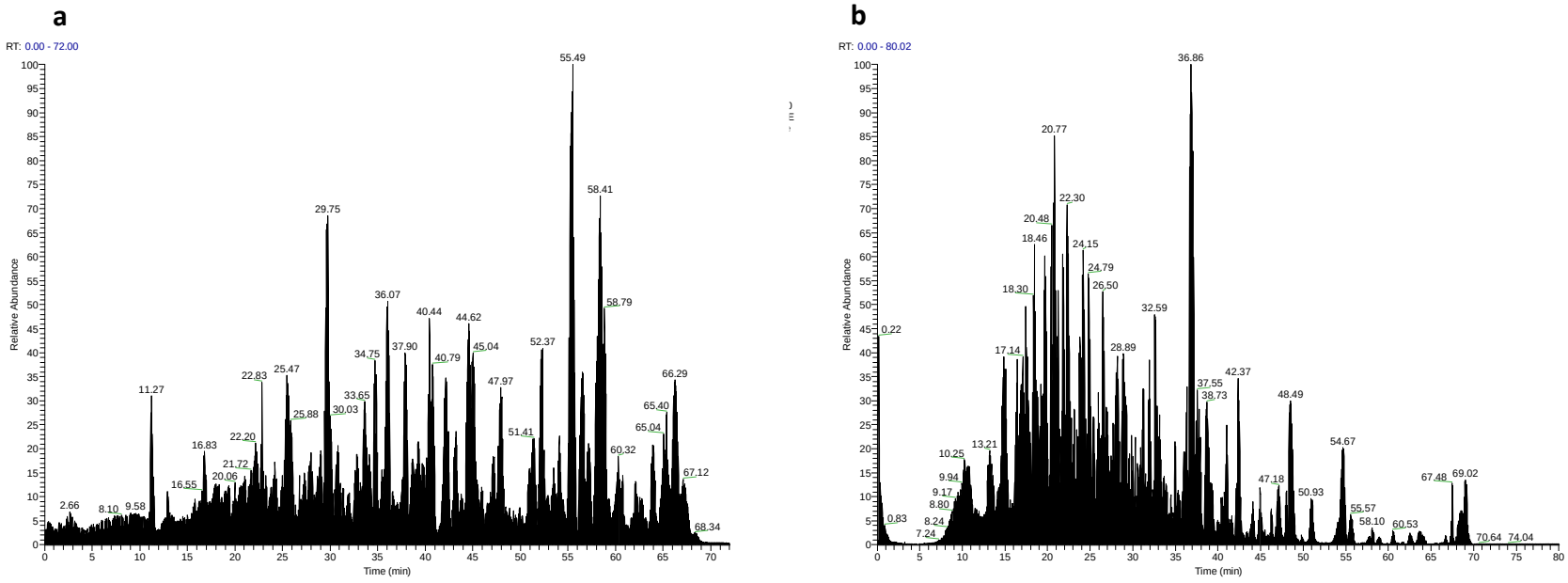
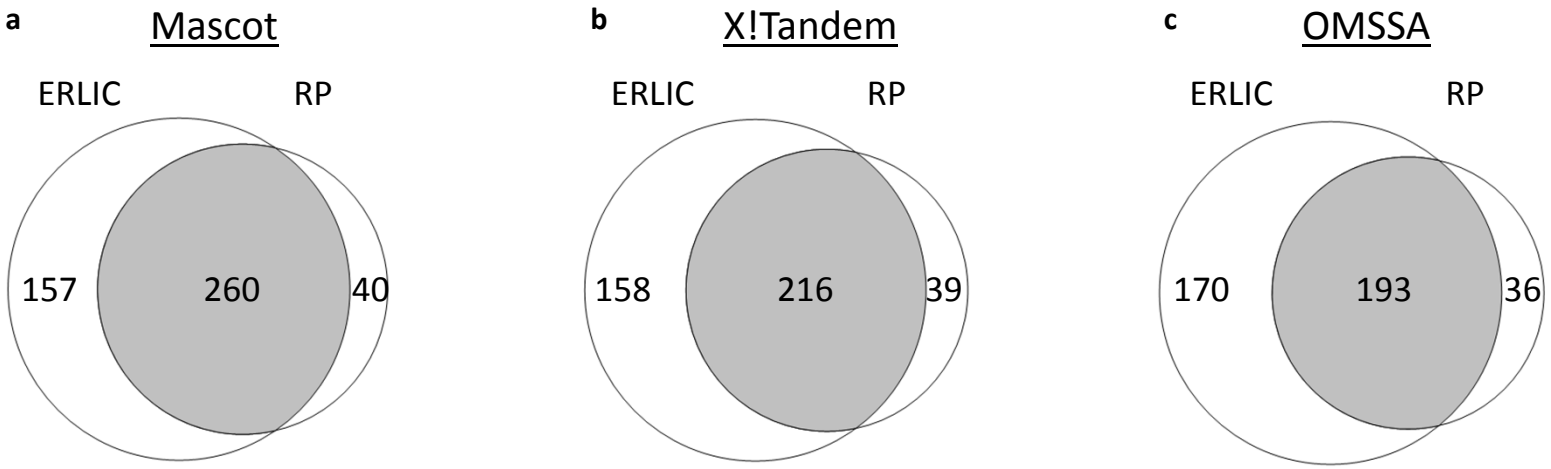
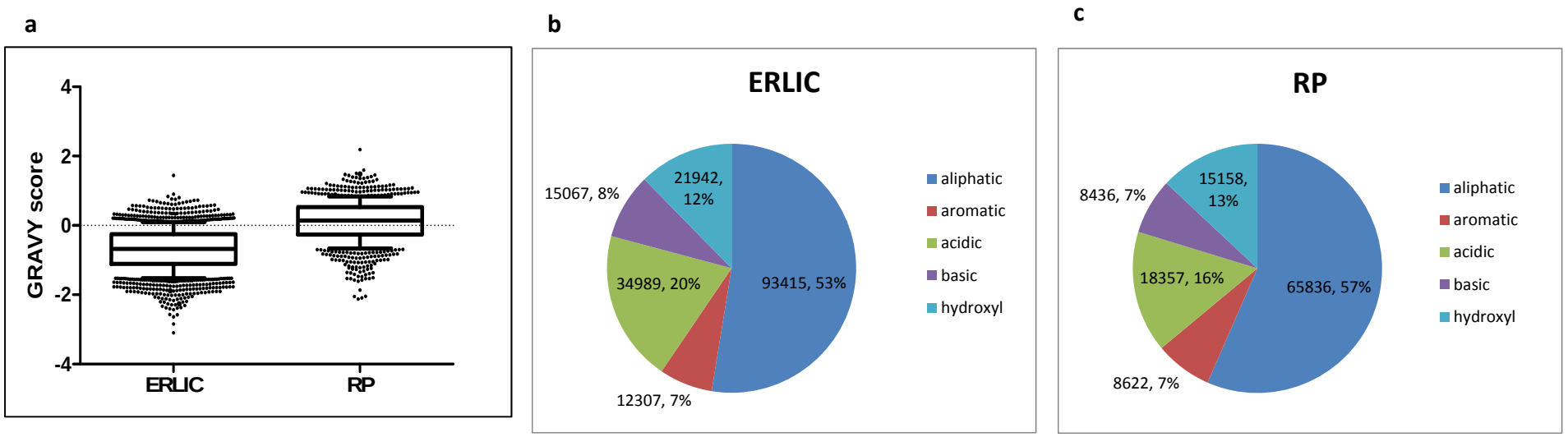


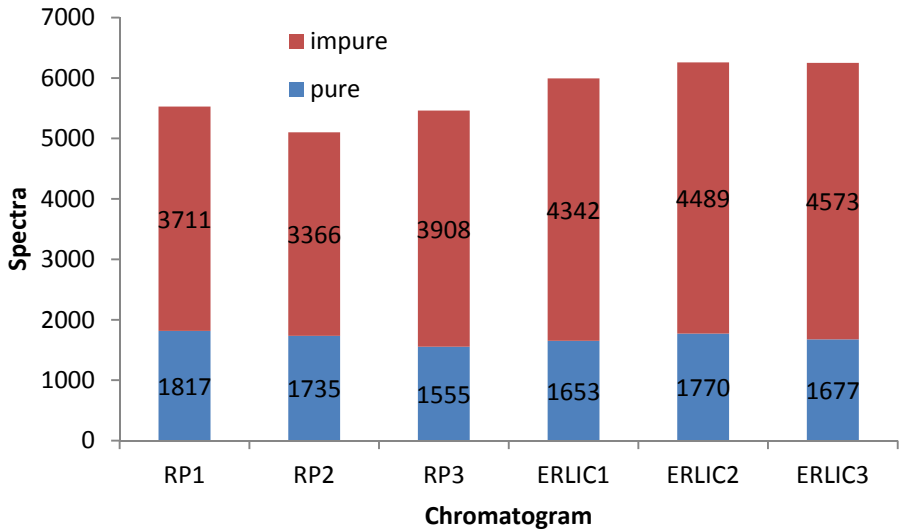
Supplementary Figure 1. Representative base peak chromatograms of yeast lysate digests. (a) online ERLIC-MS using an 11cm WAX column with a 60 min gradient from 75 to 30% ACN. (b) online RP-MS using an 11cm C18AQ column with a 60 min gradient from 2 to 40% ACN.



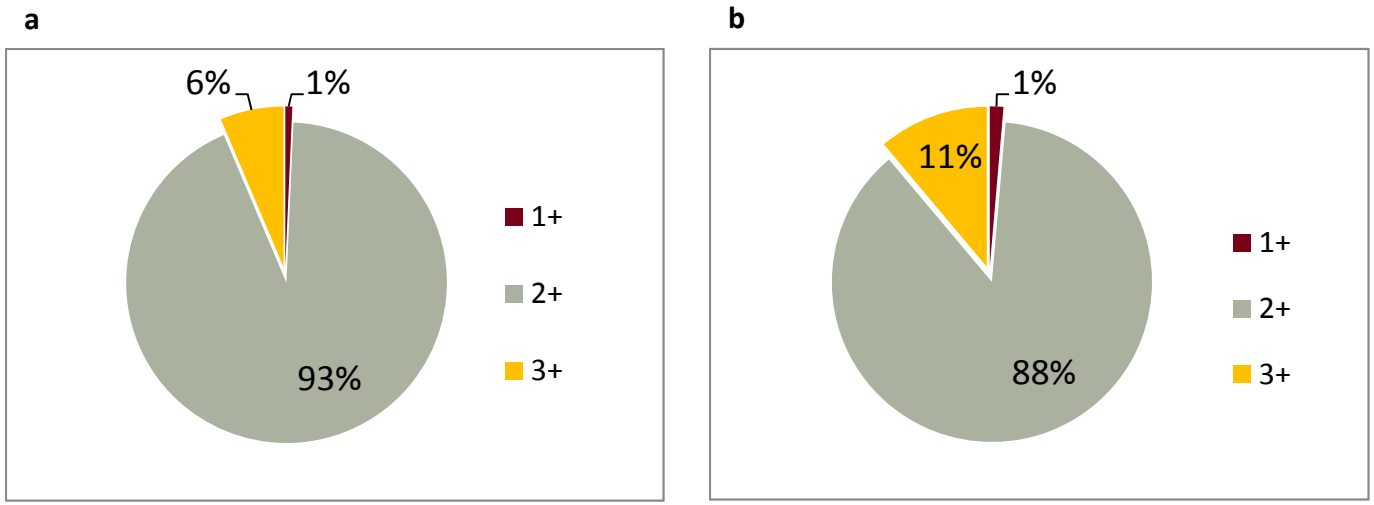
Supplementary Figure 2. Venn diagram for the number of proteins identified at the ≥2-peptide per protein level in triplicate ERLIC and RP online LC-MS runs using three alternate search engines.



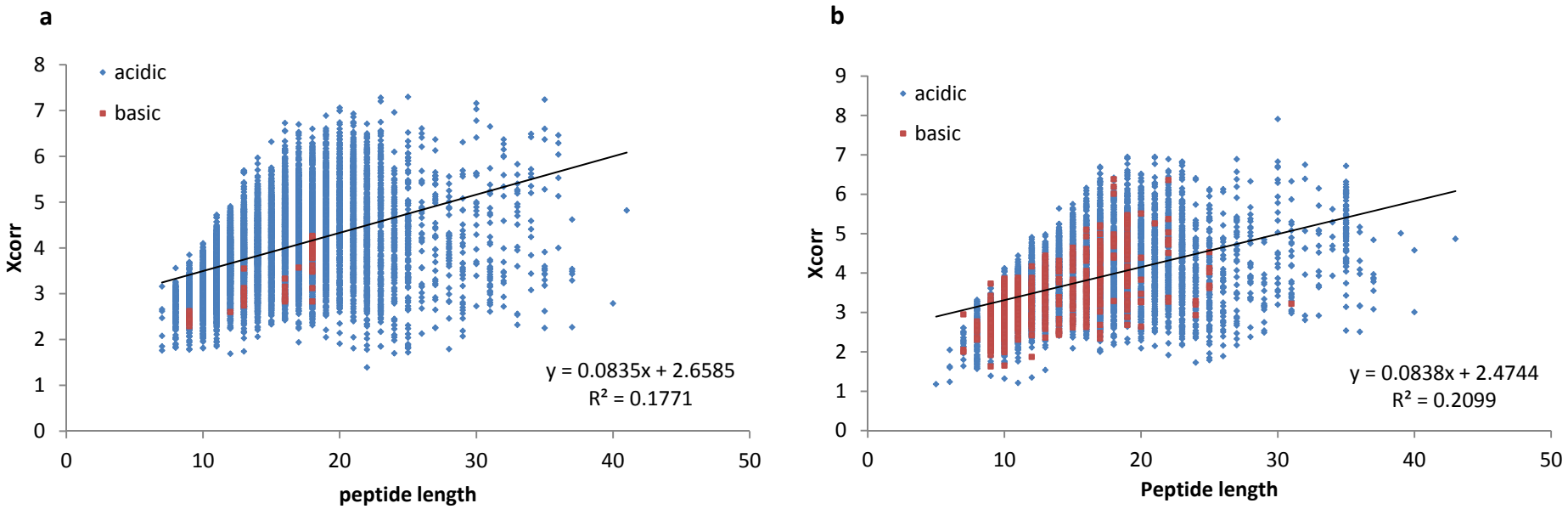
Supplementary Figure 3. Analysis of (a) the hydrophobicity of yeast peptides identified in three runs of online ERLIC-MS or RP-MS, and the distribution of amino acid side-chain types in online ERLIC-MS (b) and RP-MS (c).



Supplementary Figure 4. Isobaric tagging experiment to test precursor ion purity. Yeast and HeLa peptides were labeled with iTRAQ® labels 114 and 117 respectively, and mixed in equal amounts, by protein. The mixed sample was analyzed by RP and ERLIC and the number of MS^2 spectra containing only one reporter ion ("pure") or both reporter ions ("impure") was calculated. There is no substantial difference between ERLIC and RP runs, indicating that precursor ion purity is not improved by ERLIC's favourable distribution of peptides throughout the m/z – time space.



Supplementary Figure 5. Distribution of charge states of yeast peptides identified across triplicate runs of online ERLIC-MS (a) or RP-MS (b).



Supplementary Figure 6. Relationship between peptide identification score and peptide length (# residues) of yeast peptides identified across triplicate runs of online ERLIC-MS (a) or RP-MS (b), sorted by peptide pI. The best-fit line considers only acidic peptides, however analysis parameters are less than 5% different when considering all peptides.