Proteomics of protein post-translational modifications

Post-Translational Modifications (PTMs)

DNA encodes functions

↓

mRNA transfers functions

↓

Proteins fulfill functions

↕

PTMs regulate functions

Post-Translational Modifications (PTMs)

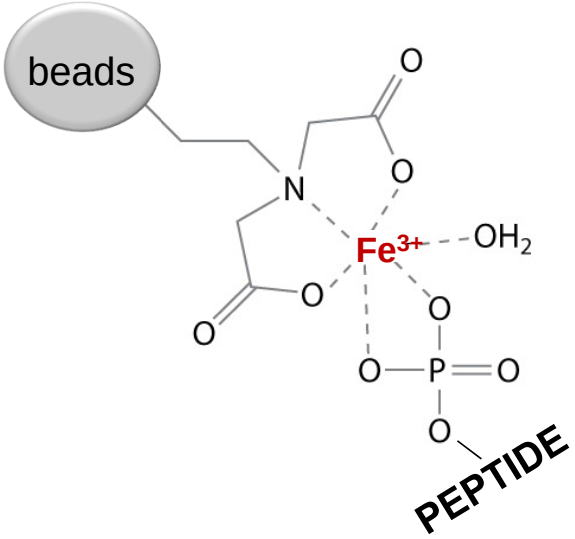
1. Phosphorylation
2. Ubiquitination
3. Lysine PTMs (acetylation, methylation, …)
4. Arginine methylation
5. Glycosylation (N-linked & O-linked)
6. Cysteine redox PTMs
7. Proteolytic processing (terminal modifications)
8. …

Phosphorylation

Immobilized Metal ion Affinity Chromatography (IMAC)

High specificity: >95%

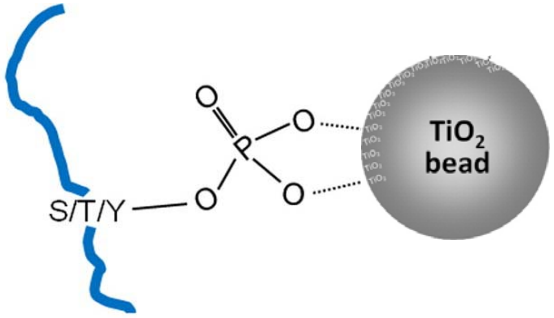
Fe^{3+} NTA-Agarose magnetic beads



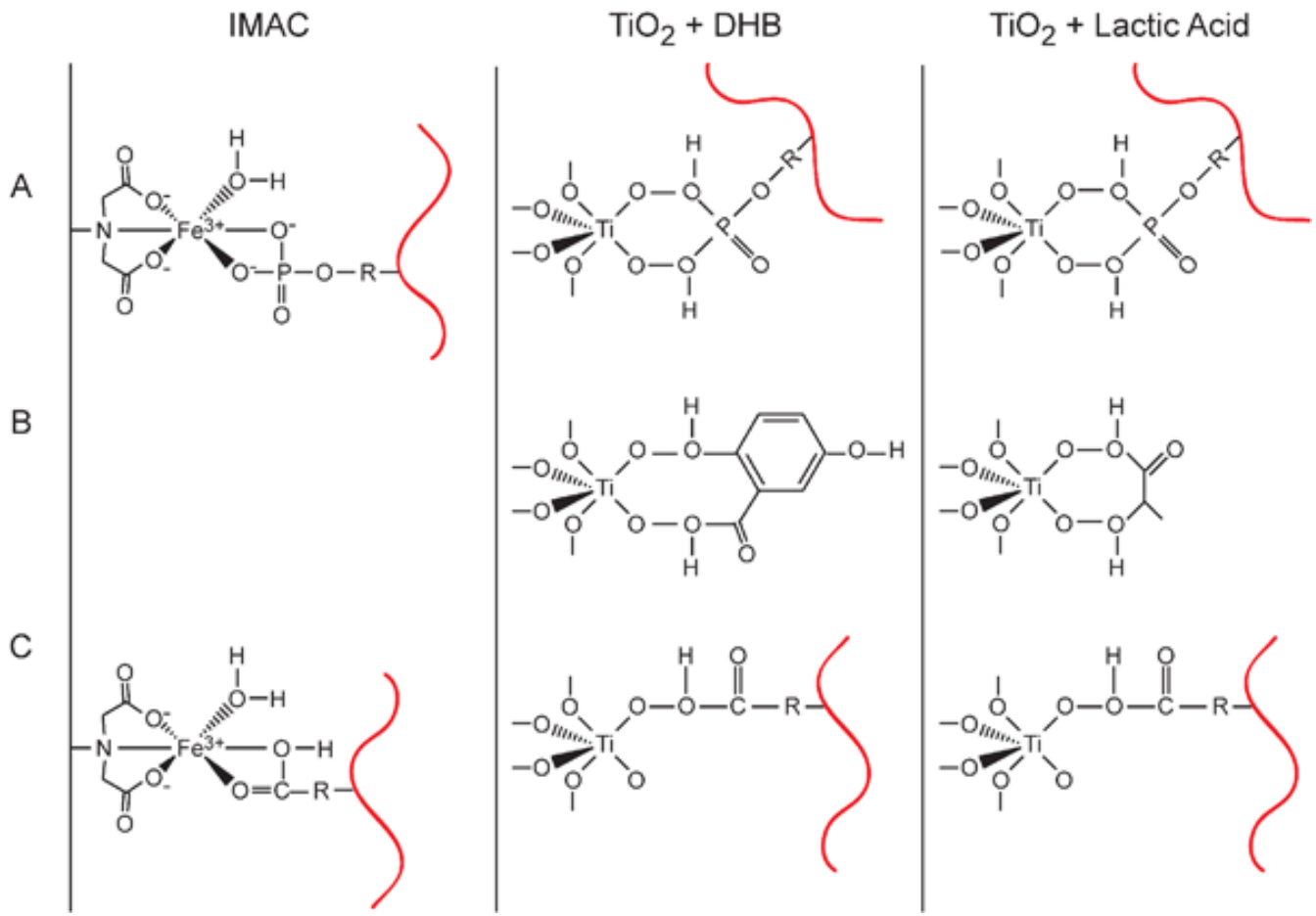
Metal Oxide Affinity Chromatography (MOAC)

High specificity: >90%

Titanium oxide beads



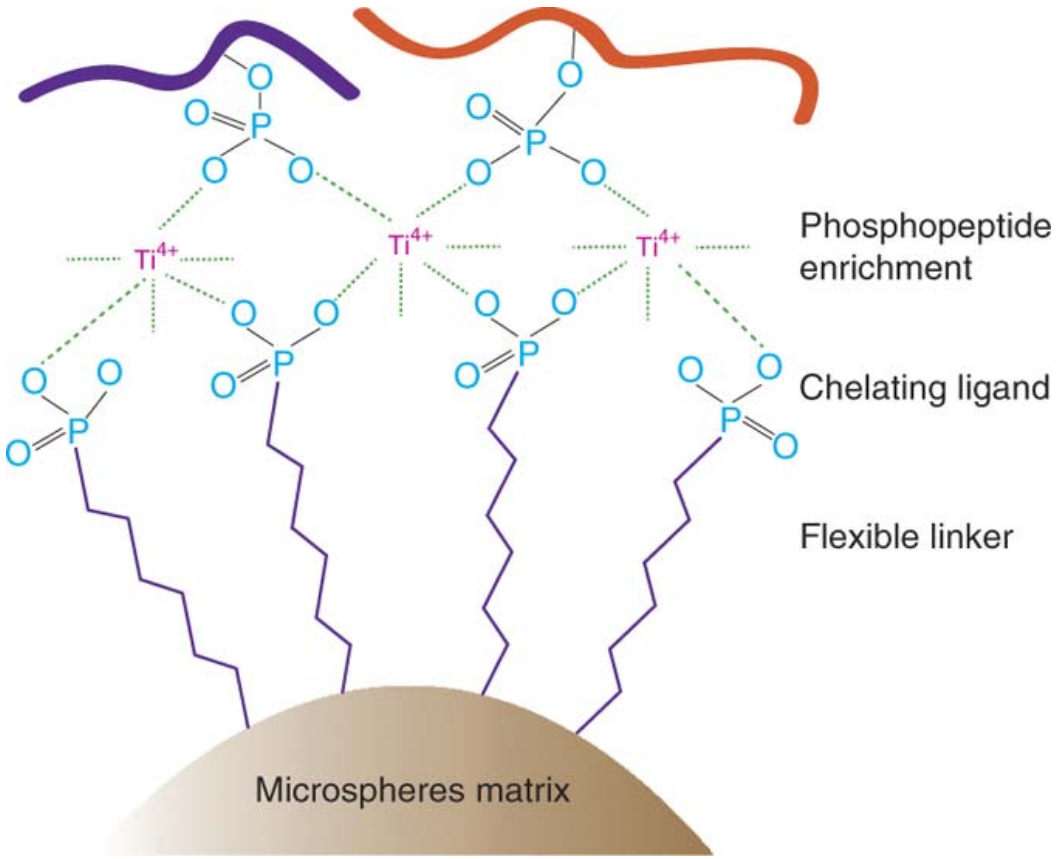
Phosphorylation



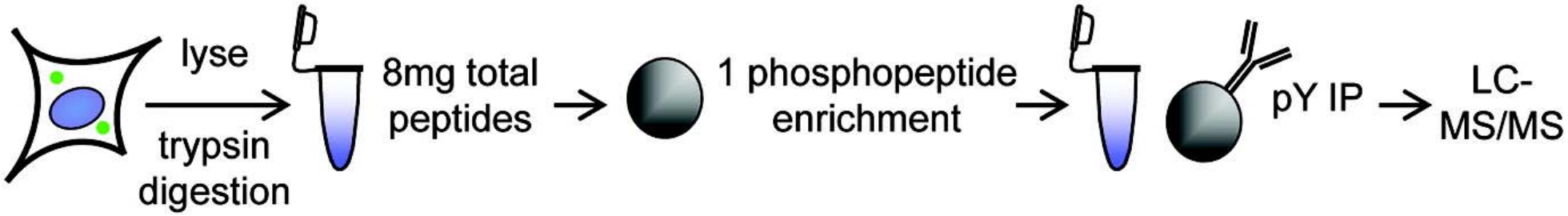
Phosphorylation

Ti (4+)-IMAC

High specificity: >98%



Phosphorylation on tyrosine



Cell Reports
Resource

OPEN ACCESS
CellPress

Ultradeep Human Phosphoproteome Reveals a Distinct Regulatory Nature of Tyr and Ser/Thr-Based Signaling

Kirti Sharma,[1] Rochelle C.J. D'Souza,[1] Stefka Tyanova,[1] Christoph Schaab,[1] Jacek R. Wiśniewski,[1] Jürgen Cox,[1,*] and Matthias Mann[1,*]
[1]Department of Proteomics and Signal Transduction, Max-Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany
*Correspondence: cox@biochem.mpg.de (J.C.), mmann@biochem.mpg.de (M.M.)
http://dx.doi.org/10.1016/j.celrep.2014.07.036
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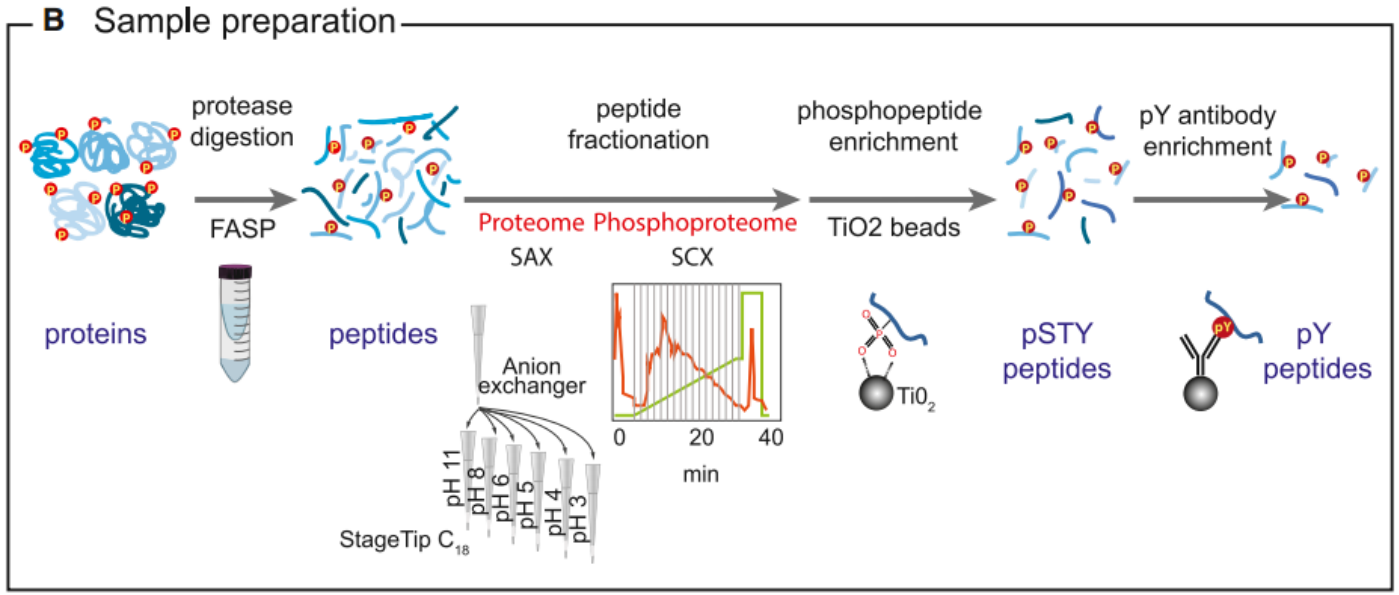
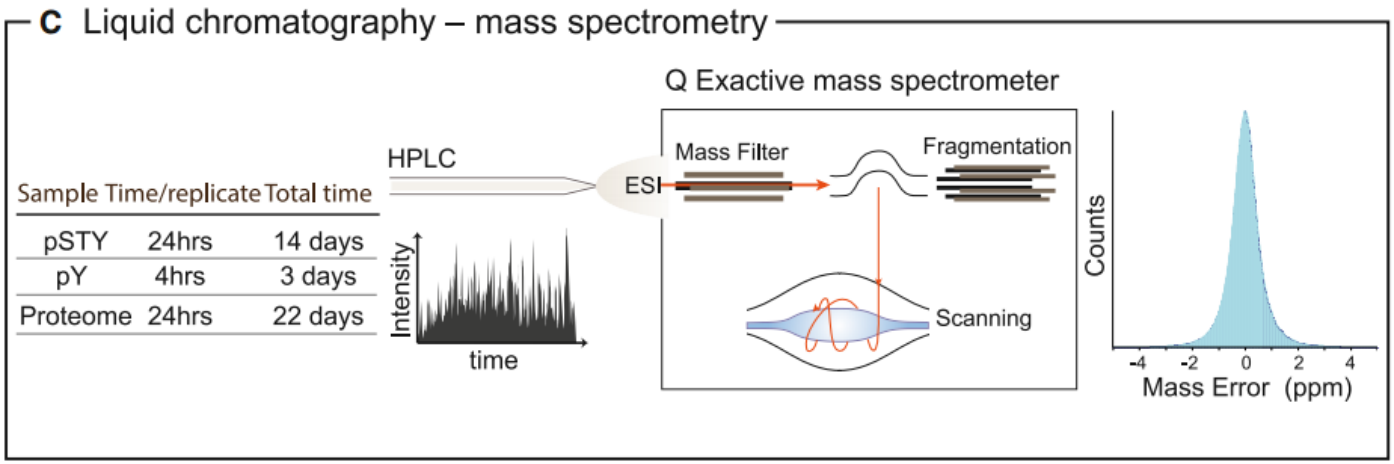
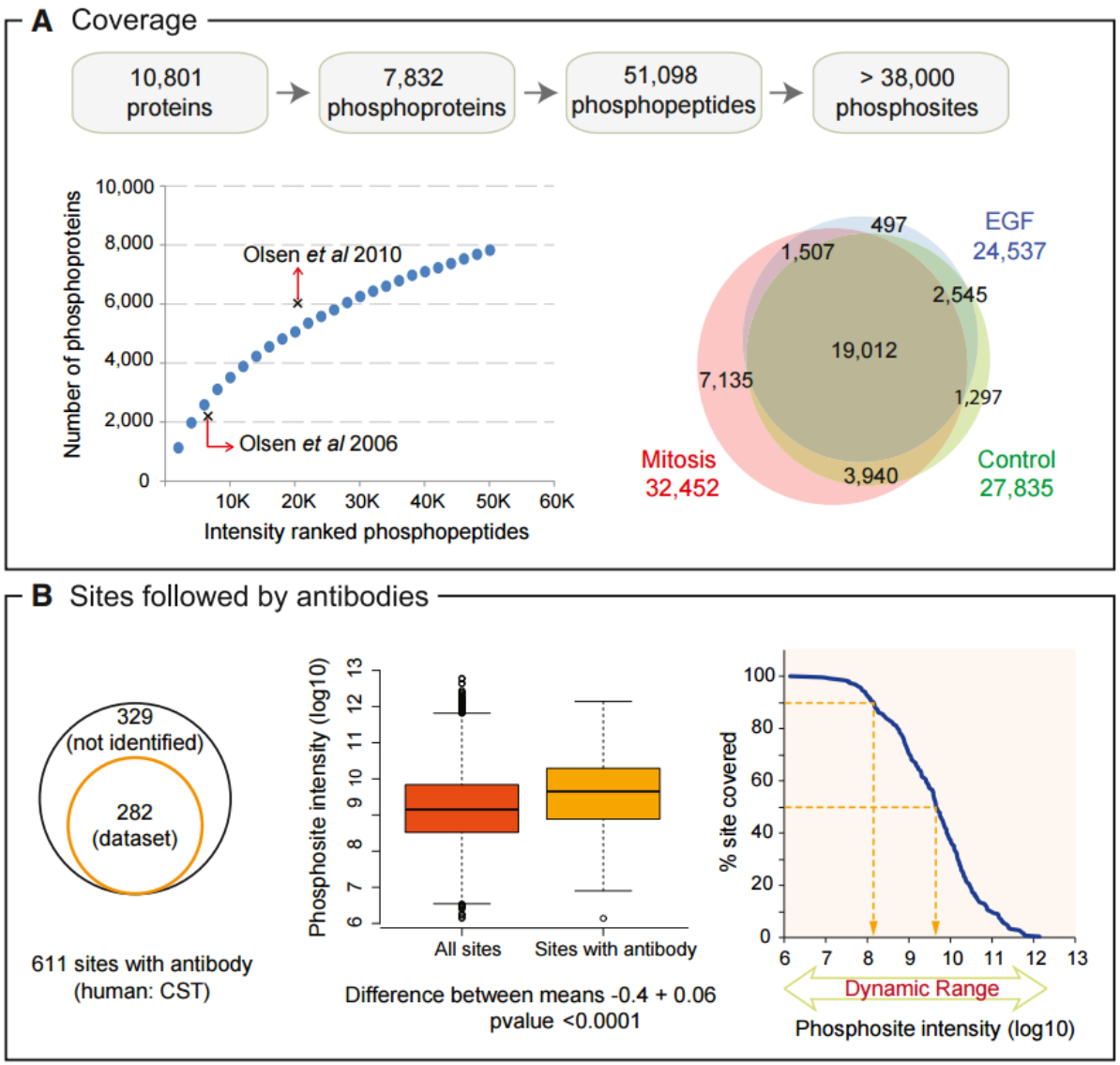
A total of 12.5 mg of protein lysate per experiment

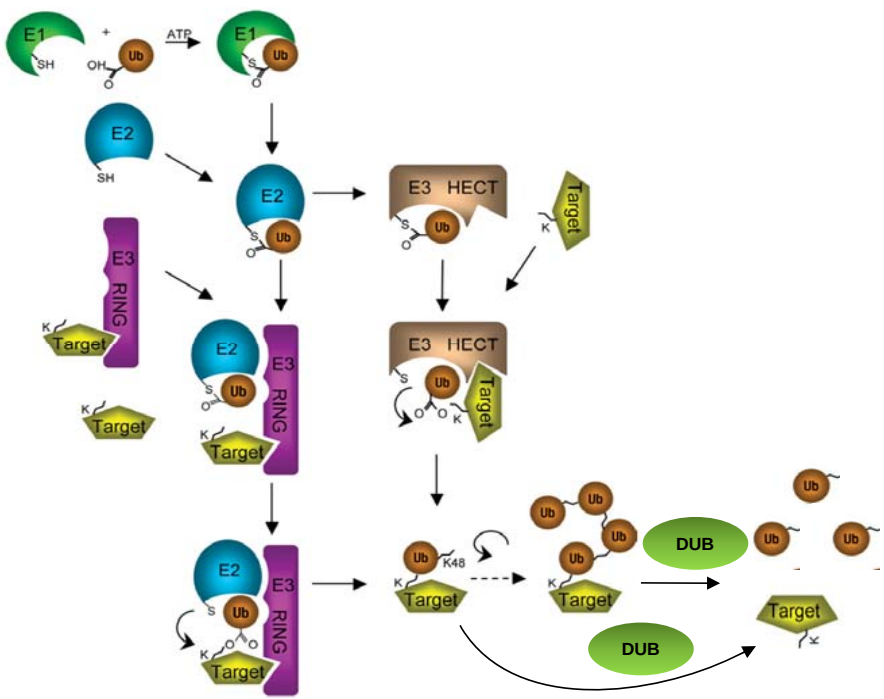





A Coverage
10,801 proteins
7,832 phosphoproteins
51,098 phosphopeptides
> 38,000 phosphosites
Number of phosphoproteins
10,000
8,000
6,000
4,000
2,000
0
Olsen et al 2010
Olsen et al 2006
10K
20K
30K
40K
50K
60K
Intensity ranked phosphopeptides
497
EGF
24,537
1,507
2,545
19,012
7,135
1,297
Mitosis
32,452
3,940
Control
27,835
B Sites followed by antibodies
329
(not identified)
282
(dataset)
611 sites with antibody
(human: CST)
Phosphosite intensity (log10)
6
7
8
9
10
11
12
13
All sites
Sites with antibody
Difference between means -0.4 + 0.06
pvalue <0.0001
% site covered
100
80
60
40
20
0
6
7
8
9
10
11
12
13
Dynamic Range
Phosphosite intensity (log10)

Ubiquitination

Ubiquitin: 76 AA polypeptide that conjugates with other proteins

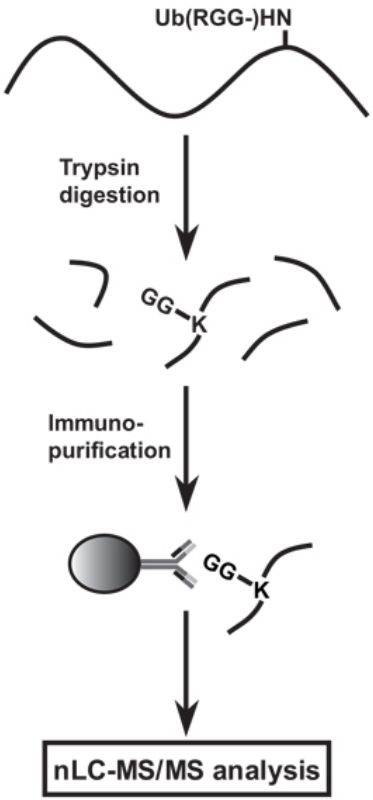


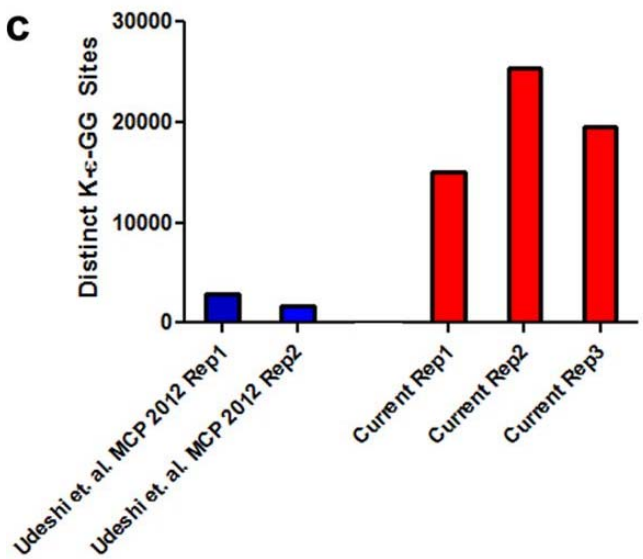
Hurley et al, BJ, 2006

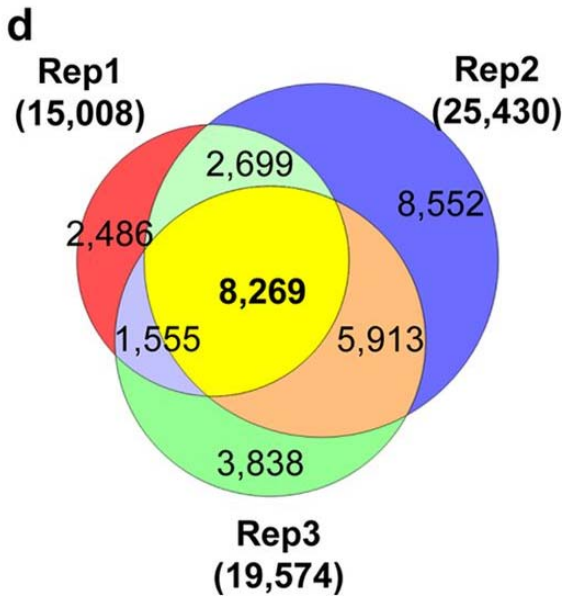
Ubiquitination	
E1	**10**
E2	**~40**
E3	**>600**
DUB	**~90**
Phosphorylation	
Kinase	**>500**
Phosphatase	**~140**

Cohen et al, Cell, 2010.

Anti-KGG antibody



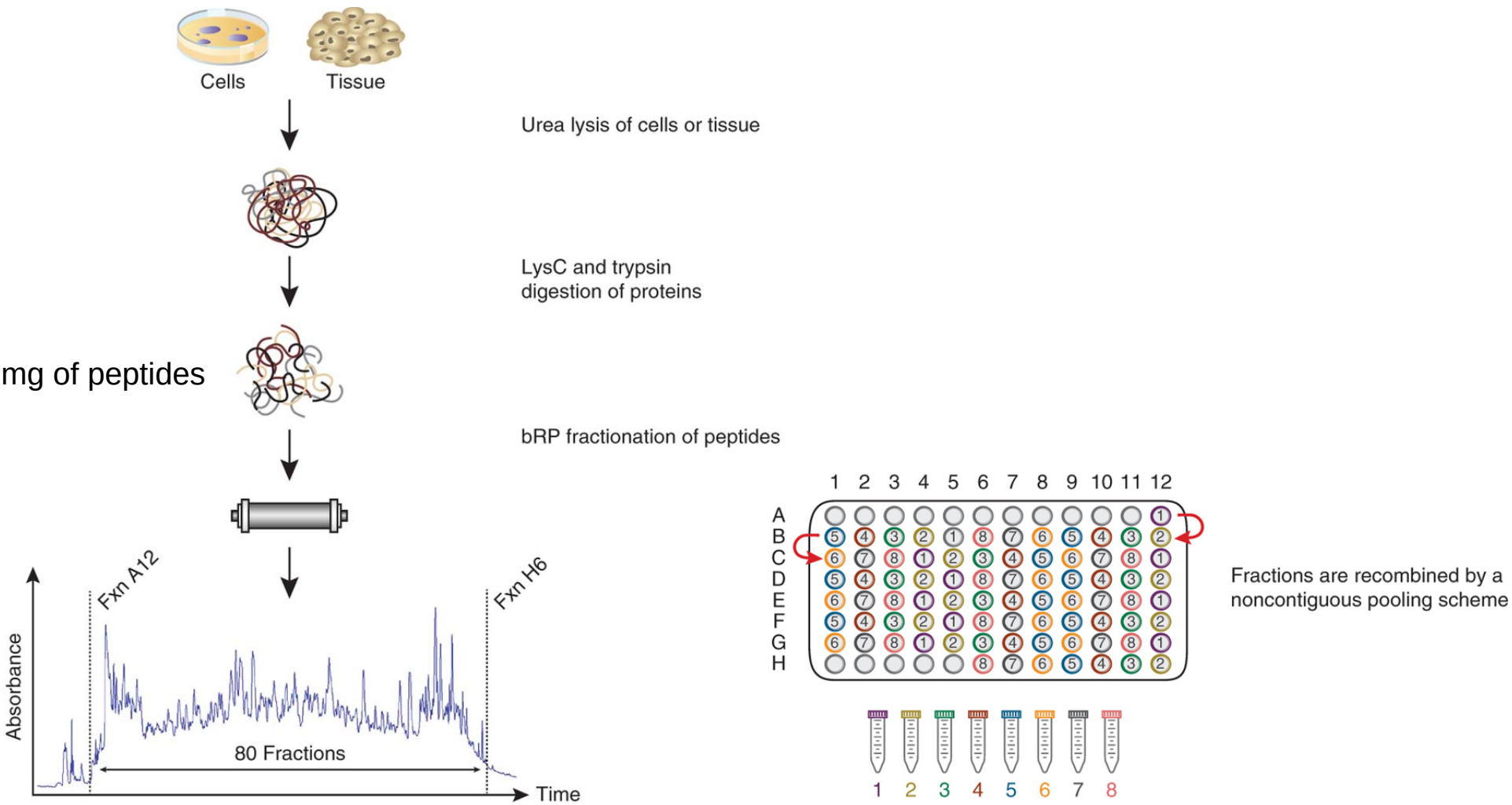




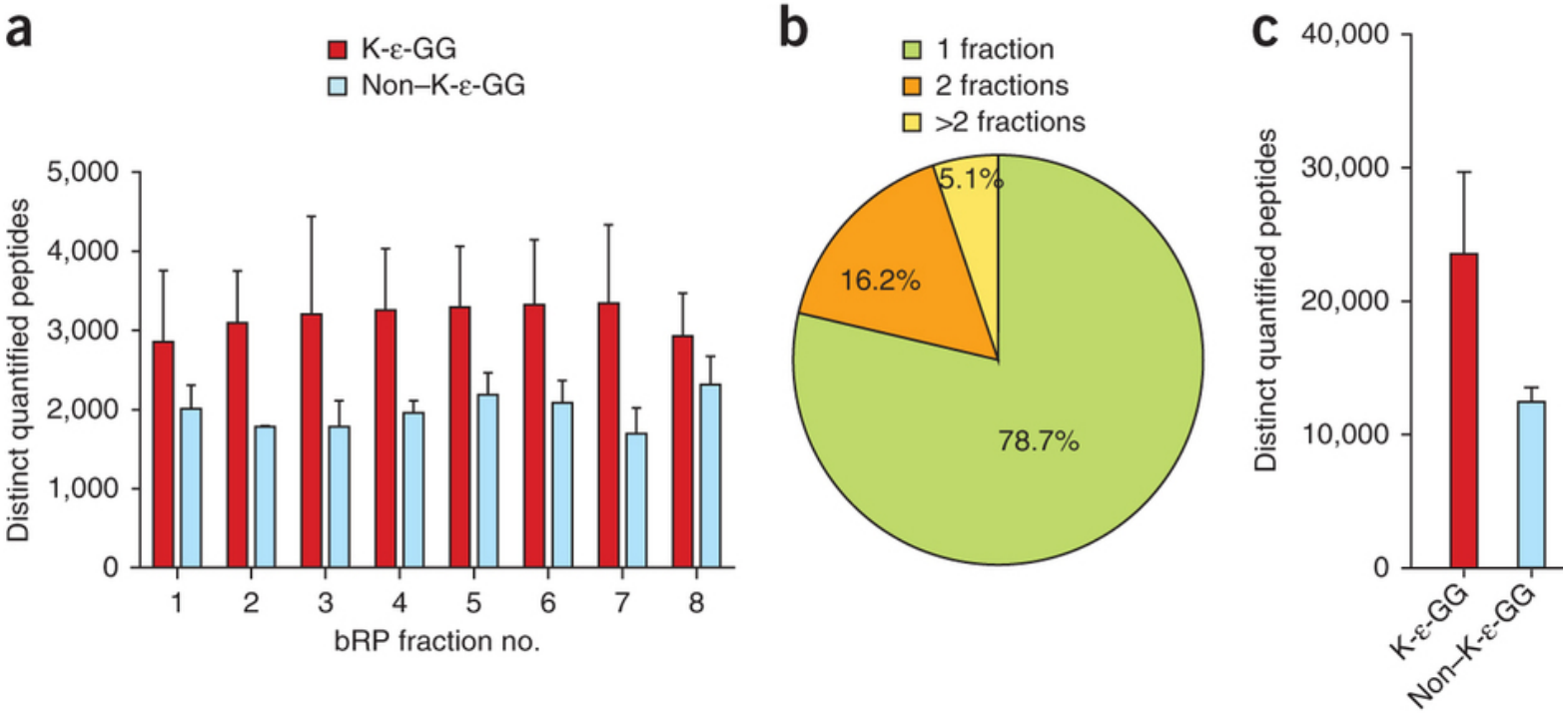
Xu et al, Nat Biotech, 2010

Mol Cell Proteomics. **2013** Mar;12(3):825-31

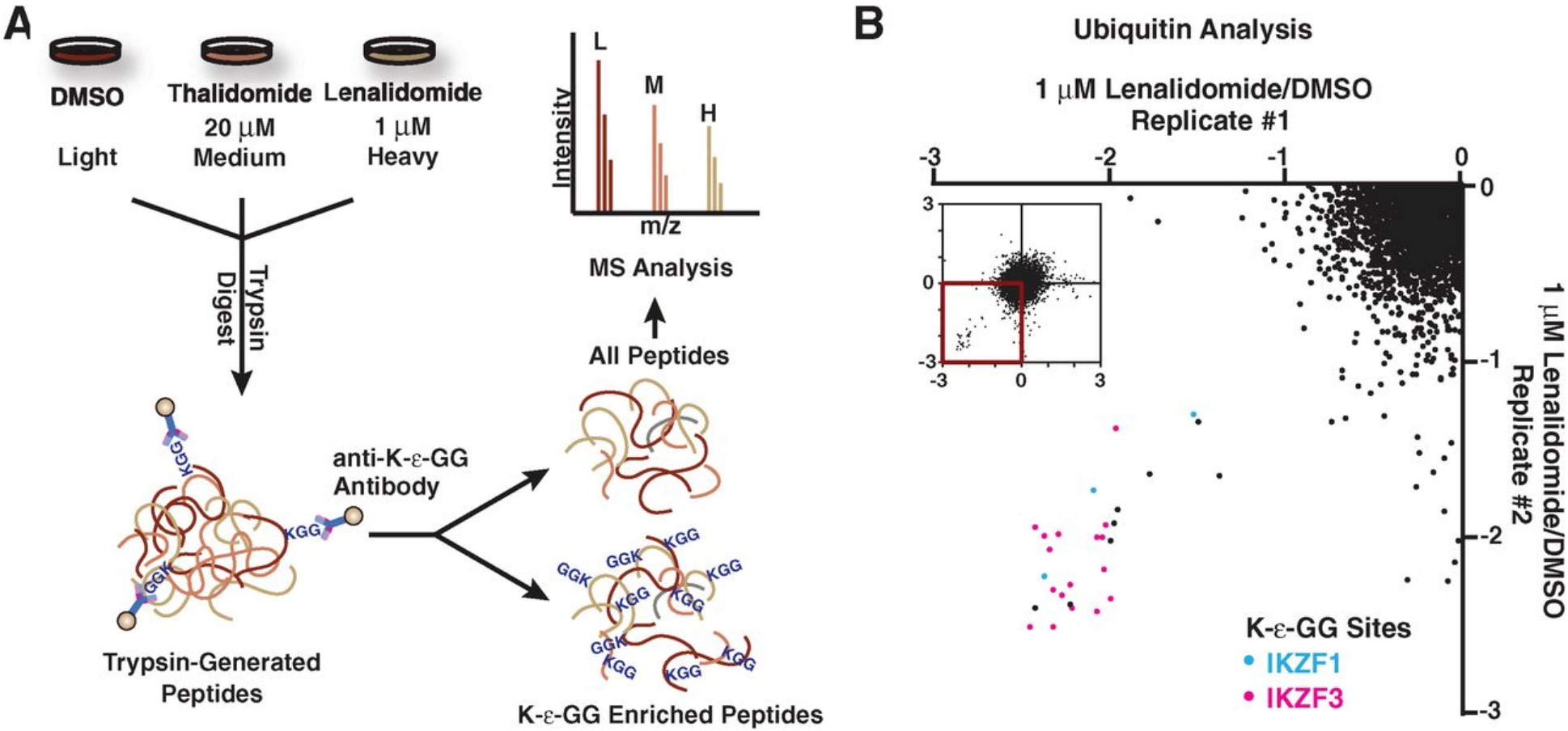
15 mg of peptides



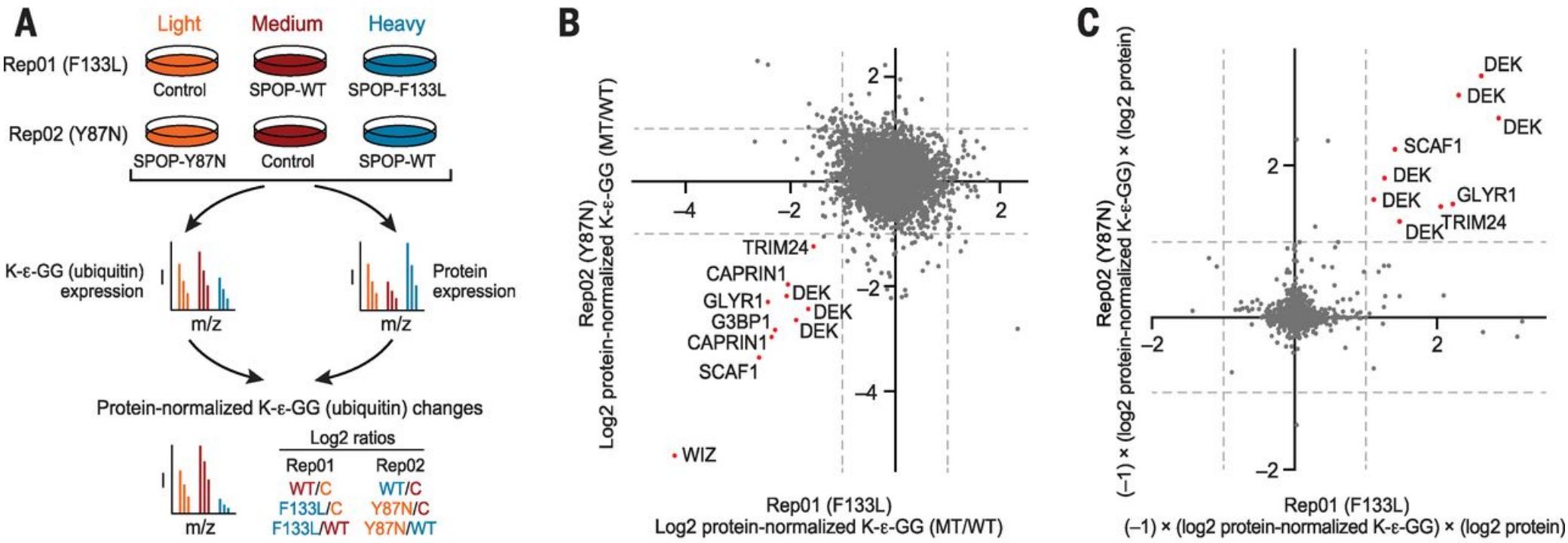
Nature Protocols 8, 1950–1960 (2013)



Nature Protocols 8, 1950–1960 (2013)

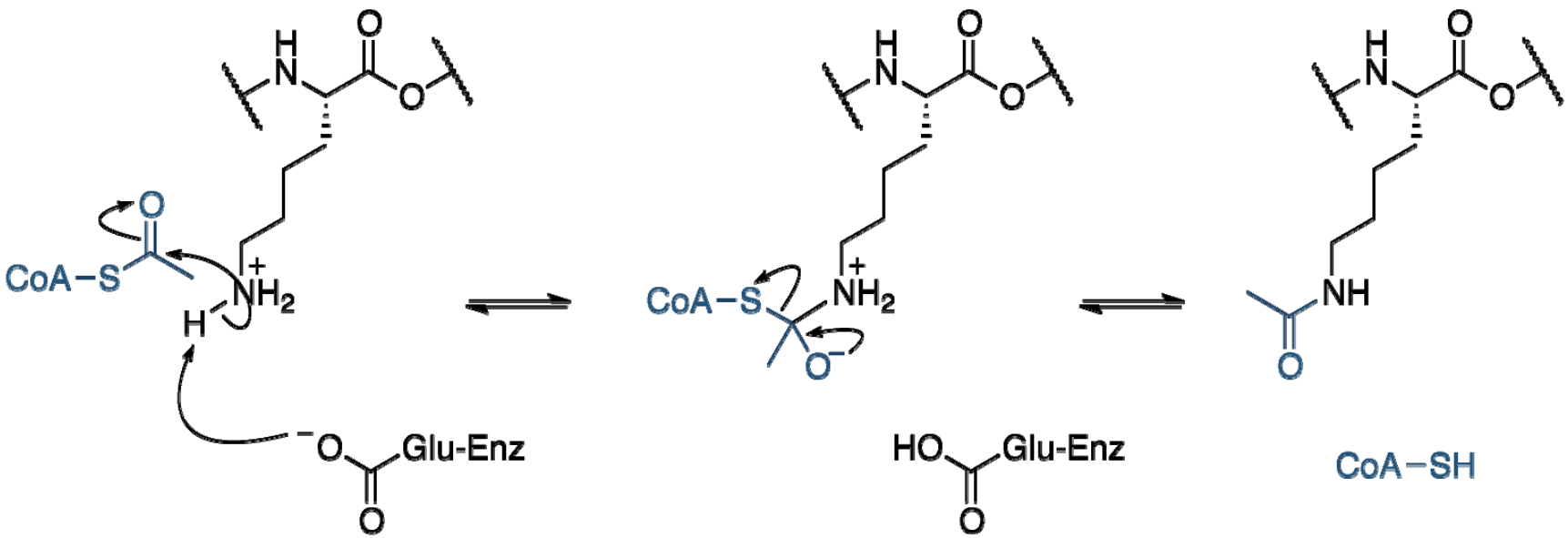


Science 17 January **2014** Vol. 343 no. 6168 pp. 301-305

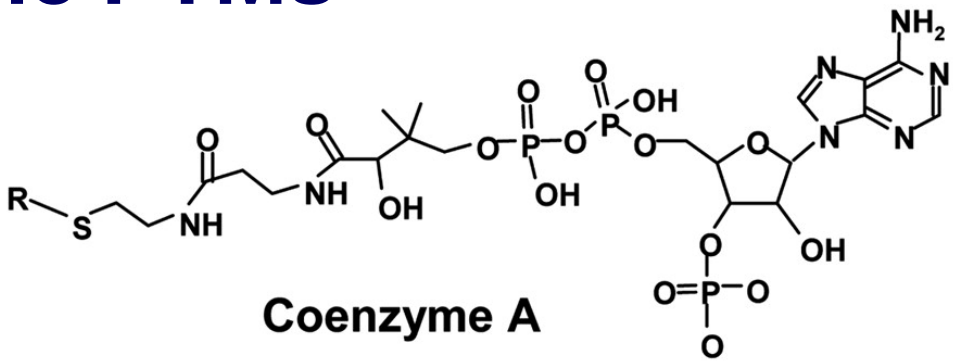


Science 3 October **2014** Vol. 346 no. 6205 pp. 85-89

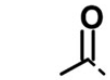
Lysine acetylation



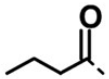
Lysine PTMs

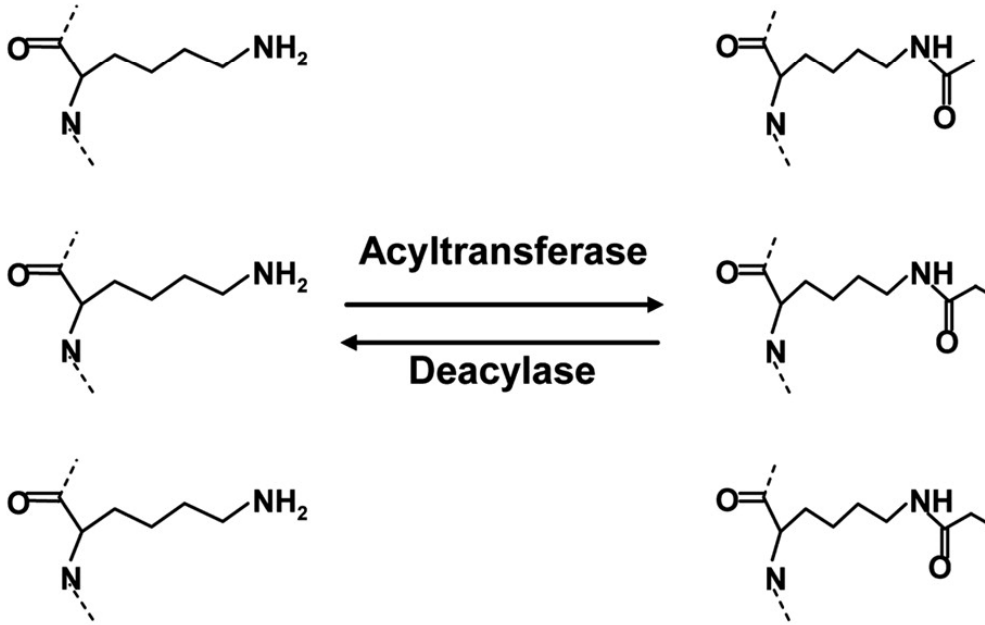


R =


Acetyl

Propionyl

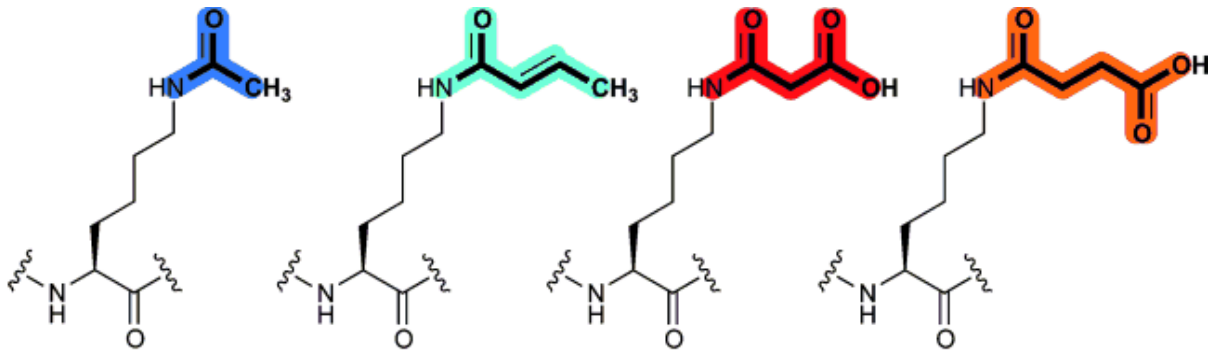

Butyryl

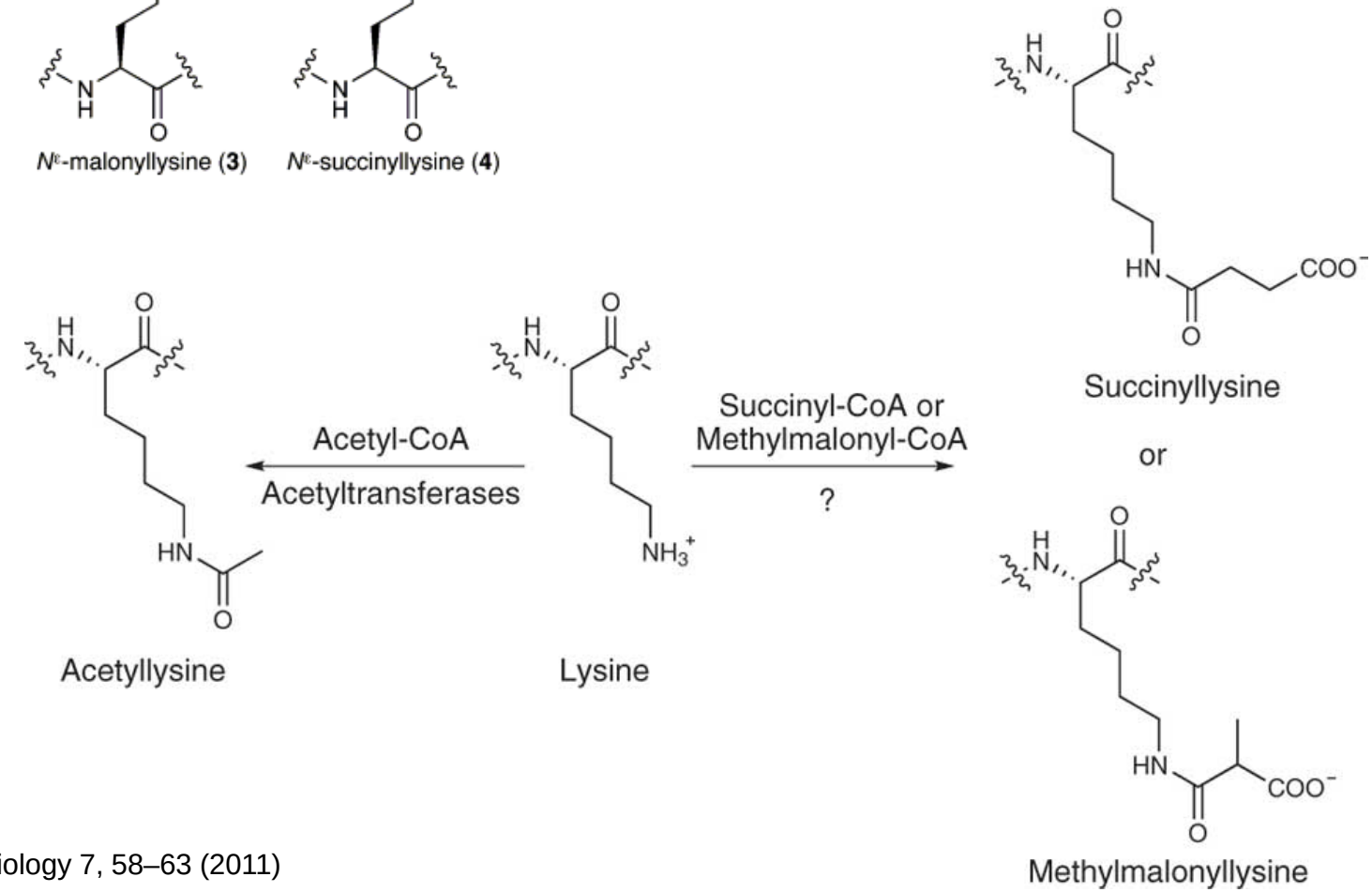


Acetyl lysine

Propionyl lysine

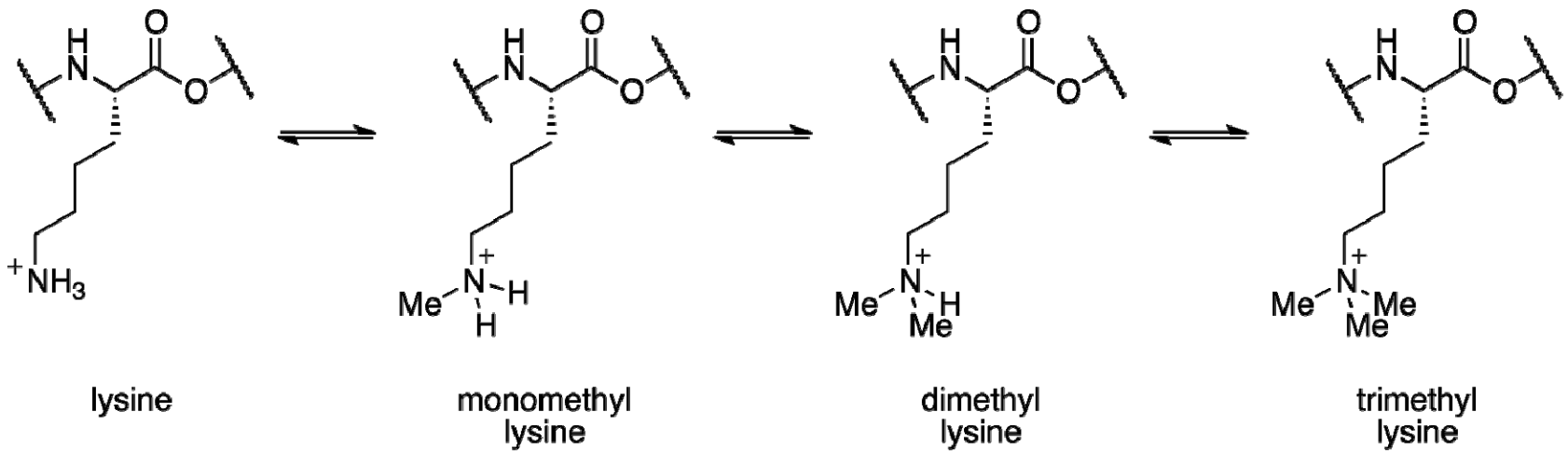
Butyryl lysine



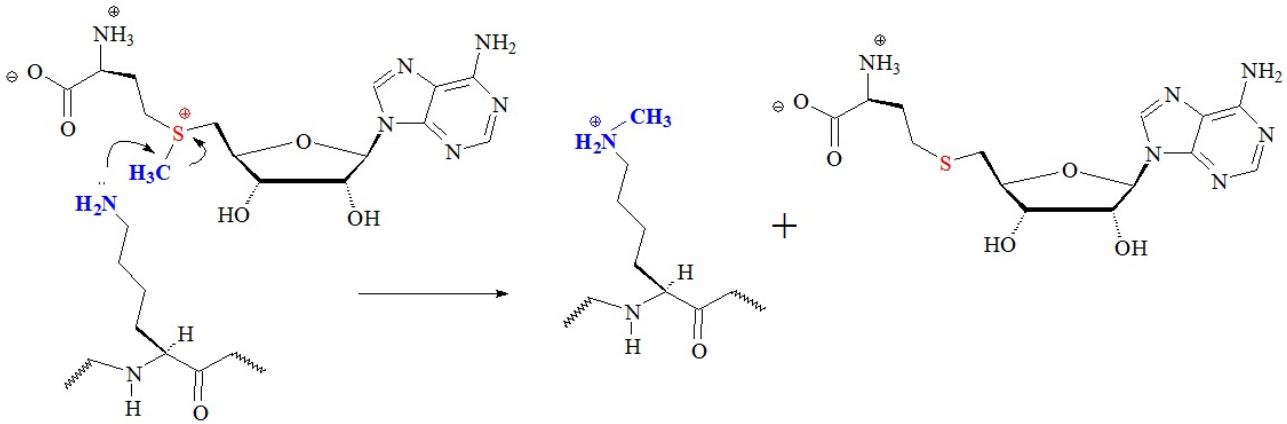


Nature Chemical Biology 7, 58–63 (2011)

Lysine methylation

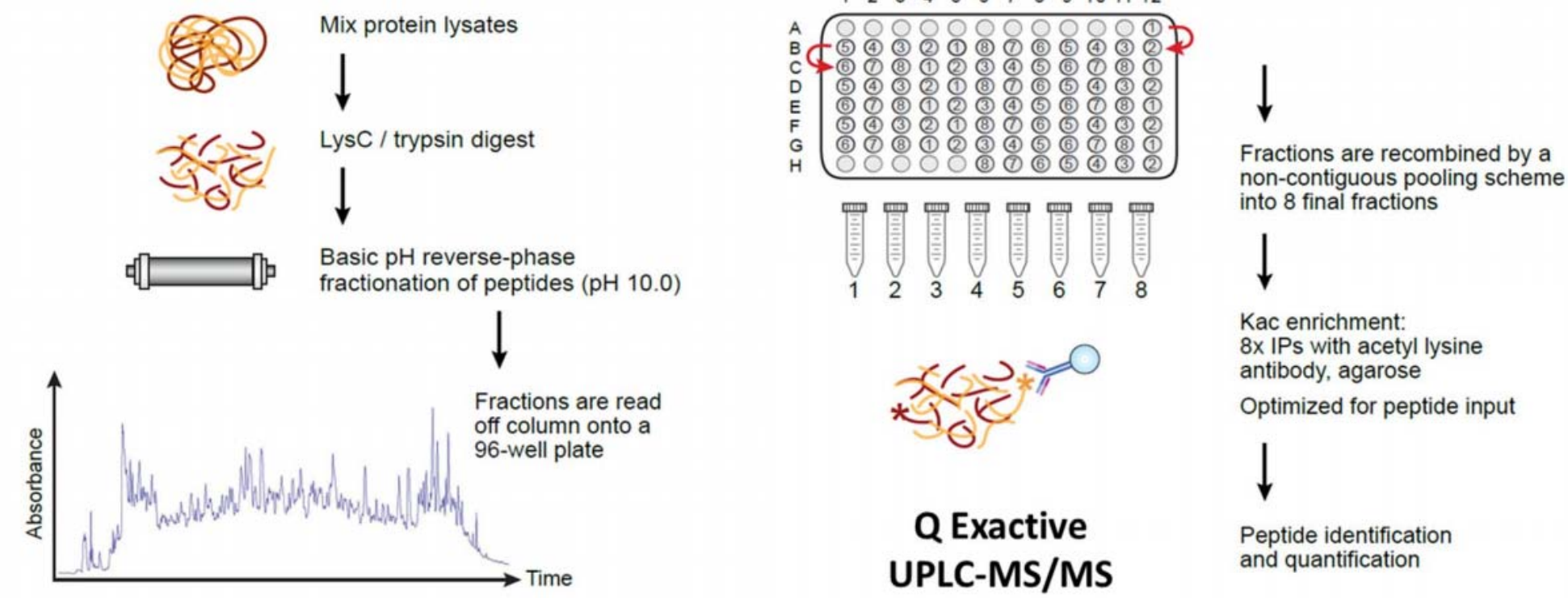


S-Adenosyl methionine (SAM)

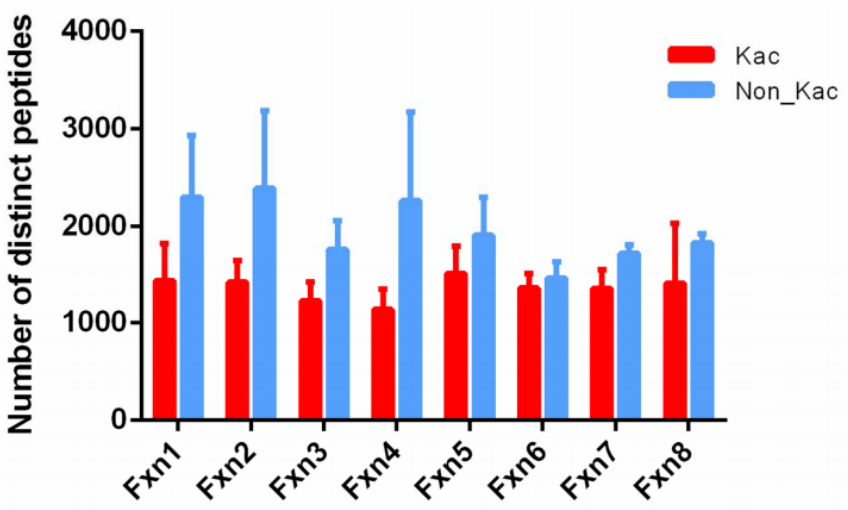


Lysine acetylation

7.5 mg of proteome



Lysine acetylation



	Rep1	Rep2	Rep3	Average
Identified proteins with Kac sites	3127	3221	3320	**3223**
Kac Identified Peptides	10409	10717	11417	**10848**
Kac Quantified Peptides	10135	10128	11030	**10431**
Kac Identified and Localized Sites	8584	8831	9379	**8863**
Kac Quantified and Localized Sites	8400	8406	9107	**8638**

Mol Cell Proteomics. 2015 May 7. pii: mcp.O114.047555

RESOURCE

nature biotechnology

Acetylation site specificities of lysine deacetylase inhibitors in human cells

Christian Schölz[1], Brian T Weinert[1], Sebastian A Wagner[1], Petra Beli[1], Yasuyuki Miyake[2], Jun Qi[3], Lars J Jensen[4], Werner Streicher[5], Anna R McCarthy[6,9], Nicholas J Westwood[7], Sonia Lain[6], Jürgen Cox[8], Patrick Matthias[2], Matthias Mann[8], James E Bradner[3] & Chunaram Choudhary[1]

Lysine deacetylases inhibitors (KDACIs) are used in basic research, and many are being investigated in clinical trials for treatment of cancer and other diseases. However, their specificities in cells are incompletely characterized. Here we used quantitative mass spectrometry (MS) to obtain acetylation signatures for 19 different KDACIs, covering all 18 human lysine deacetylases. Most KDACIs increased acetylation of a small, specific subset of the acetylome, including sites on histones and other chromatin-associated proteins. Inhibitor treatment combined with genetic deletion showed that the effects of the pan-sirtuin inhibitor nicotinamide are primarily mediated by SIRT1 inhibition. Furthermore, we confirmed that the effects of tubacin and bufexamac on cytoplasmic proteins result from inhibition of HDAC6. Bufexamac also triggered an HDAC6-independent, hypoxia-like response by stabilizing HIF1-α, providing a possible mechanistic explanation of its adverse, pro-inflammatory effects. Our results offer a systems view of KDACI specificities, providing a framework for studying function of acetylation and deacetylases.

Nat Biotechnol. 2015 Apr;33(4):415-23