**Abstract**

In mass spectrometry (MS), electron transfer dissociation (ETD) is often employed to generate fragments used for determining a peptide's sequence. ETD is a preferred fragmentation technique because labile post-translational modifications (PTMs) such as phosphorylation are left intact, which allows for straightforward localization. However, the ability to successfully utilize ETD is highly dependent on a peptide's charge state, which excludes some peptides of interest. To help get around this problem, our lab has been able to develop a carbodiimide-based derivatization strategy to covalently link histamine molecules to free carboxylic acid residues found on peptides. Once attached, the imidazole ring on histamine provides an additional site for positive charge to be retained. One drawback to using histamine is that upon derivatization peptides become substantially more hydrophilic, which can lead to problems retaining peptides on the reverse-phase chromatography columns typically utilized in conjunction with MS-based detection systems.

The work presented for this defense is an attempt to resolve the problem of increased hydrophilicity. To accomplish this task we have identified several molecules with similar functionality to histamine, but which also contain hydrophobic functionalities. Unfortunately, these chemicals contain undesirable contaminants, and in order to use them, we had to develop a new derivatization strategy. We ultimately developed a cross-coupling strategy, which made use of 1-Hydroxy-7-azabenzotriazole (HOAt) and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) to covalently attach 2-(7-Methyl-1H-benzimidazol-2-yl) ethanamine (Methyl Benzimidazole) free carboxylic acid residues. Once attached, we were able to demonstrate that methyl benzimidazole increases peptide charge while also increasing hydrophobicity. We believe this charge enhancement reagent shows great promise for improving

ETD-based sequencing of peptides containing PTMs of interest, which have previously been difficult to localize.