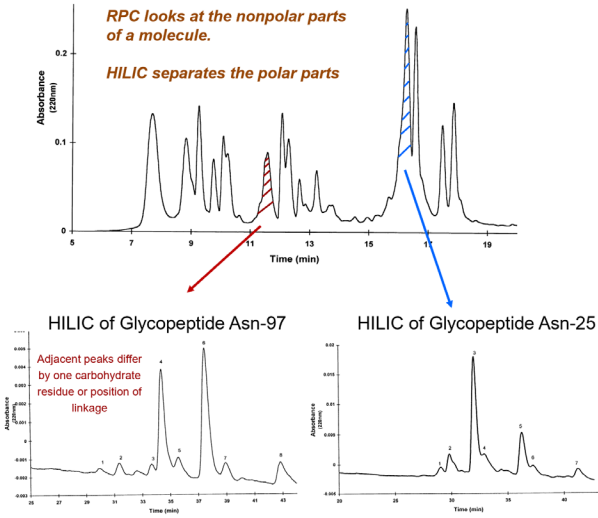


HILIC Application Note

Separate Polar Compounds and Reduce Sample Matrix Interference with Hydrophilic Interaction Liquid Chromatography (HILIC)

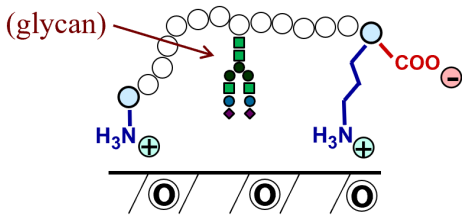
If the stationary phase is more polar than the mobile phase, then solutes will be retained in proportion to their hydrophilicity. With PolyHYDROXYETHYL A, this is usually the case if the mobile phase contains > 60% organic solvent (usually acetonitrile [ACN] or propanol). The order of elution is generally the inverse of that with reversed-phase HPLC (RPC). Chaotropes decrease retention. These include TFA, urea, guanidinium hydrochloride, and unbuffered formic acid. Avoid their use unless the objective is to accelerate elution. Retention is influenced by the solvent used:
ACN~PrOH>EtOH>MeOH.



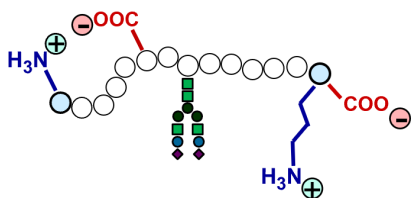
On a neutral stationary phase like **PolyHYDROXYETHYL A™**, polarity dominates retention.



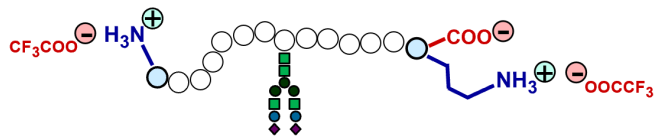
Tune Down Interaction with Basic Residues to Increase Sensitivity to the Rest of the Peptide



Basic residues are the most hydrophilic and dominate the chromatography. The addition of a glycan amounts to a small difference between large numbers. Use HILIC to selectively isolate peptides with post-translational modifications.



A) Steve Carr (1993): Run @ pH 6 →
↑ -COO⁻; less net + charge



B) Wen Ding (2009): Form a hydrophobic ion pair with TFA; tunes down retention through the basic residues

C) Andrew Alpert (2008): Perform HILIC on an anion-exchange column that selectively repels the basic residues (= ERLIC) and reorients the peptide

