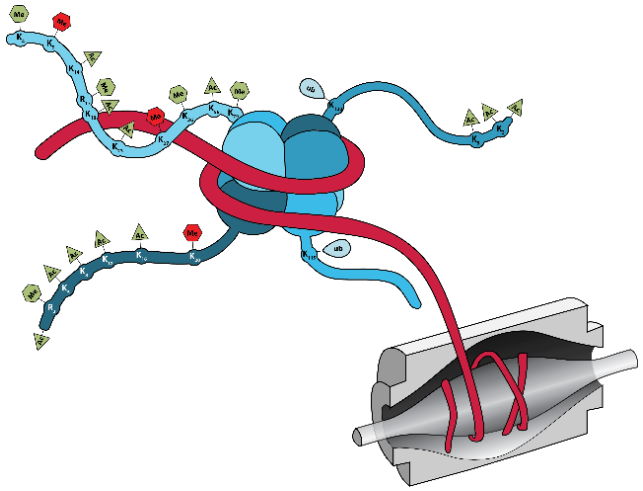
# Data independent acquisition for differential quantification of isobaric phosphopeptides and other protein post-translational modifications

**Simone Sidoli, Johayra Simithy, Benjamin A. Garcia**



06/04/2017, Skyline User Meeting, ASMS 2017

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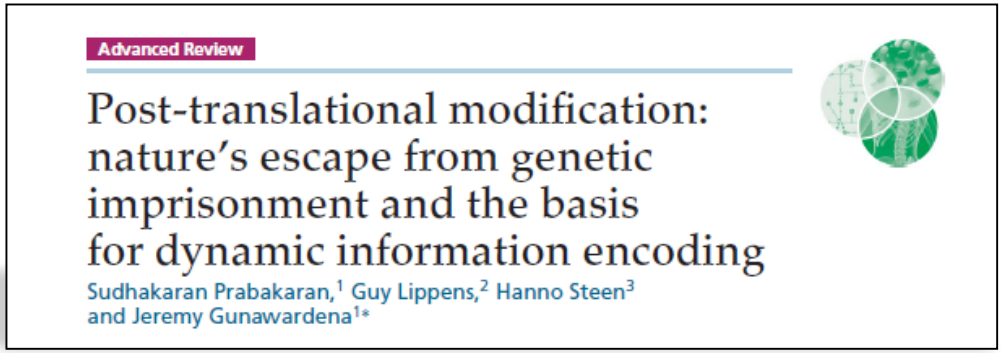
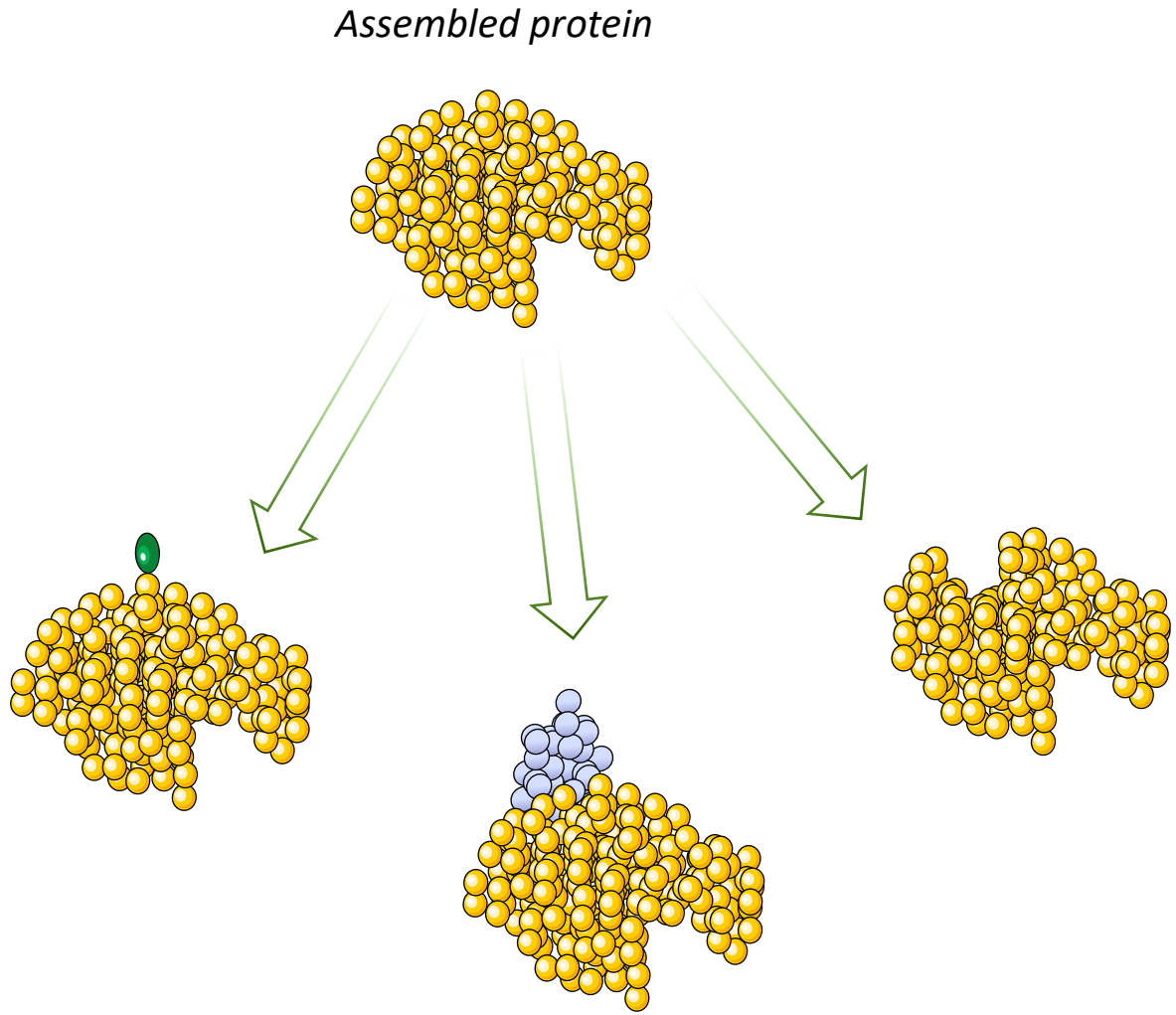
# The role of post-translational modifications




**<u>Post-translational modifications (PTMs) are critical regulators of protein function, half-life and localization</u>**

A post-translational modification is covalently bound to a protein after its translation. It can be a small chemical tag, or a big biomolecule, or a proteolytic cleavage

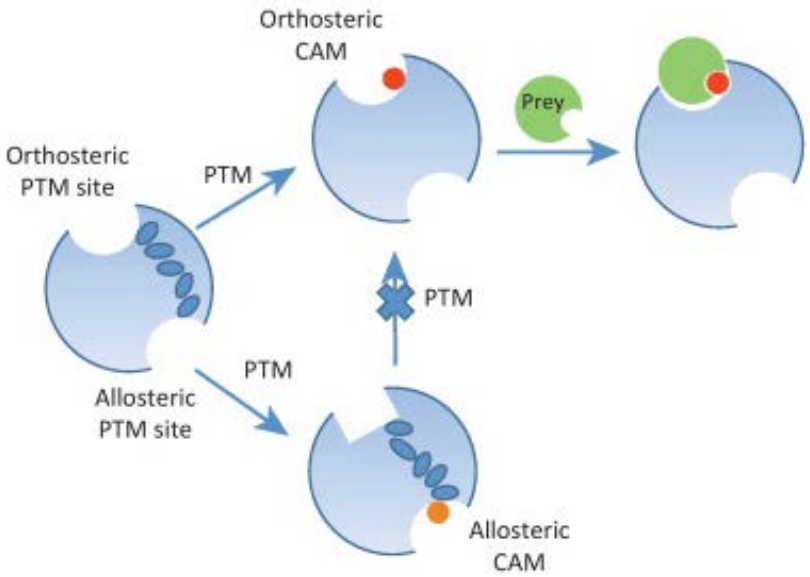
*Prabakaran et al. Wiley Interdiscip Rev Syst Biol Med (2012)*

# The role of combinatorial post-translational modifications

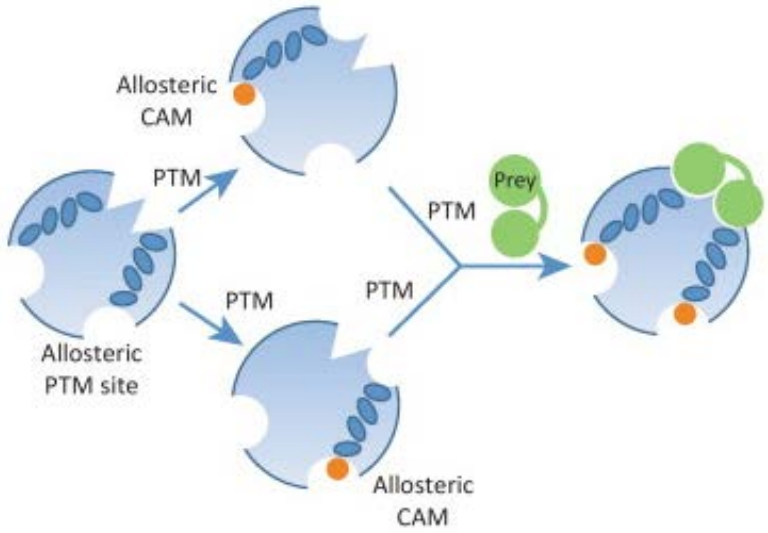
**In the Garcia lab, we work with combinatorial PTMs and investigate their "cross-talk"**

In protein science, a cross-talk between PTMs is an event where one PTM blocks or modifies the signal provided by another PTM. A cross-talk is "positive" if, for instance, a second PTM is required to fulfill a function that a single PTM cannot provide

**Negative cross-talk**




**Positive cross-talk**




*Nussinov et al. Trends in Biochemical Sciences*

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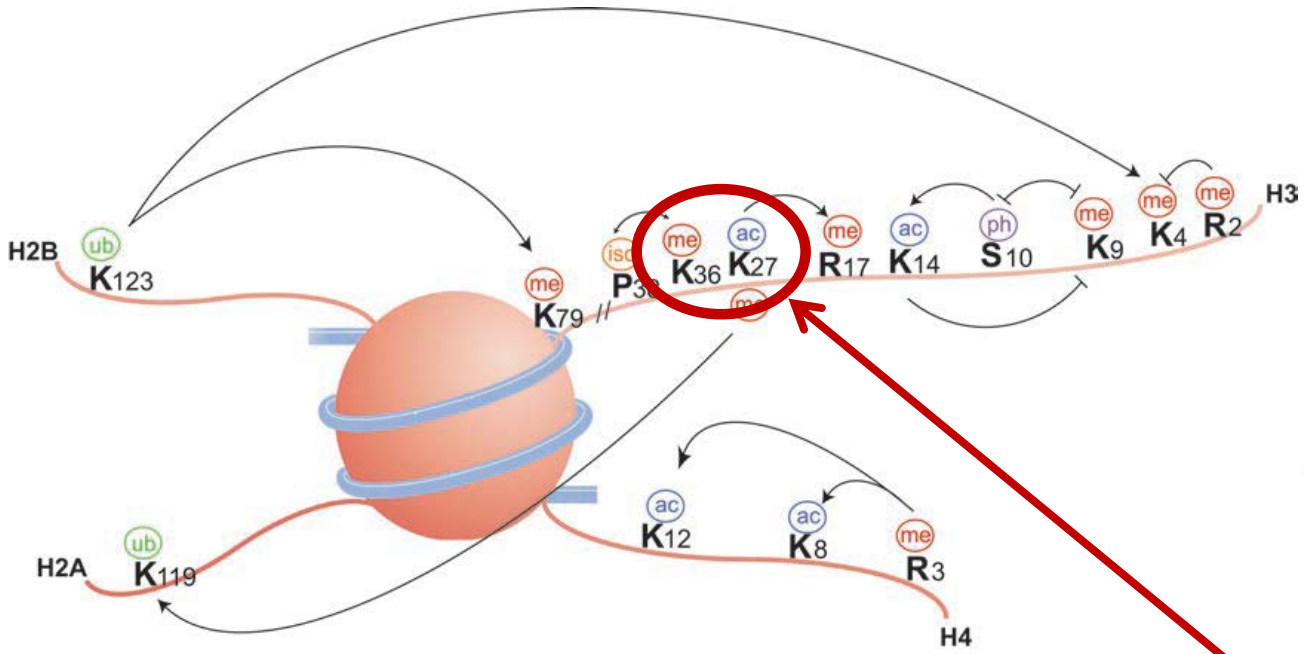
# Examples of histone PTM cross-talk




Numerous histone modifications are interdependent, i.e. the regulation of one affects the other one

CANCER

## Histone H3K36 mutations promote sarcomagenesis through altered histone methylation landscape

Chao Lu,[1] Siddhant U. Jain,[2,3] Dominik Hoelper,[2,3] Denise Bechet,[4] Rosalynn C. Molden,[5,6]* Leili Ran,[7] Devan Murphy,[7] Sriram Venneti,[8] Meera Hameed,[9] Bruce R. Pawel,[10] Jay S. Wunder,[11,12] Brendan C. Dickson,[13,14] Stefan M. Lundgren,[2,3] Krupa S. Jani,[6] Nicolas De Jay,[4] Simon Papillon-Cavanagh,[4] Irene L. Andrulis,[13,14,15,16] Sarah L. Sawyer,[17] David Grynspan,[18] Robert E. Turcotte,[19] Javad Nadaf,[4] Somayyeh Fahiminiyah,[4] Tom W. Muir,[6] Jacek Majewski,[4] Craig B. Thompson,[20] Ping Chi,[7,21] Benjamin A. Garcia,[5] C. David Allis,[1]† Nada Jabado,[4,22]† Peter W. Lewis[2,3]†

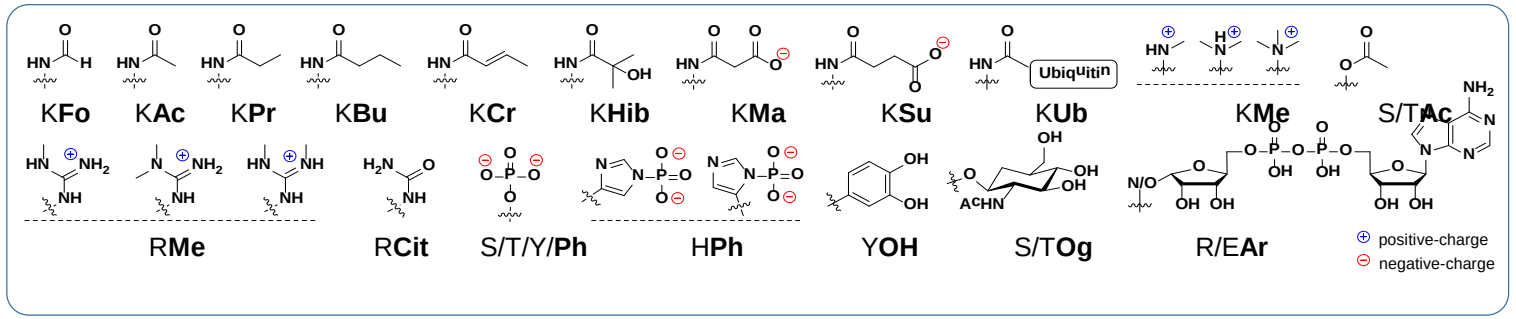
Oncohistones: histone mutations correlate with selected types of cancers

Nearby PTM sites (H3K27 and H3K36) interplay mutations and PTM levels

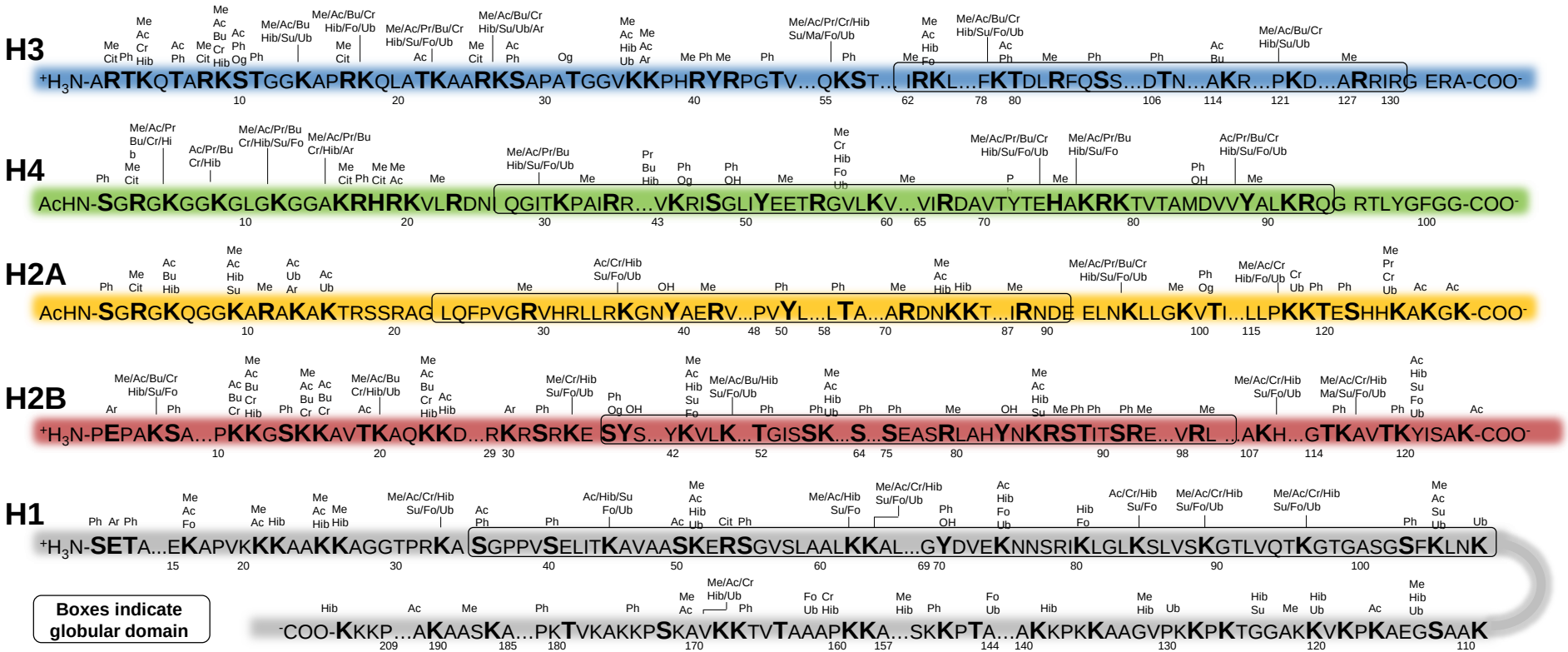
*Bannister and Kouzarides, Cell Research (2011)*

*Lu et al., Science (2016)*

# Histone modifications




| | |
|---|---|
| Me | Methylation (K,R) |
| Ac | Acetylation (K, S, T) |
| Pr | Propionylation (K) |
| Bu | Butyrylation (K) |
| Cr | Crotonylation (K) |
| Hib | 2-Hydroxyl-isobutyrylation (K) |
| Ma | Malonylation (K) |
| Su | Succinylation (K) |
| Fo | Formylation (K) |
| Ub | Ubiquitylation (K) |
| Cit | Citrullination (R) |
| Ph | Phosphorylation (S, T, Y, H) |
| OH | Hydroxylation (Y) |
| Og | *O*-glynacylation (S, T) |
| Ar | ADP ribosylation (R, E) |




Almost every known PTM occurs on histones as well

The likelihood to have cross-talking PTMs is exponentially higher in hypermodified proteins

*Huang et al. Cell (2014)*

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# Nearby PTMs is common in all proteins

Resource

Cell

## Systematic Functional Prioritization of Protein Posttranslational Modifications

Pedro Beltrao,[1,3,*] Véronique Albanèse,[4] Lillian R. Kenner,[1,3] Danielle L. Swaney,[5] Alma Burlingame,[2,3] Judit Villén,[5] Wendell A. Lim,[1,3,6] James S. Fraser,[1,3] Judith Frydman,[4] and Nevan J. Krogan[1,3,7,*]

Molecular Systems Biology 8; Article number 599; doi:10.1038/msb.2012.31
Citation: *Molecular Systems Biology* 8:599

www.molecularsystemsbiology.com

molecular systems biology

## Deciphering a global network of functionally associated post-translational modifications

Pablo Minguez[1], Luca Parca[2], Francesca Diella[1,3], Daniel R Mende[1], Runjun Kumar[4], Manuela Helmer-Citterich[2], Anne-Claude Gavin[1], Vera van Noort[1] and Peer Bork[1,5,*]

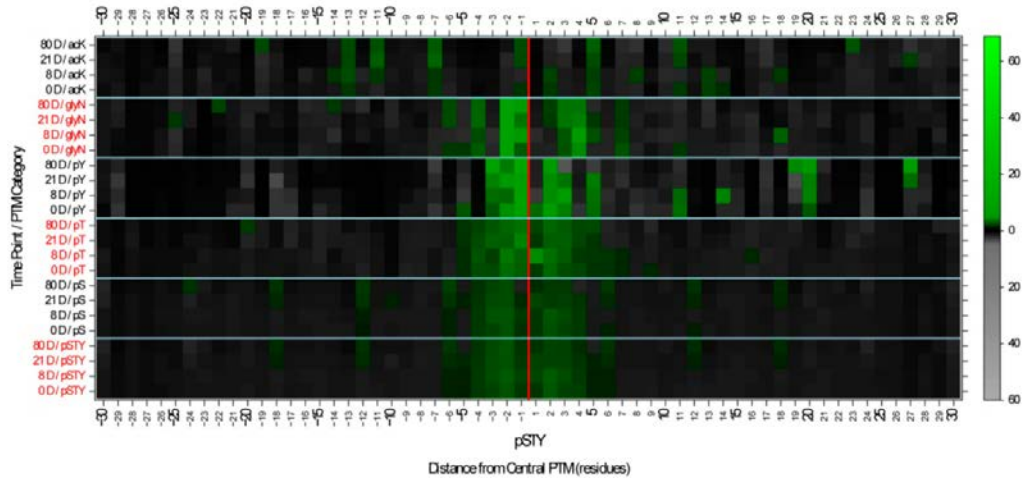
Journal of proteome research

Article

pubs.acs.org/jpr

## Spatial and Temporal Effects in Protein Post-translational Modification Distributions in the Developing Mouse Brain

Alistair V. G. Edwards,[†] Gregory J. Edwards,[‡] Veit Schwämmle,[†] Henrik Saxtorph,[§] and Martin R. Larsen*[,†]




*Density plot of phosphorylation distances on a phosphoproteome. The x-axis is the distance between phosphosites (0 in the middle)*

PTMs are more frequently found nearby as compared to random. This is mostly due to enzyme docking, limitation in accessible protein surface, and evolutionary preservation of meaningful PTM domains

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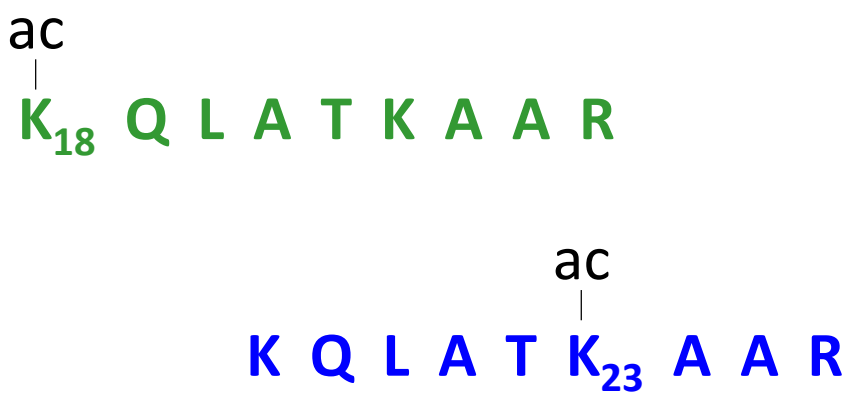
**Problem with mass spectrometry:**

**Nearby PTMs can easily co-localize on the same peptide**
**i.e.**
**Presence of isobaric peptides**

# Quantitative discrimination of isobaric peptides




**These PTMs have frequently different functions**

Discriminating their abundance is critical

Extracting the MS ion chromatogram is insufficient

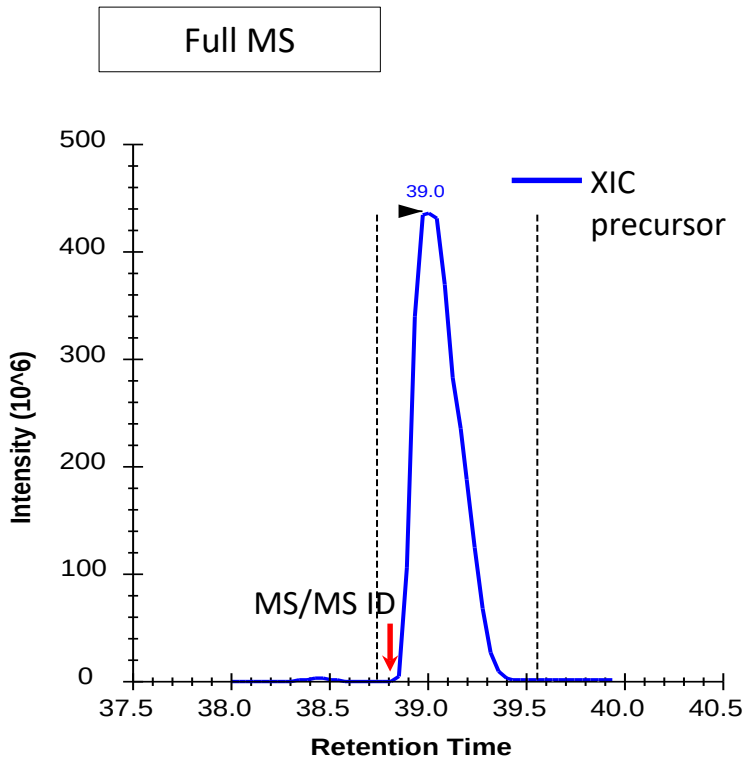
| | H3K18ac | | H3K23ac | |
|---|---|---|---|---|
| **Sequence** | ***b*** | ***y*** | ***b*** | ***y*** |
| **K** | **227.15** | 1140.71 | **241.19** | 1140.71 |
| **Q** | **355.21** | **914.57** | **369.25** | **900.53** |
| **L** | **468.30** | **786.51** | **482.34** | **772.47** |
| **A** | **539.33** | **673.42** | **553.37** | **659.38** |
| **T** | **640.38** | **602.39** | **654.42** | **588.35** |
| **K** | 824.53 | **501.34** | 824.53 | **487.30** |
| **A** | 895.56 | 317.19 | 895.56 | 317.19 |
| **A** | 966.60 | 246.16 | 966.60 | 246.16 |
| **R** | 1122.70 | 175.12 | 1122.70 | 175.12 |

*Example with histone H3 K18/K23 acetylation*

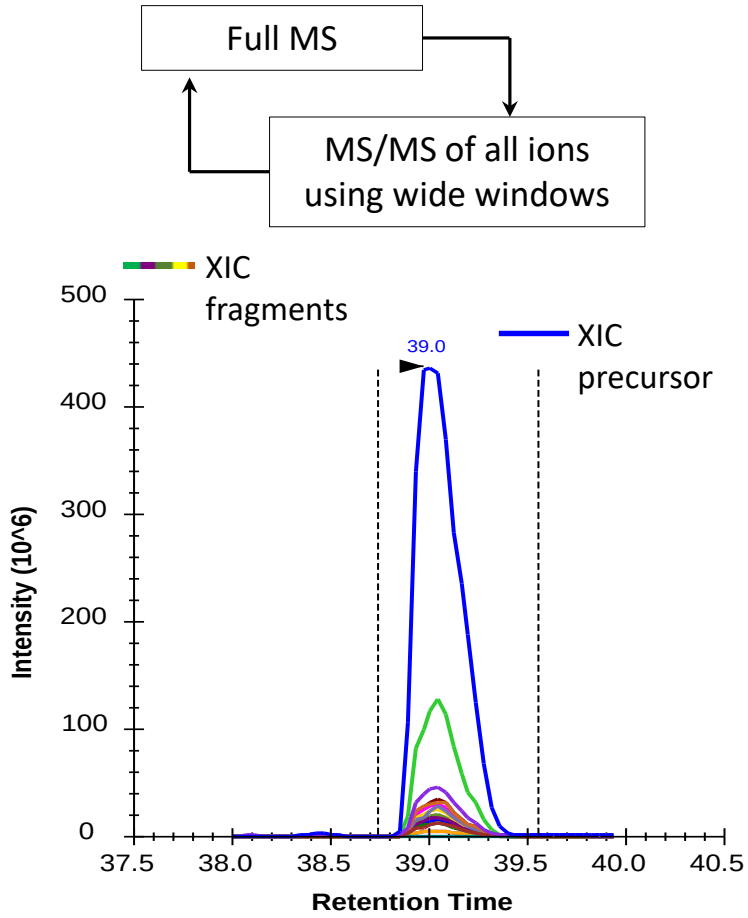
*Sidoli and Garcia, Methods in Mol. Biology (2016)*

# Data independent acquisition – profile of fragment ions

**DDA**




**DIA***




The Garcia lab has been committed in optimizing **MS methods for discriminating isobaric peptides**

** Sidoli et al. Mol Cell Proteomics (2015); * Sidoli et al. Analytical Chemistry (2015); * Sidoli et al. Proteomics (2016)*

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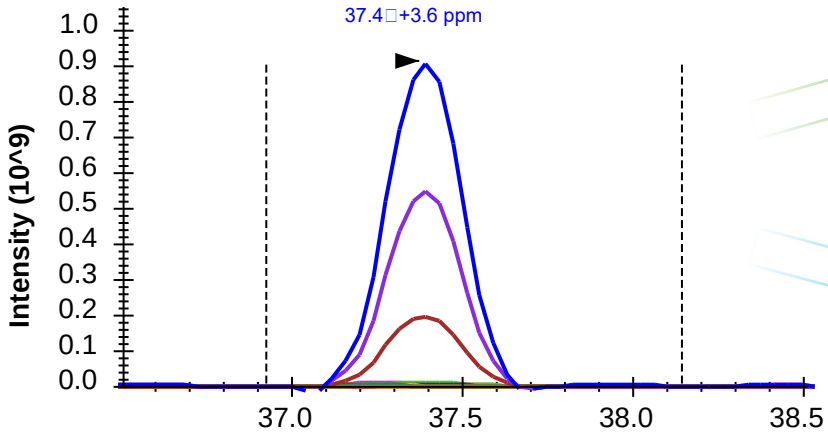
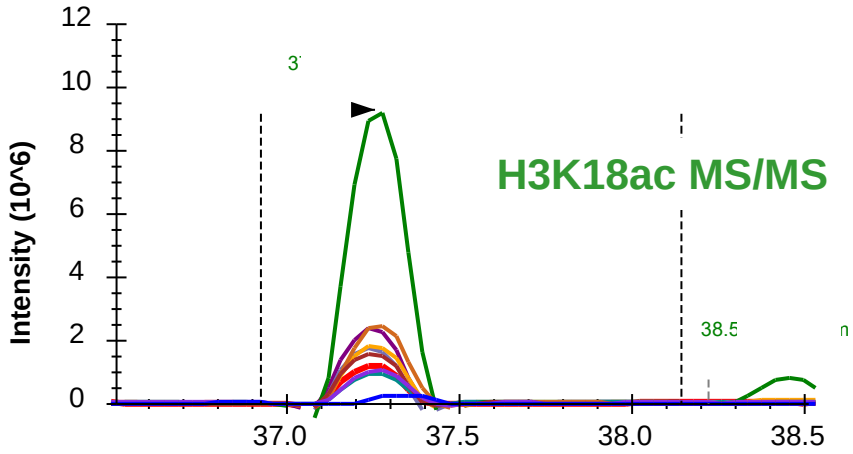
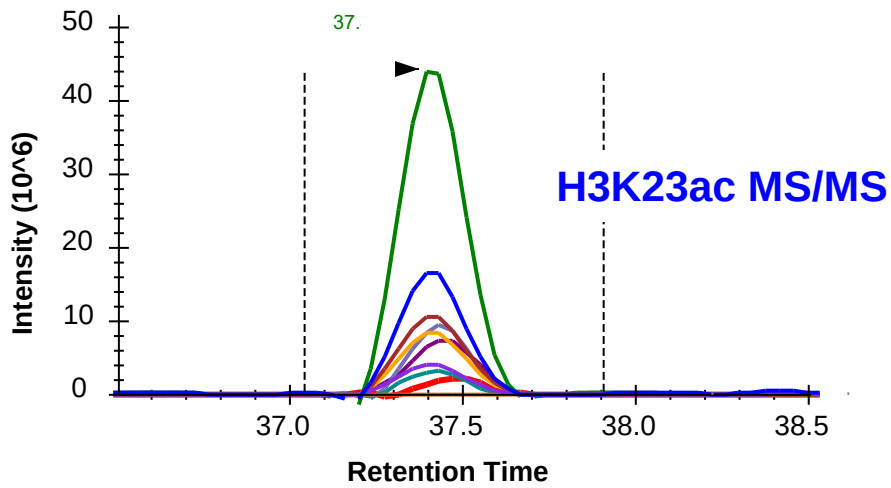
# Quantitative discrimination of isobaric peptides

(H3 aa 18-26) KQLATKAAR 1ac




Fragment ions are used to divide the precursor area intensity between the two forms

*Figure from Sidoli et al. Proteomics (2016)*

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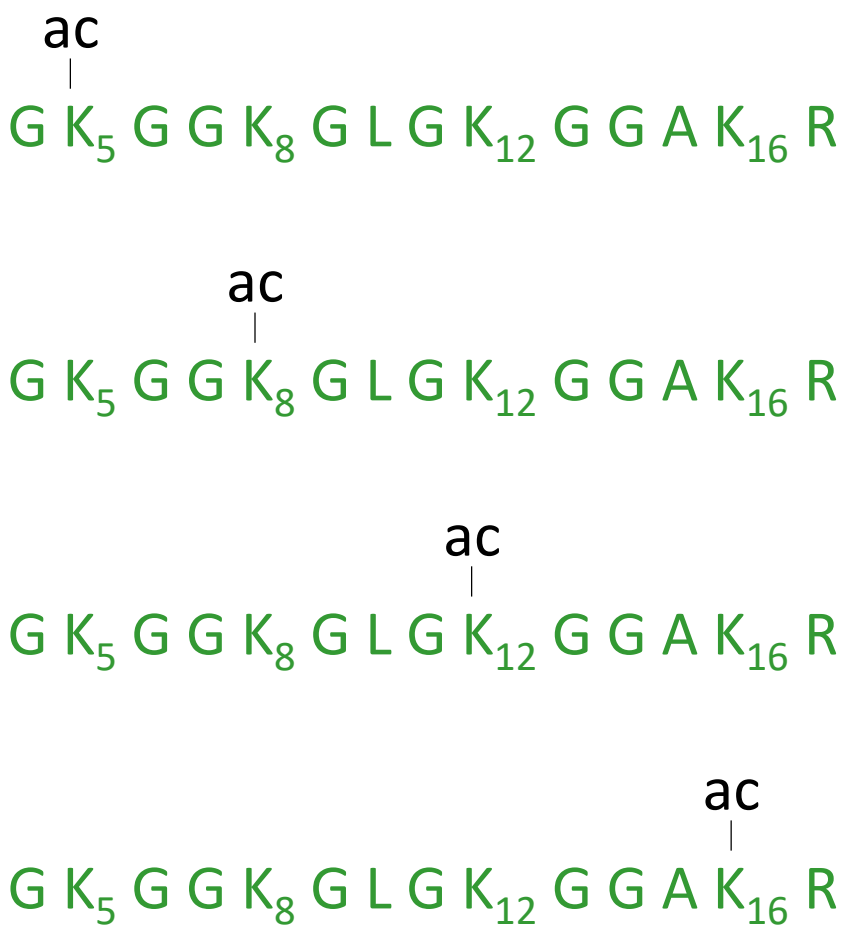
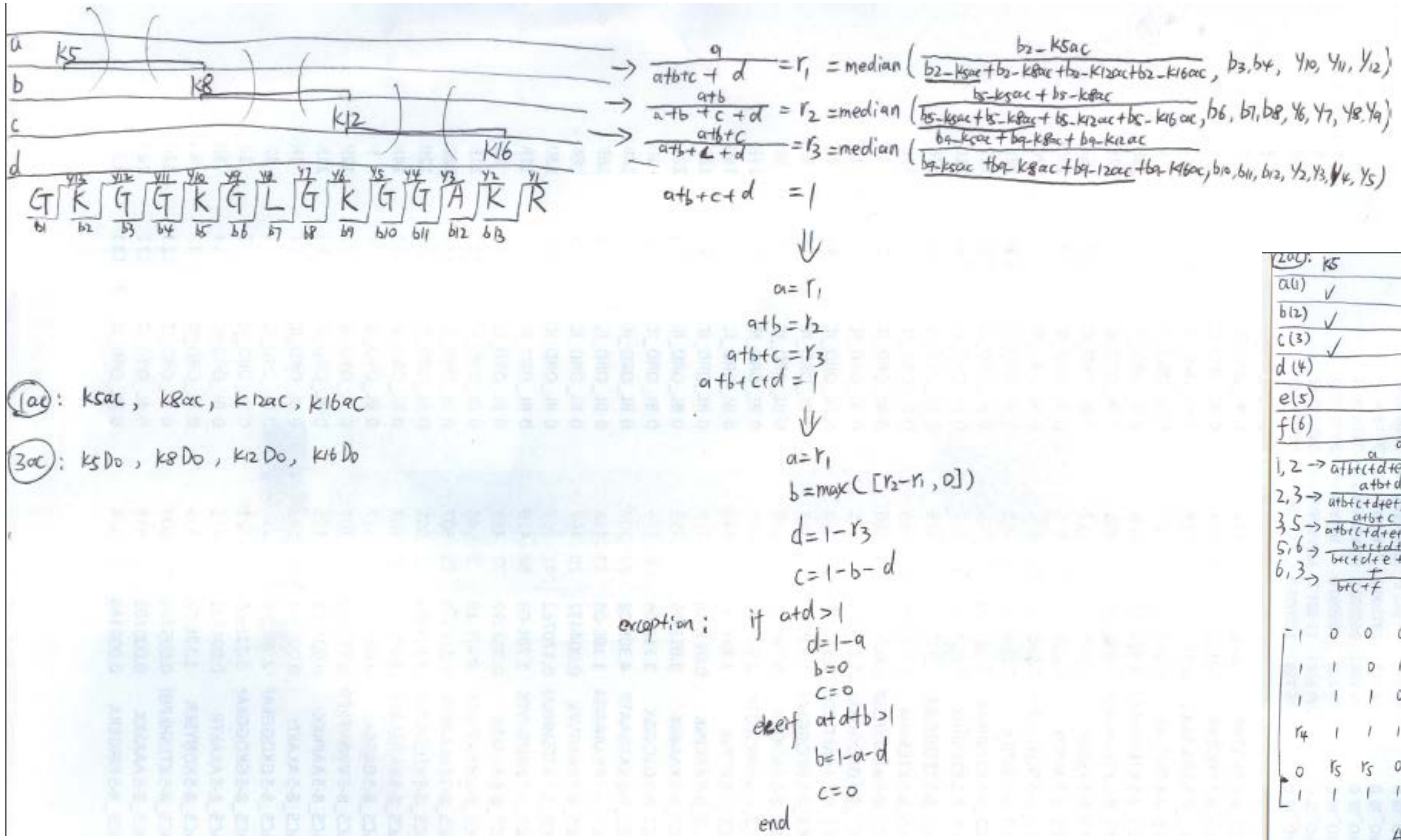
# When isobaric forms are more than two

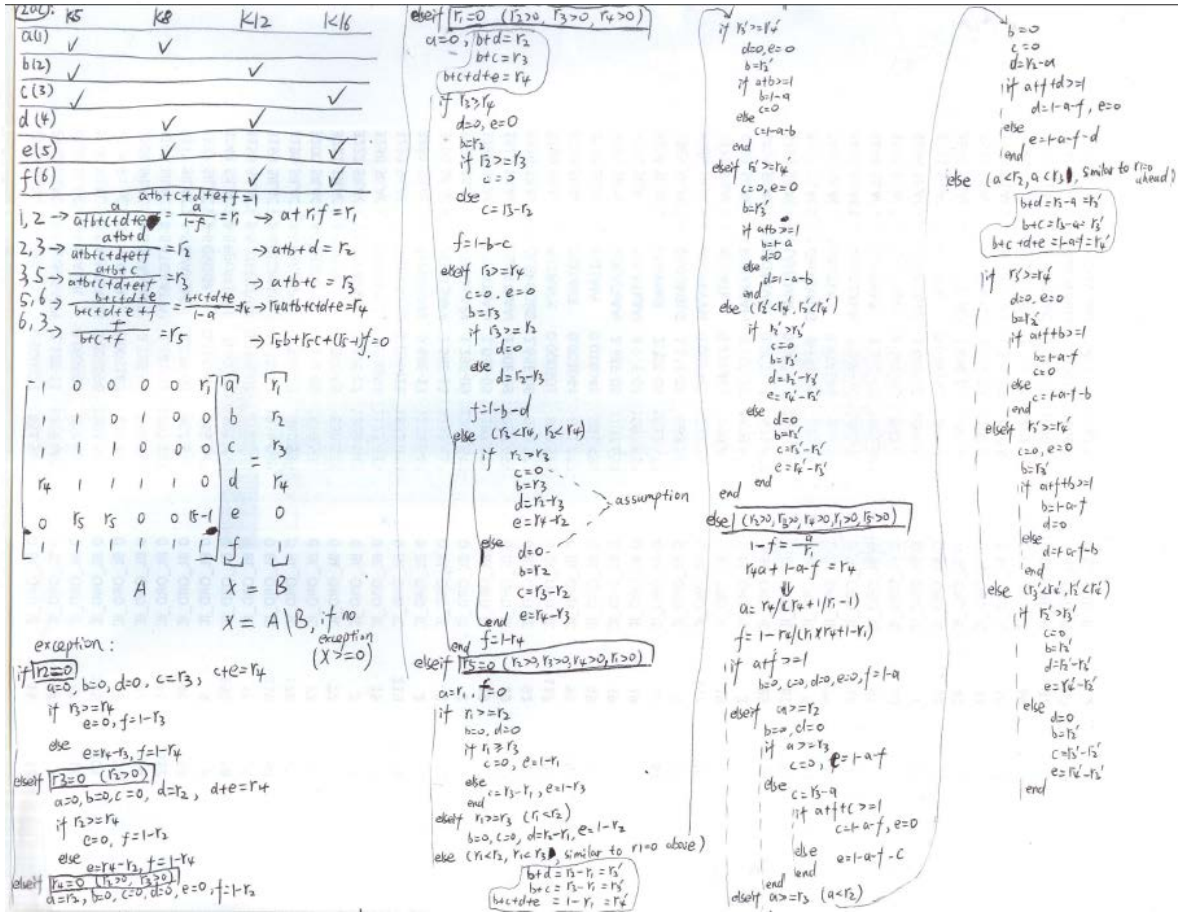



# When isobaric forms are more than two




4 isobaric forms

6 isobaric forms




*Yuan et al. Mol Cell Proteomics (2015)*

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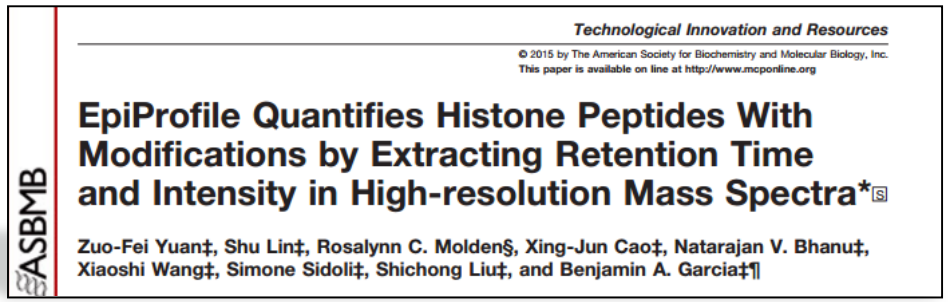
# Software for histone PTM analysis

(1) MATLAB software



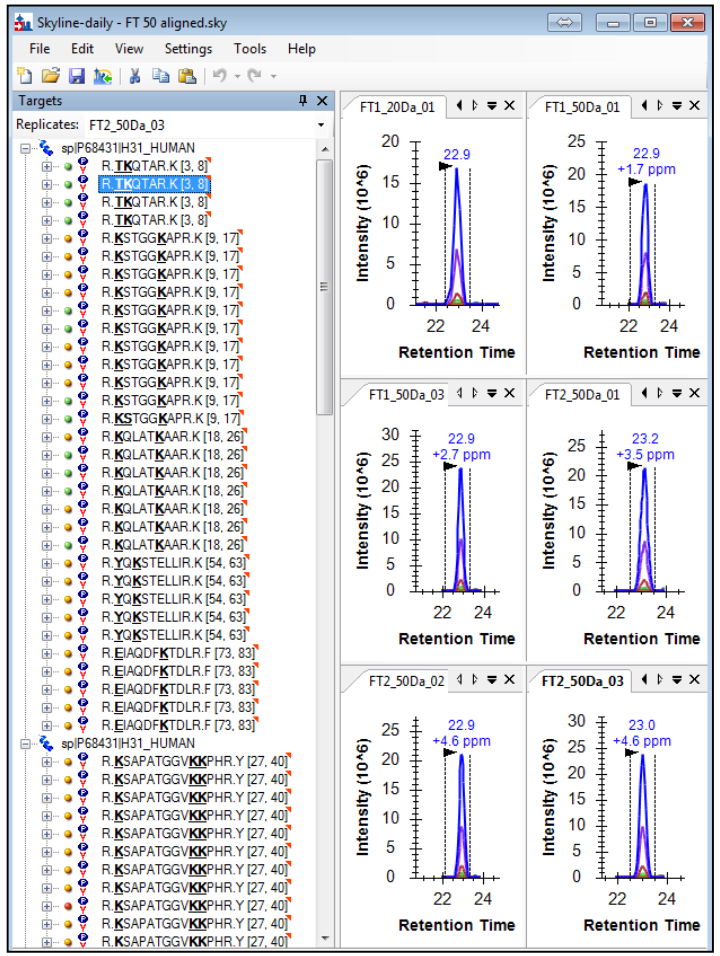
Technological Innovation and Resources



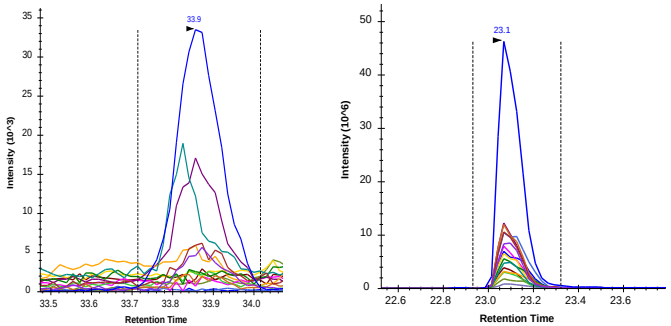
**EpiProfile Quantifies Histone Peptides With Modifications by Extracting Retention Time and Intensity in High-resolution Mass Spectra***

Zuo-Fei Yuan‡, Shu Lin‡, Rosalynn C. Molden§, Xing-Jun Cao‡, Natarajan V. Bhanu‡, Xiaoshi Wang‡, Simone Sidoli‡, Shichong Liu‡, and Benjamin A. Garcia‡¶

(2) Skyline templates




(3) New Skyline templates for rare histone PTMs




Histone crotonylation

Histone butyrylation

*(1) Yuan et al. Mol Cell Proteomics (2015); (2) Sidoli et al. Anal Chemistry (2015); (3) Simithy, Sidoli et al. (in preparation)*

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University of Pennsylvania

# Getting there also with middle-down MS

1Me 1Me

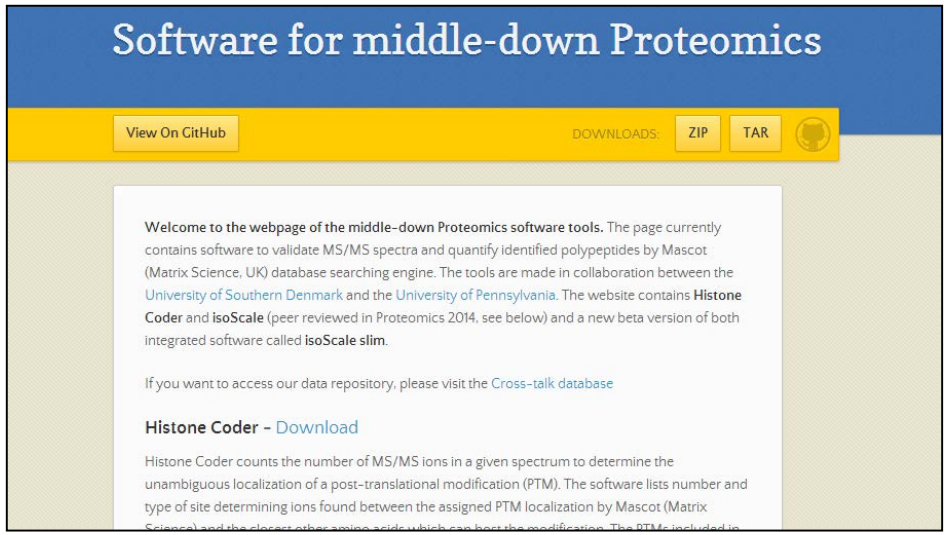
**ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALRE**

*Example of human canonical histone H3 N-terminal tail (aa 1-50) and possible modifiable sites*

The same precursor mass can be hundreds of isobaric forms (theoretically, many more!)

For quantification, we use a combination of MS/MS ion intensity (DDA) and counting # spectra corresponding to the same identification

http://middle-down.github.io/Software/




*Sidoli et al. Proteomics (2014)*

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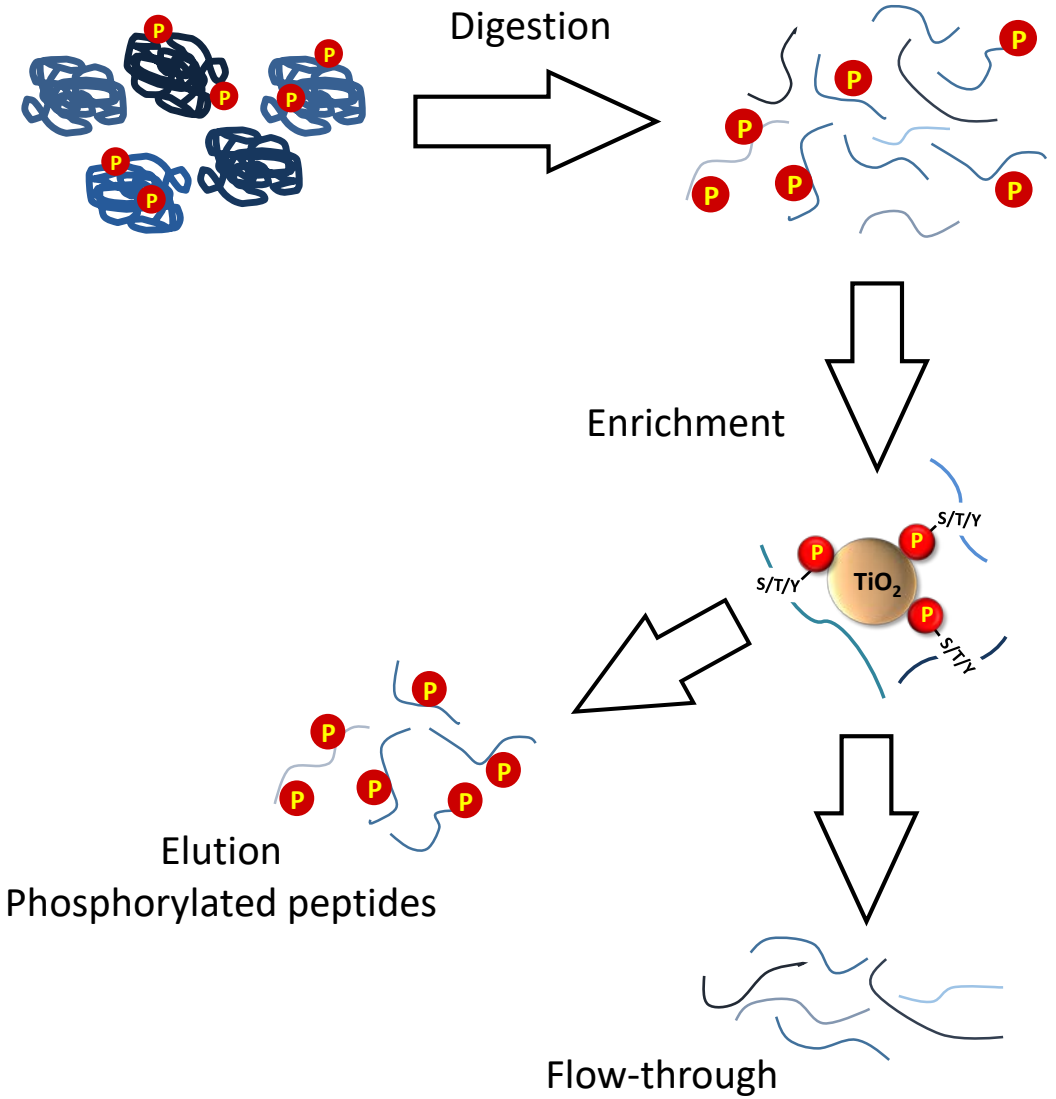
# Isobaric forms in large-scale PTM-omics

# Example with phosphoproteomics




Phosphorylation is the most frequent PTM detected on proteins (>250,000 non-redundant sites, PhosphoSitePlus)

To discriminate isobaric phosphopeptides, we:

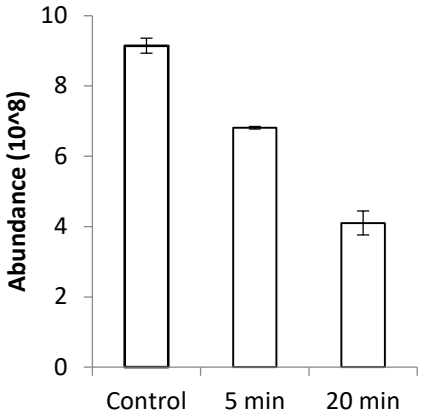
- Select isobaric phosphopeptides with same sequence and # of phospho from the spectral library (identified with DDA)
- Select fragment ions unique for each of the two forms
- Use the MS/MS extracted ion chromatogram to split the precursor MS area between the isobaric forms

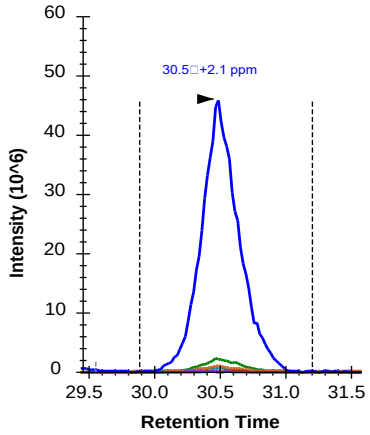
*Modified from Kulej, Sidoli et al. (in preparation)*

# Isobaric species in phosphoproteomics

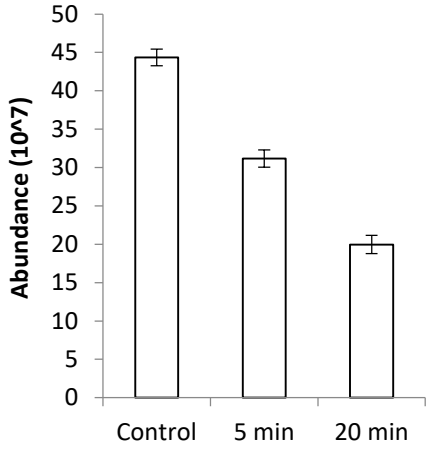
AA**S**P**S**PQSVR (aa 747,756)

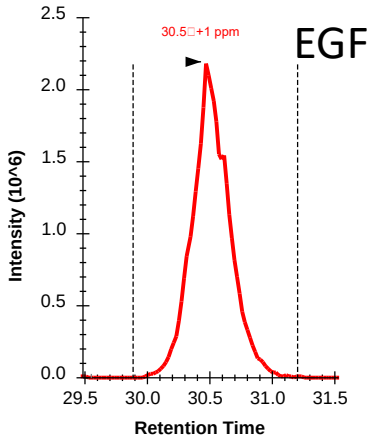
*Precursor ion*




*Fragment Ion (y5)*

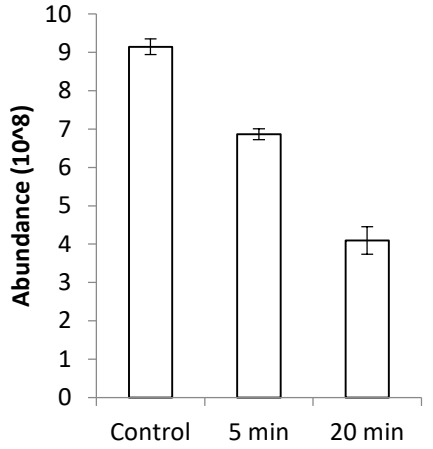


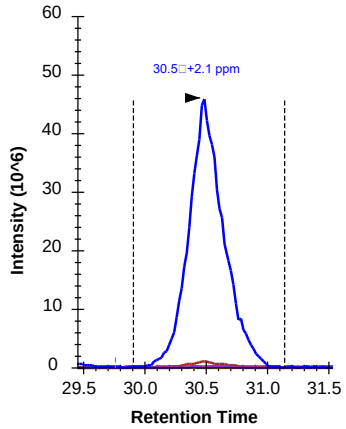




EGF stimulation of HeLa cells

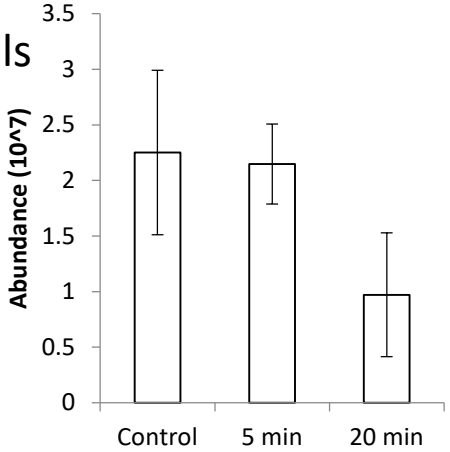
AA**S**PSPQ**S**VR (aa 747,756)

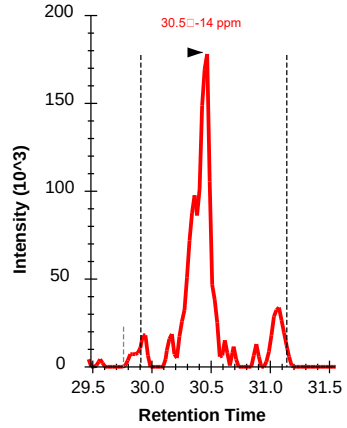
*Precursor ion*




*Fragment Ion (y5)*




*Sidoli et al., Molecular BioSystems (2016)*

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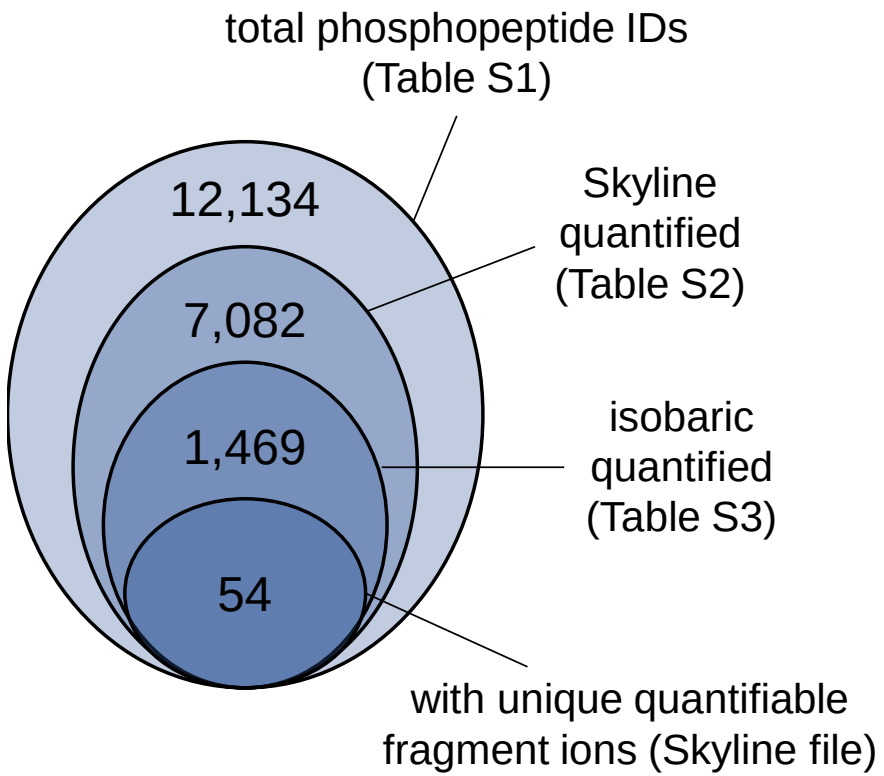
# Isobaric species in phosphoproteomics




Our first attempt was only partially successful.
Some conclusions:

1) I clearly need to get better with Skyline

2) Standard DDA methods are insufficient to detect most isobaric phosphopeptides

3) Defining unique fragment ions useful for discriminating the isobaric forms is currently the major bottleneck

*Sidoli et al., Molecular BioSystems (2016)*

# We know we are not alone ☺

Journal of proteome research

Article

pubs.acs.org/jpr

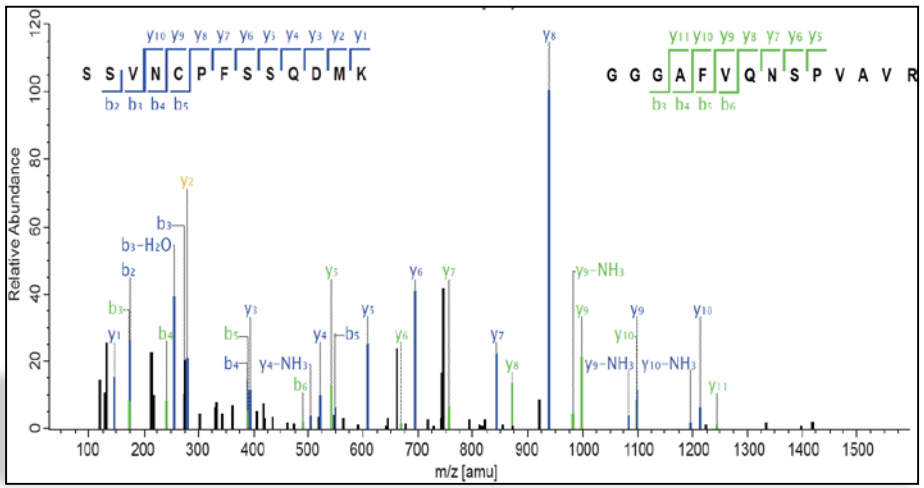
**FASIL-MS: An Integrated Proteomic and Bioinformatic Workflow To Universally Quantitate In Vivo-Acetylated Positional Isomers**

Dijana Vitko,[†] Peter Májek,[†] Erika Schirghuber,[†,‡,§] Stefan Kubicek,[†,‡] and Keiryn L. Bennett*[,†]

[†]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, A-1090 Vienna, Austria

[‡]Christian Doppler Laboratory for Chemical Epigenetics and Antiinfectives, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, A-1090 Vienna, Austria

Second peptide search, MaxQuant




From ASMS 2017

**Quantifying phosphopeptide positional isomers in DIA experiments**

**Authors**

Brian C. Searle[1, 2]; Michael J. MacCoss[1]; Judit Villén[1]

*«… we find that in any given experiment approximately 40% of phosphopeptides exist as at least two significantly localized positional isomers.»*

# Conclusions

Isobaric modified peptides have been overlooked for too long, while they hide fundamental information about biological systems and PTM cross-talk

- Independently from the quantification method (label-free, SILAC, isobaric labeling), the profile of the fragment ions is required to discriminate isobaric forms. DIA seems to be currently the only method suitable for the issue
  - *Because isobaric forms do not always completely co-elute, a single MS/MS spectrum is not sufficient to estimate their relative ratio*

- Future spectral libraries need to include isobaric peptides, and software need to cope with differential quantification of modified peptides with the same mass but different modified residues
  - *This is already a common practice in the analysis of histone peptides. It should become routine for large-scale proteomics as well*

# Acknowledgments

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Johayra Simithy

Katarzyna Kulej

Zuo-Fei Yuan

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Leukemia & Lymphoma Society

Perelman School of Medicine
University of Pennsylvania