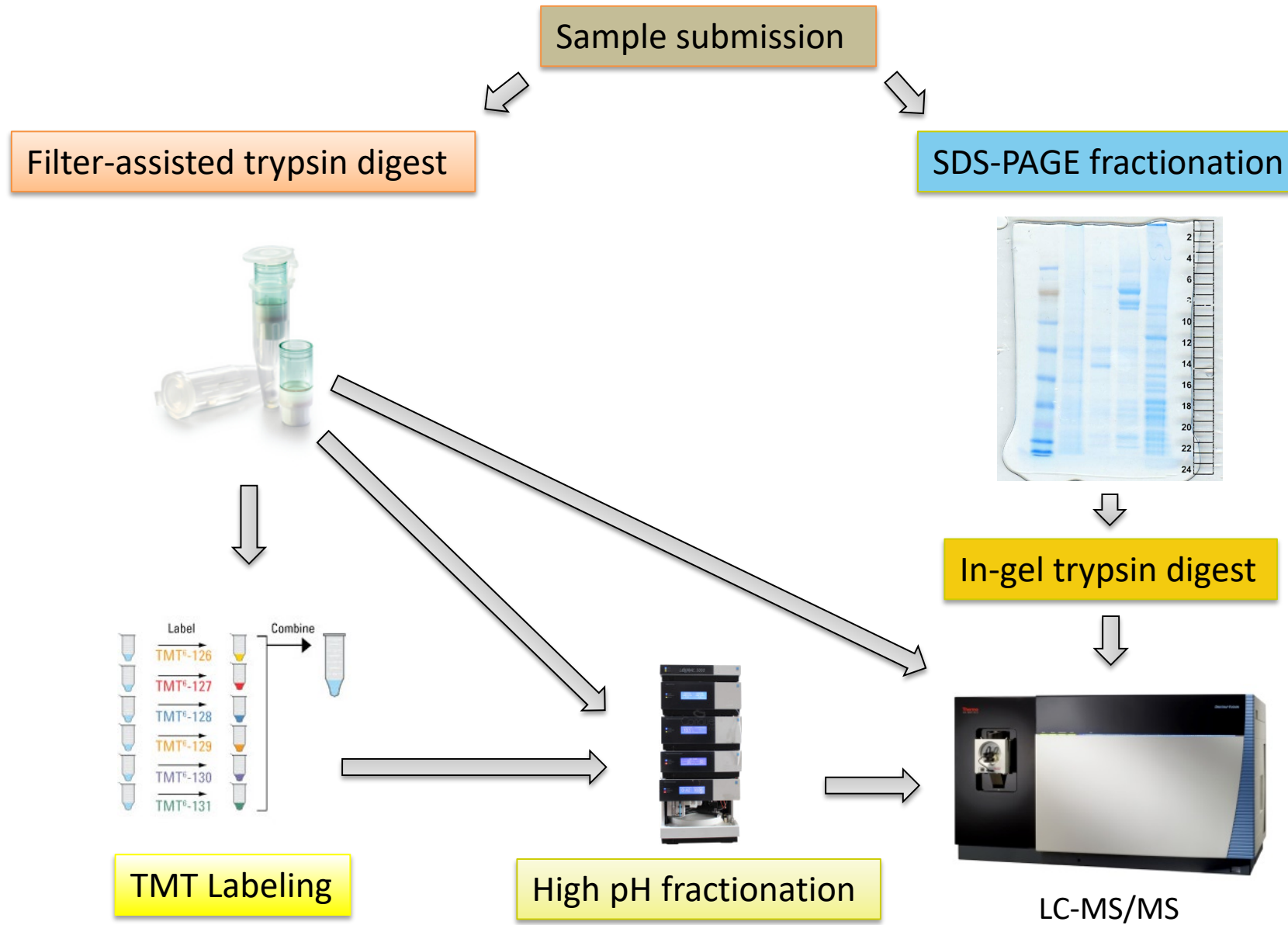


Experimental Design

IDeA National Resource
for Proteomics

Workshop for Students and Faculty
February 2020

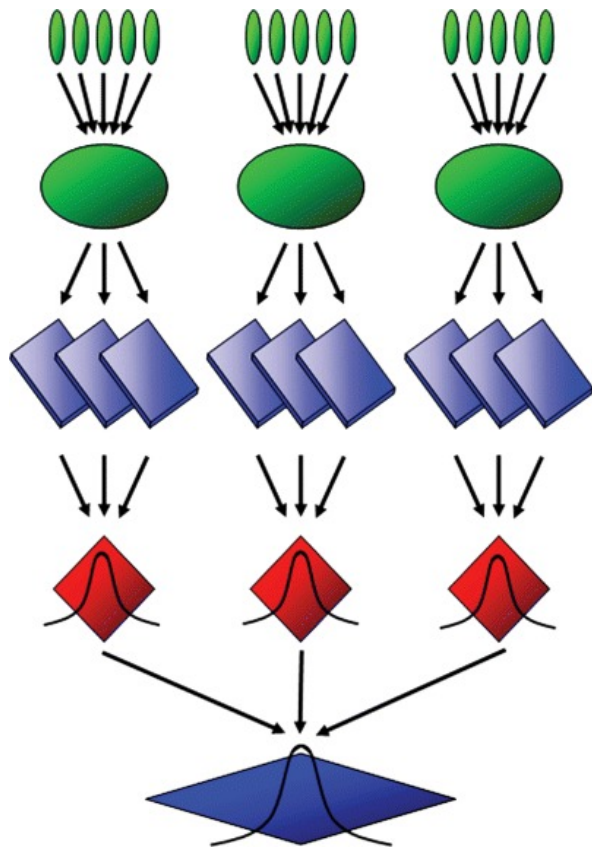
Proteomics Experimental Workflow



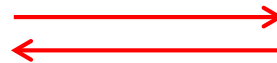
What question do you want to answer?



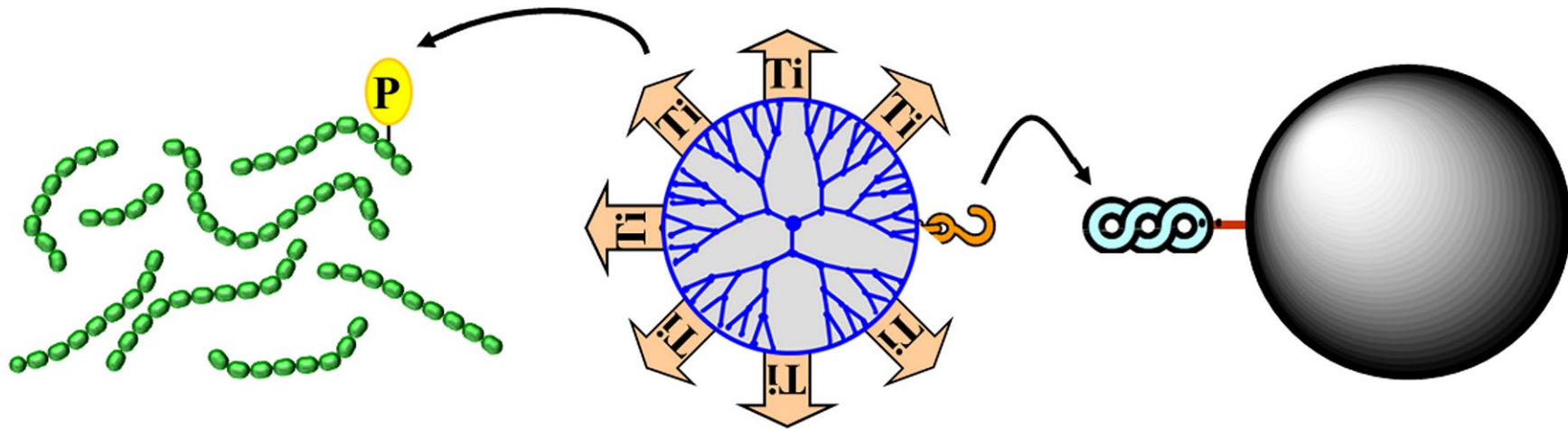
How many replicates?



**Talk to statisticians and bioinformaticians
before you do anything in the lab**



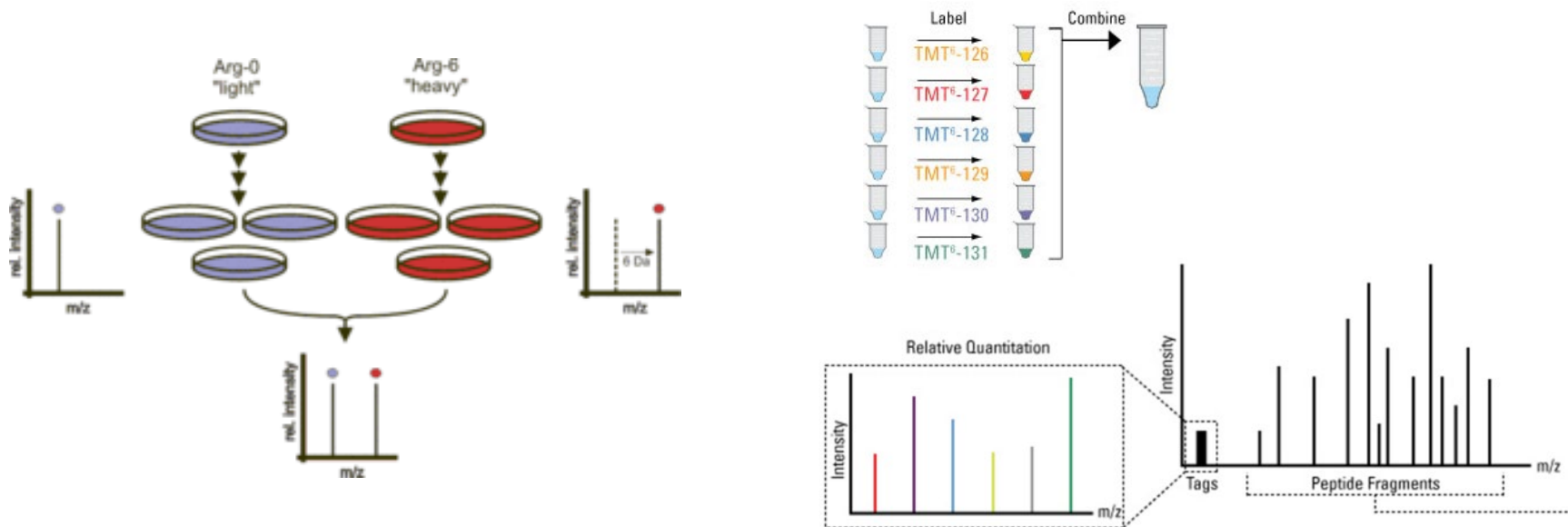
Are PTMs important?



TMT, SILAC or label-free?

Key factors:

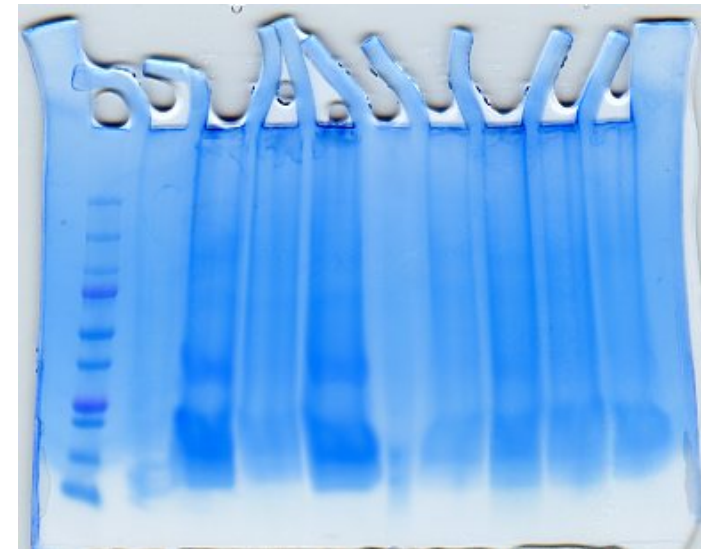
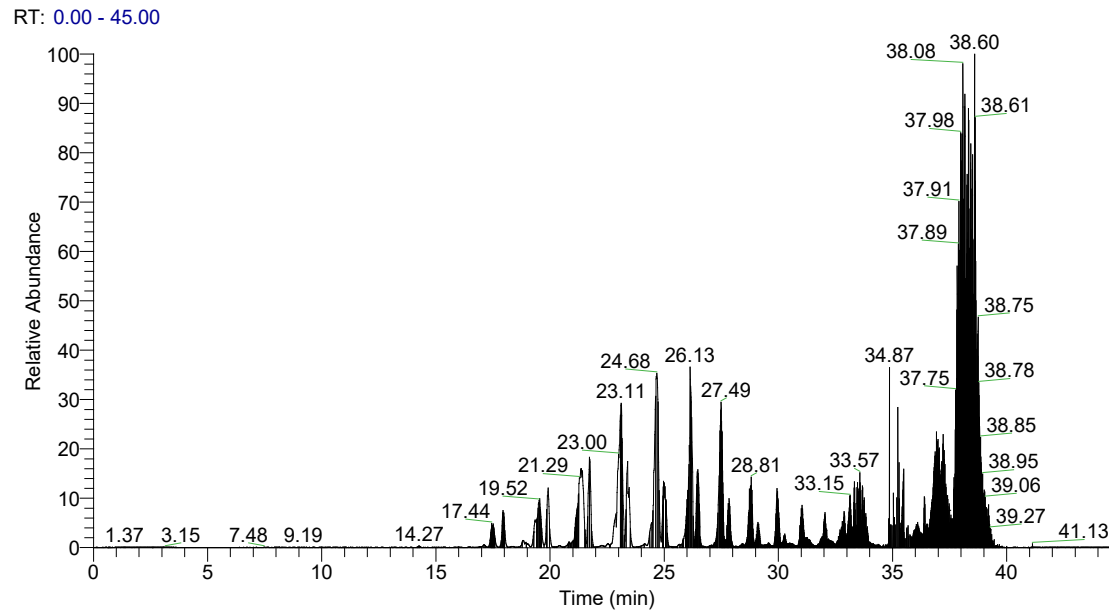
- Biological question/hypothesis
- Quantitative precision
- Time and cost



Detergents and Mass Spectrometry

Do not use:

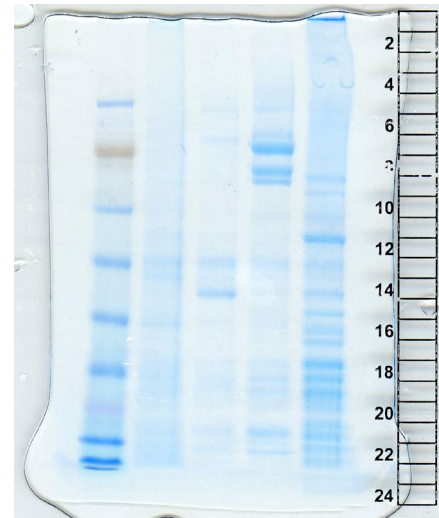
- Triton X-100
- Tween-20
- Igepal/PEG (or any derivative)



How much fractionation?

Key factors:

- Biological question/hypothesis
- Sample complexity
- Time and cost
- Experimental design

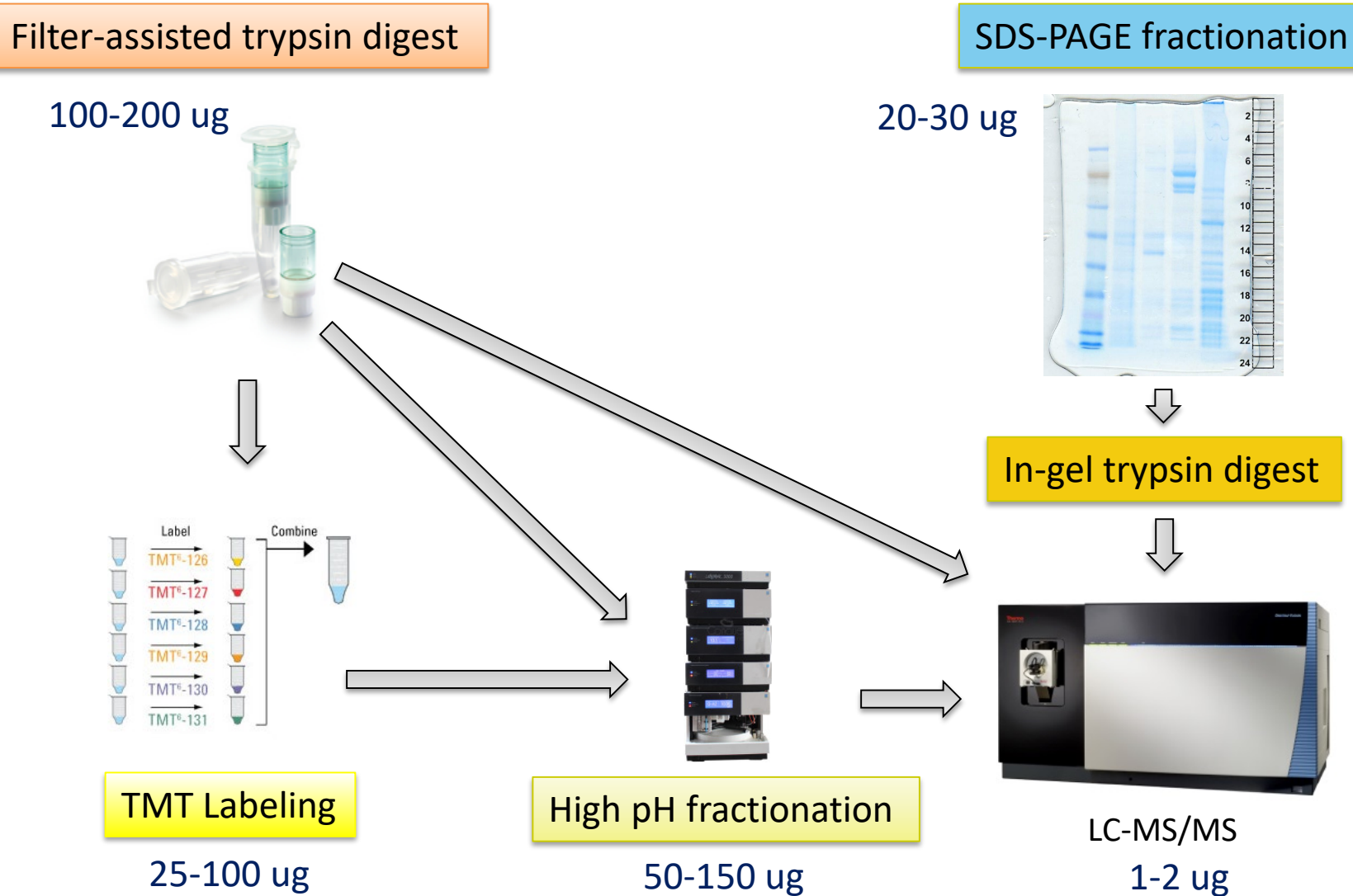


How much protein?

Cell culture:	$\sim 10^6$ cells
Tissue sample:	~ 100 mg
Serum/plasma:	~ 10 μ l
IP:	$\sim ???$



How much protein?



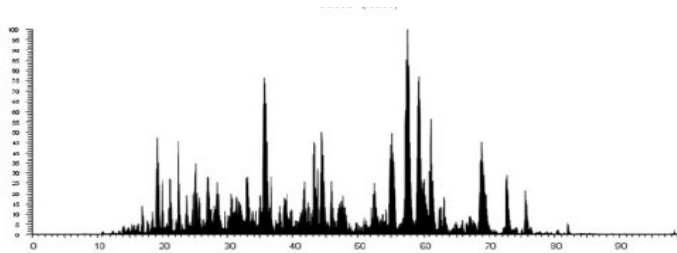
How much instrument time?

Key factors:

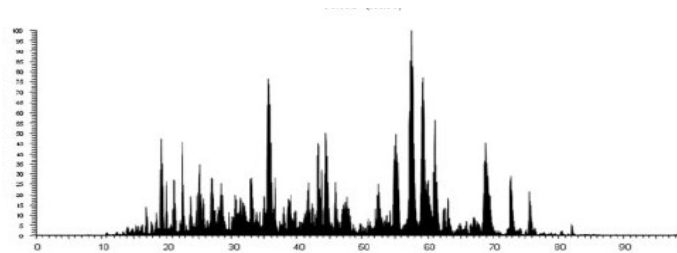
- Biological question/hypothesis
- Sample complexity
- Time and cost
- Experimental design



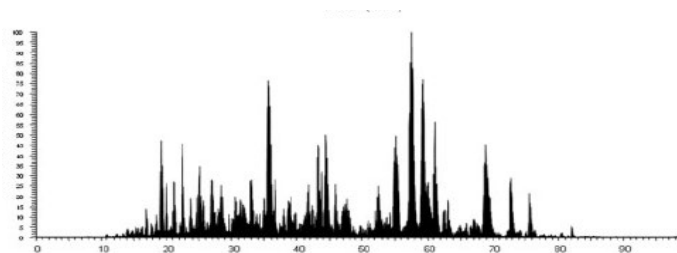
How much instrument time?



x 1 = 3000 proteins?



x 12 = 7000 proteins?



x 24 = 10000 proteins?

Where should data analysis stop?

