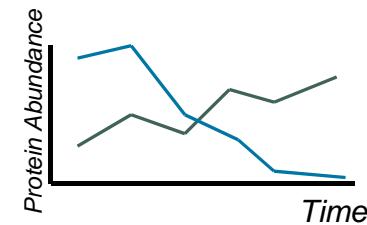
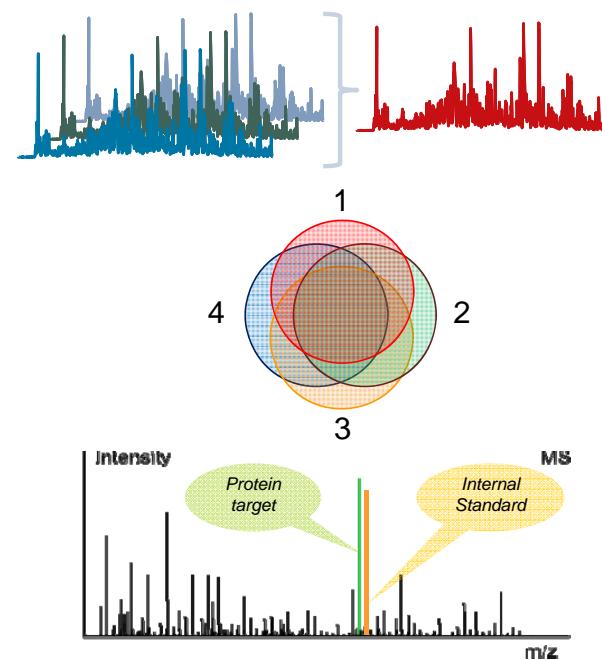


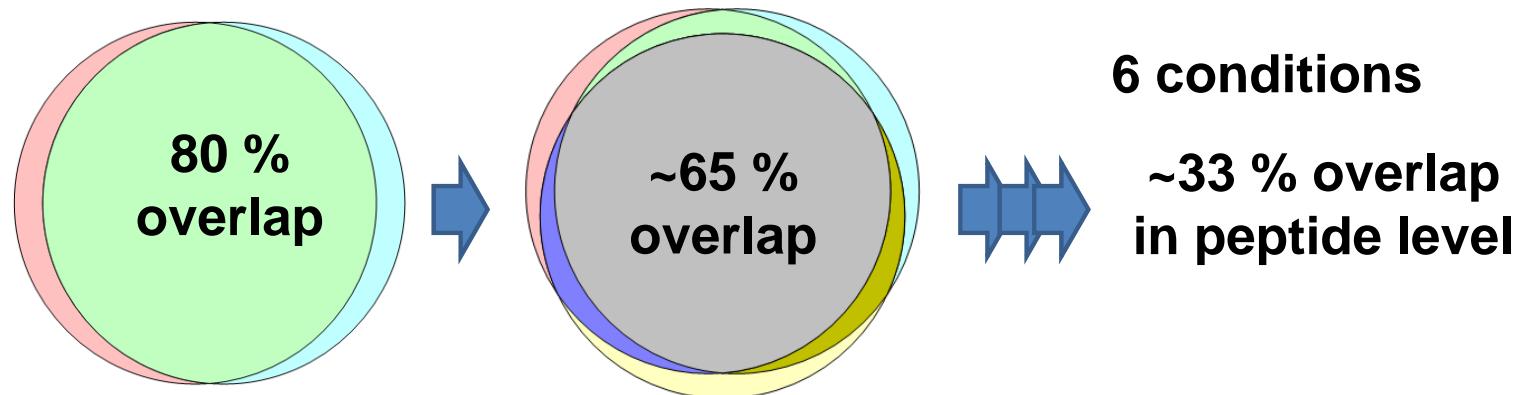
Advantages of Sample Multiplexing

- Increased Throughput
 - Combine biological samples to reduce fractionation and instrument analysis time
- Fewer Missing Values
 - Quantified peptides from independent experiments do not overlap completely
- Internal Controls
 - An internal reference sample verifies performance and improves comparisons across experiments
- Multiple Comparisons and Improved Statistics
 - Incorporate replicates with multiple conditions: dose-response, time-course, multiple tissues, subcellular fractions, etc



Importance of multiplex quantification

Under-sampling; low overlapping between LC-MS runs



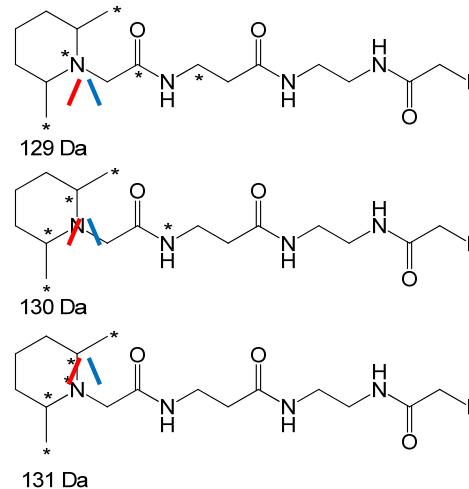
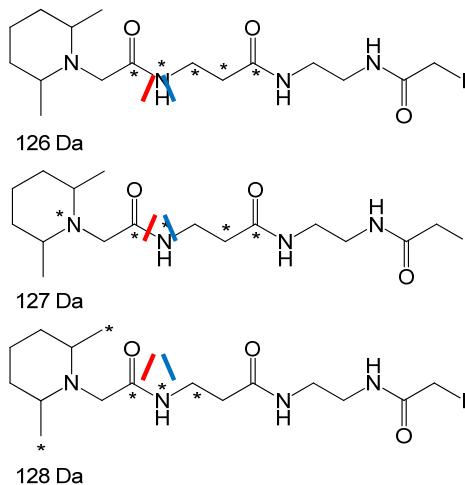
Missing data, especially in quantifiable data

Cysteine-reactive Tags for MS Quantitation

Iodoacetyl Tandem Mass Tag™ (iodoTMT™) Reagents

- Sixplex set of iodoTMT Reagents for irreversible sulfhydryl labeling
- Enrichment of cysteine-containing peptides using novel anti-TMT antibody

Thermo Scientific
iodoTMTsixplex Reagents

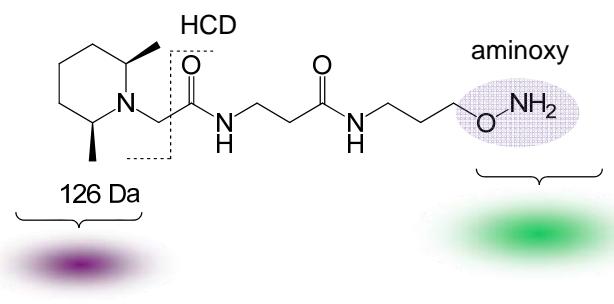


ETD cleavage site

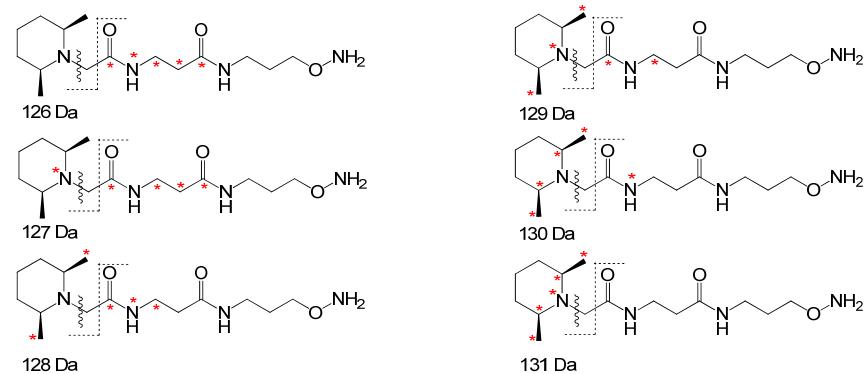
HCD cleavage site

AminoxyTMT

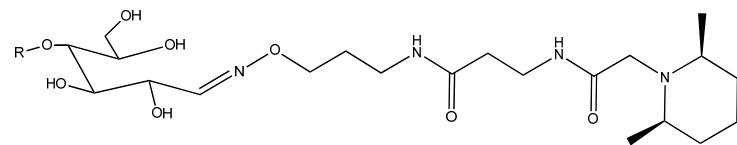
aminoxyTMT Reagent Structure



aminoxyTMTsixplex Reagent Set



A very stable oxime product



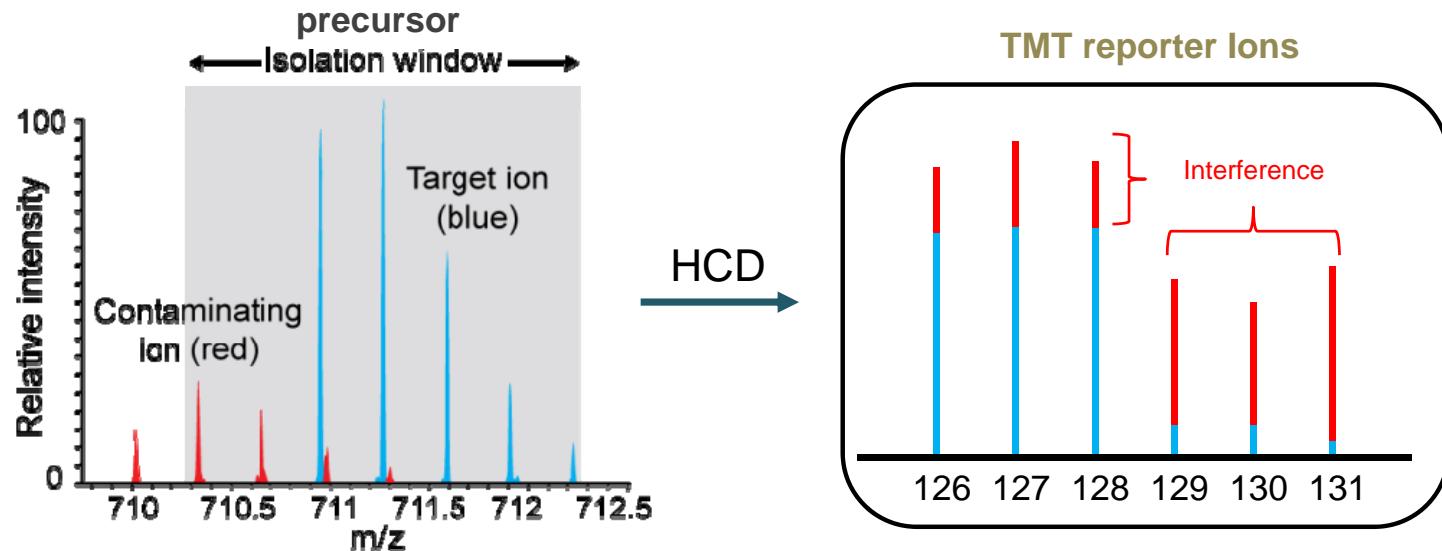
Ratio Distortion with Isobaric Multiplexing

Problem: Quantitation of low-abundance proteins in a complex background is distorted by co-isolated interfering precursor ions

Journal of
research articles **proteome**
research

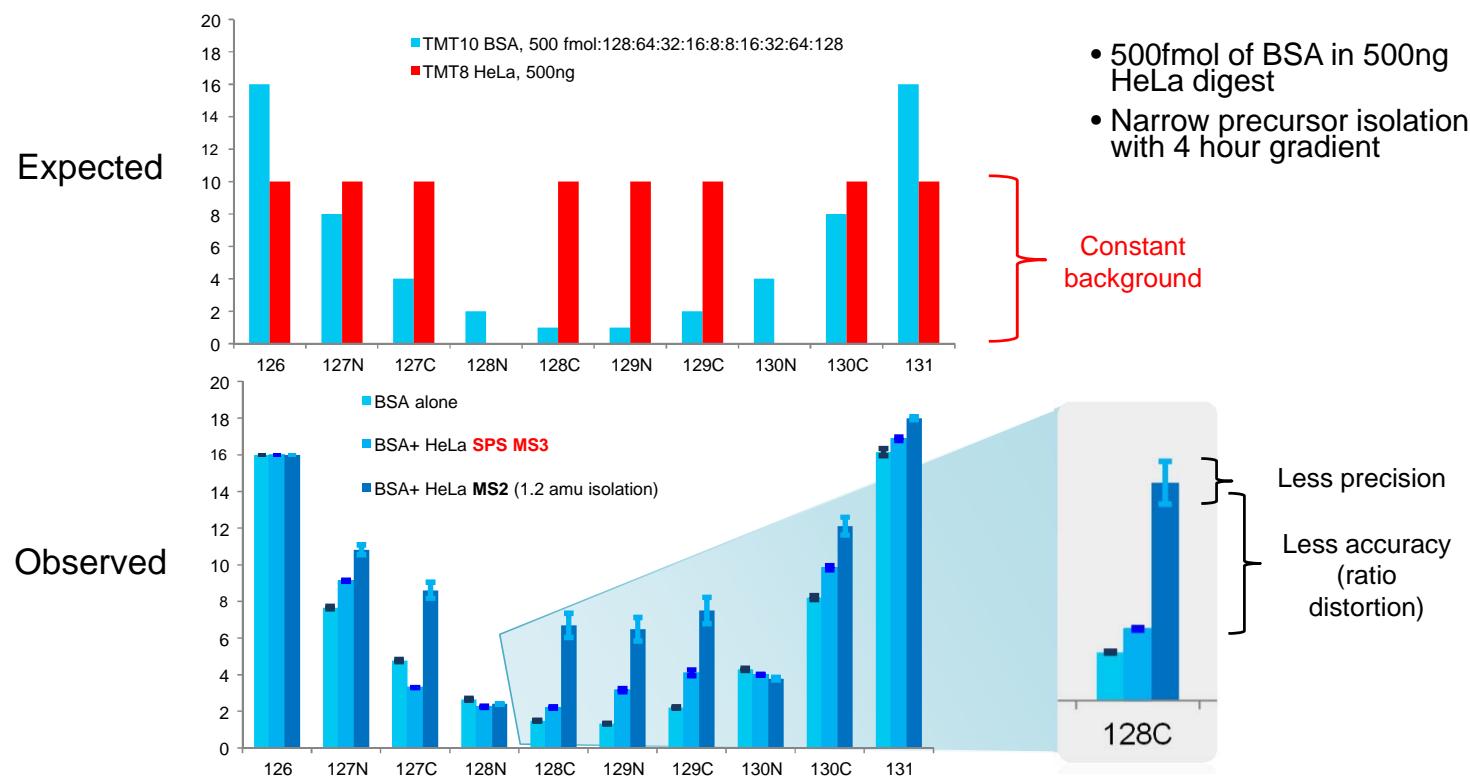
iTRAQ Underestimation in Simple and Complex Mixtures:
"The Good, the Bad and the Ugly"

Saw Yen Ow,[†] Malinda Salim,[†] Josselin Noirel,[†] Caroline Evans,^{†,‡} Ishtiaq Rehman,[†] and Phillip C. Wright^{*,†}



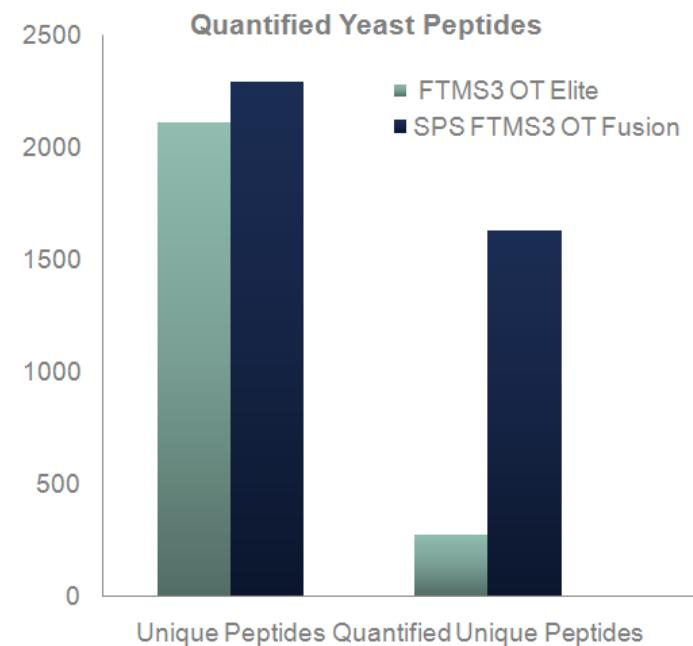
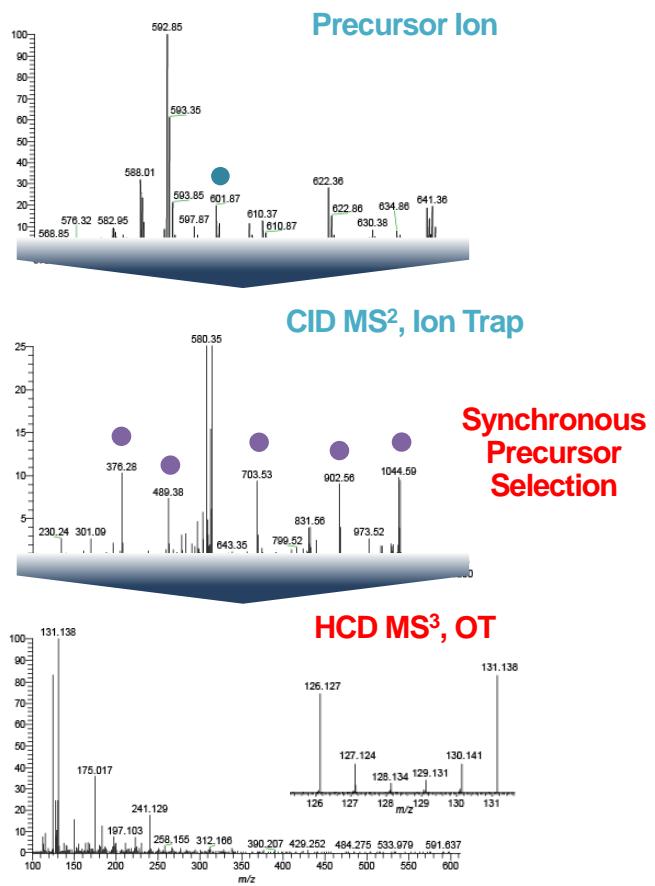
Ow, S.Y. et al. 2009. JPR 5347-5355
Ting, L. et al. 2011. *Nature Methods* 8 : 937-940

Co-isolation of Interfering Ions Affects Accuracy

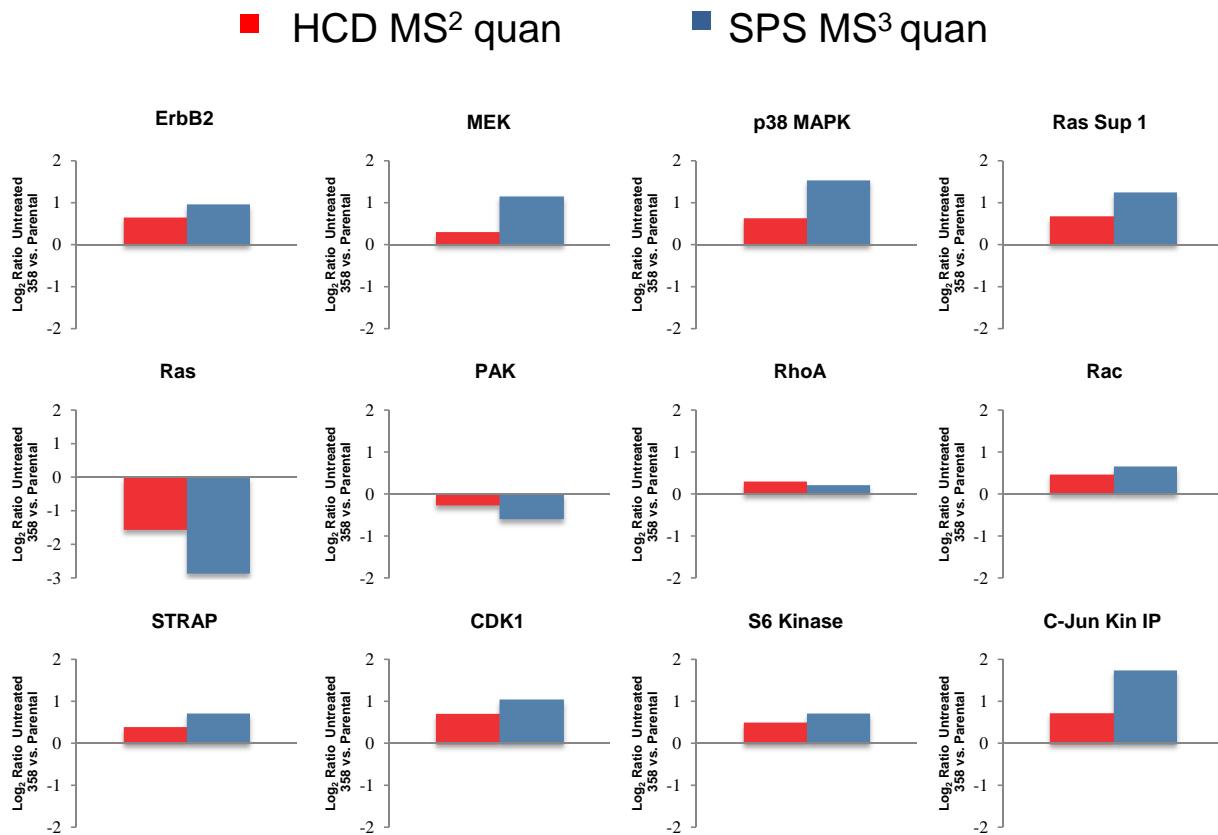


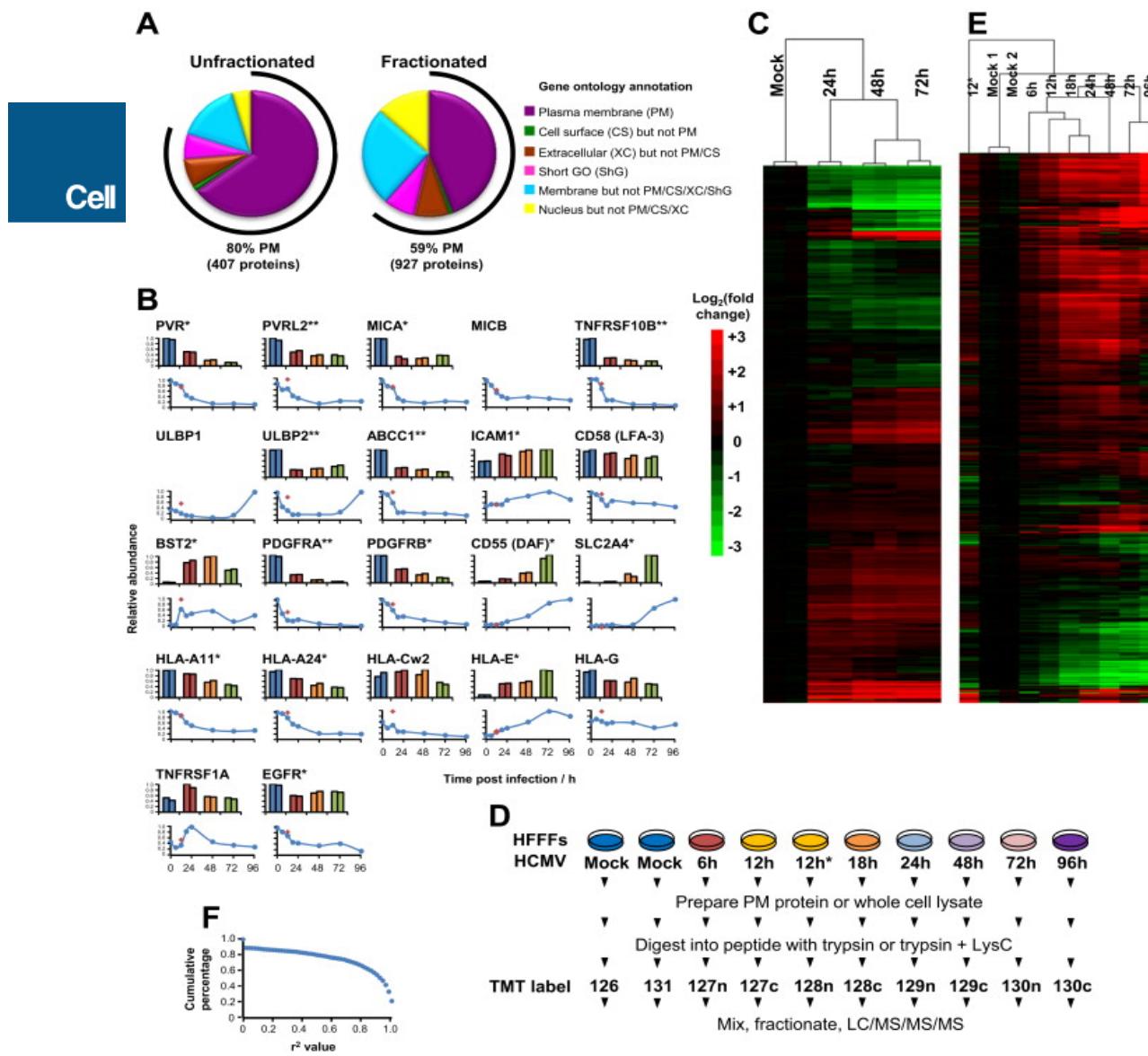
Results: Best possible accuracy and precision by reducing co-isolated interfering ions.

Synchronous Precursor Selection for Accurate Quan



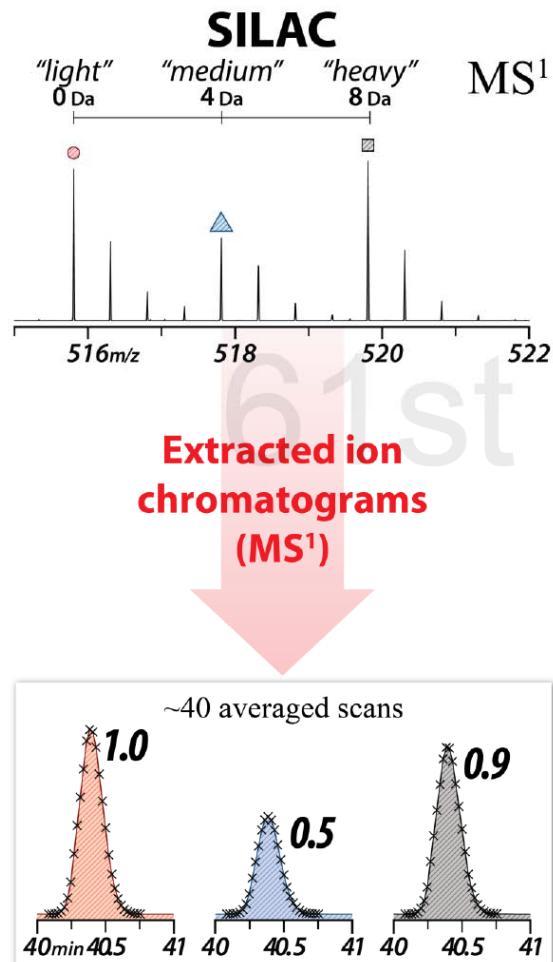
Enhanced Differences Using SPS MS³ Quantification





NeuCode labeling (SILAC or chemical labeling)

SILAC: Summary



good

***in vivo* labeling**

MS¹ quantification

limited interference

bad

increases MS¹ complexity

lowplexing capacity

Isobaric tagging: Summary

good

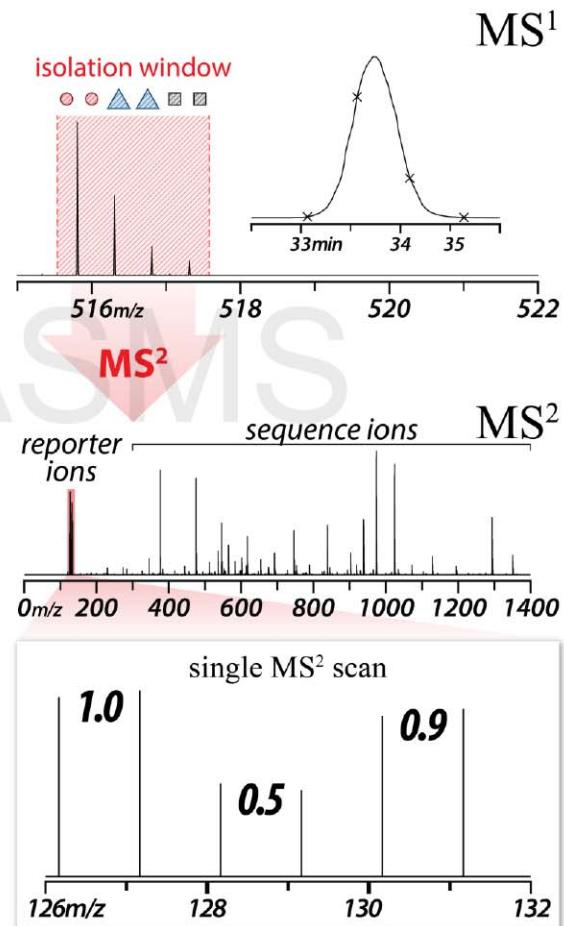
high levels ofplexing

MS¹ complexity not affected

bad

**low accuracy/
interference**

must have MS/MS



isobaric tagging

good

high levels ofplexing

**MS¹ complexity not
affected**

bad

~~low accuracy/
interference~~

~~must have MS/MS~~

SILAC

good

***in vivo* labeling**

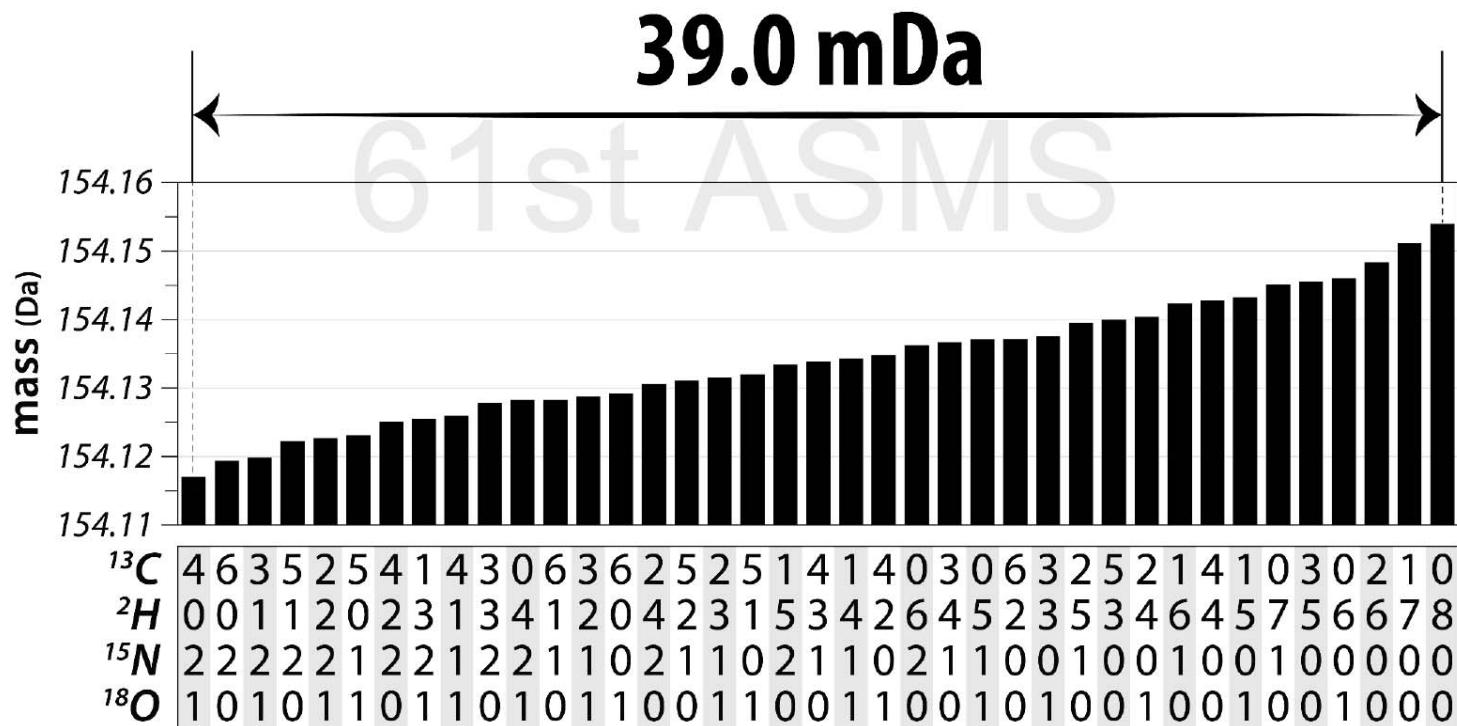
**MS¹ quantification
limited interference**

bad

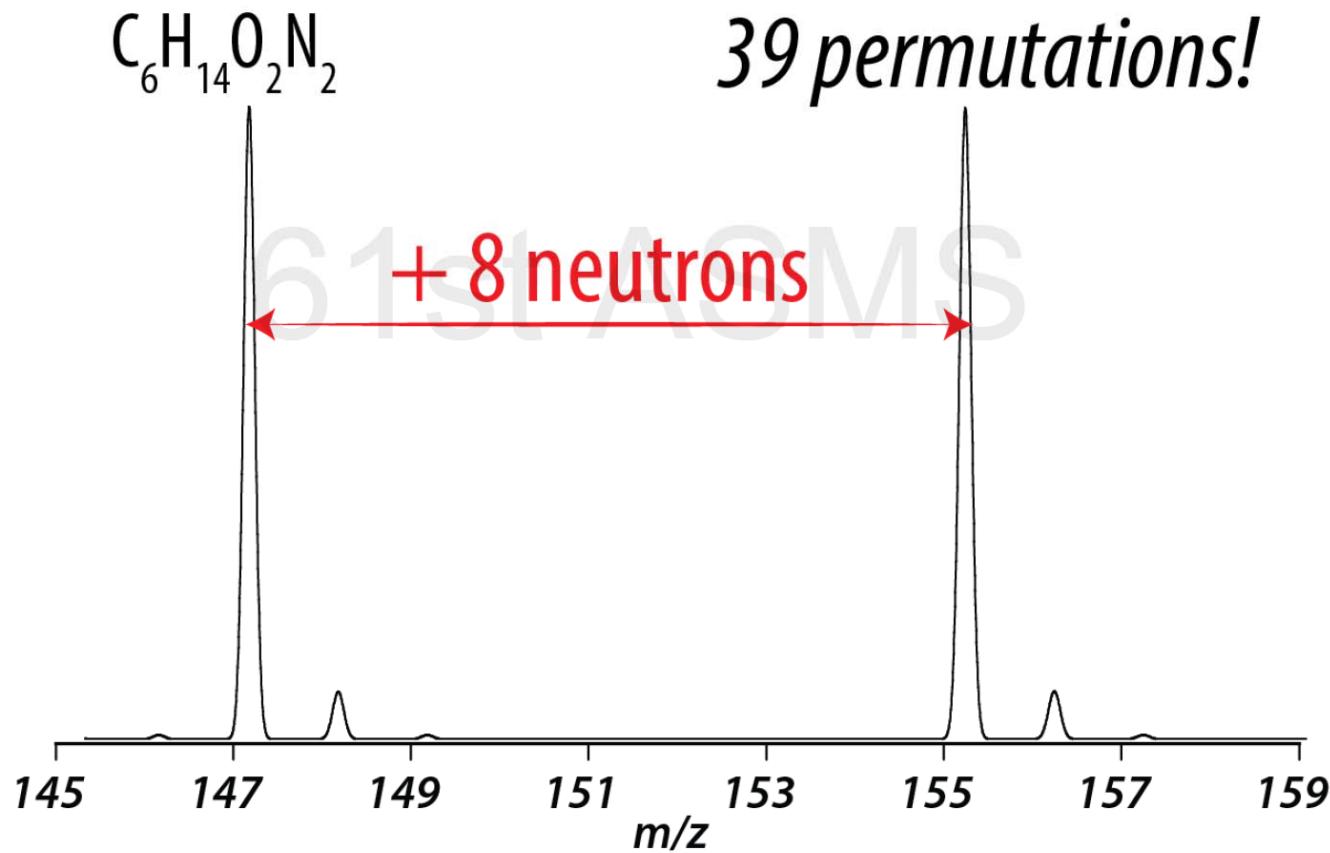
~~increases MS¹
complexity~~

~~low plexing capacity~~

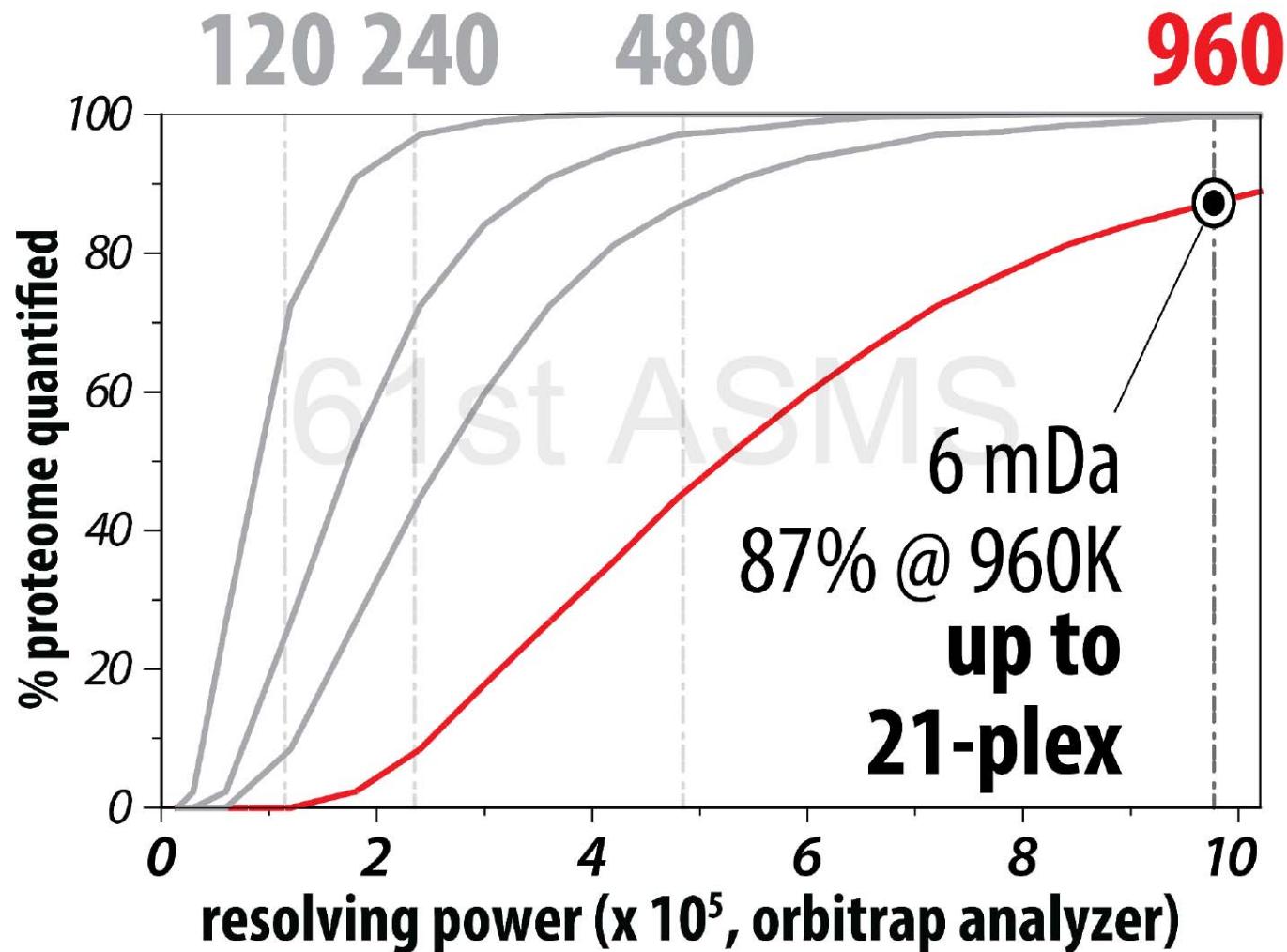
+8 Da Lysine has 39 isotopologues!



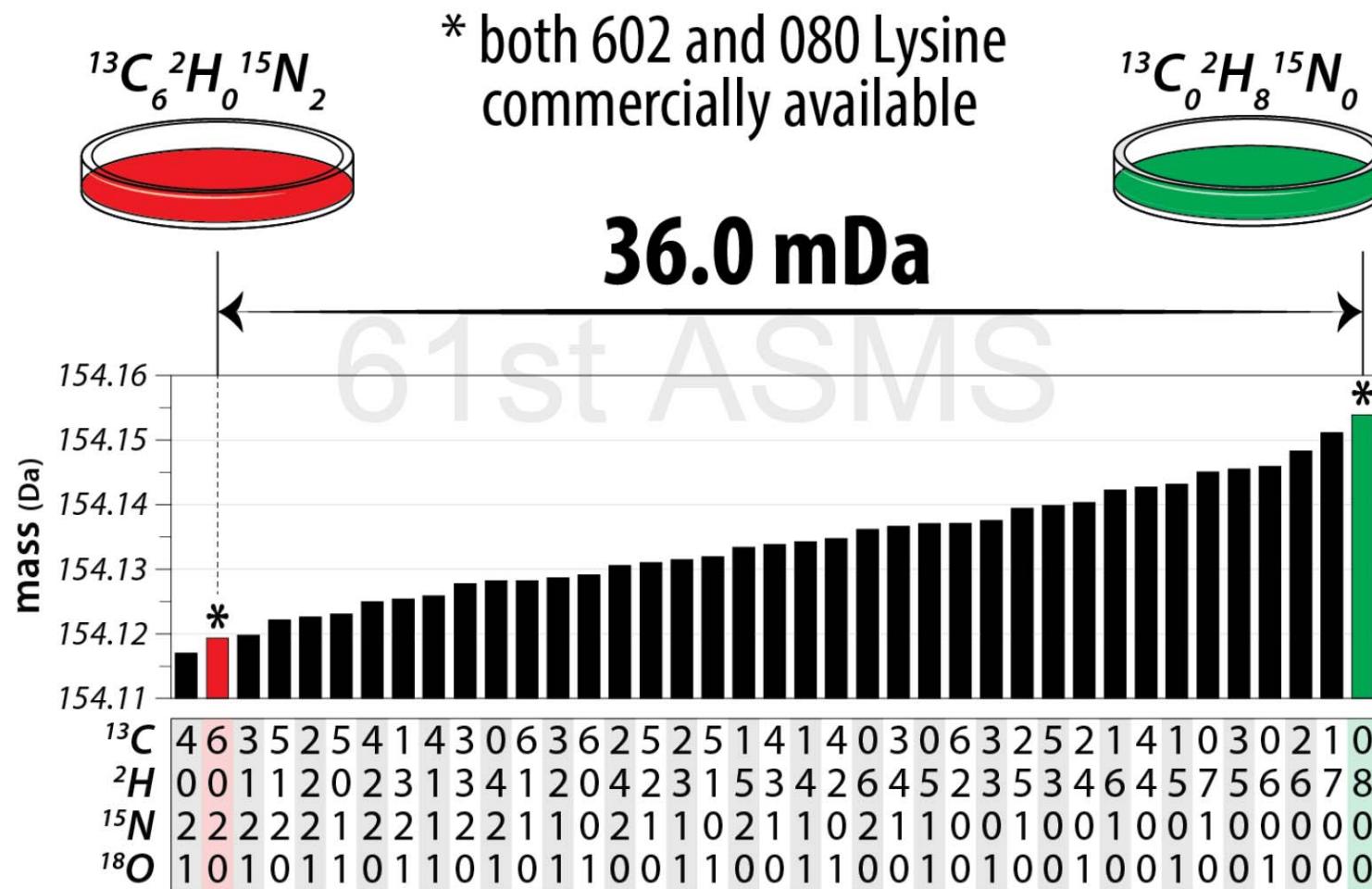
NeuCode Lysine (+8 Da) isotopologues



NeuCode plexing scales with resolution



2-Plex NeuCode SILAC



3-Plex NeuCode SILAC

