talusbio

How sweet it is: Leveraging the nuclear envelope glycome for the automated extraction of proteins from cell nuclei

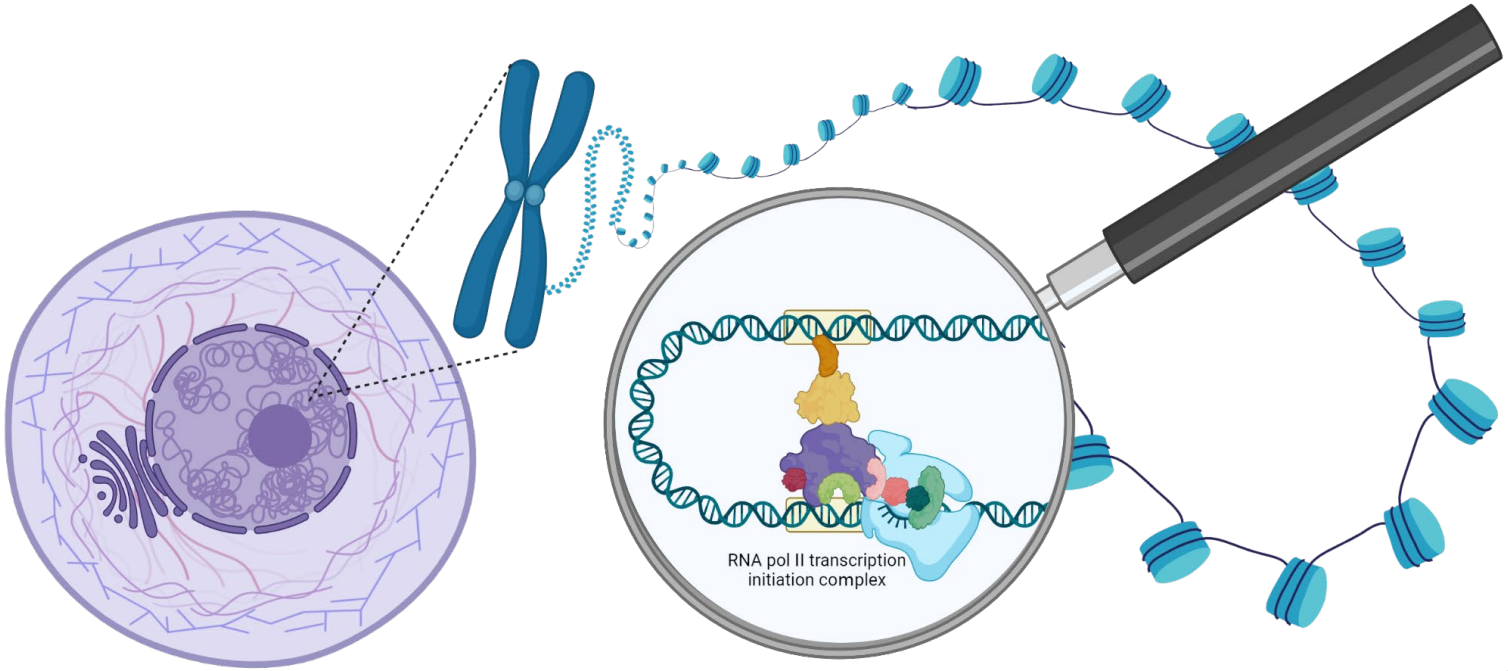
(and how Skyline helped!)

Julia Robbins

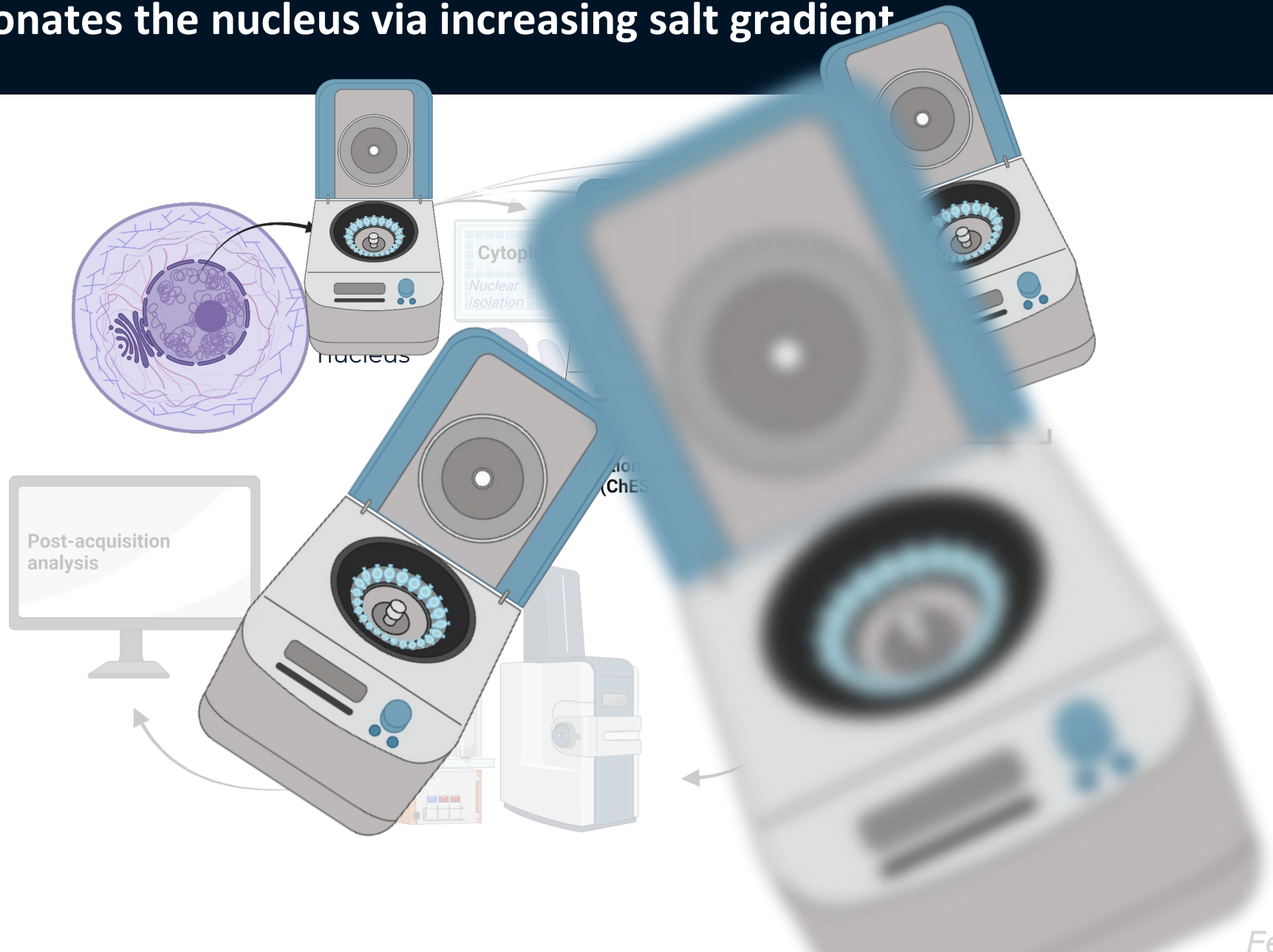
Talus is building a proteomic mass spectrometry-based platform that enables high-throughput drug screening in cancer cells

- Transcription factors (TFs) are regulatory components that turn genes on and off

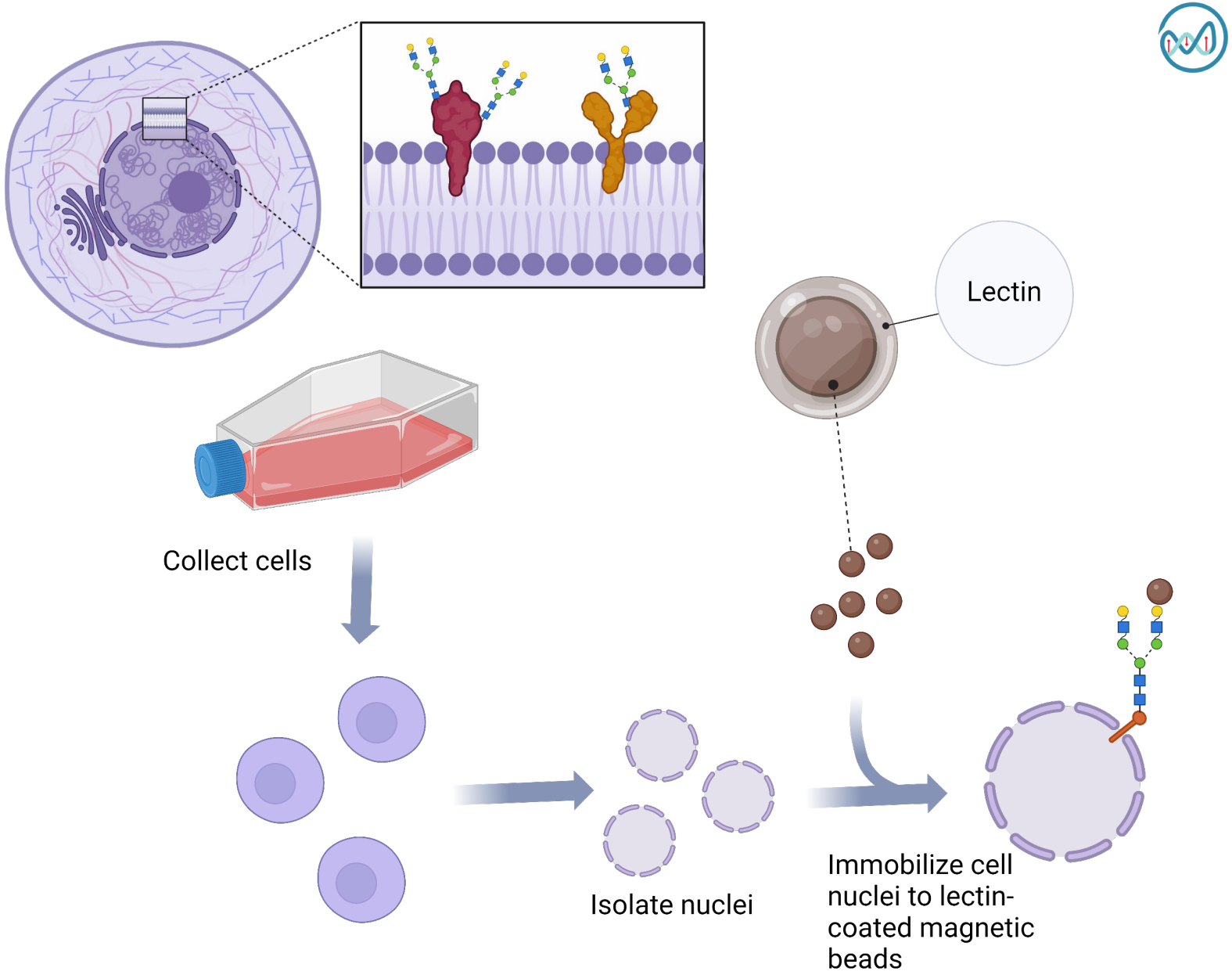
- Most TFs are found in the nucleus
- Inherent transience and low abundance mean that enrichment is usually acquired for quantitative characterization



Chromatin Enrichment by Salt Separation (ChESS) fractionates the nucleus via increasing salt gradient



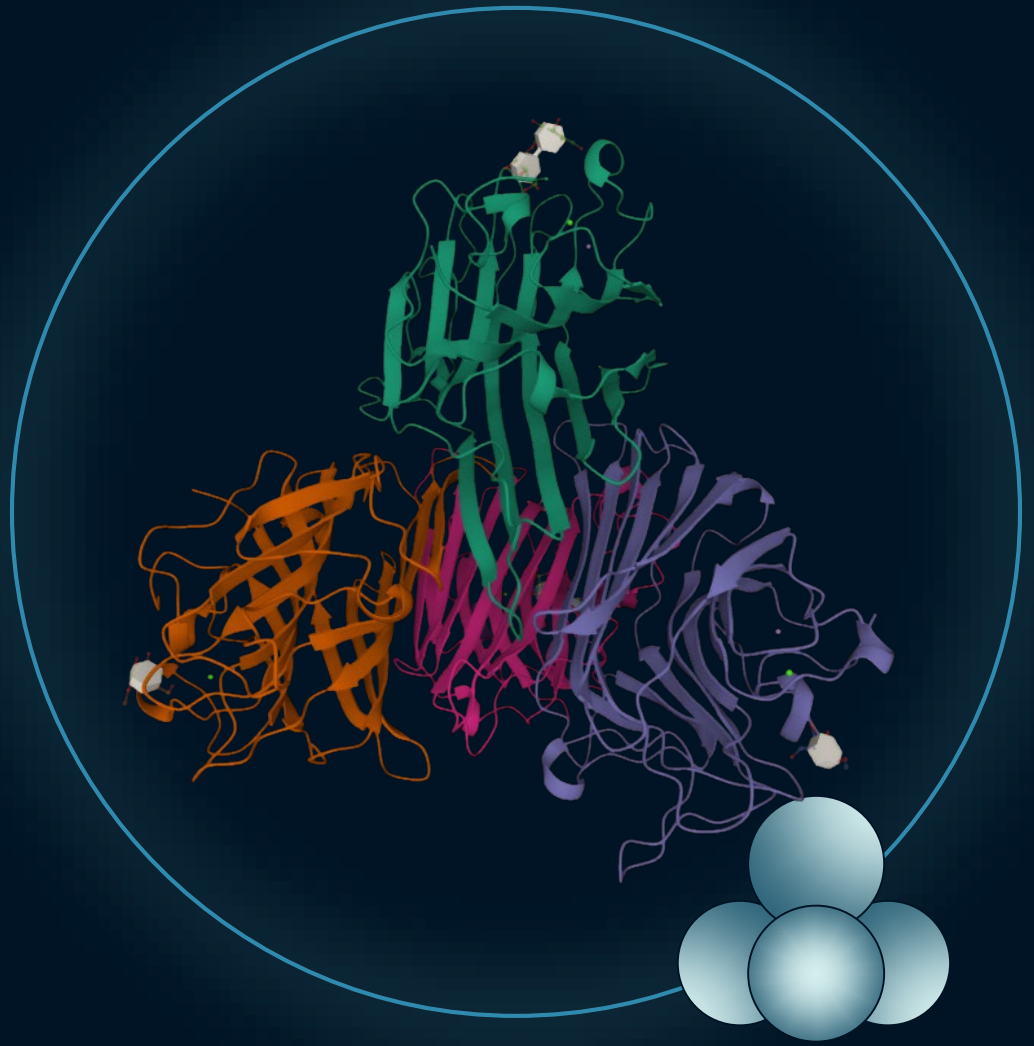
- The nuclear envelope is heavily decorated with glycoproteins
- Sugar-binding proteins (lectins) can be used to interact with the glycoproteins
- Lectins can be conjugated to magnetic beads
- Binding cell nuclei to magnetic beads grants a spin-free manipulation and/or extraction



Concanavalin A: the current gold standard

104 kDa

- A mitogenic lectin from the jack bean
- Forms a dimeric tetramer as its active form
- Binds non-covalently to D-mannose Man and D-glucose Glc
- Requires the presence of metal cations such as Ca++ or Mn+



Monneron et al, 1974

We set out to determine the best lectin based on the nuclear envelope glycome and pit it against the current standard (ConA)

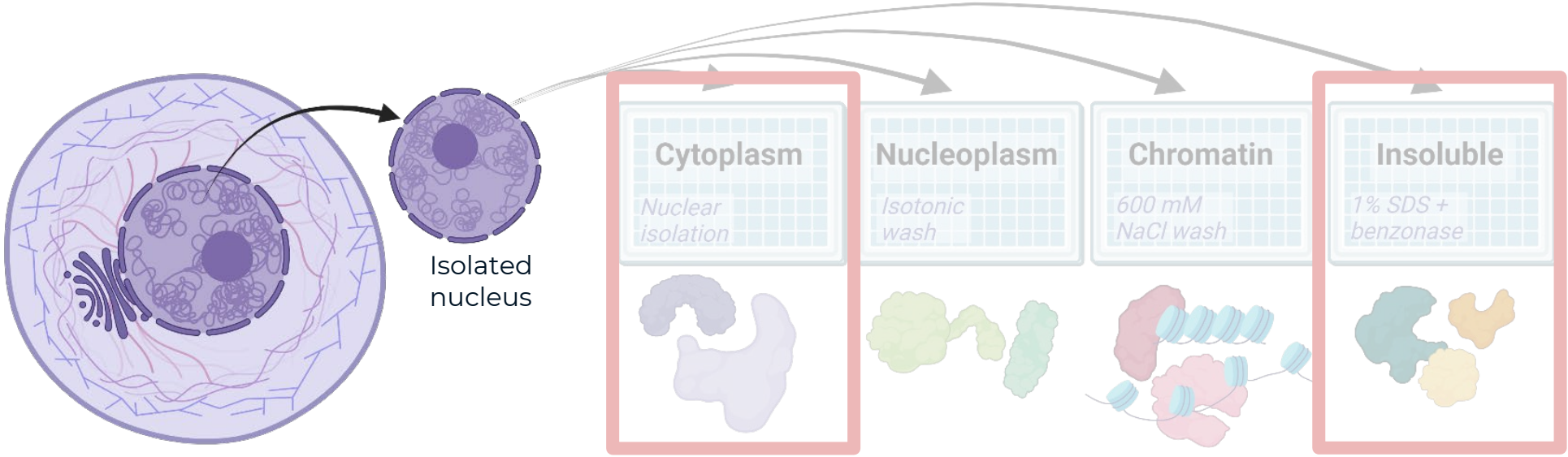
Nuclear membrane glycome profiling

Candidate lectin selection

Concanavalin A vs. Candidate lectin

MS Analysis of Lectin Thunderdome

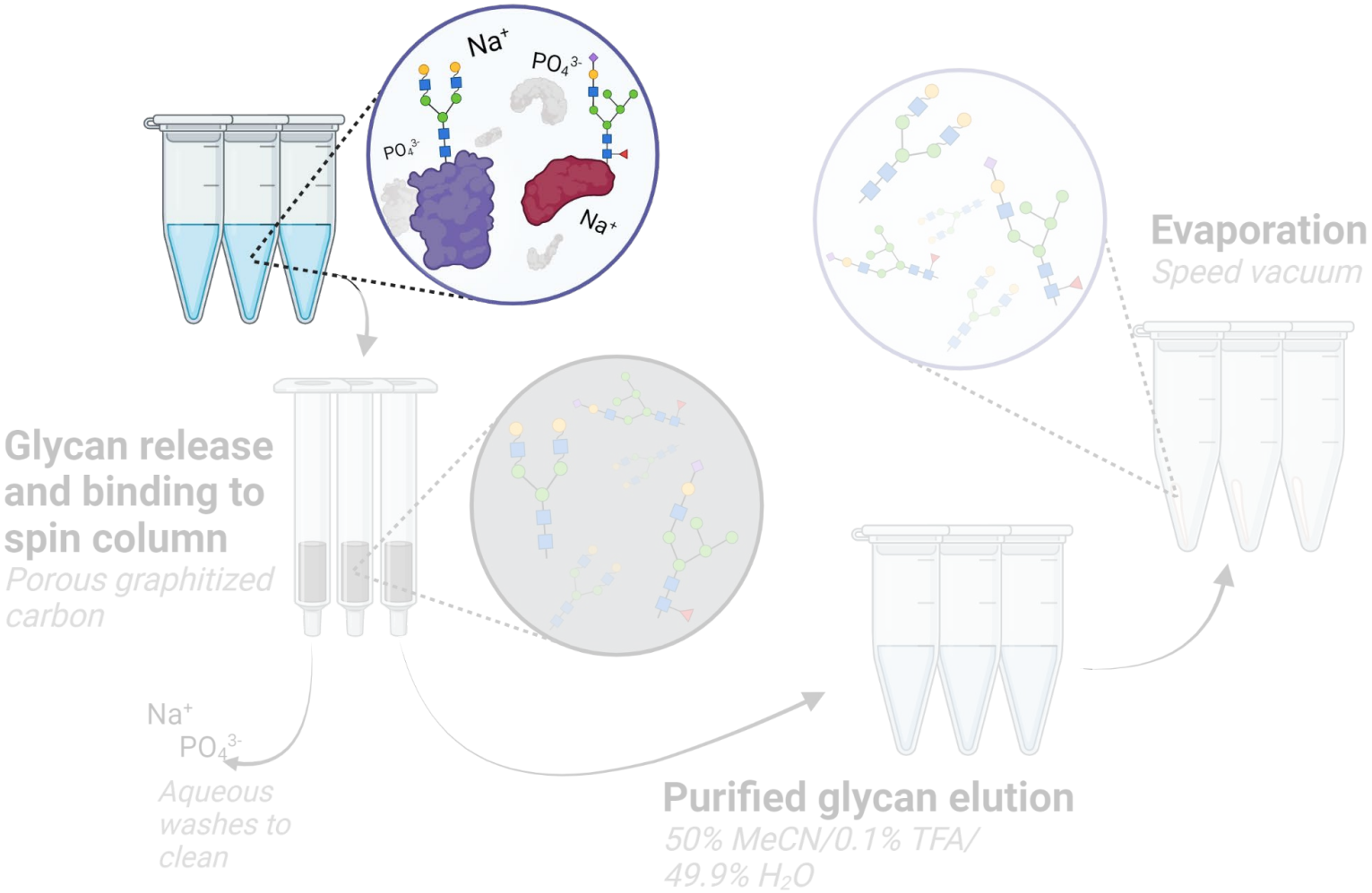
Cells were fractionated and the cytosolic and nuclear membrane (insoluble) fractions sent to Chris Ashwood for glycomic analysis



Cytoplasm = outer cell membrane and organelles other than nucleus

Insoluble = nuclear membrane and insoluble proteins

The glycoproteins were relieved of their glycans, and the glycans purified by PGC spin column



Purified glycans were directly infused and analyzed on an MS using the SUGA method

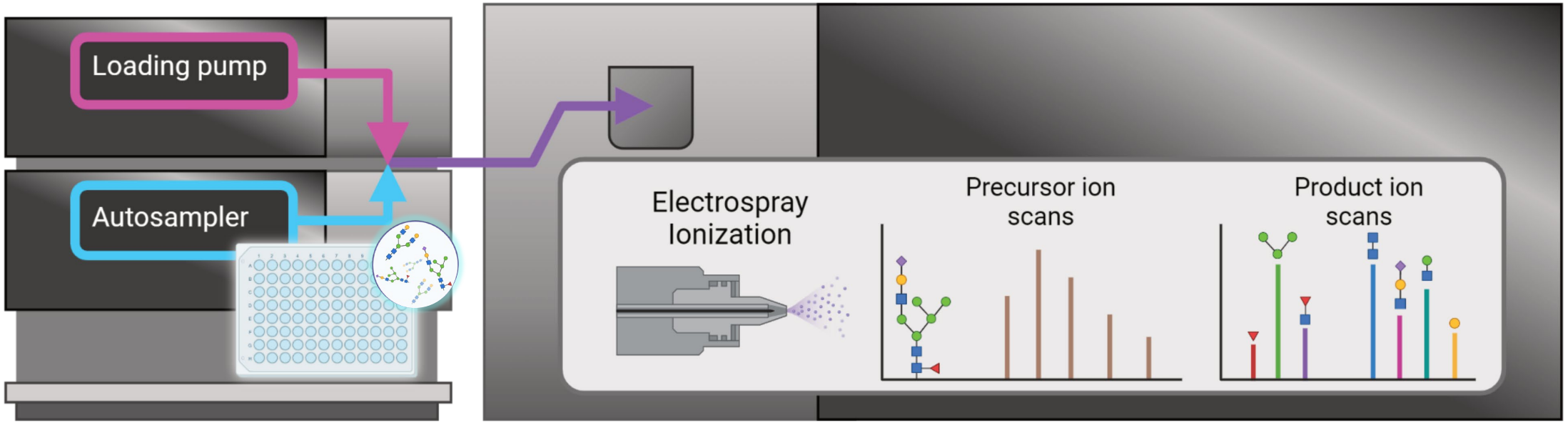
Chris Ashwood, Ph.D

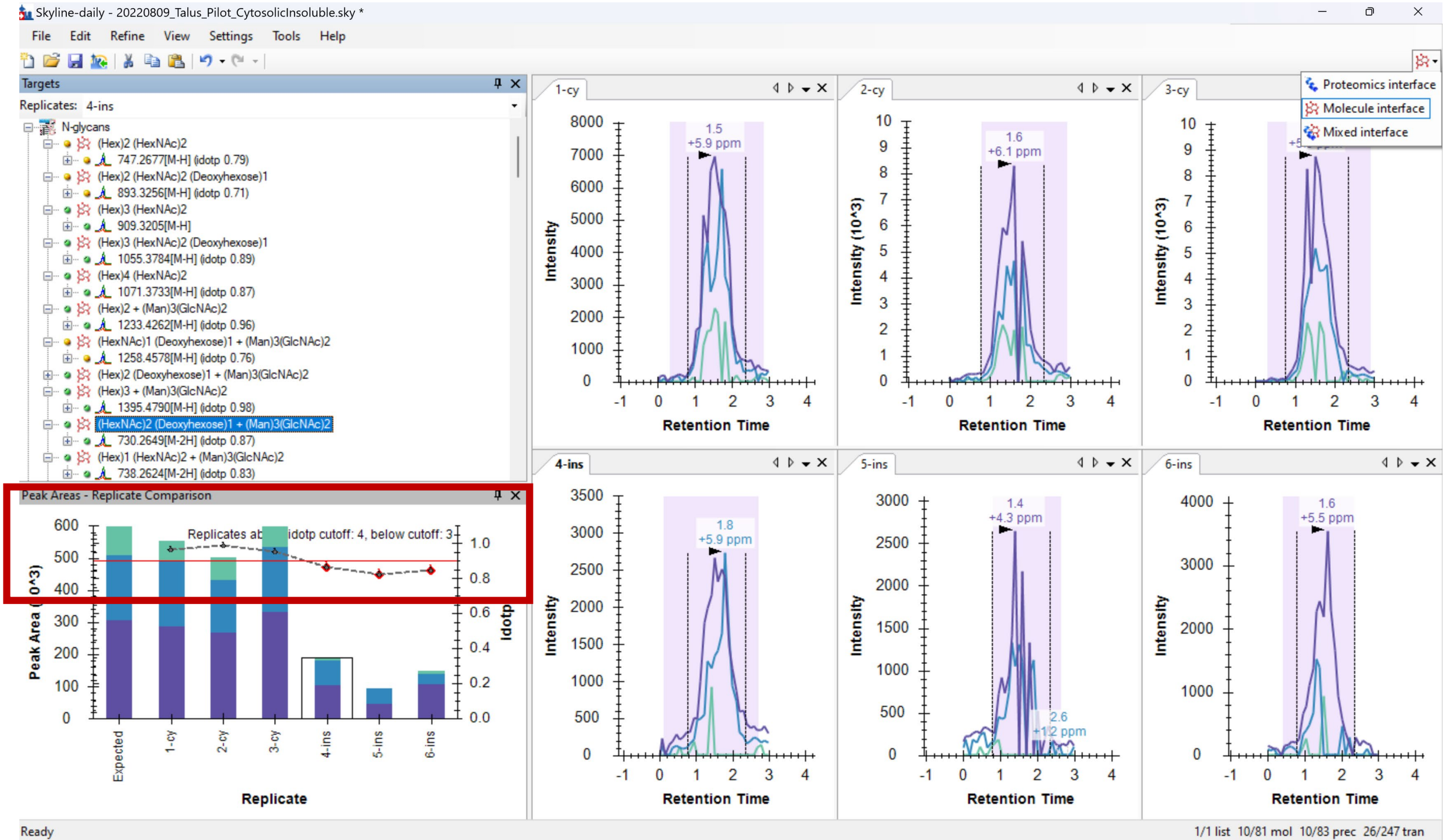
@chrashwood

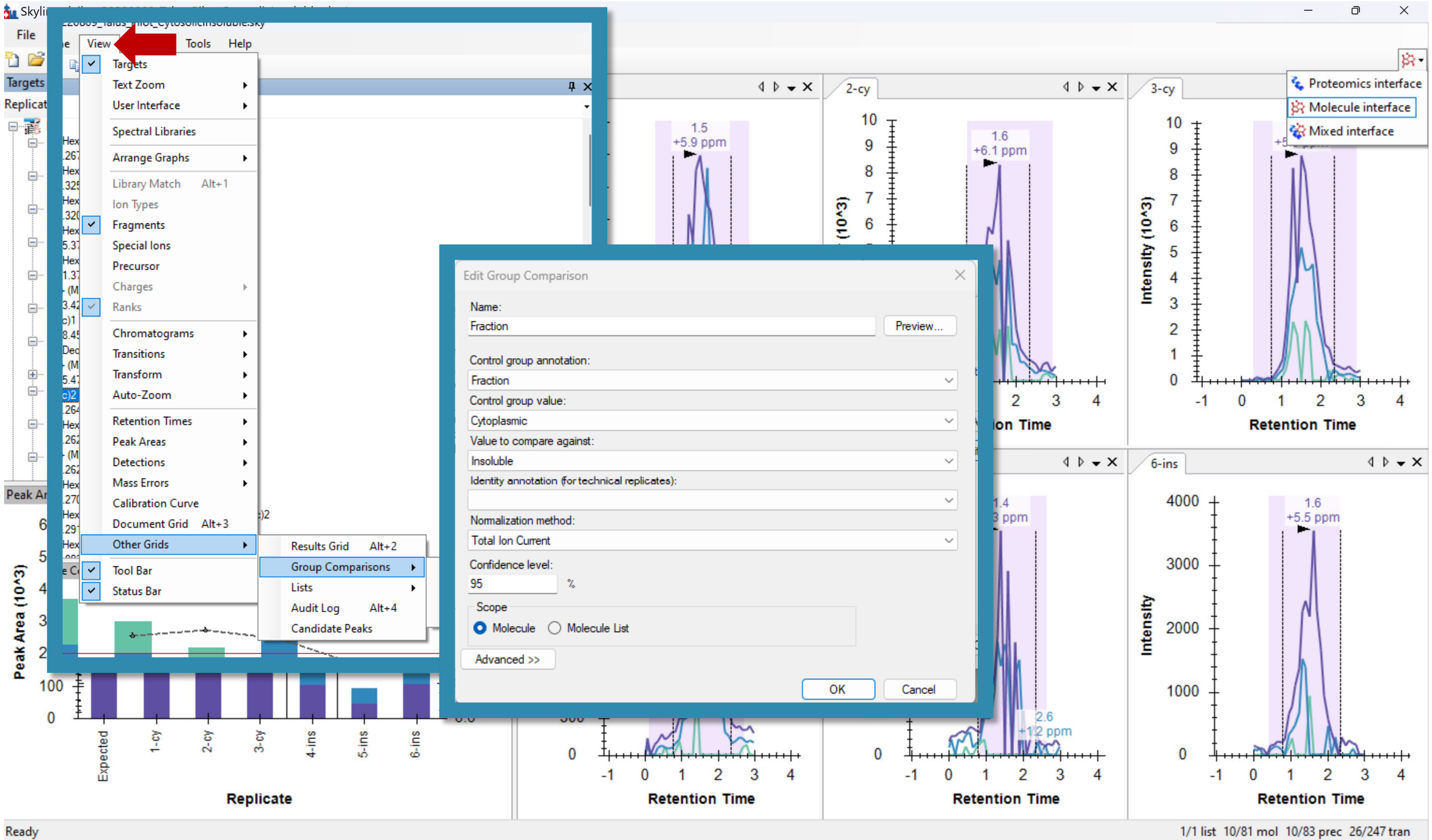
"Swift Universal Glycan Acquisition (SUGA) enables quantitative glycan composition profiling across diverse sample types"

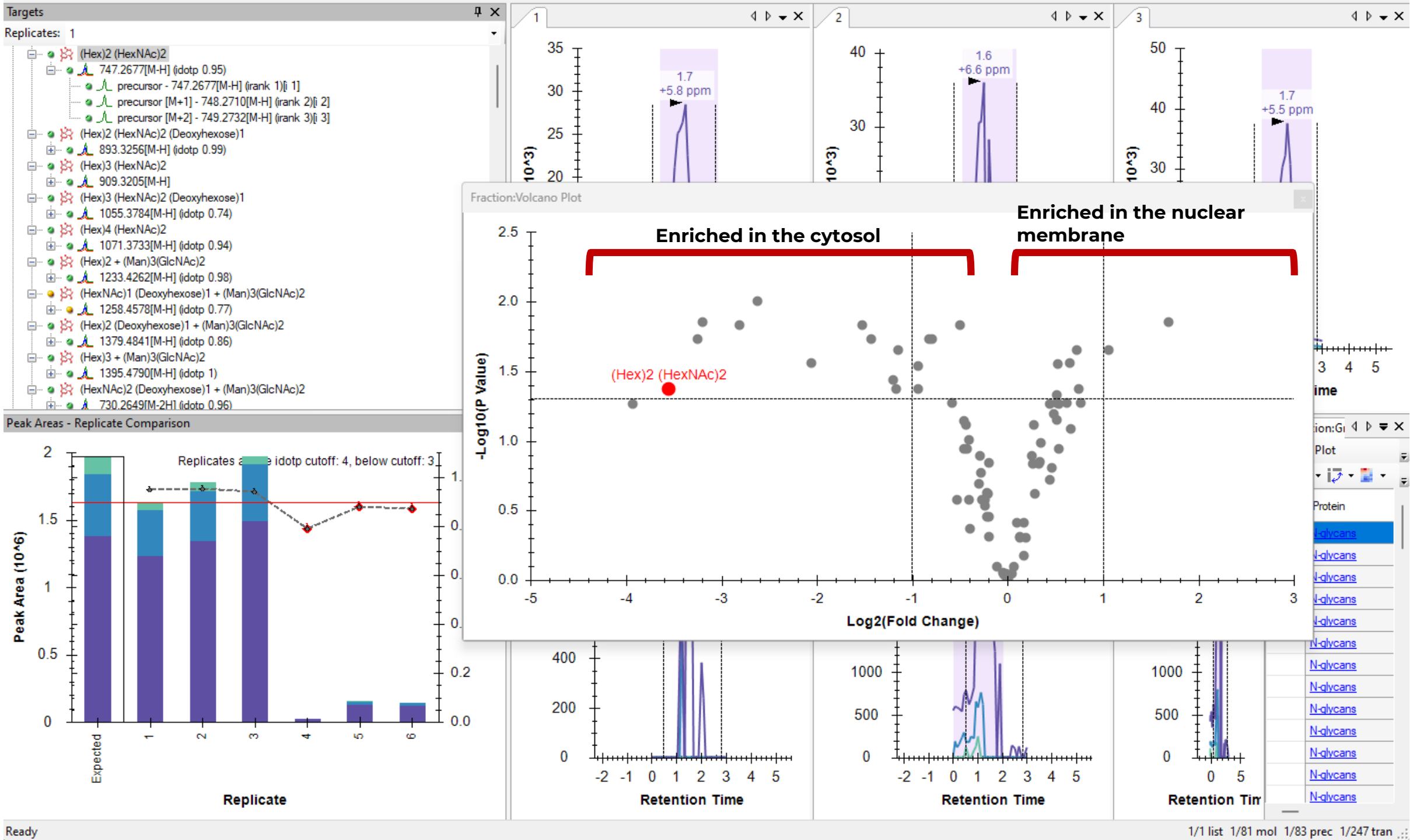
ASMS 2022

- **S**wift – 3 min per sample
- **U**niversal – native and permethylated
- **G**lycan – *N* and *O*-glycans, polysaccharides
- **A**cquisition – Precursor and product ion scans are collected in a single run



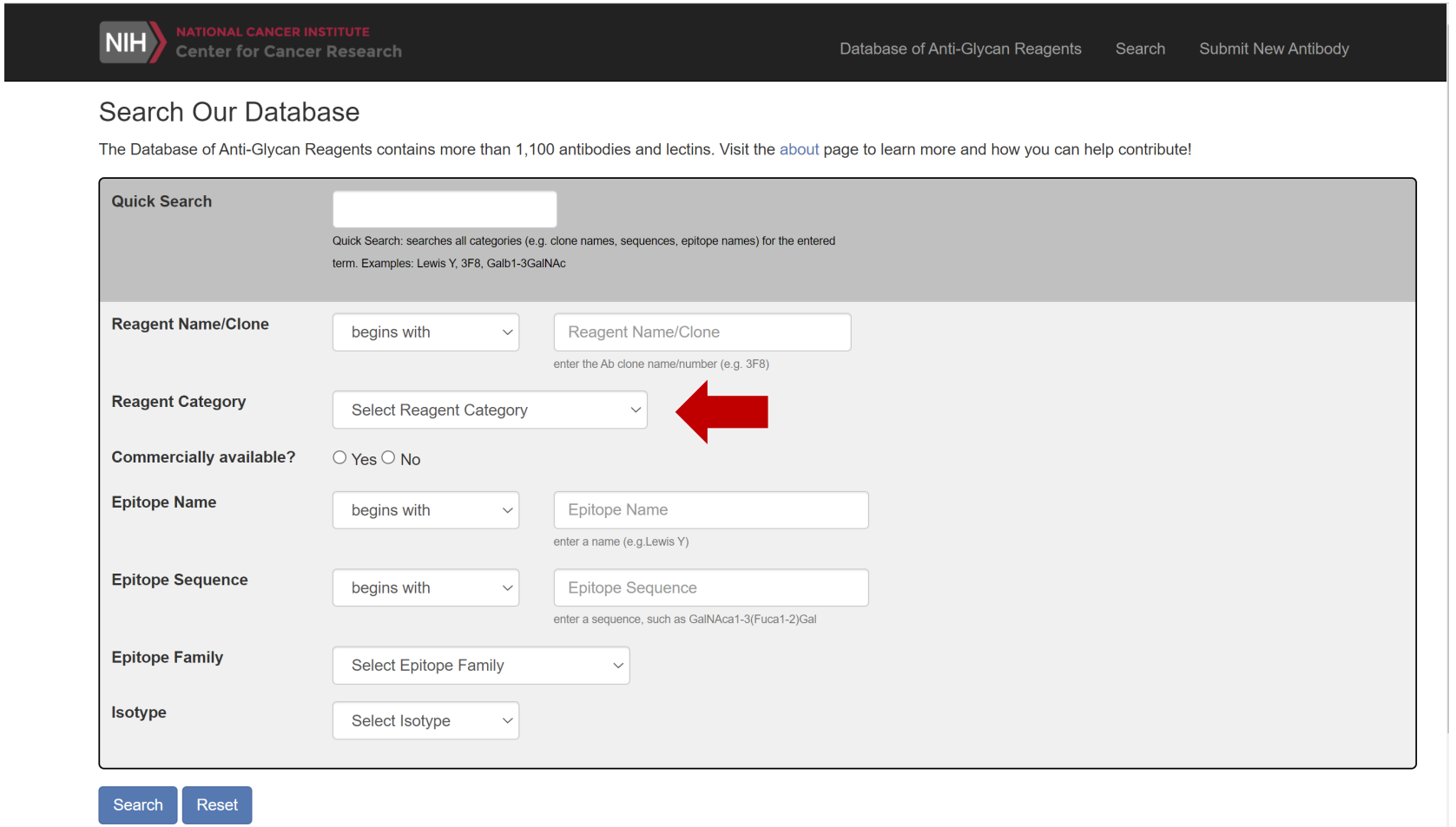

Skyline-daily - 20220809_Talus_Pilot_CytosolicInsoluble.sky *
File Edit Refine View Settings Tools Help
Targets
Replicates: 4-ins
N-glycans
(Hex)2 (HexNAc)2
747.2677[M-H] (idotp 0.79)
(Hex)2 (HexNAc)2 (Deoxyhexose)1
893.3256[M-H] (idotp 0.71)
(Hex)3 (HexNAc)2
909.3205[M-H]
(Hex)3 (HexNAc)2 (Deoxyhexose)1
1055.3784[M-H] (idotp 0.89)
(Hex)4 (HexNAc)2
1071.3733[M-H] (idotp 0.87)
(Hex)2 + (Man)3(GlcNAc)2
1233.4262[M-H] (idotp 0.96)
(HexNAc)1 (Deoxyhexose)1 + (Man)3(GlcNAc)2
1258.4578[M-H] (idotp 0.76)
(Hex)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2
(Hex)3 + (Man)3(GlcNAc)2
1395.4790[M-H] (idotp 0.98)
(HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2
730.2649[M-2H] (idotp 0.87)
(Hex)1 (HexNAc)2 + (Man)3(GlcNAc)2
738.2624[M-2H] (idotp 0.83)
Peak Areas - Replicate Comparison
Replicates ab idotp cutoff: 4, below cutoff: 3
Peak Area (10^3)
idotp
Expected
1-cy
2-cy
3-cy
4-ins
5-ins
6-ins
Replicate
1-cy
2-cy
3-cy
4-ins
5-ins
6-ins
Intensity
Intensity (10^3)
Retention Time
1.5
+5.9 ppm
1.6
+6.1 ppm
1.8
+5.9 ppm
1.4
+4.3 ppm
2.6
+1.2 ppm
1.6
+5.5 ppm
Proteomics interface
Molecule interface
Mixed interface
Ready
1/1 list 10/81 mol 10/83 prec 26/247 tran


File
View
Tools
Help
Targets
Text Zoom
User Interface
Spectral Libraries
Arrange Graphs
Library Match Alt+1
Ion Types
Fragments
Special Ions
Precursor
Charges
Ranks
Chromatograms
Transitions
Transform
Auto-Zoom
Retention Times
Peak Areas
Detections
Mass Errors
Calibration Curve
Document Grid Alt+3
Other Grids
Tool Bar
Status Bar
Results Grid Alt+2
Group Comparisons
Lists
Audit Log Alt+4
Candidate Peaks
Edit Group Comparison
Name:
Fraction
Preview...
Control group annotation:
Fraction
Control group value:
Cytoplasmic
Value to compare against:
Insoluble
Identity annotation (for technical replicates):
Normalization method:
Total Ion Current
Confidence level:
95
%
Scope
Molecule
Molecule List
Advanced >>
OK
Cancel
Proteomics interface
Molecule interface
Mixed interface
2-cy
3-cy
6-ins
1.5
+5.9 ppm
1.6
+6.1 ppm
1.6
+5.5 ppm
Peak Area (10^3)
Expected
1-cy
2-cy
3-cy
4-ins
5-ins
6-ins
Replicate
Intensity (10^3)
Retention Time
Intensity
Ready
1/1 list 10/81 mol 10/83 prec 26/247 tran

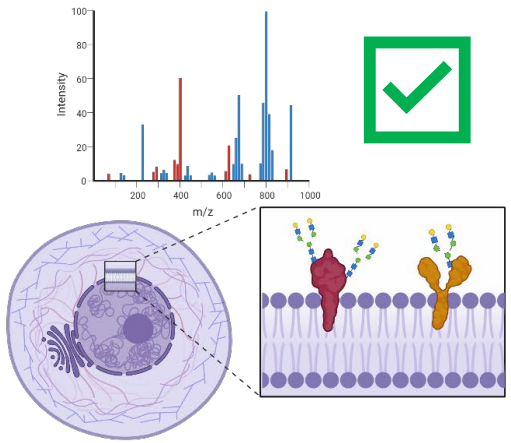

Targets
Replicates: 1
(Hex)2 (HexNAc)2
747.2677[M-H] (idotp 0.95)
precursor - 747.2677[M-H] (irank 1)[i 1]
precursor [M+1] - 748.2710[M-H] (irank 2)[i 2]
precursor [M+2] - 749.2732[M-H] (irank 3)[i 3]
(Hex)2 (HexNAc)2 (Deoxyhexose)1
893.3256[M-H] (idotp 0.99)
(Hex)3 (HexNAc)2
909.3205[M-H]
(Hex)3 (HexNAc)2 (Deoxyhexose)1
1055.3784[M-H] (idotp 0.74)
(Hex)4 (HexNAc)2
1071.3733[M-H] (idotp 0.94)
(Hex)2 + (Man)3(GlcNAc)2
1233.4262[M-H] (idotp 0.98)
(HexNAc)1 (Deoxyhexose)1 + (Man)3(GlcNAc)2
1258.4578[M-H] (idotp 0.77)
(Hex)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2
1379.4841[M-H] (idotp 0.86)
(Hex)3 + (Man)3(GlcNAc)2
1395.4790[M-H] (idotp 1)
(HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2
730.2649[M-2H] (idotp 0.96)
1
1.7
+5.8 ppm
2
1.6
+6.6 ppm
3
1.7
+5.5 ppm
10^3)
Fraction:Volcano Plot
Enriched in the cytosol
Enriched in the nuclear membrane
(Hex)2 (HexNAc)2
-Log10(P Value)
Log2(Fold Change)
Peak Areas - Replicate Comparison
Replicates a idotp cutoff: 4, below cutoff: 3
Peak Area (10^6)
Expected
Replicate
Retention Time
Protein
N-glycans
Ready
1/1 list 1/81 mol 1/83 prec 1/247 tran

The Database of Anti-Glycan Reagents (DAGR) helped us choose candidate lectins based on ligand specificity

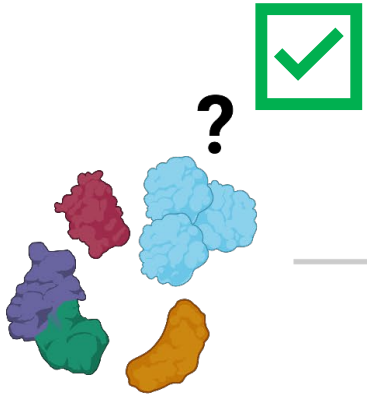
- “Public, searchable database of carbohydrate binding antibodies and lectins.”
- We set the category to “lectin” and the epitope name to “contains” “LacNAc” and got a starting list, Chris helped pare it down



After selecting our candidate lectin, all that was left was to test it



Nuclear membrane glycome profiling

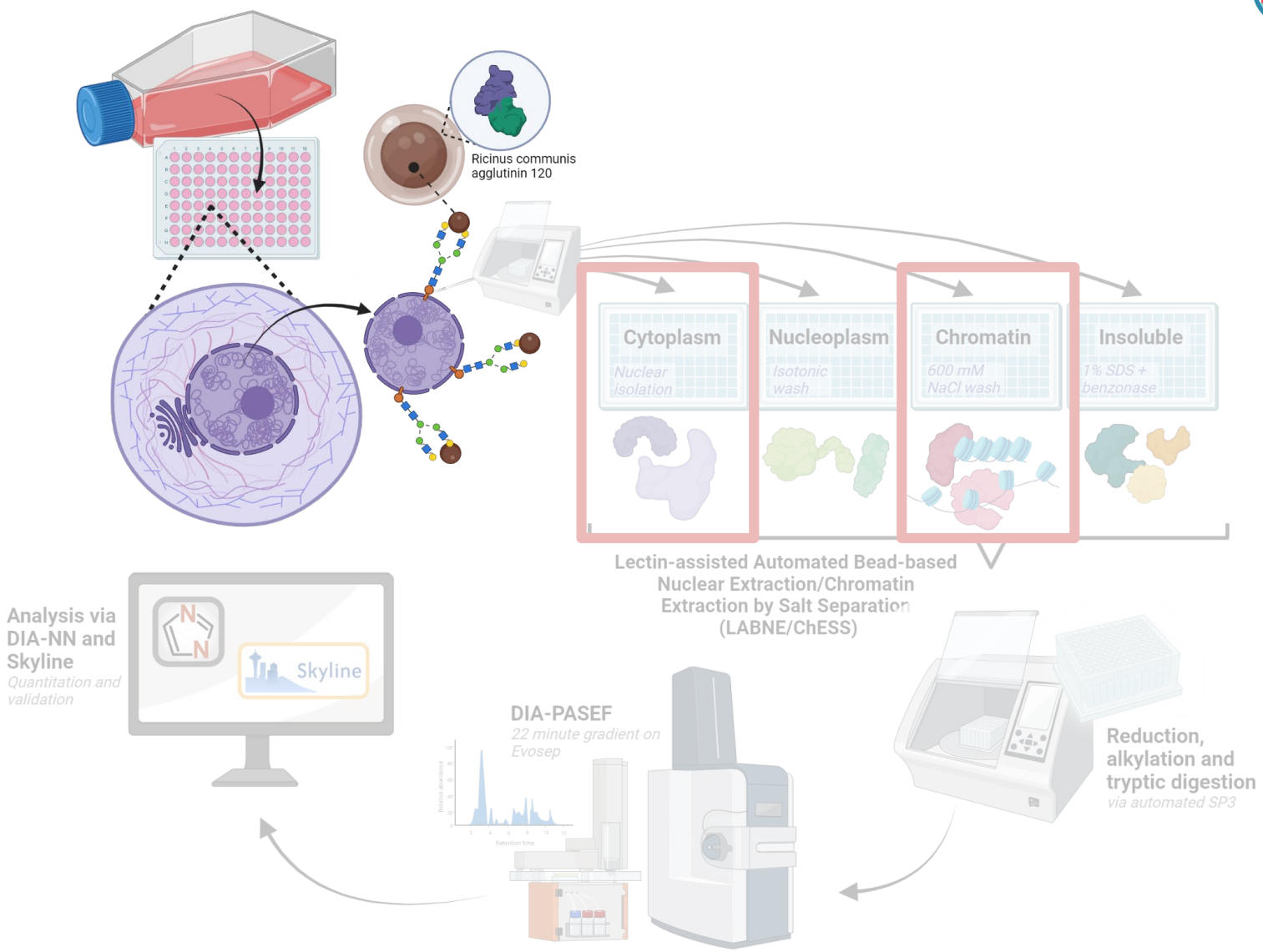


Candidate lectin selection

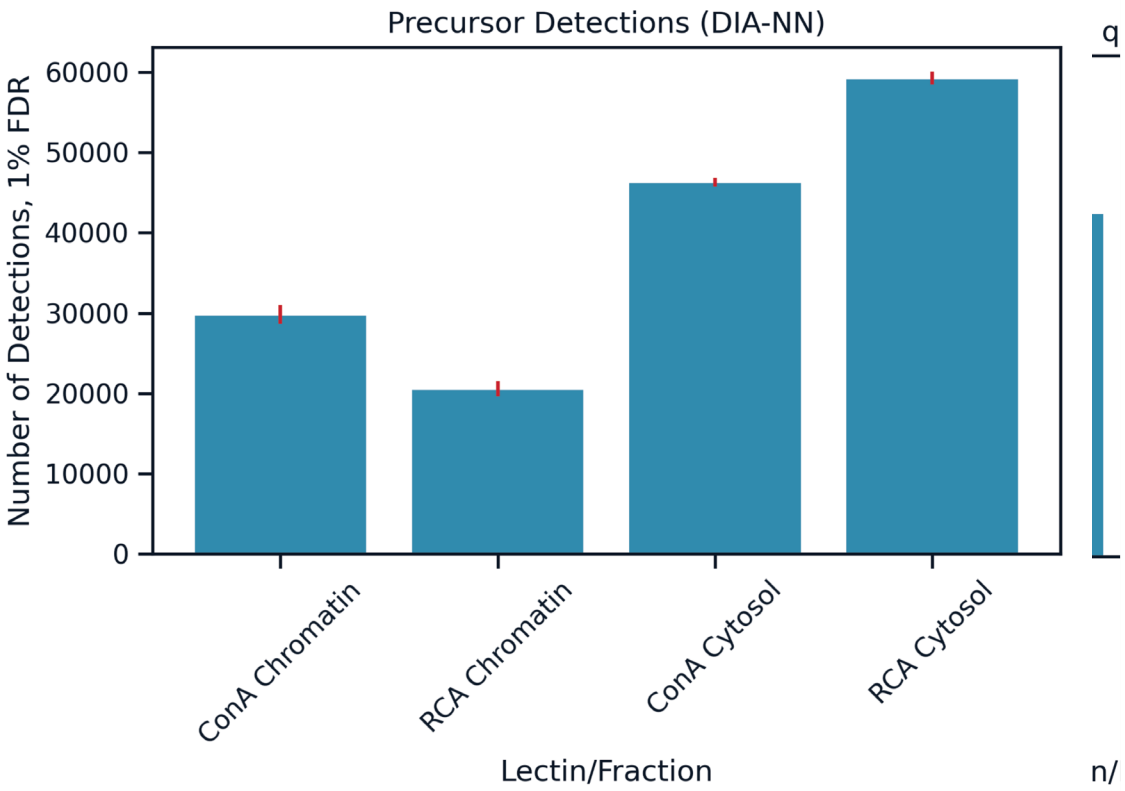
Concanavalin A vs RCA Thunderdome

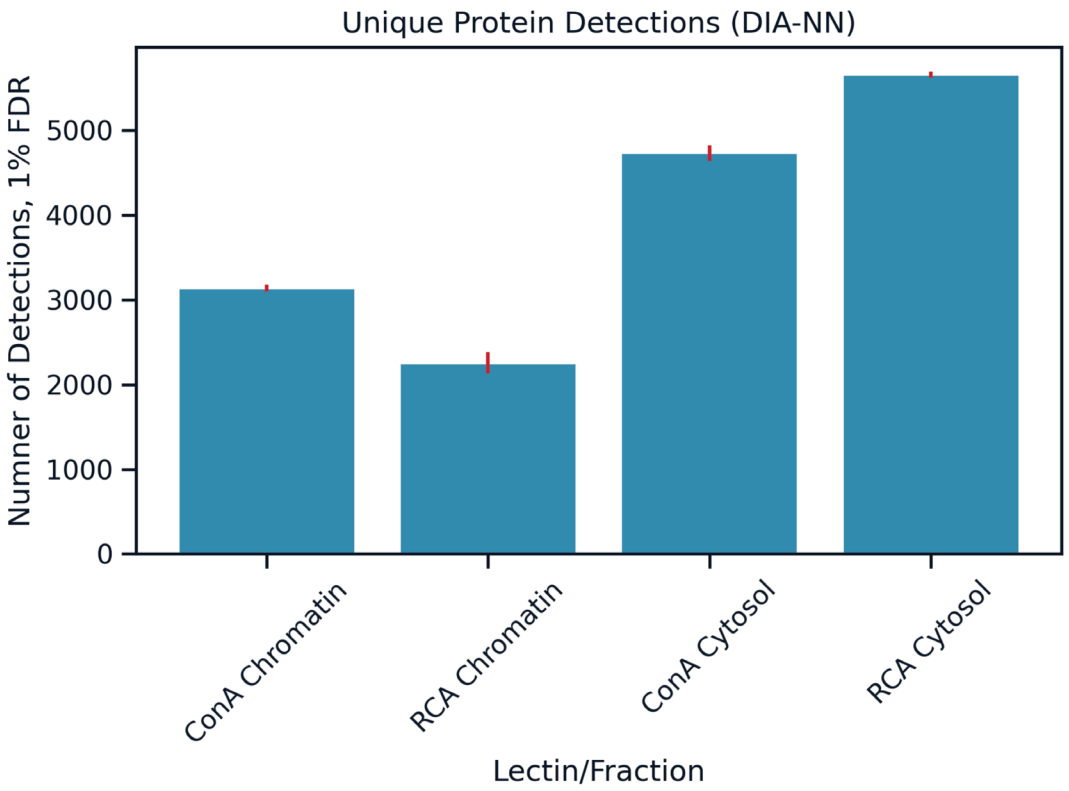
MS Analysis of Lectin Thunderdome

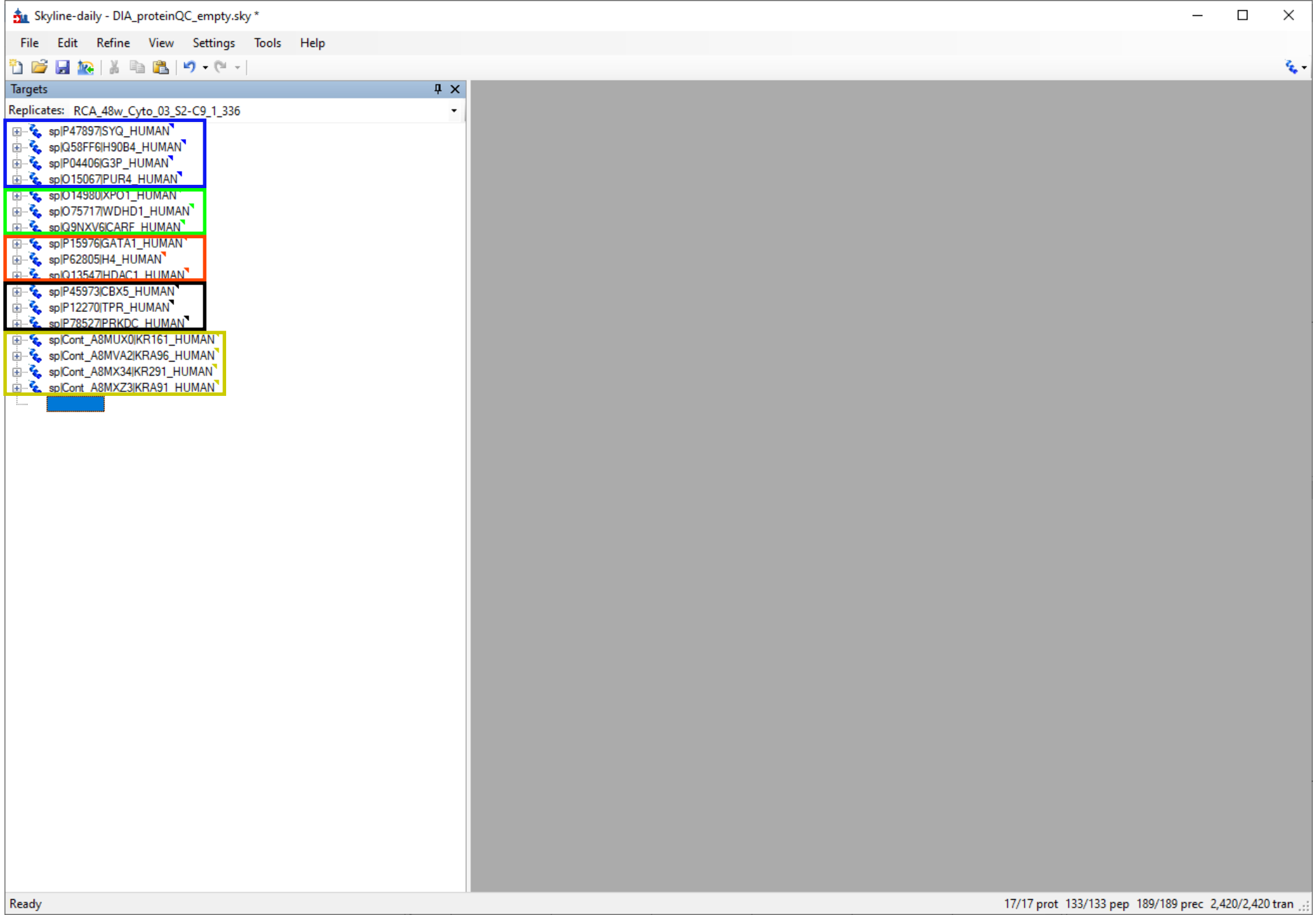
- We performed head-to-head comparison of ConA and RCA
- Lysed and extracted the nuclei in the cell culture plate
- Automated fractionation using the beads
- Automated SP3 tryptic digest
- DIA-PASEF acquisition, followed by DIA-NN for quantitative processing



The initial MS results were (initially) worrying



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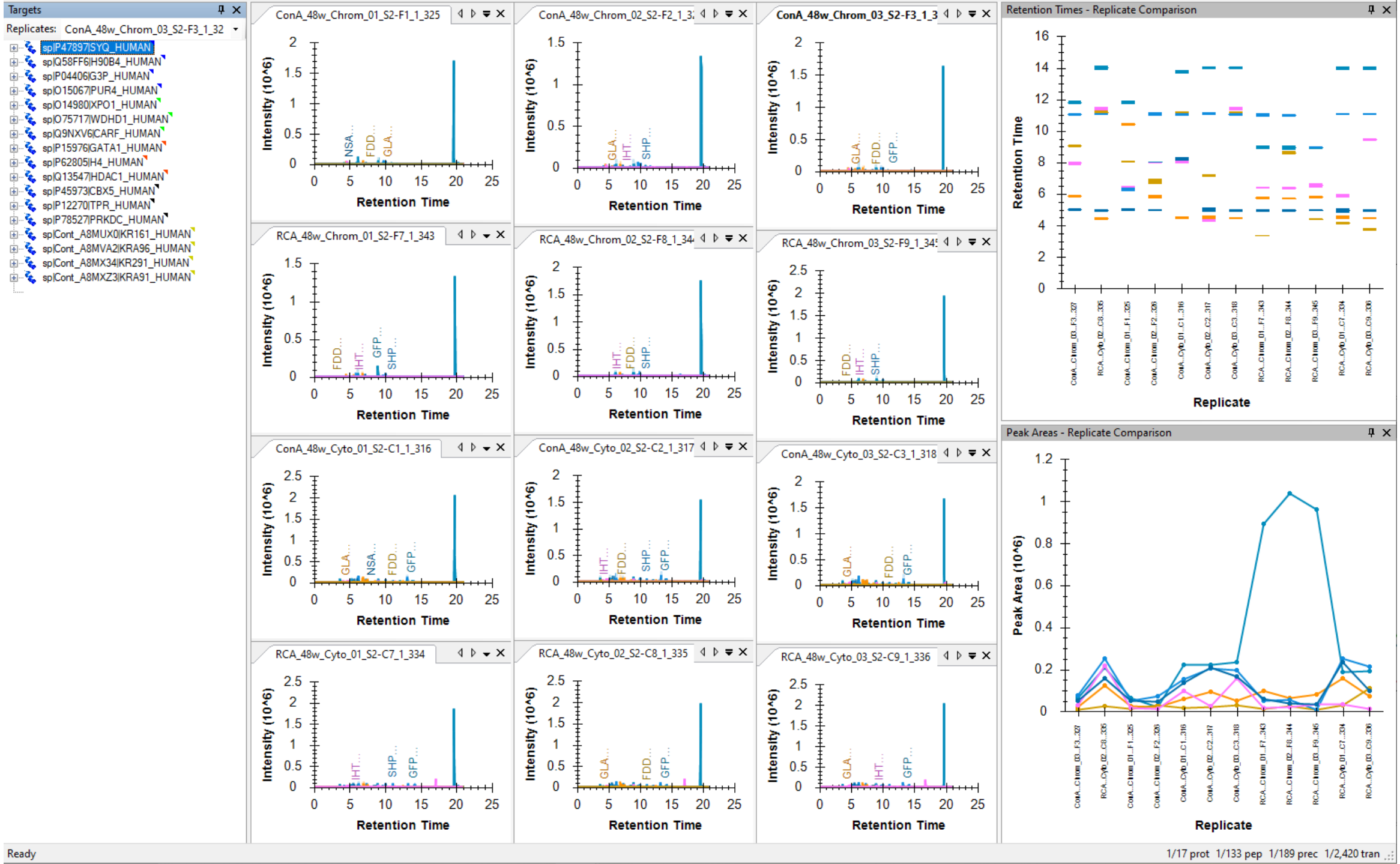
File
Edit
Refine
View
Settings
Tools
Help
Targets
Replicates: RCA_48w_Cyto_03_S2-C9_1_336
sp|P47897|SYQ_HUMAN
sp|Q58FF6|H90B4_HUMAN
sp|P04406|G3P_HUMAN
sp|O15067|PUR4_HUMAN
sp|O14980|XPO1_HUMAN
sp|O75717|WDHD1_HUMAN
sp|Q9NXV6|CARF_HUMAN
sp|P15976|GATA1_HUMAN
sp|P62805|H4_HUMAN
sp|O13547|HDAC1_HUMAN
sp|P45973|CBX5_HUMAN
sp|P12270|TPR_HUMAN
sp|P78527|PRKDC_HUMAN
sp|Cont_A8MUX0|KR161_HUMAN
sp|Cont_A8MVA2|KRA96_HUMAN
sp|Cont_A8MX34|KR291_HUMAN
sp|Cont_A8MXZ3|KRA91_HUMAN
Ready
17/17 prot 133/133 pep 189/189 prec 2,420/2,420 tran


Targets
Replicates: ConA_48w_Chrom_03_S2-F3_1_32
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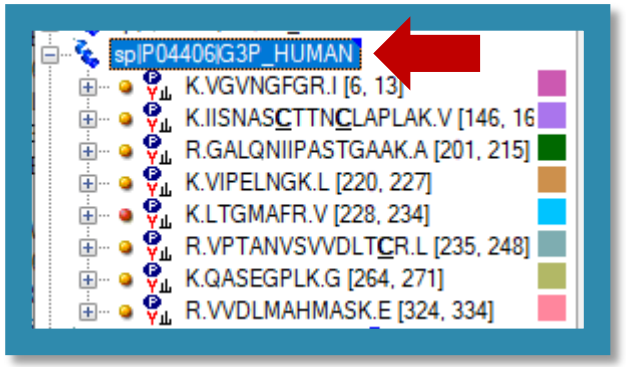
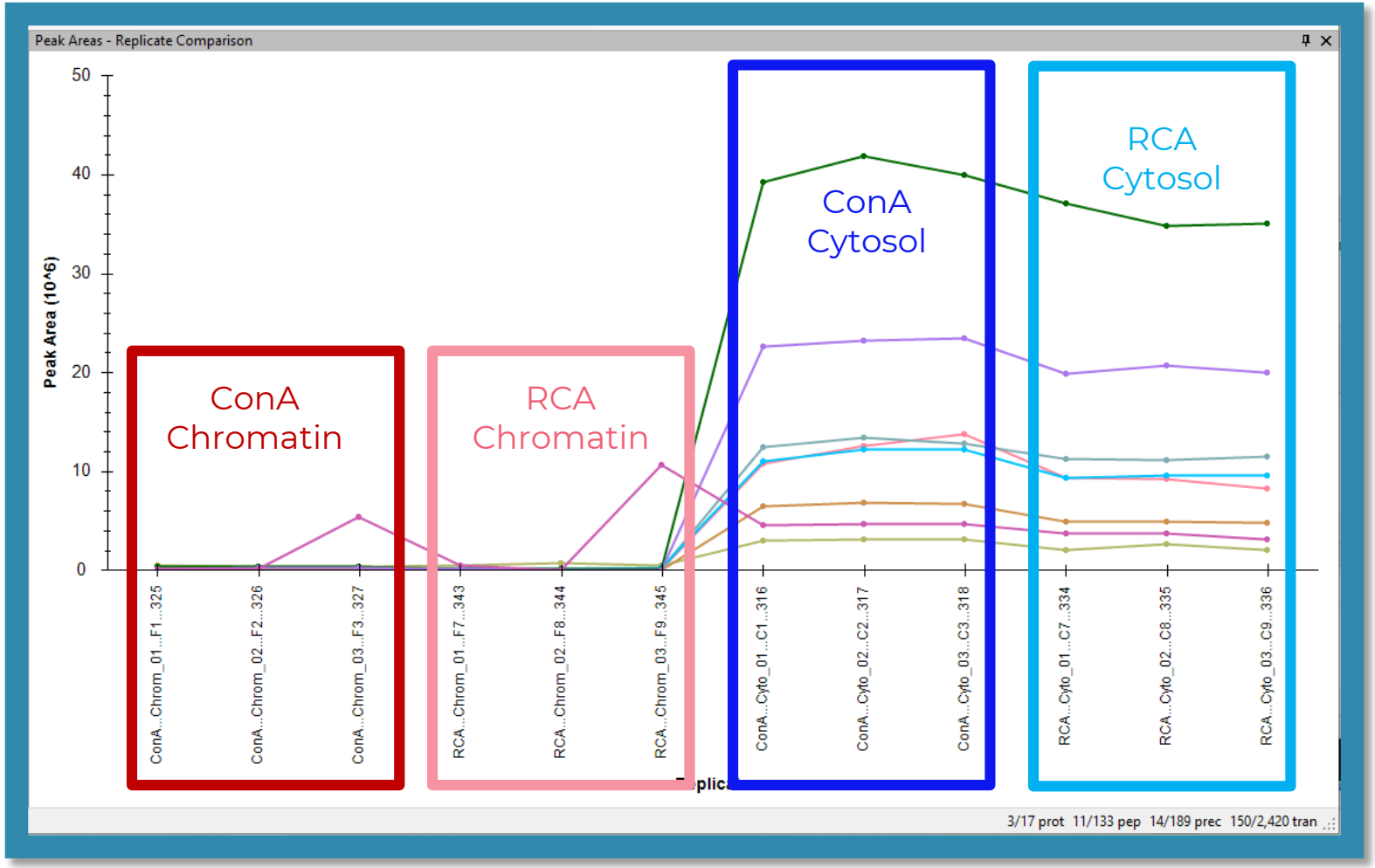
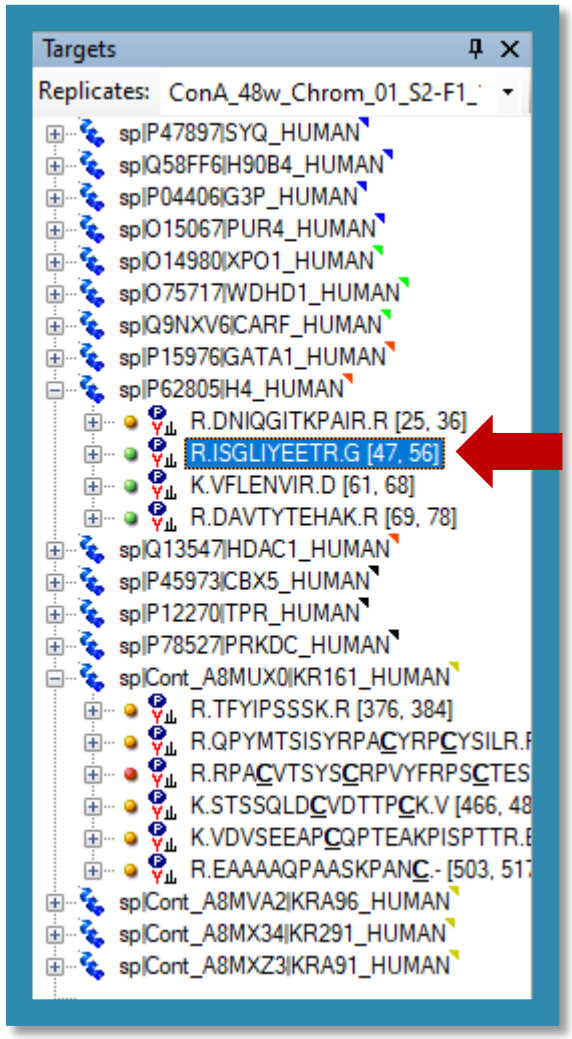
sp|P04406|G3P_HUMAN
sp|O15067|PUR4_HUMAN
sp|O14980|XPO1_HUMAN
sp|O75717|WDHD1_HUMAN
sp|Q9NXV6|CARF_HUMAN
sp|P15976|GATA1_HUMAN
sp|P62805|H4_HUMAN
sp|Q13547|HDAC1_HUMAN
sp|P45973|CBX5_HUMAN
sp|P12270|TPR_HUMAN
sp|P78527|PRKDC_HUMAN
sp|Cont_A8MUX0|KR161_HUMAN
sp|Cont_A8MVA2|KRA96_HUMAN
sp|Cont_A8MX34|KR291_HUMAN
sp|Cont_A8MXZ3|KRA91_HUMAN
ConA_48w_Chrom_01_S2-F1_1_325
ConA_48w_Chrom_02_S2-F2_1_3
ConA_48w_Chrom_03_S2-F3_1_3
RCA_48w_Chrom_01_S2-F7_1_343
RCA_48w_Chrom_02_S2-F8_1_34
RCA_48w_Chrom_03_S2-F9_1_345
ConA_48w_Cyto_01_S2-C1_1_316
ConA_48w_Cyto_02_S2-C2_1_317
ConA_48w_Cyto_03_S2-C3_1_318
RCA_48w_Cyto_01_S2-C7_1_334
RCA_48w_Cyto_02_S2-C8_1_335
RCA_48w_Cyto_03_S2-C9_1_336
Intensity (10^6)
Retention Time
Retention Times - Replicate Comparison
Retention Time
Replicate
Peak Areas - Replicate Comparison
Peak Area (10^6)
Replicate
Ready
1/17 prot 1/133 pep 1/189 prec 1/2,420 tran

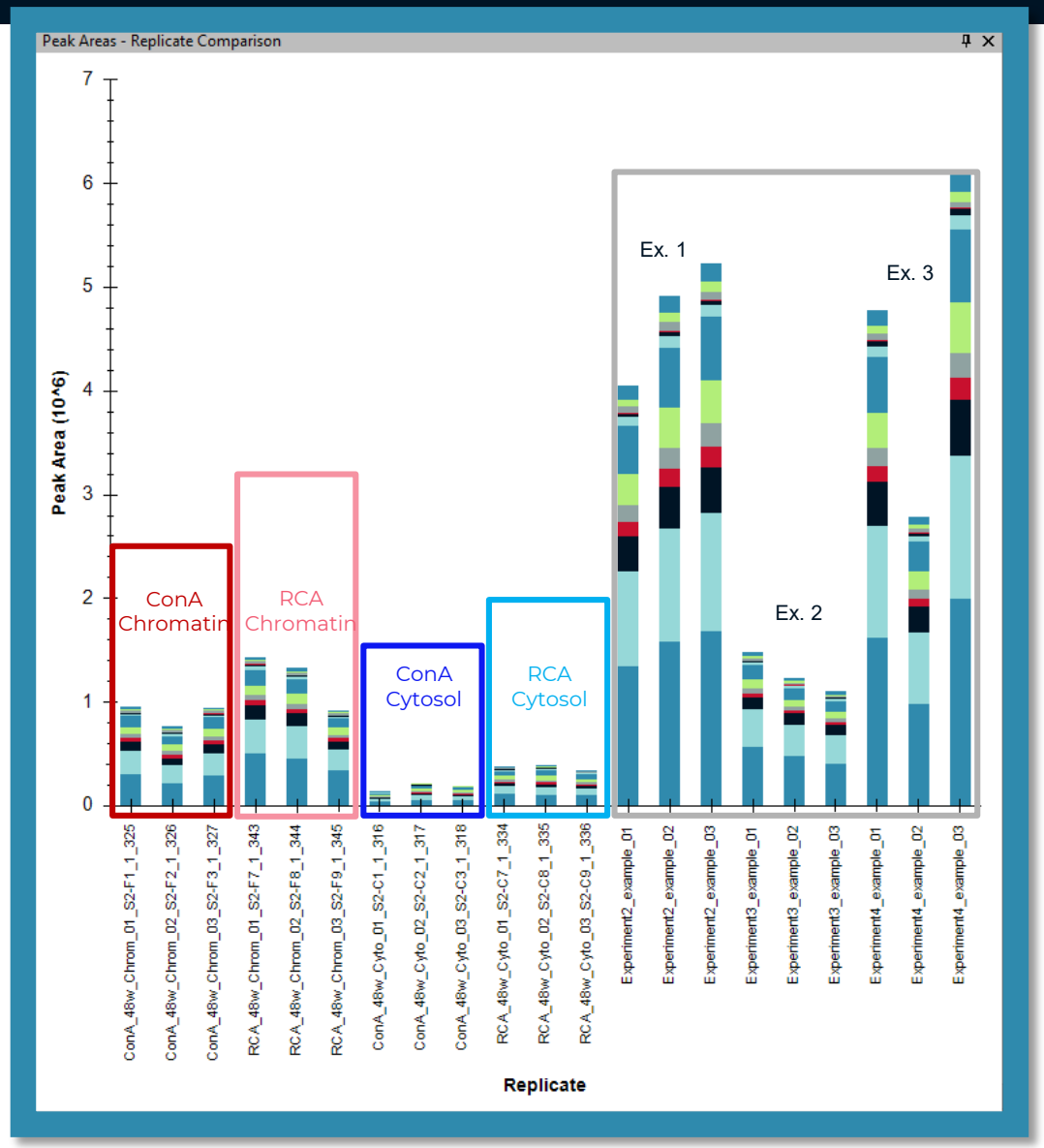
QC Skyline document allows a quick assessment of the success of the enrichment



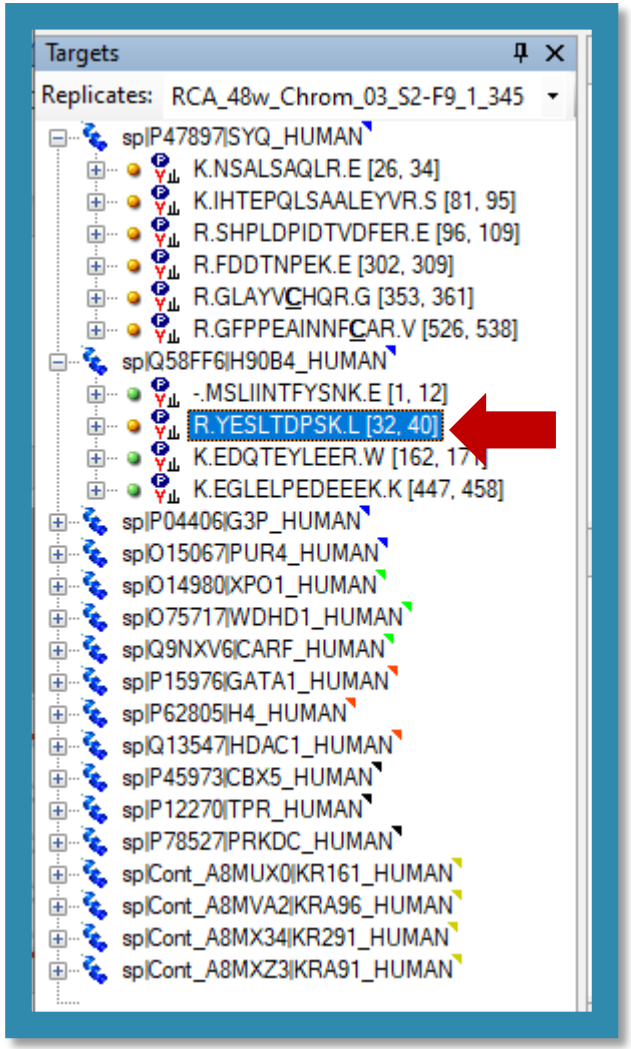


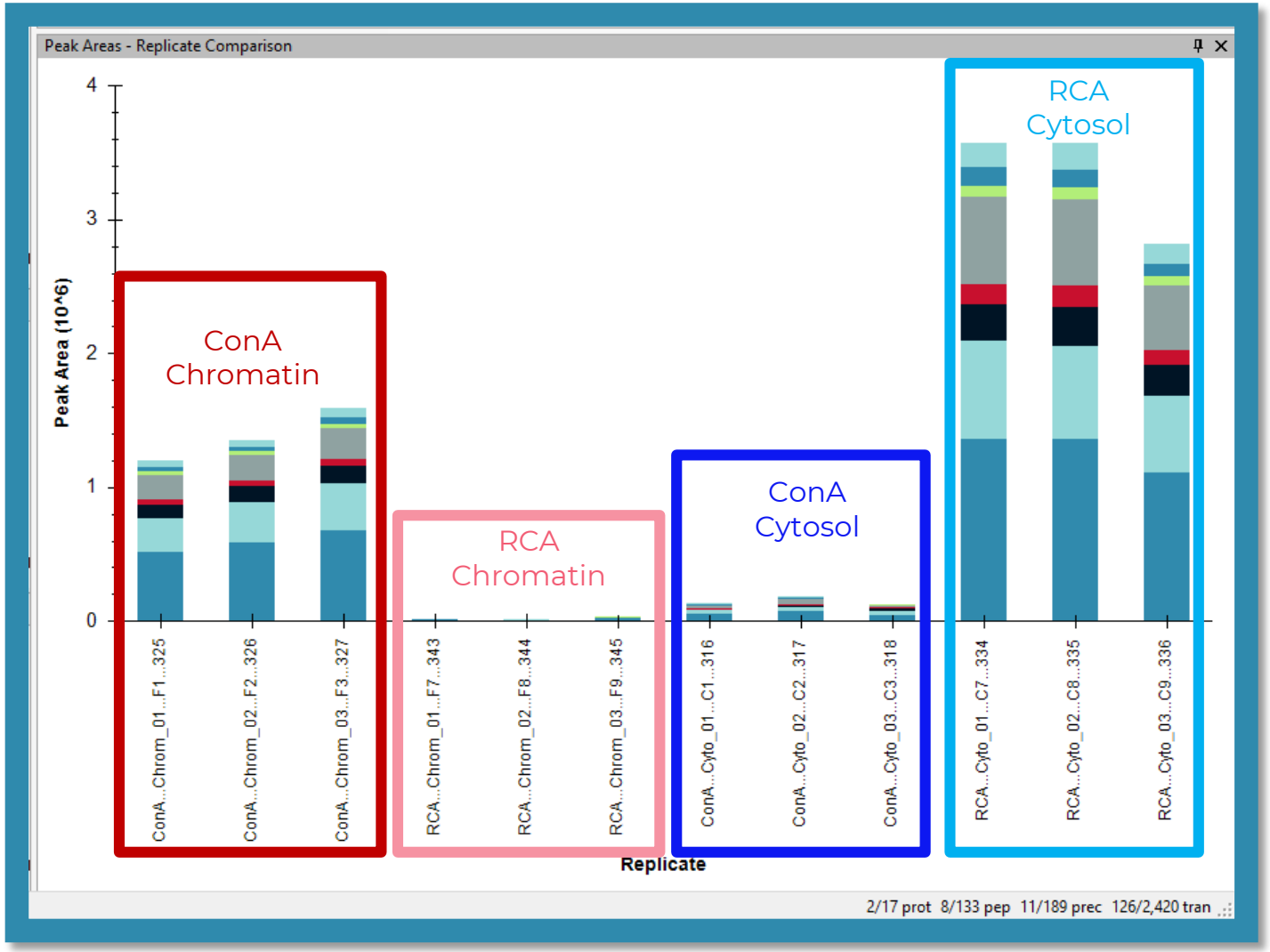
Importing results from different experiments visualizes intra and inter experiment enrichment success



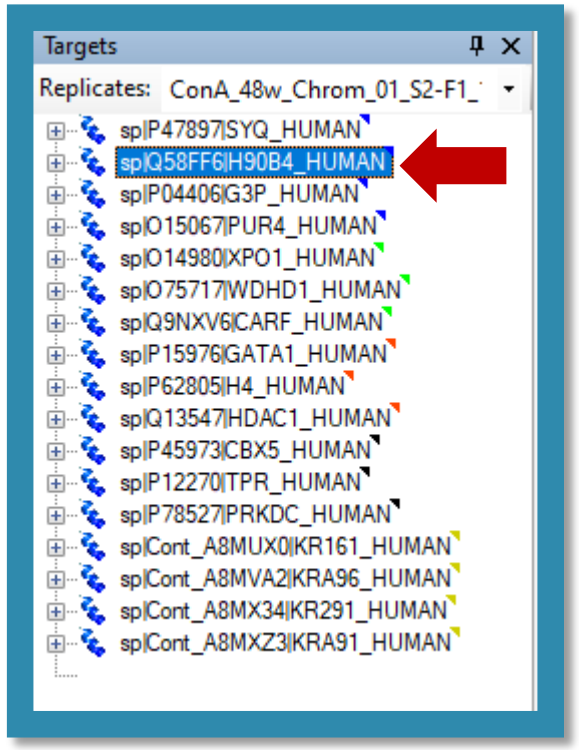


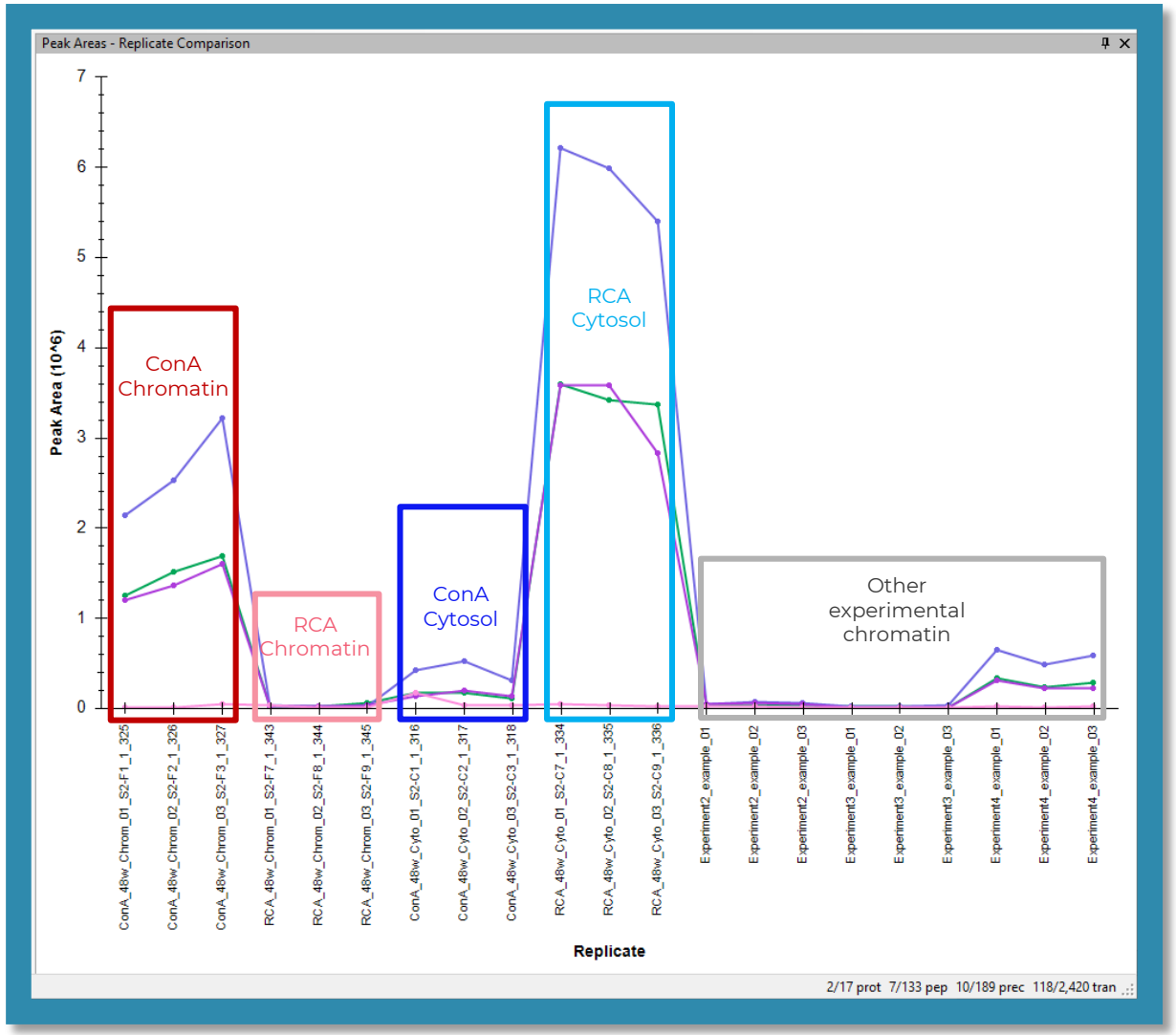
Looking closer at cytoplasm QC proteins reveals that all was not lost…



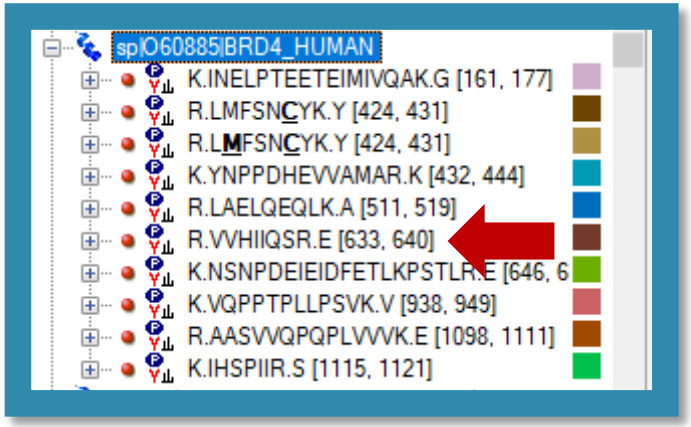


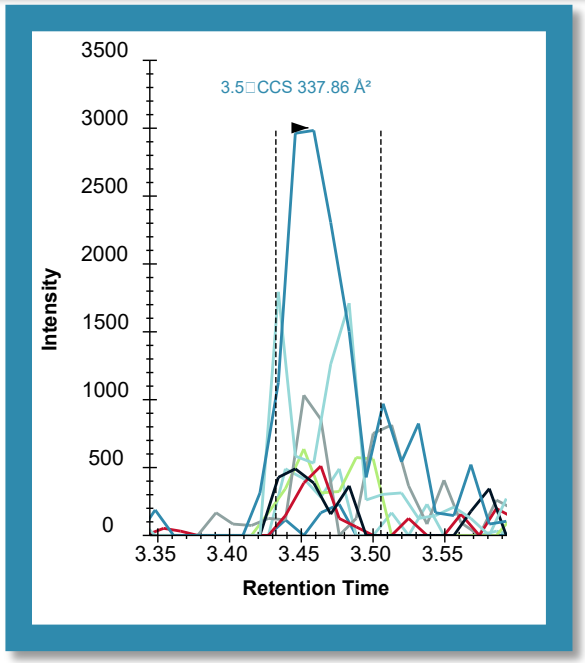
Higher cytoplasmic contamination in the ConA samples suggests more nonspecific binding

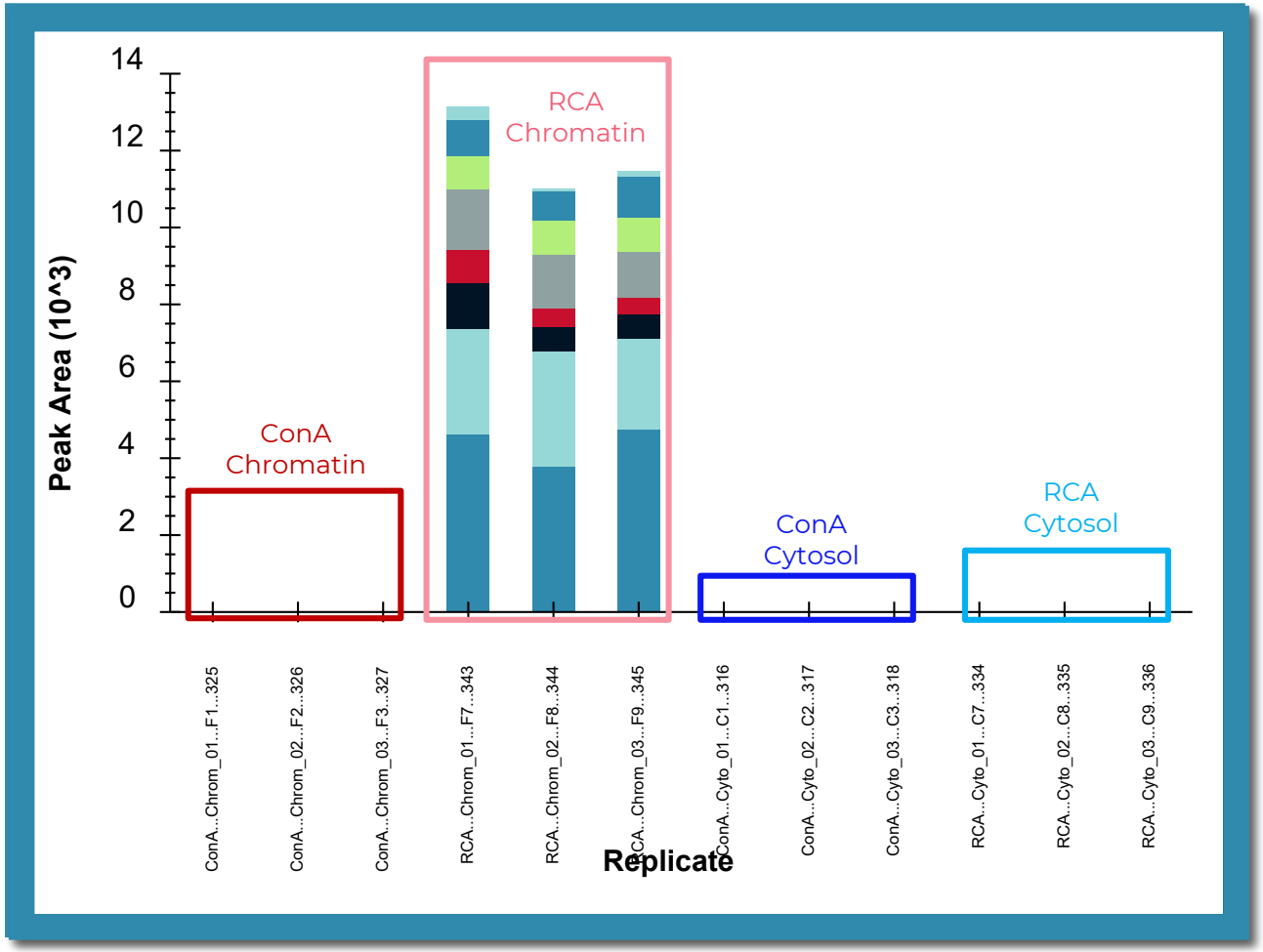




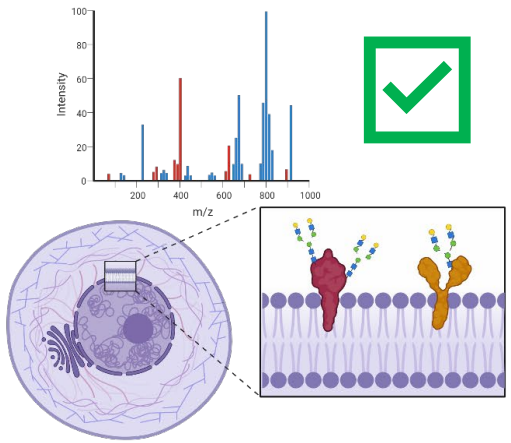
Customizing target list with proteins of interest along with QC proteins allows for a more specific assessment



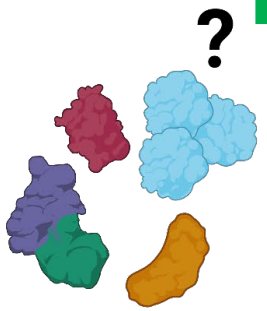




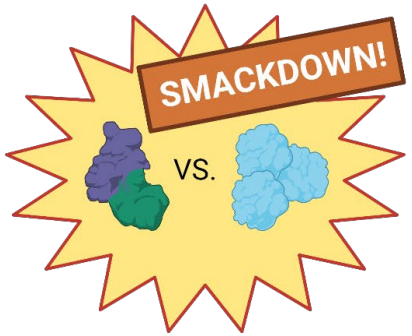
Skyline enabled key steps in this protocol optimization workflow



Nuclear membrane glycome profiling



Candidate lectin selection

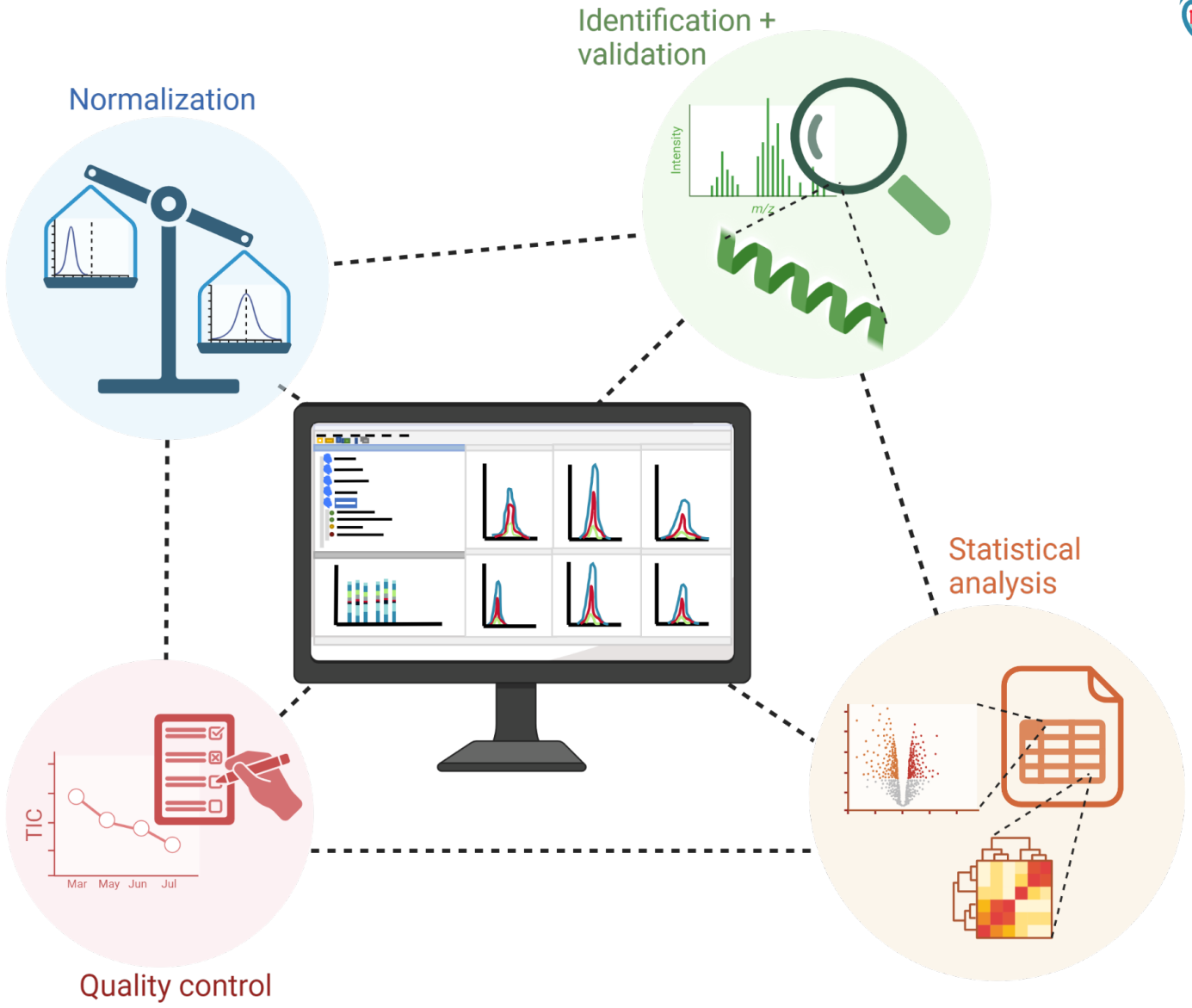


Concanavalin A vs RCA Thunderdome

MS Analysis of Lectin Thunderdome

Conclusion:

Skyline offers a unique, multi-omic approach for technology development and optimization



Thank you!

Skyline Grouped Comparisons Tutorial

DAGR Website

Chris Ashwood

Talus Bio ASMS Menu

Thanks to:

- Carolyn Allen
- Andrea Gutierrez
- Sebastian Paez
- Erin Broderick
- Daniele Canzani
- Alex Federation

Special thanks to:

- Chris Ashwood for his Glyco Wizardry
- Lindsay Pino for encouraging me to submit an abstract

Come and see more cool stuff from Talus:

- Mon, June 5:
  - **MP 144** *An atlas of reactive and functional chromatin-associated cysteines in cancer* (Daniele Canzani)
  - **MP 389** *Gopher: Fast Gene Ontology Enrichment Analysis for Quantitative Proteomic Data* (Carolyn Allen)
  - **MP 586** *Characterizing the effects of kinase inhibitors on the phosphorylation of transcription factors using subcellular fractionation* (Andrea Gutierrez)
- Tues, June 6:
  - **TOD** *Library-free analysis of DIA experiments using a feature-centric approach (*J. Sebastian Paez)
    - Ballroom C, 09:10am - 9:30am
  - **TP 043** *Diving deeper with depthcharge: A transformer deep learning framework for modeling mass spectrometry data* (William Fondrie)
- Wed, June 7:
  - **WOC** *Simultaneous In situ Pharmacological Profiling of Transcription Factors for Cancer Therapy* (Lindsay K Pino)
    - Ballroom B, 09:30am - 9:50am
- Thurs, June 8:
  - **ThP 679** *How sweet it is: Leveraging the nuclear envelope glycome for the automated extraction of proteins from cell nuclei* (Me!)