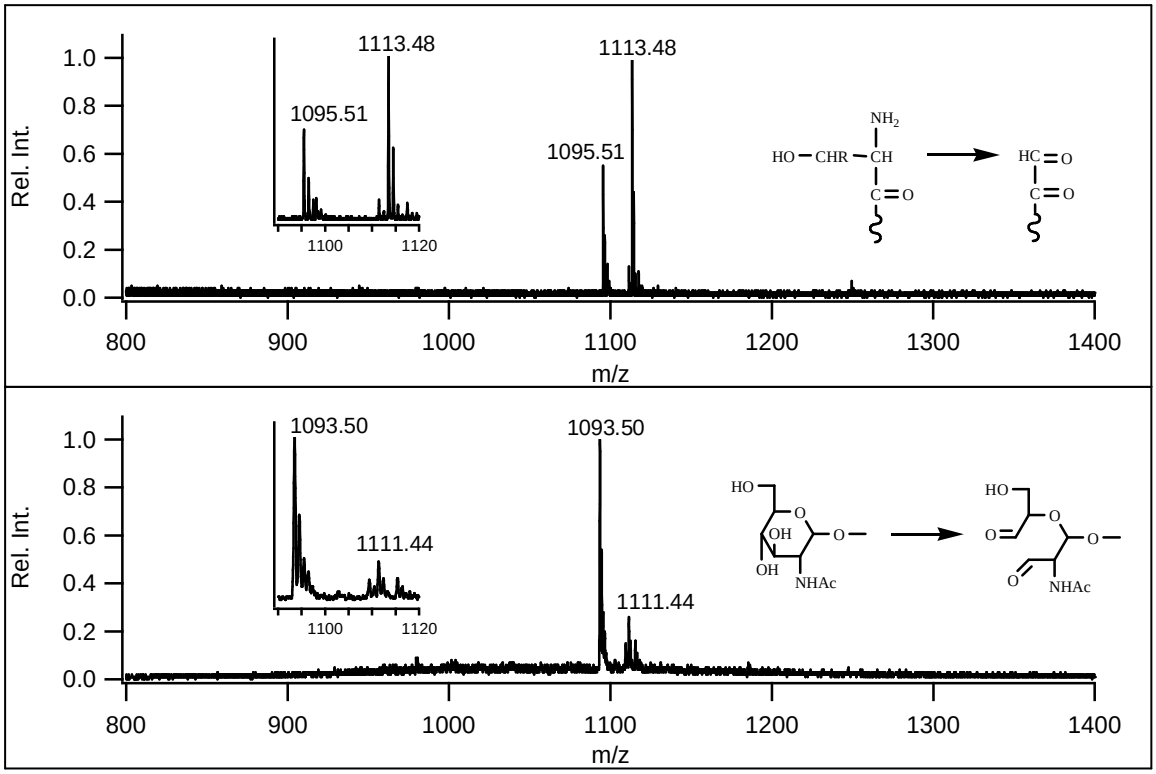
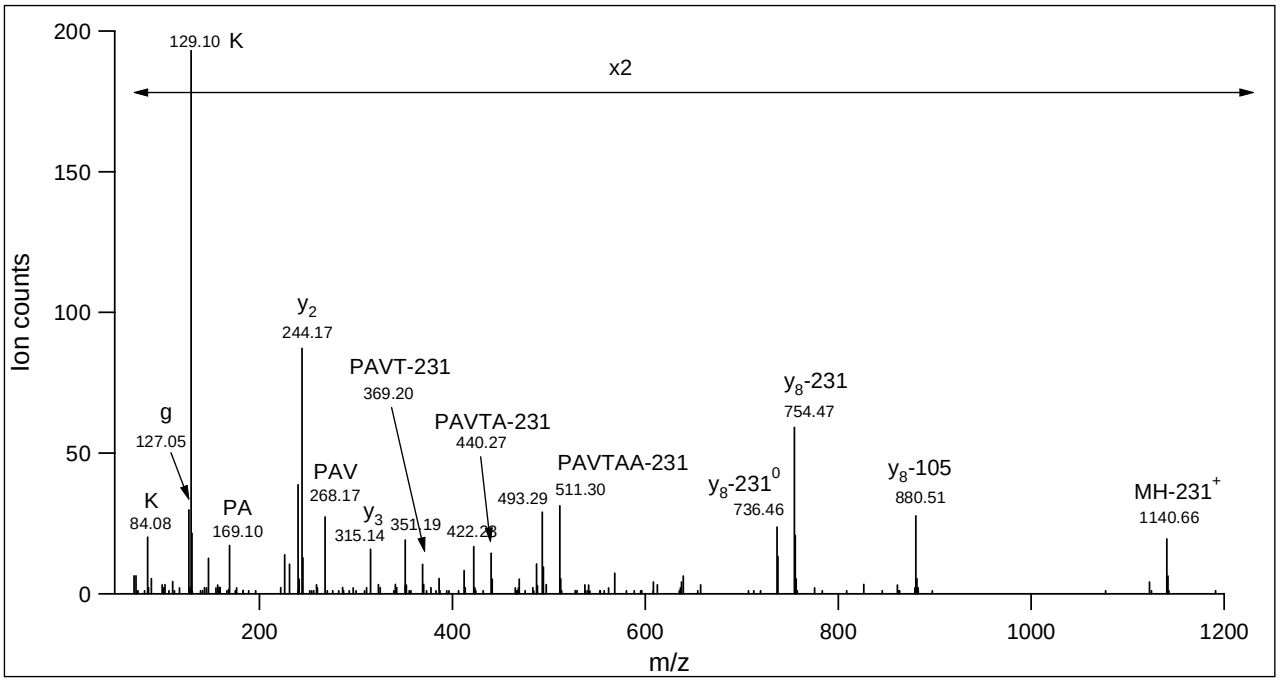
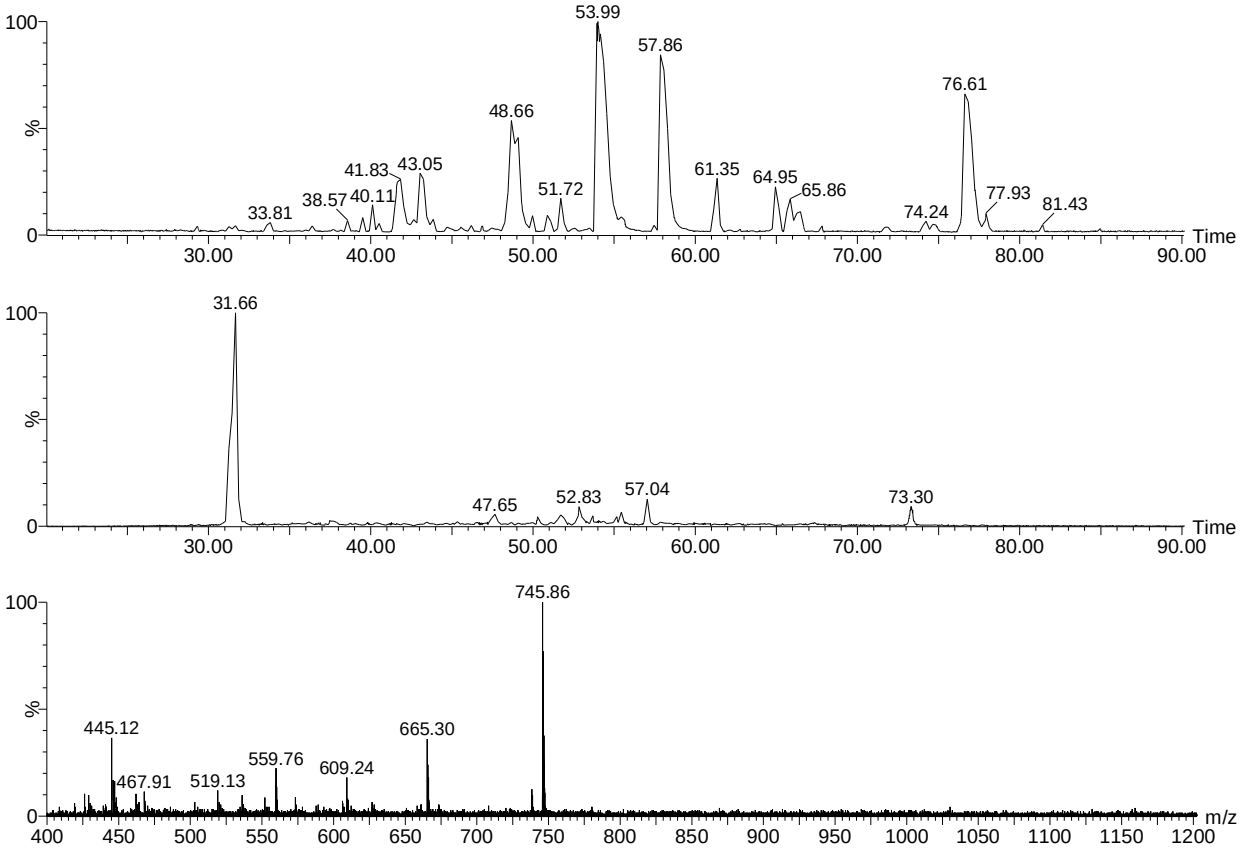


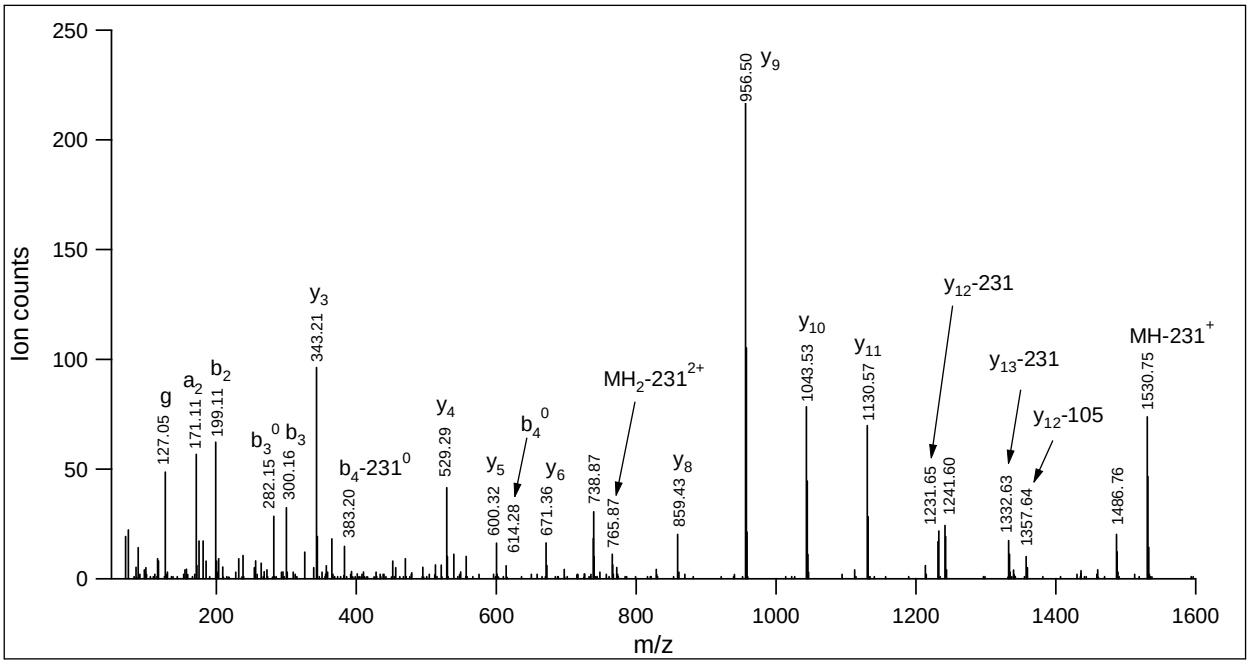
Supplemental Figure 1. MALDI-TOF mass spectra of the periodate oxidation products of the O-GlcNAc modified peptide standard TAPTS^{g}TIAPG (m/z 1118.50). A) Initial oxidation (-45 Da) occurs at the peptide N-terminus. B) The carbohydrate ring is split open (-2 Da) in a longer exposition (4.5 h) at elevated temperatures (37 ºC). The oxidation products are observed as sodium/potassium adducts. Masses observed with 18 Da mass difference correspond to the glyoxylyl hydrates.



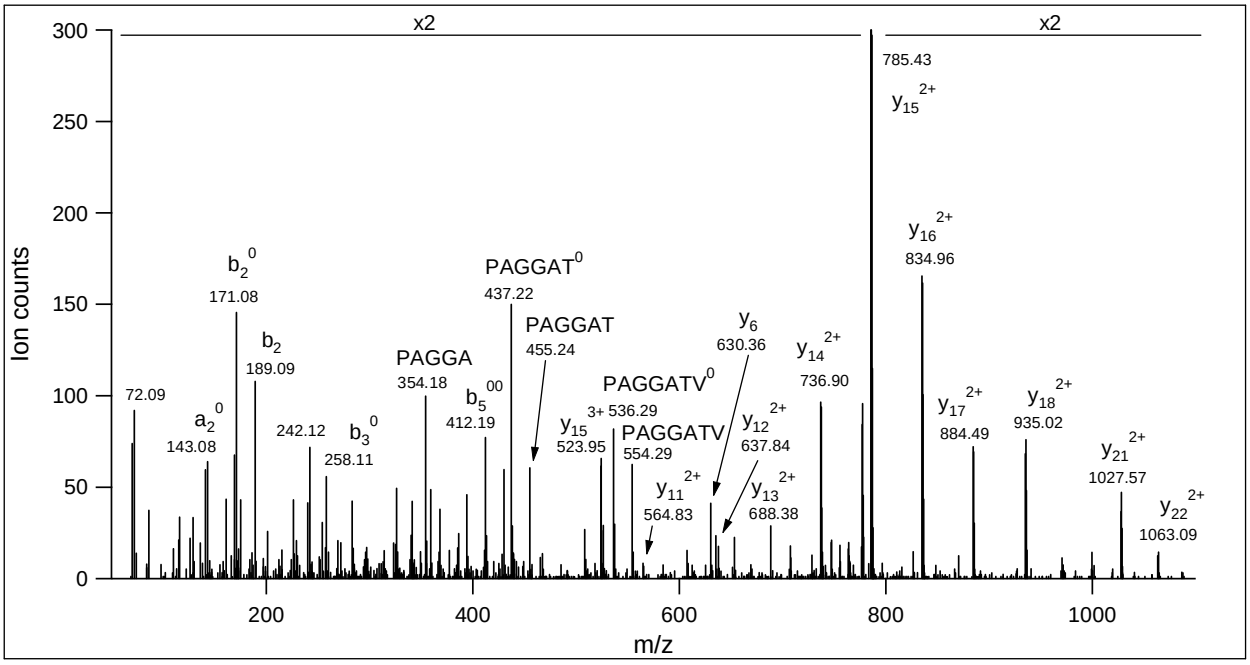
Supplemental Figure 2. CID spectrum of the oxime derivative of the O-GlcNAc modified α-crystallin B peptide EEKPAVT^{g}AAPK; precursor ion at m/z 686.38 (2+); acquired in a Q-TOF mass spectrometer; g denotes a sugar related product ion; ^{0} stands for water loss.



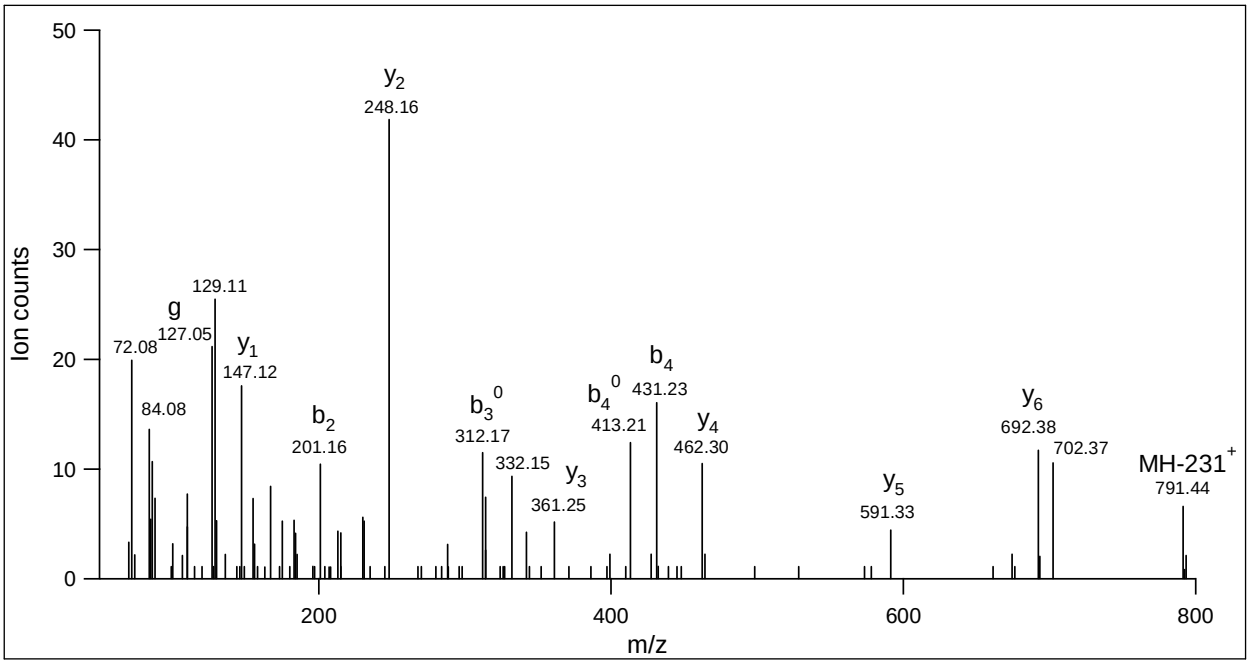
Supplemental Figure 3. Enrichment of GlcNAc modified peptides from the *Drosophila* proteasome complex. Upper panel: Base peak chromatogram of the enriched fraction. The abundant peaks correspond to the oxime derivatives of N-terminally oxidized proteasome subunits. Middle panel: Extracted ion chromatogram of m/z 745.85. The peak eluting at 31.66 min corresponds to the GlcNAc modified qDTEAKN^{g}ETIK peptide, where q stands for pyroglutamic acid, and 'g' indicates the site of glycosylation. Lower panel: full-scan mass spectrum at the elution of the GlcNAc modified qDTEAKN^{g}ETIK peptide (m/z 745.85).



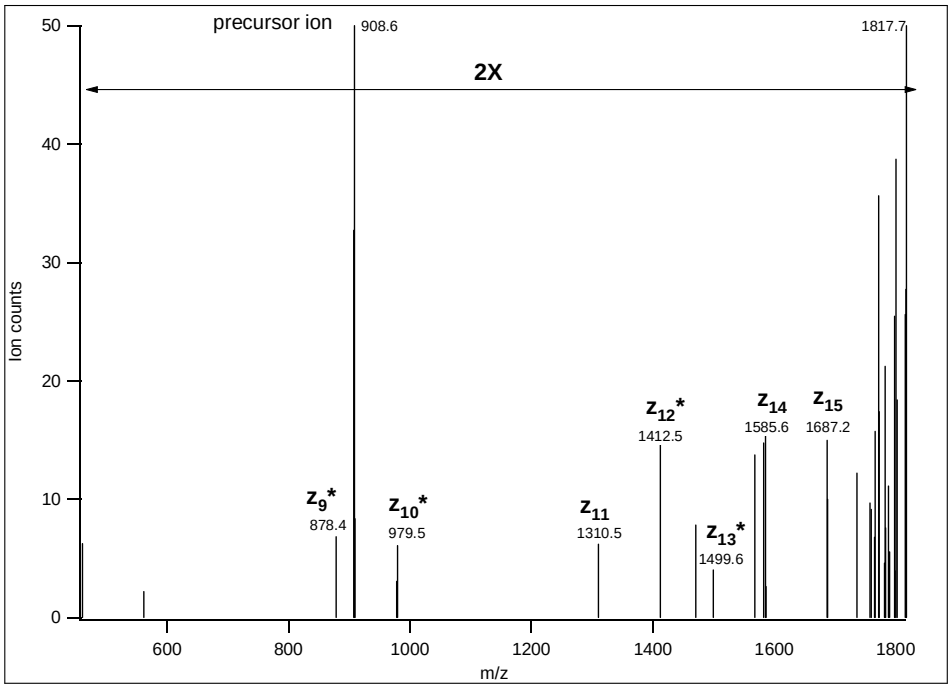
Supplemental Figure 4. CID spectrum of the "O-GlcNAc-oxime" modified ^{2614}TPTT^{g}SSPSTAAWPAR^{2628} peptide; precursor ion at m/z 881.43 (2+); Thr-2617 was identified as the modification site; g denotes a sugar related product ion; ^{0} stands for water loss.



Supplemental Figure 5. CID spectrum of the BEMAD derivative of the ^{776}STSTAPAGGATVVPPTS^{g}AAVTRPVTTGR^{803} peptide; precursor ion at m/z 668.10 (4+); Ser-792 was identified as the modification site; ^{0} stands for water loss.



Supplemental Figure 6. CID spectrum of the "O-GlcNAc-oxime" modified ^{1209}VTETITK^{1215} peptide; precursor ion at m/z 511.77 (2+); g denotes a sugar related product ion; ^{0} stands for water loss.



Supplemental Figure 7. ETD spectrum of the "O-GlcNAc-oxime" modified ^{1026}NTSSTT^{g}TSTATATITK^{1041} peptide; precursor ion at m/z 908.9448 (2+); Thr-1031 was identified as the modification site. Asterisks indicate ions produced by hydrogen migration, i.e. z+1 fragments.