Electronic Supplementary Material (ESI) for Analyst.
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Supporting Information

Simplified Identification of Disulfide, Trisulfide, and Thioether Pairs with 213 nm UVPD

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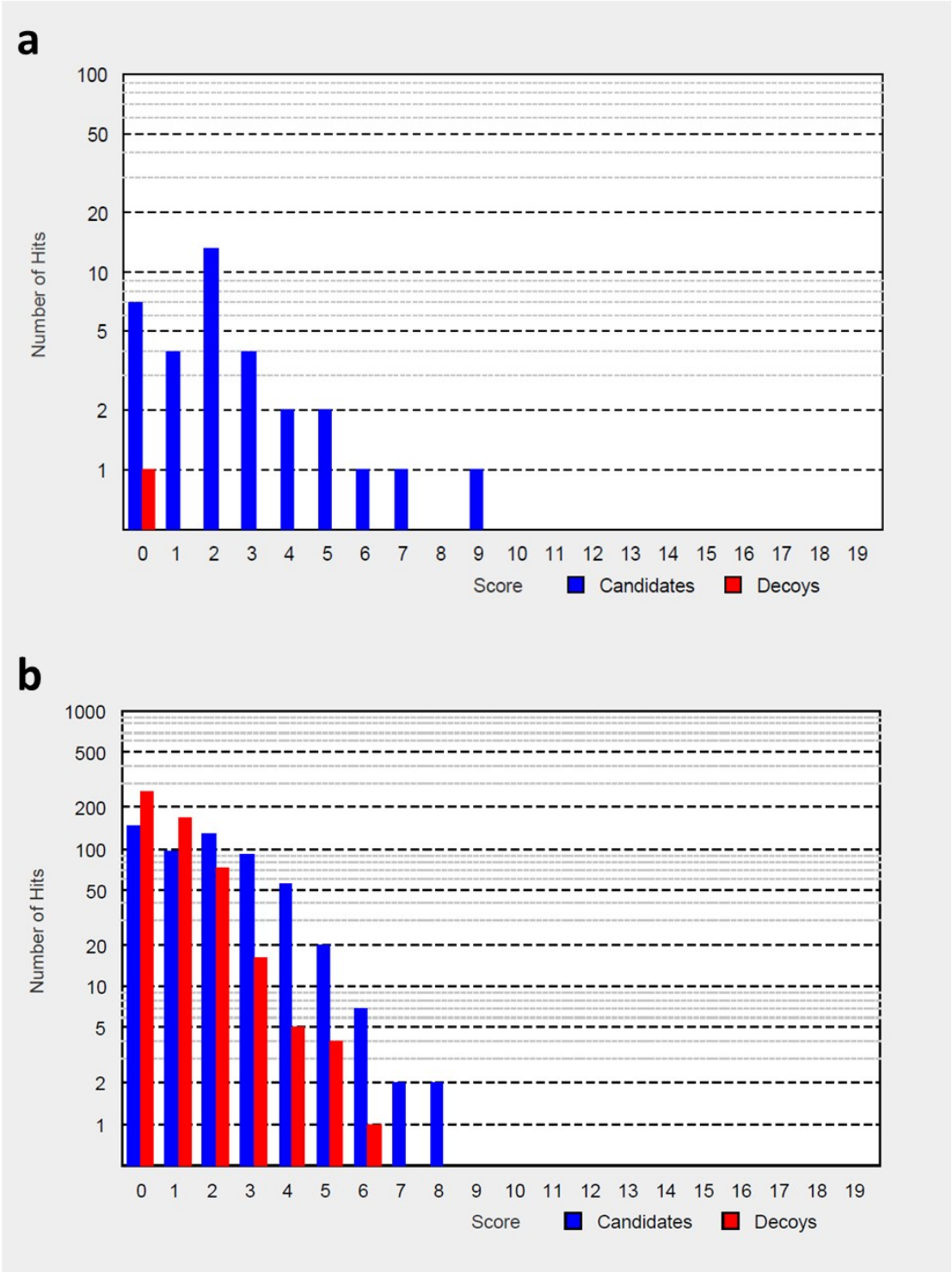

Figure S1. Decoy analysis using a) RISE mode (two-tiered) and b) without RISE mode enabled. Red bars indicate decoy peptides derived from a reversed Rituximab sequence and blue bars are for actual candidate peptides. Matches scoring 1 or higher have FDR below 5% in a), while a score of 7 or higher is required in b). When two-tiered data analysis is used, far more confident matches are obtained.

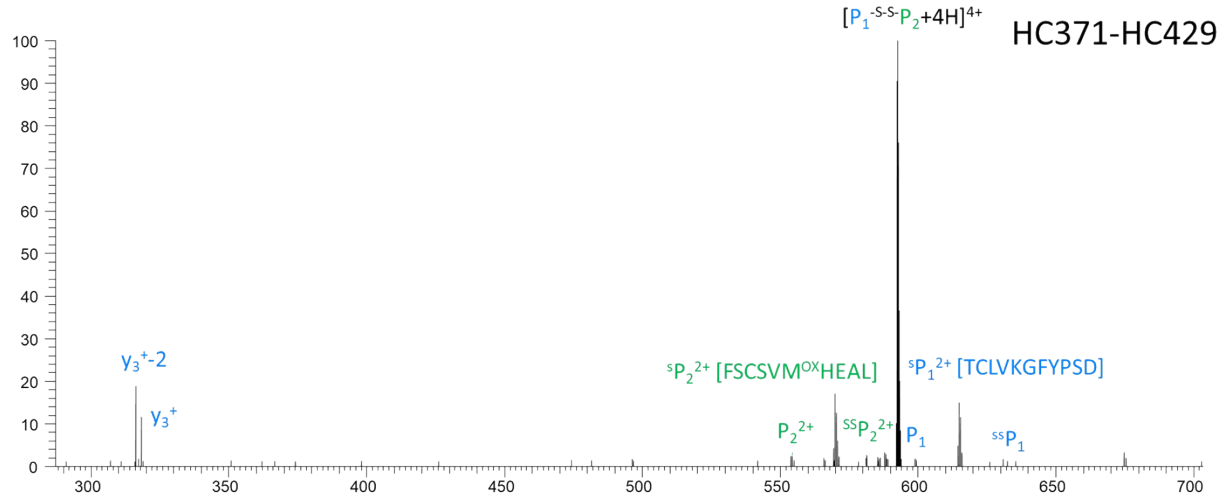

Figure S2. MS^2 of a disulfide link found between Cys371 and Cys429 of the heavy chain. Here it is noteworthy to point out the high UVPD efficiency near proline, which rivals that of the disulfide bond. The y_3 fragment is split into two ions as previously noted, a canonical y_3 ion and also the unusual y_3-2 Da ion.

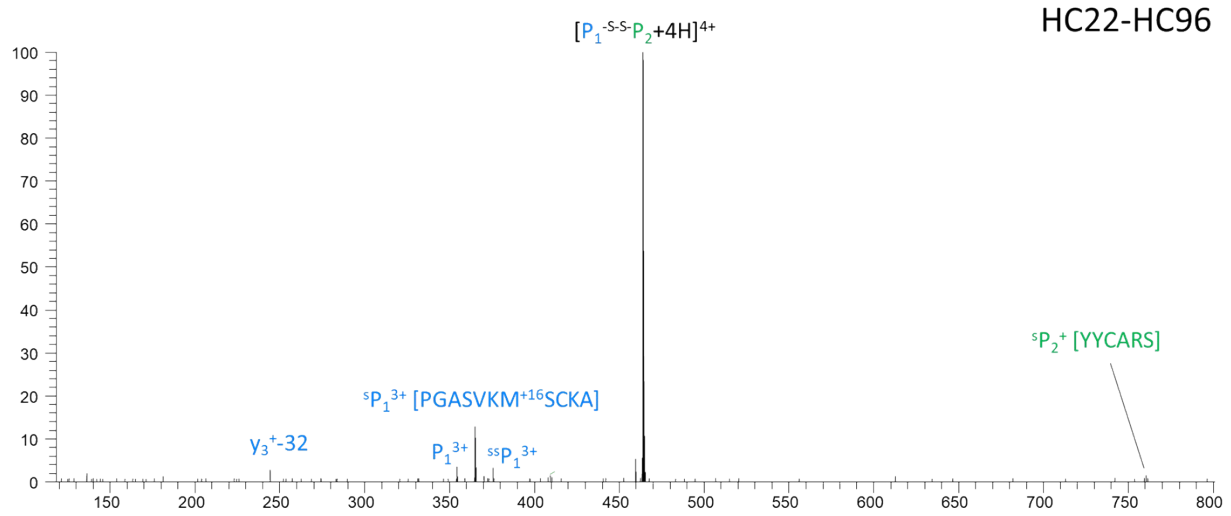

Figure S3. MS^2 of a disulfide link found between Cys22 and Cys96 of the heavy chain. P1 produces a triplet while only the homolytically dissociated ion is observed for P2, (presumably due to low ion intensity). Adding the single sequence ion from P1 is enough to produce a high confidence match (FDR below 5%).

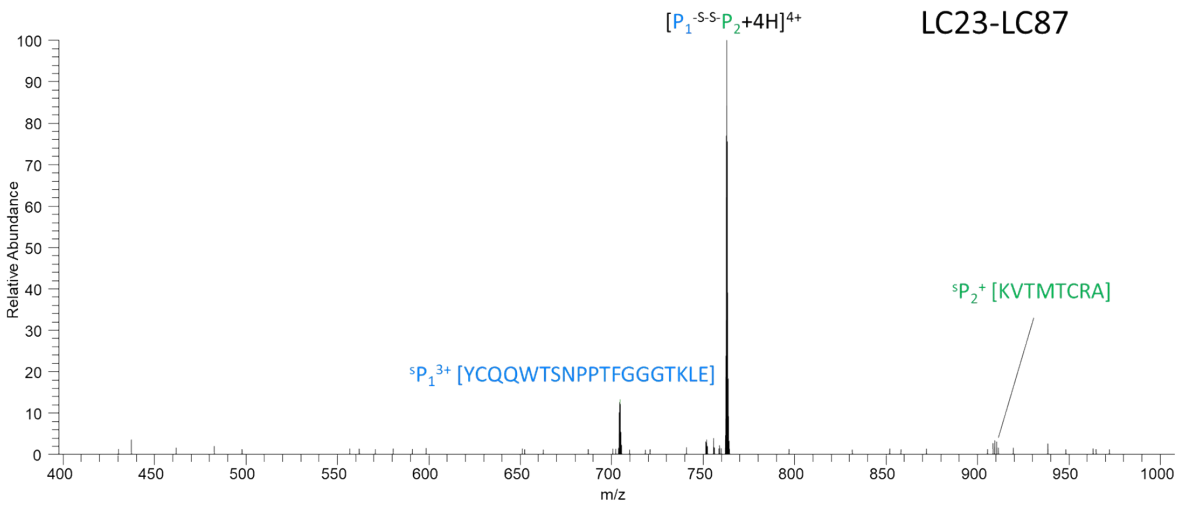

Figure S4. MS^2 of a disulfide link found between Cys23 and Cys87 of the light chain. Noticeably (and rather unusually for 213 nm UVPD), neither P1 nor P2 produces a triplet in this particular spectrum.

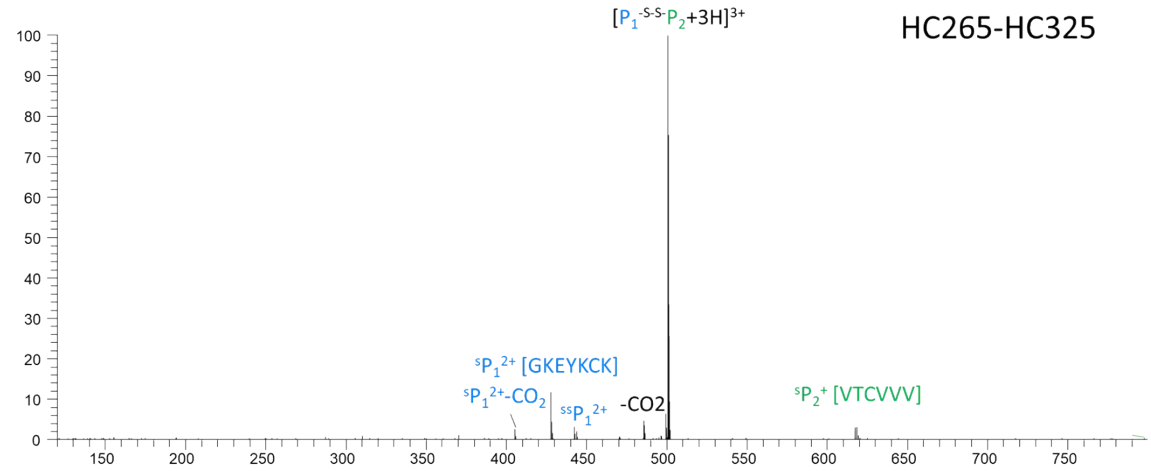

Figure S5. MS^2 of a disulfide link found between Cys265 and Cys325 of the heavy chain. There is an intense loss of CO_2 observed the parent ion and also for homolytically disulfide cleaved fragment P_1. This intense neutral loss might be caused in part due to the close sequence proximity of the UVPD generated radical sulfur species in P1 to the peptide's C-terminus.

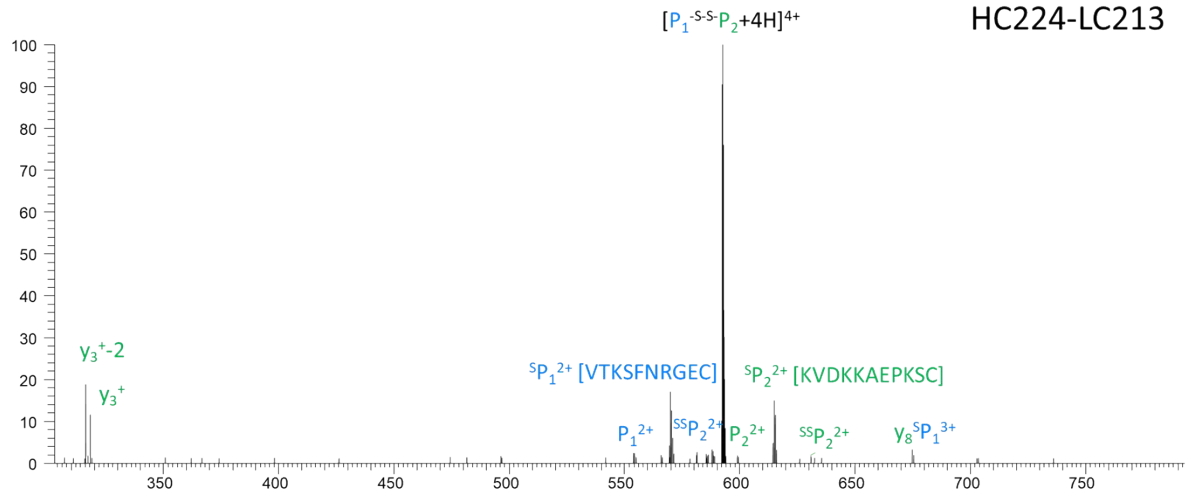

Figure S6. MS^2 of a disulfide link found between interchain disulfide pair Cys224 (heavy) and Cys213(light). Here again an intense doublet is observed corresponding to fragmentation at proline, specifically, y_3 and y_3-2 Da.

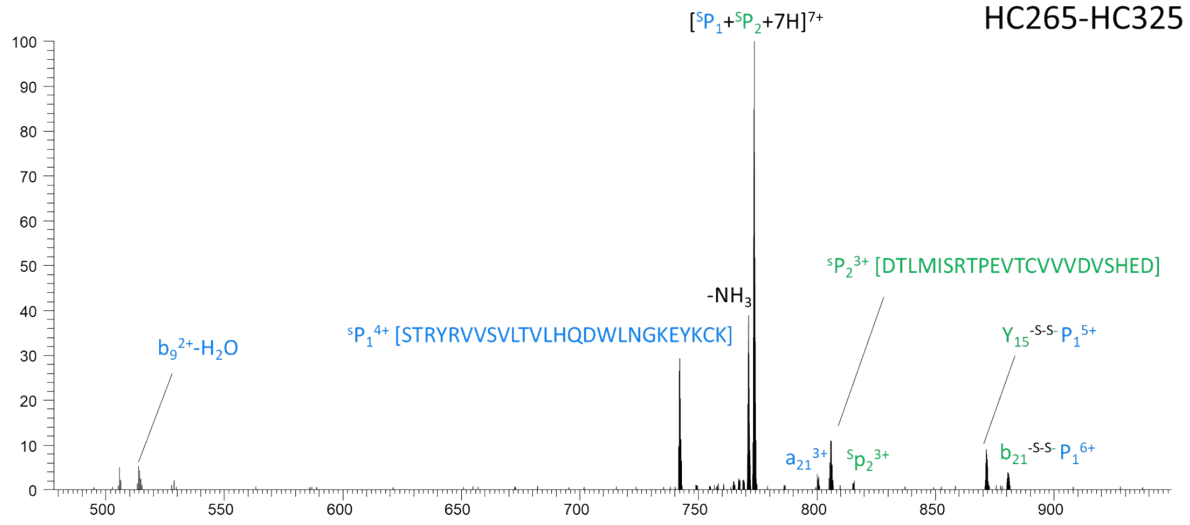

Figure S7. Additional MS^2 of a disulfide link found between Cys265 and Cys325 of the heavy chain. Despite the large size of these peptide fragments, both are still sufficiently activated by 50 ms of UVPD to produce important sequence information in addition to crosslink fragments. This result is not uncommon of UVPD and highlights the flexibility and utility of the technique.

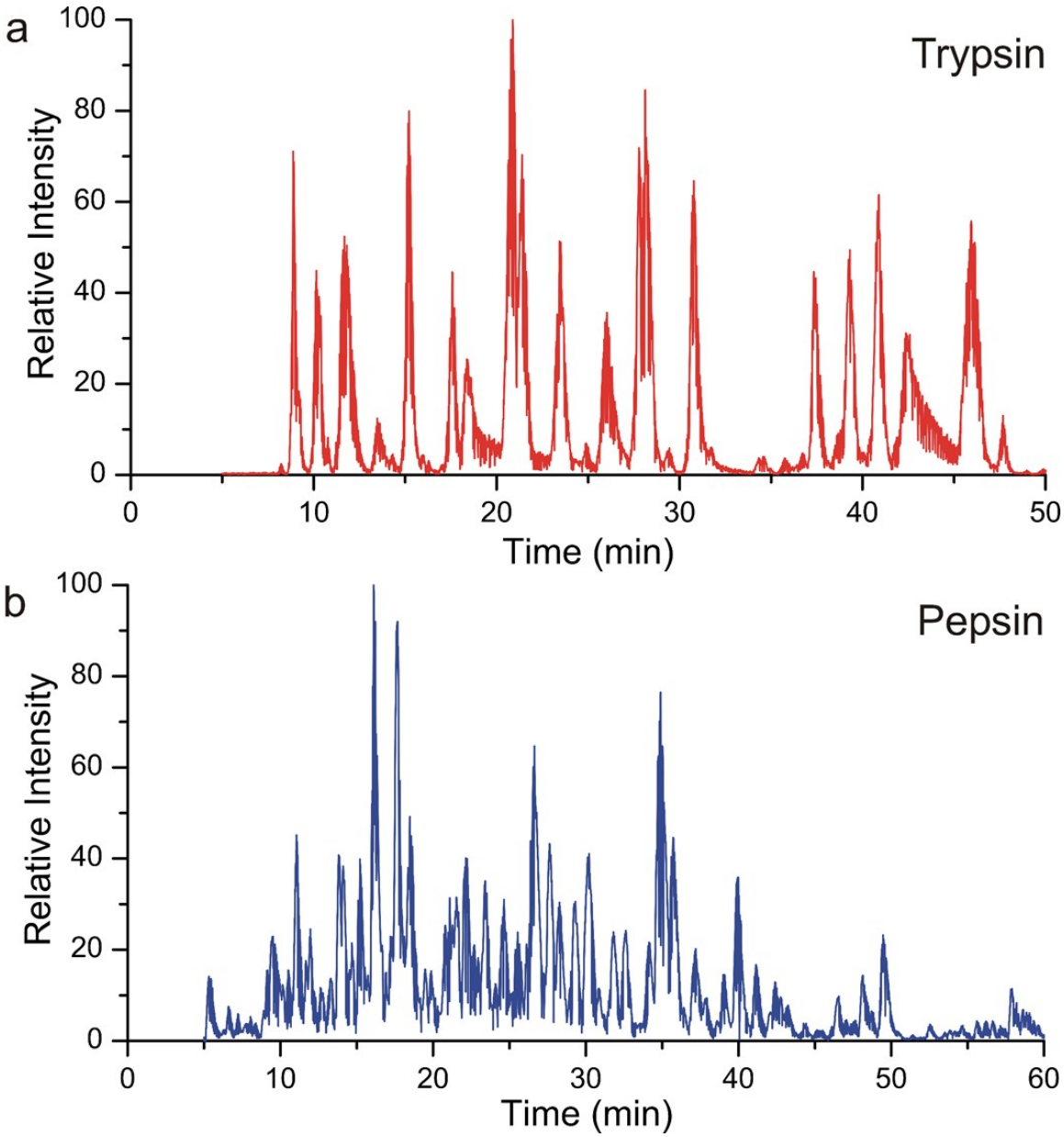

Figure S8. LCMS chromatogram of two Rituximab digests using a) trypsin and b) pepsin. The peptide map obtained from the tryptic data yields only 32% sequence coverage while the dataset from pepsin provides 65% sequence coverage.