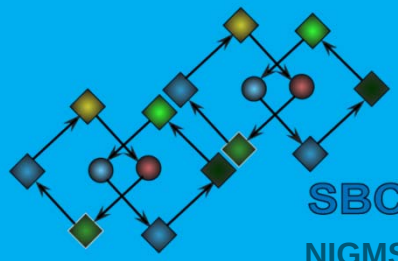


NIGMS funded Center

Experimental Methods in Systems Biology

Part of the Coursera Certificate in Systems Biology

Marc Birtwistle, PhD
Department of Pharmacology & Systems Therapeutics
Fall 2014, Week 3 Mass Spectrometry-Based Proteomics

Icahn School of Medicine at Mount Sinai

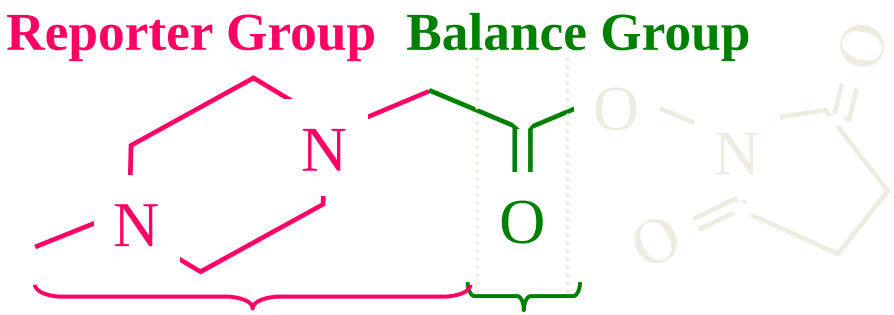
Quantification

MS-based protein quantification

- Stable isotope-labeling methods
  - SILAC: Stable isotope labeling with amino acids in cell culture
  - iTRAQ: Isobaric Tags for Relative and Absolute Quantification
- Selective Reaction Monitoring
  - Need to know what you're looking for and typically employs internal standards
  - We're focusing more on omic-level proteomics so we won't cover this
- Label-free method
  - Spectral counting—counts of peptides that align to a protein, analogous to aligned reads in mRNA seq
  - iBAQ—intensity based absolute quantification

iTRAQ reagents

(Isobaric Tags for Relative and Absolute Quantification)

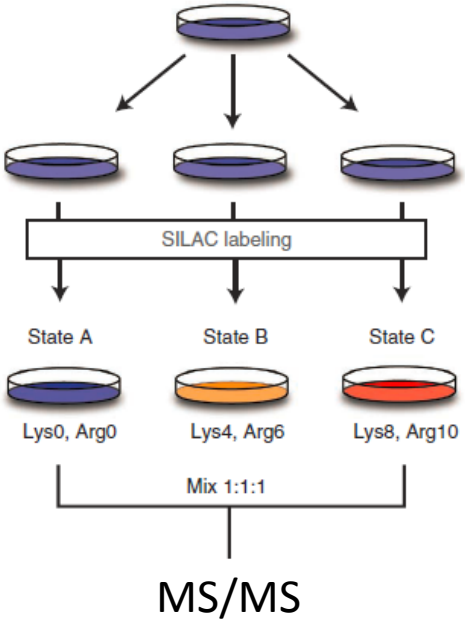


Amine-reactive Group

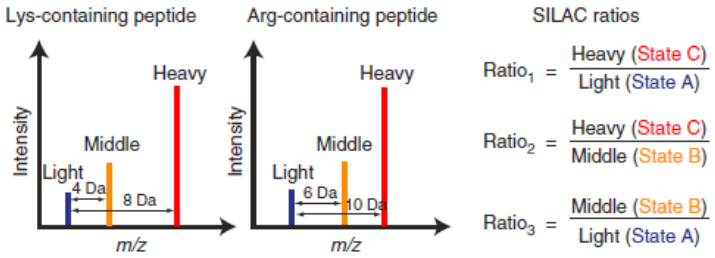
1	^{13}C	114.1	^{13}C, ^{18}O	31.0
2	$^{13}C_2$	115.1	^{18}O	30.0
3	$^{13}C_2$, ^{15}N	116.1	^{13}C	29.0
4	$^{13}C_3$, ^{15}N	117.1		28.0

- Allows multiplexing
  - 4 or 8
- Labeled peptides co-elute on LC separation
- Differences in mass only apparent after fragmentation

SILAC—Stable Isotope Labeling with Amino Acids in Cell Culture

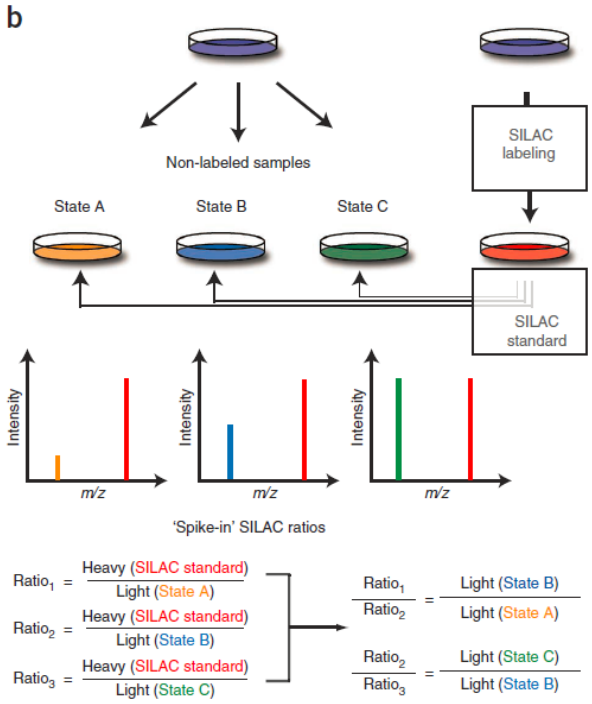


Geiger et al., Nat Protocols, 2011



- Heavy isotopes of lysines and arginine residues are incorporated into proteome
- The mass shift allows relative quantification

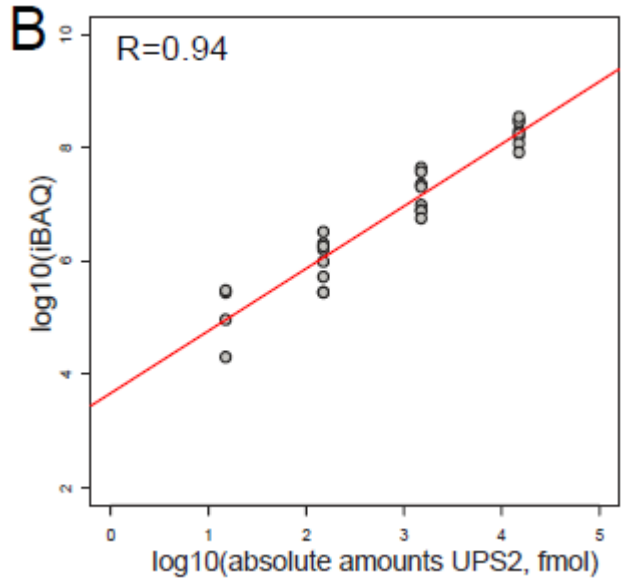
Spike-in SILAC



Geiger et al., Nat Protocols, 2011

- Some samples very expensive and/or not possible to label
  - E.g. mice, human tissues
- The spike-in method of labeled standards can solve that
- Not all tissues have similar expression profiles as a single standard
  - There is a "super SILAC standard" that addresses this issue by mixing many standards (Geiger et al., 2010, Nat Methods)

iBAQ—Intensity-Based Absolute Quantification



Schwanhausser et al., 2011, Nature

- Sum all identified peptide intensities
  - Maximum detector peak intensities of the peptide elution profile
- Divide by the number of theoretically observable peptides
  - Similar to mRNA seq divide by transcript length
- Seems to perform (slightly) better than other label-free methods (Arike et al., J Proteomics, 2012)
- Can be converted into absolute copy number / cell by keeping track of how much protein is kept from lysate until mass spec or with known spike-ins