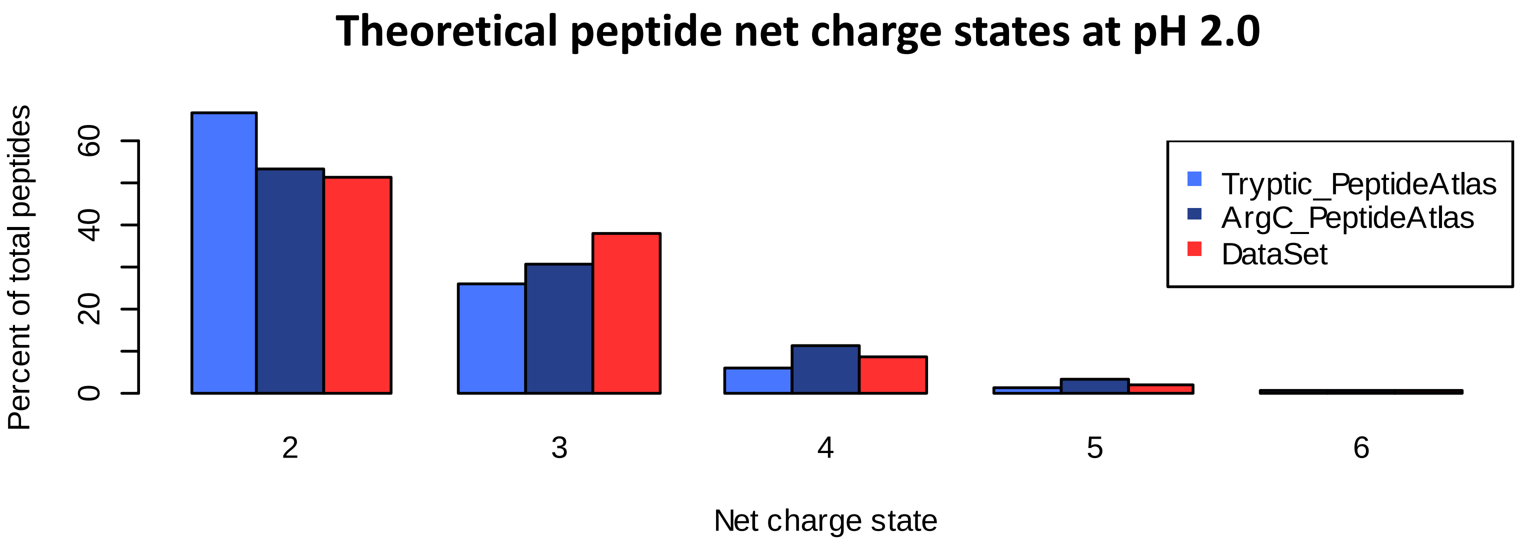

Supplemental Figure 3: For all proteins covered by the PeptideAtlas (PA) repository (October 2017) the most N-terminal peptides were extracted if they had semi-trypsin or Arg-C specificity (C-terminal specific, N-terminal flexible), corresponding to 11,282 PA trypsin and 7,868 PA Arg-C peptides.

We calculated theoretical charge states at pH 2.0 (Arg, His and Lys are positively charged, acidic amino acids not charged). In comparison with our dataset (3,086 unique quantified N-terminal peptides with semi Arg-C specificity) the theoretical net charge states show two differences: *(i)* As expected, tryptic peptides have predominantly +2 and +3, whereas ArgC specificity leads to an increasing number of peptides with higher charge states. *(ii)* The distribution in our dataset is shifted to slightly lower charge states compared to the Arg-C PA data. One of the reasons may be that we only collected the fraction +1 till +4 in our ChaFRA*tip* procedure. However, the bulk of PA data derives from qualitative ion-trap MS/MS which are better suited for identifying long peptids, whereas it has been demonstrated that iTRAQ labeling leads to increased charge states and concurrently to reduced numbers of identified and quantified peptides in reporter ion-based quantification.[1]

1 Thingholm, T. E., Palmisano, G., Kjeldsen, F., and Larsen, M. R. (2010) Undesirable Charge-Enhancement of Isobaric Tagged Phosphopeptides Leads to Reduced Identification Efficiency. Journal of Proteome Research 9, 4045-4052.)