each peptide after seven days. All experiments were conducted at 60°C.



Supplemental Figure 12) The rate of PE crosslink formation of when a 5 fold molar excess of PE was incubated with either a C-terminal Asn or Asp-amide. Incubations in 80% acetonitrile**.**