Skyline: Everyday Tool for Protein Quantification

Skyline User Meeting

June 14, 2014

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Outline

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

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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

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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

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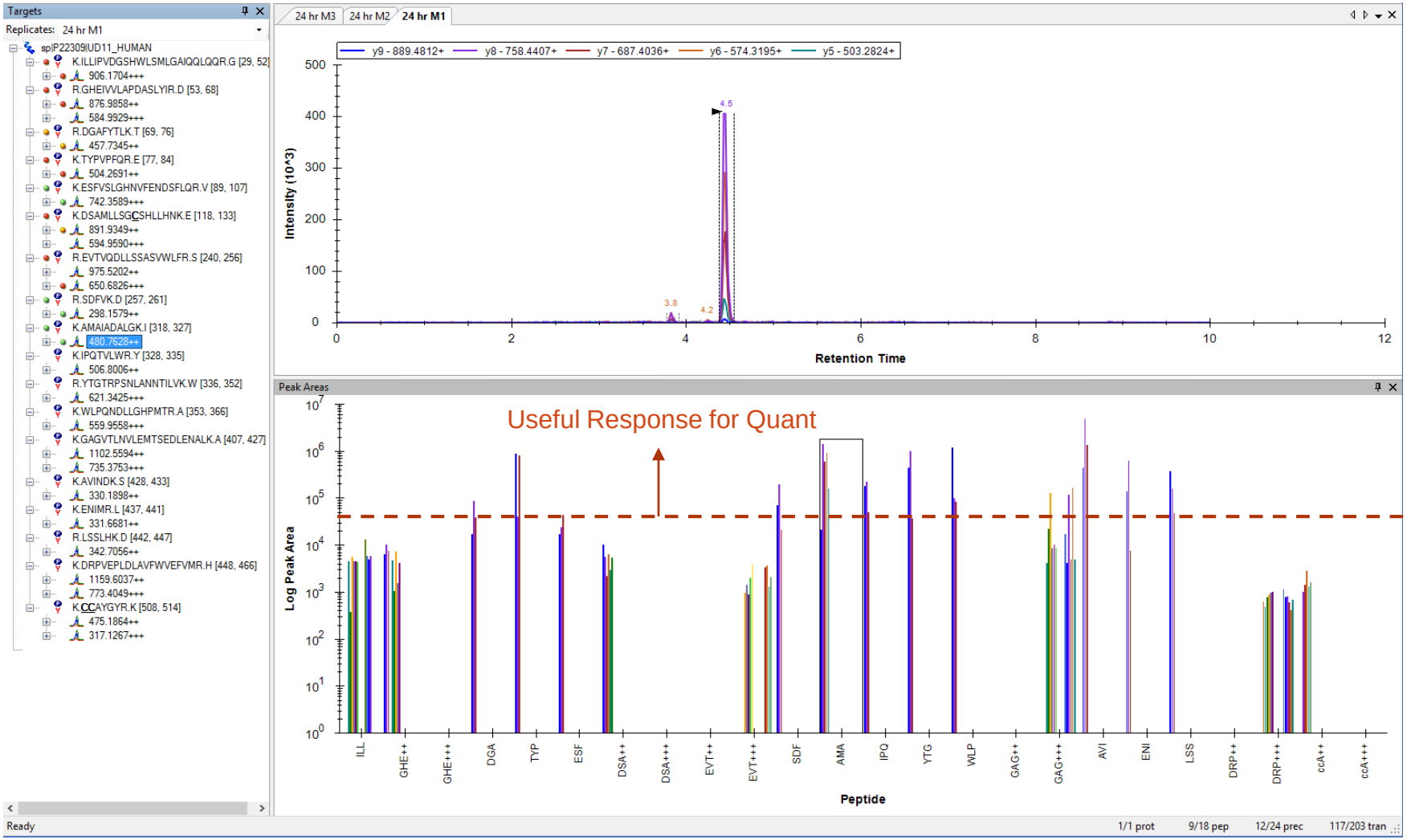
Skyline SRM Screens *All Tryptic Peptides*



Digest of 50 ug of recombinant UGT1A1 microsomes (Supersomes)

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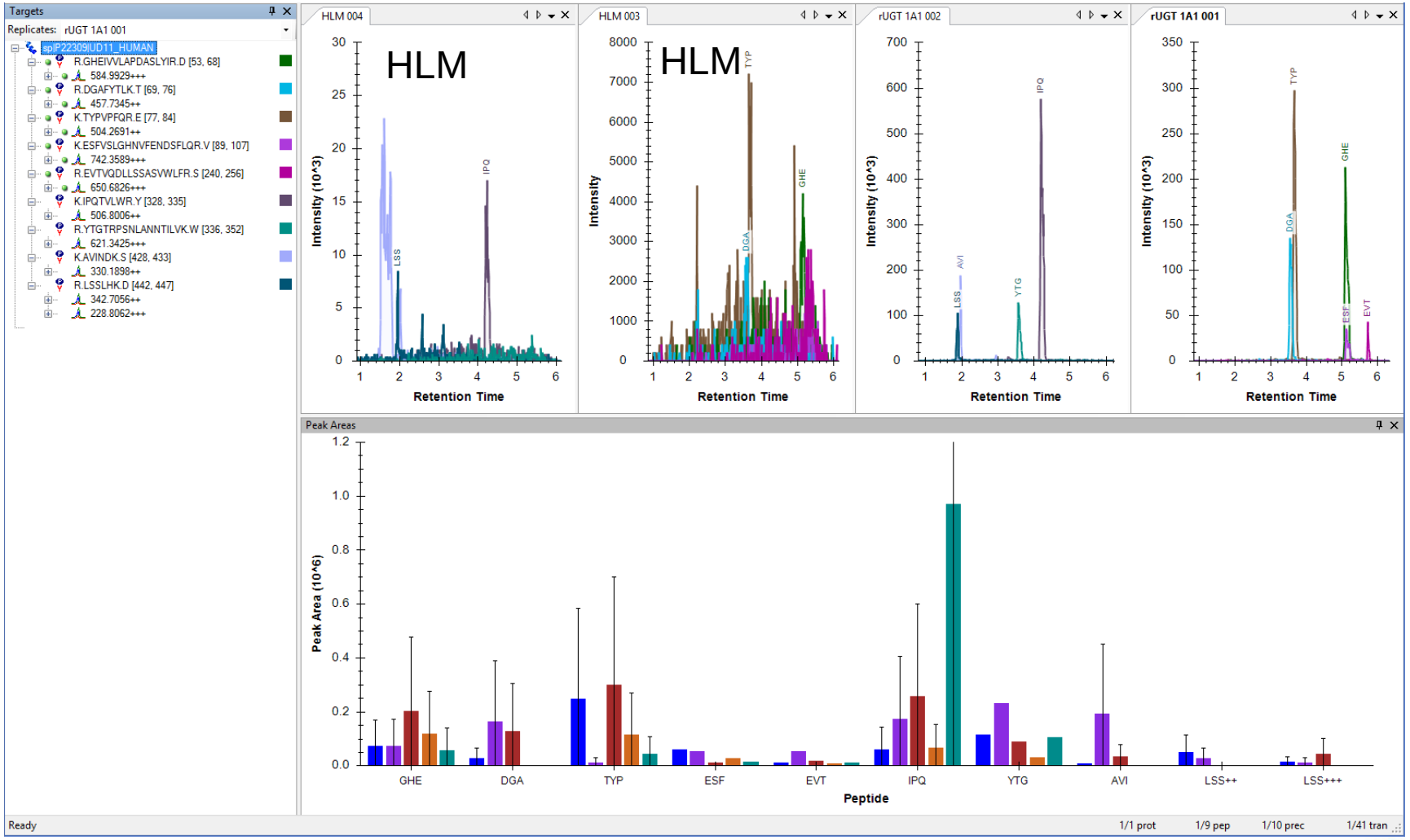
Skyline SRM Screens *Matrix Check*



Digest of 50 ug of rUGT1A1 microsomes, 200 ug HLM

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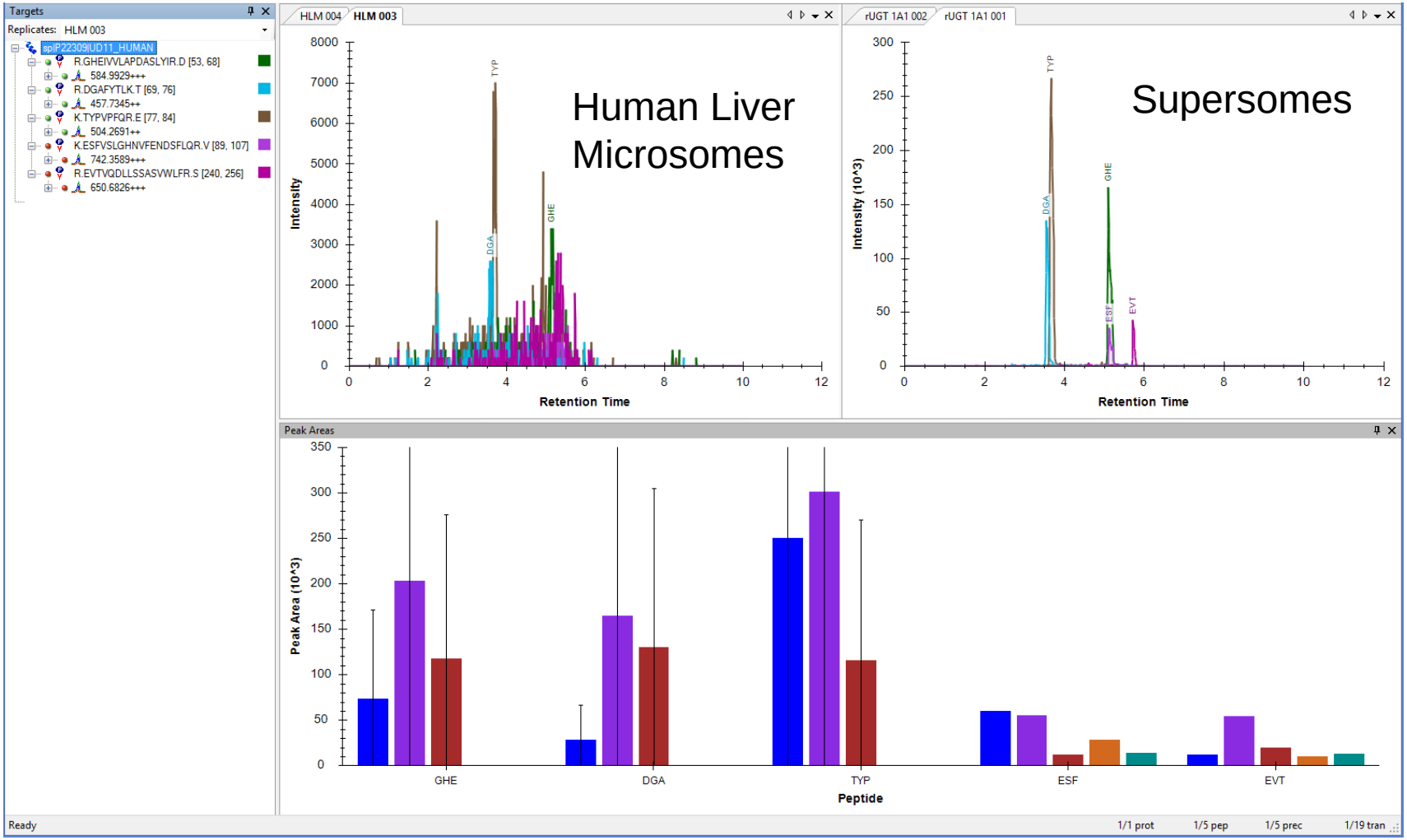
Skyline SRM Screens *Unique Peptides*



Digest of 50 ug of rUGT1A1 microsomes, 200 ug HLM

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Peptide Catalog

Resulting peptide sequence (see explanations)	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		
TGWTSPIPLCVSLLLTCGFAEAGK	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		
SLFSLFLSSSNGFFNLFFSHCR	22	2557.909	125						
SLFNDR	6	750.809	131				x		
K	1	146.189	132	x	x		x		
LVEYLK	6	763.932	138				x		

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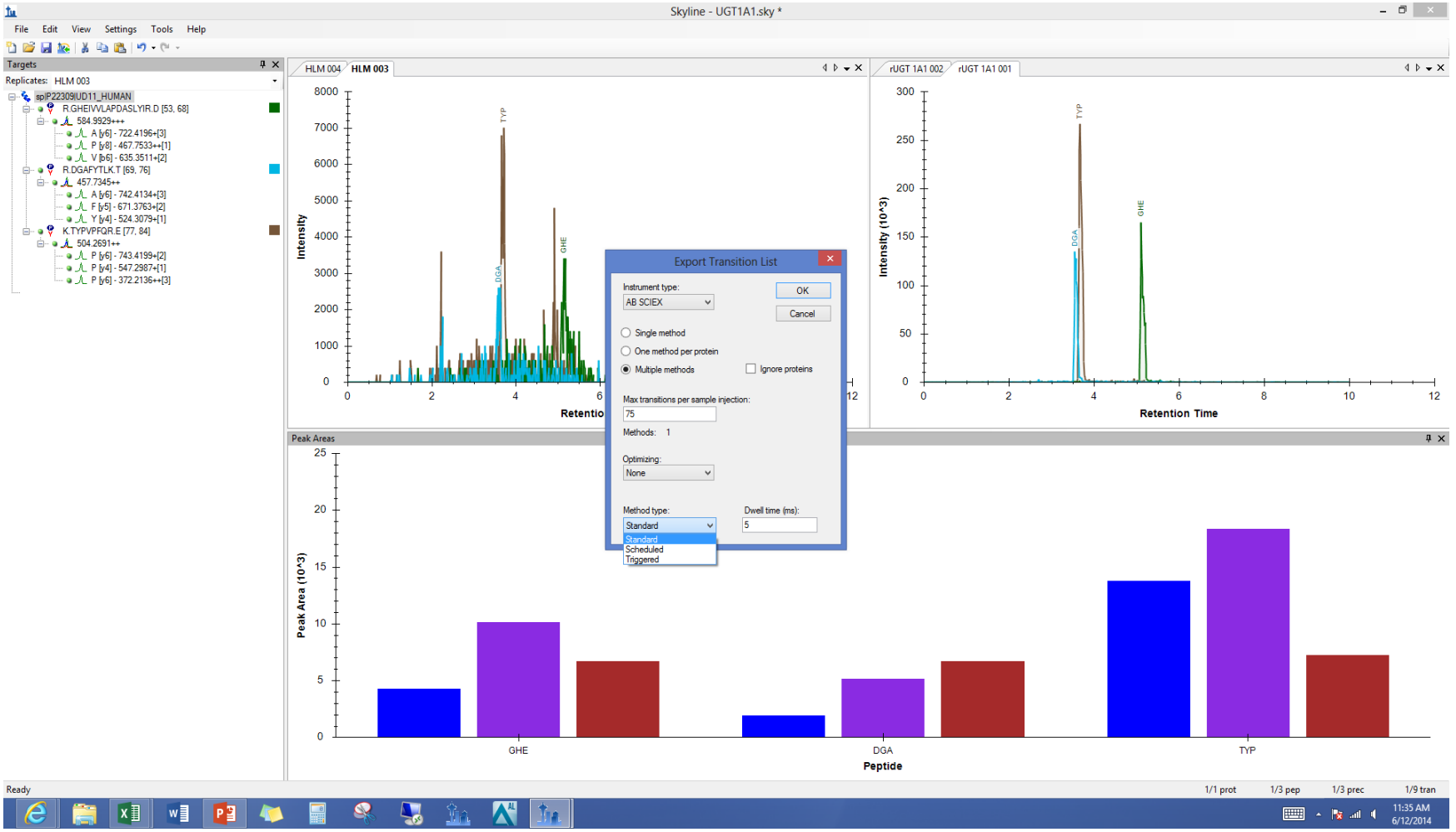
Export SRMs of Chosen Peptides



Digest of 50 ug of rUGT1A1 microsomes, 200 ug HLM

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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

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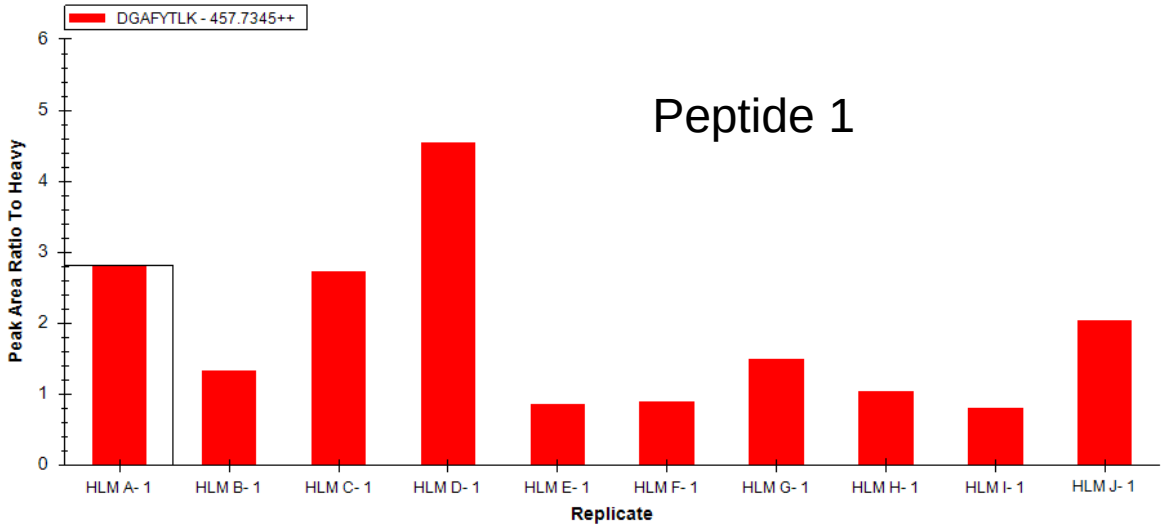
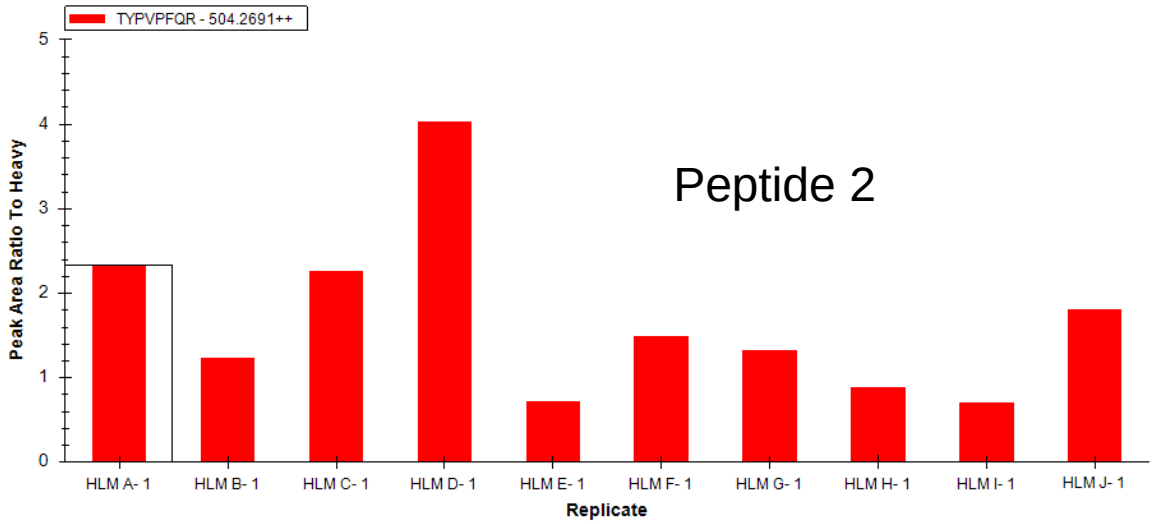
10 Individual Donor Liver Microsomes
UGT 1A1 Relative Abundance





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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

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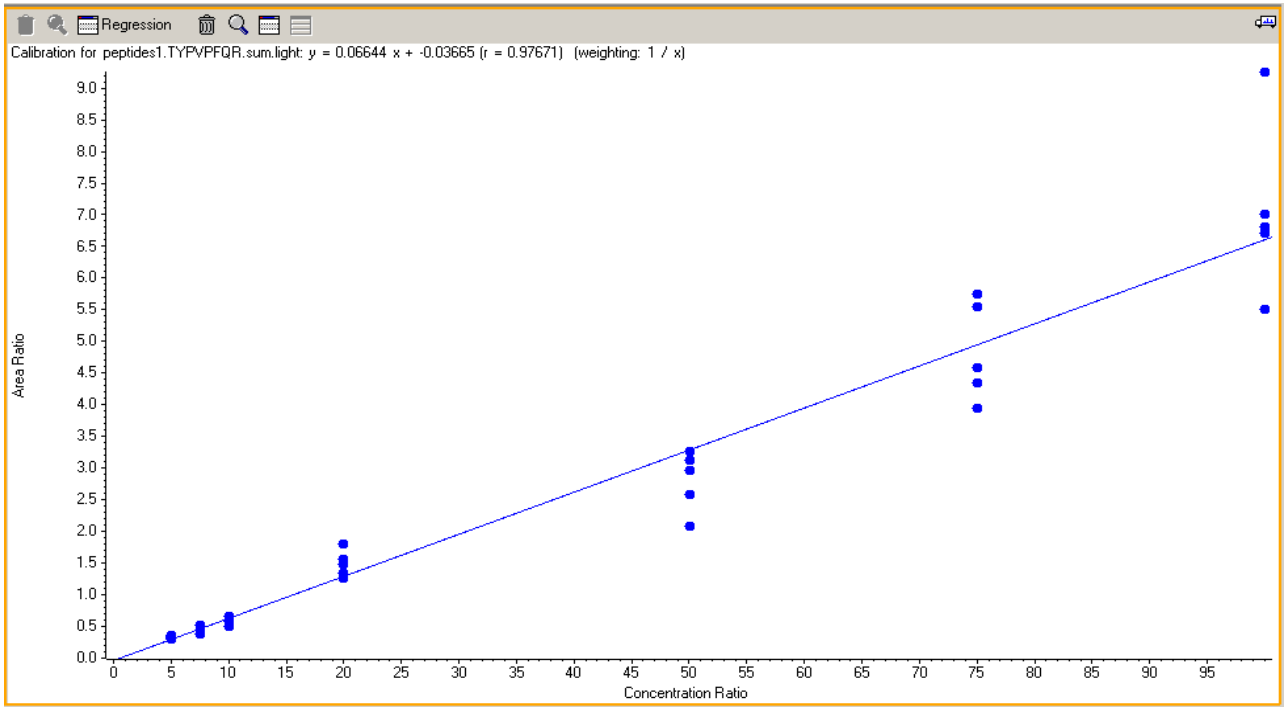
UGT1A1 Calibration Curve



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

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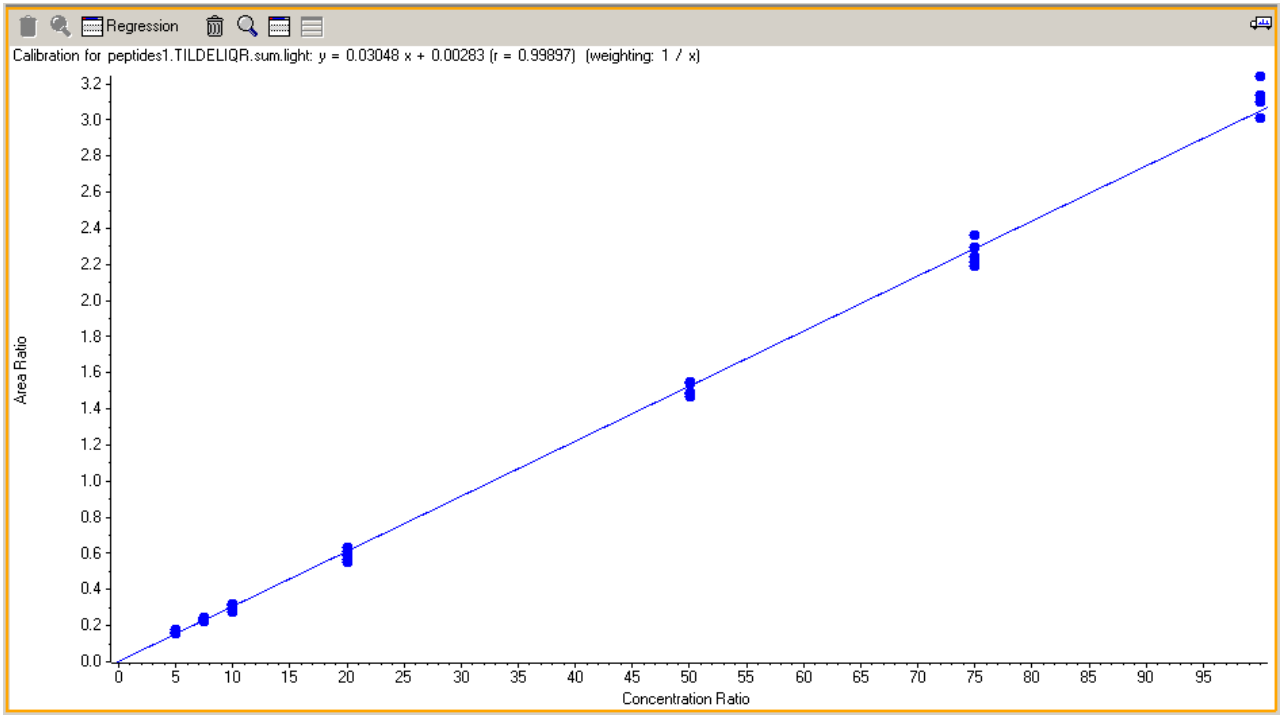
UGT2B7 Calibration Curve



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

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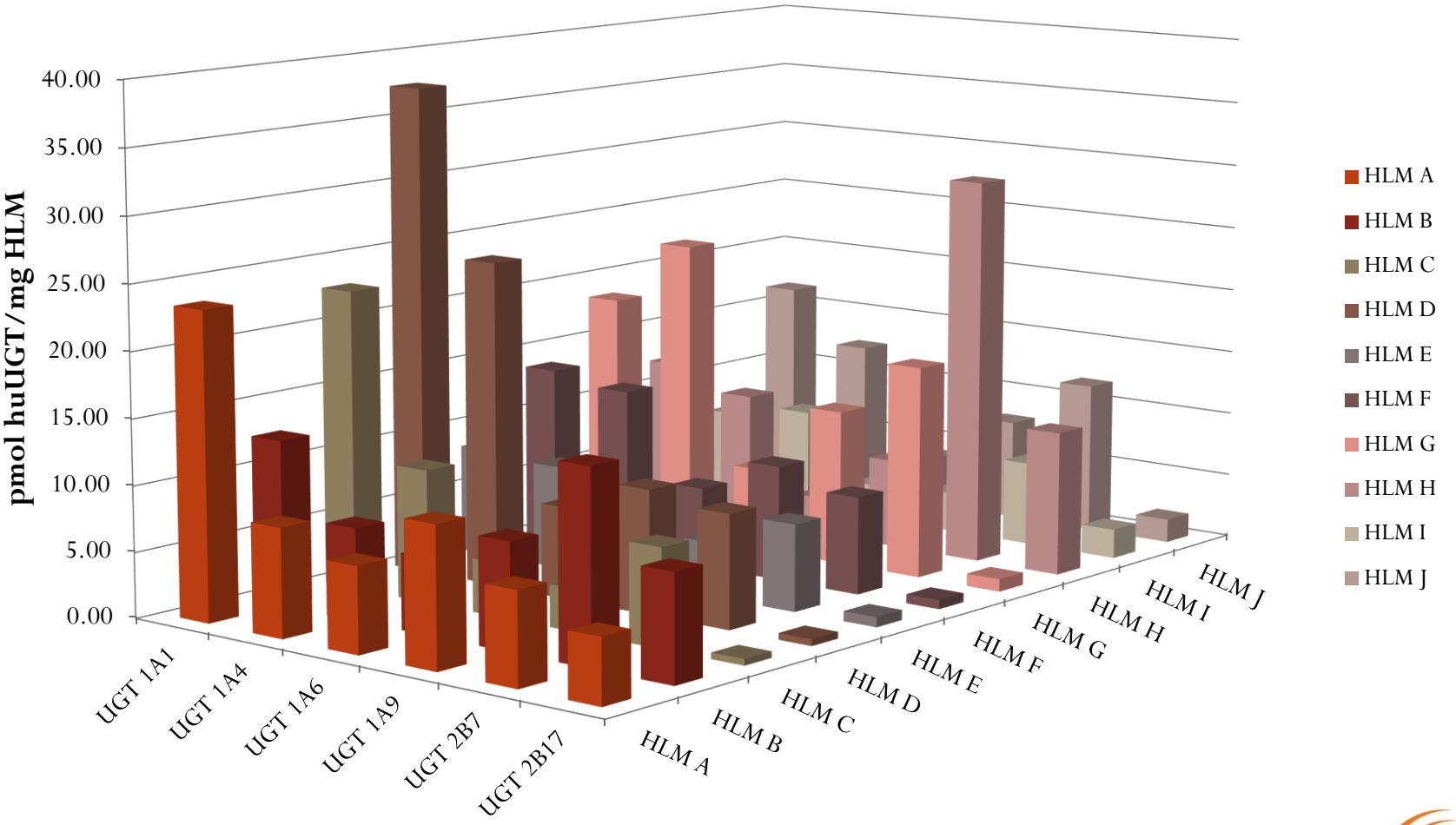
Abundance of huUGT in 10 Individual Human Liver Microsomes



Protein Modification Characterization

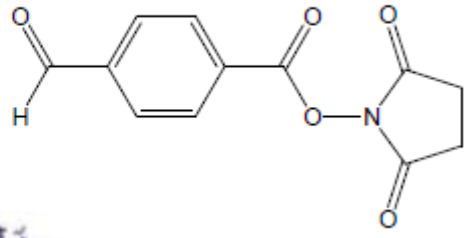
Solulink Chemistry

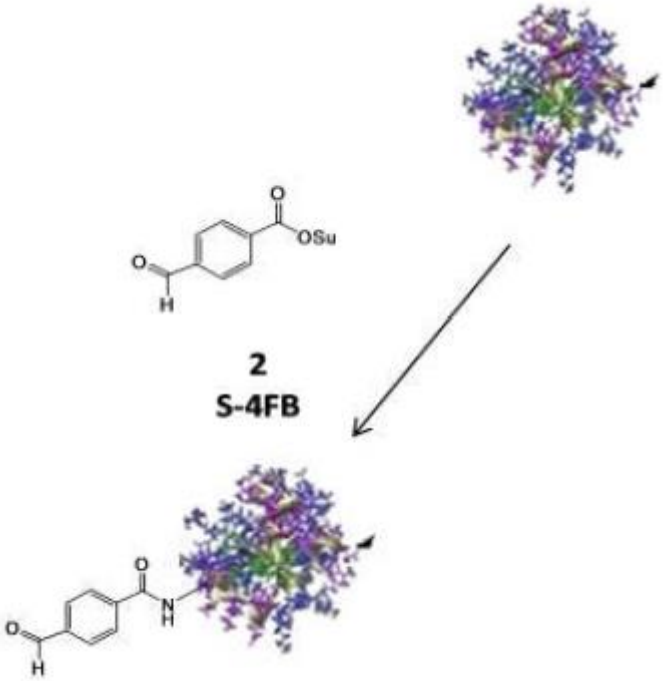
- 4-formylbenzamide – amine derivative

S-4FB

$C_{12}H_9NO_5$ MW 247.20

Catalog# S-1004





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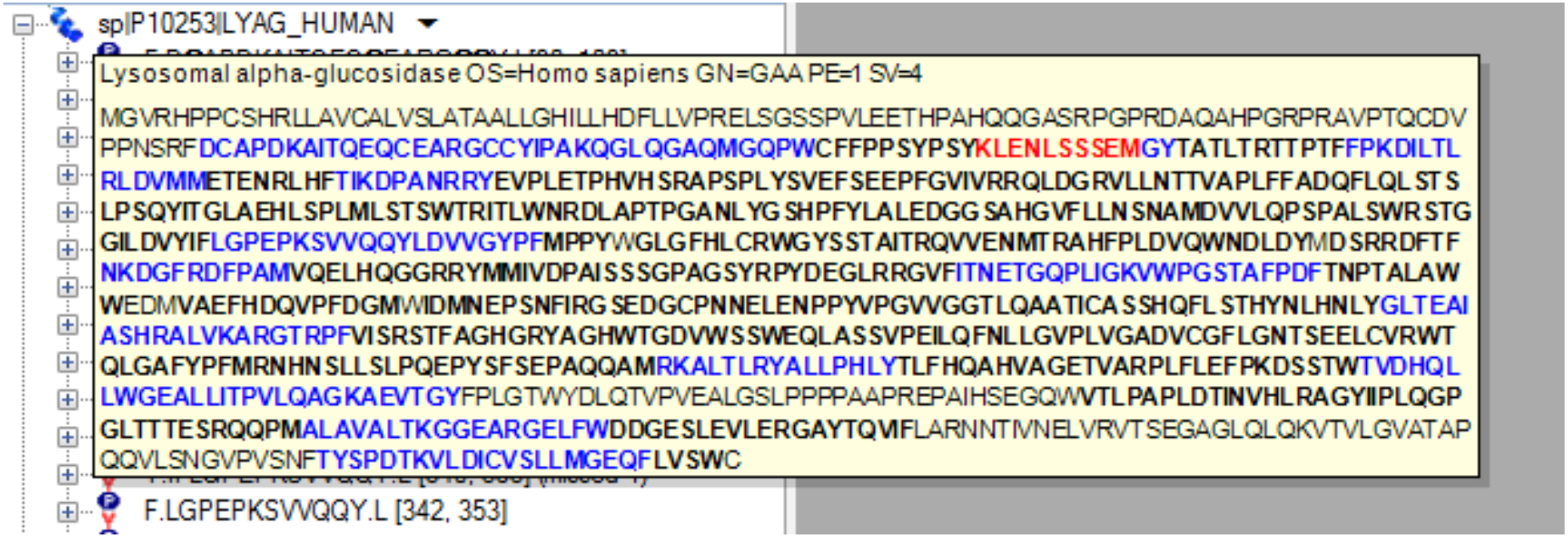
Problem: Identify the Modified Lysines



- 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

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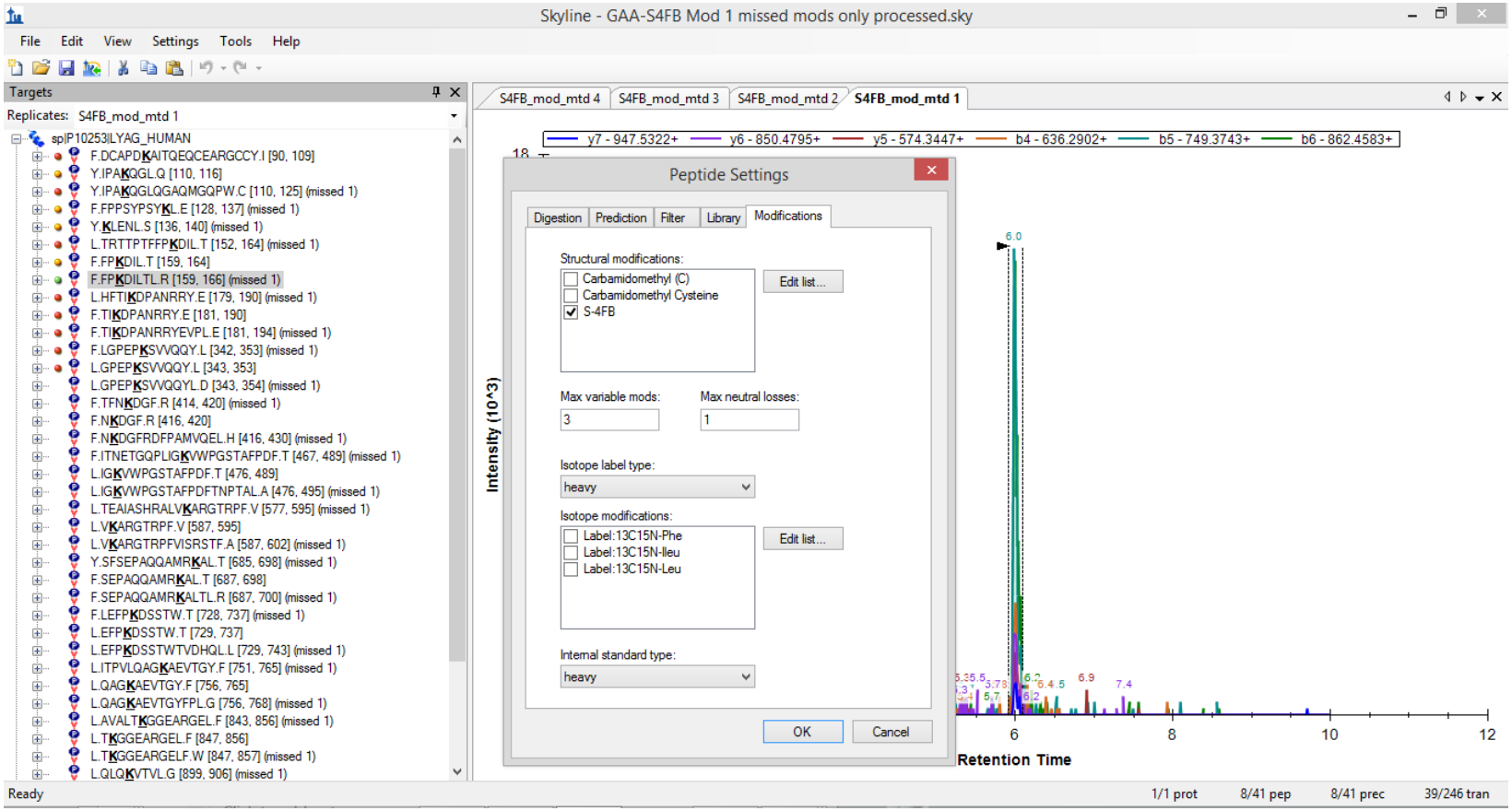
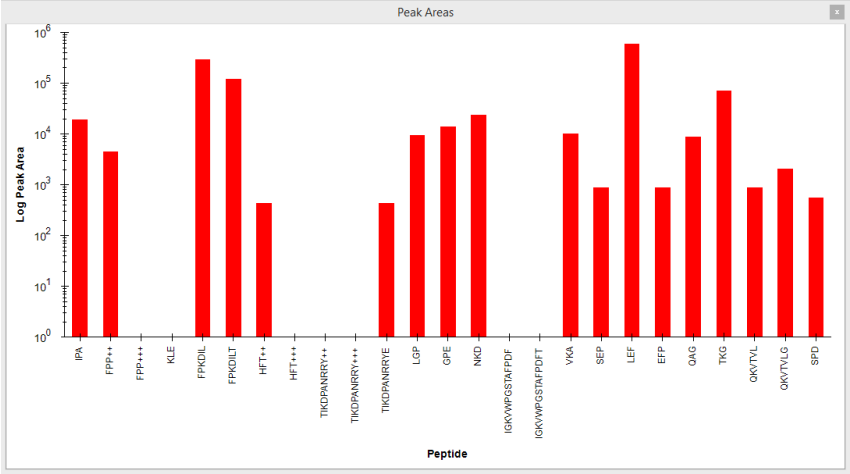
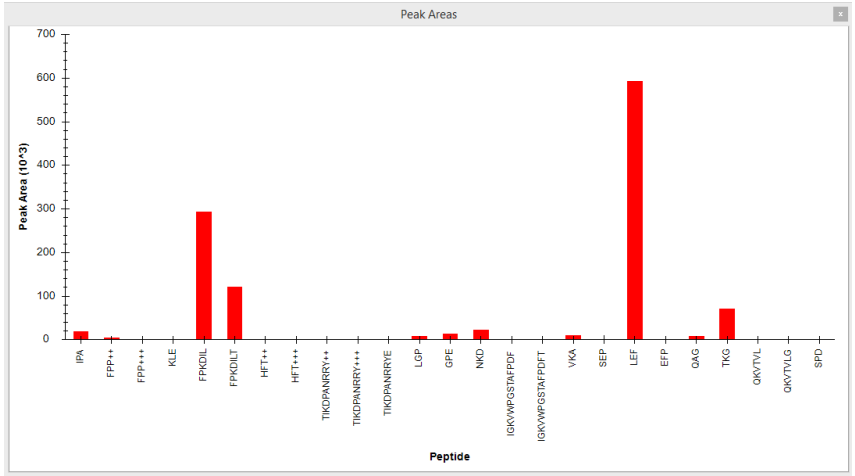
Skyline Peptide Prediction and Screening



Modified Lysine Containing Peptides





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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.

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