Applications of Skyline for Method Development and Quantification of Histone PTMs

Josue Baeza, Lindsay K Pino, Joseph Cesare, Hee Jong Kim, Benjamin A Garcia

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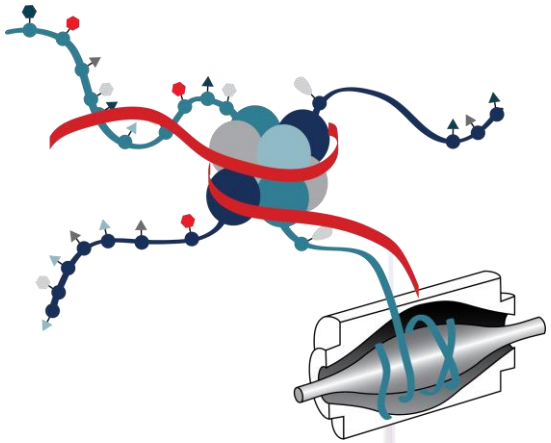
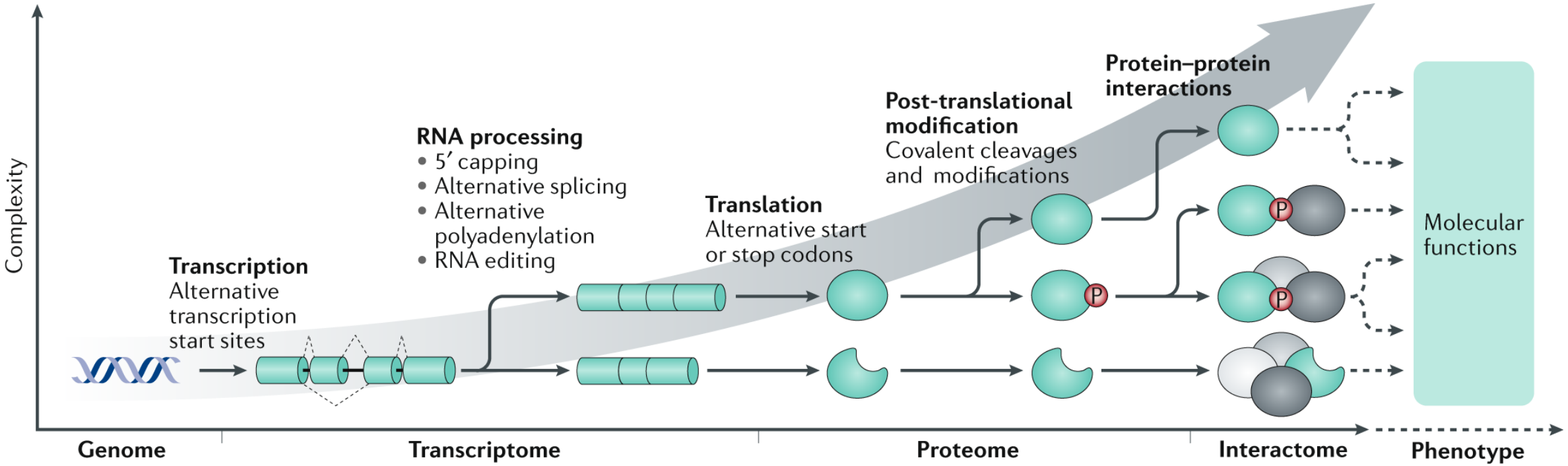
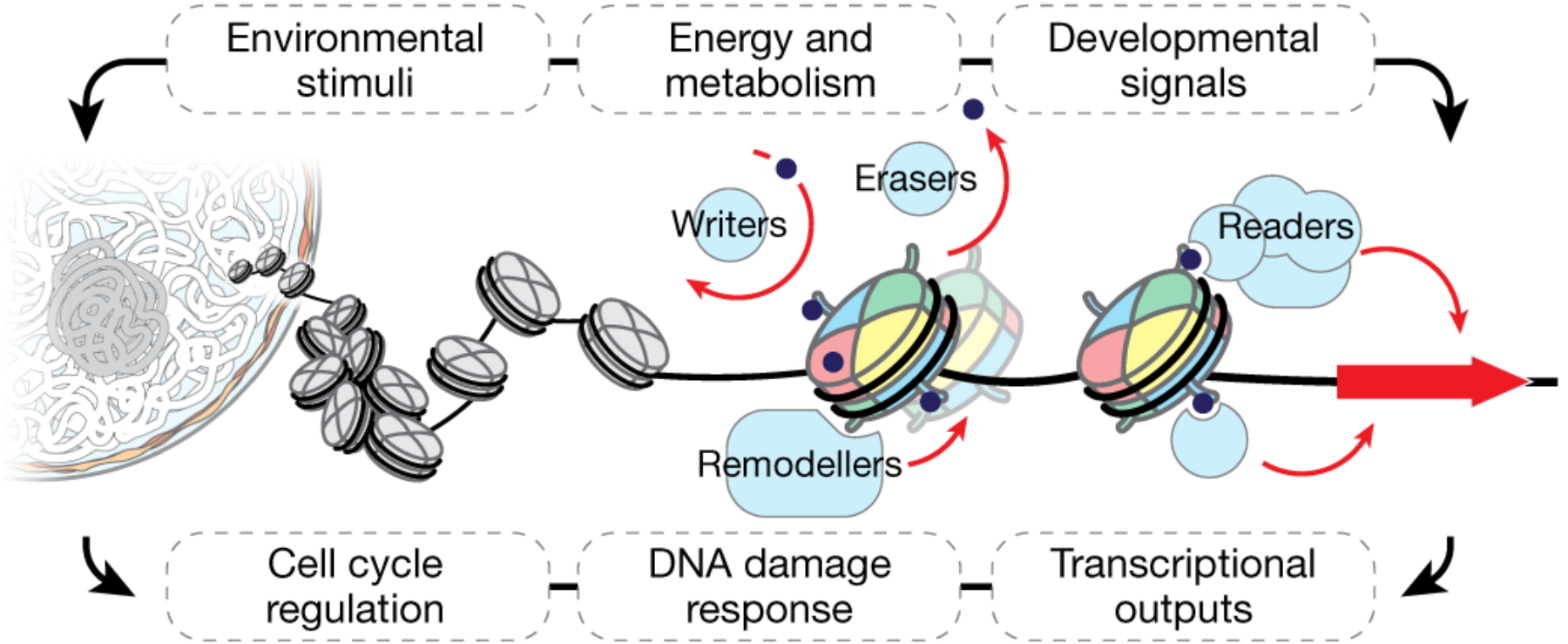


Molecular diversity in the cell



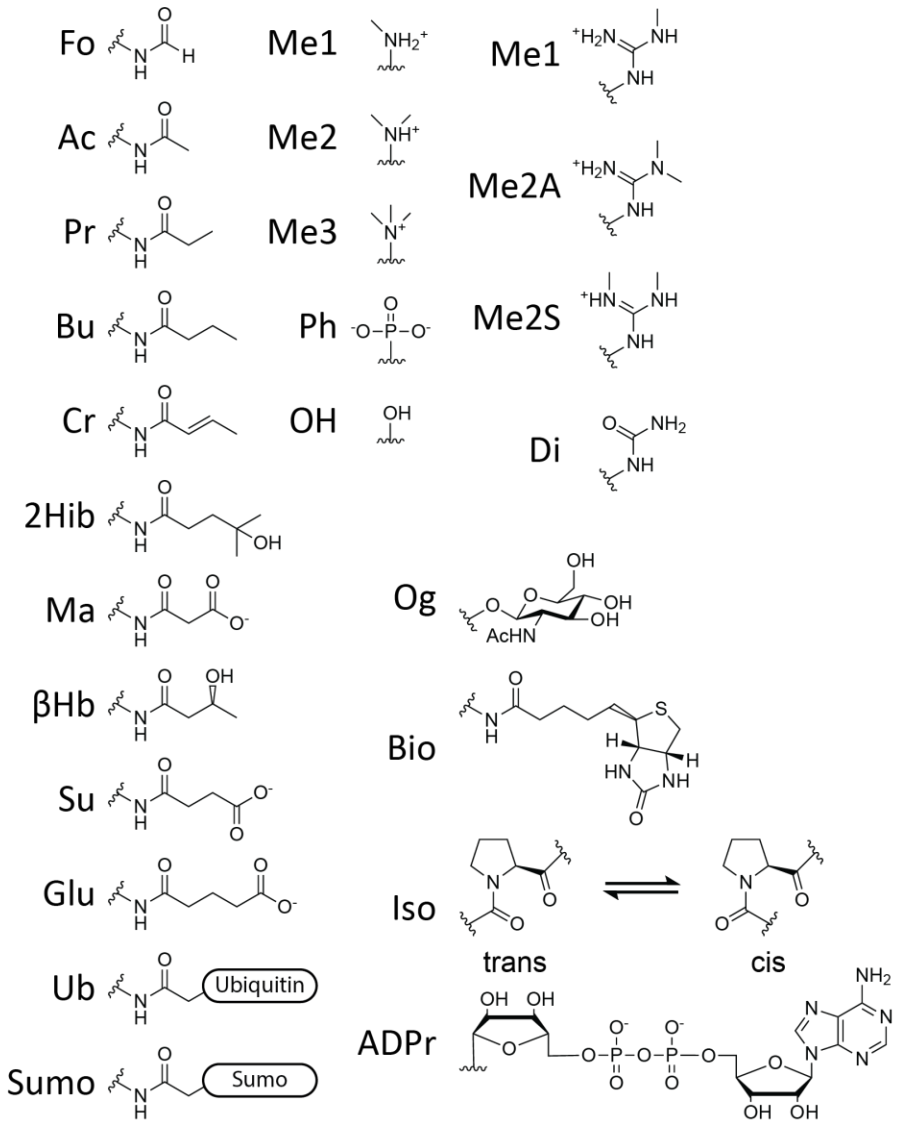
Bludau et.al. (2020) https://doi.org/10.1038/s41580-020-0231-2

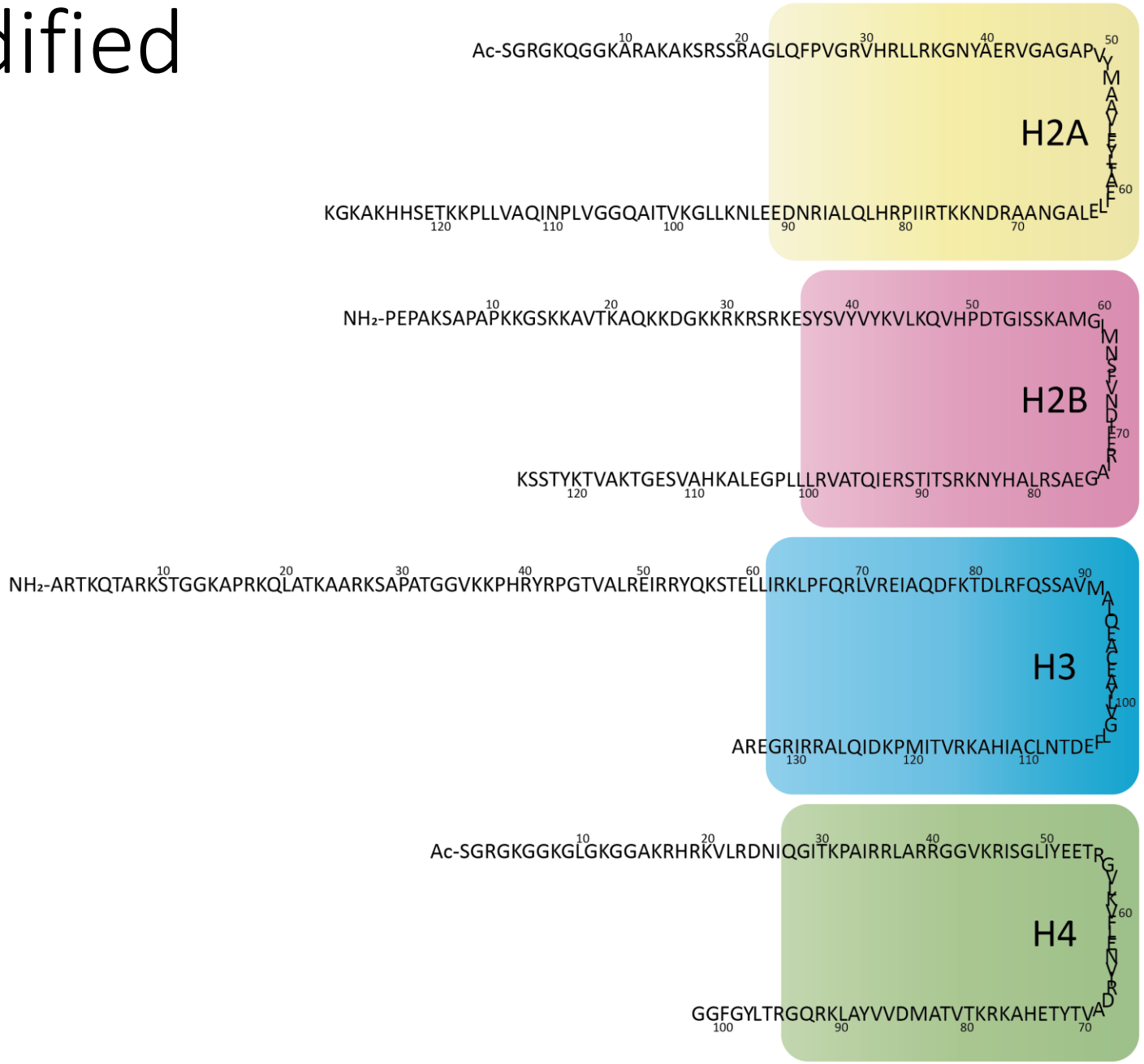
Histones are the gatekeepers of gene expression



Nacev et.al. (2019) https://doi.org/10.1038/s41586-019-1038-1

Histones are highly modified





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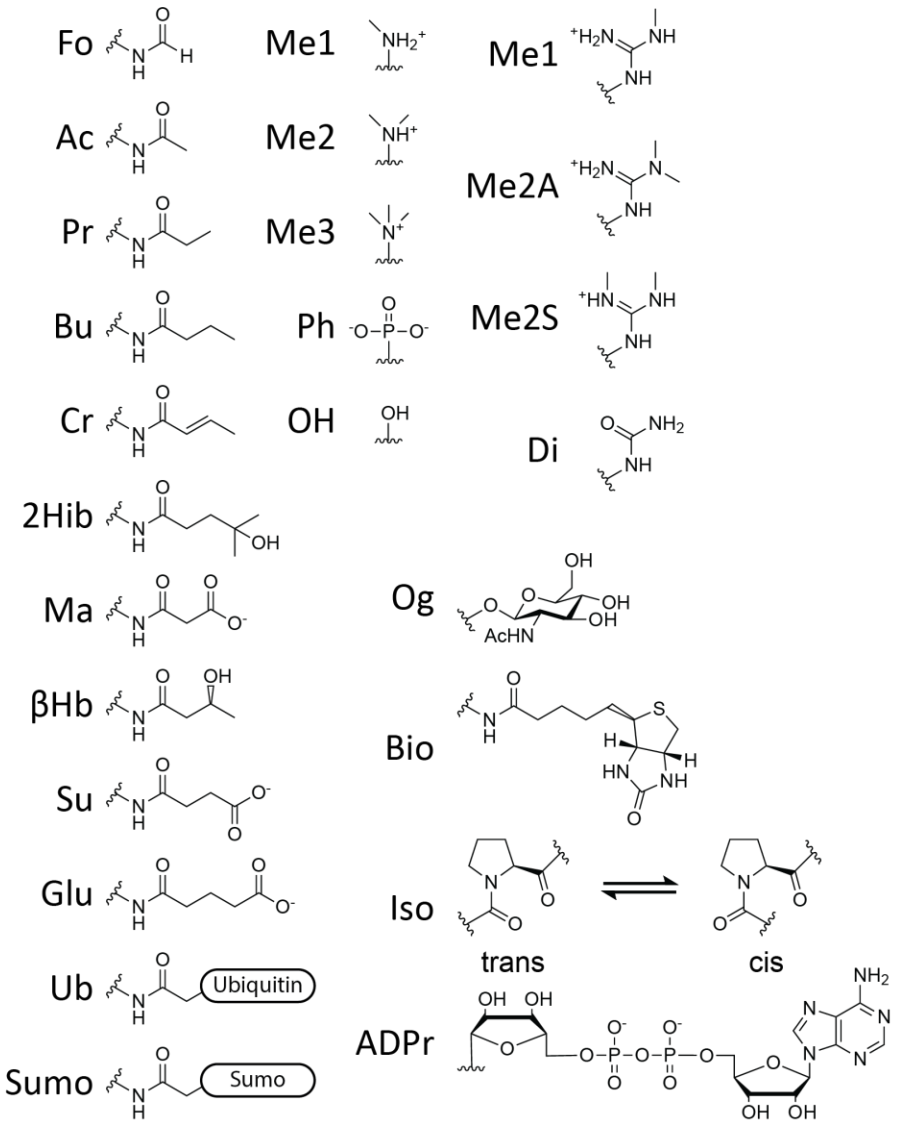

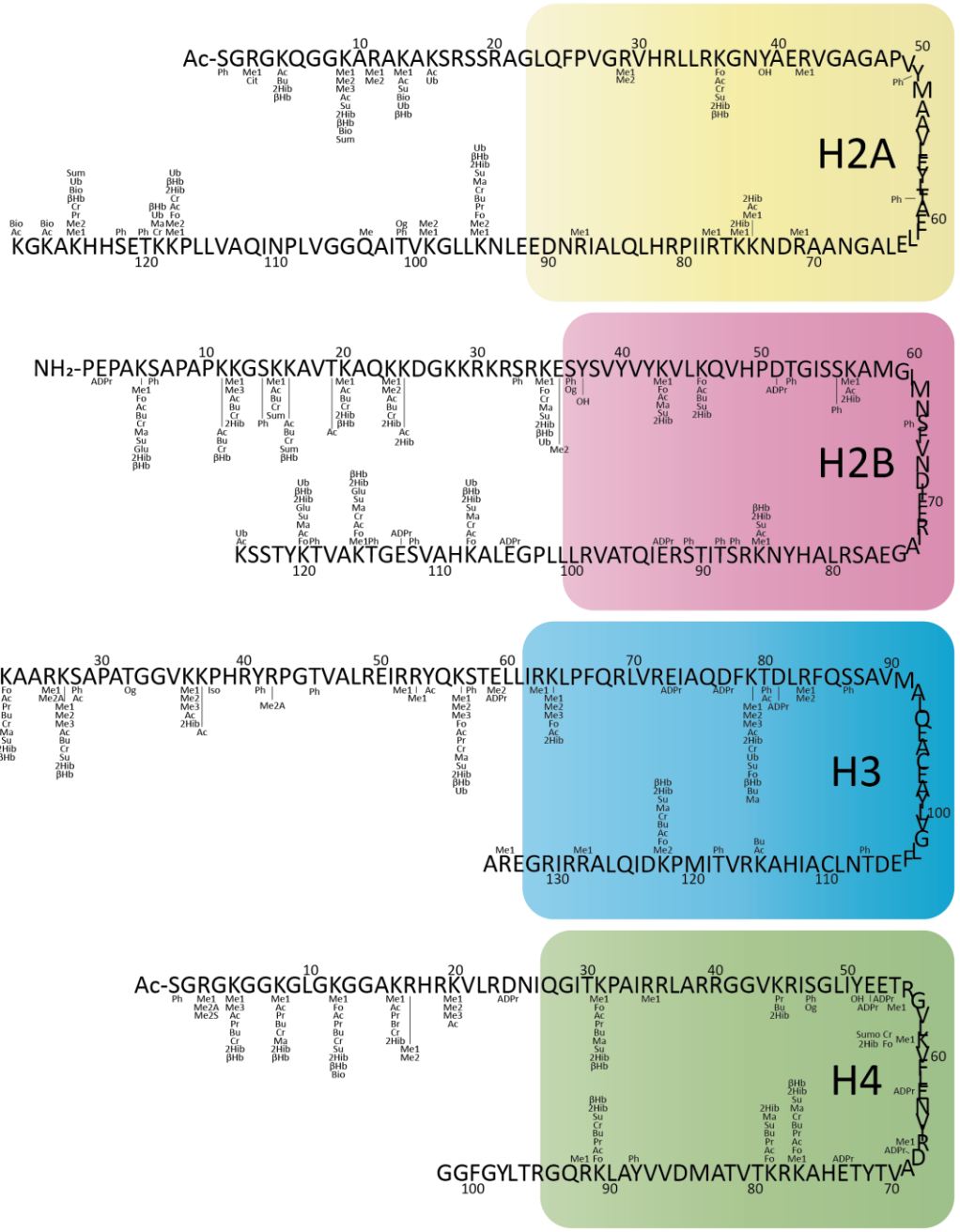

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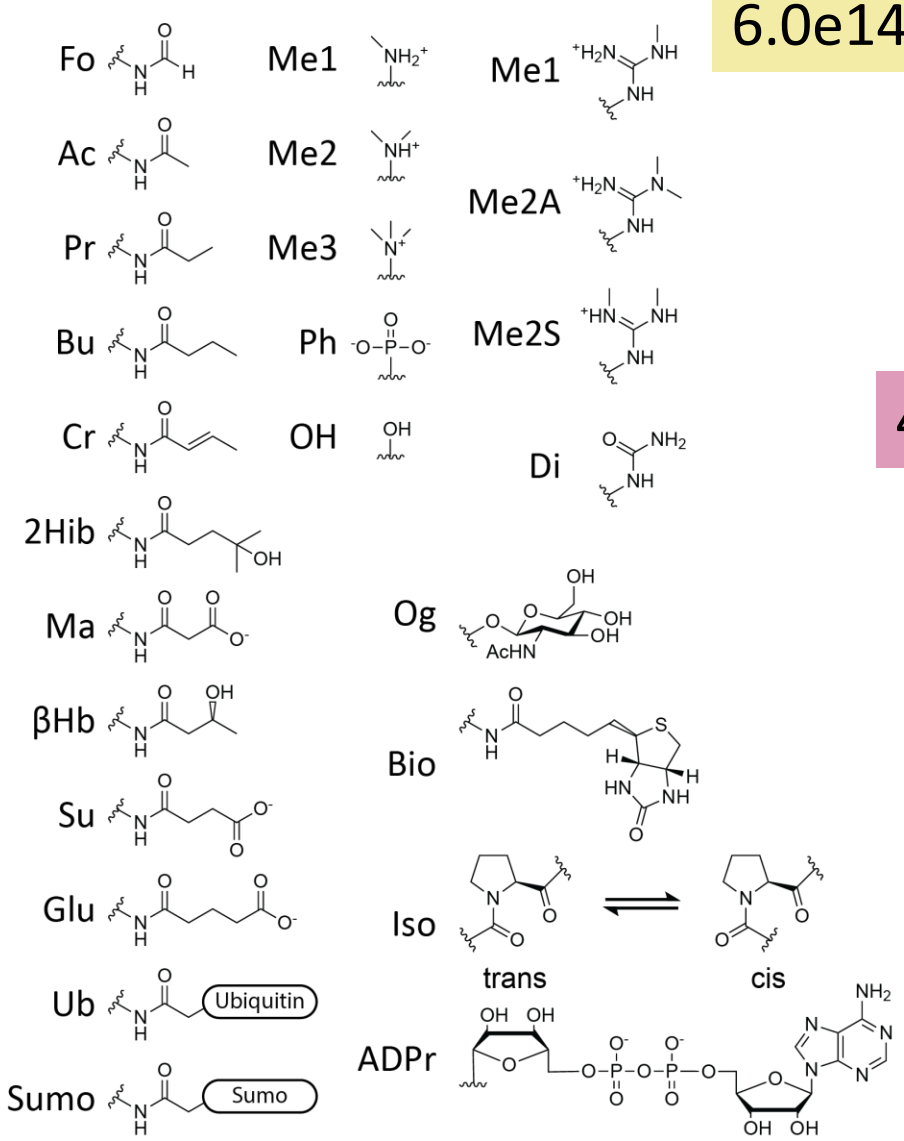
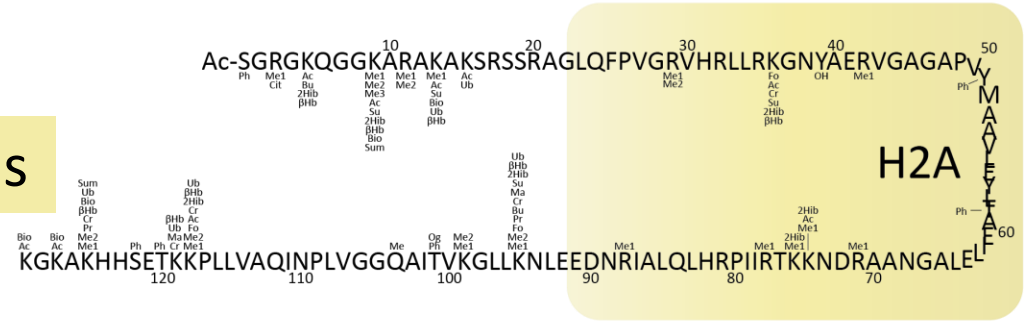
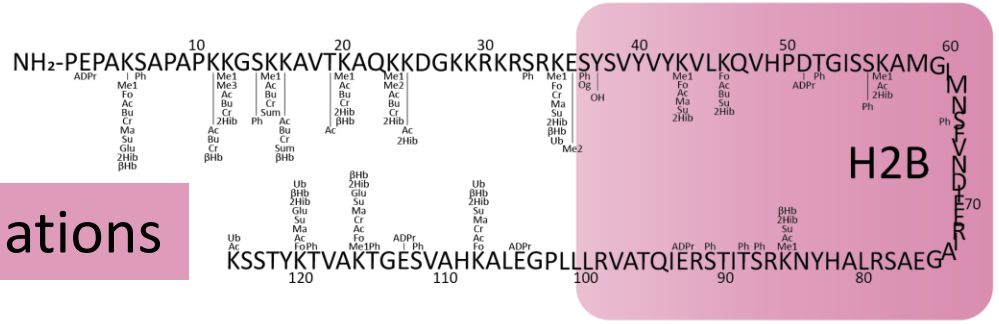


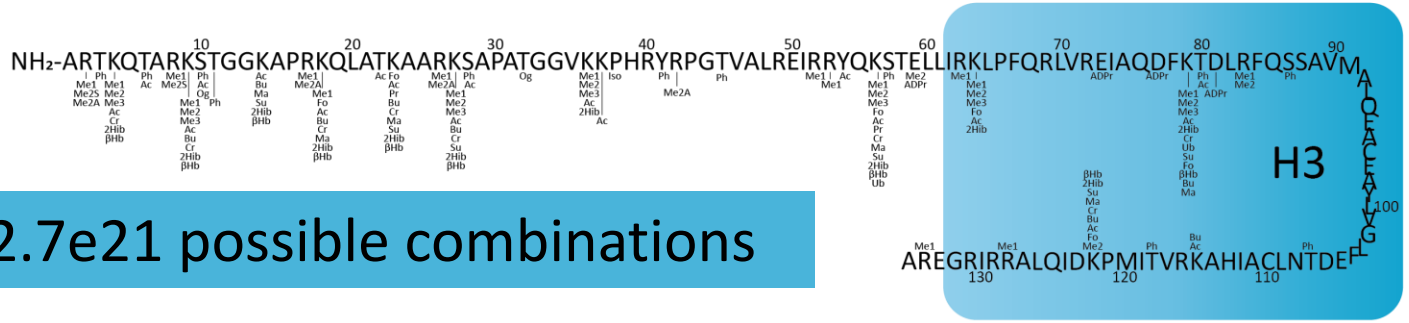
6.0e14 possible combinations



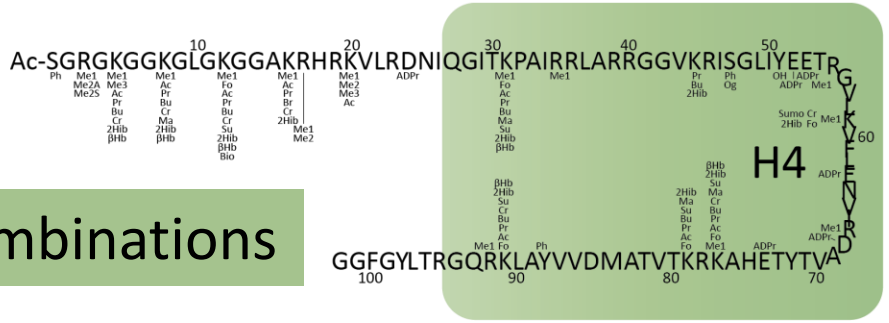
4.4e19 possible combinations



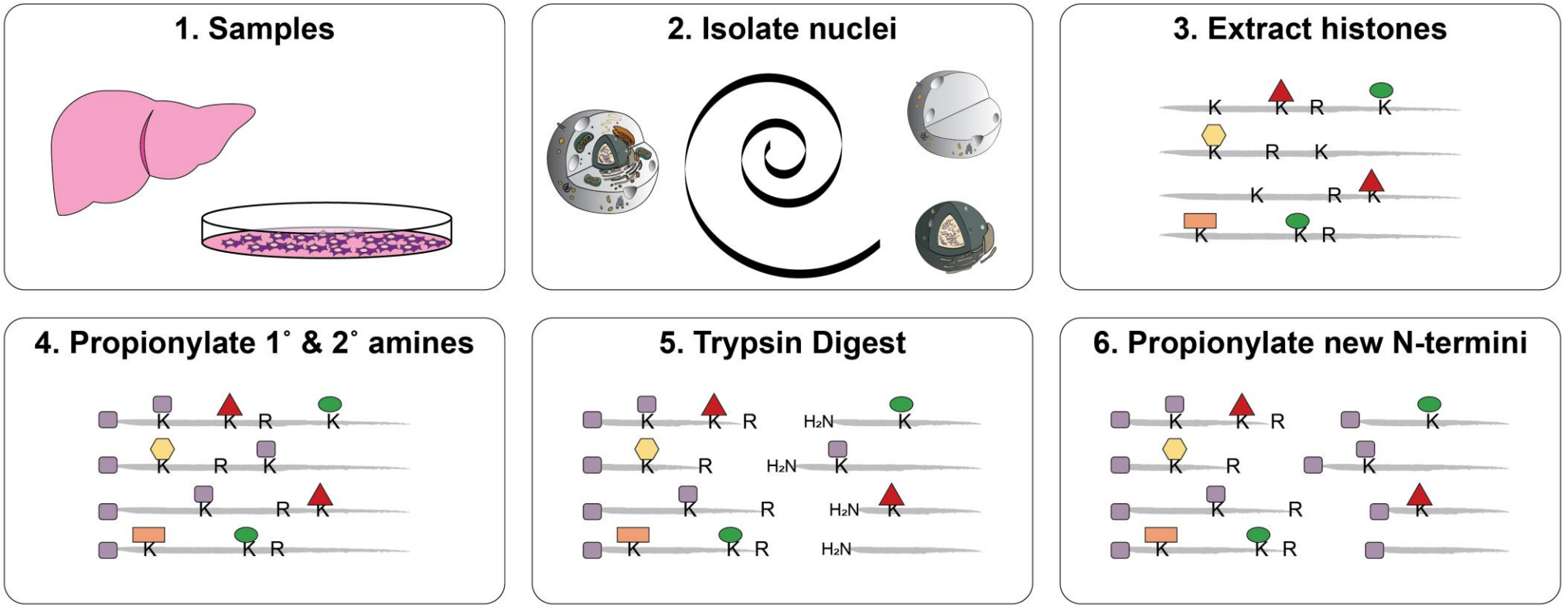
2.7e21 possible combinations



4.4e14 possible combinations



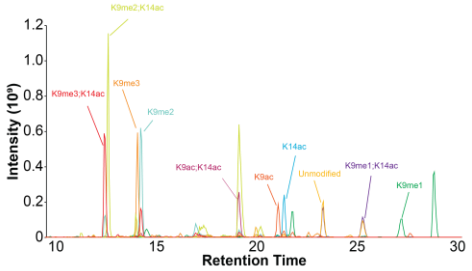
Preparing histone samples for Mass Spectrometry analysis



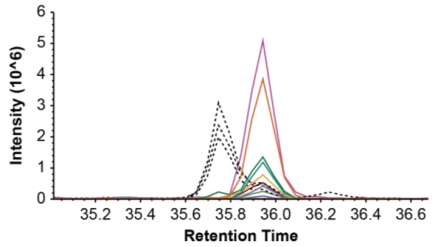
Detailed protocol at: Sidoli et.al. (2016) http://dx.doi.org/10.3791/54112

Improving histone PTM method development and quantification with Skyline

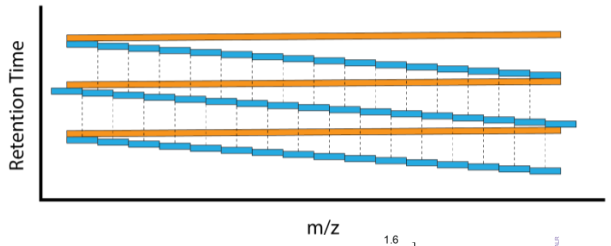
Challenges with quantifying histone PTMs



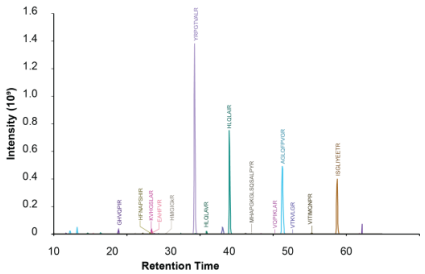
Site-localizing transitions for PTM quantification.



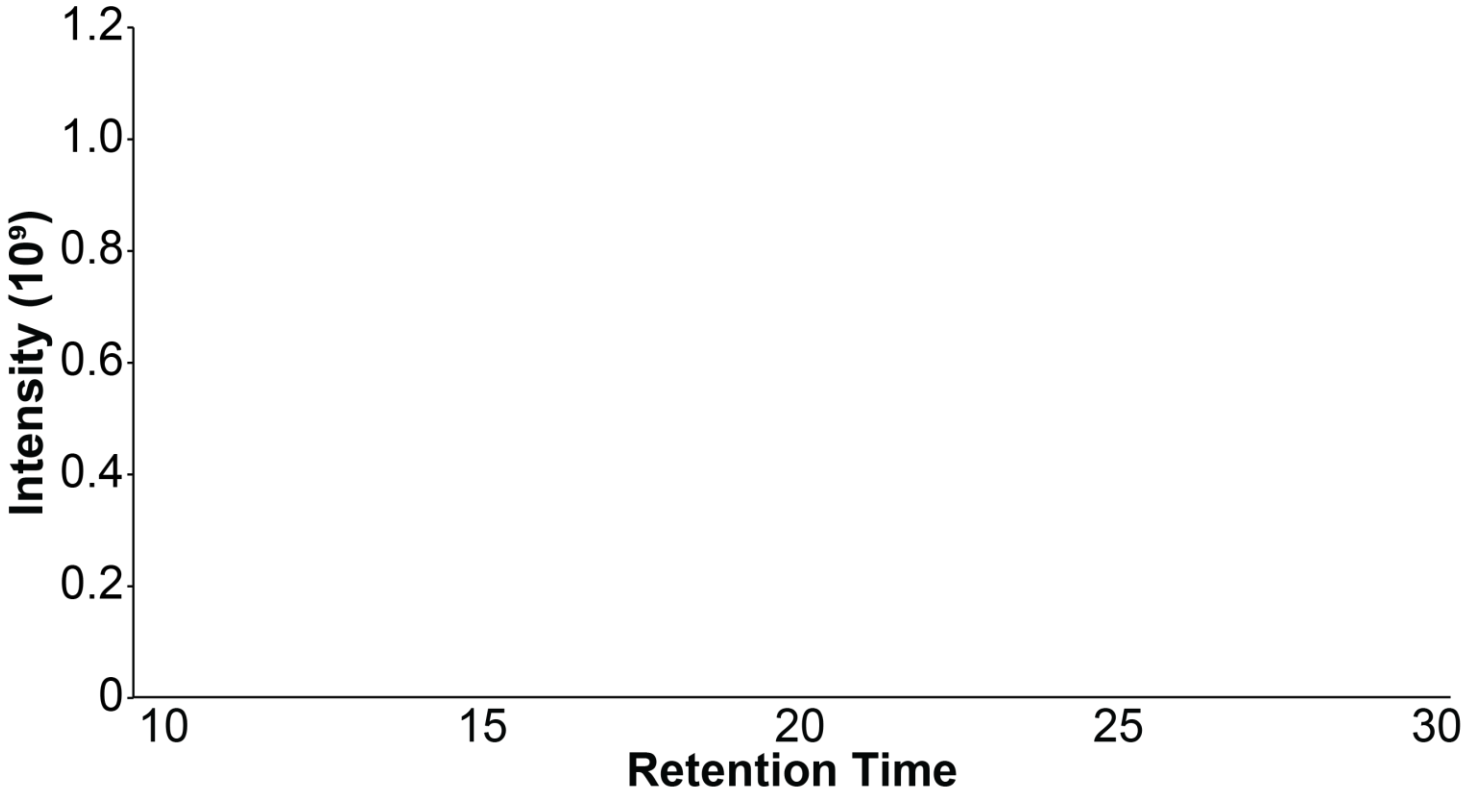
Staggered windows increases precursor selectivity



Co-enriched histone iRT peptides for retention time calibration.



Challenges in quantifying histone PTMs



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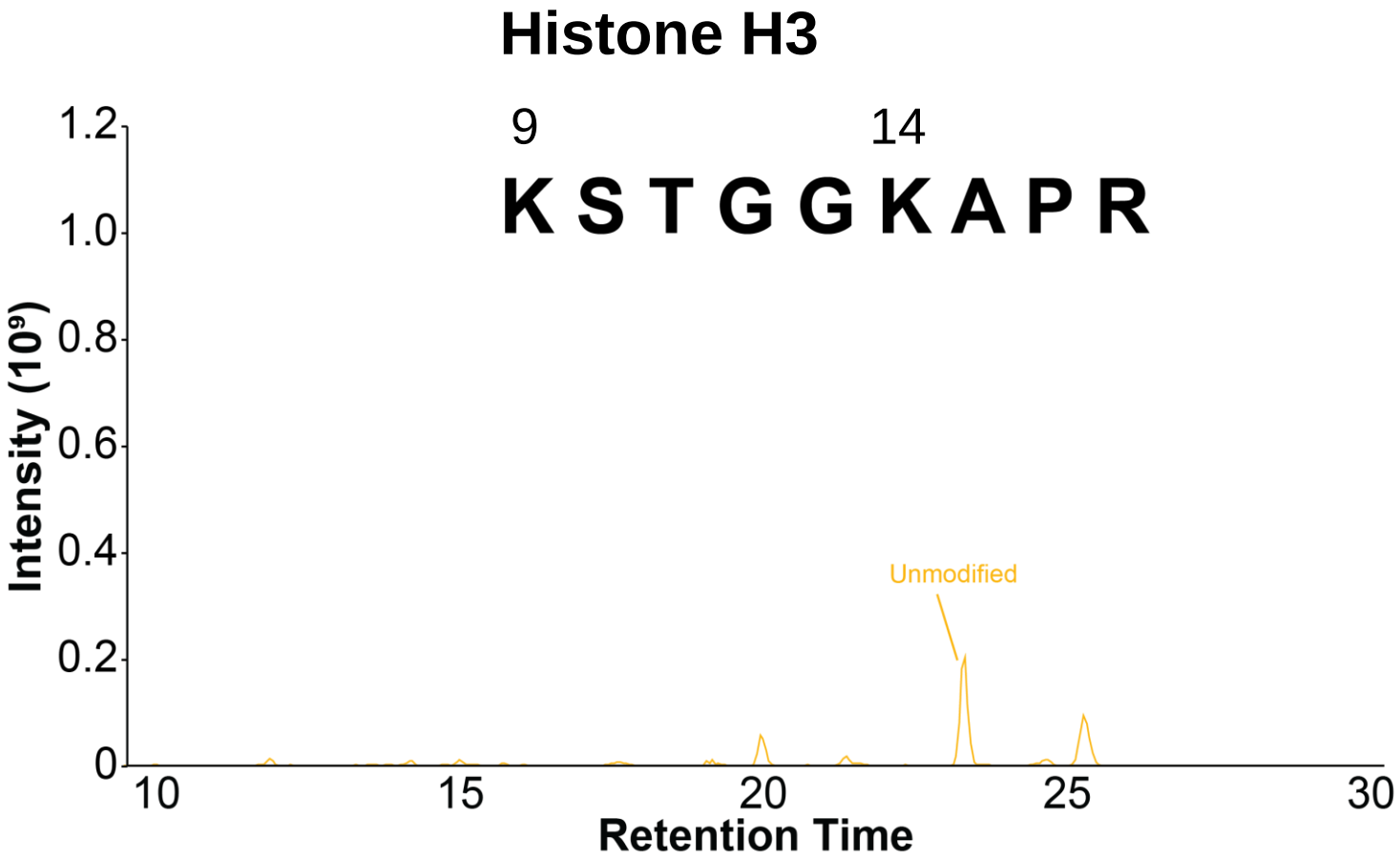
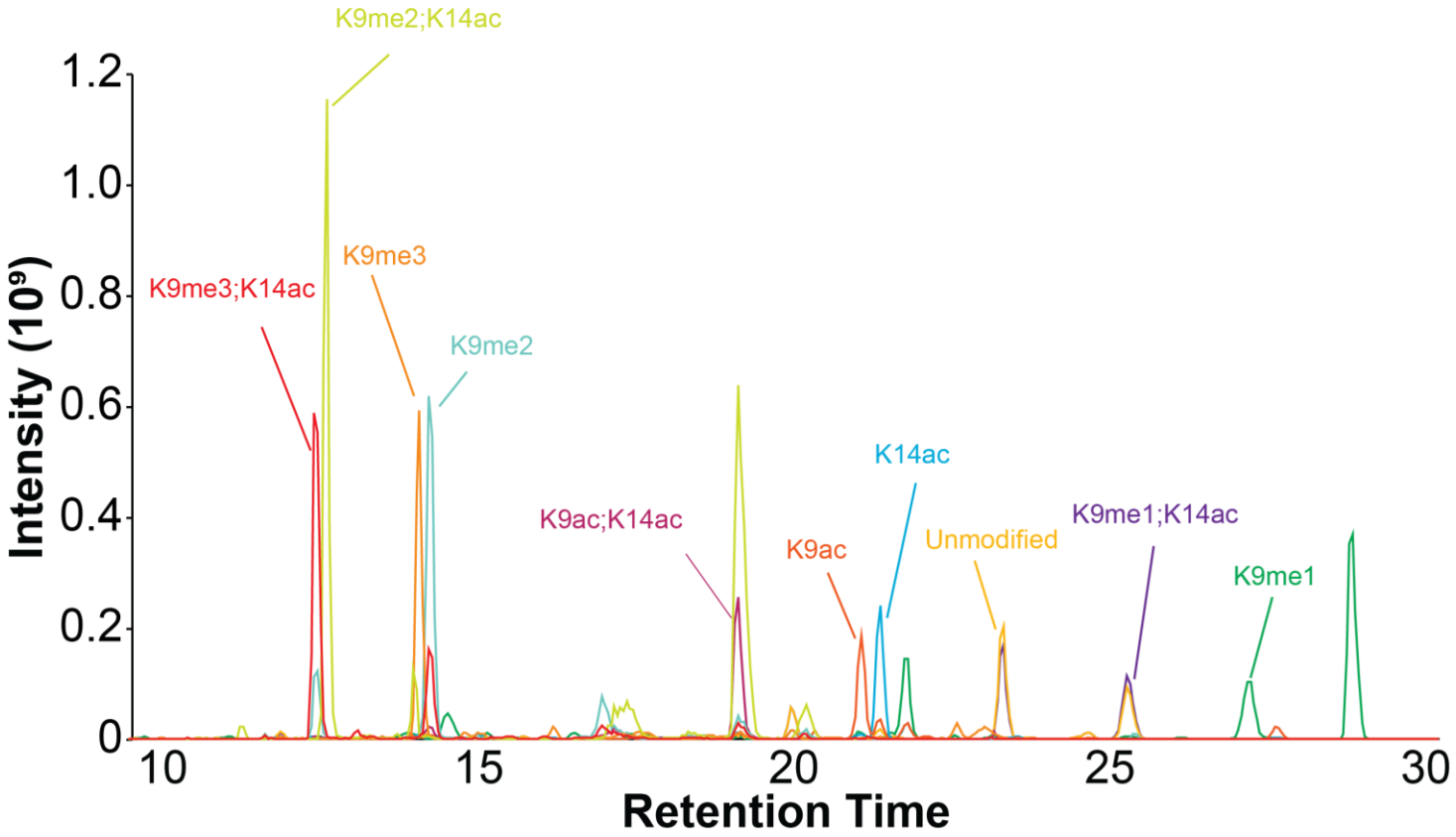


Challenges in quantifying histone PTMs

10 modified forms are shown
Serine phosphorylation also occurs

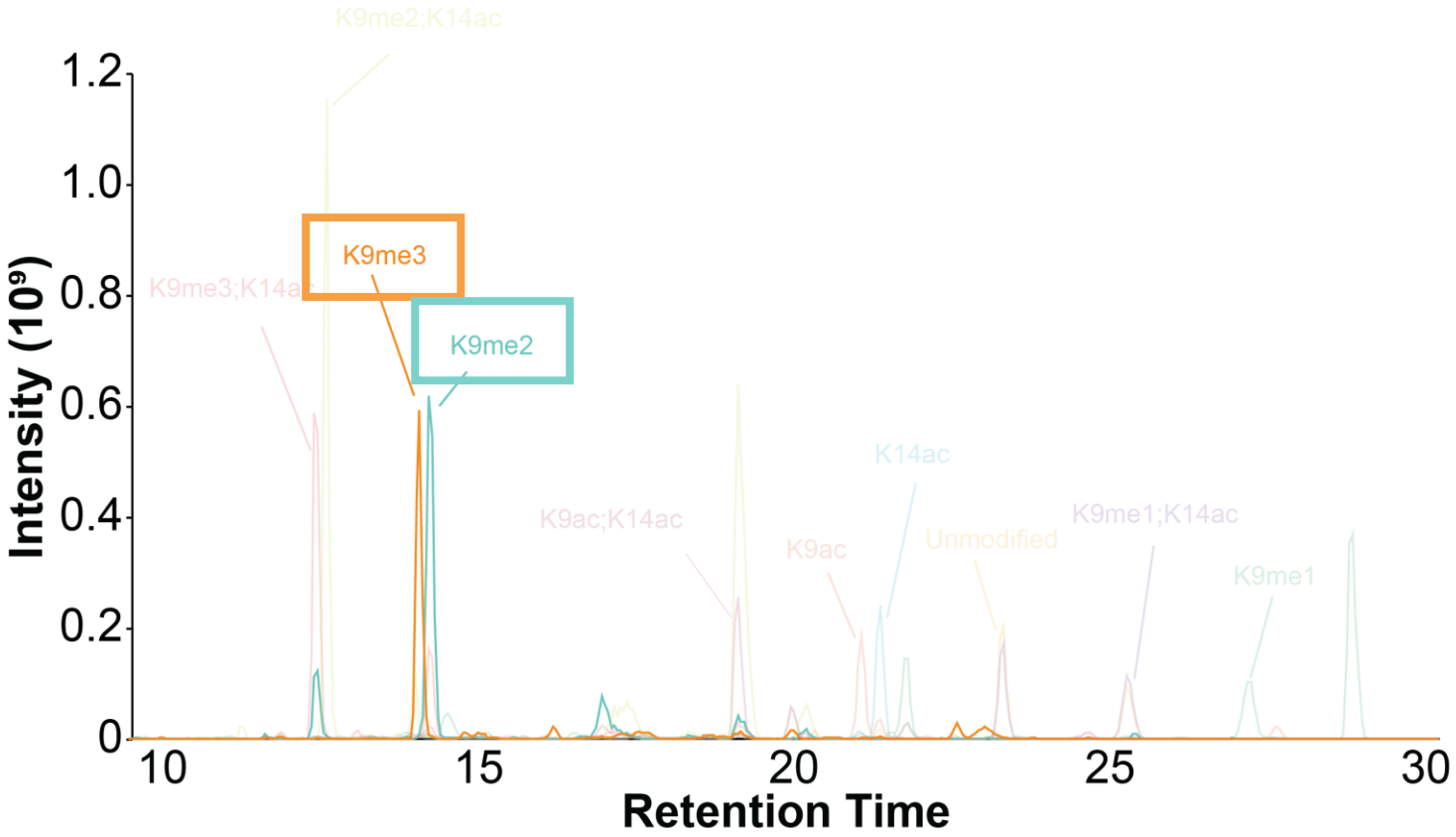


- Highly modified peptides

pr K S T G G K A P R pr	unmodified
ac K S T G G K A P R ac	K9ac;K14ac
ac K S T G G K A P R pr	K9ac
pr K S T G G K A P R ac	K14ac
me1 K S T G G K A P R pr	K9me1
me2 K S T G G K A P R pr	K9me2
me3 K S T G G K A P R pr	K9me3
me1 K S T G G K A P R ac	K9me1;K14ac
me2 K S T G G K A P R ac	K9me2;K14ac
me3 K S T G G K A P R ac	K9me3;K14ac

Challenges in quantifying histone PTMs

- Highly modified peptides
- Co-eluting/overlapping RT



Challenges in quantifying histone PTMs

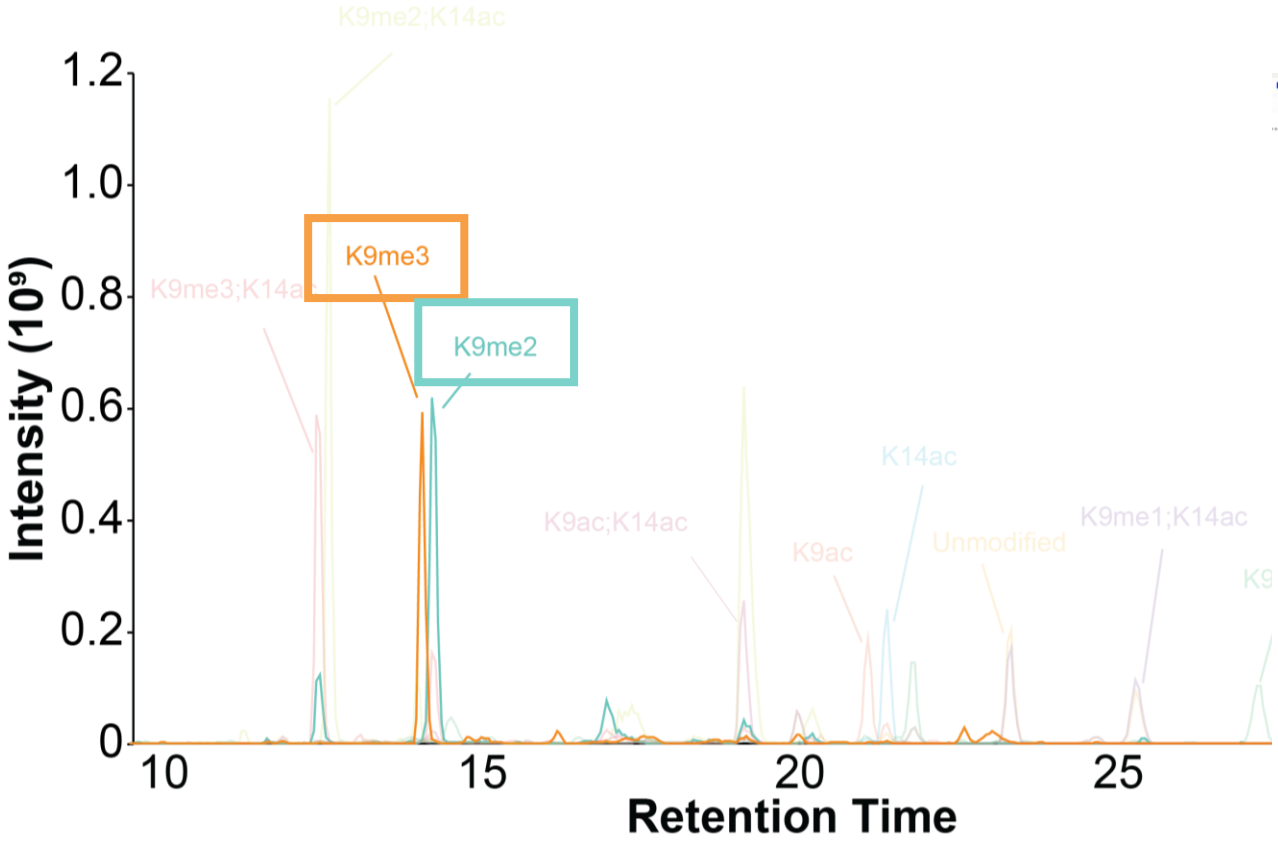
$K_{me2}STGGK_{pr}APR$

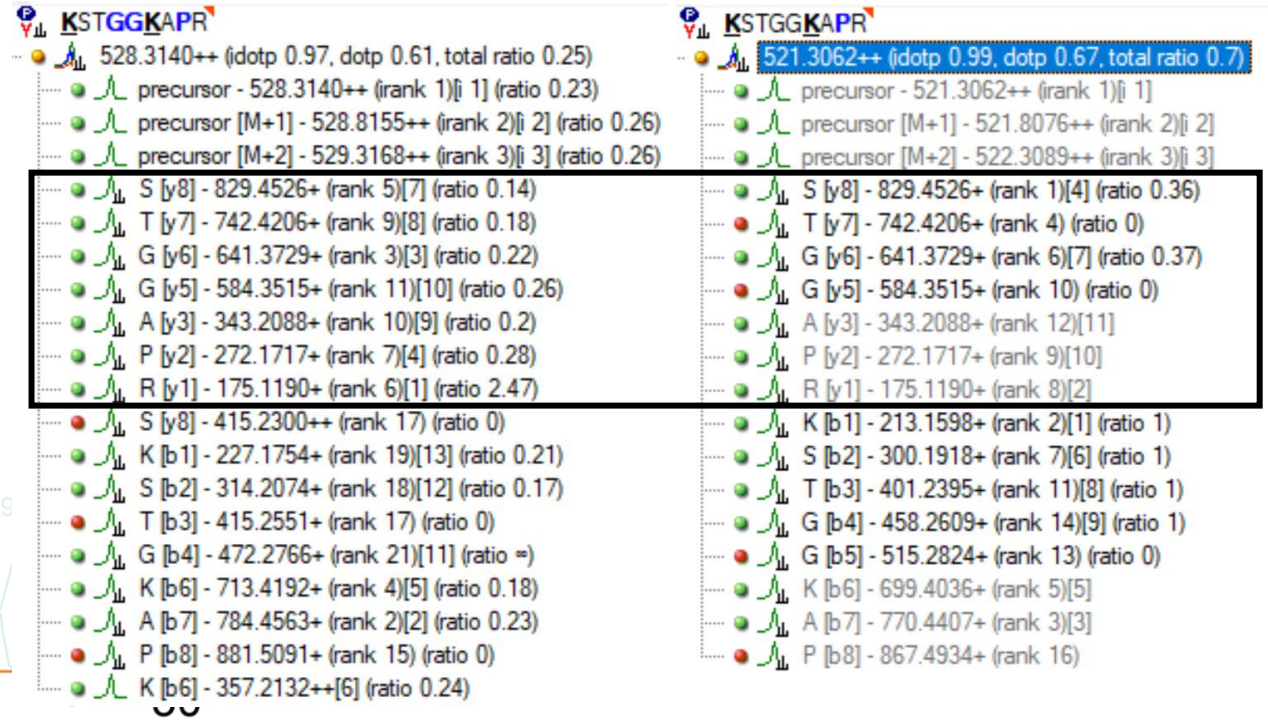
$K_{me3}STGGK_{pr}APR$

Shared transitions

PTM localizing transitions

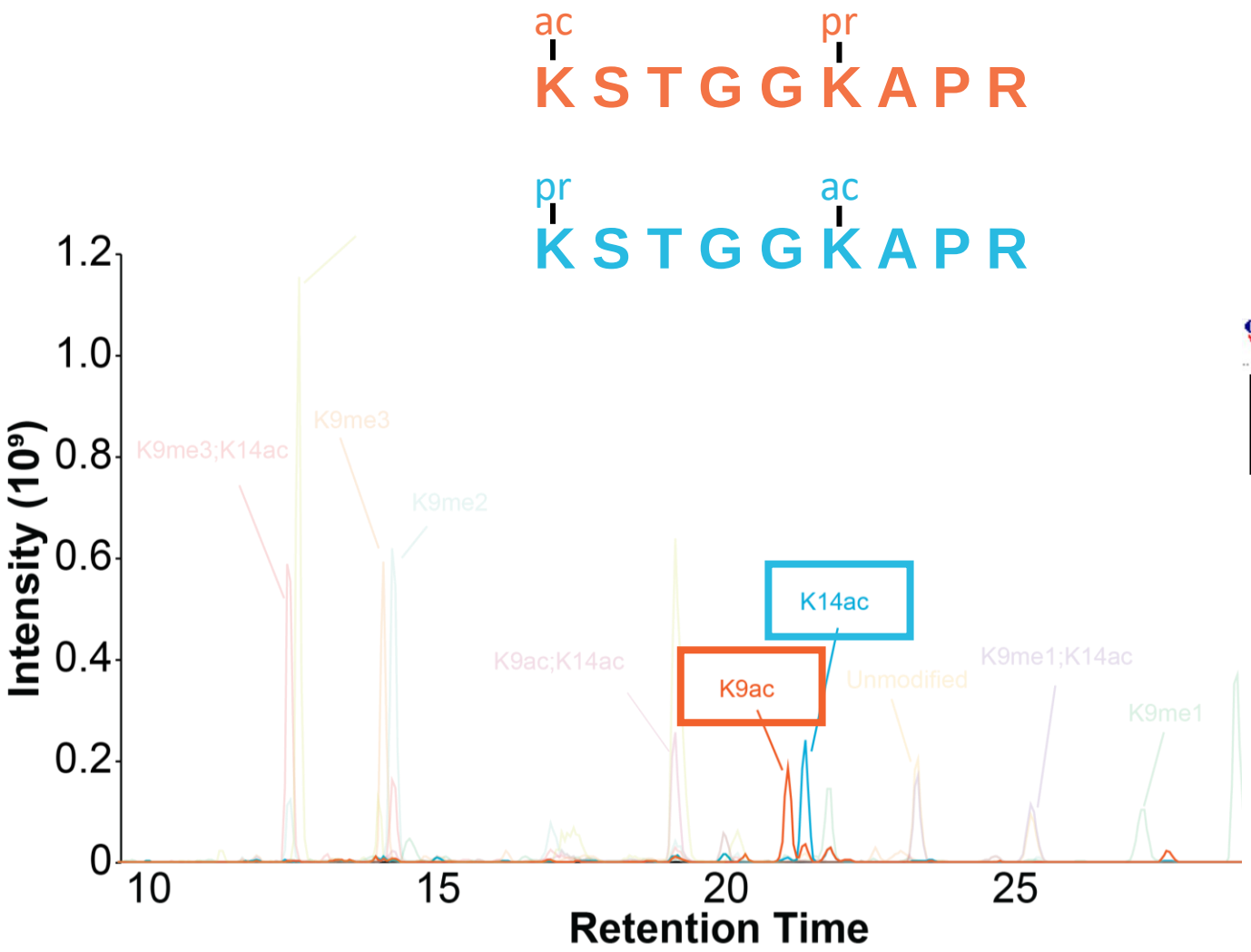
- Highly modified peptides
- Co-eluting/overlapping RT
- Shared transition ions

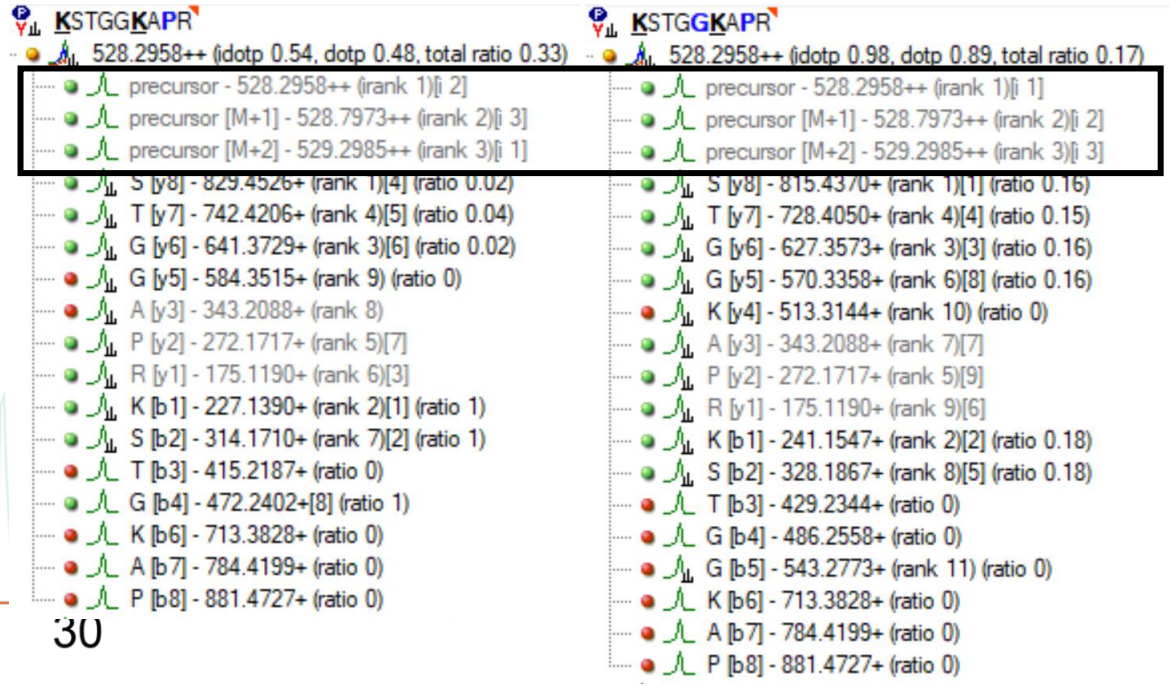




Challenges in quantifying histone PTMs

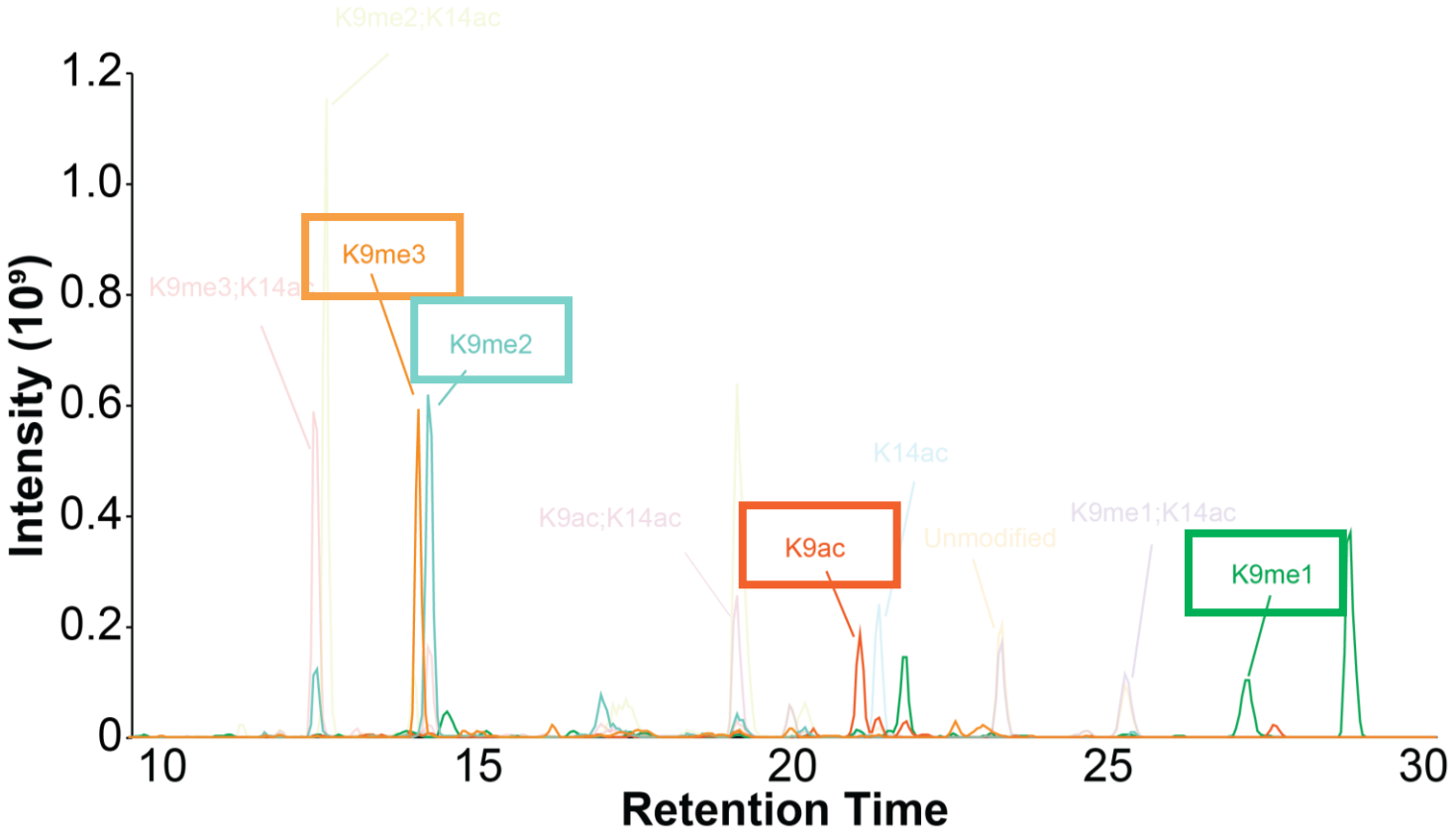
- Highly modified peptides
- Co-eluting/overlapping RT
- Shared transition ions
- Positional isomers





Why is this elution profile observed?

K9me3 < K9me2 < K9ac < K9me1



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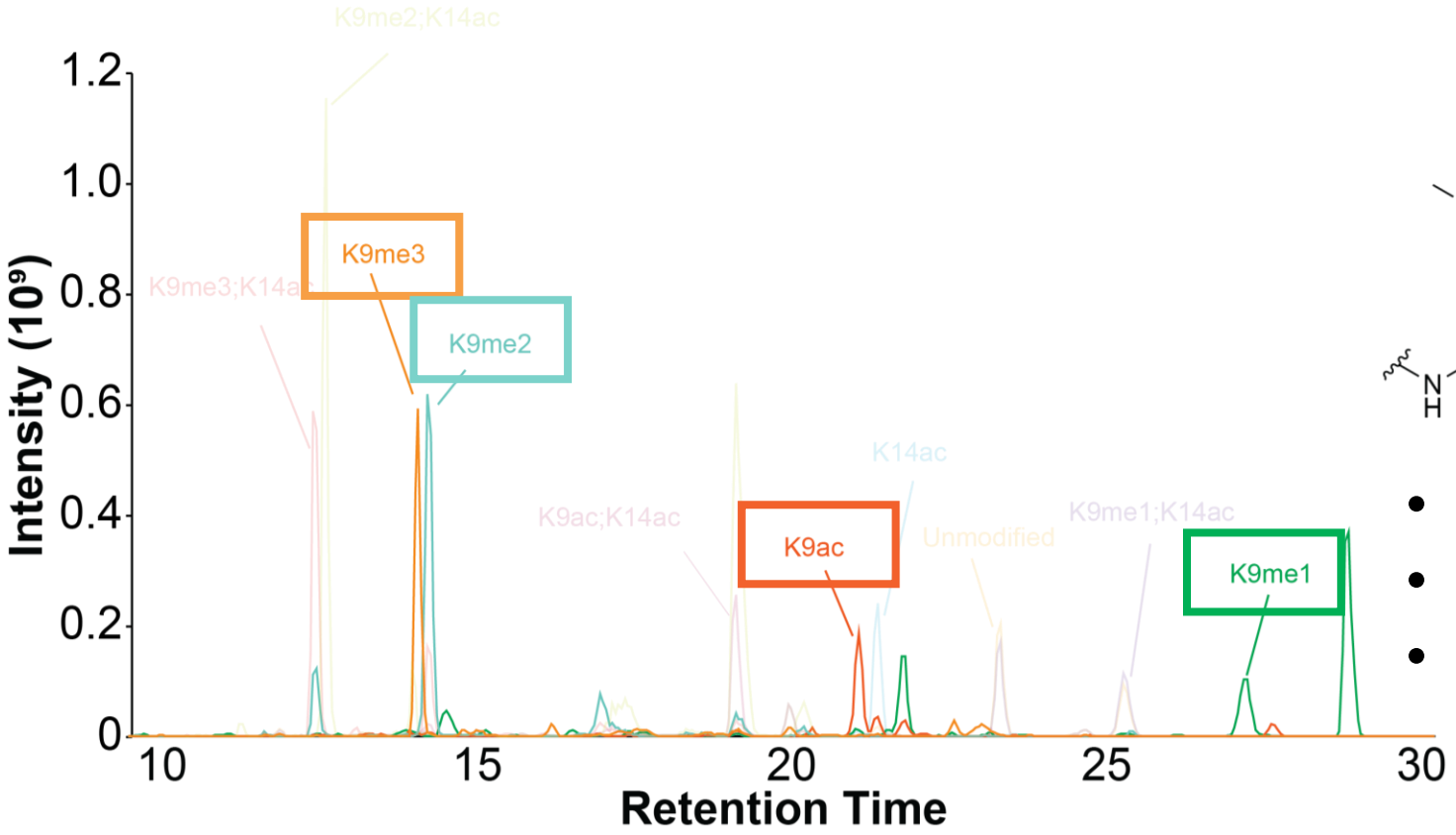
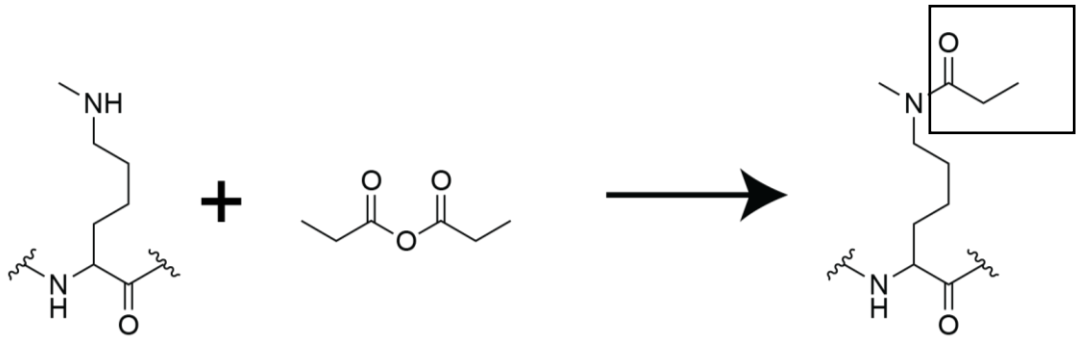


Not biological



- Lysine me1 is a 2° amine
- 2° amine can also be propionylated
- Forms an N-methyl,N-proprionyl lysine

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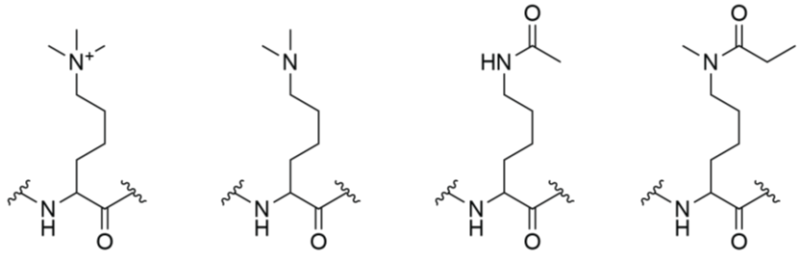
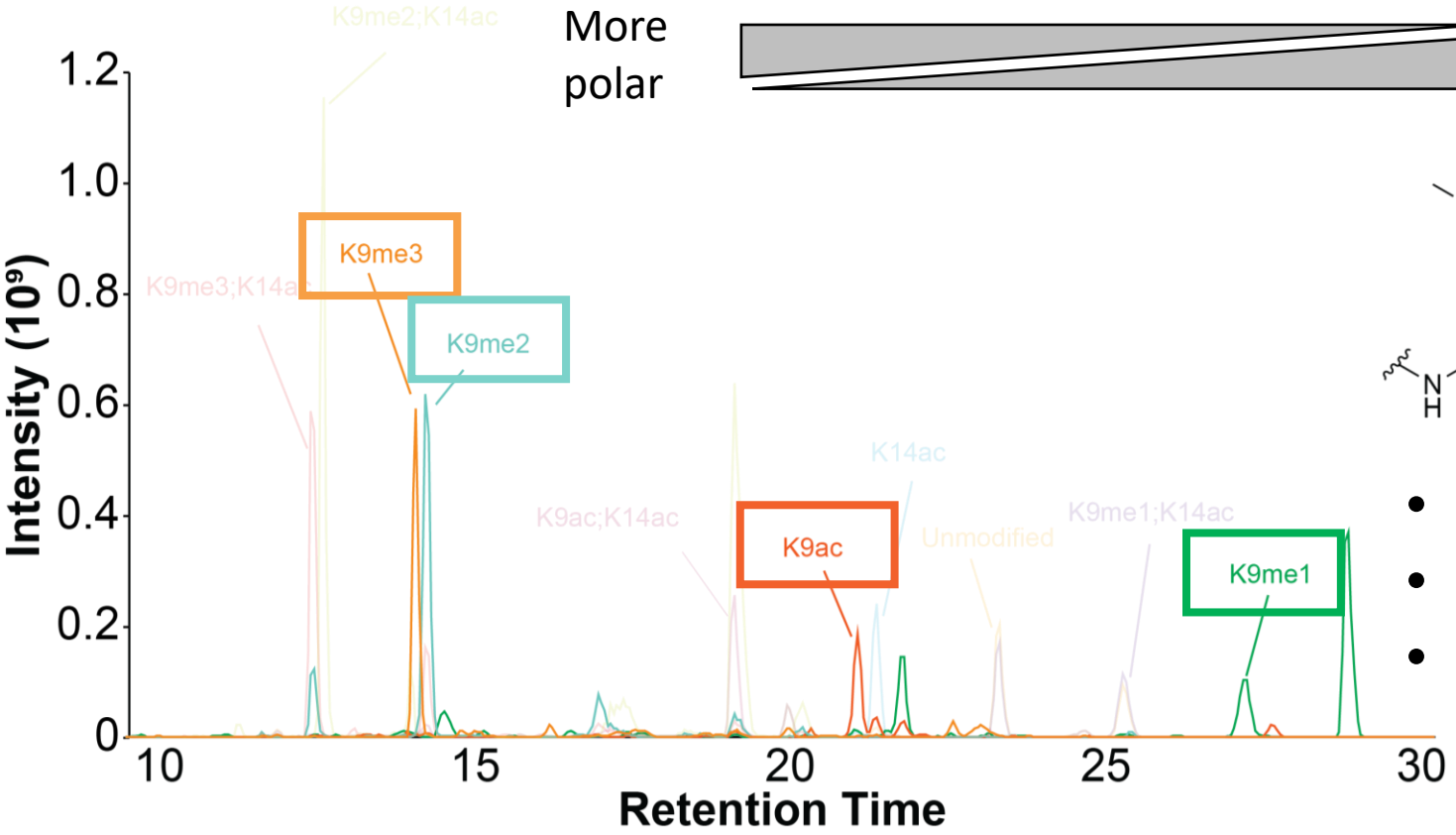


More polar

More hydrophobic



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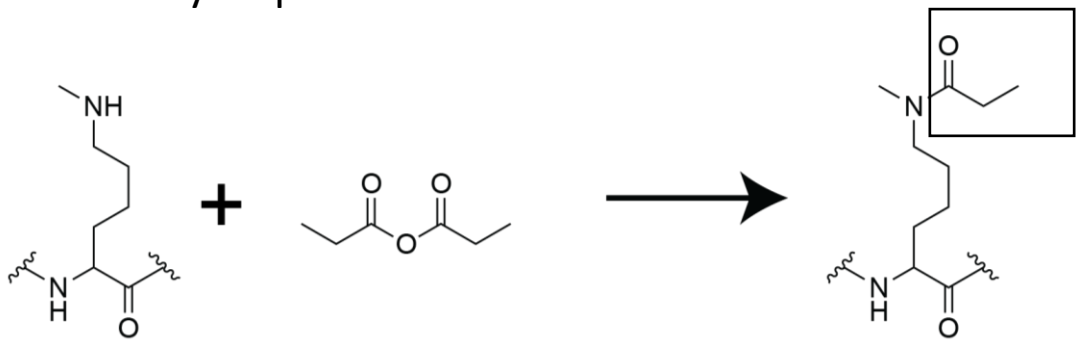

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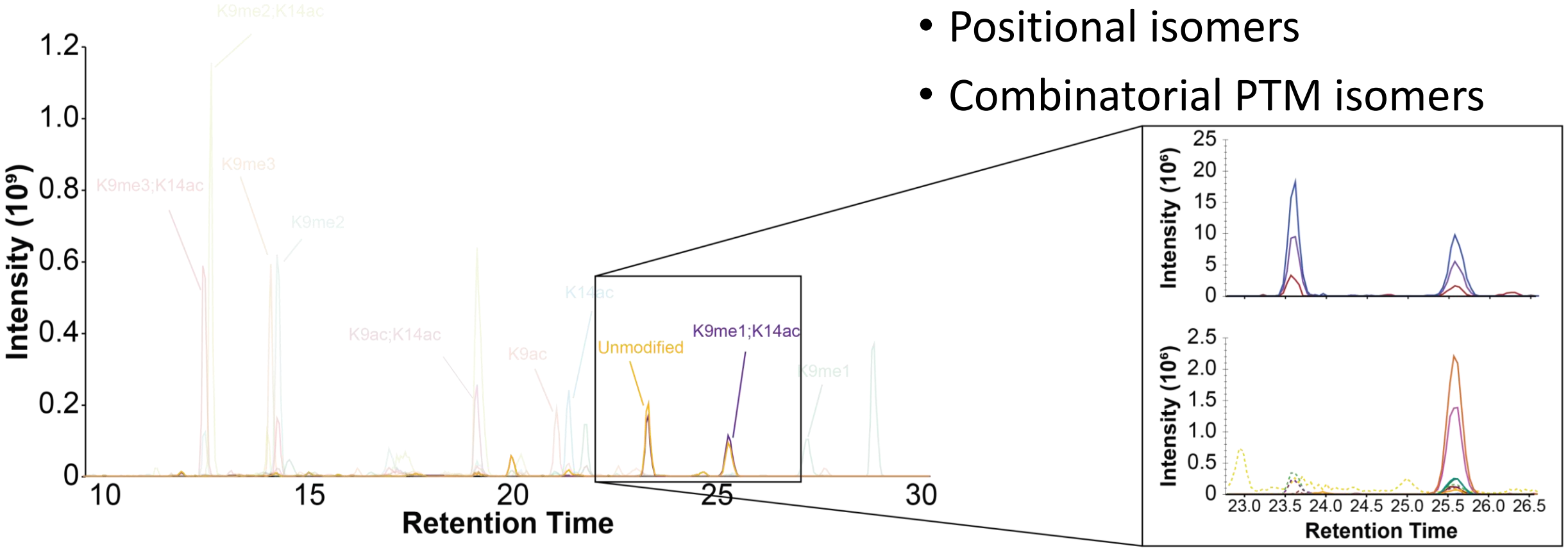
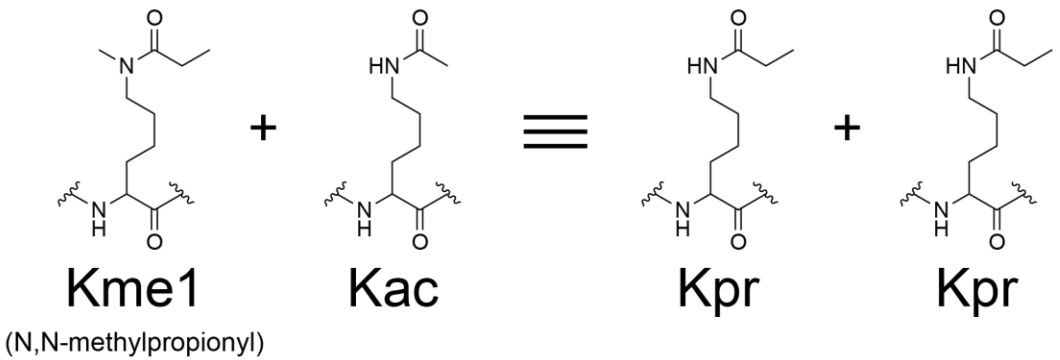
Challenges in quantifying histone PTMs

- Highly modified peptides
- Co-eluting/overlapping RT
- Shared transition ions
- Positional isomers
- Combinatorial PTM isomers



Challenges in quantifying histone PTMs



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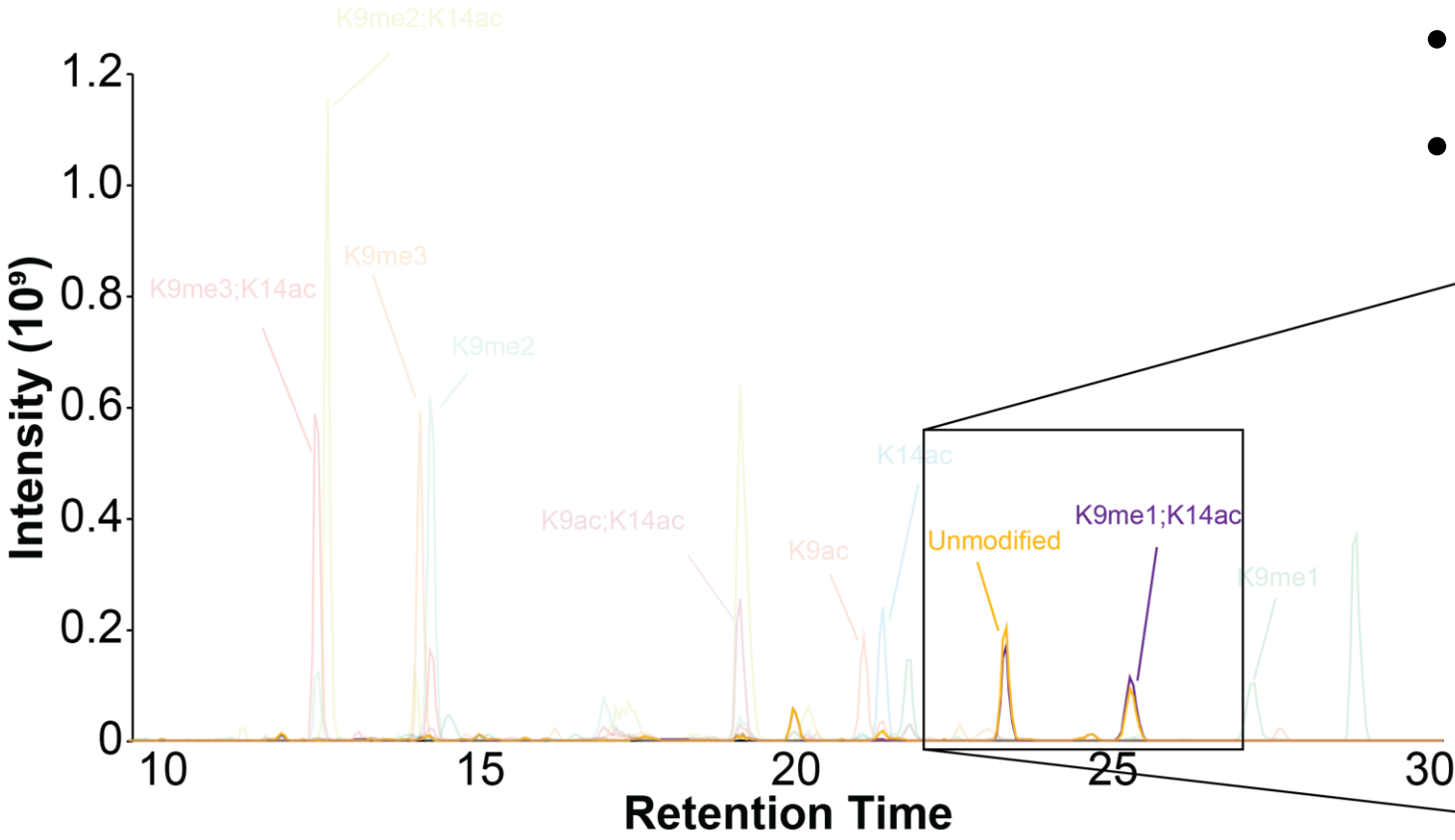
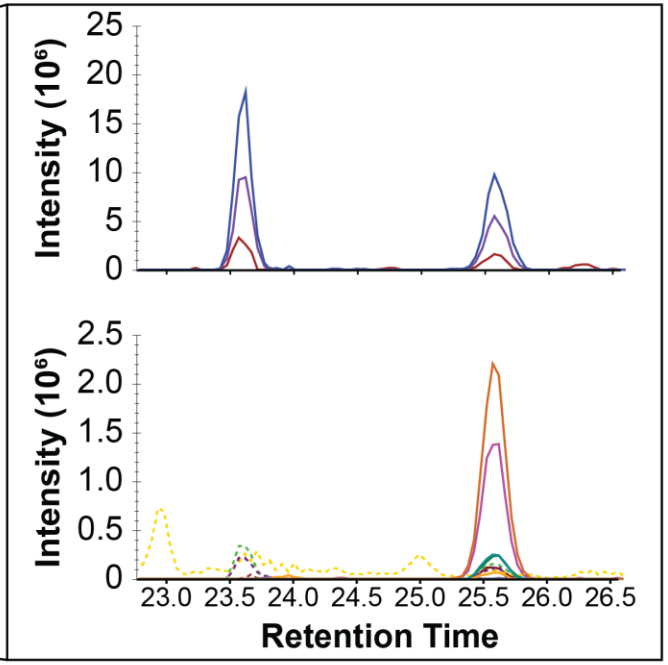
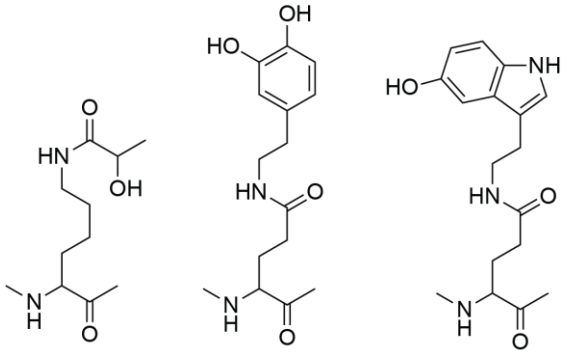




Using the Skyline ecosystem to improve histone PTM quantification

Using the Skyline ecosystem to improve histone PTM quantification

Newly discovered PTMs



Zhang et.al https://doi.org/10.1038/s41586-019-1678-1
Lepack et.al. https://doi.org/10.1126/science.aaw8806
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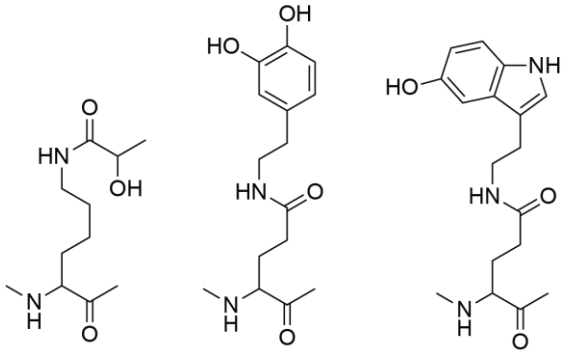

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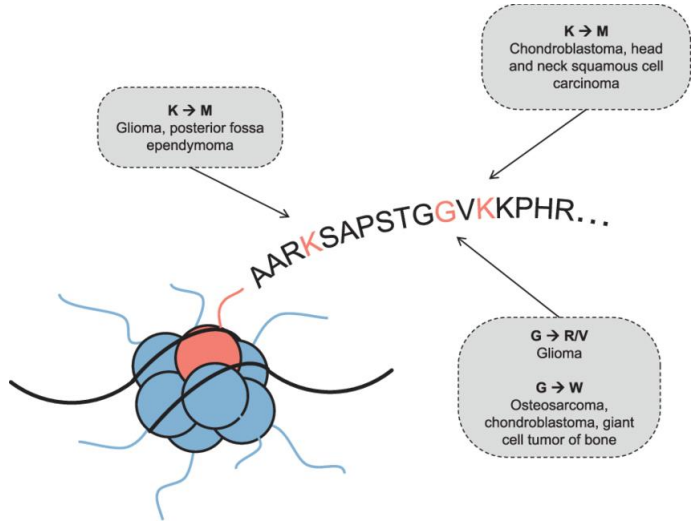
Skyline

Single Amino Acid Substitutions



Marchione et.al. https://doi.org/10.1080/14789450.2019.1550363

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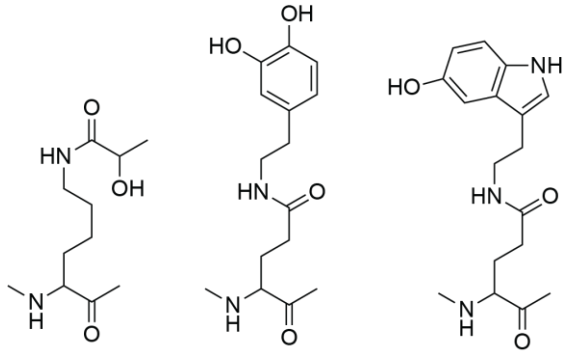
Newly discovered PTMs



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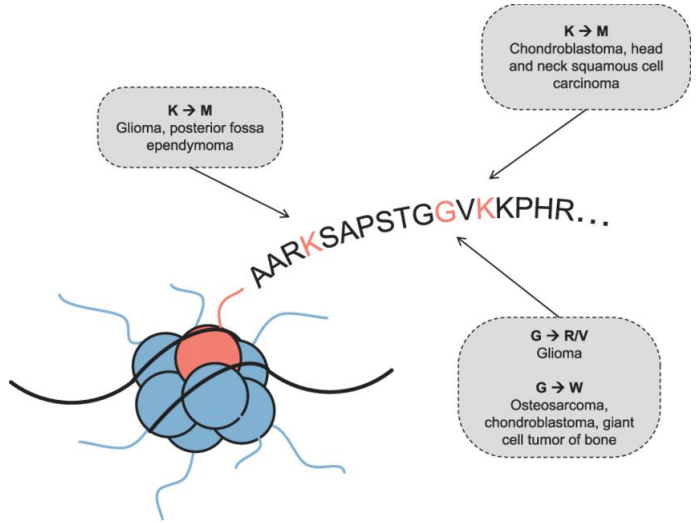

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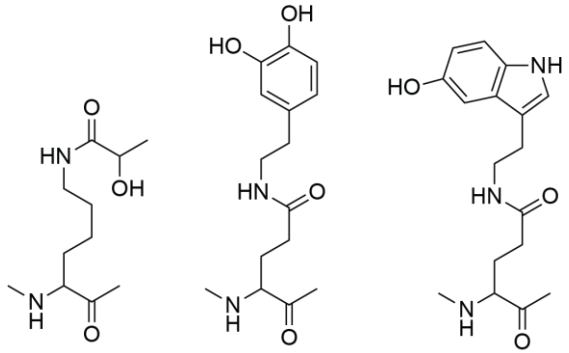
Non-model organisms

Image courtesy of Michael Gilbert

Using the Skyline ecosystem to improve histone PTM quantification

Newly discovered PTMs



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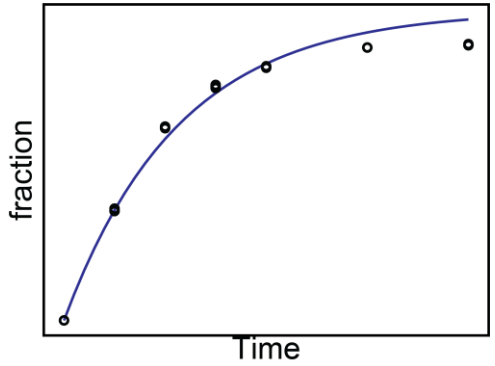
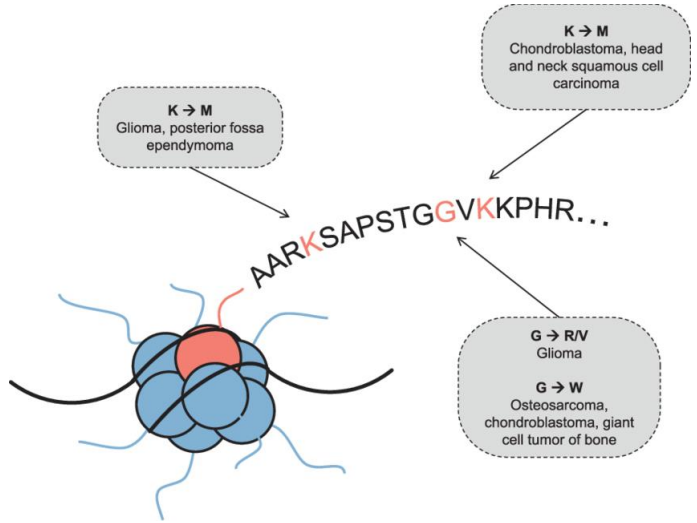
Isotopic labels – turnover studies



Zee et.al. https://doi.org/10.1186/1756-8935-3-22

Single Amino Acid Substitutions



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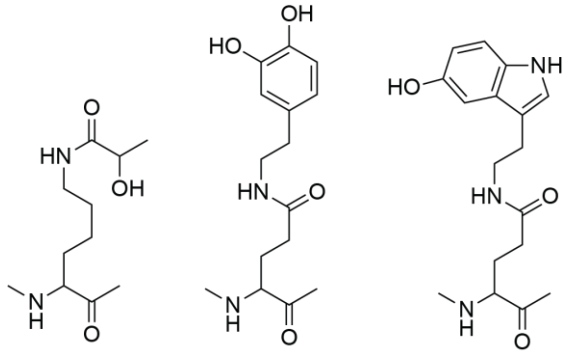

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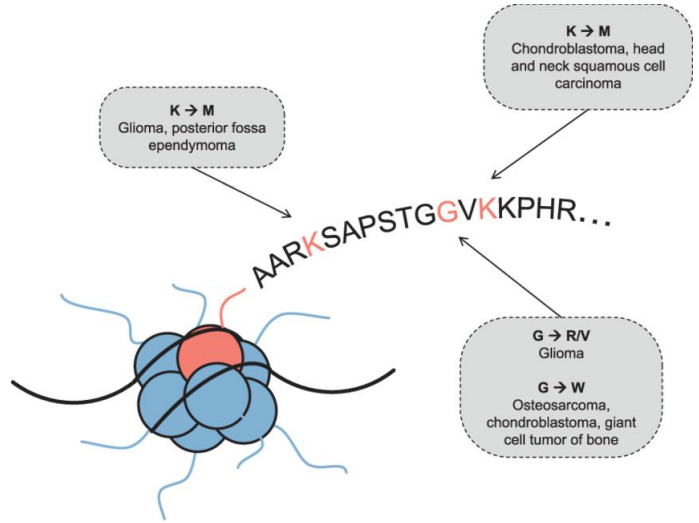

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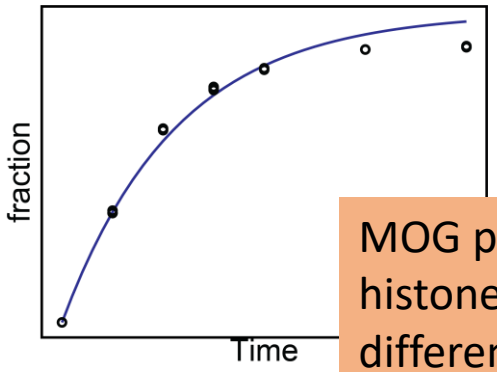


Zee et.al. https://doi.org/10.1186/1756-8935-3-22

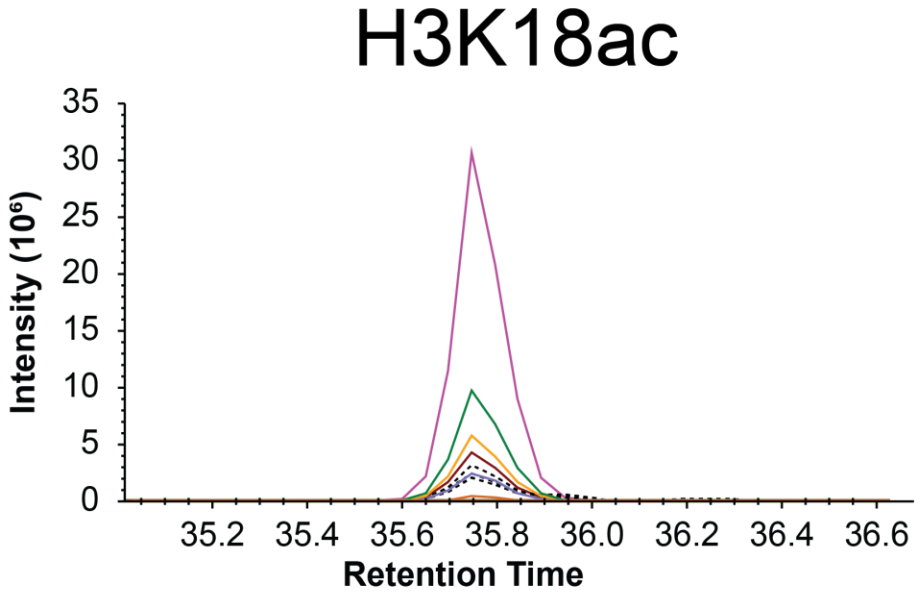
MOG pm 03:30 Methylation dynamics of histone H3.3K27me3 in pluripotency and differentiation of embryonic stem cells

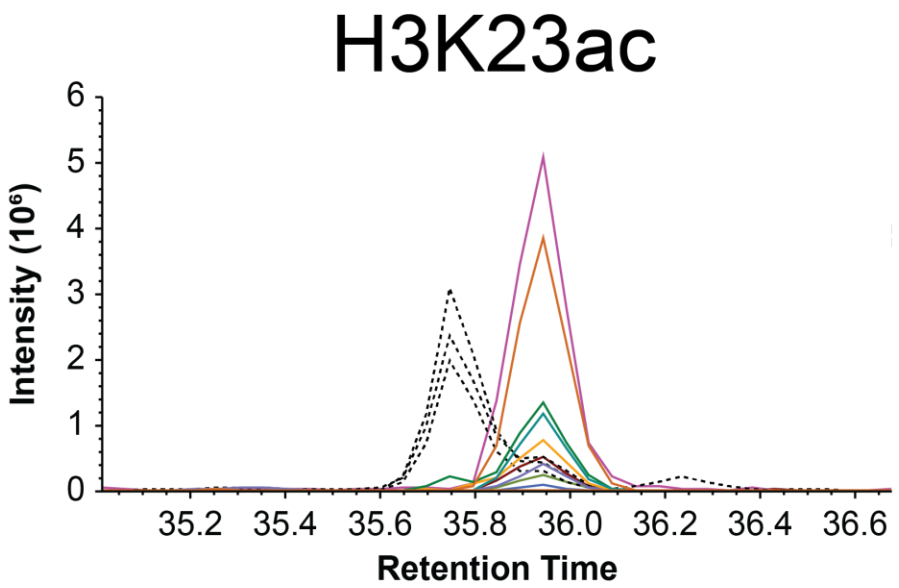
Non-model organisms

Image courtesy of Michael Gilbert

WP 574 Epigenetic Signatures that Regulate Caste Plasticity of Leafcutter Ants

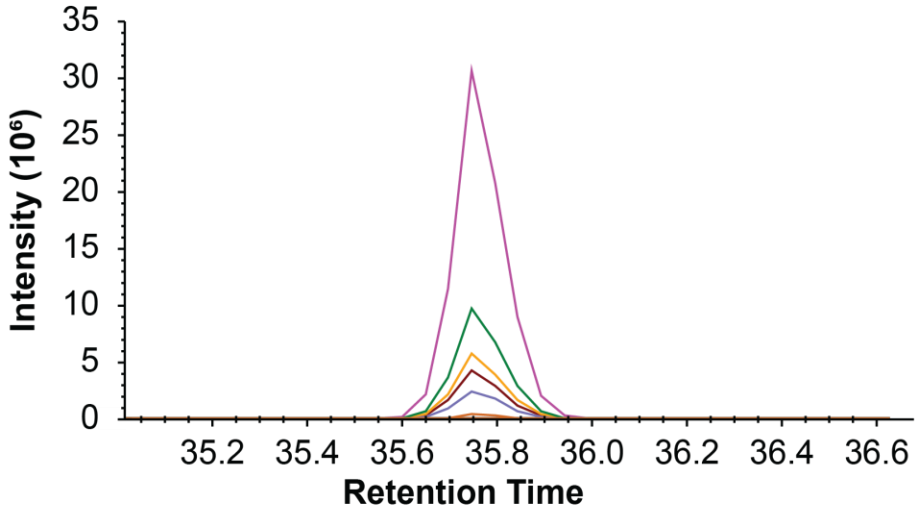
Site-localizing fragment ions improves quantification of isobaric peptides





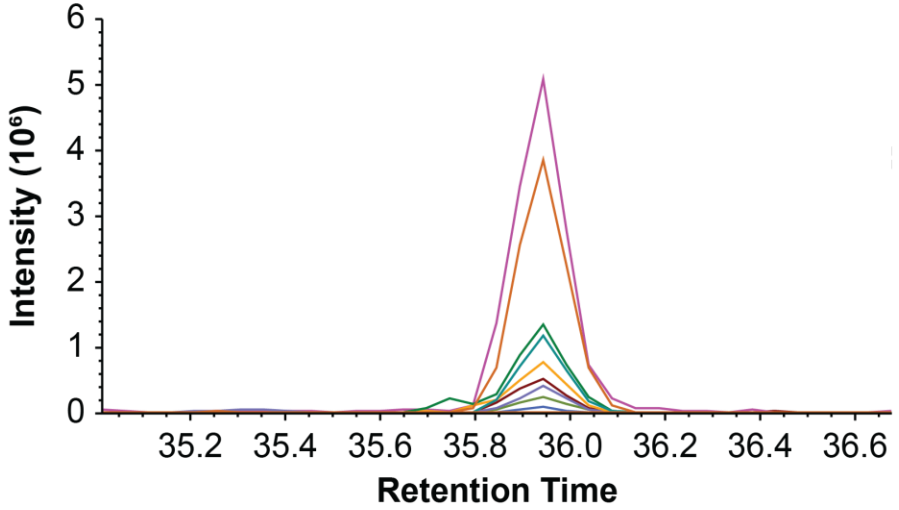
Site-localizing fragment ions improves quantification of isobaric peptides

H3K18ac

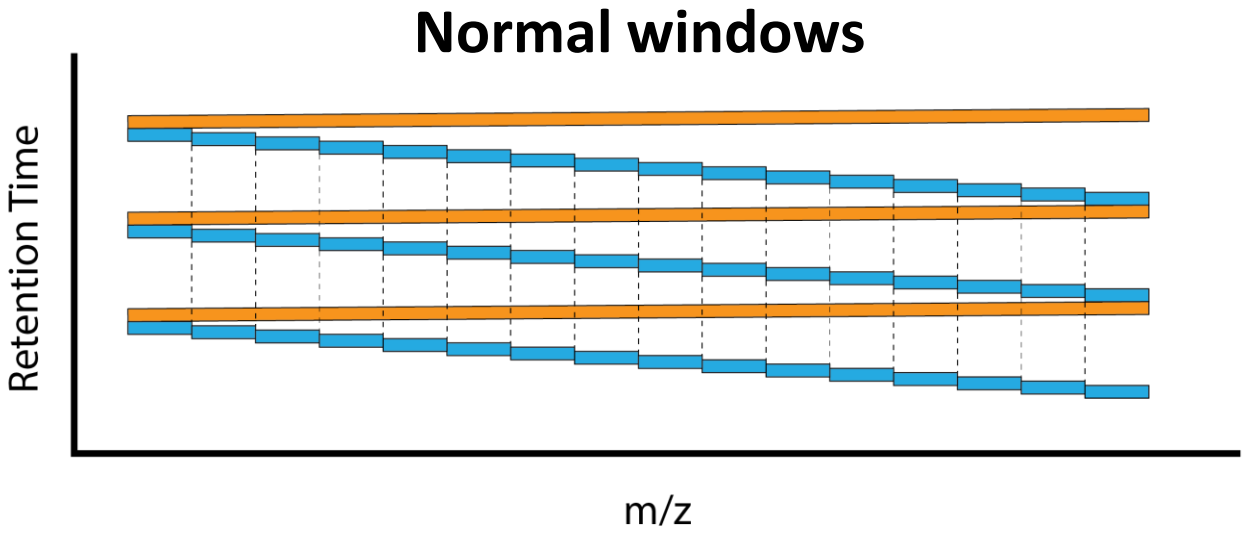


$K_{ac}QLATK_{pr}AAR$

H3K23ac



$K_{pr}QLATK_{ac}AAR$



<u>Scan range: 300 m/z – 1100 m/z</u>

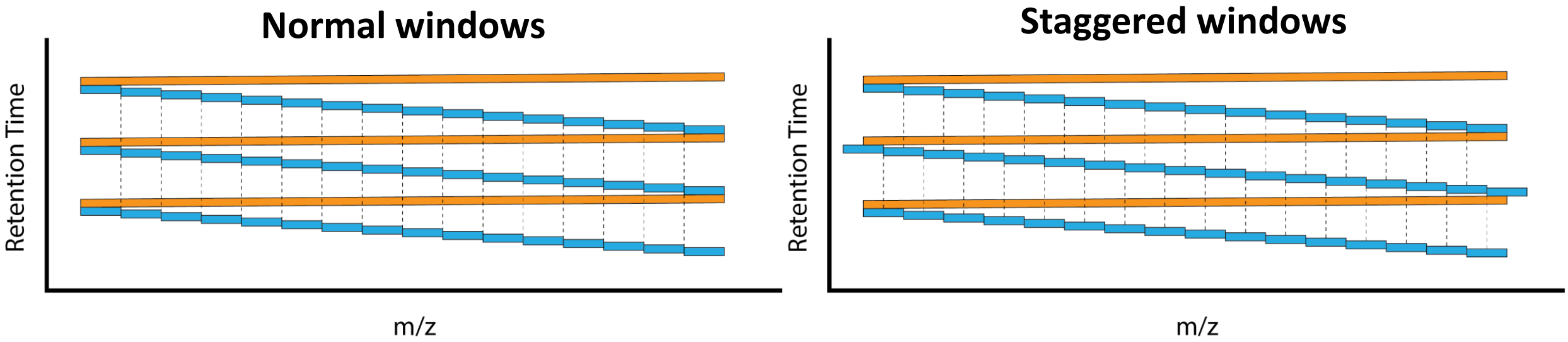
Constrained by m/z range of histone peptides

H4K20me2 – 300.2156 m/z

H2AZK4-K15 – 1080.1068

Pino et.al. (2020) https://doi.org/10.1074/mcp.P119.001913

Staggered windows ≡ 2x faster instrument



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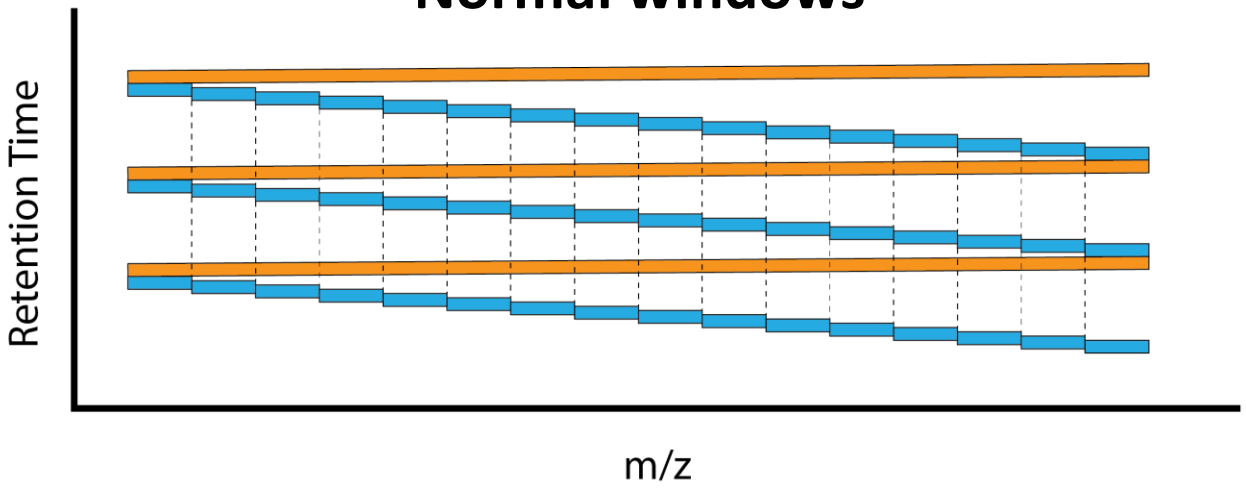
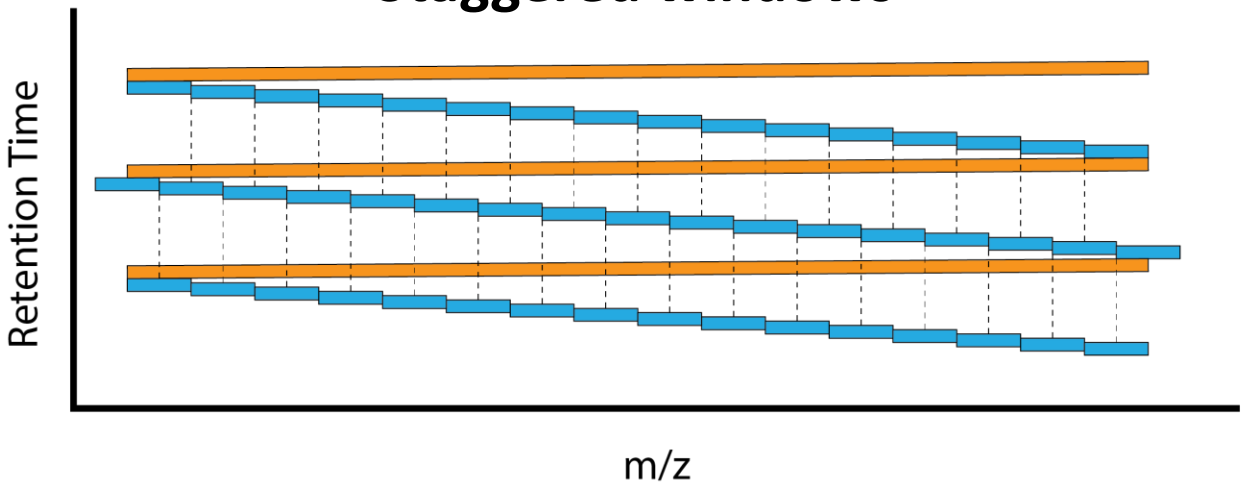
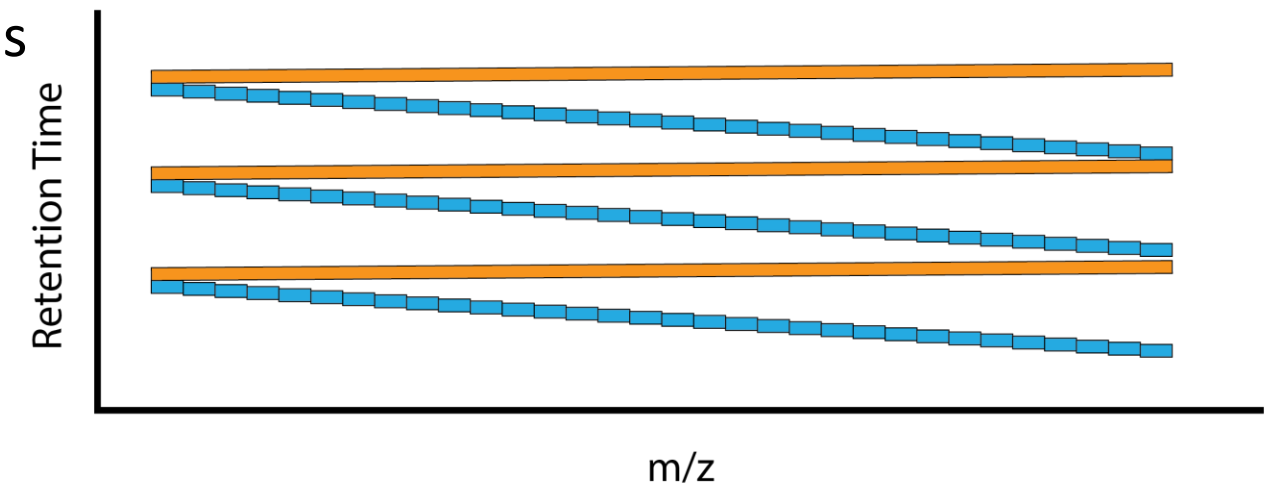
Normal windows



Staggered windows



After Demultiplexing



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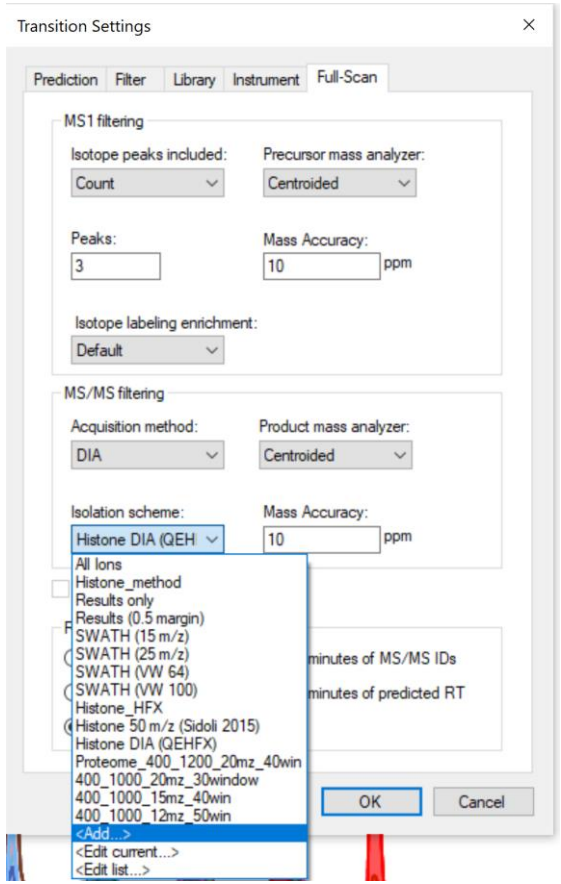
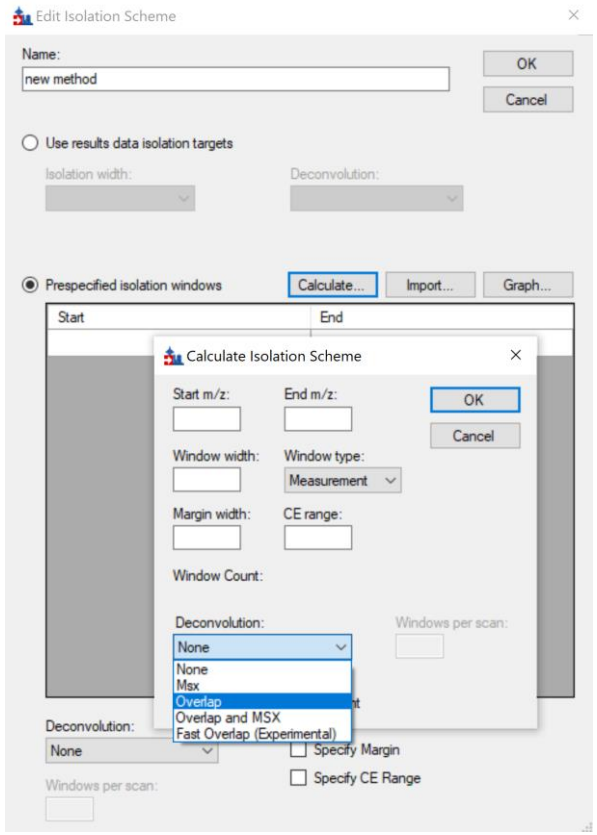
Using staggered windows in Skyline

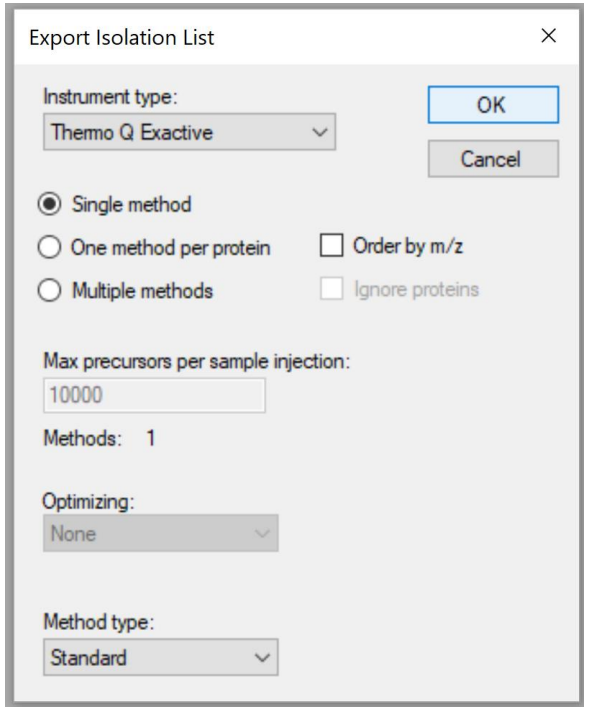
1. Settings > transition settings > Full-Scan
Isolation scheme dropdown menu: Add a new method



2. Calculate isolation windows.
Deconvolution is set to **Overlap**

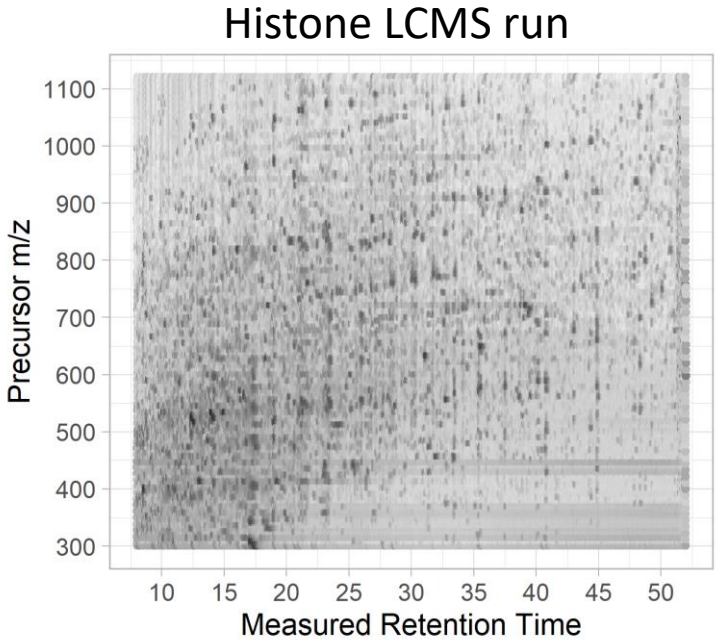


3. Export Isolation list



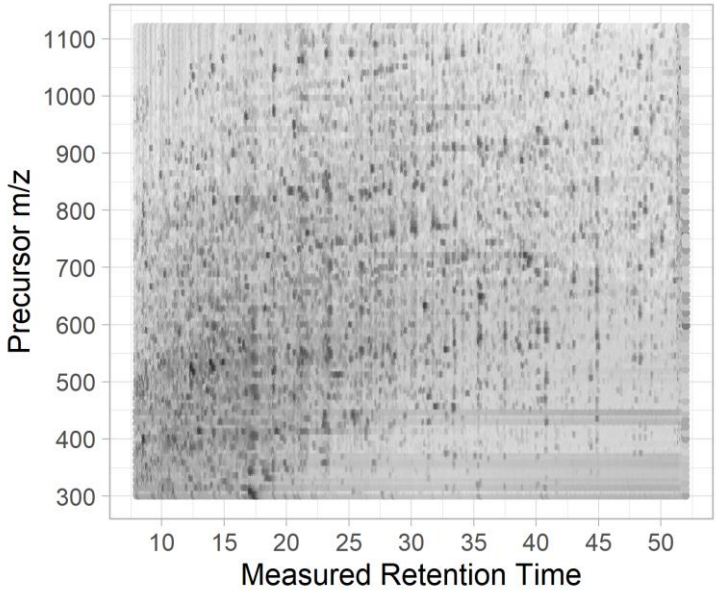
Overlapping windows (staggered) are used with orbitraps.
Margins are used with TOFs.
Do not combine the two!

Smaller windows increases precursor selectivity

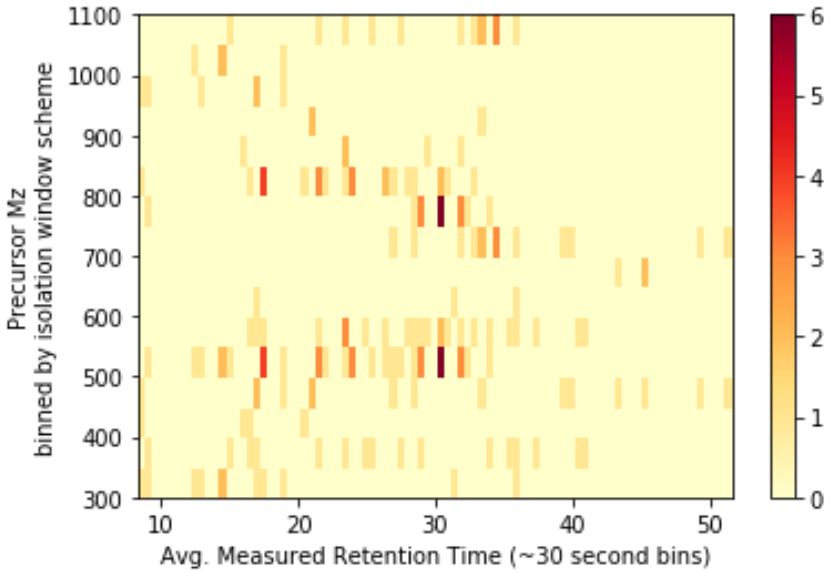


Smaller windows increases precursor selectivity

Histone LCMS run



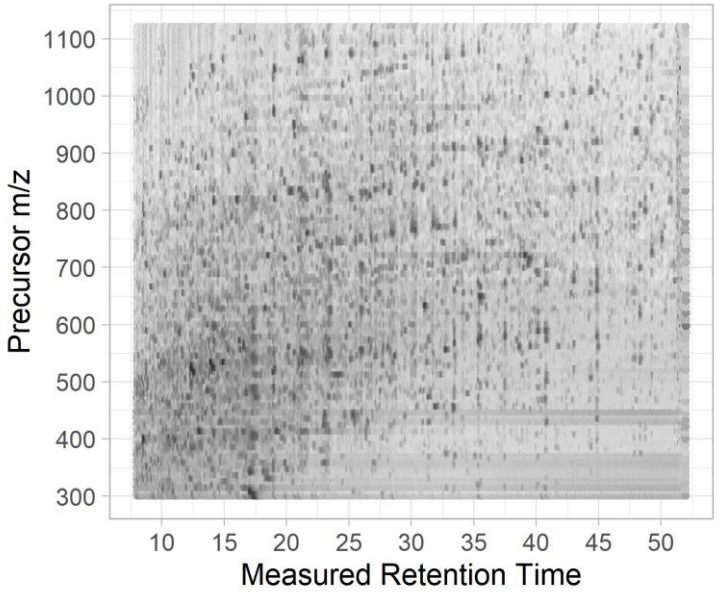
50 m/z isolation windows



The color represents the number of unique precursors that fall within a given isolation window.
Problematic for isobaric peptides.

Smaller windows increases precursor selectivity

Histone LCMS run



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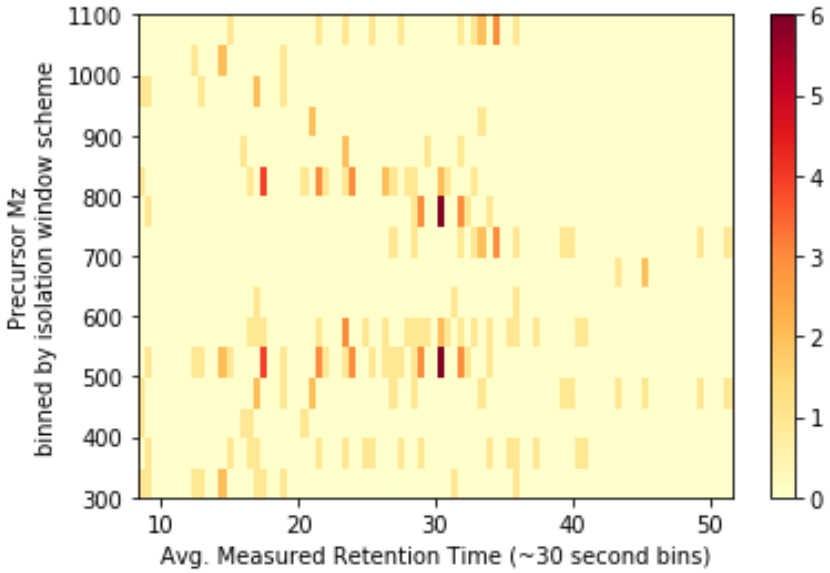
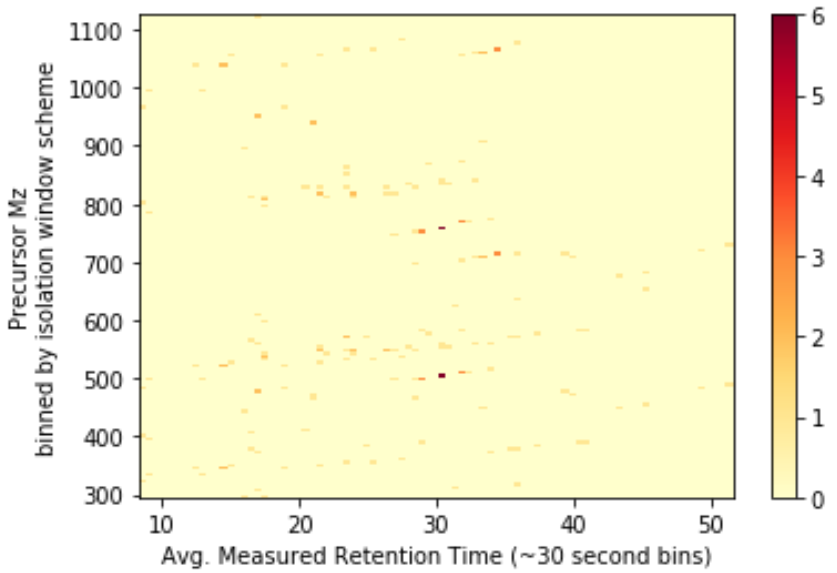
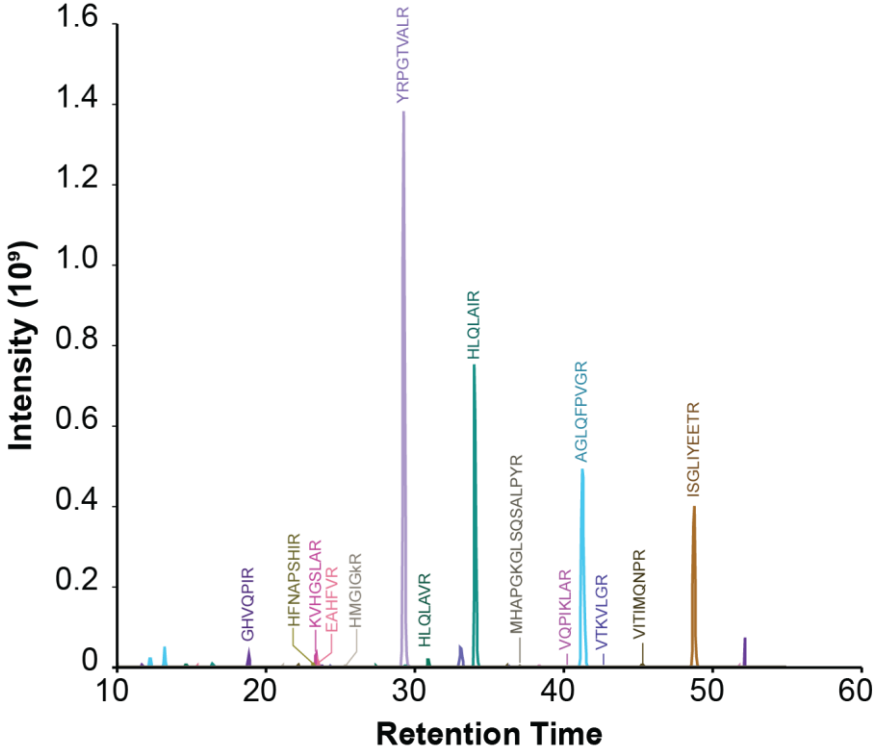


5 m/z effective isolation windows



The color represents the number of unique precursors that fall within a given isolation window.
Problematic for isobaric peptides.

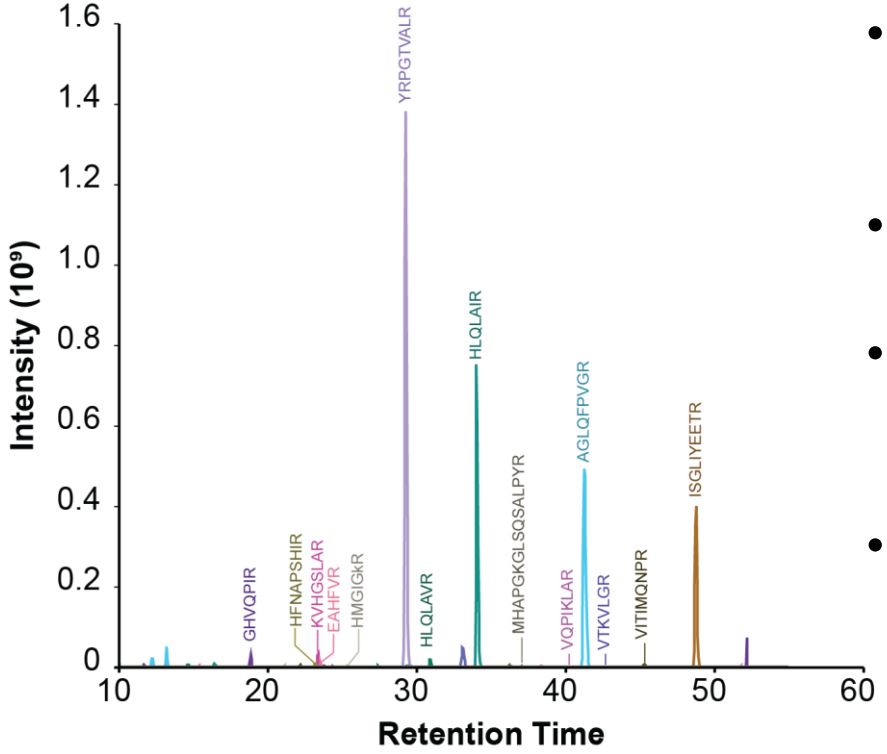
Using co-enriched peptides for iRT calibration

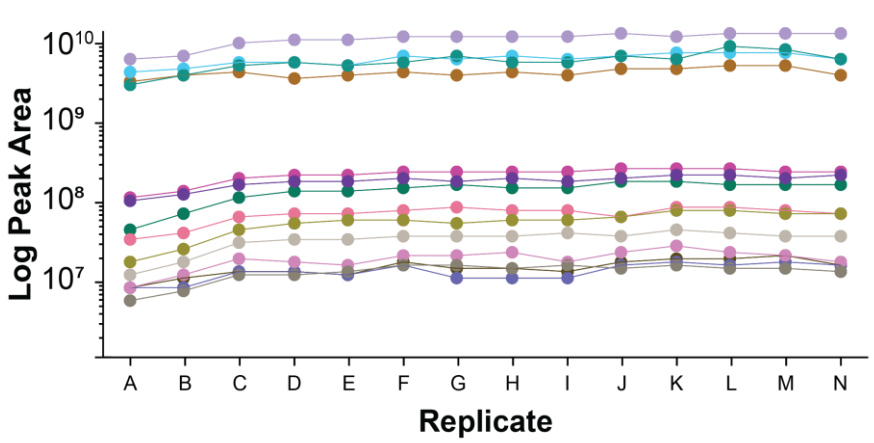


- iRT is a unit-less indexed retention time value for each peptide which are empirically derived
- The iRT is defined using a set of reference peptides
- iRT values can be transferred across labs and C18 chromatographic systems
- Improved peak detection and quantification

Escher et.al. (2012) https://doi.org/10.1002/pmic.201100463

Using co-enriched peptides for iRT calibration





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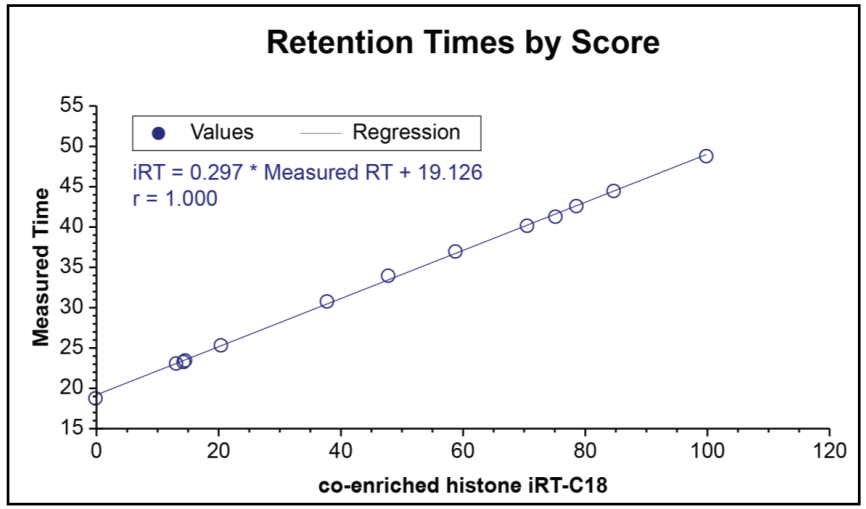
Unmodified histone peptides

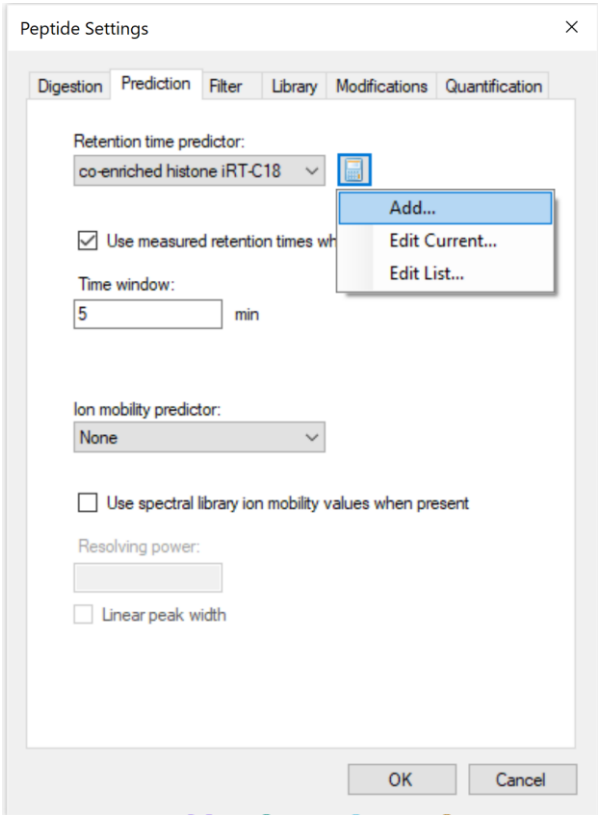
Non-histone peptides derived from co-enriched proteins.

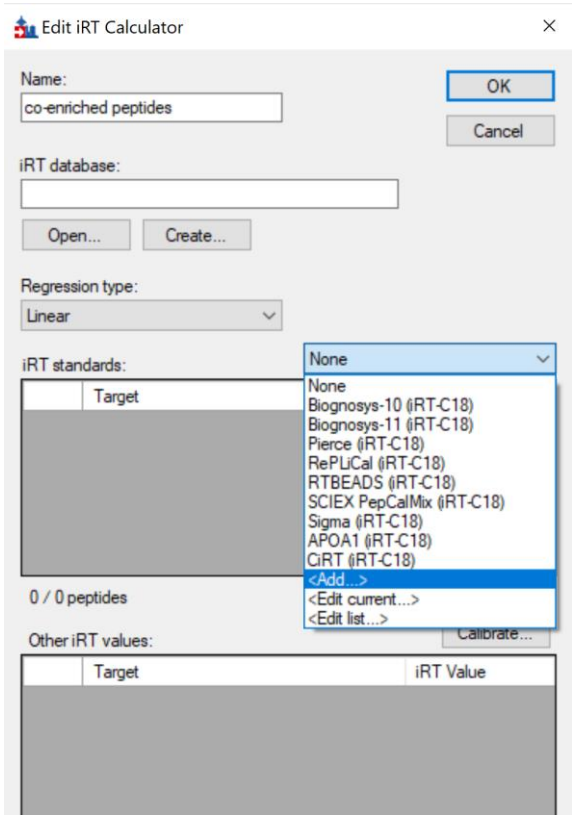


Escher et.al. (2012) https://doi.org/10.1002/pmic.201100463

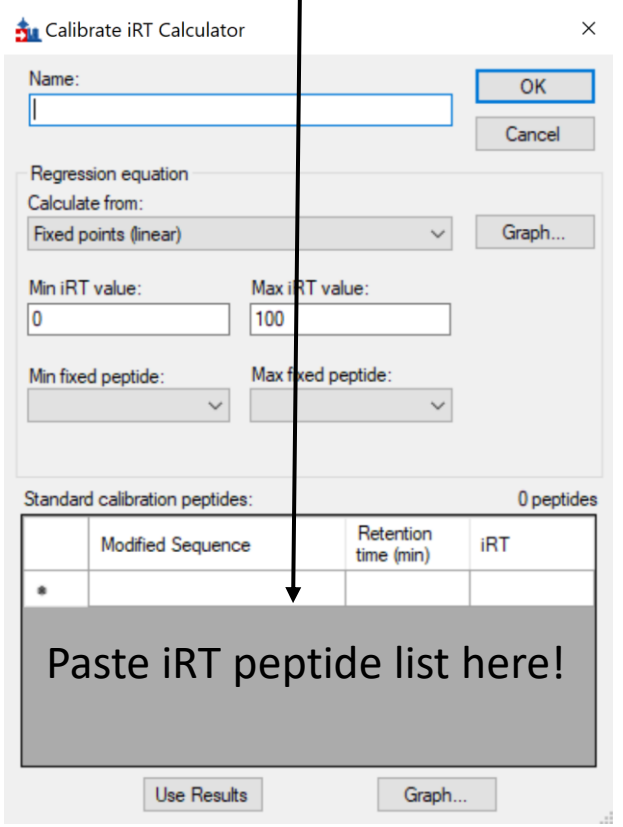
Creating custom iRT database in Skyline

1. Settings > Peptide settings > Predictions
2. Retention time predictor: Add a new method

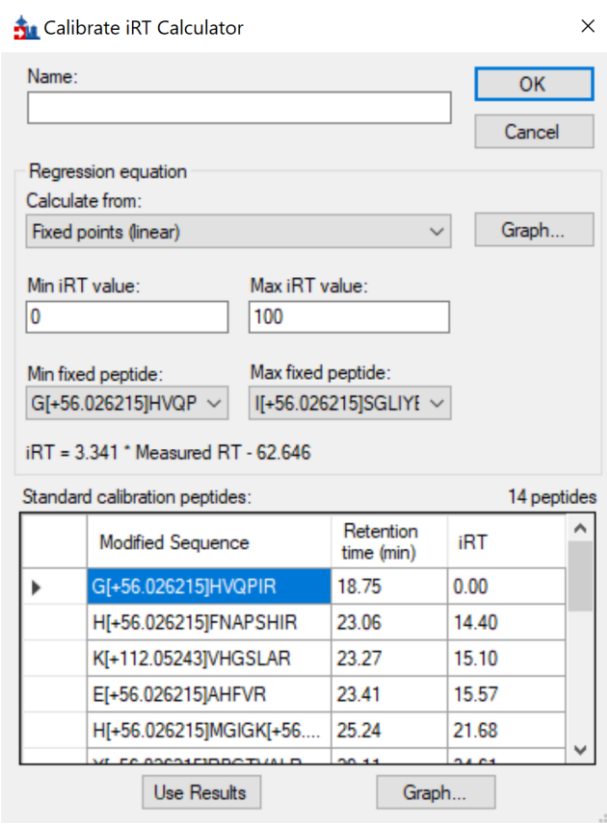




3. Paste reference peptides and observed retention time.



4. Skyline calculates iRT automatically



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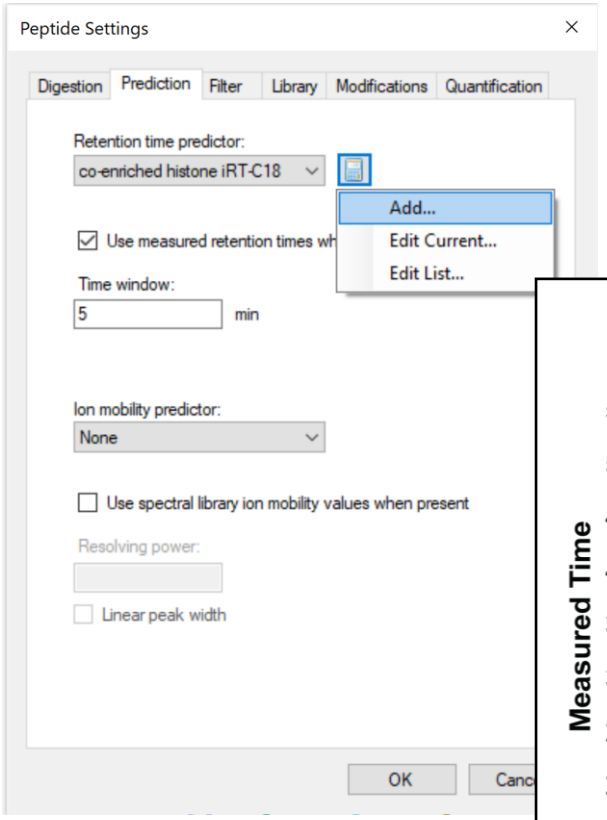
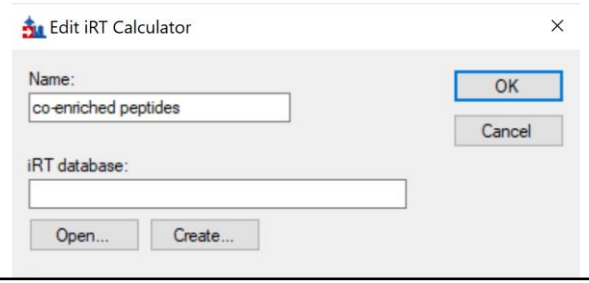
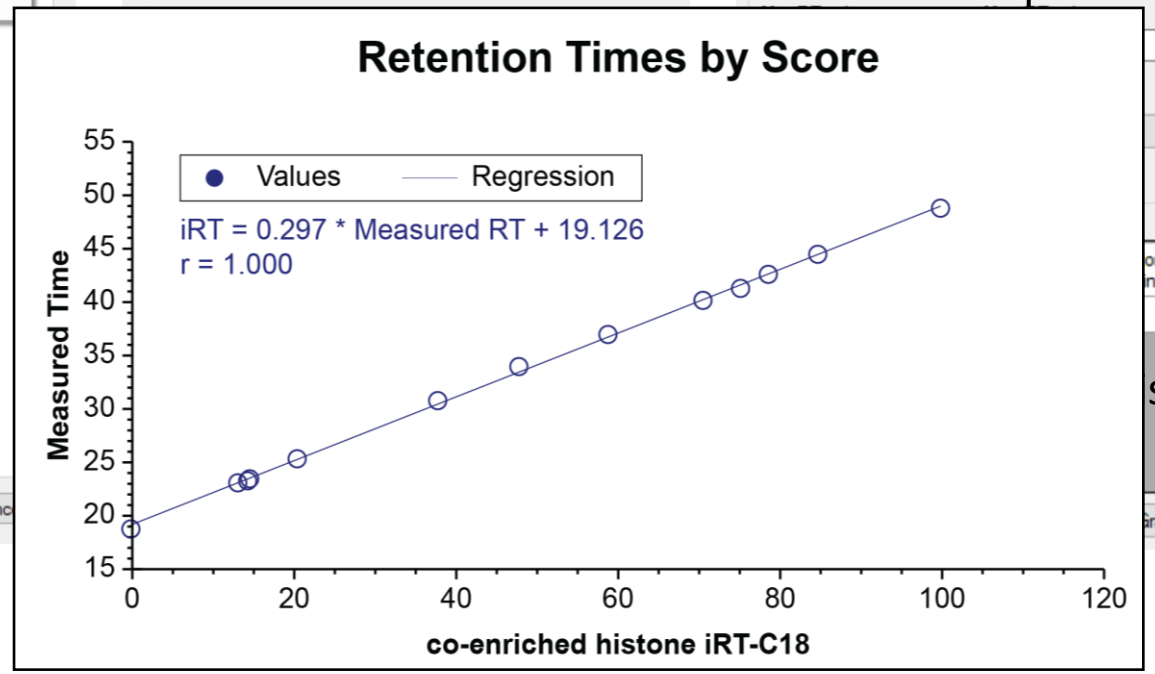
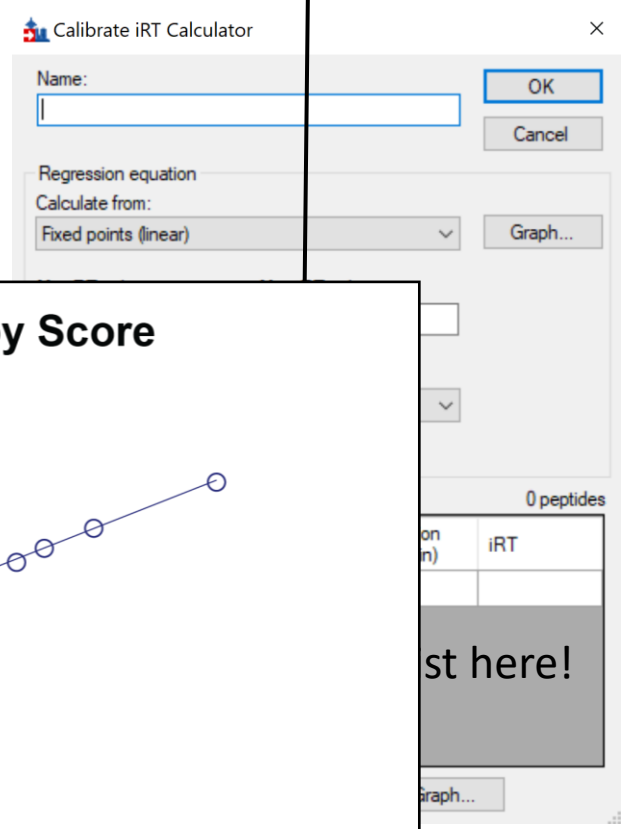
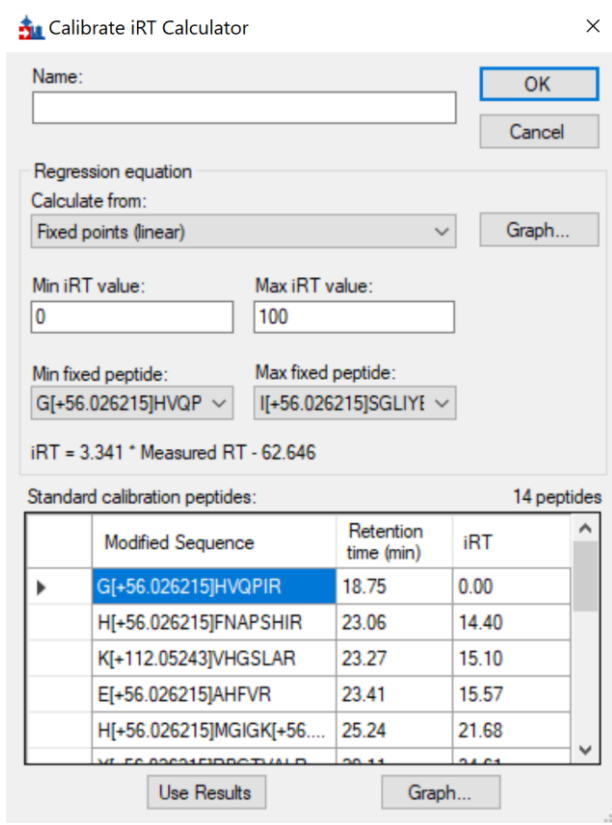




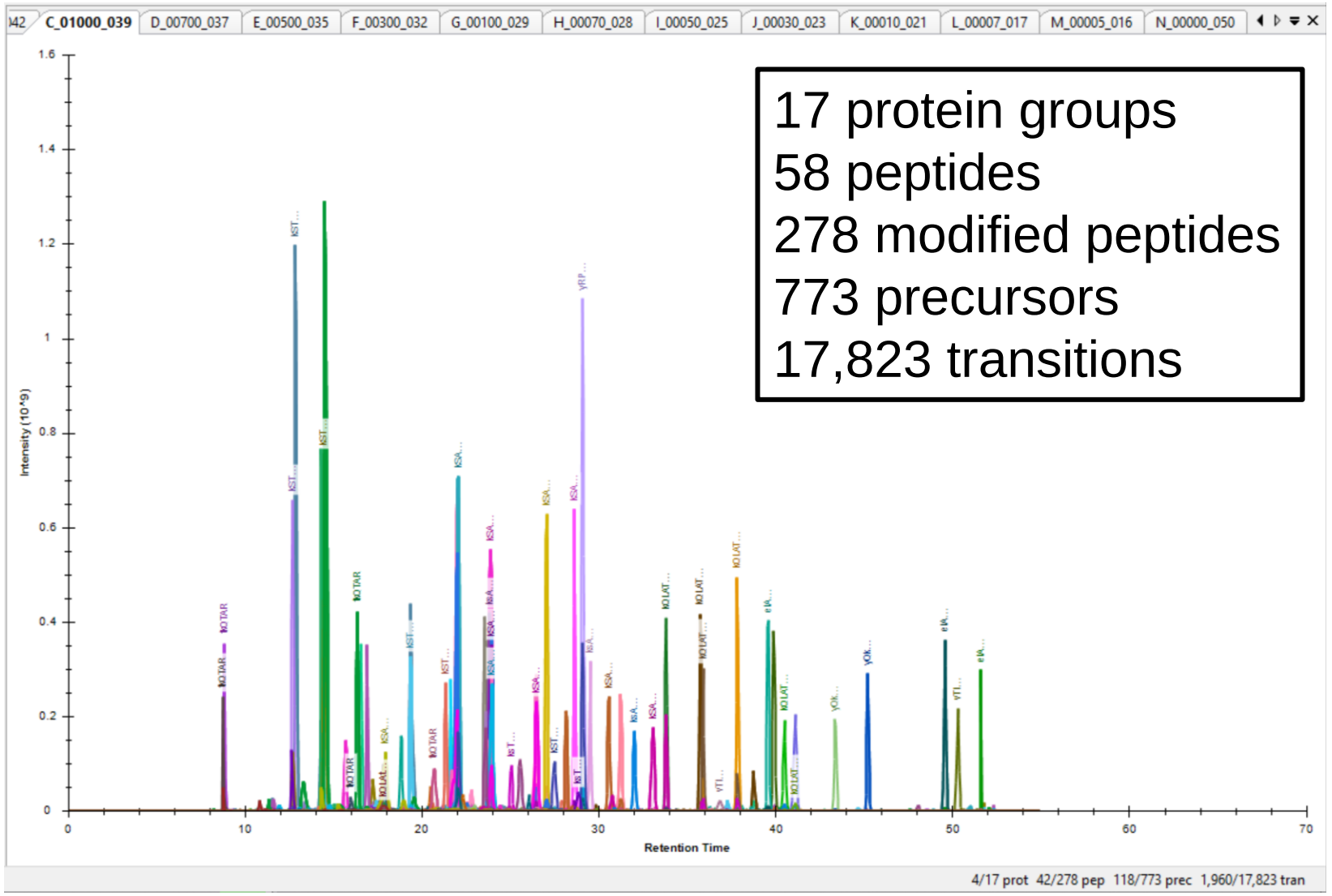






Skyline document for Histone PTM analysis

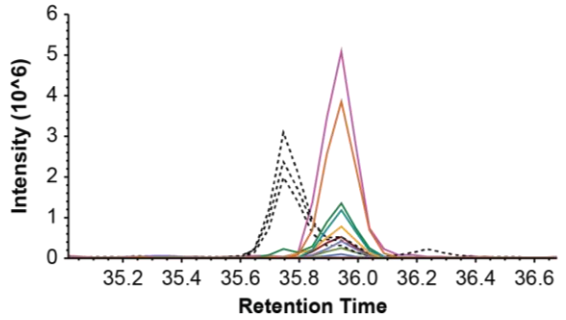
- H1
- H2A
- H2B
- H3
- H4
- sp|P84243|H33_HUMAN
- sp|Q6NXT2|H3C_HUMAN
- sp|P16402|H13_HUMAN
- sp|P16401|H15_HUMAN
- sp|Q9BTM1|H2AJ_HUMAN
- sp|Q71UI9|H2AV_HUMAN
- sp|P16104|H2AX_HUMAN
- sp|P0C0S5|H2AZ_HUMAN
- SP|O75367|H2AY_HUMAN
- sp|P0C0S8|H2A1_HUMAN
- sp|Q96A08|H2B1A_HUMAN
- Co-enriched iRT peptides



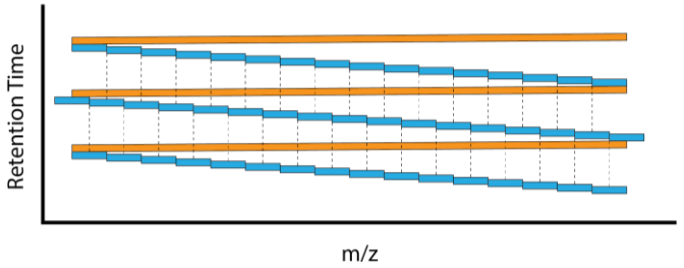
17 protein groups
58 peptides
278 modified peptides
773 precursors
17,823 transitions

Improving histone PTM method development and quantification with Skyline

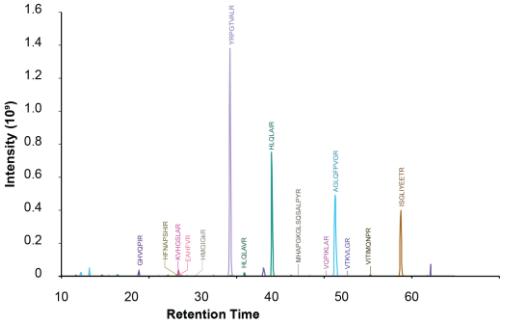
Site-localizing transitions for PTM quantification.



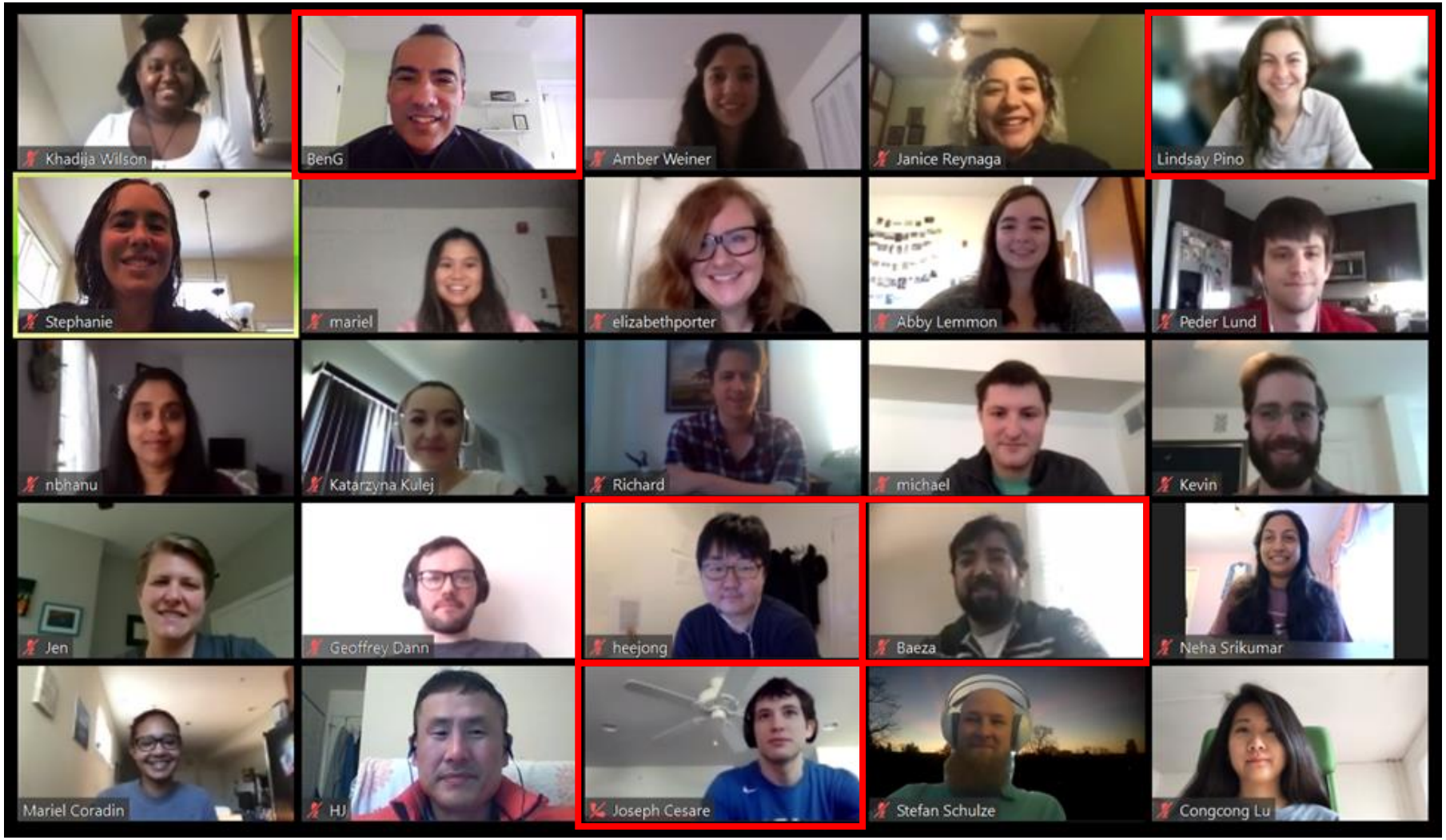
Staggered windows increases precursor selectivity



Co-enriched histone iRT peptides for retention time calibration



Acknowledgements



Thank you!

Histone PTM Skyline document soon to be available on Panorama

Want to try the Histone PTM Skyline document before the official release? Contact us.

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Josue Baeza – baeza@pennmedicine.upenn.edu
Lindsay Pino – lindsay.pino@pennmedicine.upenn.edu