This poster is not available as a reprint but will likely be published in a refereed journal in '95. Steve and I will be happy to talk to any chemists interested

This poster is by far our most sophisticated, including kinetics of dephosphoroylation during hydrolysis. This poster is best used when phospho-peptide/protein analysis is at issue. This is another example of work which lends credibility to our more academic endeavors

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High Sensitivity Analysis of Phosphoamino Acids by HPLC with Pre-column Derivatization

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Protein phosphorylation is an important control mechanism for many intracellular functions such as signal transduction. Most often, these enzymatically catalyzed reactions target the amino acids serine, threonine or tyrosine, resulting in PSer, PThr, or PTyr, respectively. Consequently, methods to identify and quantify these modified amino acids are of significant interest. We have studied the derivatization and analysis of phosphoamino acids using the reagent 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate which has been recently shown to effectively label amino acids prior to reversed phase HPLC analysis with high sensitivity fluorescence detection. Favorable reaction kinetics in aqueous buffer systems gave excellent yields for the derivatized phosphoamino acids. Good resolution of the standards from each other as well as from the hydrolysate amino acids was obtained using previously studied conditions, but small peptides produced by the short hydrolysis of intact polypeptides or proteins required for phophoamino acid recovery can interfere with analysis under these conditions. Alternate chromatographic systems exploiting favorable retention selectivity with modifications in eluent pH, ionic strength and gradient profile have been developed that allow quantitation of PSer, PThr and PTyr in a single analysis. Samples hydrolyzed for varying times (2-10h) have been derivatized and analyzed with this method to provide quantitative information on the presence of High Sensitivity Analysis of Phosphoamino Acids by HPLC with Pre-column Derivatization