

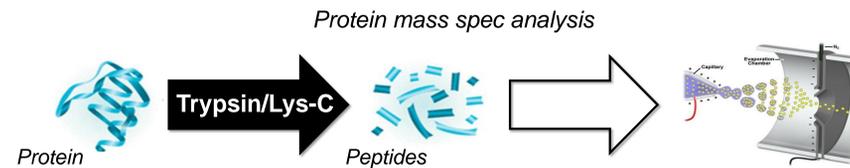
# Enhanced protein mass spectrometry analysis with Trypsin/Lys-C mix

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## 1. Introduction

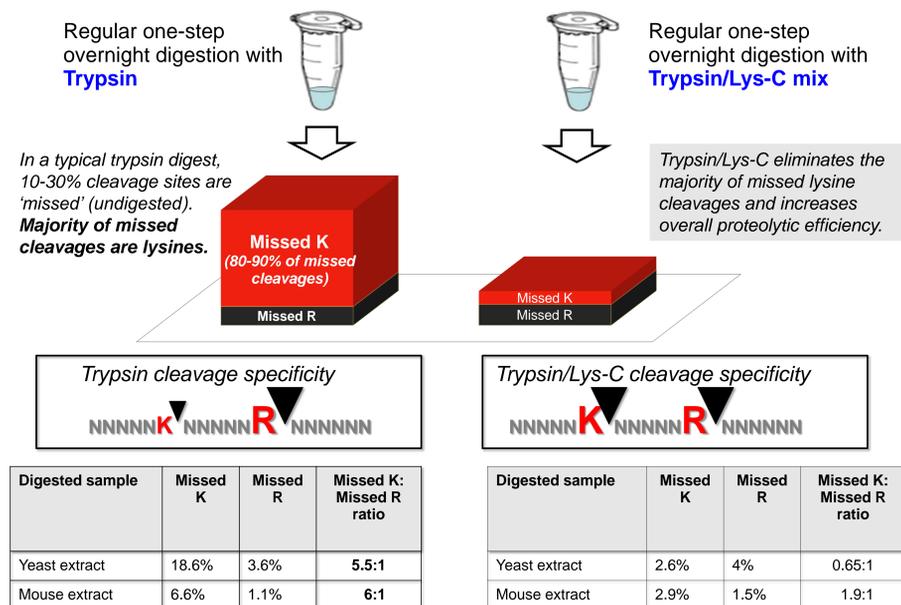


Efficient proteolysis is required for protein mass spectrometry analysis. Here we describe a Trypsin/Lys-C mix, which dramatically improves proteolysis:

- the majority of missed cleavages, commonly occurring in trypsin digests, are largely eliminated
- protease-inhibiting impurities are tolerated
- proteolytically resistant proteins are efficiently digested

We show here how enhanced proteolysis with Trypsin/Lys-C improves protein mass spectrometry analysis. These benefits include an increase in identified peptides and proteins, higher signal intensity of individual peptides and higher analytical reproducibility.

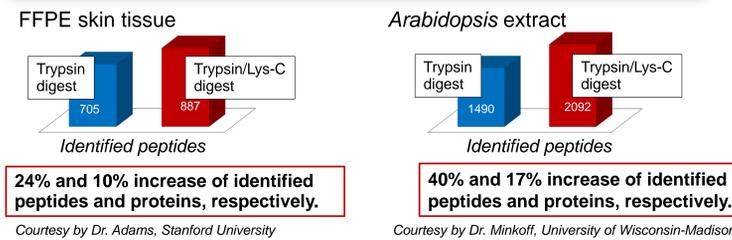
## 2. Proteolytic enhancement with Trypsin/Lys-C



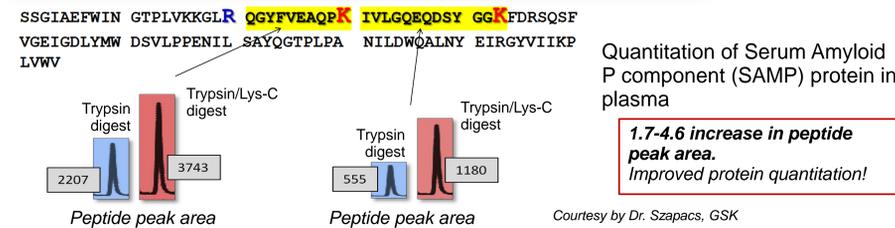
Similar ratios of missed lysine and arginine cleavages sites were observed in Trypsin and Trypsin/Lys-C digests of extracts prepared from other sources including human and E.coli.

## 3. Trypsin/Lys-C advantage for mass spec analysis

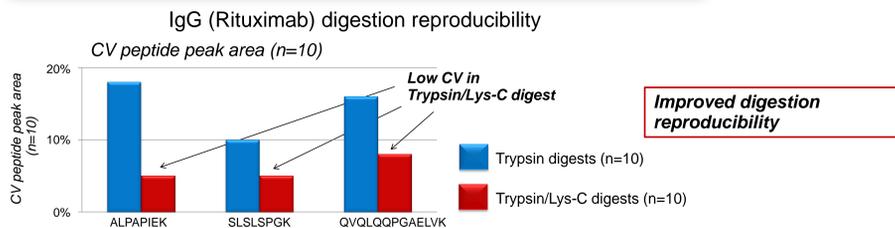
### Increase in peptide and protein identifications



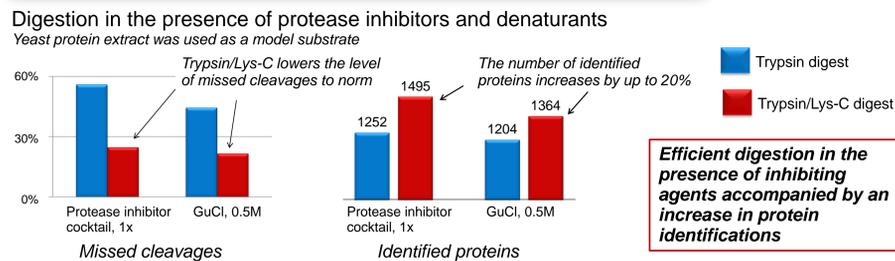
### Higher peptide recovery



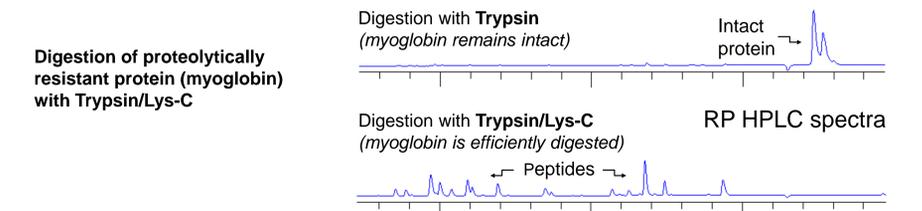
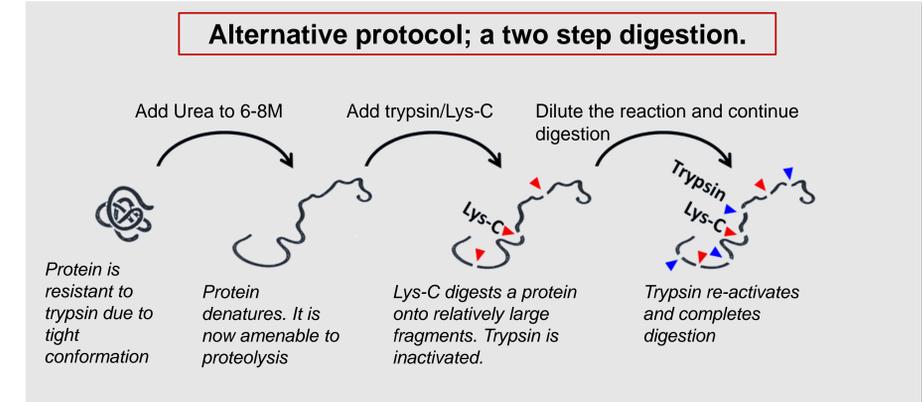
### Higher digestion reproducibility



### Tolerance to protease inhibiting agents



## 4. An additional advantage: digestion of proteolytically resistant proteins



## 5. Conclusions

- Incomplete proteolysis in trypsin digests is largely attributed to trypsin proteolytic inefficiency at lysine sites. Missed K and missed R ratio in tryptic digests equals to 5:1-6:1
- Supplementing trypsin with Lys-C leads to elimination of the majority of missed lysine sites and overall increase in proteolytic efficiency.
- Trypsin/Lys-C mix tolerates protease inhibiting impurities and digests tightly folded, proteolytically resistant proteins\*
  - \*Digestion of proteolytically resistant proteins requires a specialized, two-step procedure
- Enhanced digestion with Trypsin/Lys-C provides critical benefits for protein mass spec analysis:
  - Increase in peptide and protein identifications
  - Higher analytical reproducibility
  - More accurate protein quantitation through more complete digestion