

Prospects for Proteomics by Data-Independent-Acquisition Mass Spectrometry to Advance the Characterization of CAR-T cell Therapies

Camille Lombard-Banek, Edward J. Kwee, Sumona Sarkar, John T. Elliott, and John E. Schiel

Email: clombard@umd.edu

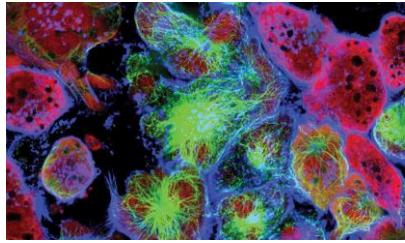


UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE
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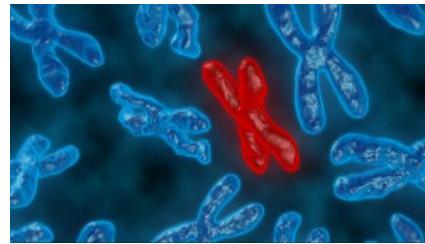
The role of NIST in advancing biomanufacturing

**Measure to
Understand and Diagnose**

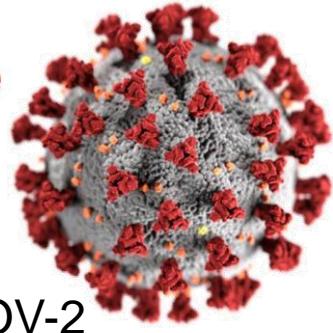
Cancer



Genetic Disorders



autoimmune disease

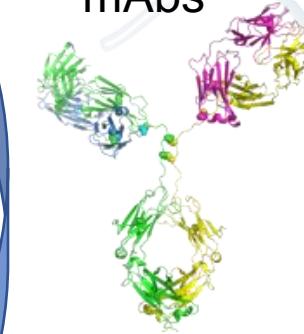


SARS COV-2

**NIST
Measurement Tools
&
Reference Materials
are central to
Innovation & Quality**

**Measure to
Accelerate & Improve
Treatment Options**

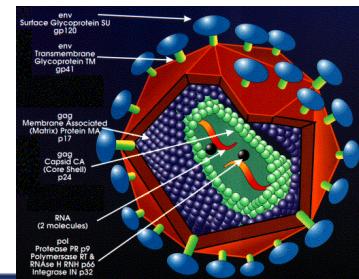
mAbs



Cell Therapies



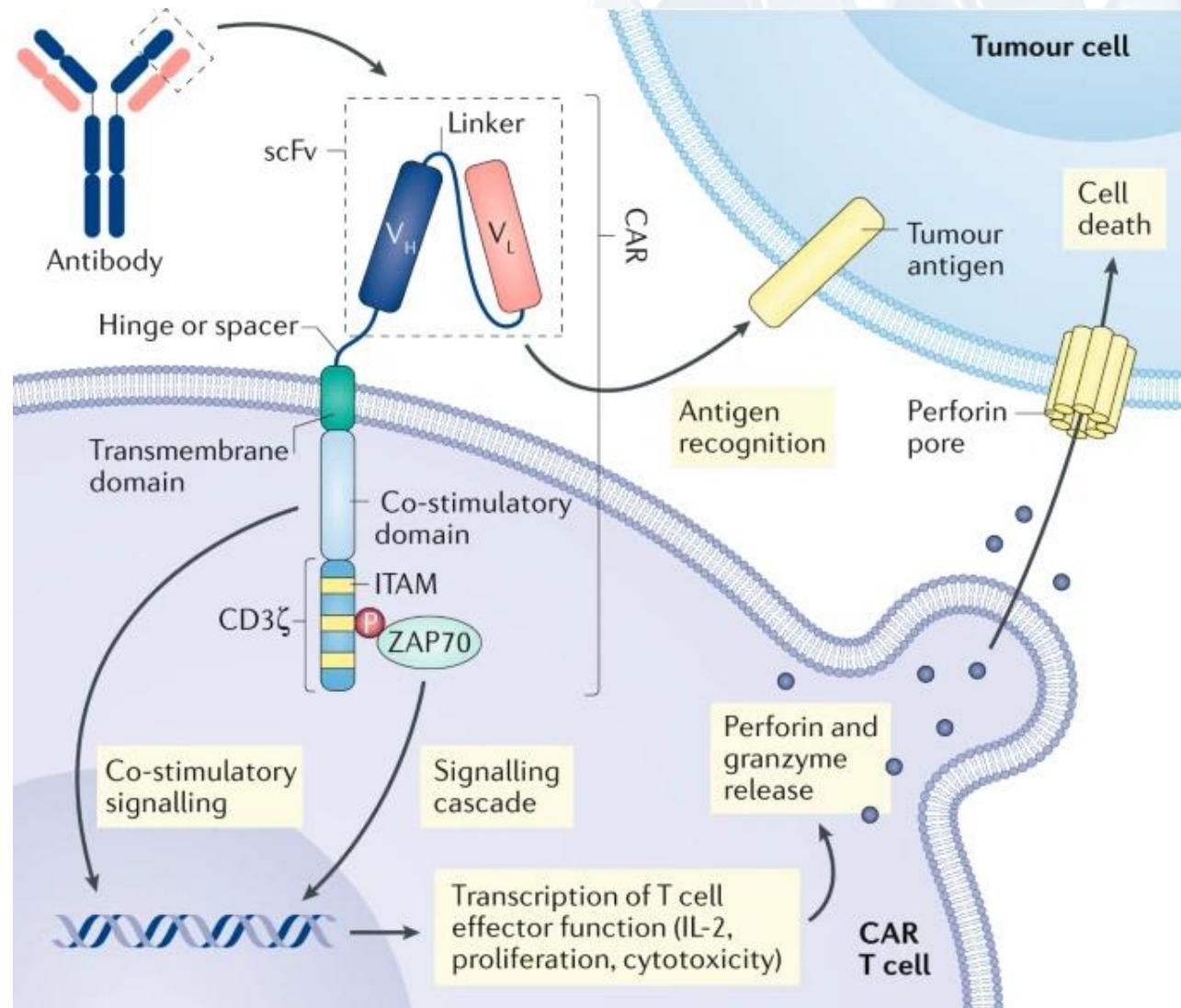
Gene Therapy &
Viral Vectors



RNA Vaccines



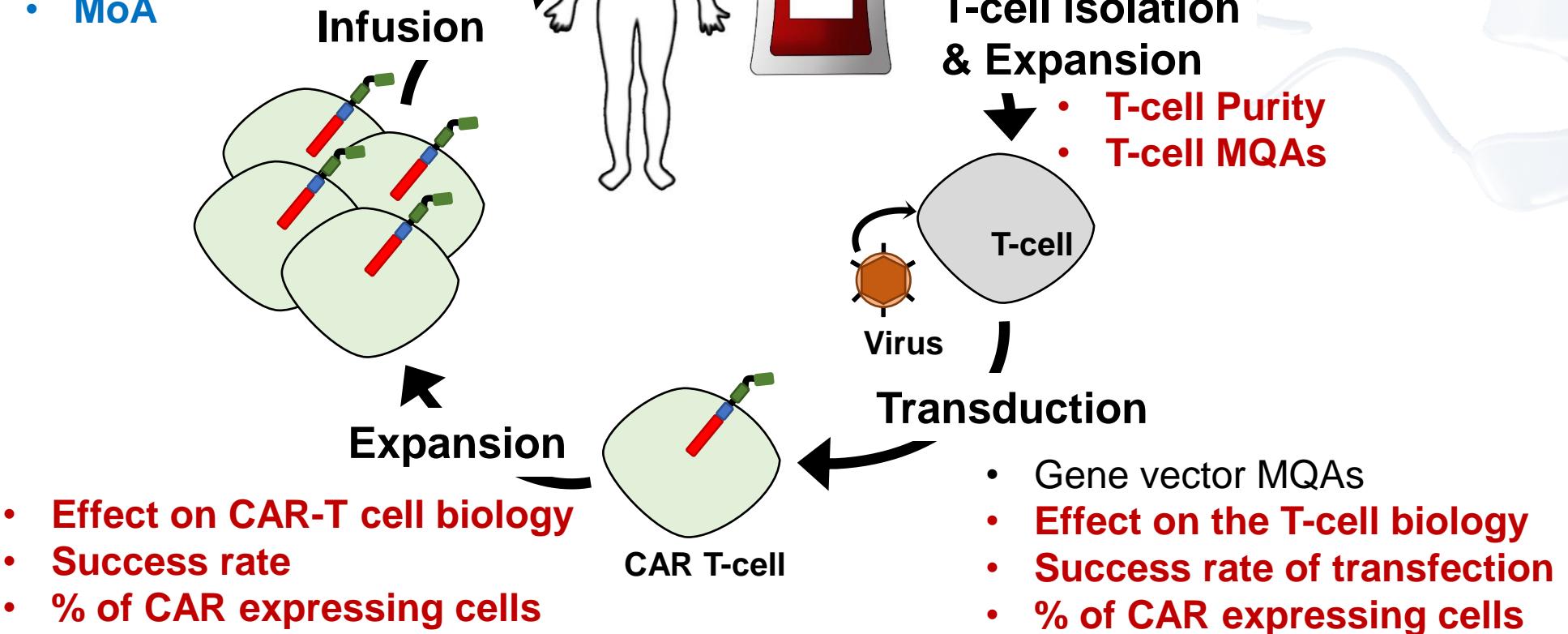
What are CAR T-cells?



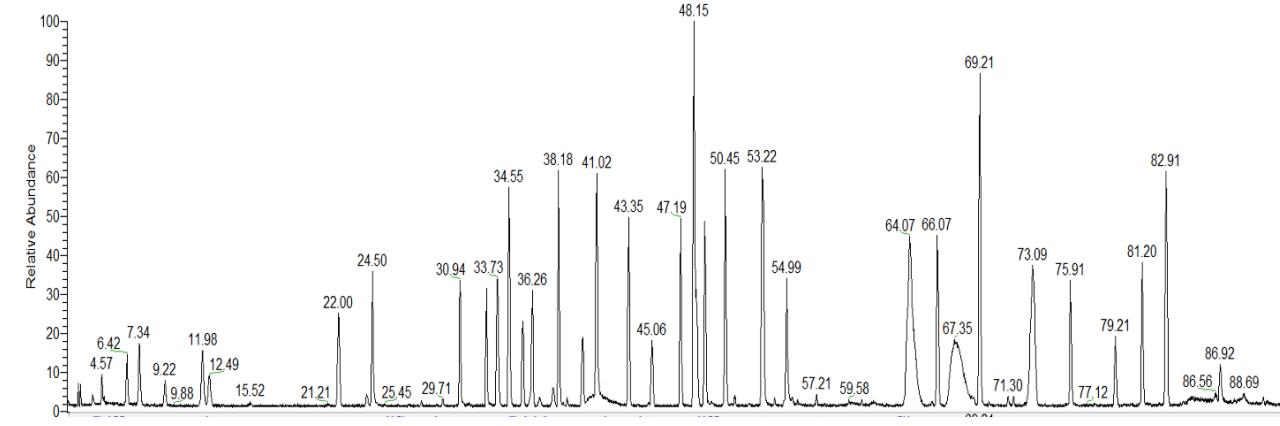
- Engineered T-cells to target circulating cancer cells
 - Autologous: T-cell from the patient
 - Allogenic: T-cell from a donor
- 5 FDA approved therapies (all autologous)

Opportunities and challenges for proteomics on CAR T-cells

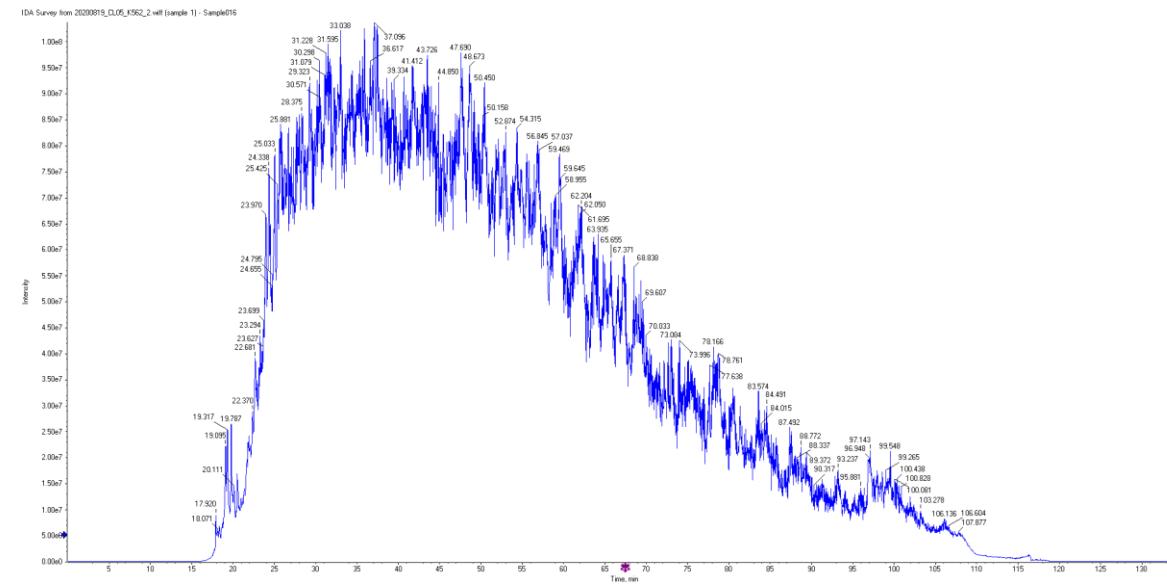
- PK & PD evaluation
- Toxicity monitoring
- **MoA**



Expanding MAM principles to CAR T-cell therapies

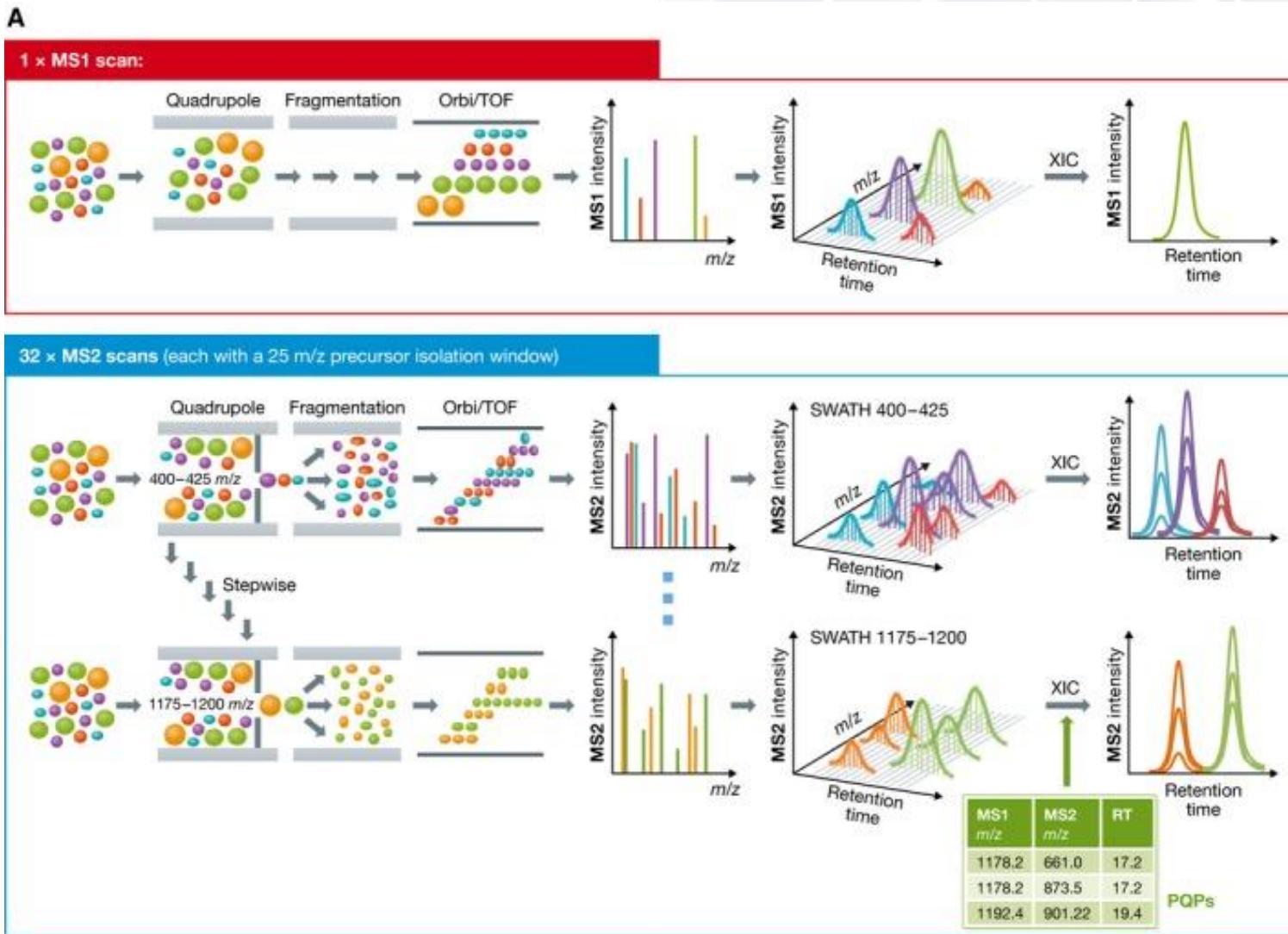


- Secreted
- 1 protein → 200 peptides
- Historical product knowledge
- Not limited in quantities
- NISTmAb as system suitability



- Live cell → Complex material extraction
- “Active ingredient” = membrane protein
- 10,000+ proteins → 10⁶ peptides
- Limited starting material
- No system suitability available

Protein quantification from SWATH® Acquisition



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

Ludwig, C, Aebersold R. et al., Mol Syst Biol. 2018 Aug; 14(8): e8126.

Implementing DIA/SWATH-MS for Cell Therapies

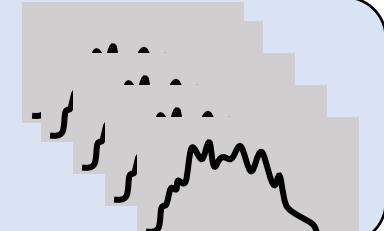
1 Control Strategy



2 Generating PQPs

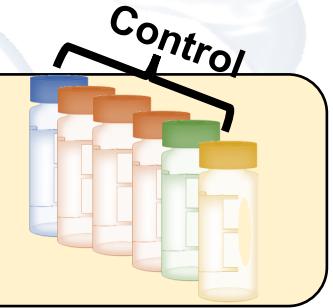
	MS1	MS2	RT
555.8	200.1	21	
555.8	355.4	21	
798.5	800.5	50	

3 SWATH-MS Measurements

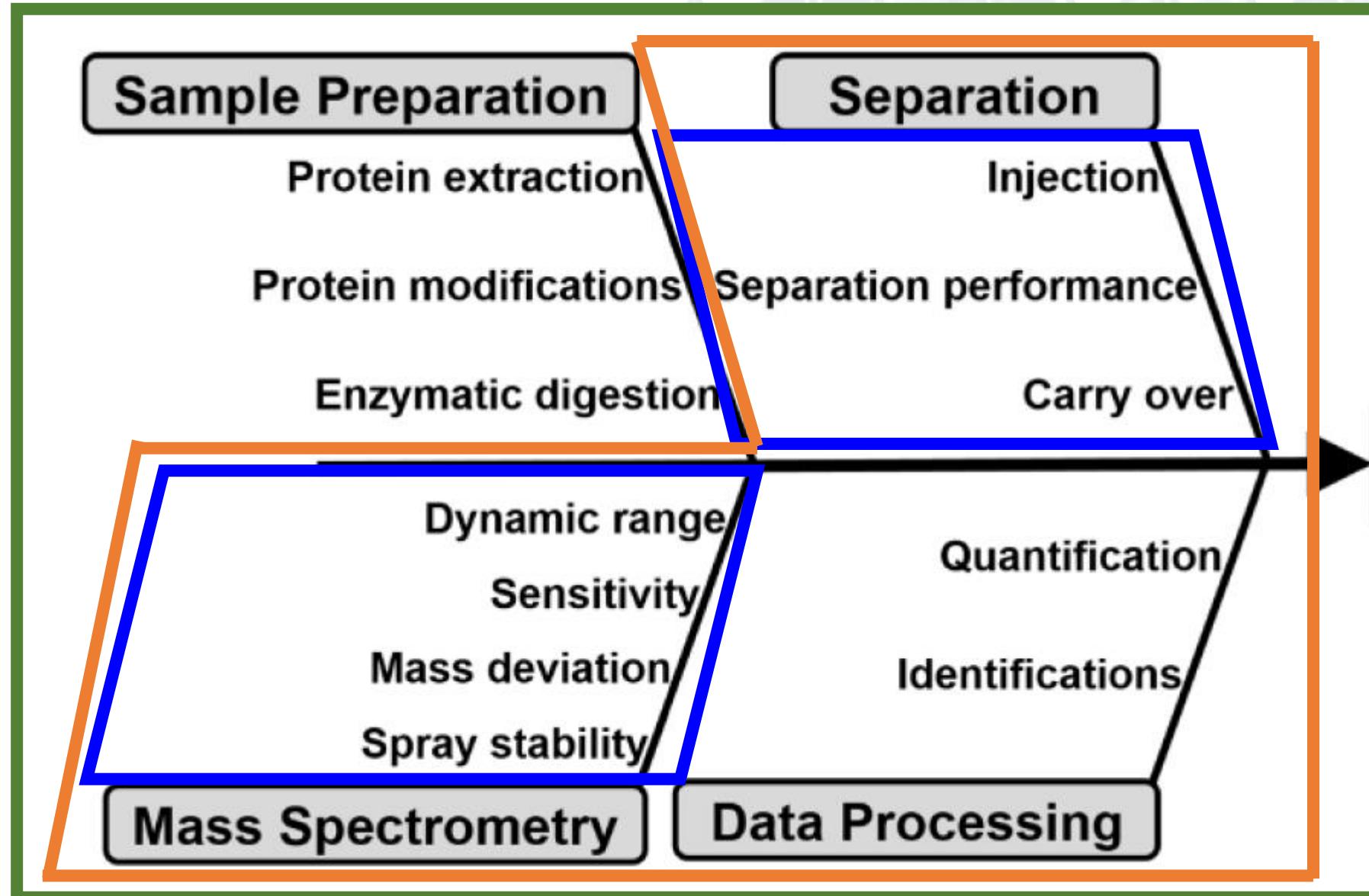


Establishing an instrument performance qualification

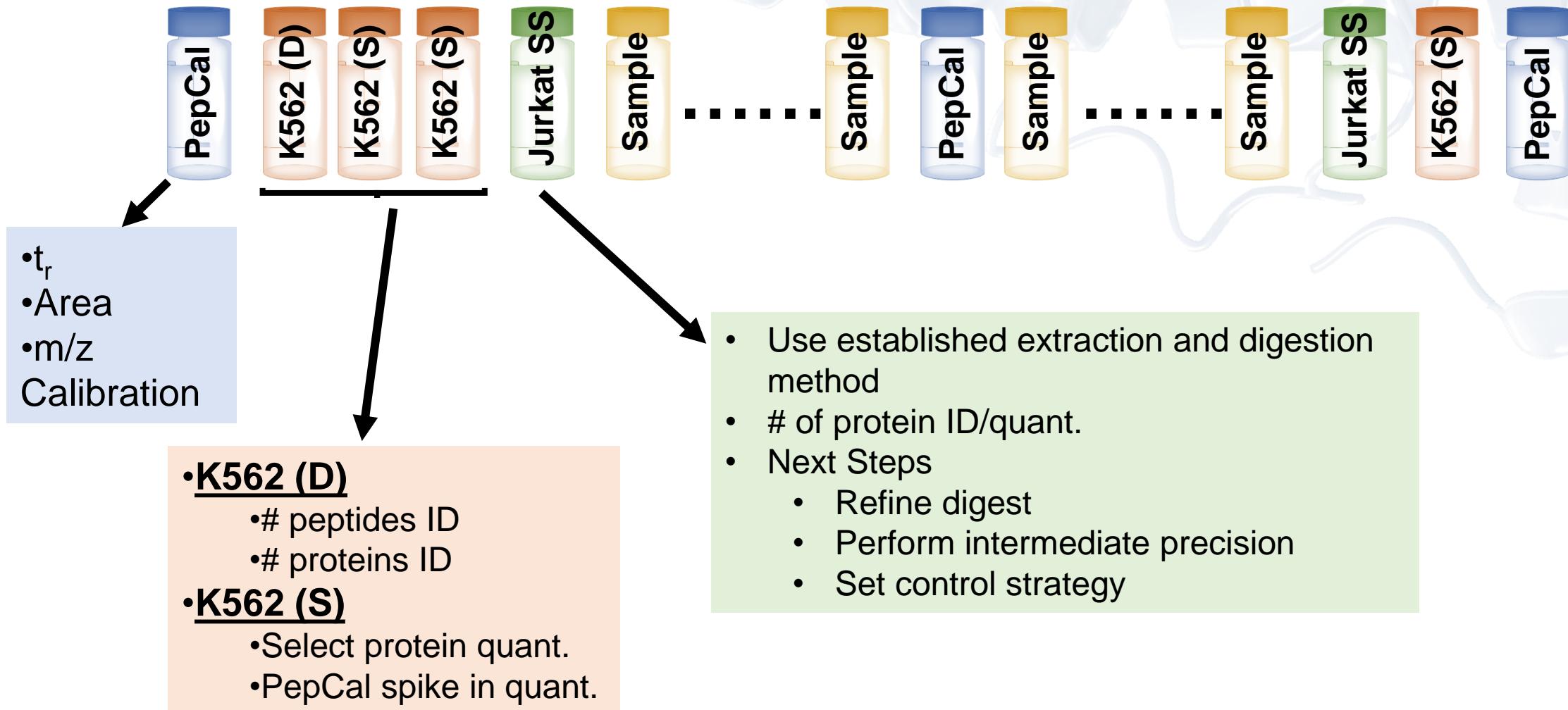
1 Control Strategy



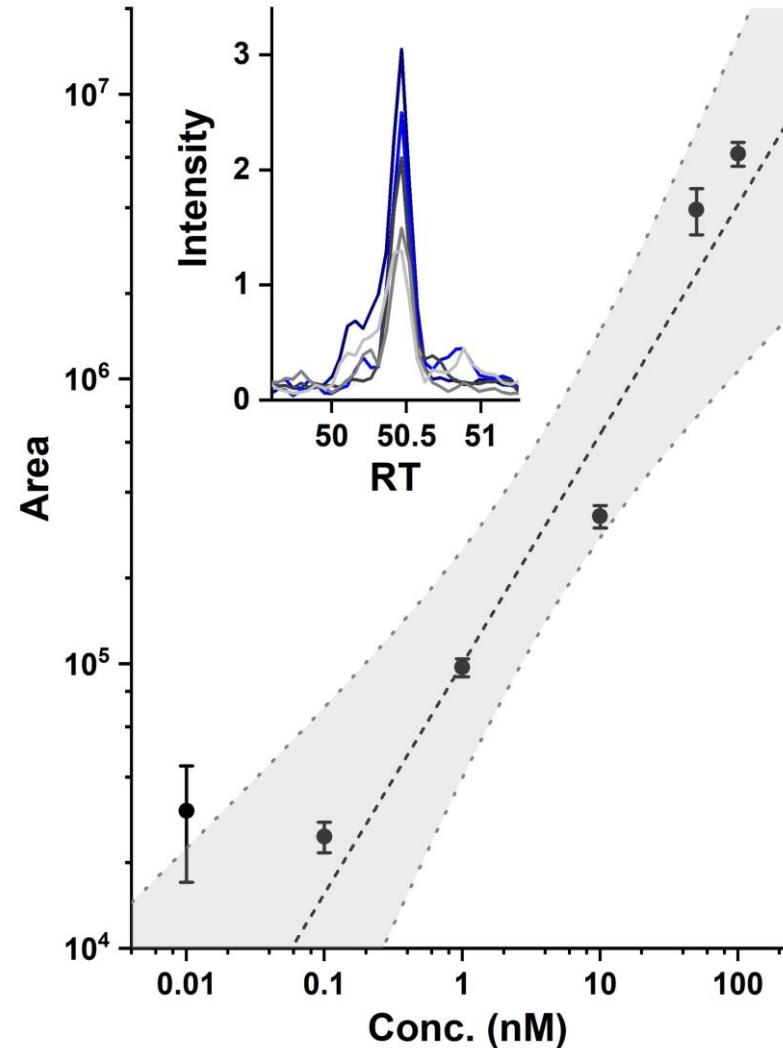
Technical Variability and Devising Method Control



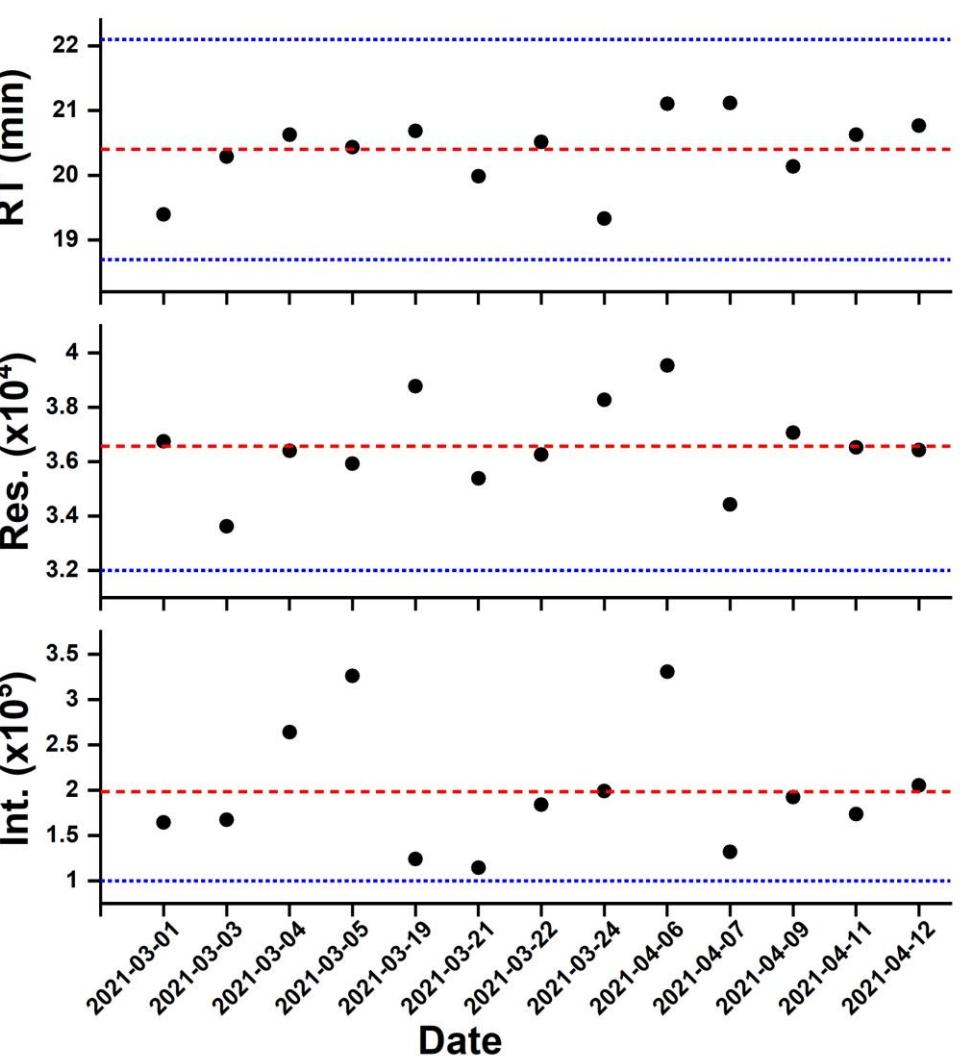
Instrument and measurement control design scheme



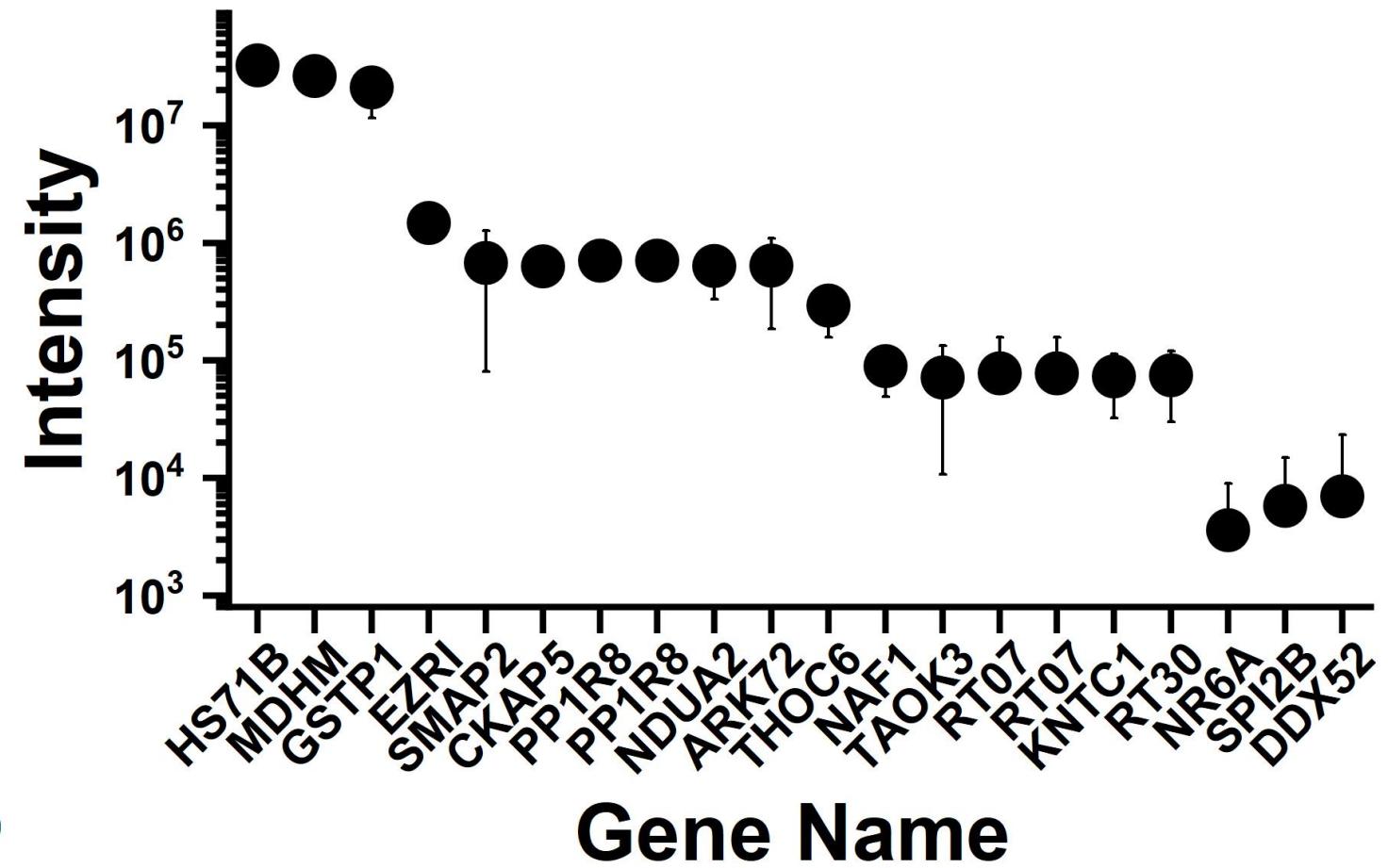
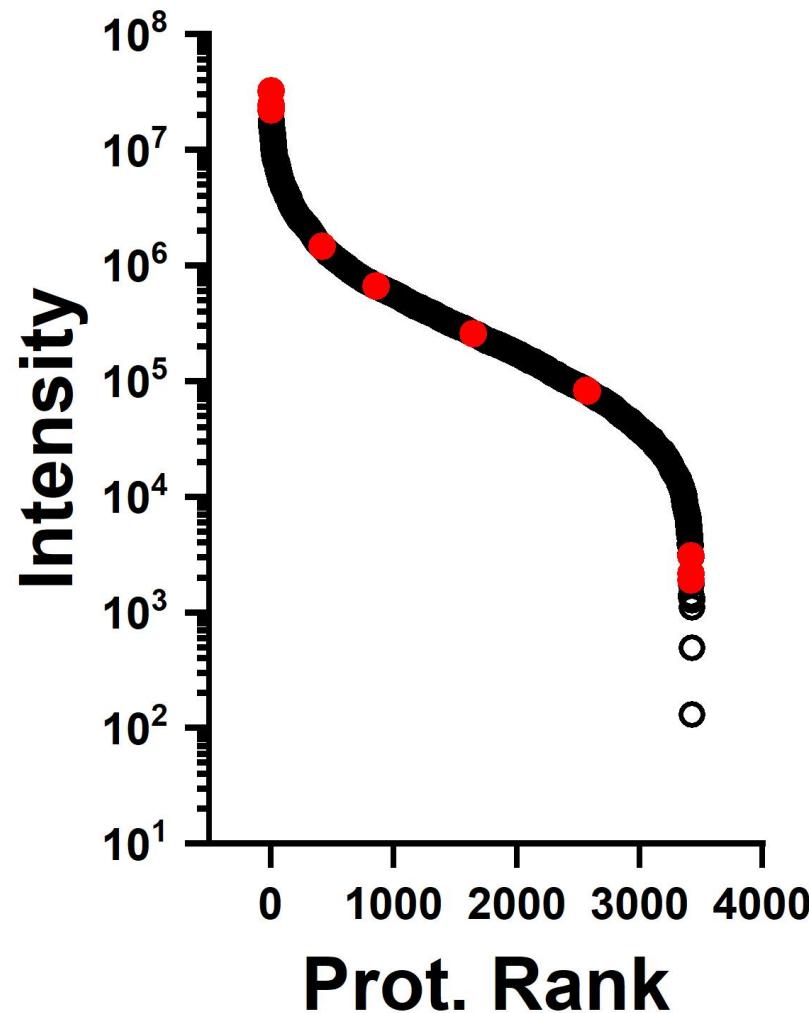
Some specific values for the sample: PepCal Mix



Lombard-Banek, C., et al., *in preparation*, 2021



Some specific values for SWATH® on K562



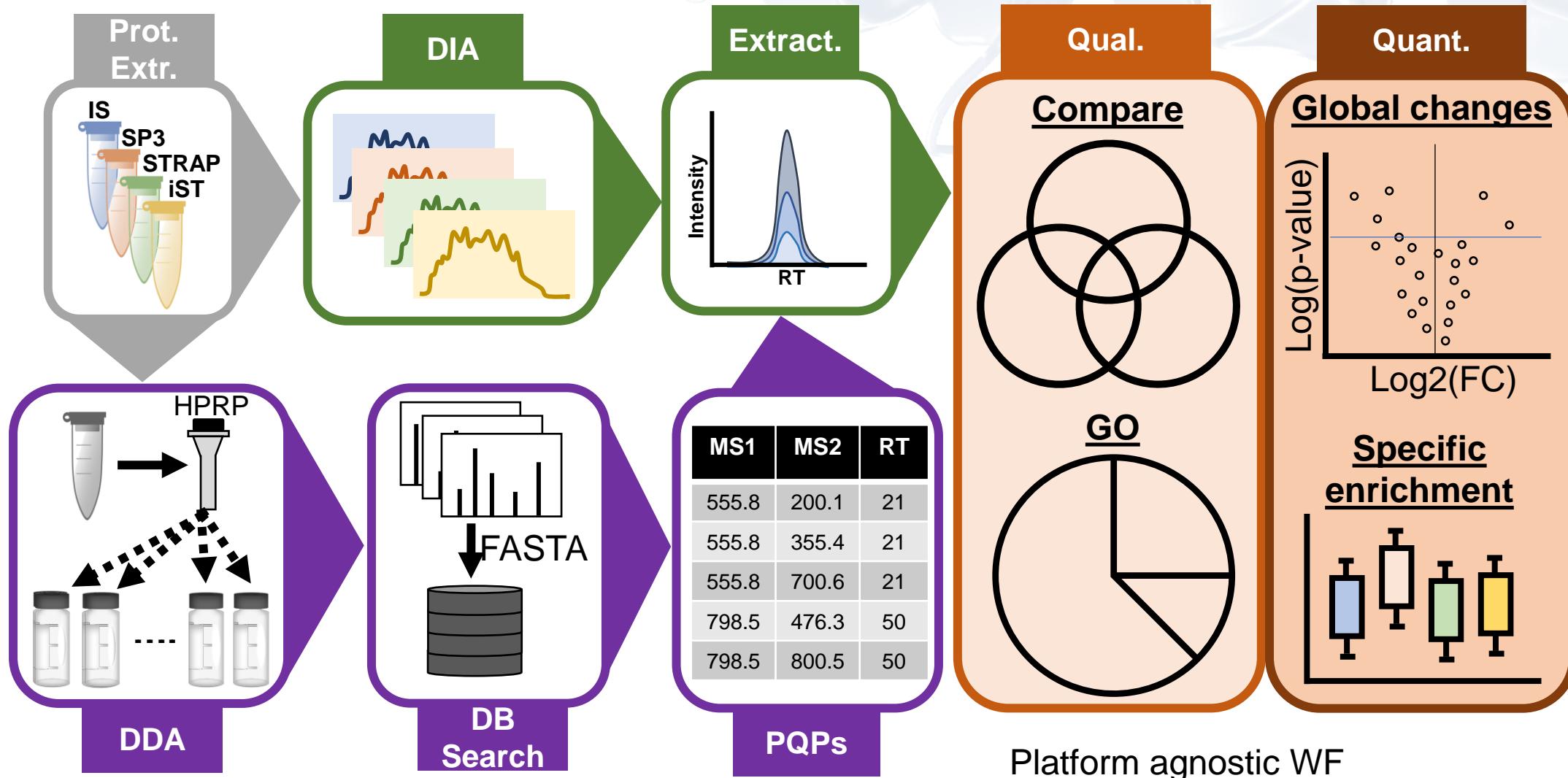
Extracting information from SWATH-MS data

2

Generating PQP Libraries

	MS1	MS2	RT
	555.8	200.1	21
	555.8	355.4	21
	798.5	800.5	50

General workflow for SWATH-MS data interpretation



Protein identification and quantification from SWATH® 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

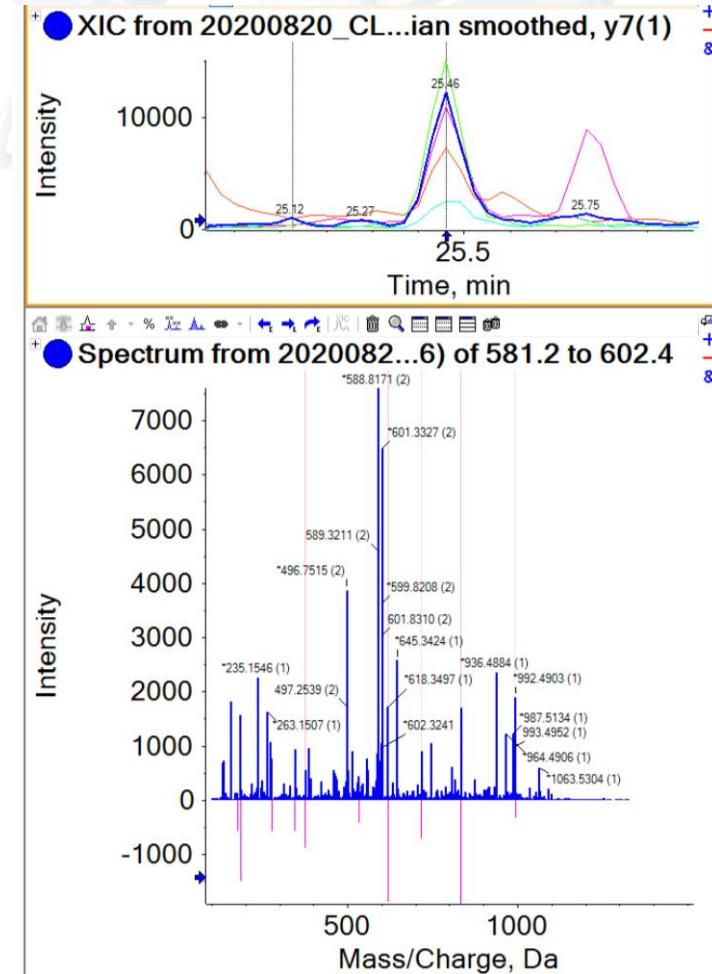
Current Sample: TIC from 20200820_CL04_SWATH-K562-1ug_2.wiff (sample 1) - Sample013

Proteins Show Selected Only Text Search:

N	Accession	Name
1	sp P78527 PRKDC_HUMAN	DNA-dependent protein kinase catalytic subunit OS=Homo sapiens OX=9606 GN=PRKDC PE=1 SV=3
2	sp Q14204 DYHC1_HUMAN	Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens OX=9606 GN=DYNC1H1 PE=1 SV=5
3	sp P49327 FAS_HUMAN	Fatty acid synthase OS=Homo sapiens OX=9606 GN=FASN PE=1 SV=3
4	sp Q15149 PLEC_HUMAN	Plectin OS=Homo sapiens OX=9606 GN=PLEC PE=1 SV=3
5	sp Q92616 GCN1_HUMAN	eIF-2-alpha kinase activator GCN1 OS=Homo sapiens OX=9606 GN=GCN1 PE=1 SV=6
6	sp P21333 FLNA_HUMAN	Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4
7	sp Q9Y490 TLN1_HUMAN	Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3
8	sp Q00610 CLTC_HUMAN	Clathrin heavy chain 1 OS=Homo sapiens OX=9606 GN=CLTC PE=1 SV=5
9	sp P35579 MYH9_HUMAN	Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9 PE=1 SV=4
10	sp Q6P2Q9 PRPF8_HUMAN	Pre-mRNA-processing-splicing factor 8 OS=Homo sapiens OX=9606 GN=PRPF8 PE=1 SV=2
11	sp O75643 U520_HUMAN	U5 small nuclear ribonucleoprotein 200 kDa helicase OS=Homo sapiens OX=9606 GN=SNRNP200 PE=1 SV=2
12	sp Q5T4S7 UBR4_HUMAN	E3 ubiquitin-protein ligase UBR4 OS=Homo sapiens OX=9606 GN=UBR4 PE=1 SV=1
13	sp P42704 LPPRC_HUMAN	Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens OX=9606 GN=LPPRC PE=1 SV=3
14	sp P49792 RANBP2_HUMAN	E3 SUMO-protein ligase RanBP2 OS=Homo sapiens OX=9606 GN=RANBP2 PE=1 SV=2
15	sp P07814 SYEP_HUMAN	Bifunctional glutamate/proline-tRNA ligase OS=Homo sapiens OX=9606 GN=EPERS1 PE=1 SV=5
16	sp Q25580 MYH10_HUMAN	Myosin-10 OS=Homo sapiens OX=9606 GN=MYH10 PE=1 SV=2

Peptides Text Search:

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

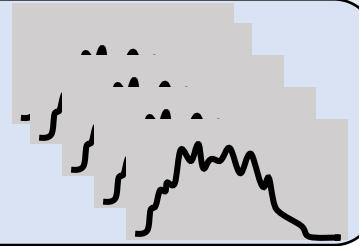


PeakView®
Software

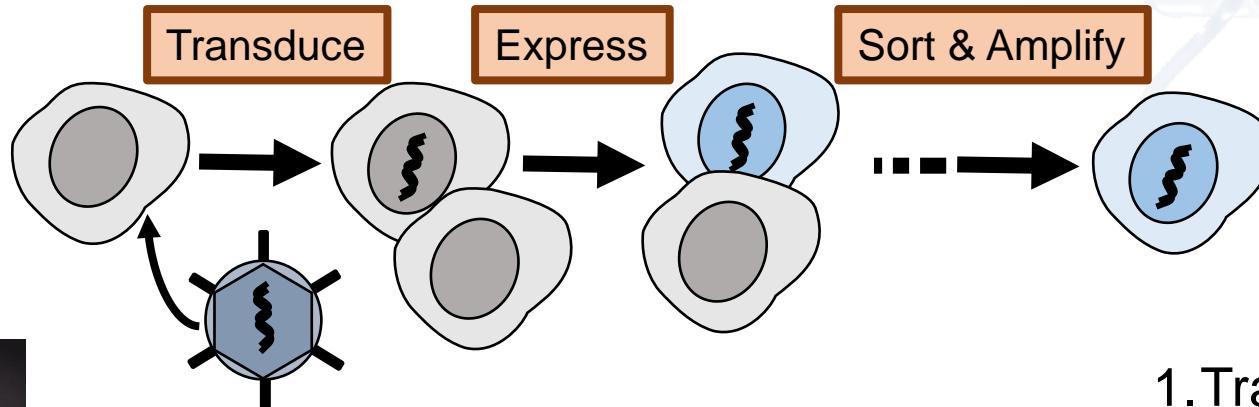
Example of SWATH-MS measurements for a CAR-T model

3

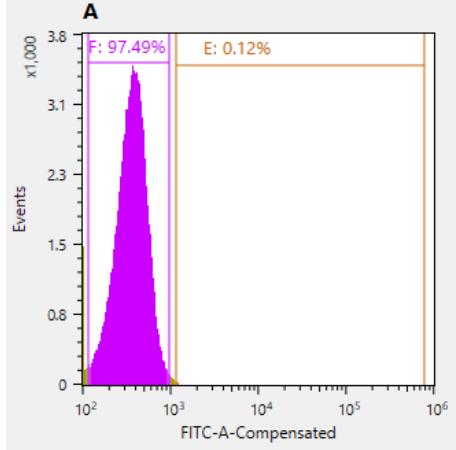
SWATH-MS Measurements



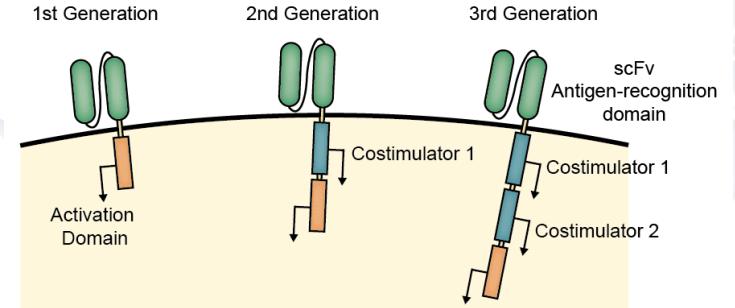
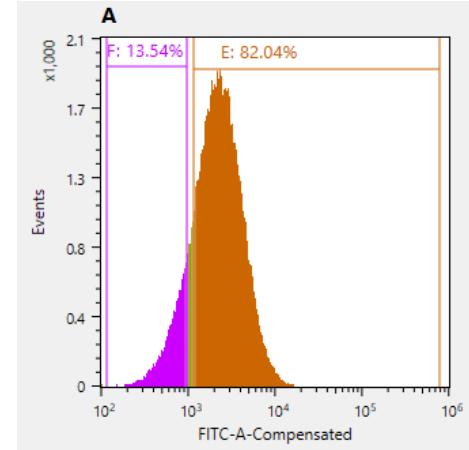
CAR-Jurkat model to advance analytics



Before transduction



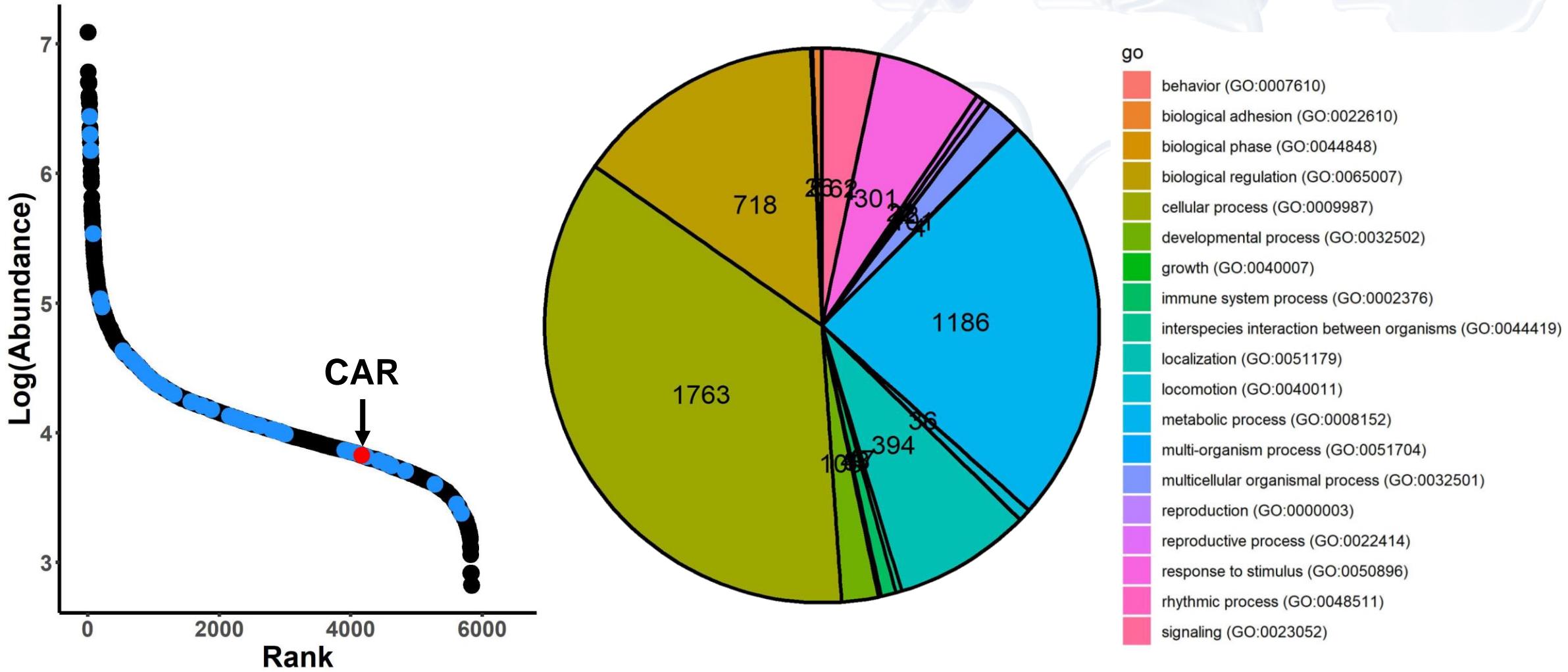
2nd sorting



1. Transduced to express anti-HER2 CAR (Gen 2)

2. Future application of this model:
1. Pre/post transduction
 2. MQAs assessment
 3. In process samples for process development/monitoring
 4. PQAs identification and product control

Example of SWATH-MS measurements of CAR-Jurkat cells

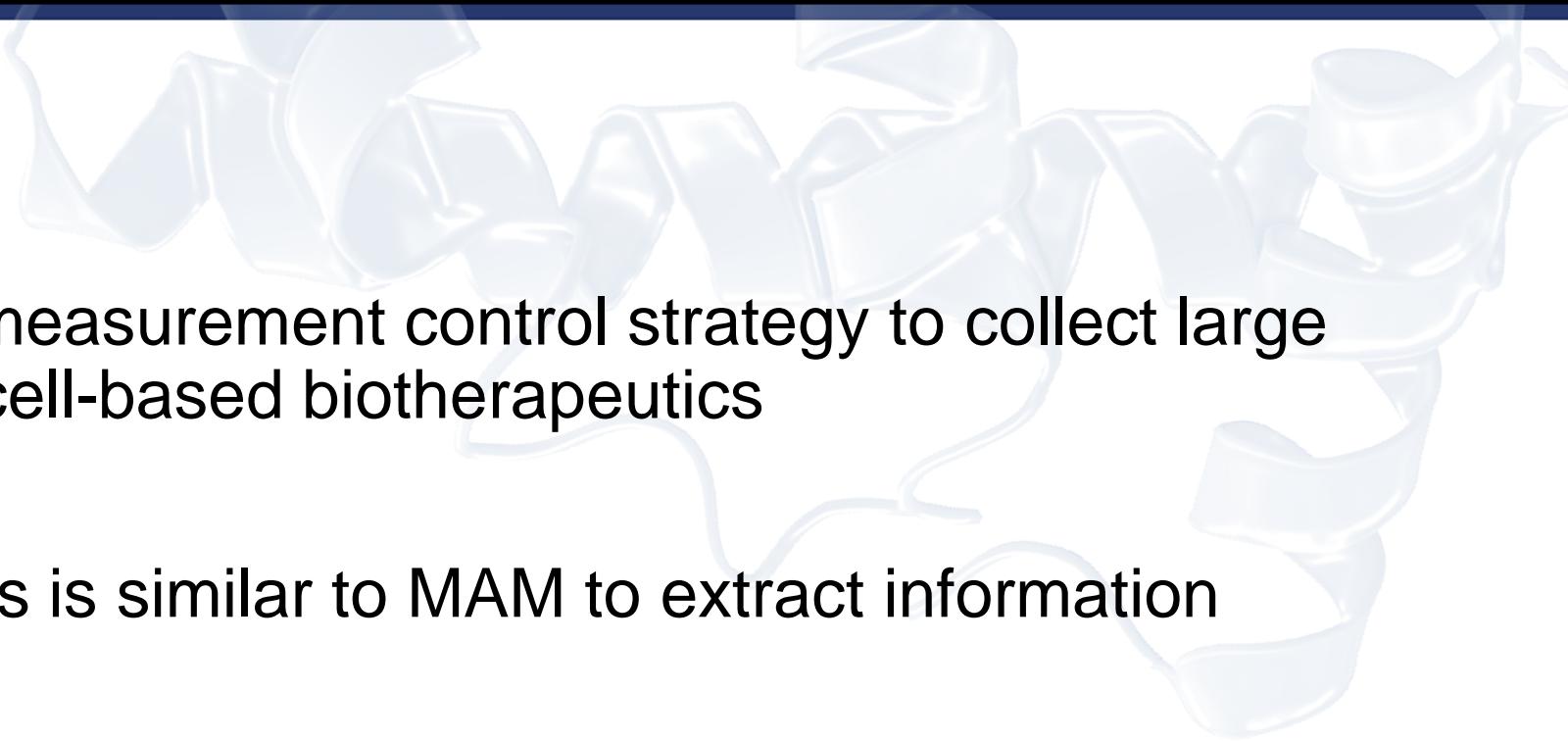


Coverage of the CAR protein with targeted peak extraction

MALPVTALLLPLALLLHAARPDIQMTQSPSSLASVGDRVTI **T**CRA **S**QDVNTAVAWYQOK
PGKAPKLLI **I**Y **S**ASF**L**Y SGVP SRF SG SR SG TDF TL TI **S**SLQPEDFATYYCQQHY TPPPTFG
QG TKVE IKGGGG SGGGG SGGGG SEVQLVE SGGGLVQPGGSLRLSCAA SGFNIKD TY I HWV
RQAPGKGLEWVARI **I**Y PTNGY TRYAD SVKGRFTI **S**AD TSKN TAYLQMNSLRAED TAVYYCS
RWGGDGFYAMDYWGQGTLVTV **S**STTPAPRPP TPAP TIA **S**QPL **S**LRPEACRPAAGGAVH T
RGLDFACDFWVLVVVGGVLACY **S**LLTVAFII FWVR SKR **S**RLLH **S**DYMNMTPRRPGP **TR**K
HYQPYAPPRDFAA **Y**RSRVKF **S**R SADAPAYKQGQNQLYNELN **N**LGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELOQDKMAEAYSEIGMKGERRGKGHDGLYQGLSTATKD **T**YDAL
HMQALPPR

37.7% sequence coverage

Conclusions

- 
1. We have established a measurement control strategy to collect large scale proteomic data for cell-based biotherapeutics
 2. PQP-based data analysis is similar to MAM to extract information from RAW data
 3. SWATH-MS measurements of CAR-Jurkat showed broad dynamic range and possibility to identify/quantify the CAR protein
 - Identification of the CAR protein with ~38 % coverage from large scale data.

Disclaimer: Instruments identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology

Acknowledgements

- Schiel's laboratory
 - Trina Mouchahoir
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 - Sumona Sarkar

