Chemistry 240
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Name: _____

# The Logic of Chemical Sequencing of Proteins

Imagine you have a small protein you'd like to sequence using chemical methods. The logic, NOT the chemistry, goes like this:

1. Inventory the amino acids
2. Cleave the chain into smaller chains using a peptidase
3. Sequence the smaller chains
4. Repeat from steps 2 & 3 using different enzymes
5. Construct overlaps and finally the full sequence

There are many possible complications (more than one chain in a protein, you have to purify each piece as you go, etc) but there are ways to deal with that, and there are other steps you can take to get more information. However, the steps above represent the core steps one needs to sequence a protein chemically.

Remember, sequences are always written N-terminus $\rightarrow$ C-terminus, and thus pro – leu $\neq$ leu – pro

Here's an example:

1. The inventory shows the following counts: ala 1, asp 1, gly 2, ile 1, lys 1, phe 1, ser 2, val 1 (total 10).
2. Cleave the original peptide using the enzyme trypsin which cleaves on the carboxyl side of positively charged amino acids. Sequence the pieces. In this case, you get two peptides:
   (a) ser – ile – gly – asp – phe – gly – ser
   (b) ala – val – lys

   In this simple example, this is actually enough information to get the entire sequence! However, continuing...
3. Cleave the original peptide using the enzyme chymotrypsin which cleaves on the carboxyl side of aromatic amino acids. Sequence the pieces. In this case, you get two peptides:
   (a) ala – val – lys – ser – ile – gly – asp – phe
   (b) gly – ser
4. Figure out how the fragments from each cleavage experiment overlap and construct the final sequence by aligning the fragments: