

# Skyline: Everyday Tool for Protein Quantification

Skyline User Meeting

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# Outline

- Introduction
- Rapid Development of Protein Quantification Assays
- Protein Characterization by Targeted SRM
- Conclusions

# PharmaCadence Analytical Services, LLC

Engineered Solutions for Complex Analytical Challenges



Protein Quantification

Targeted MRM Assays

Biomarker Validation

Pharmacokinetics

MRM Peptide Mapping

Chemoplexing

Small Molecule

Quantification by LC-MS/MS



[www.pharmacadence.com](http://www.pharmacadence.com)

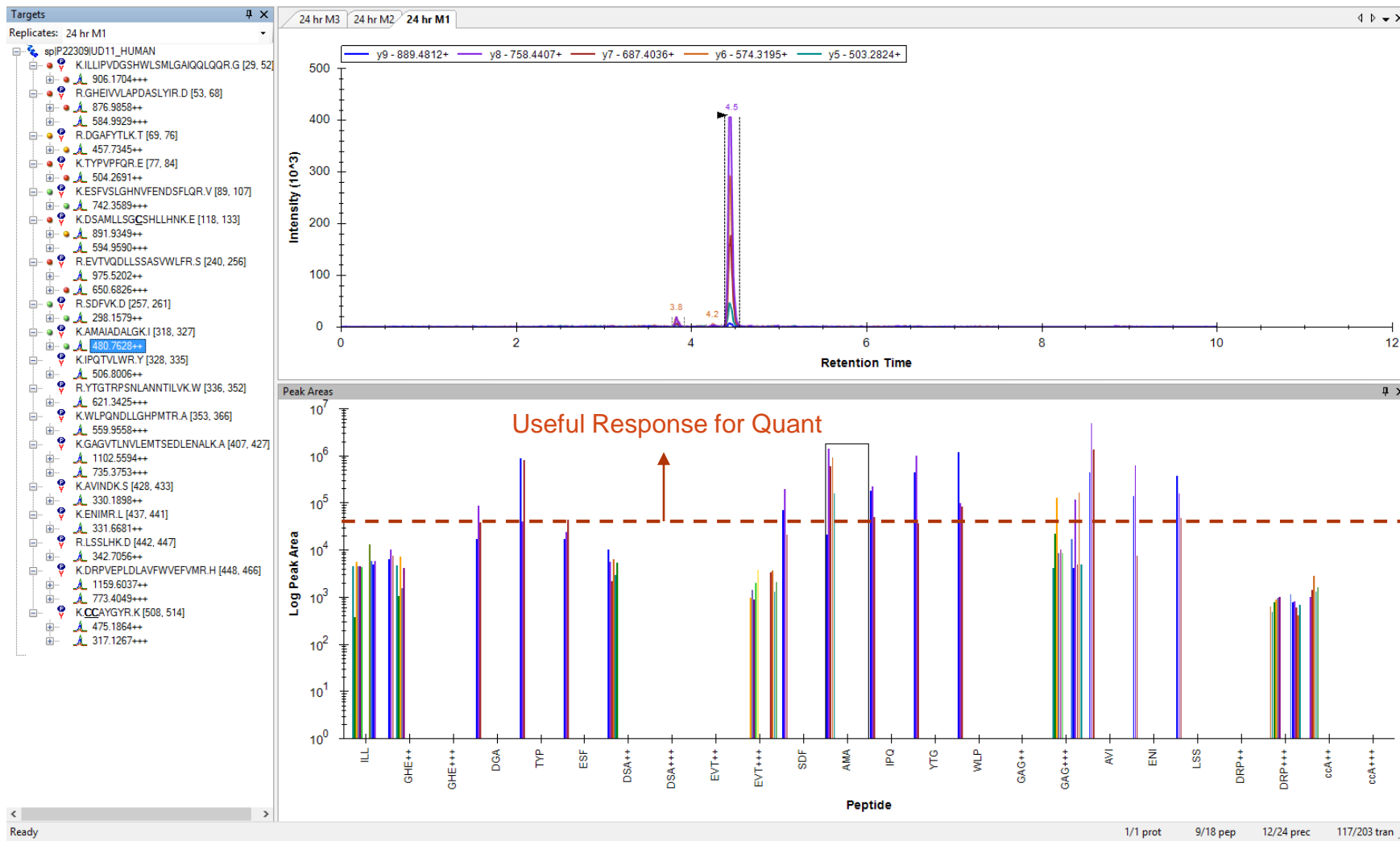
# Development of Protein Quantification Assays

Example: UGT Isoform – Specific Protein  
Quantification

# Process

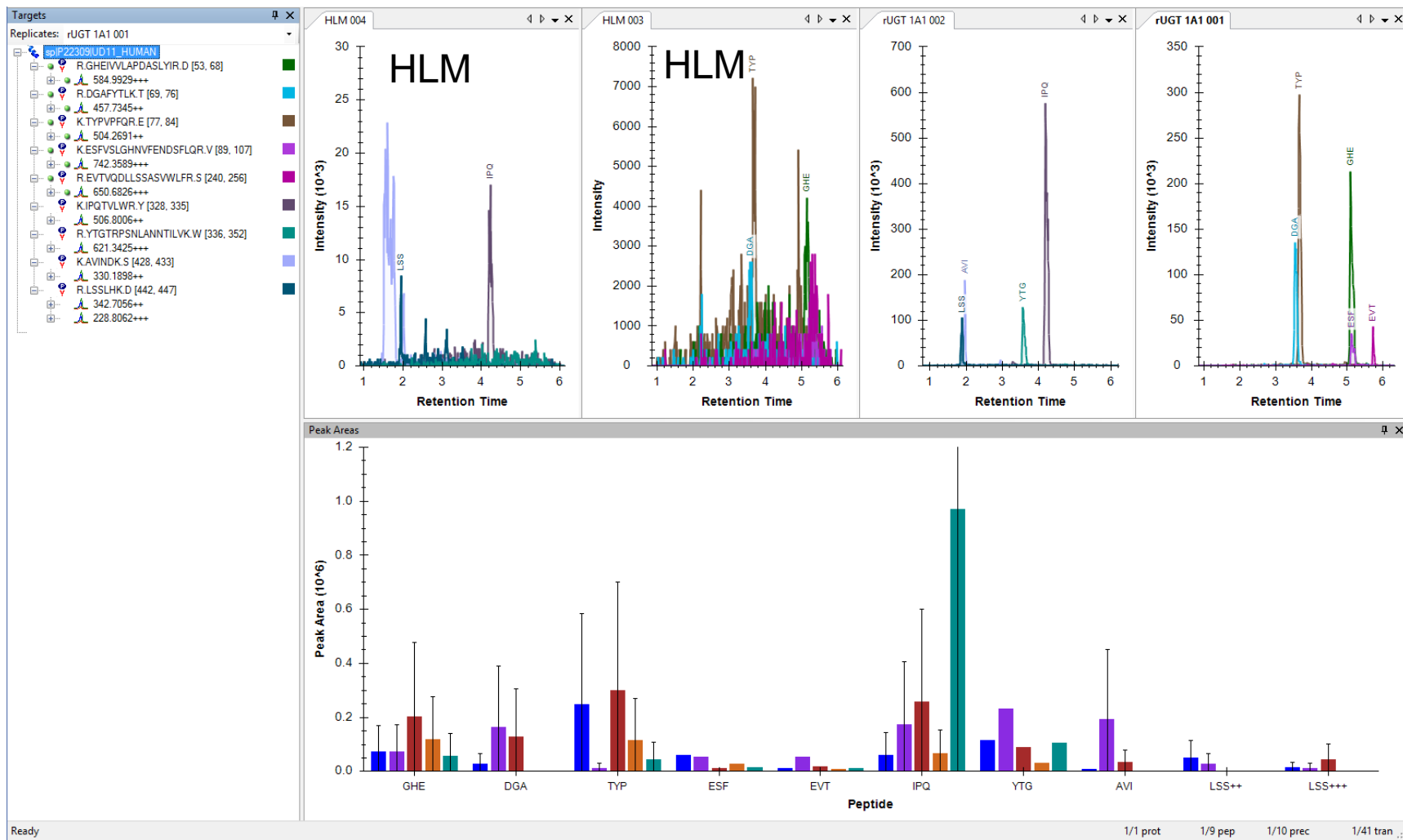
- Identify and procure appropriate recombinant protein
- Digest recombinant protein in buffer
- Screen protein digests for predicted peptides
- Check for peptide uniqueness in the background proteome
- Research known and predicted PTMs, and variants
- Choose best peptides and transitions
- Estimate LOD by serial dilution of digested protein in buffer, and digested matrix (plasma, CSF, tissue, etc)
- LOQ determination by serial dilution of recombinant protein into matrix followed by digestion
- Choose peptides and transitions for final method

# Skyline SRM Screens *All Tryptic Peptides*



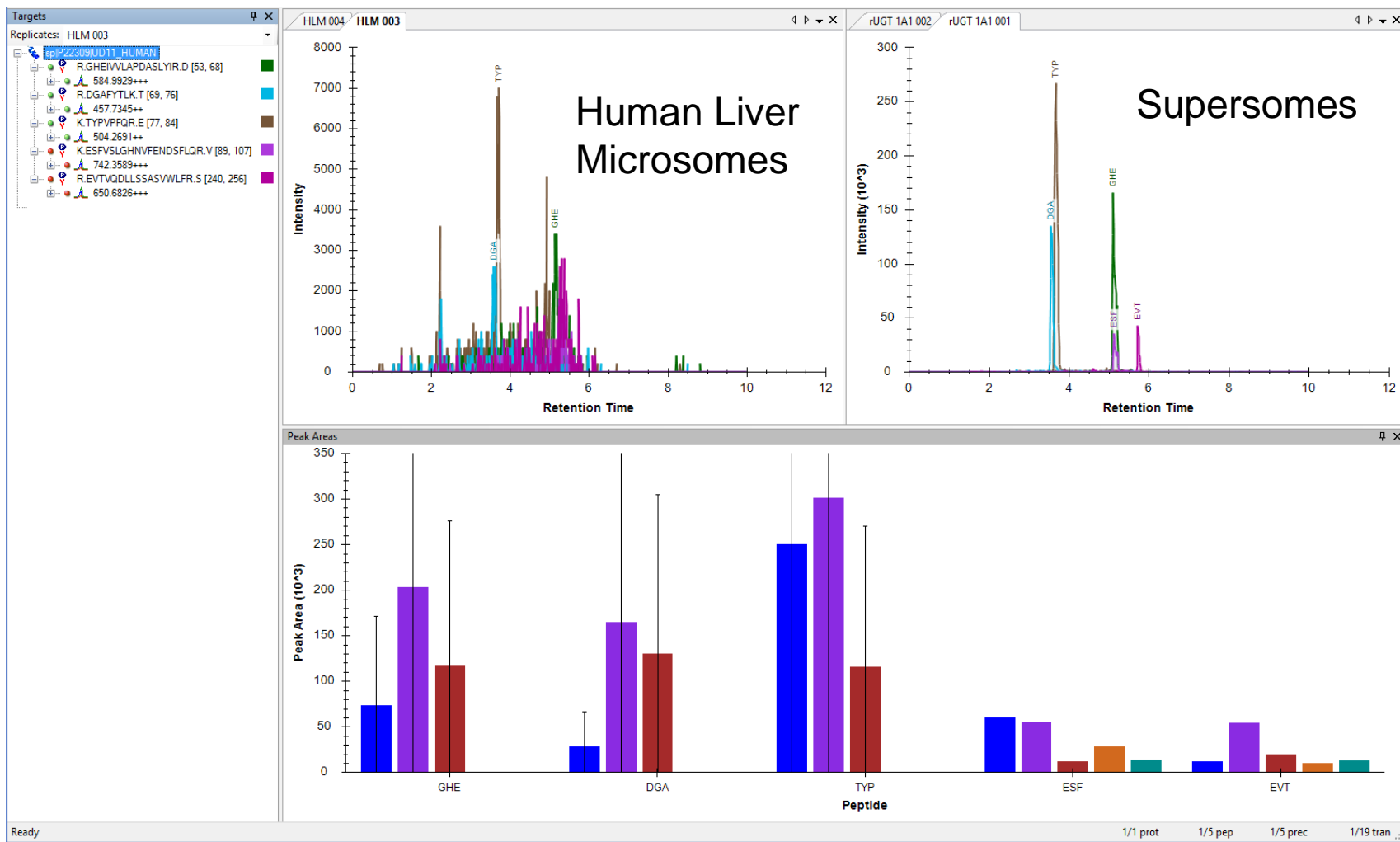
Digest of 50 ug of recombinant UGT1A1 microsomes (Supersomes)

# Skyline SRM Screens *Matrix Check*



Digest of 50 ug of rUGT1A1 microsomes, 200 ug HLM

# Skyline SRM Screens *Unique Peptides*



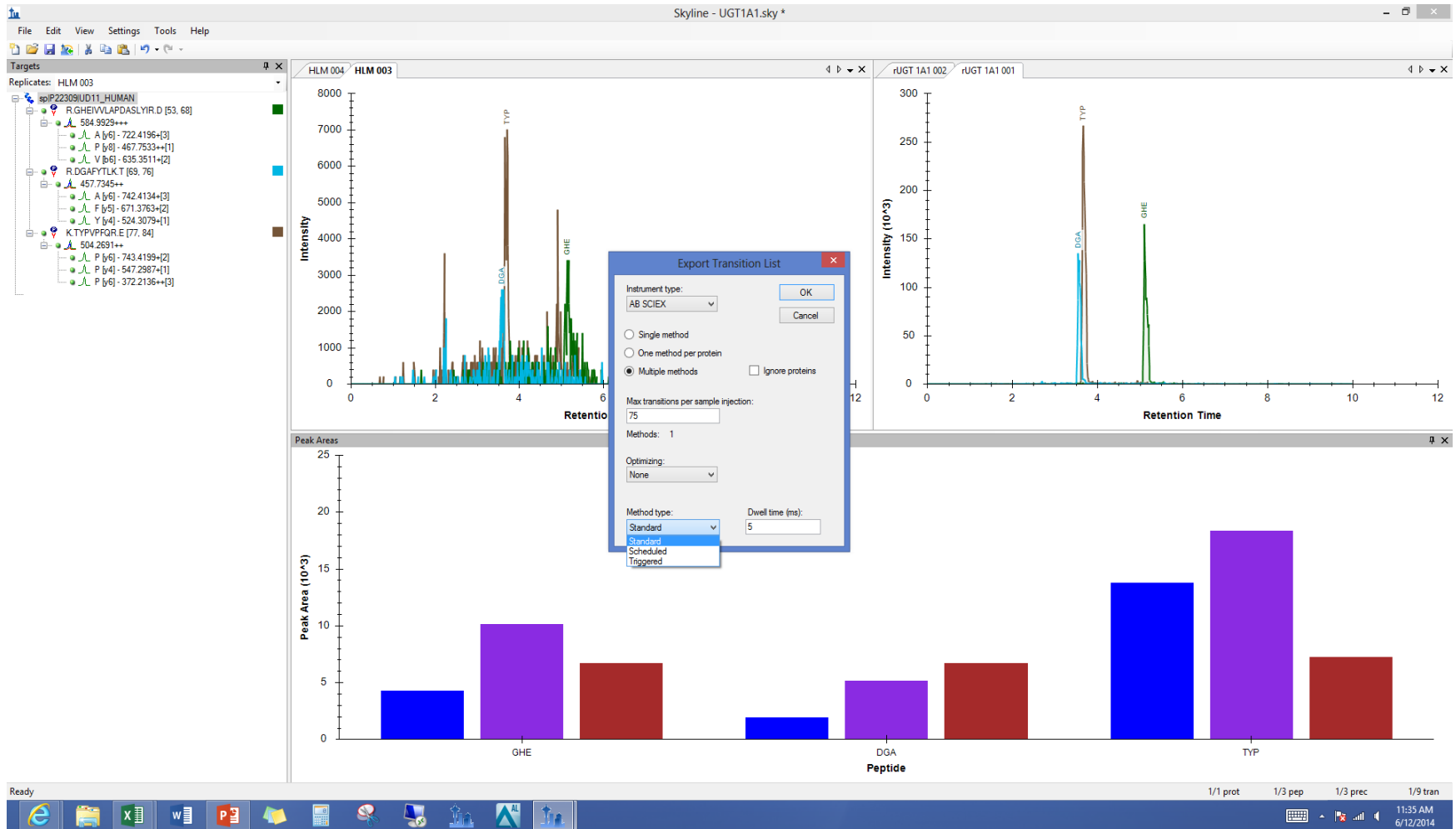
Digest of 50 ug of rUGT1A1 microsomes, 200 ug HLM



# Peptide Catalog

<a href="#">Resulting peptide sequence (see explanations)</a>	Peptide length [aa]	Peptide mass [Da]	Position of cleavage site	SNP	4-24 aa	Met	Not Unique	PTM (known)	PTM (predicted)
MAR	3	376.474	3		x	x	x		
TGWTSPILCVSLLLTCGFAEAGK	24	2464.92	27						
LLVVPMDGSHWFTMQSVVEK	20	2303.72	47			x	x		
LILR	4	513.681	51				x		
GHEVVVVMPEVSWQLGK	17	1894.219	68	x		x			
SLNCTVK	7	763.907	75				x		
TYSTSYTLEDLDR	13	1563.638	88				x	x	
EFMDFADAQWK	11	1387.529	99			x			
AQVR	4	472.545	103				x		
SLFSLFLSSSNGFFNLFESHCR	22	2557.909	125						
SLFNDR	6	750.809	131				x		
K	1	146.189	132	x	x		x		
LVEYLK	6	763.932	138				x		

# Export SRMs of Chosen Peptides



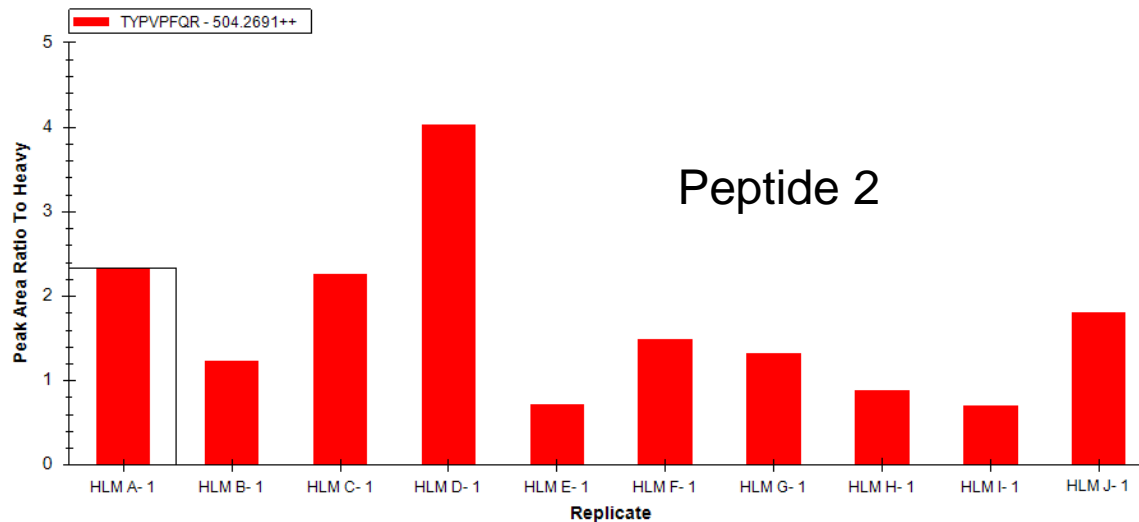
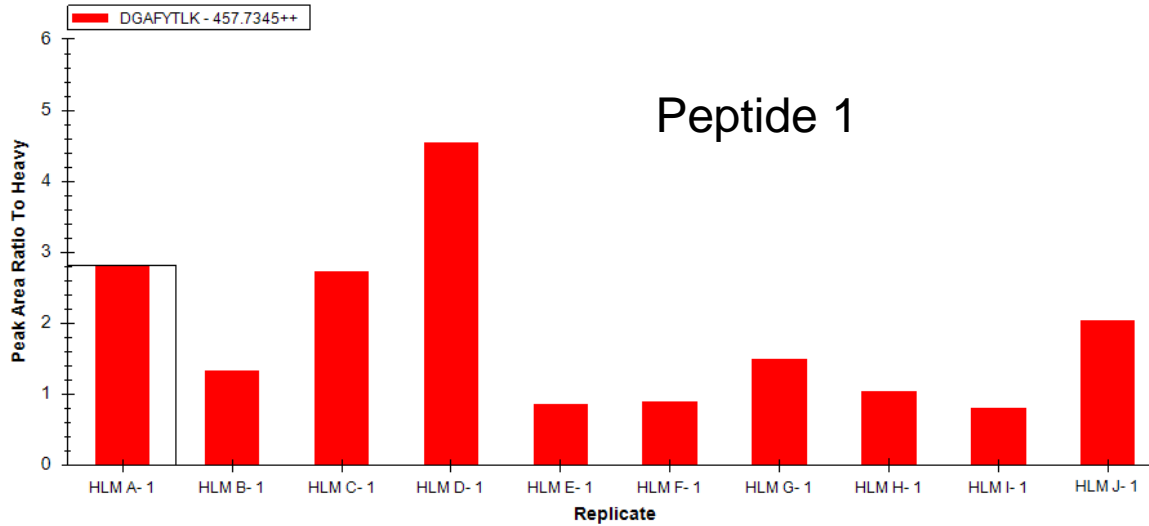
Digest of 50 ug of rUGT1A1 microsomes, 200 ug HLM

# Next Steps

- Procure stable isotope labeled versions of the chosen peptides
- Add the SIL peptides to the Skyline document
- Generate acquisition methods containing both labeled and unlabeled peptides

# 10 Individual Donor Liver Microsomes

## UGT 1A1 Relative Abundance



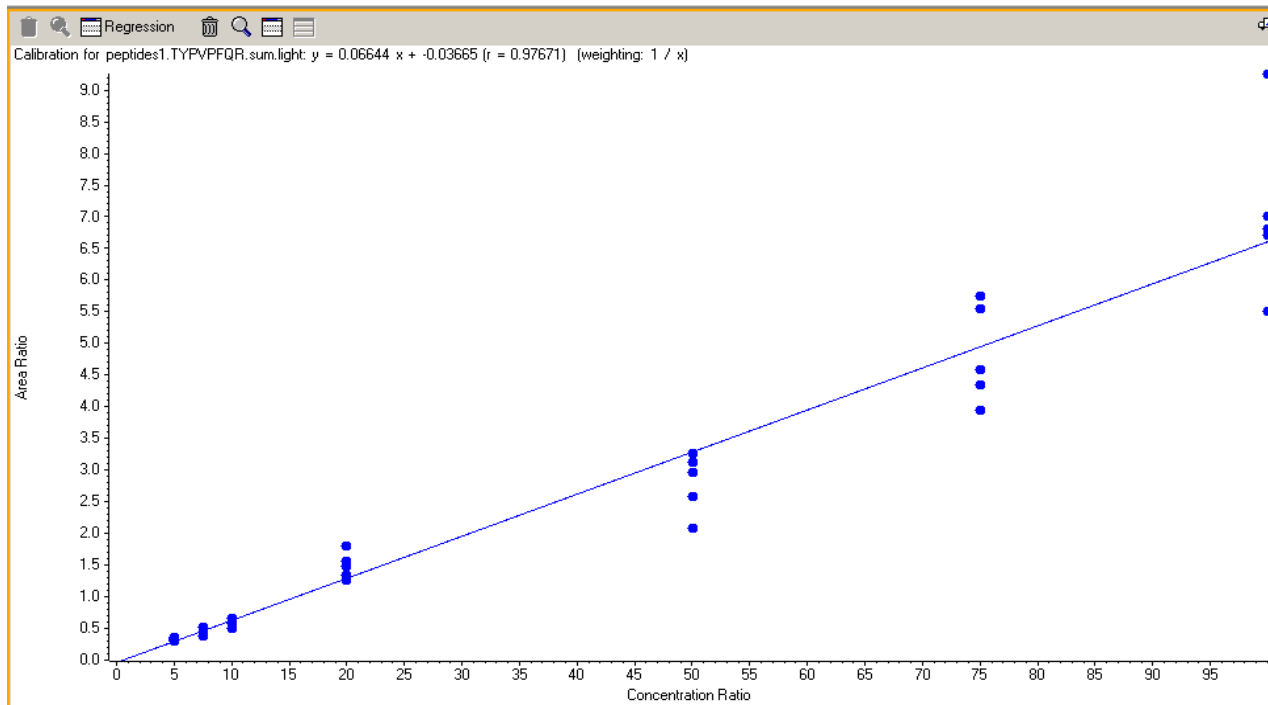
# Absolute Quantification of UGT

**Quantify  
amount of UGT  
in recombinant  
cell system  
microsome by  
isotope dilution**

**Use the  
qualified rUGT  
microsomes to  
generate the  
protein  
standard curve  
in rat liver  
microsomes**

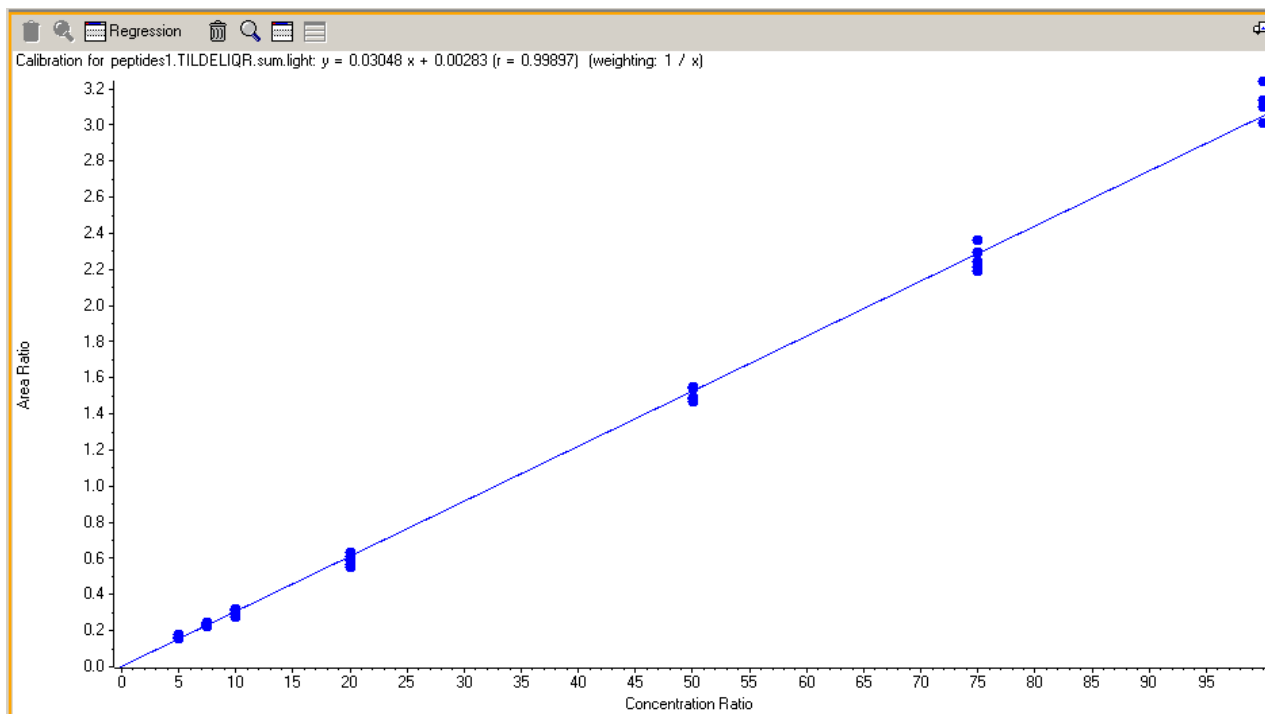
**Quantify UGT  
protein in  
human liver  
microsomes  
using surrogate  
peptides and SIL  
internal  
standards**

# UGT1A1 Calibration Curve



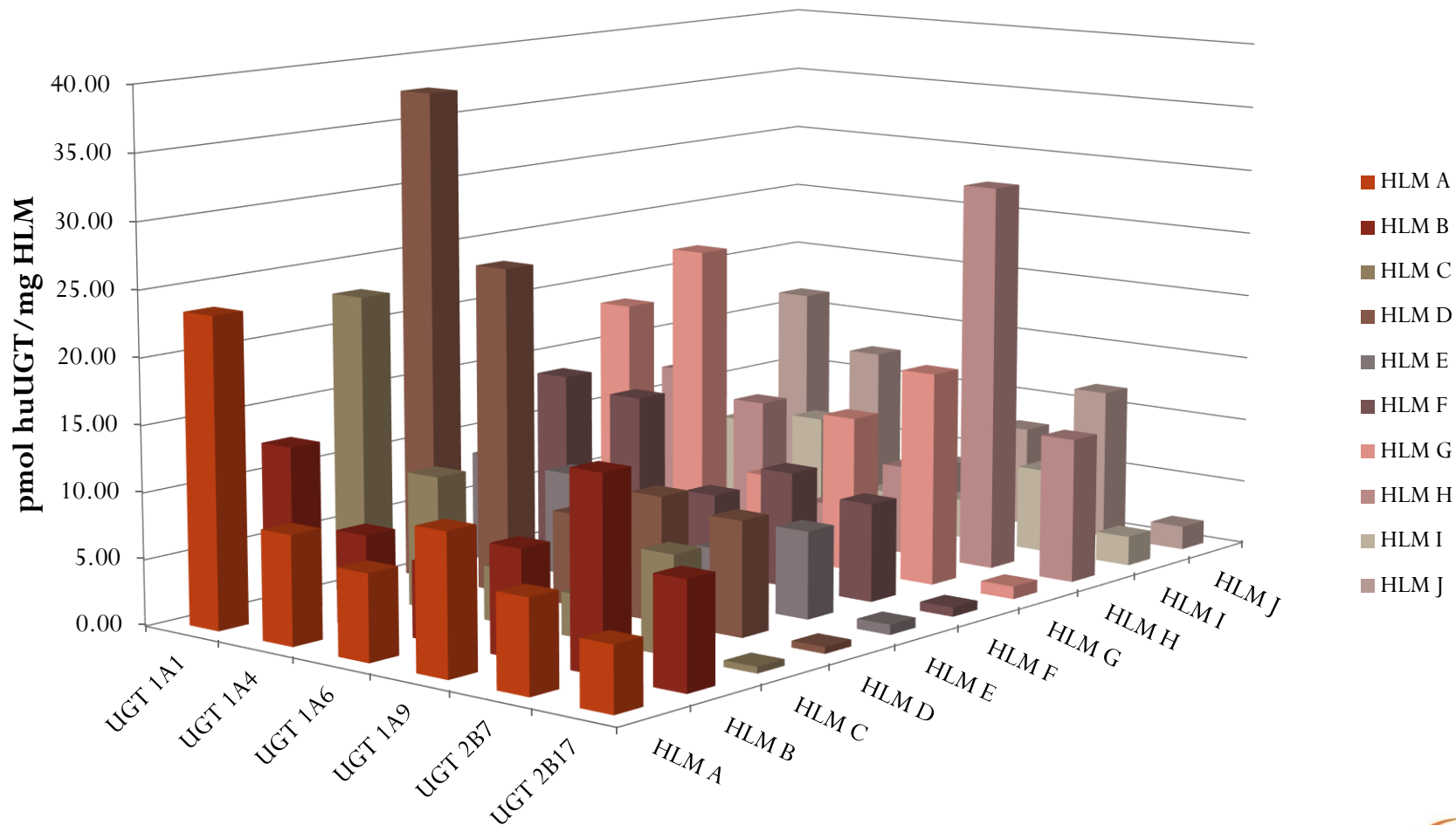
UGT Conc (ug rUGT/mg RLM)	N	Mean	Stdev	Percent CV	Accuracy
1.25	5 of 5	5.51	0.39	7.16	110.19
1.88	5 of 5	7.07	0.92	12.99	94.26
2.5	5 of 5	9.04	1.10	12.19	90.38
5.0	5 of 5	23.02	3.10	13.45	115.09
12.5	5 of 5	42.79	7.19	16.80	85.59
18.75	5 of 5	73.28	11.78	16.07	97.70
25	5 of 5	106.80	20.47	19.17	106.80

# UGT2B7 Calibration Curve



UGT Conc (ug rUGT/mg RLM)	N	Mean	Stdev	Percent CV	Accuracy
1.25	5 of 5	5.20	0.31	5.89	103.97
1.88	5 of 5	7.73	0.31	4.02	103.04
2.5	5 of 5	9.71	0.55	5.63	97.07
5.0	5 of 5	19.25	1.13	5.88	96.27
12.5	5 of 5	49.35	1.19	2.41	98.70
18.75	5 of 5	74.04	2.32	3.13	98.73
25	5 of 5	102.20	2.74	2.68	102.22

# Abundance of huUGT in 10 Individual Human Liver Microsomes





# Protein Modification Characterization

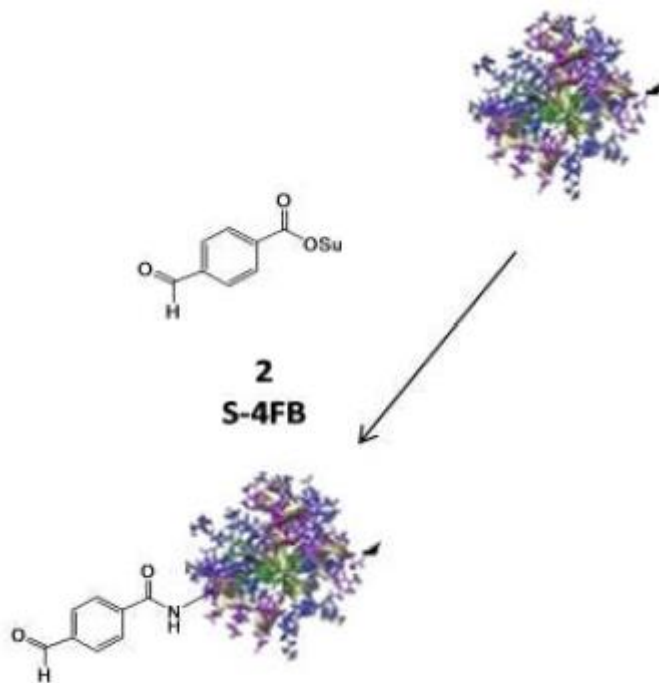
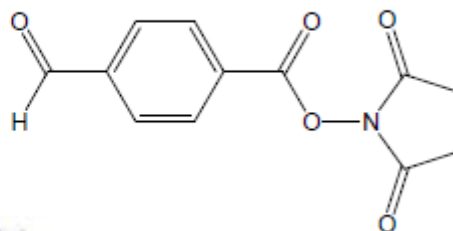
# Solulink Chemistry

- 4-formylbenzamide – amine derivative

## S-4FB

$C_{12}H_9NO_5$  MW 247.20

Catalog# S-1004



# Problem: Identify the Modified Lysines

sp|P10253|LYAG\_HUMAN

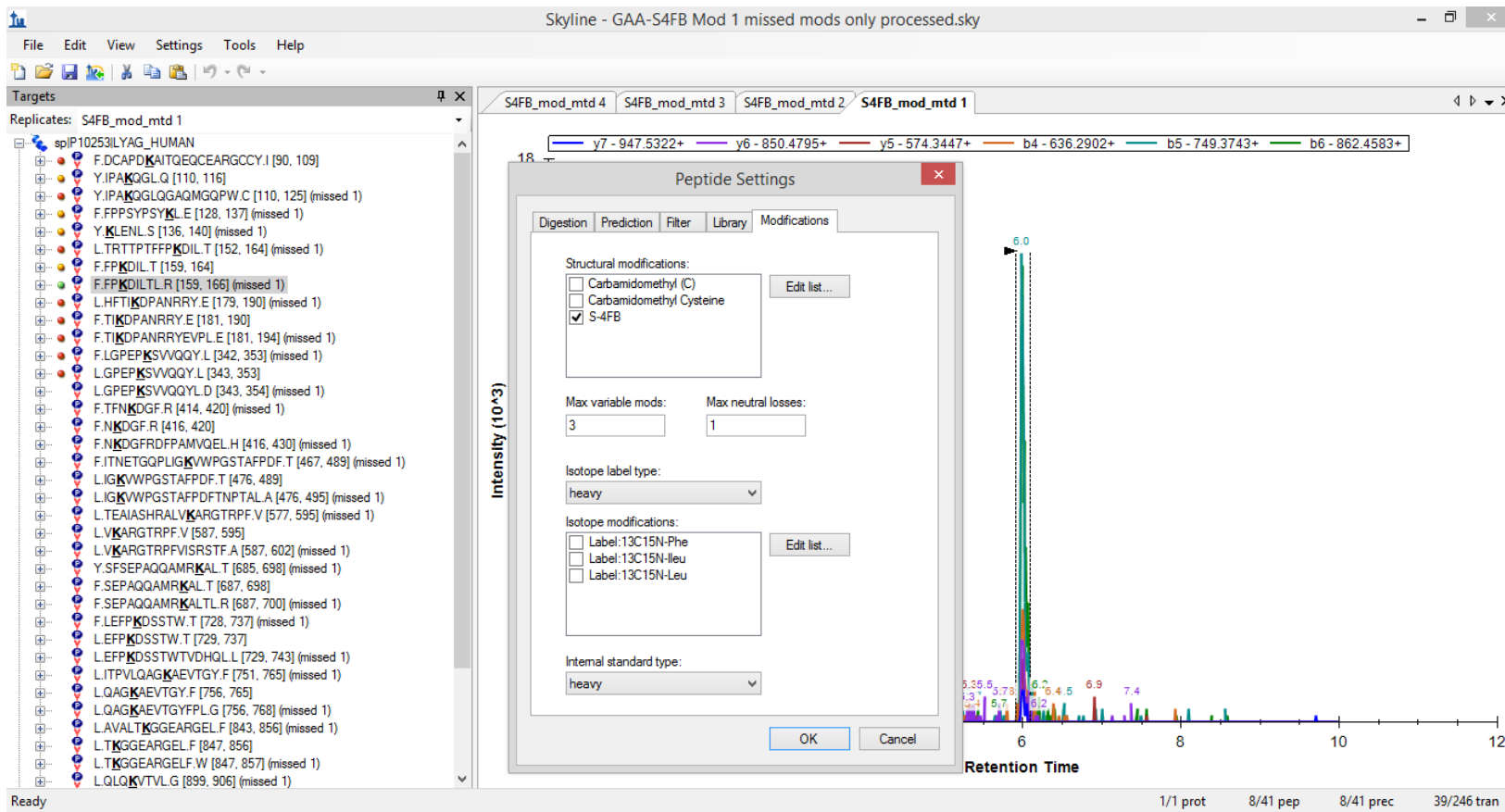
Lysosomal alpha-glucosidase OS=Homo sapiens GN=GAA PE=1 SV=4

MGVRHPPCSHRLAVCALVSLATAALLGHILLHDFLLVPRELSGSSPVLEETHPAHQQGASRPGPRDAQAHGPRPRAVPTQCDV  
PPNSRF DCAPDKAITQEQCEARGCCYIPAKQGLQGAQMGQPWCFFPPSYPSYKLENLSSSEMGYATLTRTTPTFFPKDILTL  
RLDVMMETENRLHFTIKDPANRRYEVPLETPHVH SRAPSPLYVEFSEEPFGVIVRRQLDGRVLLNTTVAPLFFADQFLQLST S  
LP SQYITGLAEHL SPLMLST SWTRITLWNRDLAPTGANLYG SHPFYLALEDGG SAHG VLLN SNAMDVVLQP SPAL SWRSTG  
GILDVYIFLGPEPKSVVQQYLDVVGYPFMPYWG LGFHL CRWGYSTAITRQVVENMT RAHFPLDVQWNDLDYMD SRRDFTF  
NKDGFRDFPAMVQELHQGRRYMMIVDPAISSSGPAGSYRPYDEGLRRGVFITNETGQPLIGKVVWPGSTAFPDFTNPTALAW  
WEDMVAEFHDQVPFDGMWIDMNEP SNFIRG SEDGCPNNELENPPYVPGVGGT LQAATICA SSHQFL STHYNLHNLYGLTEAI  
ASHRALVKARGTRPFVISRSTFAGHGRYAGHWTGDVWSSWEQLASSVPEILQFNLLGVPLVGADVCGFLGNTSEELCVRWT  
QLGAFYPFMRNHN SLLSLPQEPYSEPAQQAMRKALTLRYALLPHLYTLFHQAHVAGETVARPLFLEFPKDSSTWTVDHQL  
LWGEALLITPVLQAGKA EVTGYFPLGTWYDLQTVPEALGSLPPPPAAPREPAIHSEGQWVTLPAPLDTINVHLRAGYIIPLQGP  
GLTTESRQQPMAVALTKGGEARGELFWDDGESLEVLERGAYTQMFLARNNTMNELVRVTSEGAGLQLQKVTVLGVATAP  
QQVLSNGVPVSNFTYSPDKVLDICV SLLMGEQFLVSWC

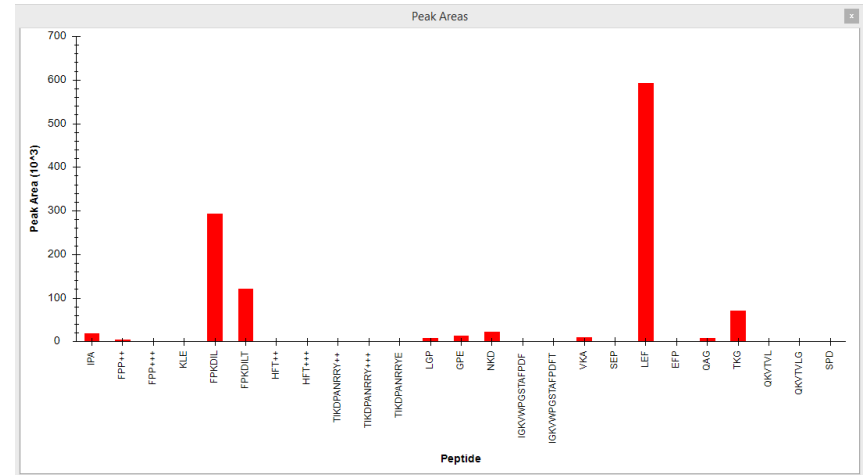
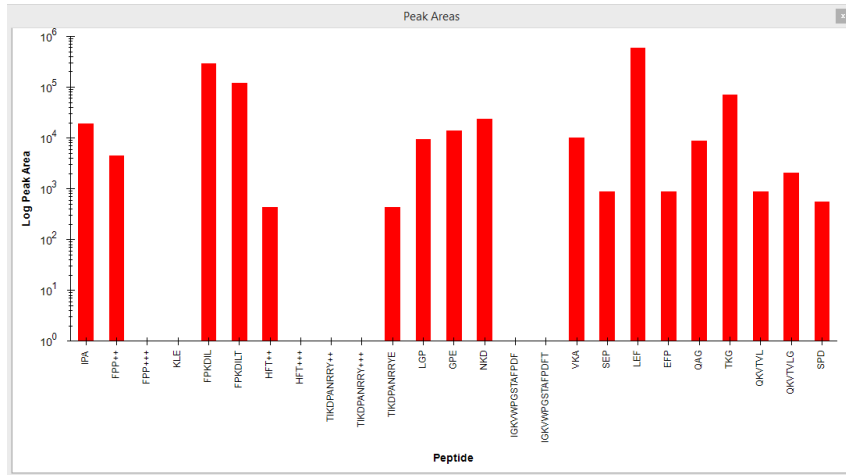
F.LGPEPKSVVQQY.L [342, 353]

- Purified protein de-glycosylated and digested with chymotrypsin
- SRM methods built for modified and unmodified lysine containing peptides
- Native and modified protein samples analyzed by SRM

# Skyline Peptide Prediction and Screening



# Modified Lysine Containing Peptides



# Conclusions

- Skyline is an integral component of our protein analysis workflow
- It provides the flexibility and capability to support both qualitative and quantitative experiments
- Skyline facilitates the use of SRM methods for protein characterization
- Establishing SRM detection methods for protein digests manually required several days to weeks. The same work can be done in less than a day using Skyline.