Poster α This poster shows how to increase the information of a peptide map in a number of ways. An updated version of this poster is #50, presented at ISPPP '94. This poster is available only as a FREELANCE presentation from either Steve or myself

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Sub-Picomole Peptide Mapping Using HPLC with Fluorescence Detection -14

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Experiments with model peptides have demonstrated quantitative conversion of intact peptides to fluorescently labeled urea-type compounds using the reagent 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate. No significant evidence of multiple products has been observed, with >95% of the fluorescence found in single peaks after reversed phase HPLC. Multiply derivatized species are formed with lysine-containing peptides, but other potential sites for reaction, such as the histamine imidazole and serine hydroxyl functions, are apparently unreactive. The tyrosine phenol forms a moderately unstable carbamate derivative that is quantitatively converted back to the phenol by a short heating step. Because fluorescence detection is sensitive to eluent pH, with highest sensitivity occurring at pH > 6, standard TFA-containing eluents are not optimal for these samples, and acetate/phosphate buffers at pH 5-7 were routinely employed. We have applied this chemistry to the analysis of tryptic digests of Cytochrome c to provide a complementary set of information to the usual reversed phase map of the underivatized digest. Less than 100fmol of digest can be detected using standard HPLC equipment with either 2 or 4mm diameter wide-pore columns, thus allowing both maps to be performed with essentially the same amount of sample that the underivatized one requires. The derivatization also increases retention for short, polar peptides, which are poorly retained in their underivatized state, and provides