

Extending Targeted Mass Spectrometry to Monitor Neuropeptide Signaling and Phosphorylation



netherlands
proteomics
centre

Thierry Schmidlin

Fifth Annual Skyline User Group Meeting

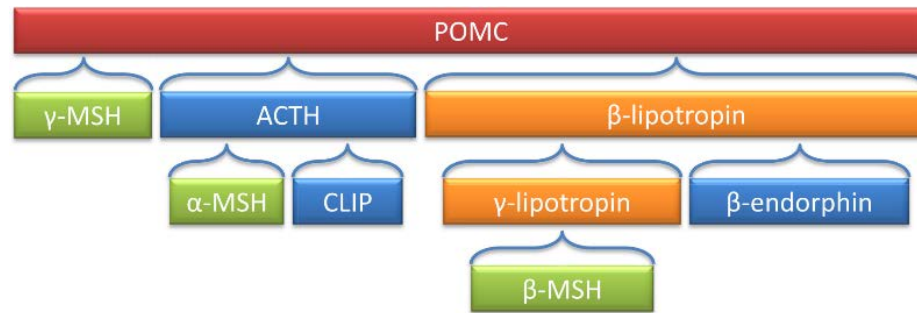
June 5, 2016



Example study 1:

Diet-induced neuropeptide expression profiling in rat

1) Biosynthesis



2) Physicochemical Properties

YGGFM

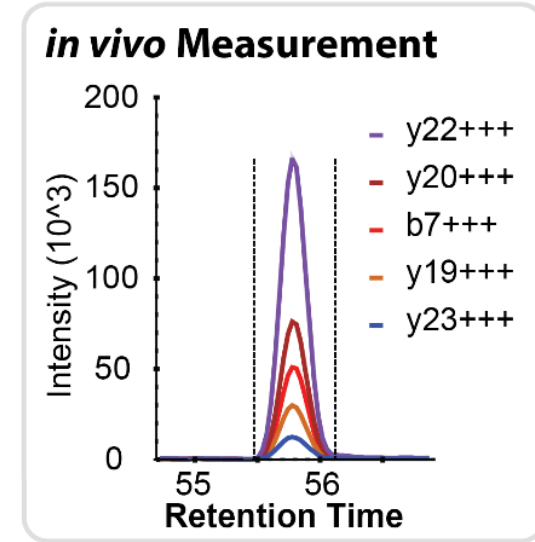
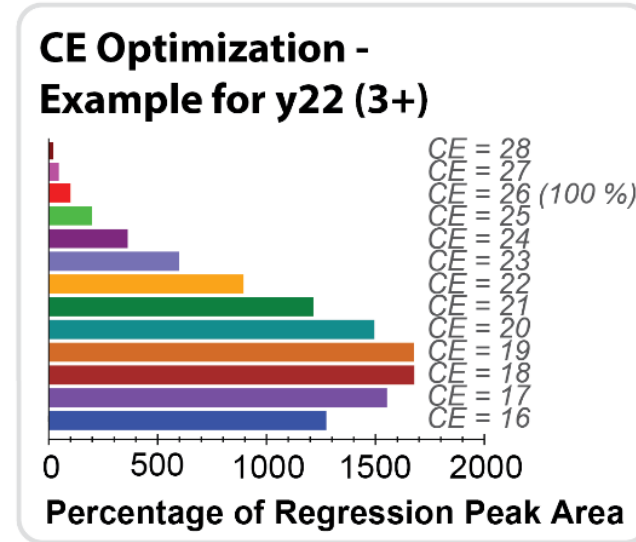
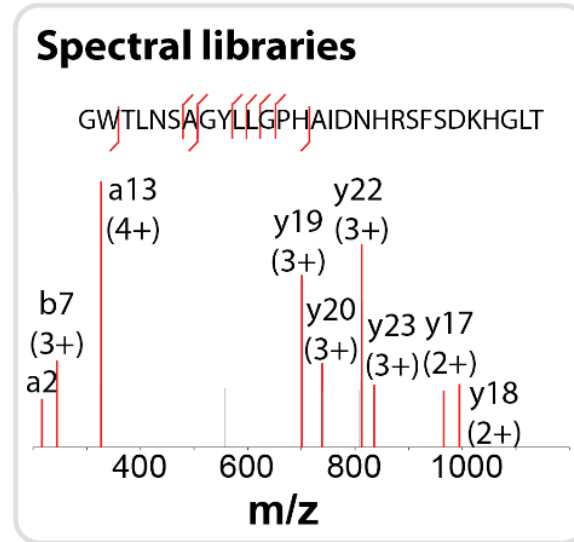
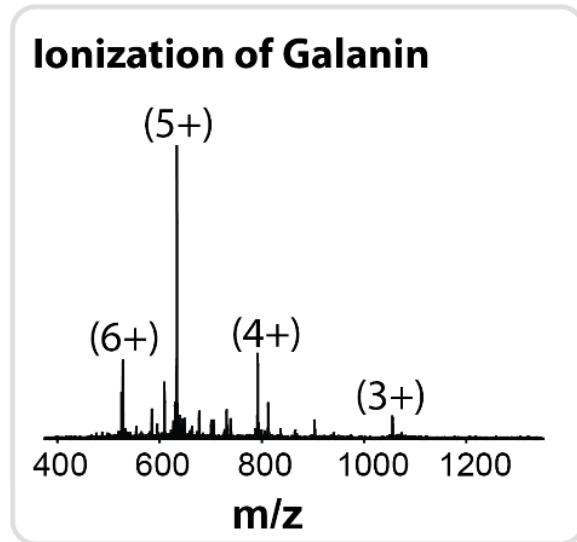
TLQPPASSRRRHFFHHALPPARRHHPDLEAQR
 RAQEEADAEERRLQEQEELENYIEHVLLHRP

3) Data Analysis



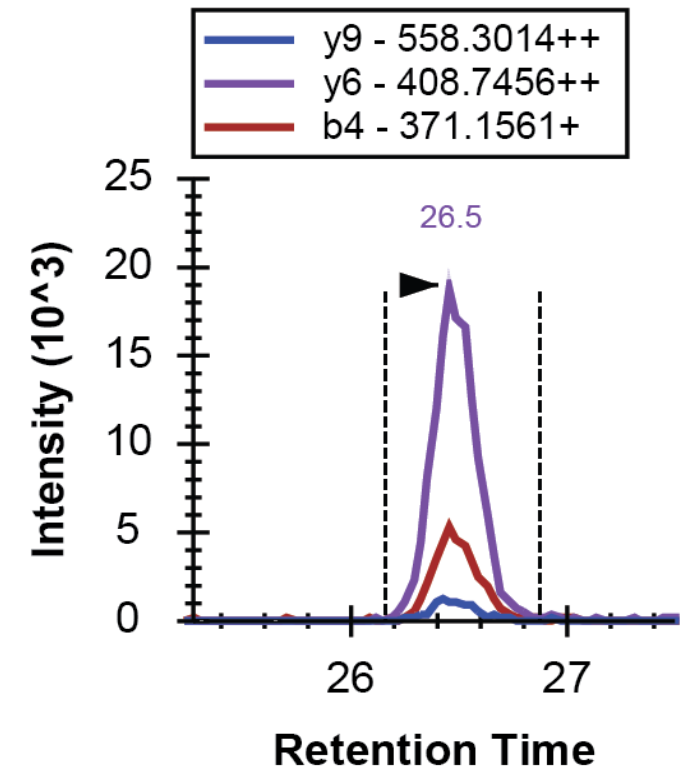
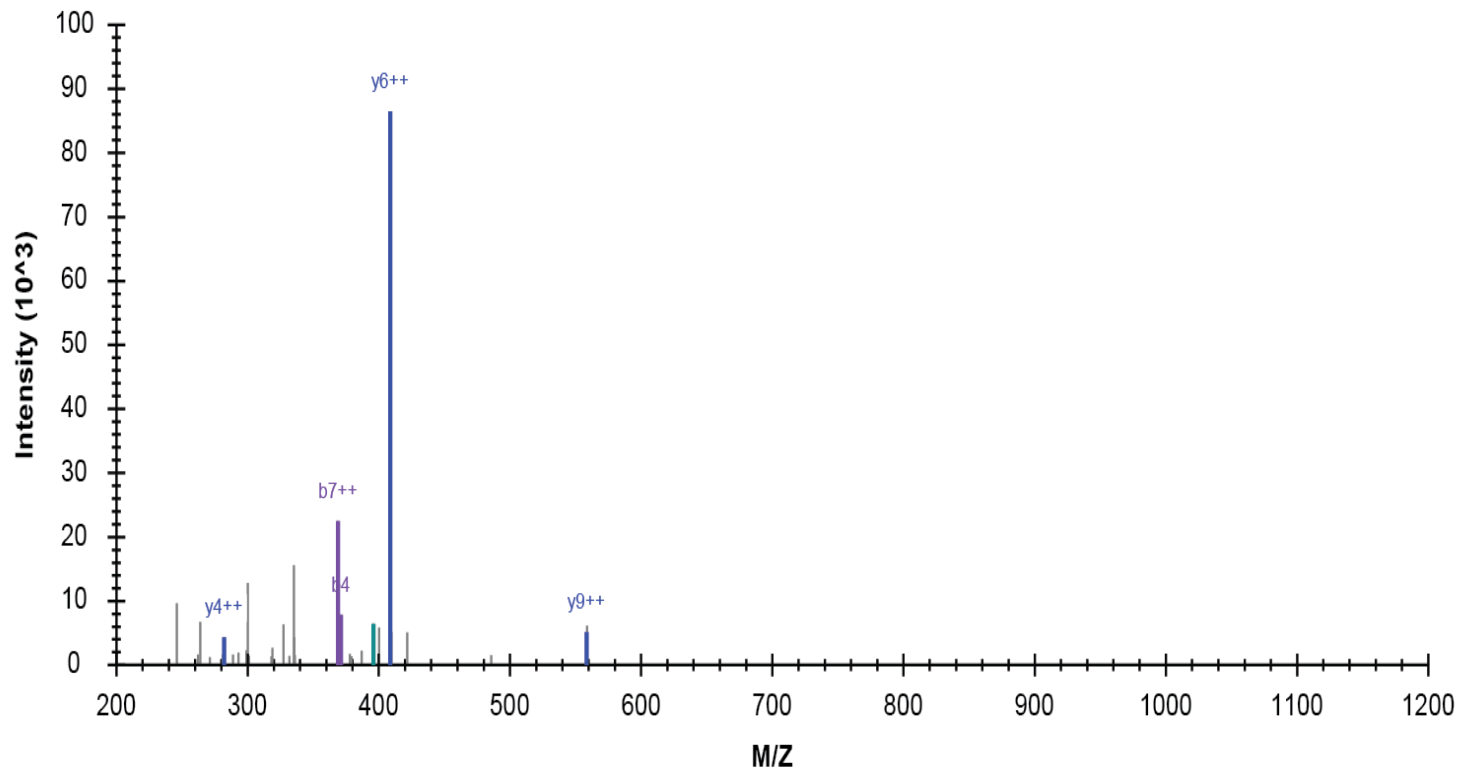
SRM Assay development for neuropeptides - Galanin

GWTLNSAGYLLGPH AIDNHR SFSDKHGLT



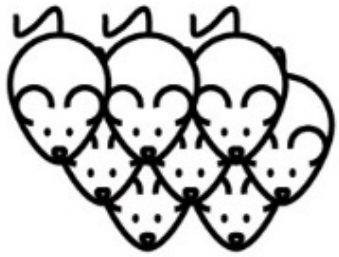
SRM Assay development for neuropeptides - Anthrin

APSDPRLRQF



Neuropeptide profiling in a rat obesity model

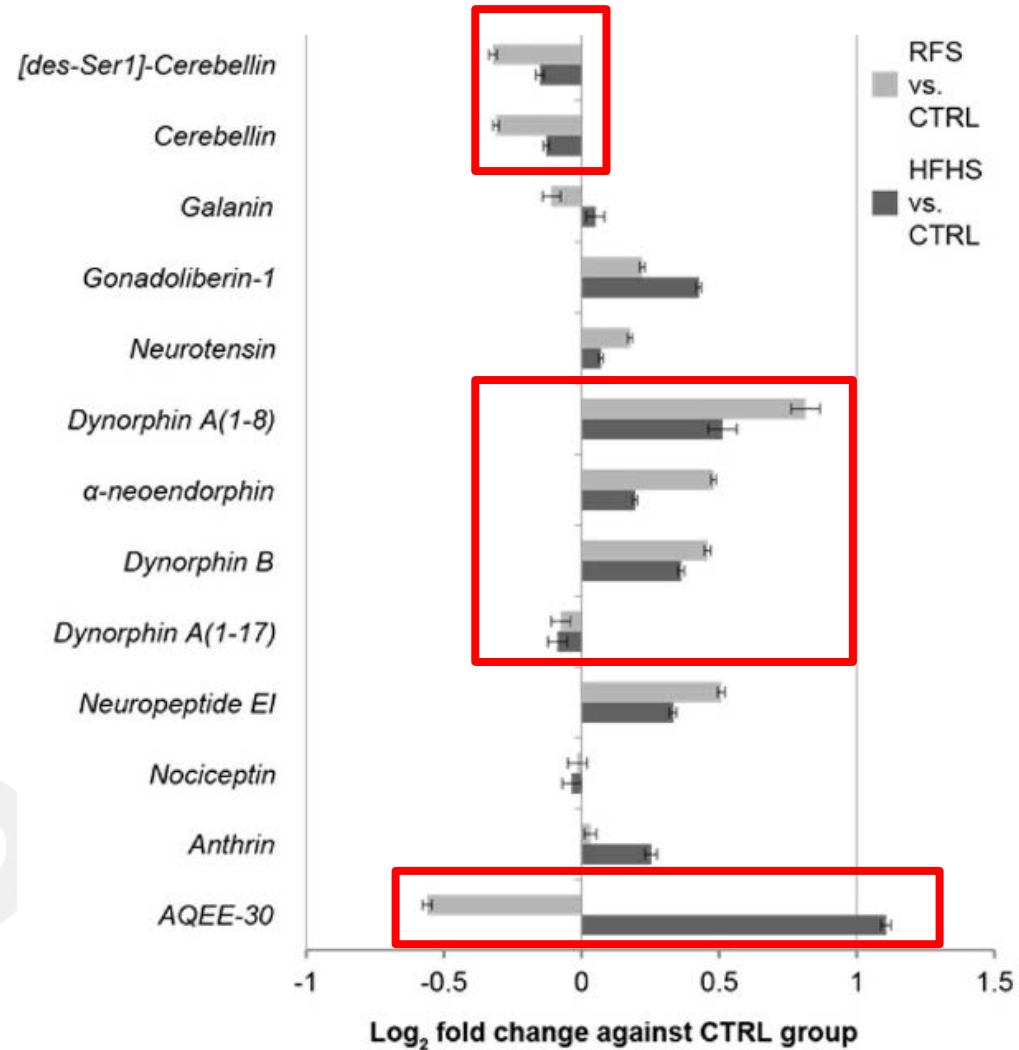
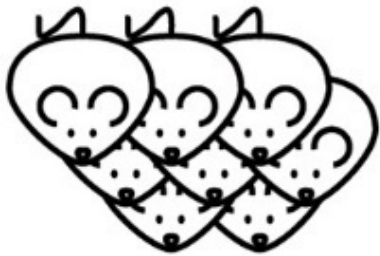
Control (CTRL)



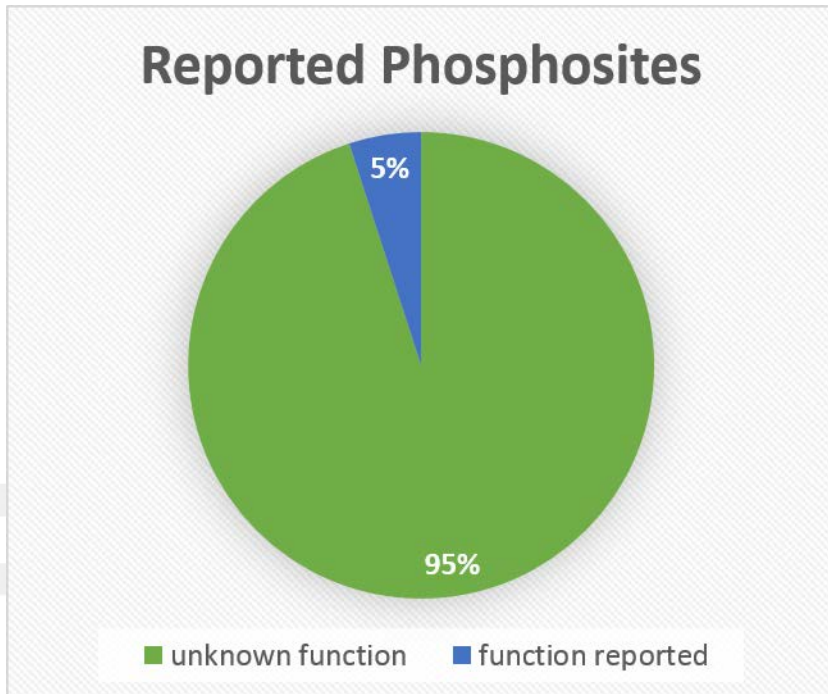
Restricted food (RFS)



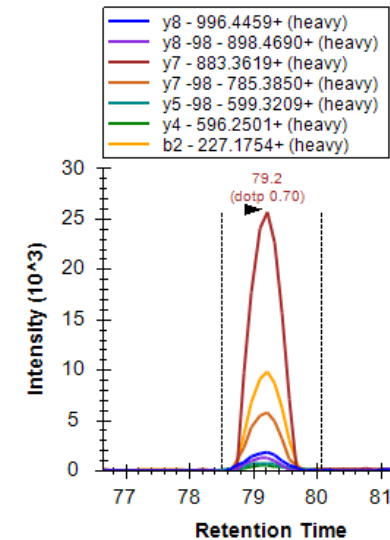
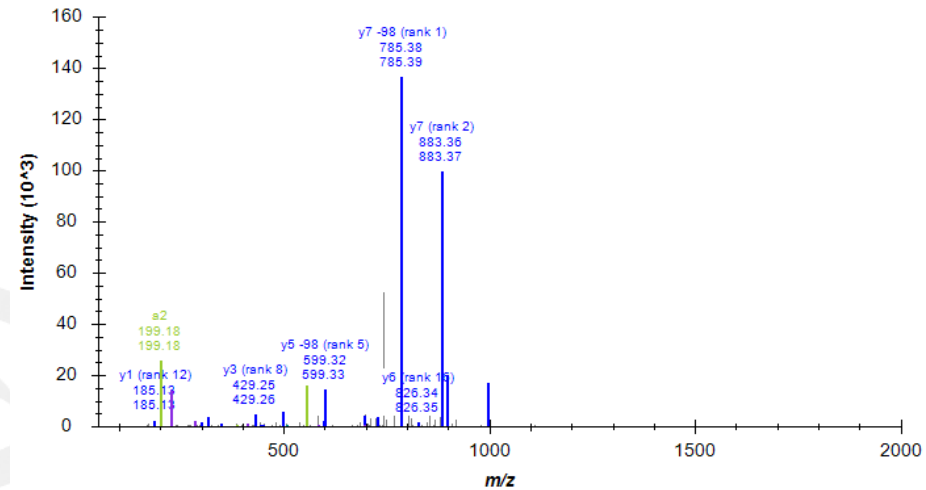
High fat / high sucrose (HFHS)



Example study 2: Applying SRM to Phosphoproteomics

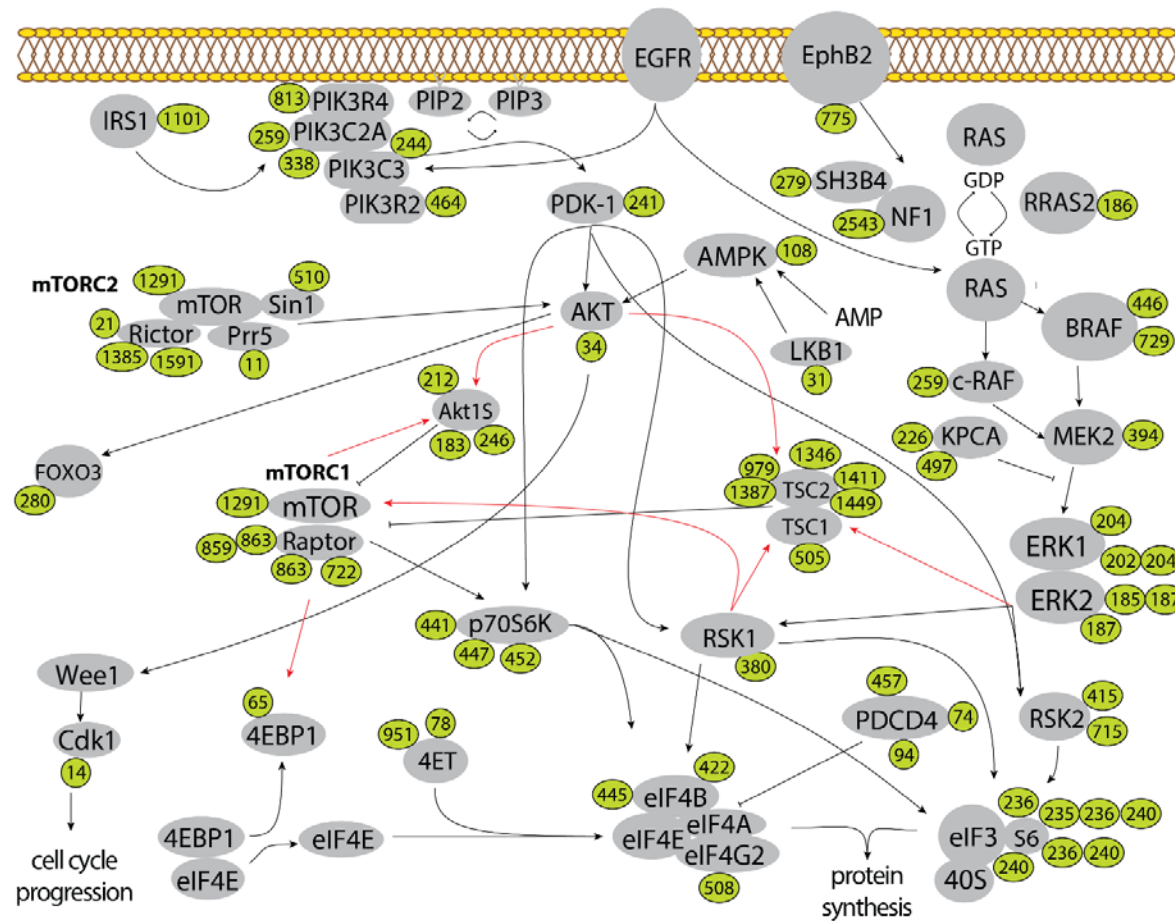


ILGETSLMR

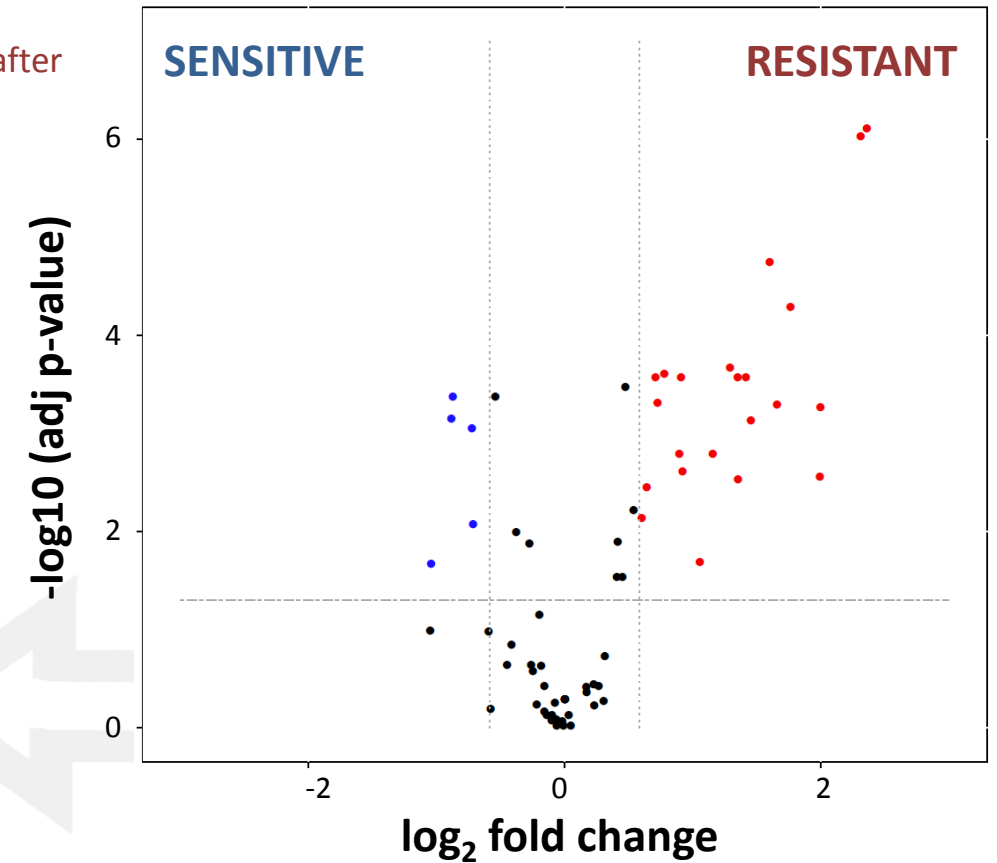
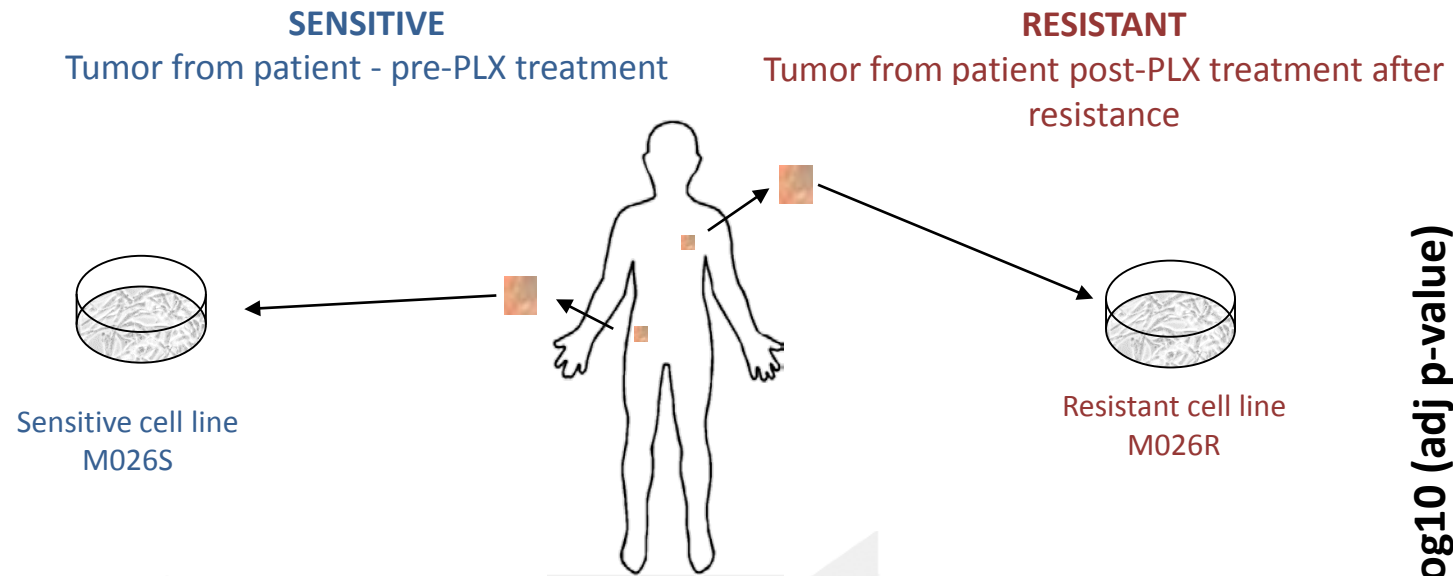


Example study 2:

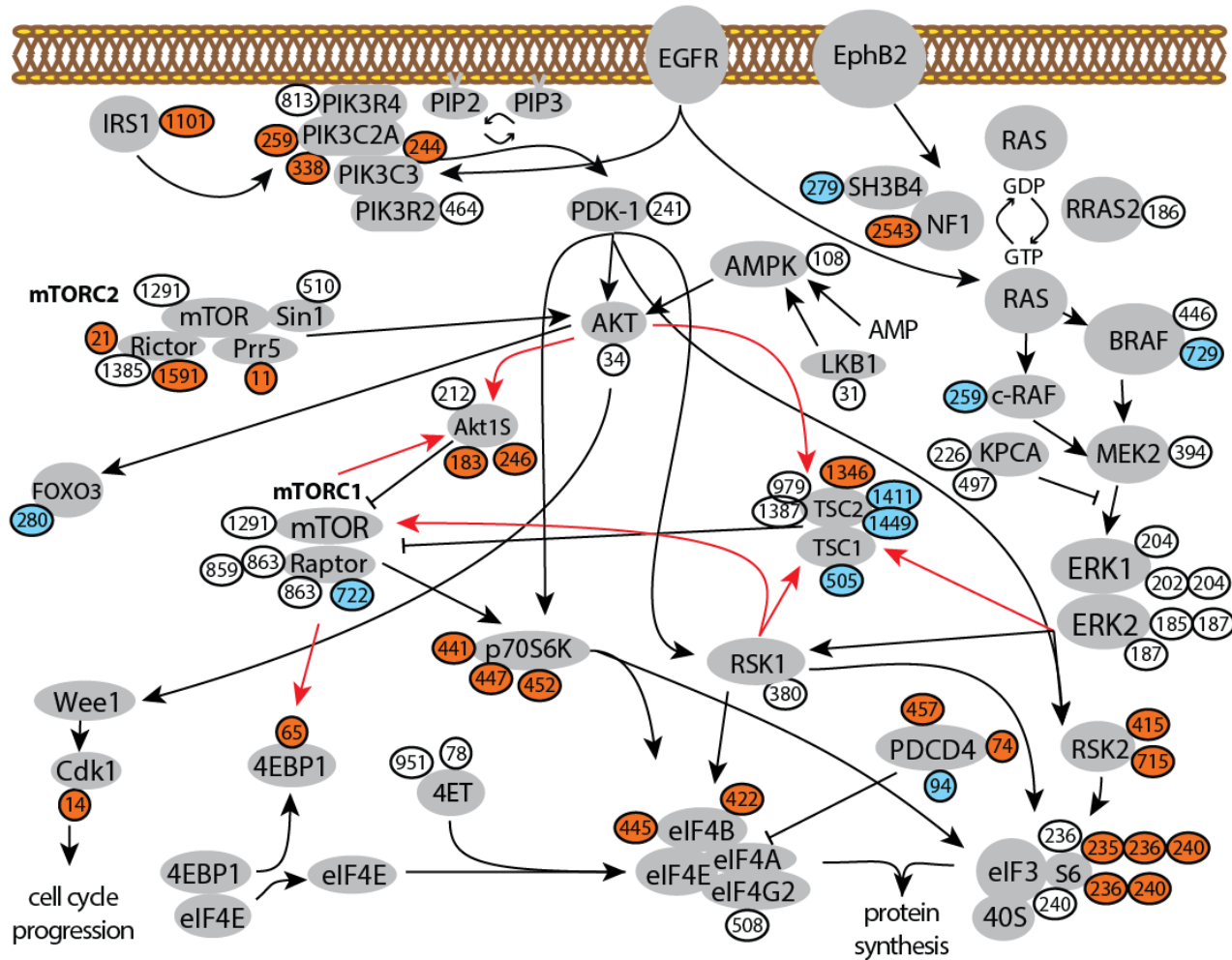
Comprehensive monitoring of PI3K-mTOR/MAPK signaling



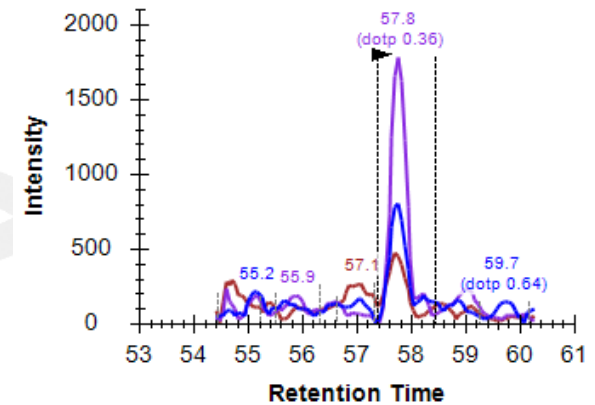
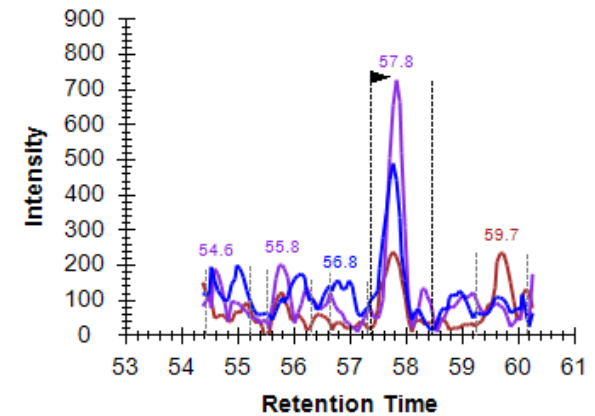
PLX resistance in Melanoma



PLX resistance in Melanoma

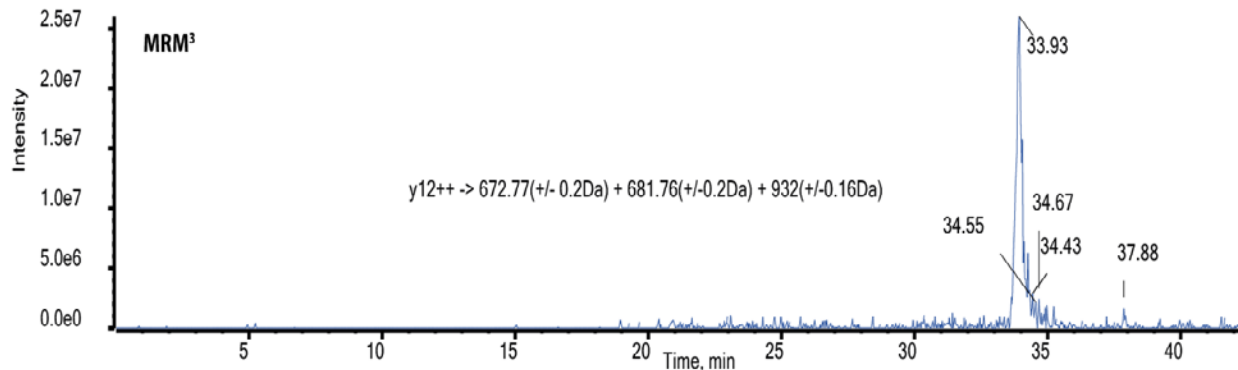
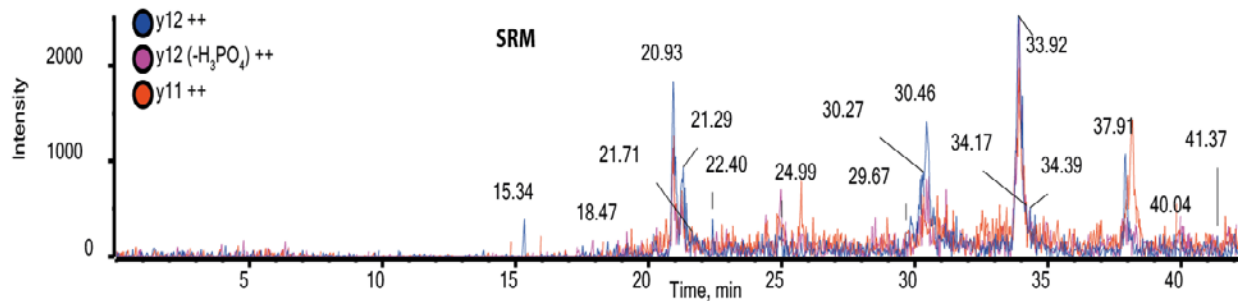


RSK1 – phosphorylation at S380



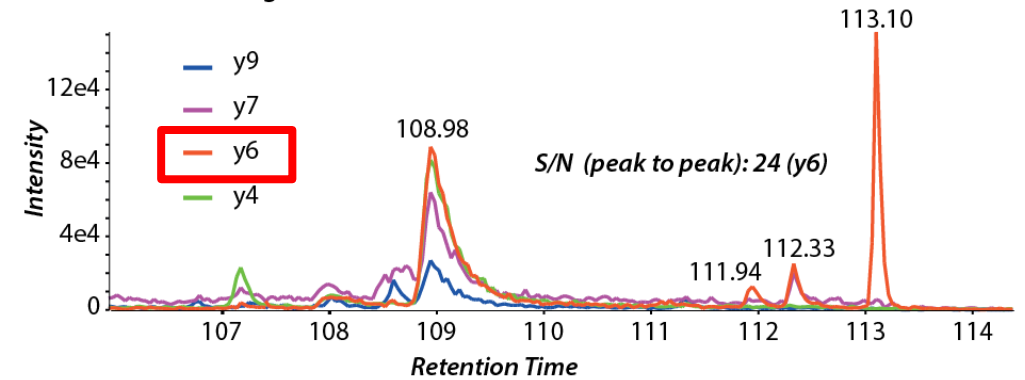
Example study 3: Applying MRM³ to increase assay sensitivity

A) ANIVDQSHLHDS(pho)SQK

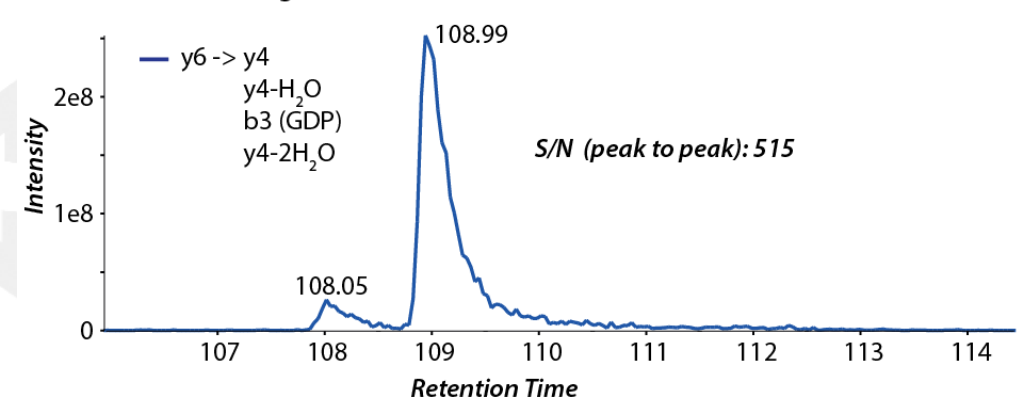


SRM versus MRM³ - TSC2 (S1387) : SS-(pho)S-SPELQTLQDILGDPGDK

A) SRM (endogenous - QTRAP measurement)



B) MRM³ (endogenous)



Conclusions

- Selected Reaction Monitoring is more versatile than how it is currently used
 - endogenous peptides
 - phosphopeptides
- Sensitivity for low S/N analytes can be improved by MRM³



Acknowledgments

- Arjen Boender
- Roger Adan
- Luc Garrigues
- Violette Gautier
- Erik de Graaf
- Cathy Lane
- Maarten Altelaar
- Albert Heck
- The Heck Lab

