

# Building a Sensitive MS-Based Proteomic Approach to Better Characterize CAR-T Cell Therapies

Camille Lombard-Banek, Kerstin Pohl, J.E. Schiel

September 17<sup>th</sup>

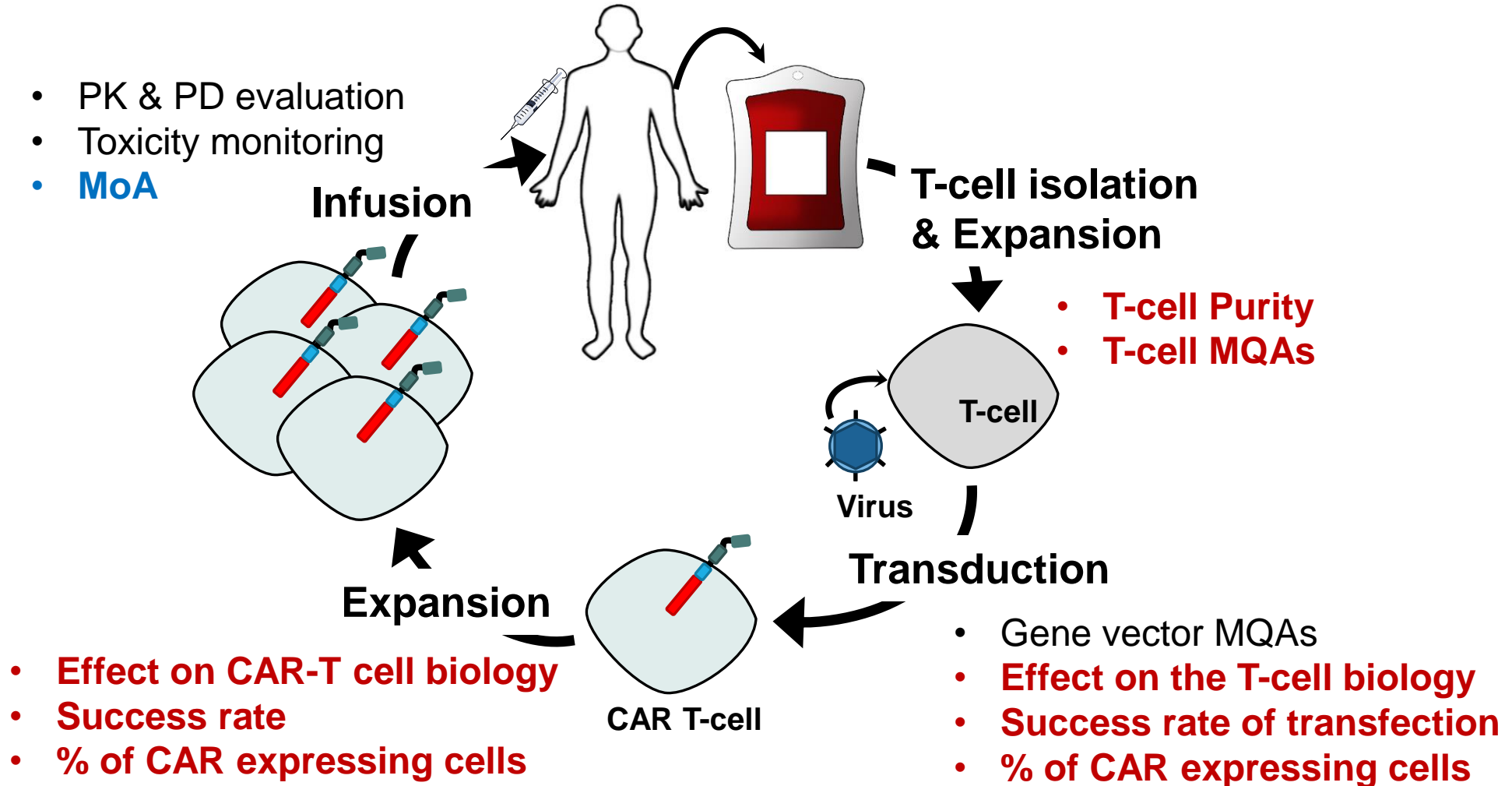
CASSS Mass Spec

**NIST**

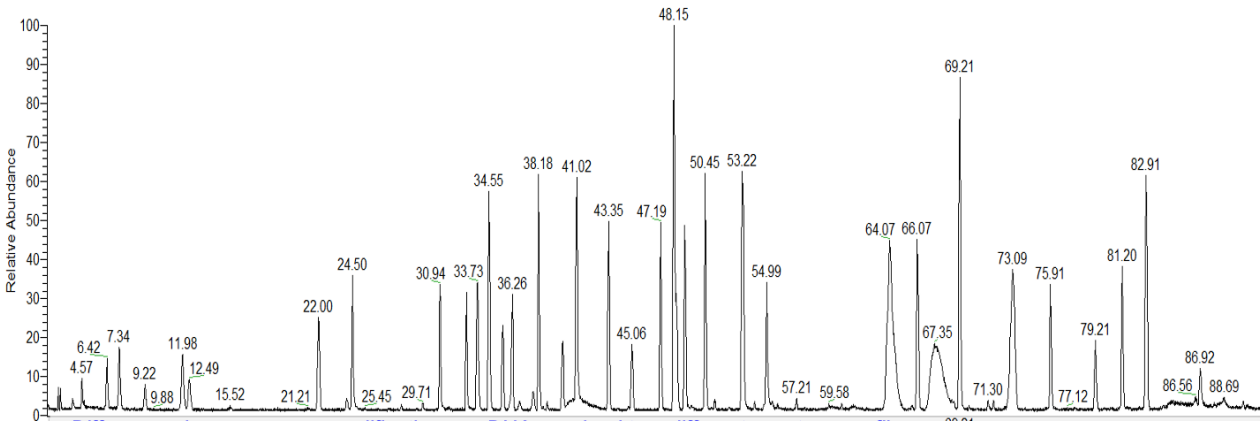


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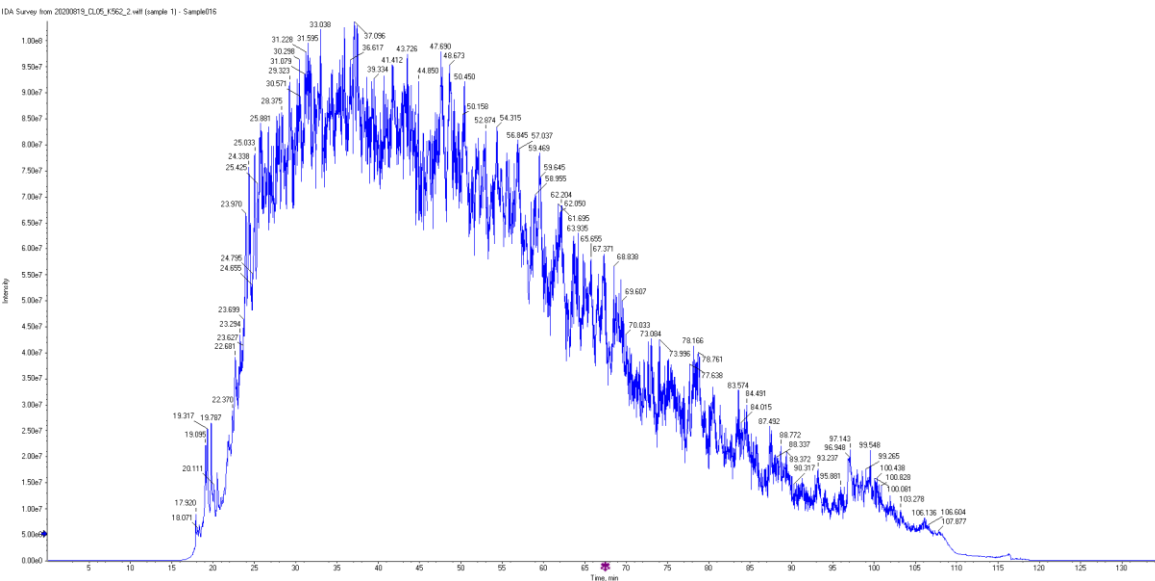
# What are CAR-T cells therapies?



# Expanding MAM principles to CAR T-cell therapies



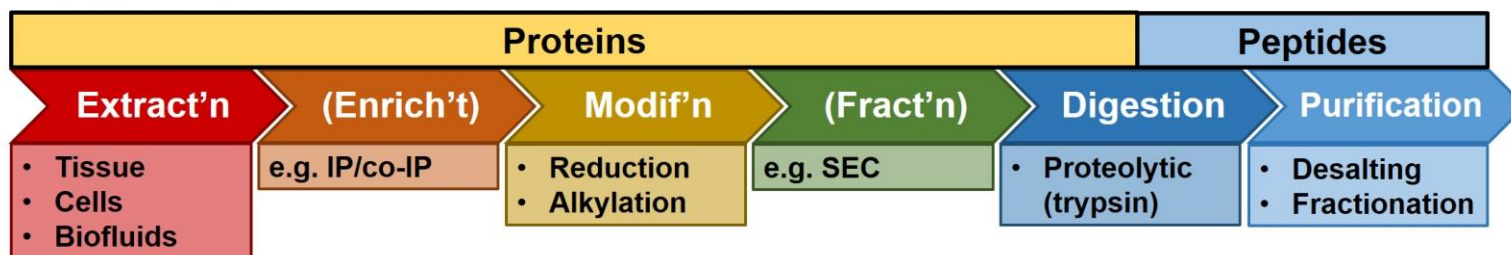
- Secreted
- 1 protein → 200 peptides
- Historical product knowledge
- Not limited in quantities
- NISTmAb as system suitability



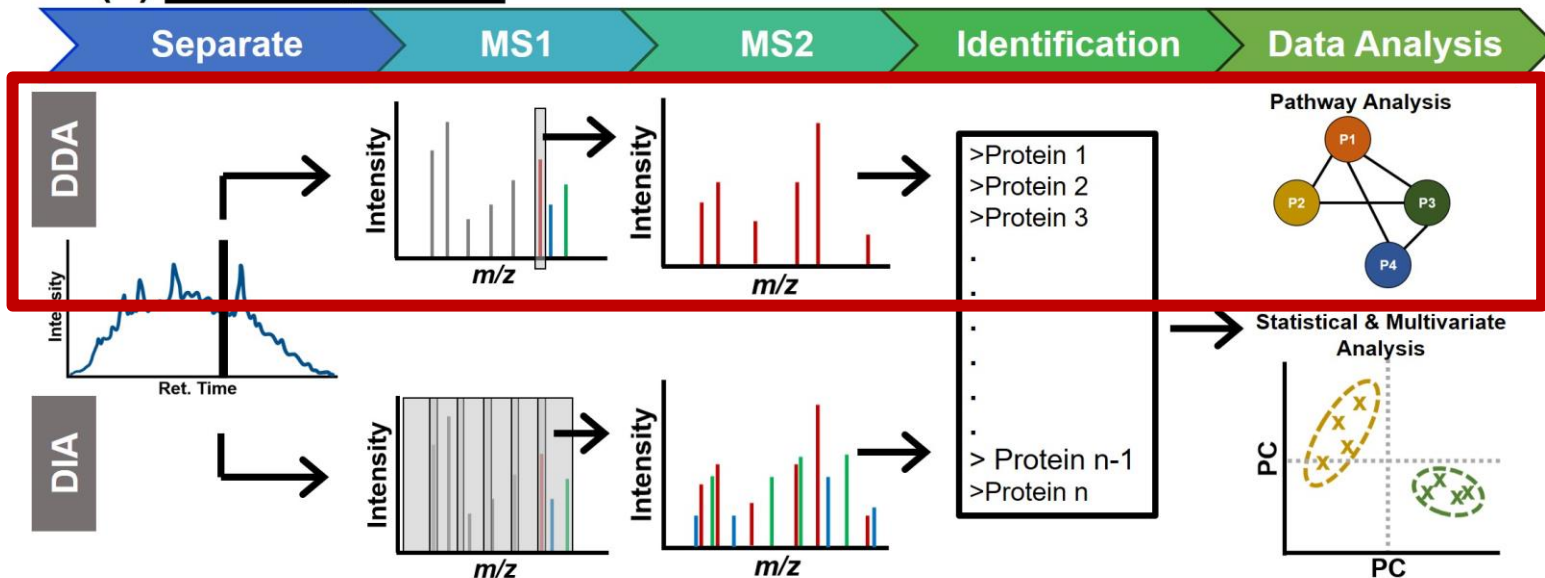
- Live cell → Complex material extraction
- “Active ingredient” = membrane protein
- 10,000+ proteins → 10<sup>6</sup> peptides
- Limited starting material
- No system suitability available

# Steps to enable large-scale proteomics implementation for CAR T-cell therapies

## (a) Sample Preparation



## (b) Data Acquisition



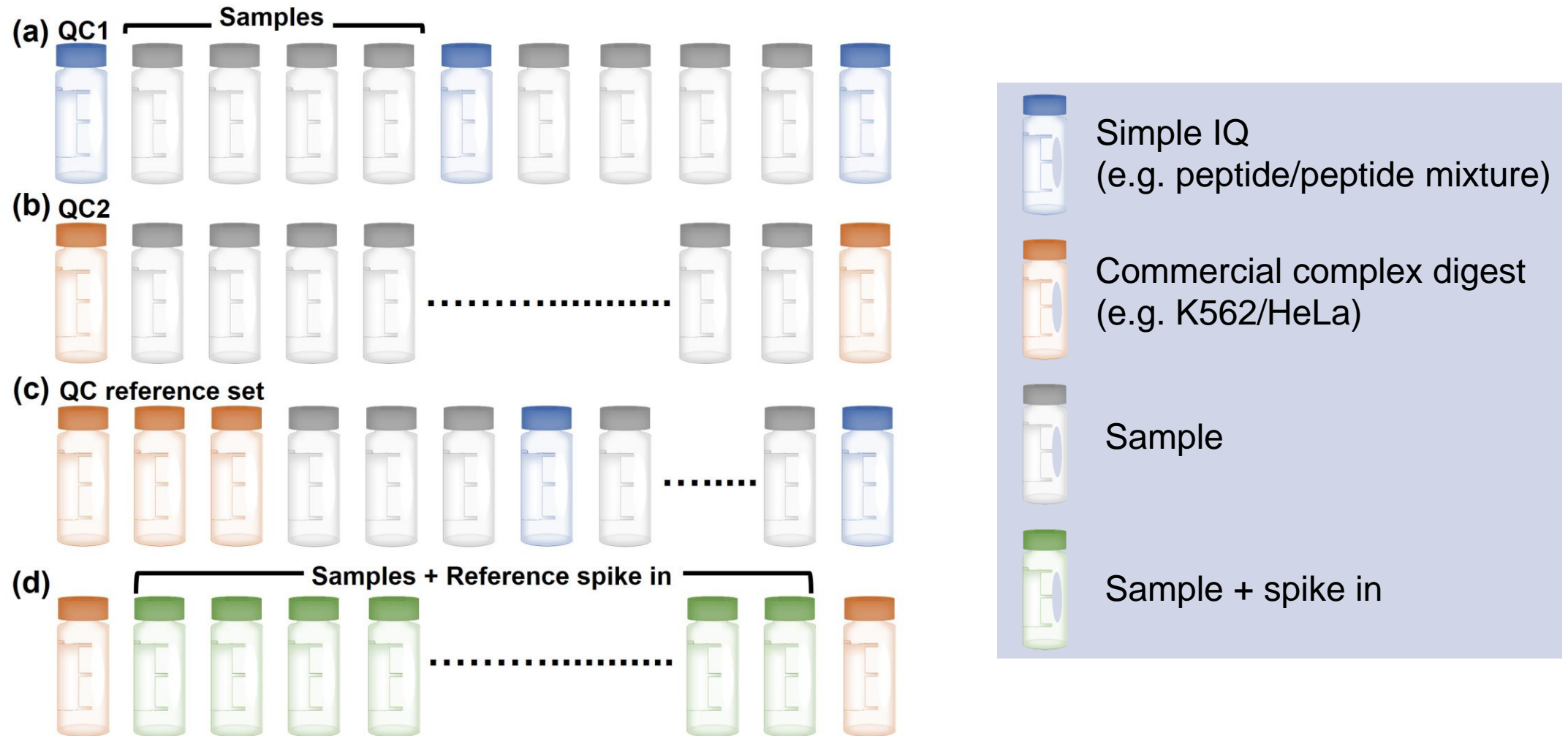
## Required performance metrics

- Enhanced protein extraction
- Scalable to low amounts of samples

- Sensitive:
  - Number of peptides and proteins identified
  - Dynamic range
- Reproducible



# Development of IQ/PQ baseline for CAR T-cell analysis



# Evaluating different columns to test selectivity and performances

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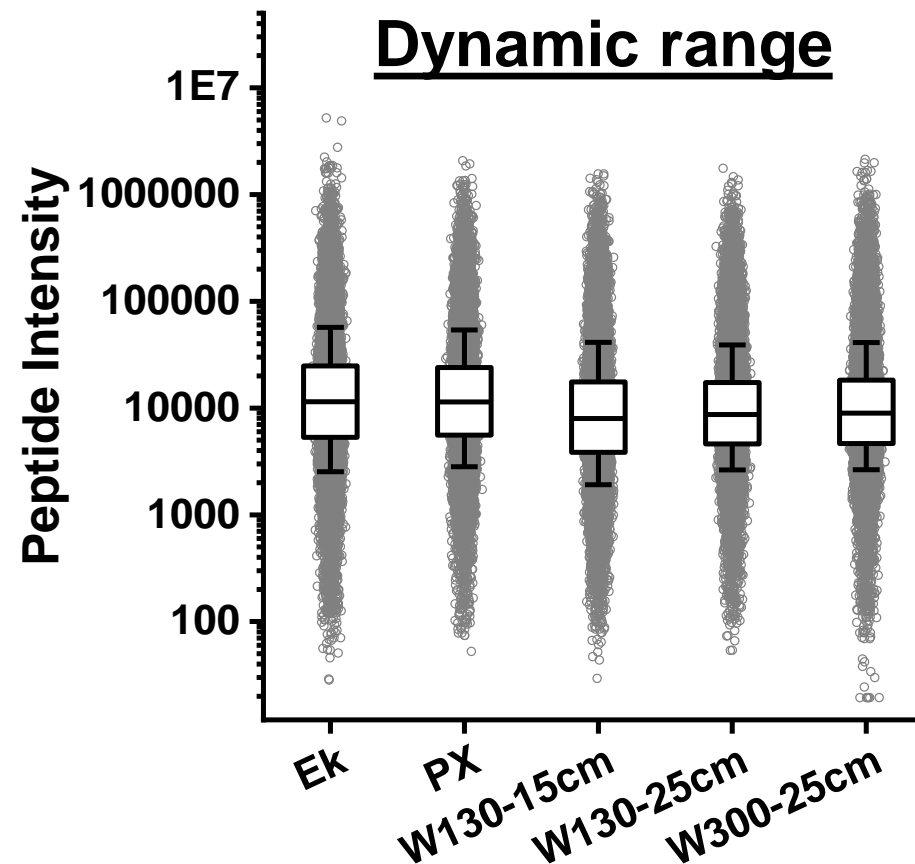
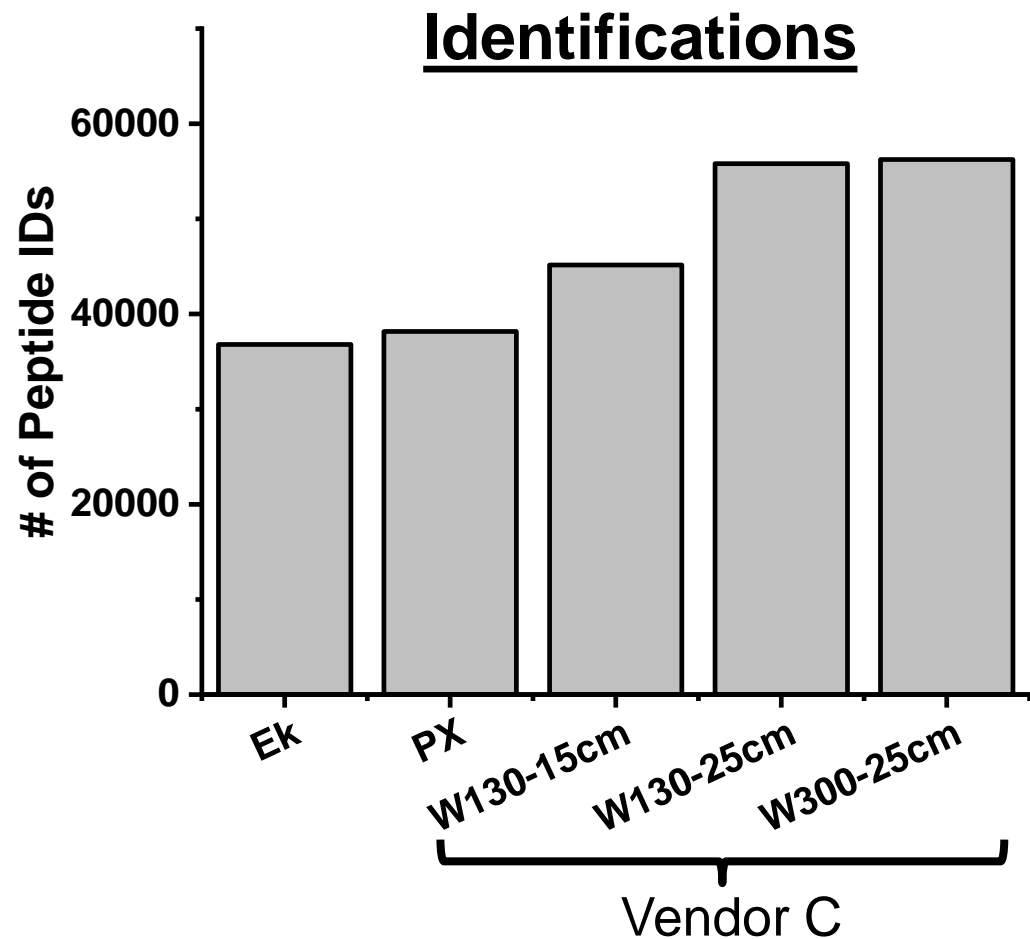
Column	Vendor	Particle size ( $\mu\text{m}$ )	Pore size (Å)	Length (cm)
Ek	A	3	120	15
PX	B	3	300	15
W130-15cm	C	1.7	130	15
W130-25cm	C	1.7	130	25
W300-25cm	C	1.7	300	25

## Metrics to evaluate:

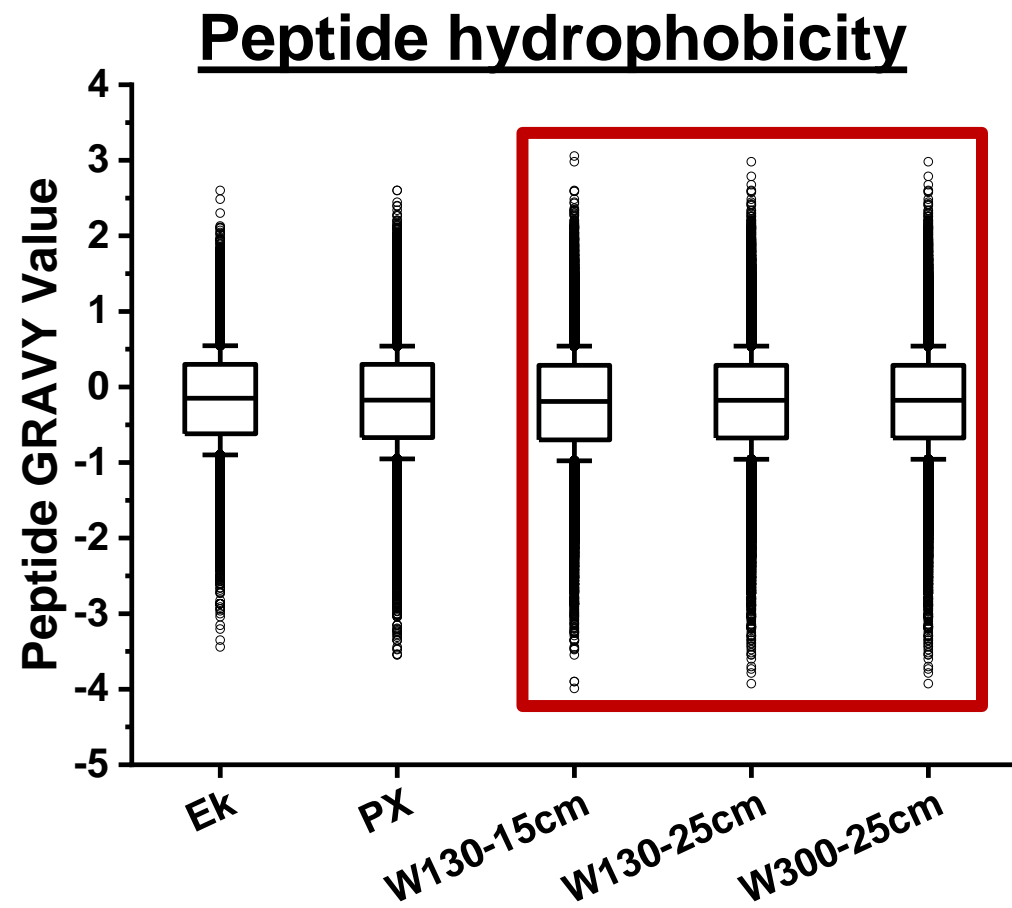
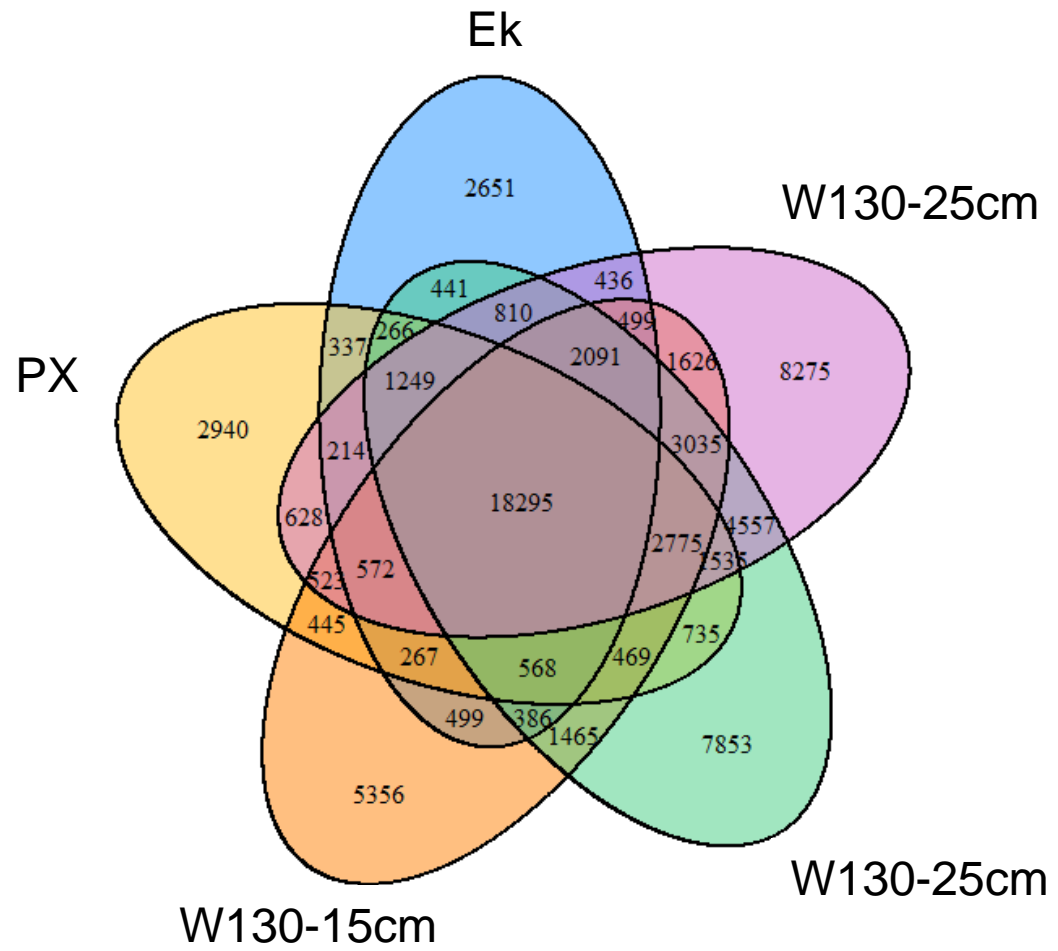
- Number of peptide identified
- Types of peptides identified
- Number of peptides quantified
- Quality of quantification (e.g. %CV)

→ Use commercial K562 digest

# Vendor C columns provided the most peptide identifications

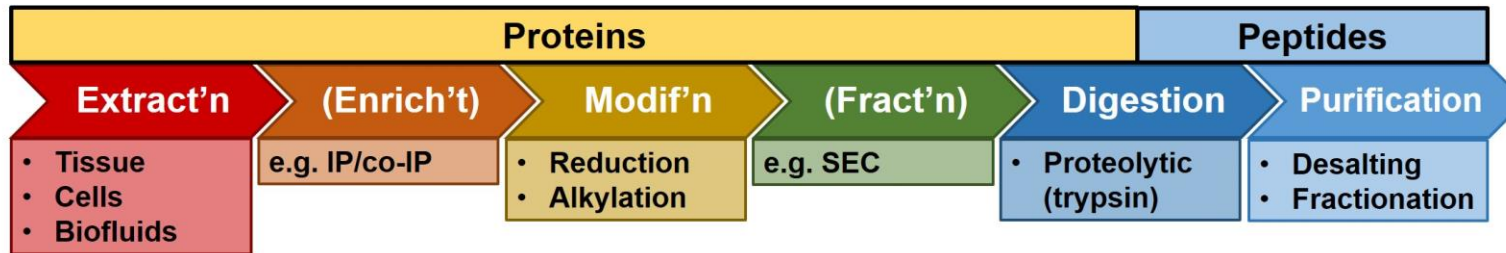


# Peptide identifications are complementary

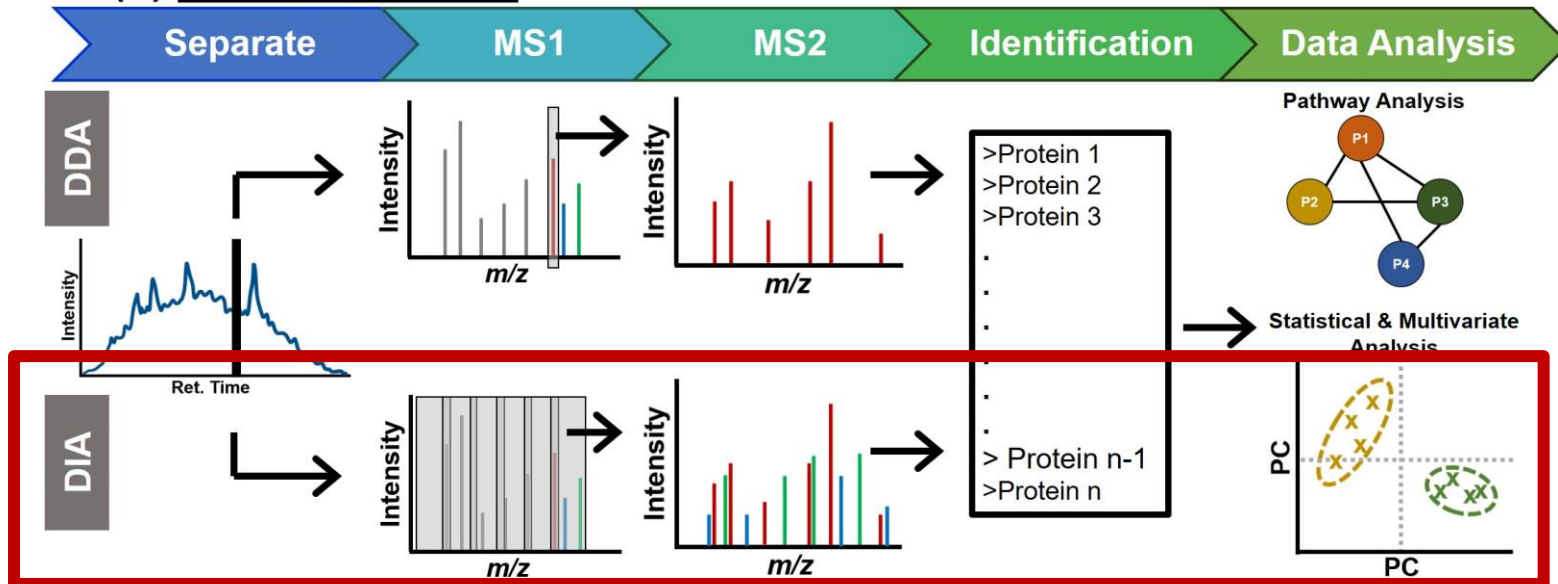


# Steps to improve the proteomic workflow

## (a) Sample Preparation

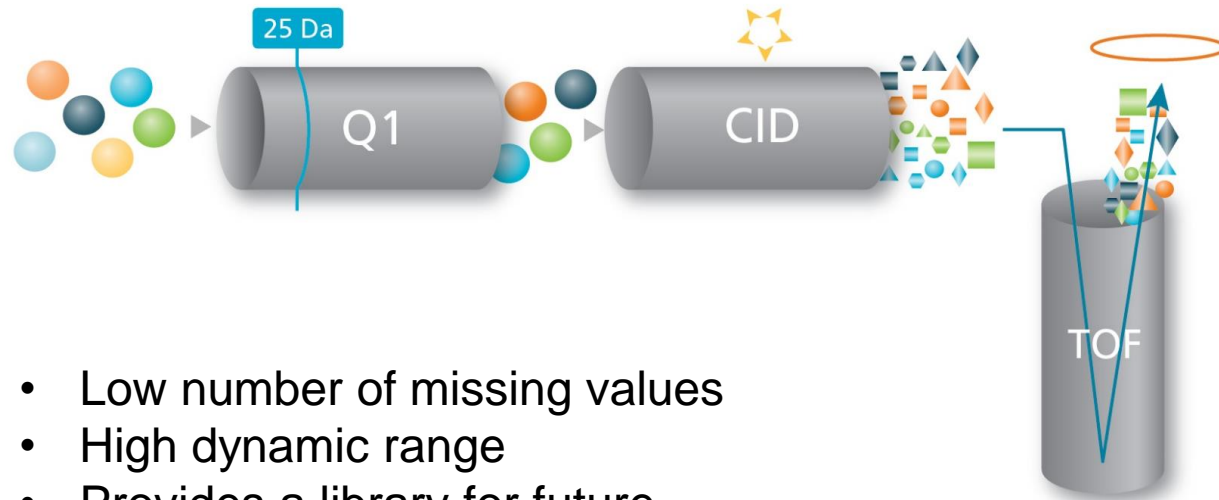


## (b) Data Acquisition

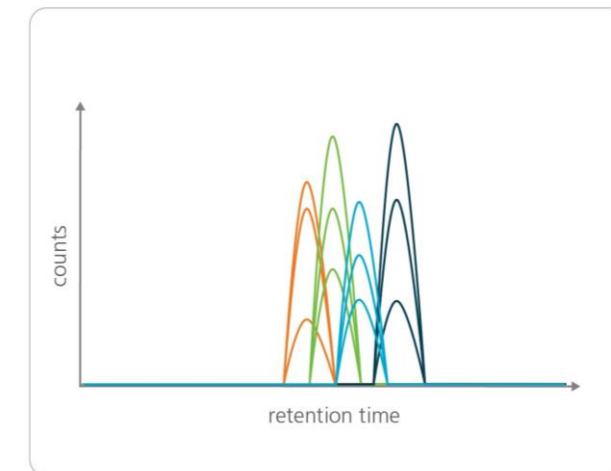
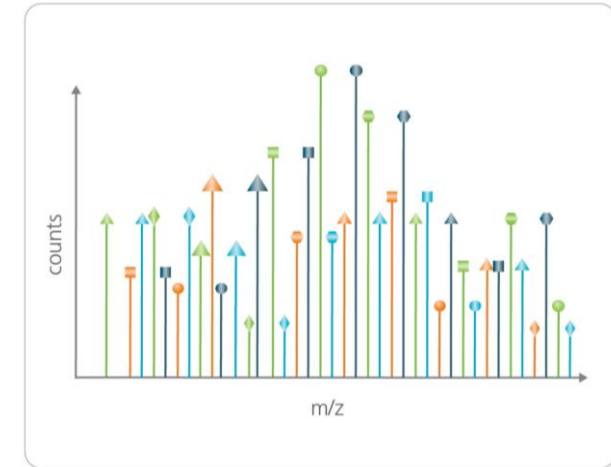


- Sensitive:
  - Number of peptides and proteins identified
  - Dynamic range
- Reproducible

# Protein quantification from SWATH<sup>®</sup> Acquisition



- Low number of missing values
- High dynamic range
- Provides a library for future targeted assays



# Protein identification and quantification from SWATH®

## Acquisition data: Generation of PQPs

**SWATH™ Processing** Detection rate 17.4% (6370 of 36510) for 12170 targeted peptides ≤ 1.0% FDR threshold over 3... Processing Settings Process

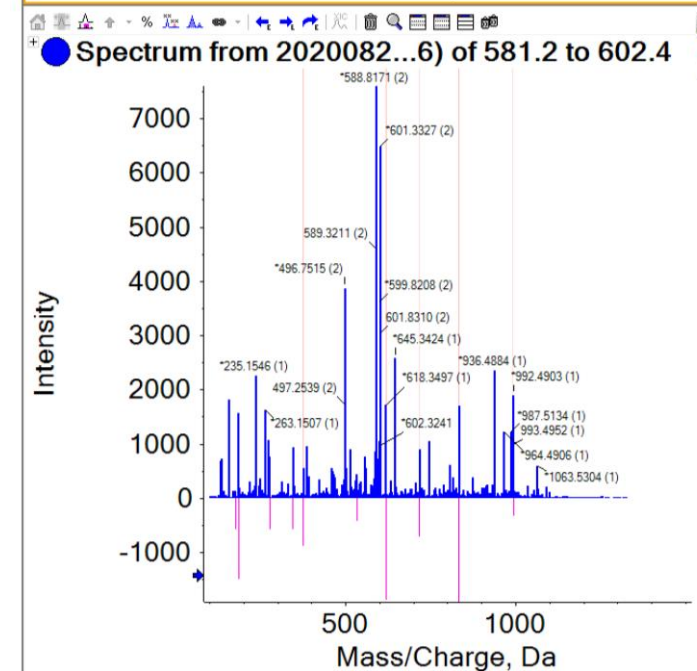
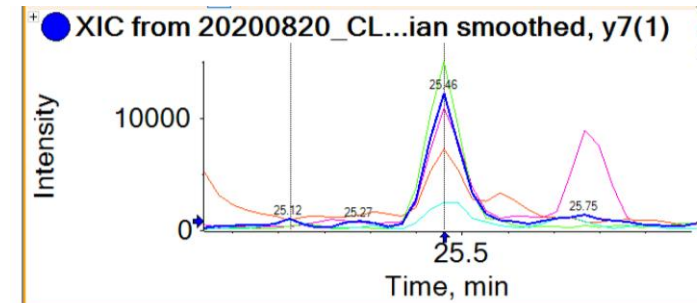
Current Sample: TIC from 20200820\_CL04\_SWATH-K562-1ug\_2\_wiff (sample 1) - Sample013

**Proteins**  Show Selected Only Text Search:

<input checked="" type="checkbox"/>	N	Accession	Name
<input checked="" type="checkbox"/>	1	splP78527 PRKDC_HUMAN	DNA-dependent protein kinase catalytic subunit OS=Homo sapiens OX=9606 GN=PRKDC PE=1 SV=3
<input checked="" type="checkbox"/>	2	splQ14204 DYHC1_HUMAN	Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens OX=9606 GN=DYNC1H1 PE=1 SV=5
<input checked="" type="checkbox"/>	3	splP49327 FAS_HUMAN	Fatty acid synthase OS=Homo sapiens OX=9606 GN=FASN PE=1 SV=3
<input checked="" type="checkbox"/>	4	splQ15149 PLEC_HUMAN	Plectin OS=Homo sapiens OX=9606 GN=PLEC PE=1 SV=3
<input checked="" type="checkbox"/>	5	splQ92616 GCN1_HUMAN	eIF-2-alpha kinase activator GCN1 OS=Homo sapiens OX=9606 GN=GCN1 PE=1 SV=6
<input checked="" type="checkbox"/>	6	splP21333 FLNA_HUMAN	Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4
<input checked="" type="checkbox"/>	7	splQ8Y490 ITLN1_HUMAN	Talin-1 OS=Homo sapiens OX=9606 GN=ITLN1 PE=1 SV=3
<input checked="" type="checkbox"/>	8	splQ00610 ICLH1_HUMAN	Clathrin heavy chain 1 OS=Homo sapiens OX=9606 GN=CLTC PE=1 SV=5
<input checked="" type="checkbox"/>	9	splP35579 MYH9_HUMAN	Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9 PE=1 SV=4
<input checked="" type="checkbox"/>	10	splQ6P2Q9 PRP8_HUMAN	Pre-mRNA-processing-splicing factor 8 OS=Homo sapiens OX=9606 GN=PRPF8 PE=1 SV=2
<input checked="" type="checkbox"/>	11	splO75643 U520_HUMAN	U5 small nuclear ribonucleoprotein 200 kDa helicase OS=Homo sapiens OX=9606 GN=SNRNP200 PE=1 SV=2
<input checked="" type="checkbox"/>	12	splQ5T4S7 UBR4_HUMAN	E3 ubiquitin-protein ligase UBR4 OS=Homo sapiens OX=9606 GN=UBR4 PE=1 SV=1
<input checked="" type="checkbox"/>	13	splP42704 LPPRC_HUMAN	Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens OX=9606 GN=LPPRC PE=1 SV=3
<input checked="" type="checkbox"/>	14	splP49792 RBP2_HUMAN	E3 SUMO-protein ligase RanBP2 OS=Homo sapiens OX=9606 GN=RANBP2 PE=1 SV=2
<input checked="" type="checkbox"/>	15	splP07814 SYEP_HUMAN	Bifunctional glutamate/proline-tRNA ligase OS=Homo sapiens OX=9606 GN=EPRS1 PE=1 SV=5

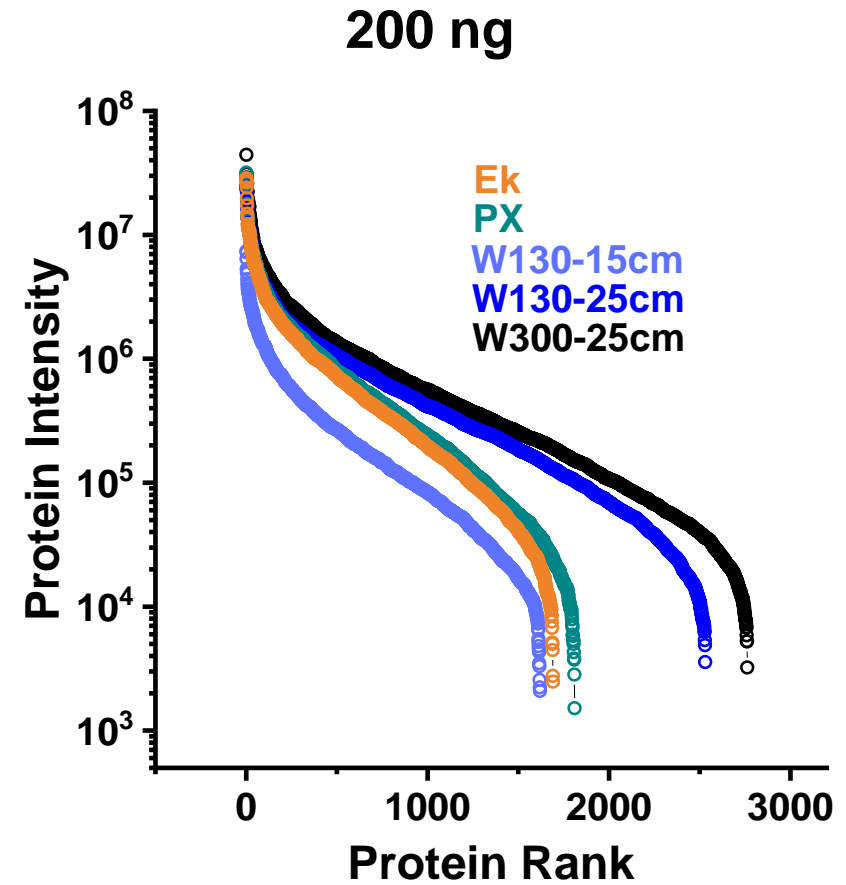
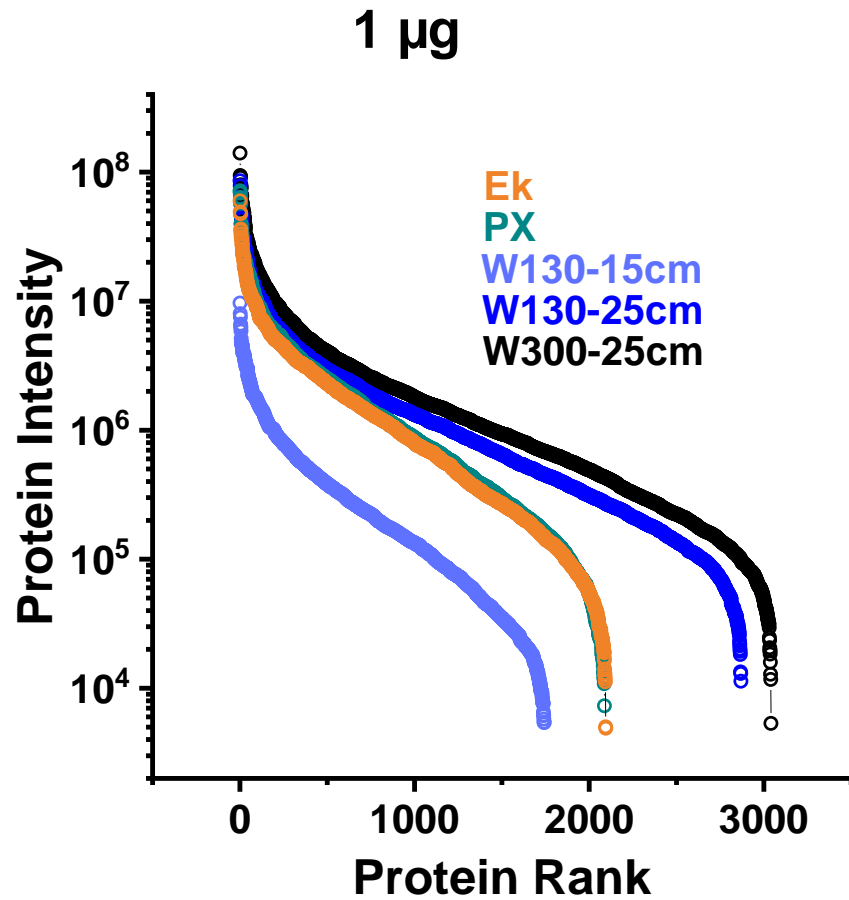
**Peptides** Text Search:

<input type="checkbox"/>	Rank	Peptide Sequence	Charge	Confidence	Intensity	Expected RT	Parent m/z	Observed RT	Score	FDR	% Gaps
<input checked="" type="checkbox"/>	1	LAC[CAM]DQVQVTR	2	99	57269.75	25.70	588.79	25.46	4.760	0.0	0.0
<input checked="" type="checkbox"/>	2	LQETLSAADR	2	99	48011.73	25.30	552.29	25.06	4.817	0.0	6.7
<input checked="" type="checkbox"/>	3	INQVFIGSC[CAM]ITEGNETLK	3	99	36843.37	36.39	683.00	36.40	1.480	7.3	6.7
<input checked="" type="checkbox"/>	4	WC[CAM]AHTNVELK	3	99	33041.68	31.57	419.87	27.26	-1.973	92.9	20.0
<input checked="" type="checkbox"/>	5	LGLPGDEVDNK	2	99	32400.85	31.38	578.80	31.22	3.069	0.0	20.0
<input checked="" type="checkbox"/>	6	SLGTIQQC[CAM]C[CAM]DAIDHLC[CAM]R	3	99	31496.1	44.92	682.98	44.89	2.940	0.0	6.7



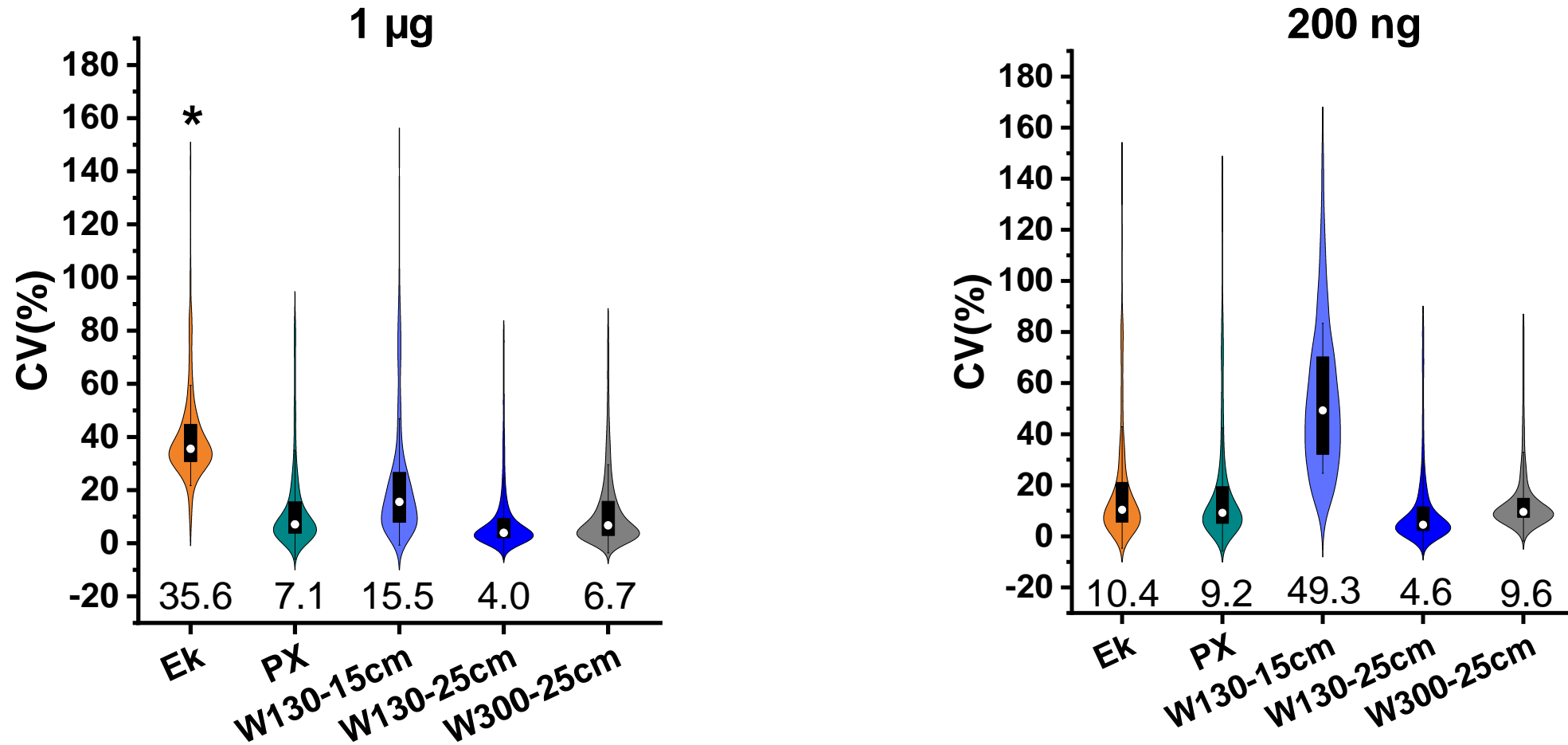
PeakView®  
Software

# Column W300-25cm led to more sensitive quantification





# Column W130-25cm led to more reproducible protein quantification



# Building a master library for SWATH® Acquisition based quantification using NIST developed tools

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## SWATH Mass Spectrometry Performance Using Extended Peptide MS/MS Assay Libraries

Jemma X. Wu, Xiaomin Song, Dana Pascovici, Thiri Zaw, Natasha Care, Christoph Krisp and Mark P. Molloy ✉

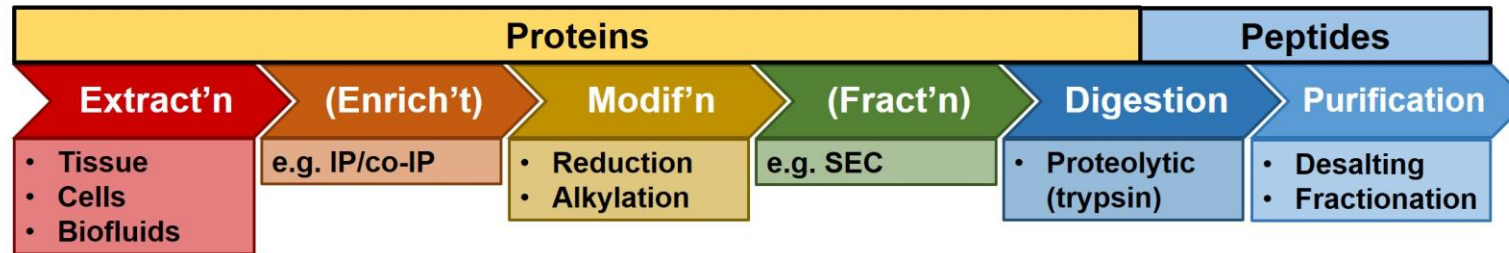
Molecular & Cellular Proteomics July 1, 2016, First published on May 9, 2016, 15 (7) 2501-2514; <https://doi.org/10.1074/mcp.M115.055558>

Iterate for each column specific library

ess  
H data

# Steps to improve the proteomic workflow

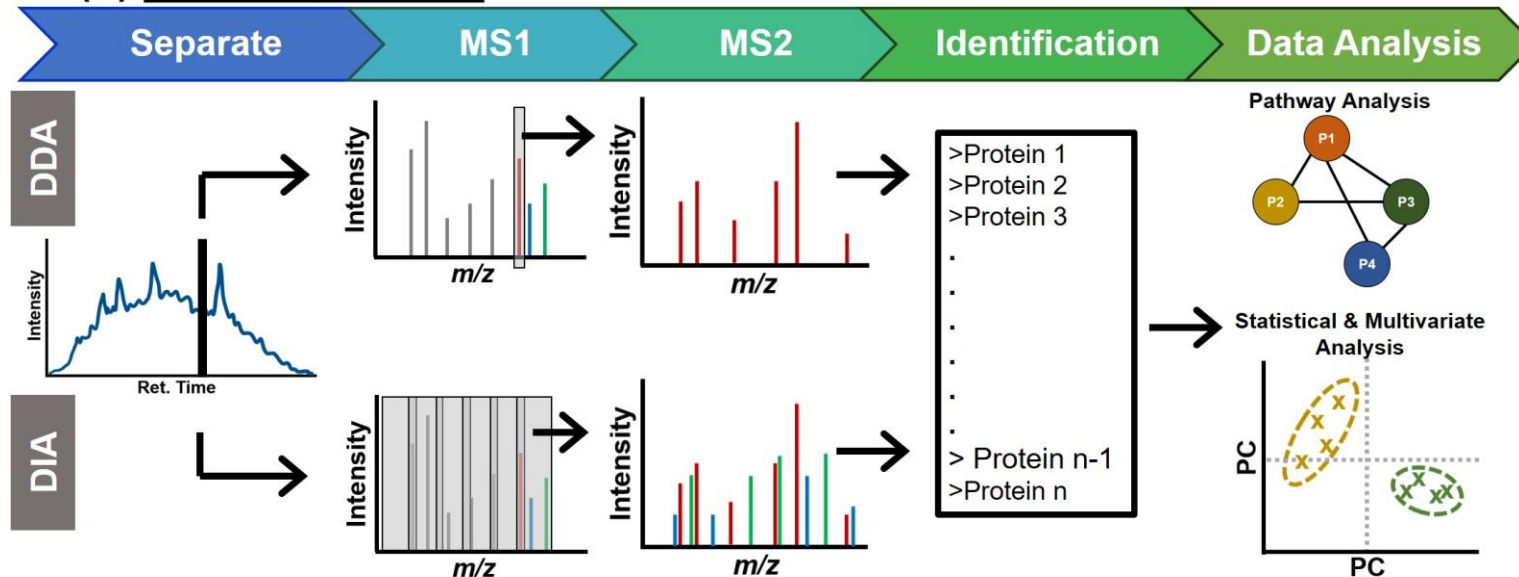
## (a) Sample Preparation



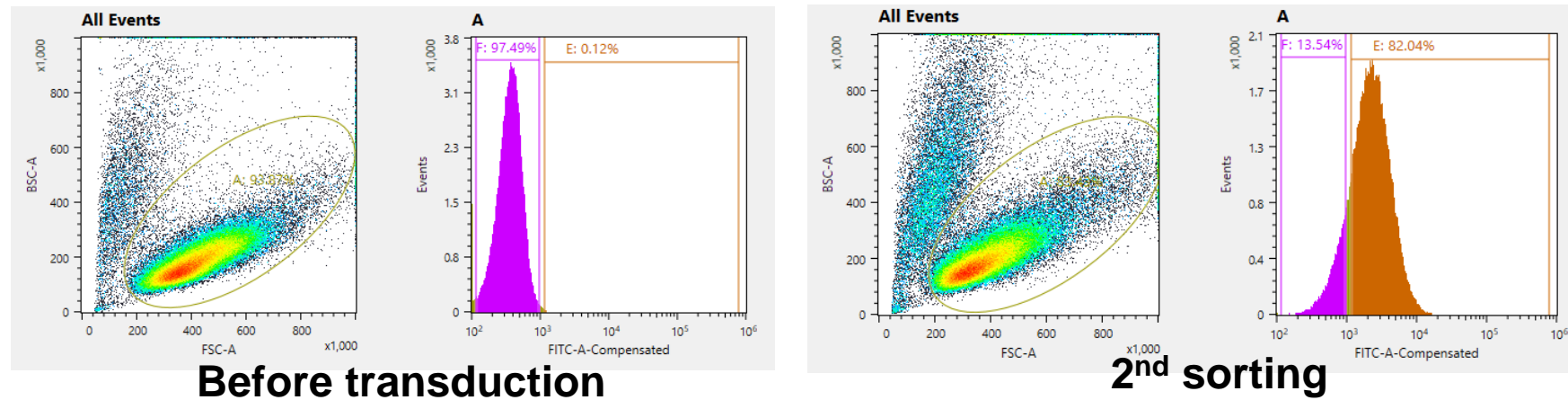
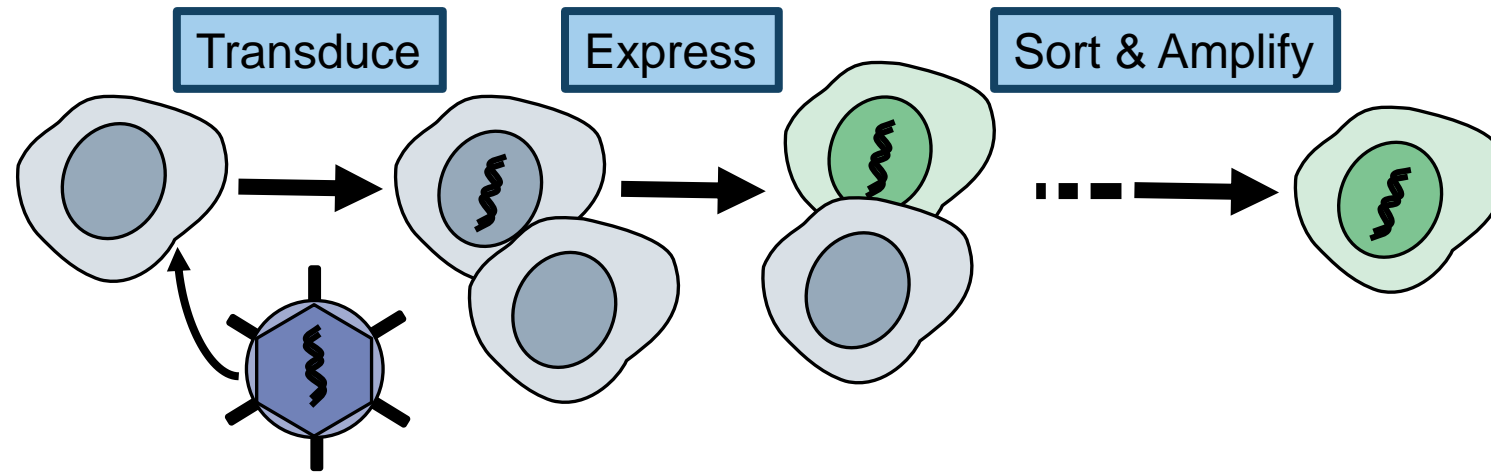
- Enhanced protein extraction
- Scalable to low amounts of samples

→ **Development a model**

## (b) Data Acquisition



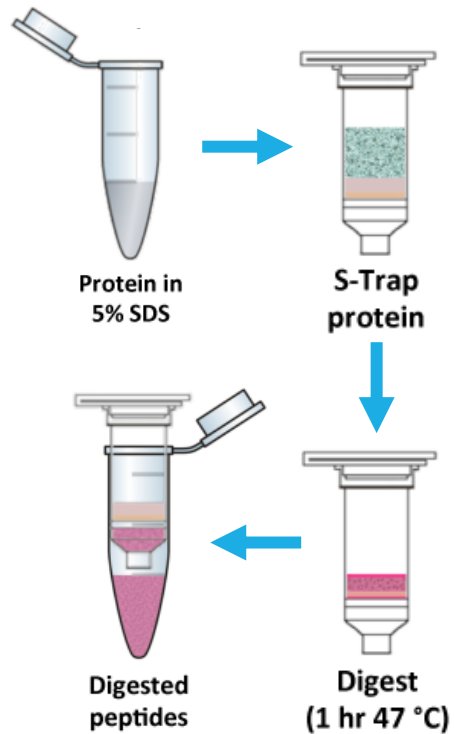
# Developed a biological model: CAR-Jurkat



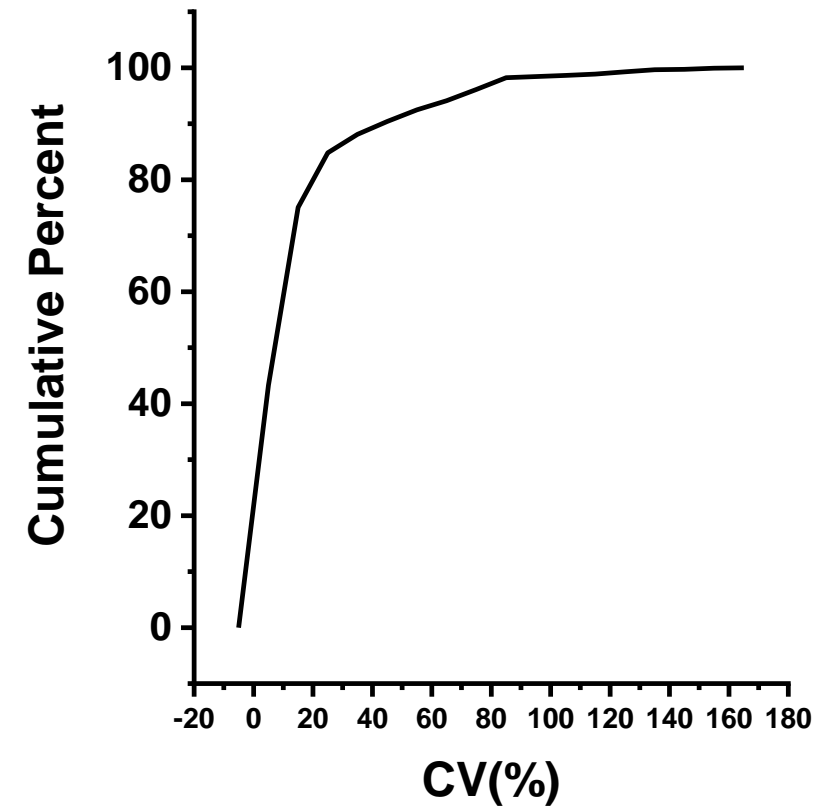
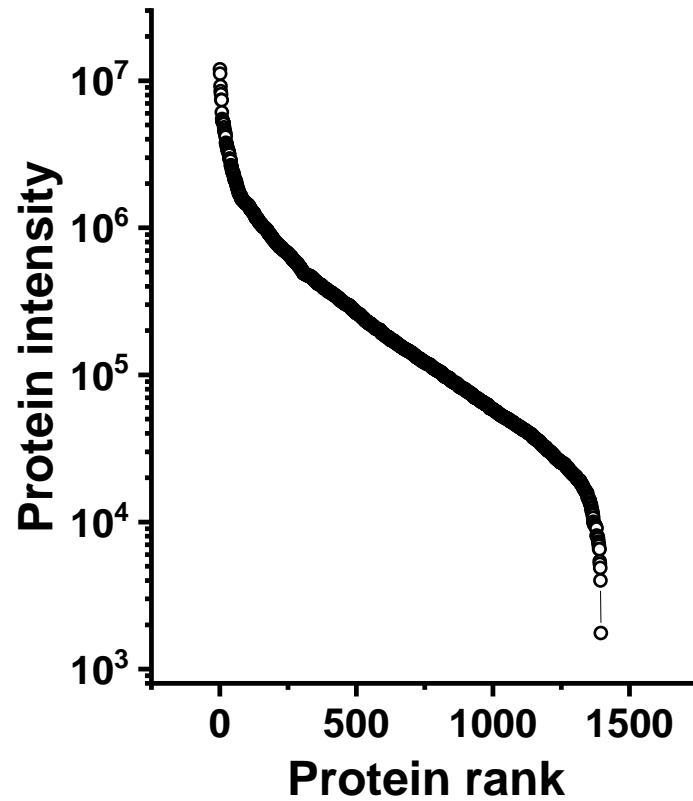
- We can obtain 80+% purity after 2 FAC sortings

# Identification and quantification of proteins from Jurkat cells

## S-Trap sample preparation



- Measured on W130-15cm



→ ~1,400 proteins quantified

# Relating proteins to cell biology

■ Cvtosol

<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">Wnt signaling pathway, planar cell polarity pathway</a>	RT	41	2.9	3.3E-20	6.9E-18
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent</a>	RT	34	2.4	4.7E-20	9.4E-18
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">mRNA export from nucleus</a>	RT	42	3.0	1.5E-19	2.9E-17
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">RNA export from nucleus</a>	RT	31	2.2	5.1E-19	9.2E-17
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition</a>	RT	36	2.6	7.5E-19	1.3E-16
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">mRNA 3'-end processing</a>	RT	29	2.1	3.0E-18	4.9E-16
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">anaphase-promoting complex-dependent catabolic process</a>	RT	36	2.6	3.5E-18	5.6E-16
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">tRNA aminoacylation for protein translation</a>	RT	26	1.9	4.8E-18	7.2E-16
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">T cell receptor signaling pathway</a>	RT	49	3.5	1.1E-17	1.6E-15
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">tricarboxylic acid cycle</a>	RT	22	1.6	2.3E-17	3.2E-15
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">stimulatory C-type lectin receptor signaling pathway</a>	RT	40	2.9	6.7E-17	1.5E-14

- DAVID
- PantherDB

■ biological phase  
■ locomotion

# Conclusions

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1. Identifications for the different columns are complementary
  - Will be used to expand our master library
2. Based on overall performance metrics the 25 cm columns are best suited for our analytics
  - W300-25cm will be chosen for future studies
3. Jurkat provided promising preliminary data
4. CAR Jurkat will be used for future application of our method:
  1. MQAs assessment
  2. In process samples for process development/monitoring
  3. PQAs identification and possibly control

# Acknowledgements

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- Schiel's laboratory

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- Sumona Sarkar

