FLR for site assignment outline

1. Introduction
   1. No current accepted method for FLR – tricky point
      1. Why do Delta scores not count? Not actually doing FLR, using fixed thresholds is inappropriate.
      2. AScore and PTMscore/MaxQuant don’t even try to correct for multiple hypothesis testing
      3. PhosphoRS?
      4. Our method
2. Methods
   1. Calculate ion detectibility
      1. Use unmodified spectra
      2. Use spectral libraries
      3. Use MassAnalyzer
   2. Generate variants + LP for site assignment
   3. Grouping
      1. Ambiguity increases with increased grouping (supplemental pie charts)
   4. FLR
      1. Scaled for # of incorrect groups vs. # of correct groups (consider all possible groups)
3. Results
   1. Yeast Phosphorylation Data
      1. Comparison with Ascore
         1. Missing pieces from current scoring methods
            1. Need to run scoring twice to generate “decoy” aa assignments
            2. Cannot handle ambiguity well.
         2. Overlap of both
         3. Ambiguous assignments where Ascore gives sure results
      2. No comparison with Mascot Delta because PSM-level IDs would be different so comparison is difficult – assess on synthetic peptides only.
   2. Human Synthetic Data
      1. Comparison with Mascot Delta Score
         1. Small # of hits so FLR difficult
         2. Well crossover, show example
         3. FLR vs. empirical
   3. Lens dataset
      1. Small DB results
      2. Multi mods
4. Discussion
   1. First FLR method that doesn’t require second localization step for “decoys”
      1. Avoids issues with aa frequencies where some sequences don’t have both aas.
   2. Comment on possible AScore extensions based on Decoy + inter-site aa distance