Boehringer Ingelheim Fonds
Stiftung für medizinische
Grundlagenforschung

Ubiquitin proteomics

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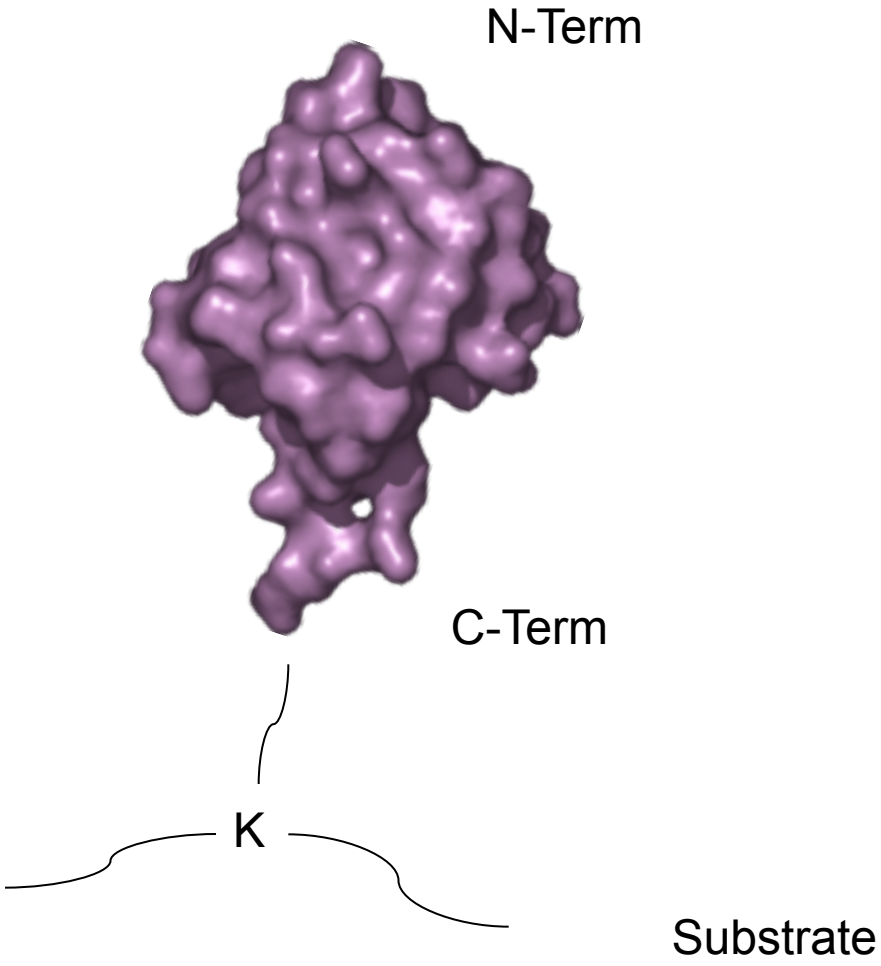
Innomol Proteomics Workshop Zagreb

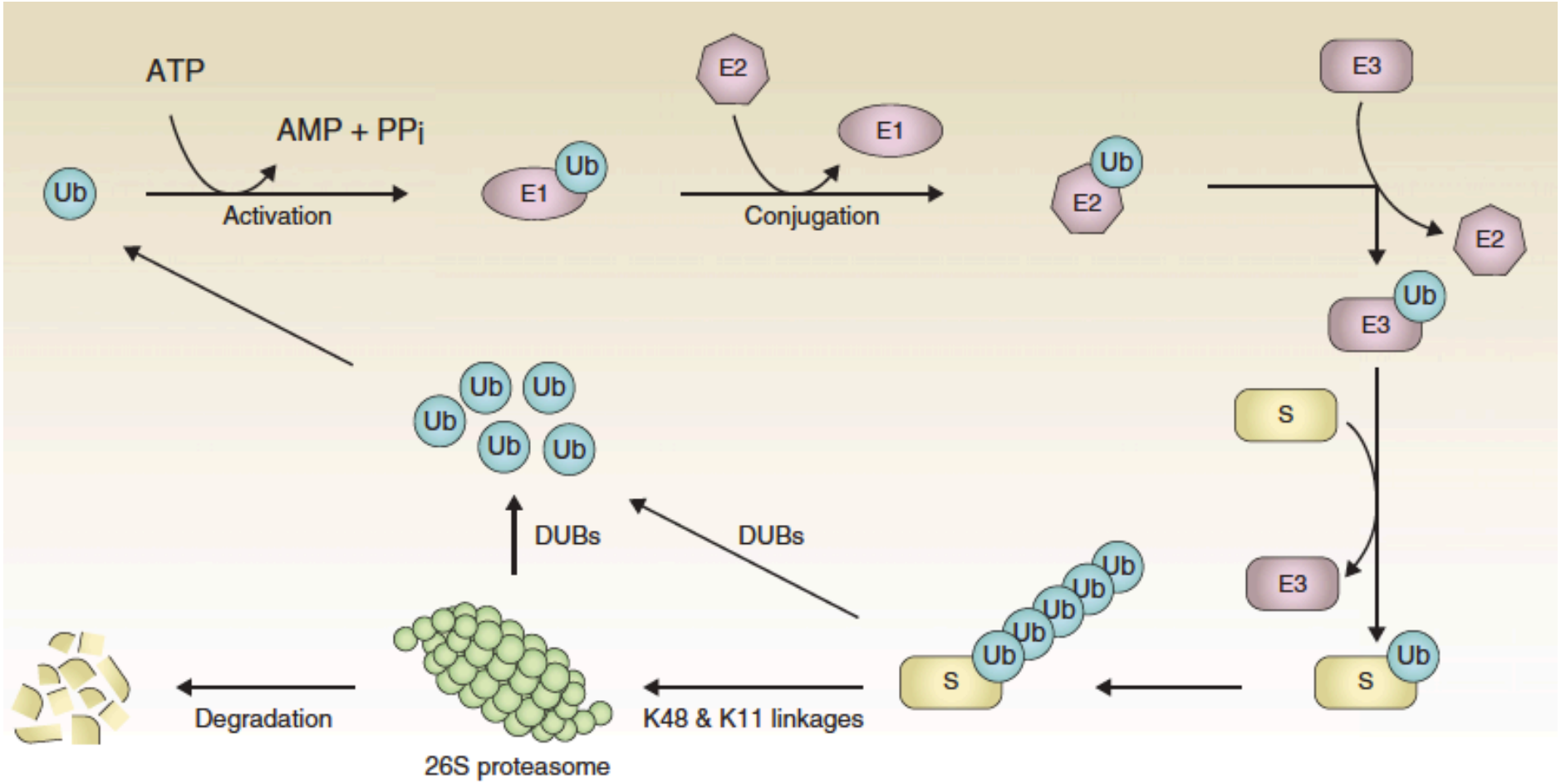
08.04.14

The Ubiquitin System I

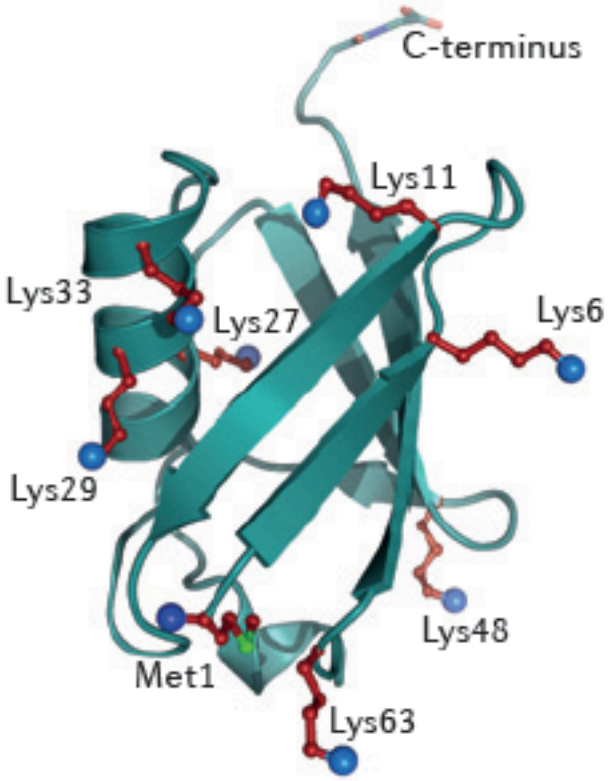
76 amino acids

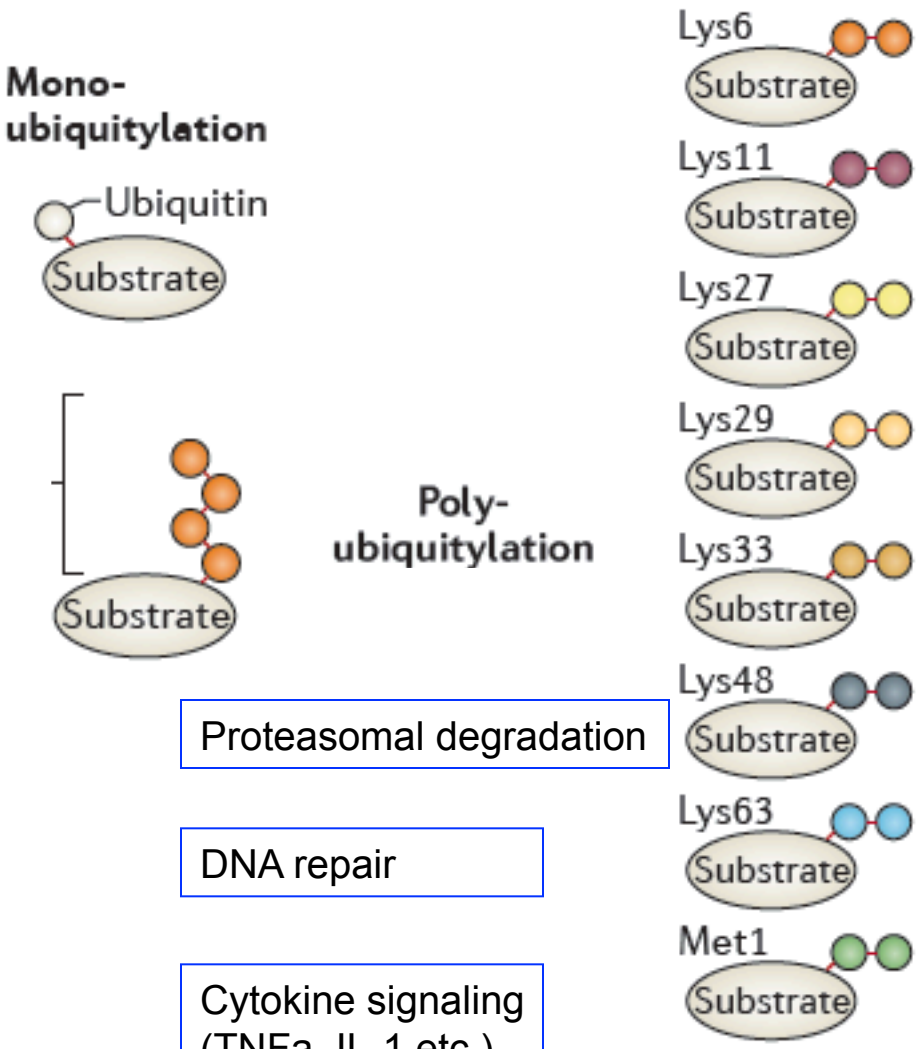


The Ubiquitin System II



Various Ubiquitin signals – various functions





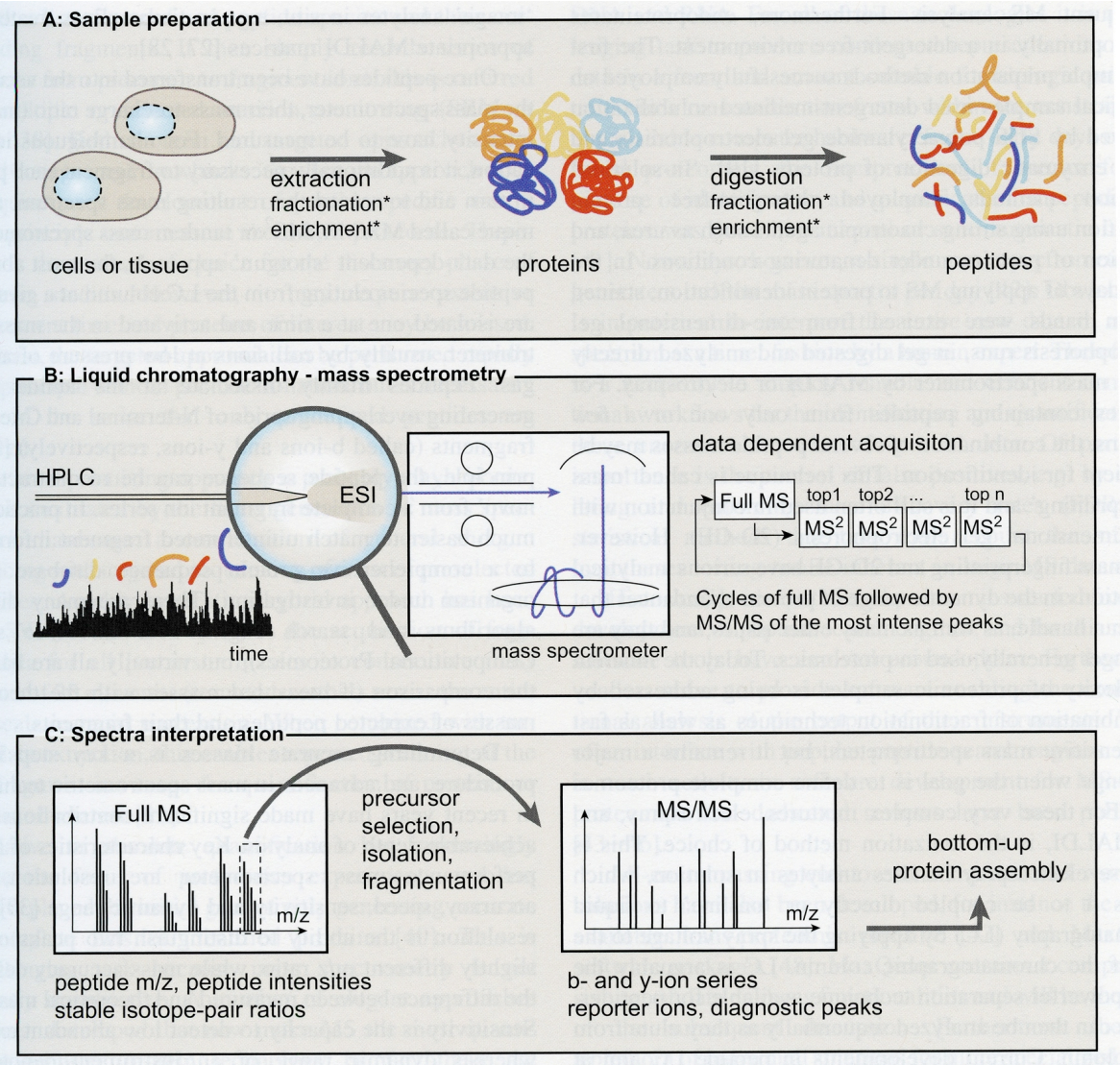
Proteasomal degradation

DNA repair

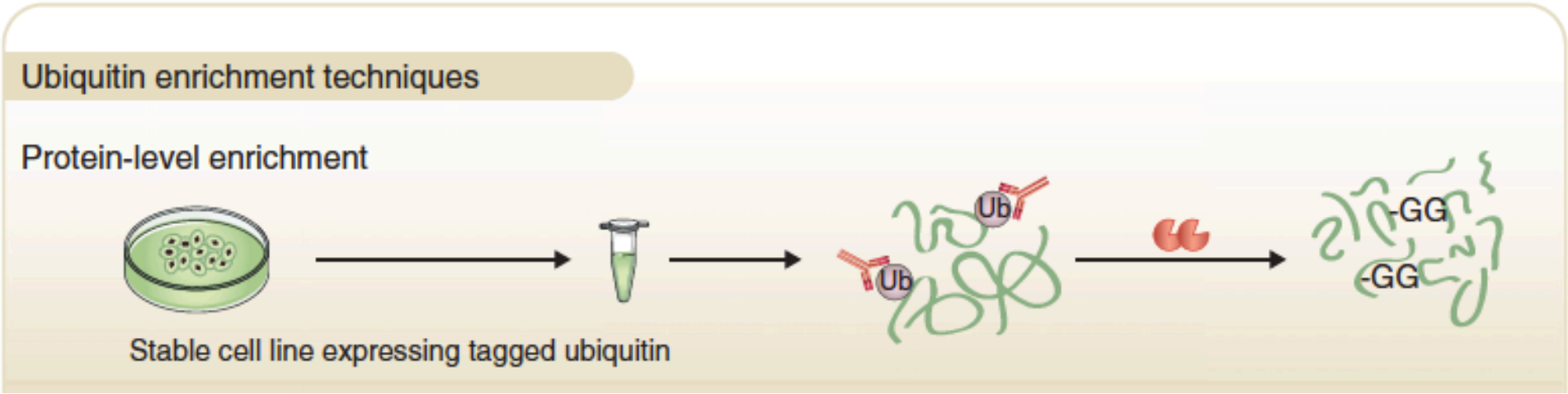
Cytokine signaling (TNFa, IL-1 etc.)

Kulathu and Komander (2012), *Nat.Rev.Mol.Cell.Biol*

Shotgun proteomics and need for PTM enrichment



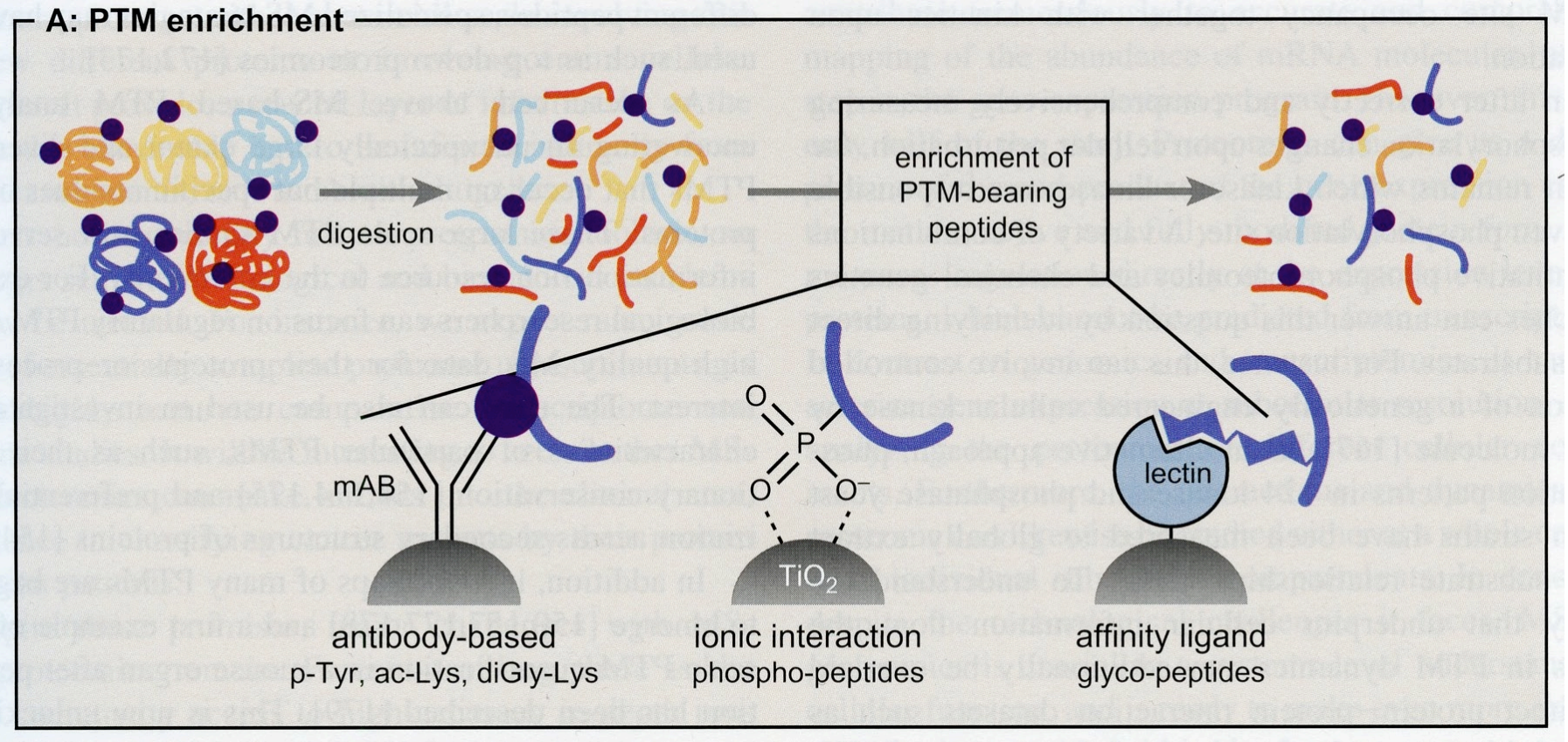
Protein-level PTM enrichment



With this method around 800 ubiquitination sites were identified

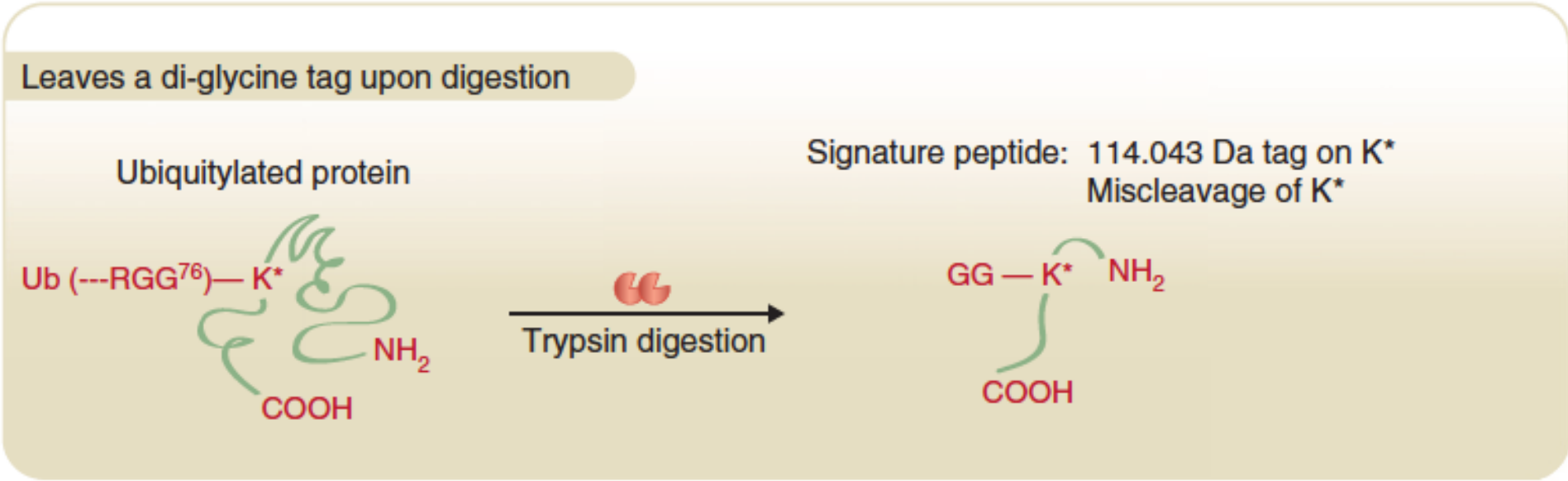
Sylvestersen KB, Young C and Nielsen ML et al (2013), *Current opininon in chemical biology*

Peptide-level PTM enrichment



Handbook of Systems Biology, Chpt1

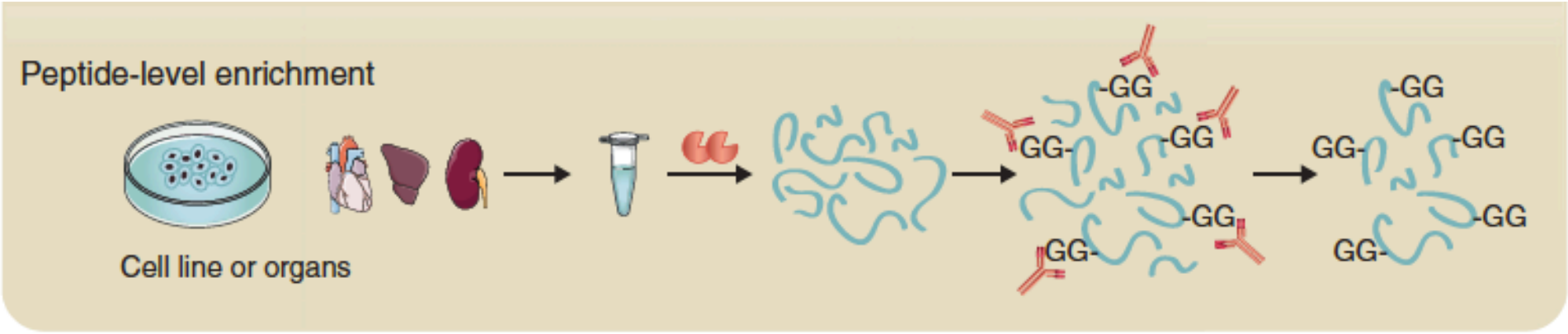
Antibodies against tryptic ubiquitin remnant

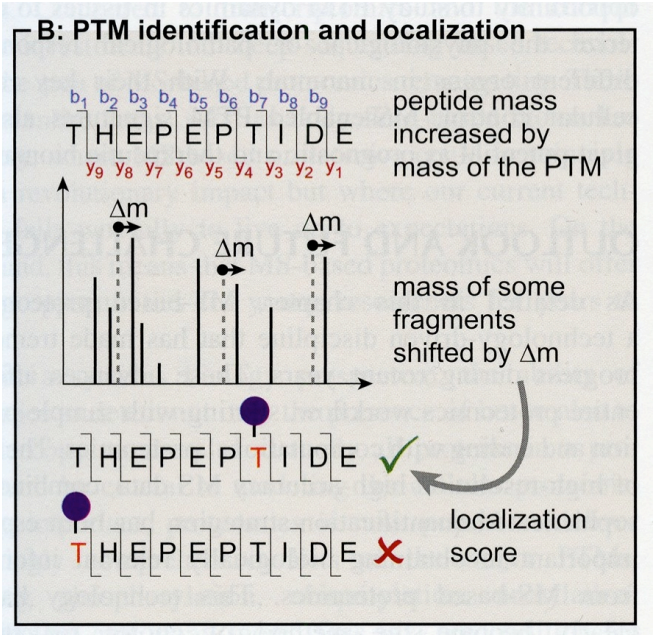


other Ubiquitin like proteins Nedd8 and ISG15 leave the same remnant and can not be distinguished

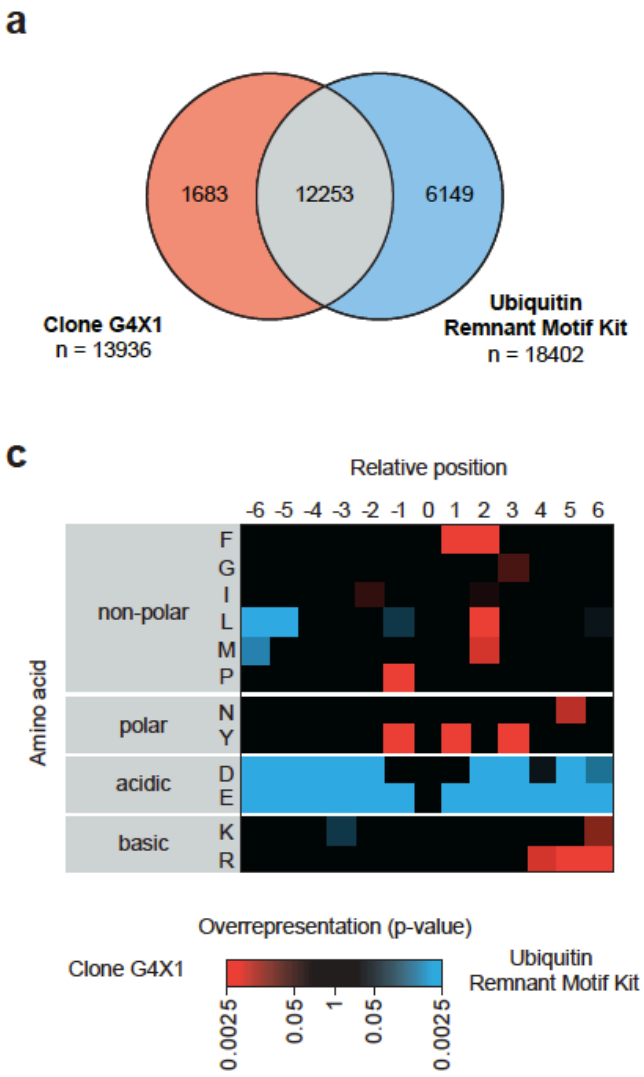
Could make up tp ~6% of identified sites

Peptide-level diGly-tag enrichment



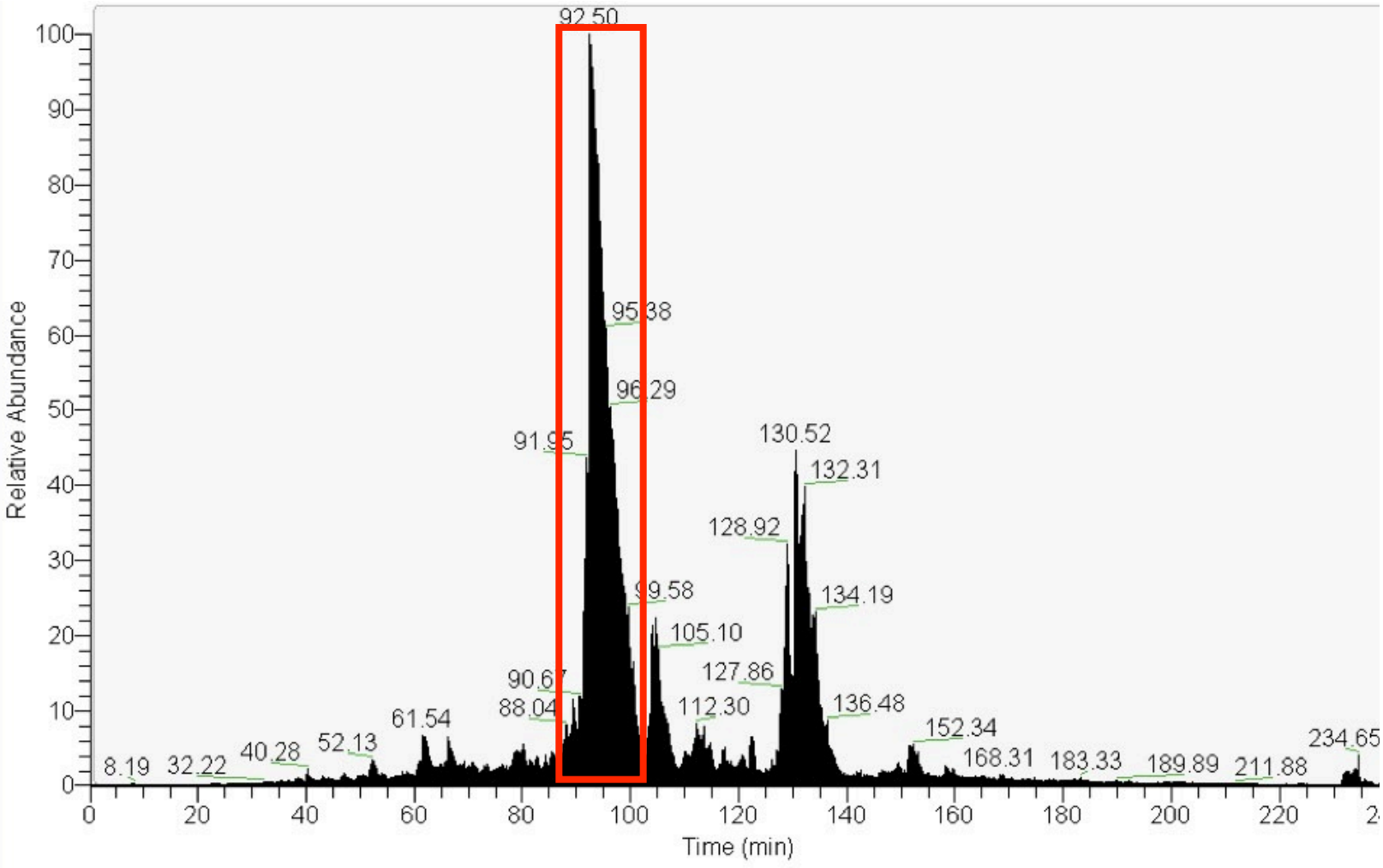


Sequence preferences of di-Gly antibodies

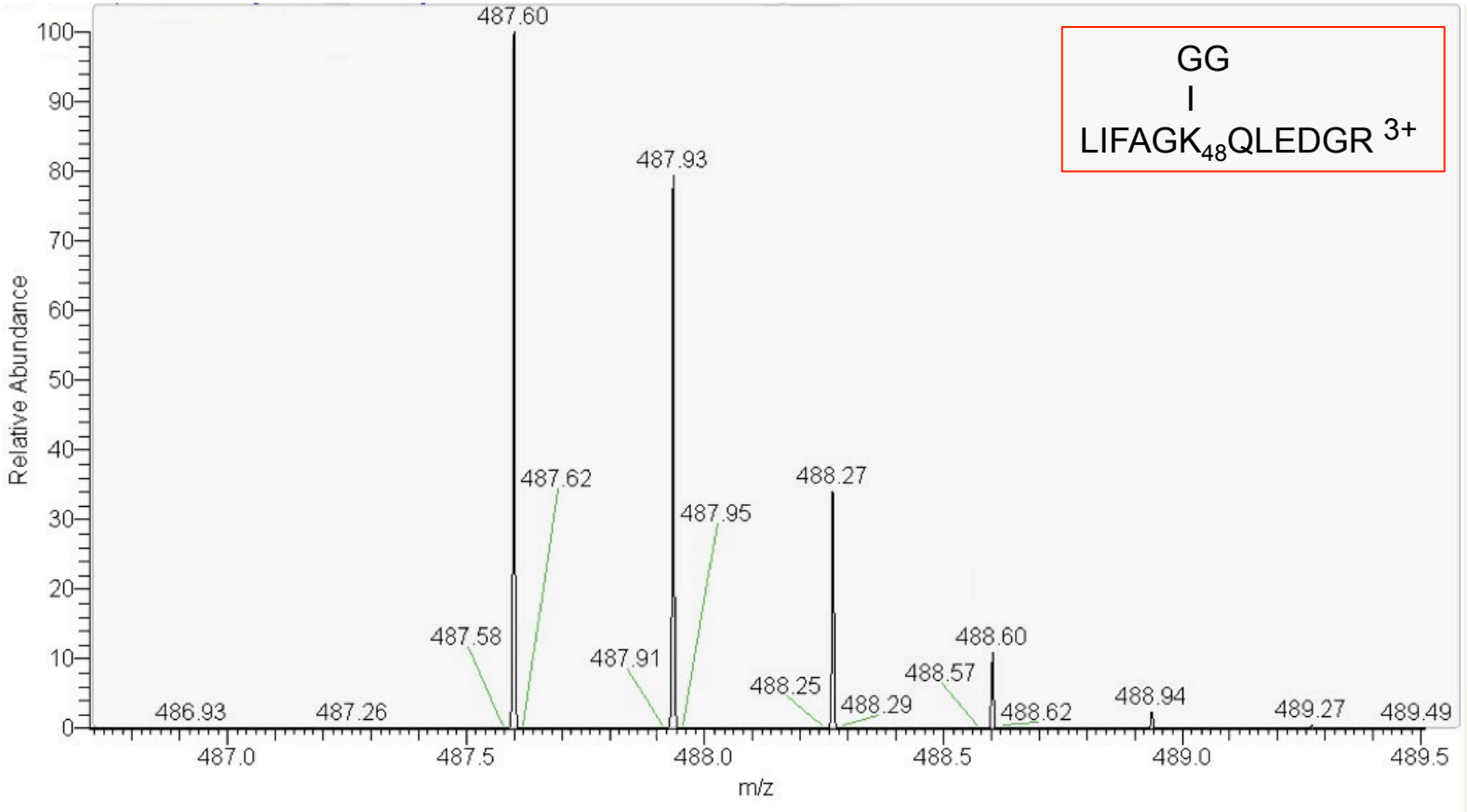


Wagner SA et al (2012), *MCP*

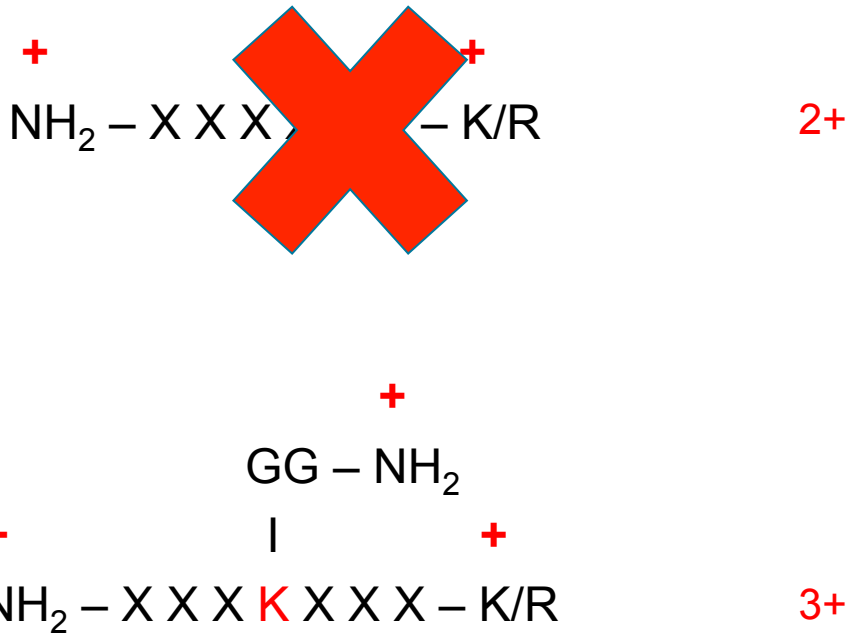
Example of GG-IP mass spec run



Example Ubiquitin K48-GG site

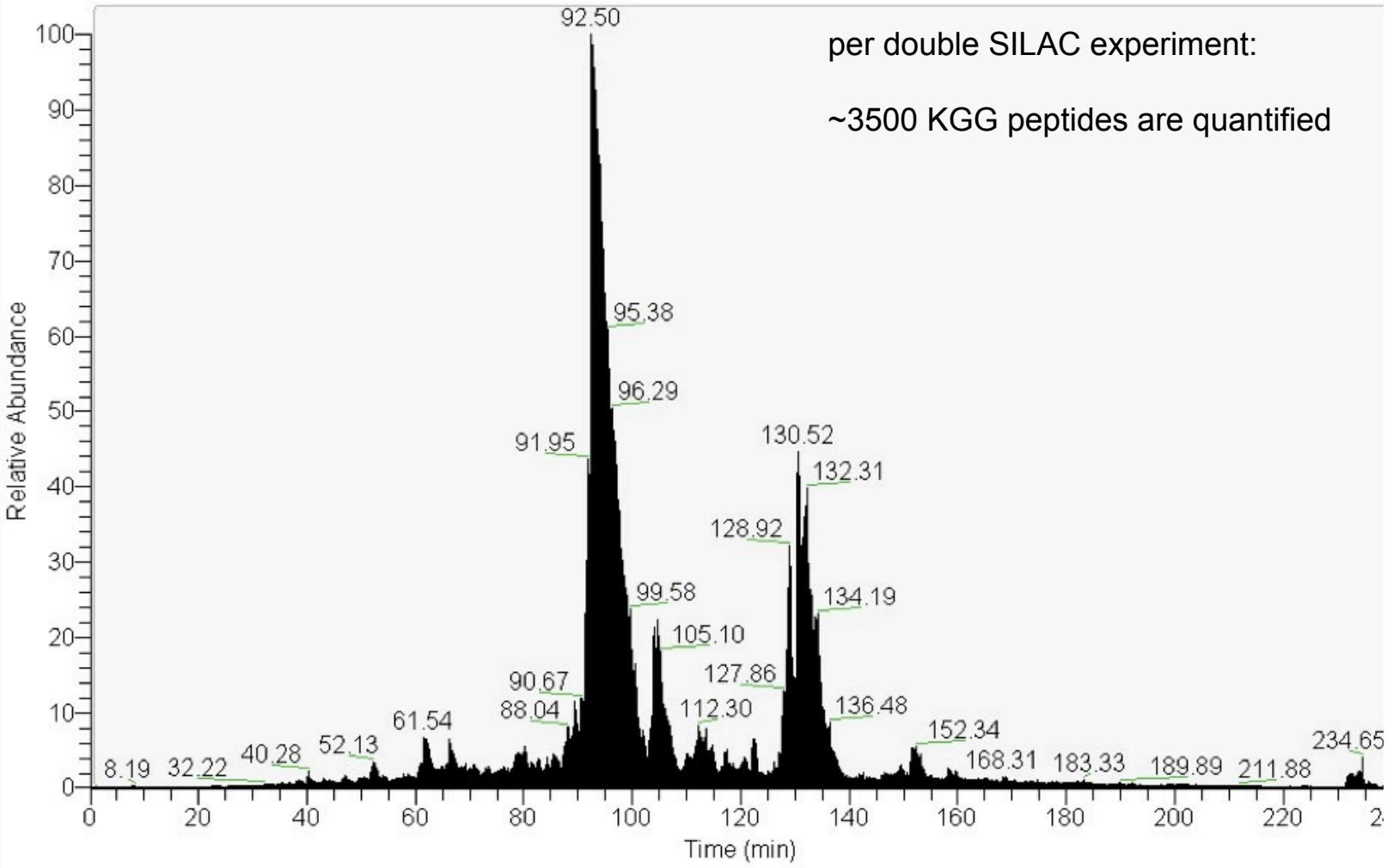


Charge state of GG- vs non-GG-peptides

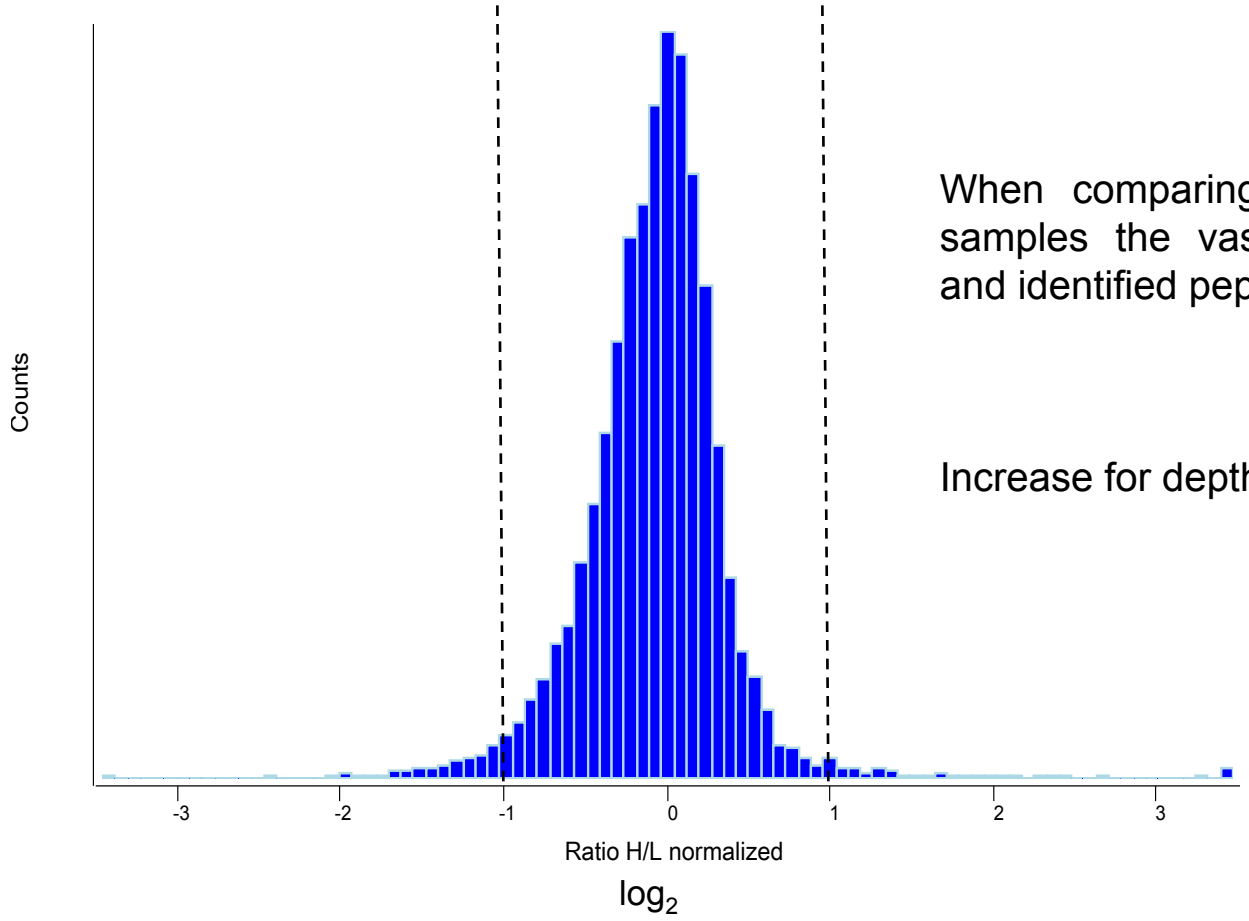


- Charge exclusion of 2+ charged ions to increase coverage of KGG containing peptides

Depth of analysis



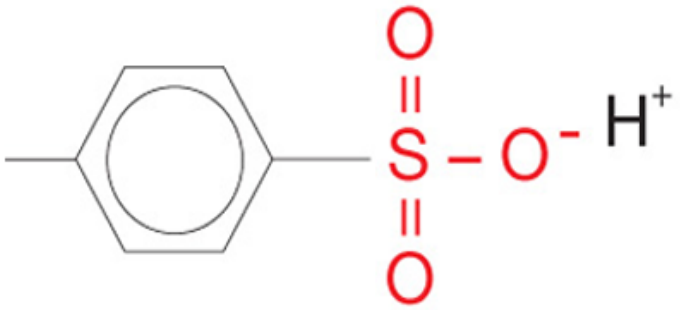
Depth of analysis



When comparing treated vs untreated samples the vast majority of detected and identified peptides are unchanged

Increase for depth of analysis is desired

Strong cation exchange (SCX) chromatography



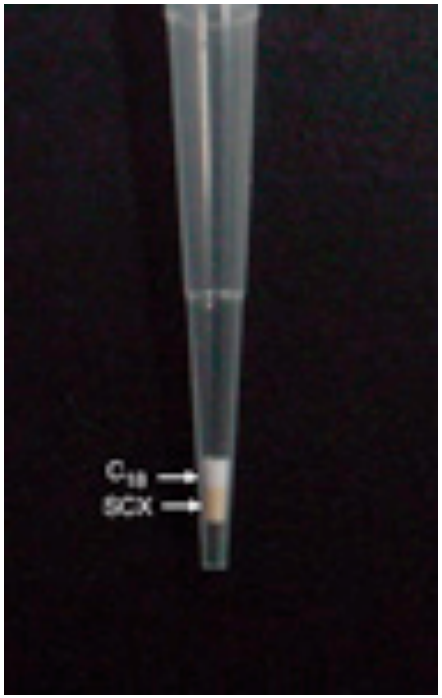
pKa < 1

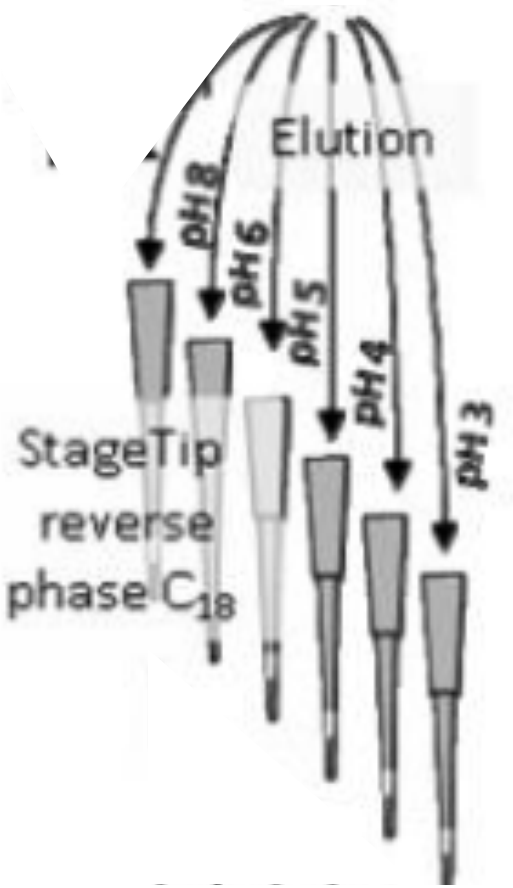
Benzenesulfonic acid functionalized matrix

Stepwise pH elution

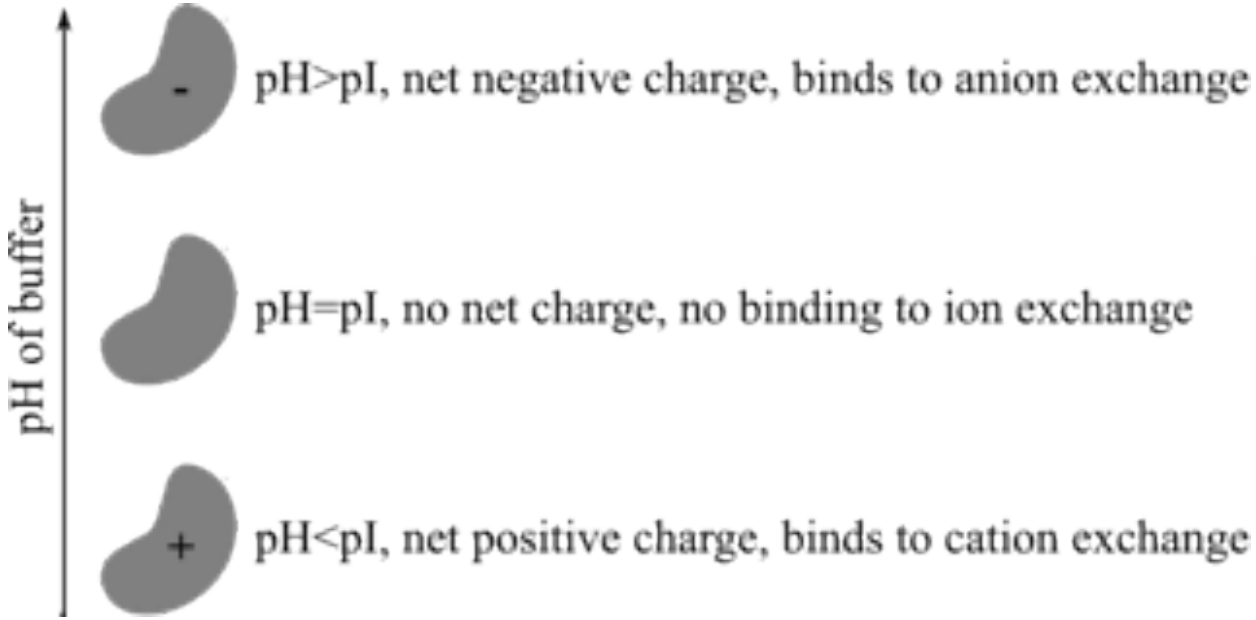
Separation of peptides according to isoelectric point

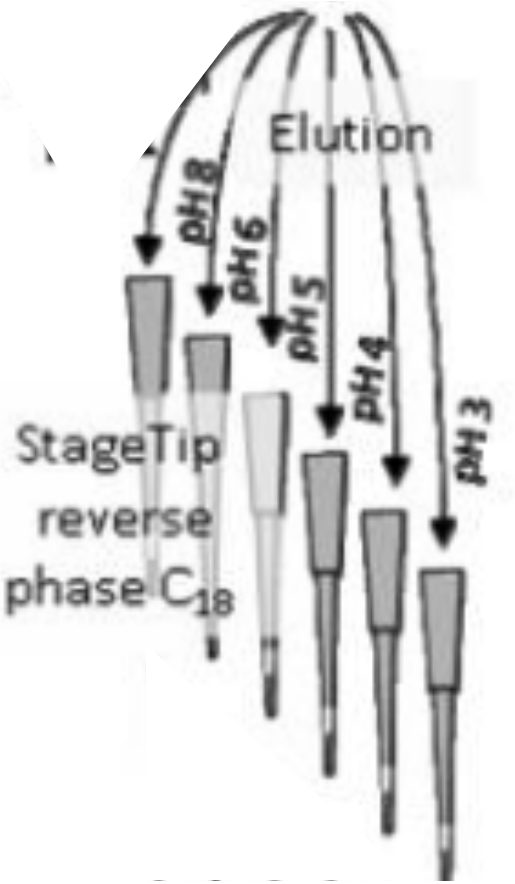
microSCX based fractionation





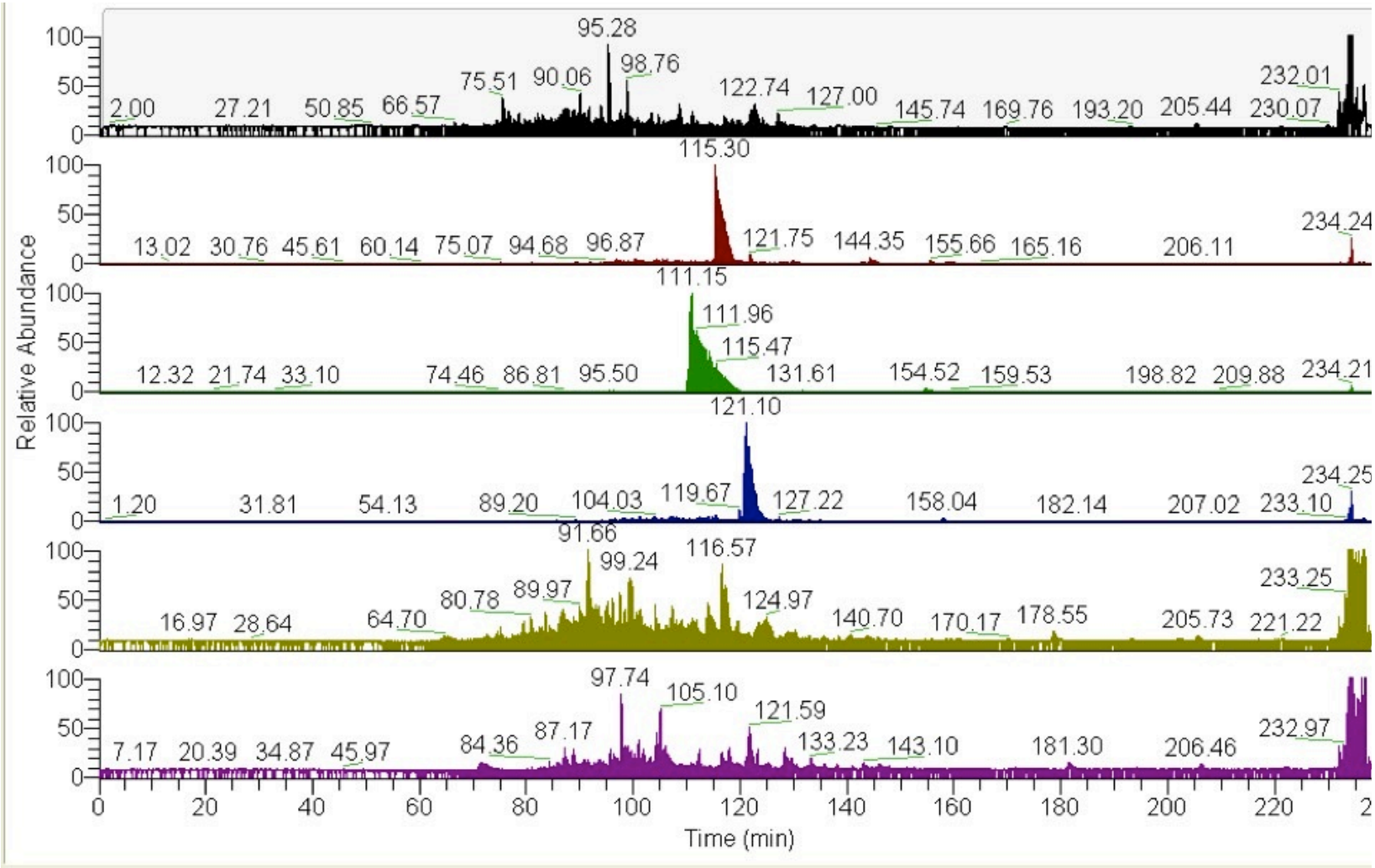
microSCX based fractionation





Micro-SCX based fractionation

per double SILAC experiment: ~7000 KGG peptides are quantified



Summary

- Ubiquitin as a dynamic and versatile post-translational modification
- PTM enrichment and identification with shotgun proteomics workflow
- Protein- vs. peptide-level enrichment strategies
- Sequence preferences of available diGly remnant antibodies
- microSCX as a fractionation method post-peptide enrichment

Acknowledgement

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