



MAG-NET

Bead based capture of membrane particles from plasma

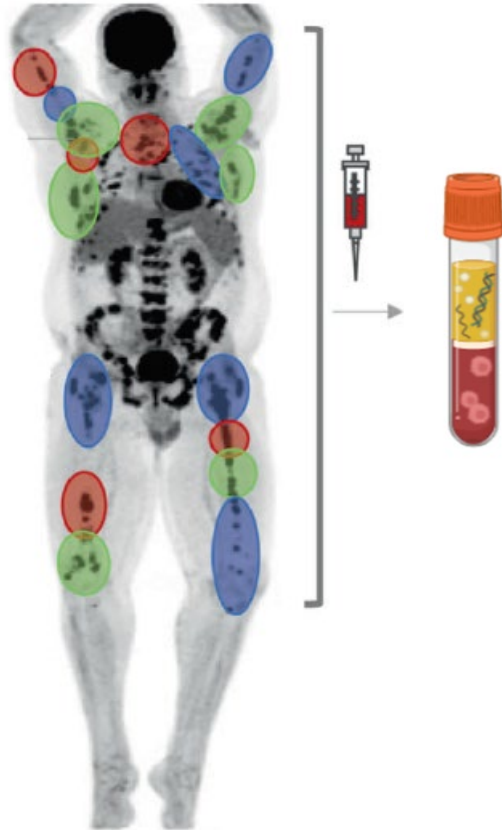


ReSynBio



Stoyan Stoychev
Head of Proteomics, ReSynBio
Senior Researcher, Evosep

Plasma: A Rich Source of Disease Relevant Information



Easy to obtain / Low cost

**Physiological / pathological
relevance**

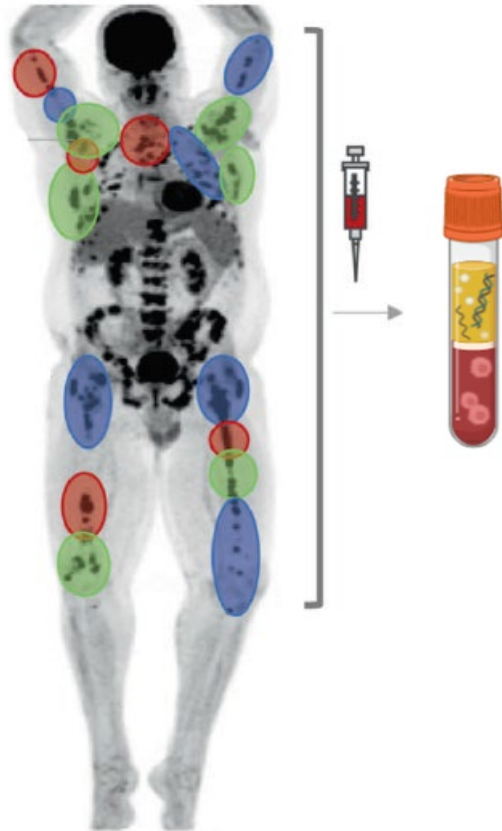
Mithraprabhu *et al.*, 2023. Nature. DOI: 10.1038/s41375-021-01339-6

Arancio *et al.*, 2017. Liquid biopsy in cancer patients. DOI: 10.1007/978-3-319-55661-1_4



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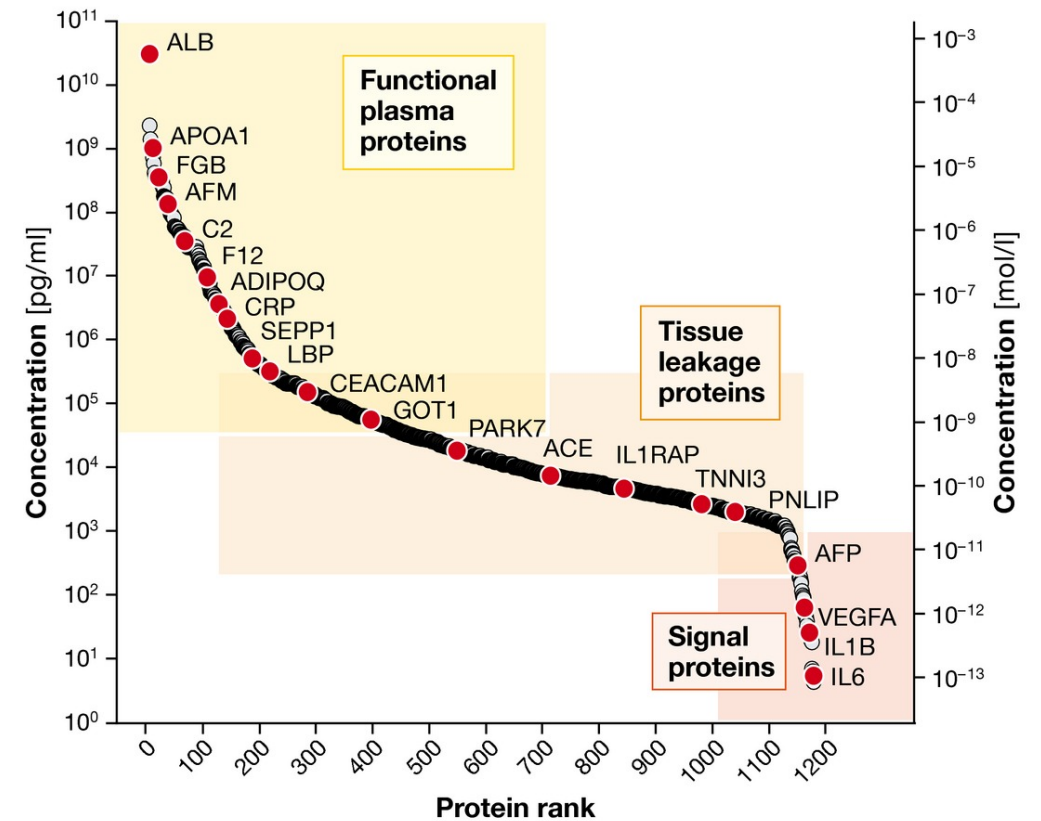
Plasma: Challenging to profile



Easy to obtain / Low cost

Physiological / pathological relevance

The challenge of plasma proteomics



Mithraprabhu *et al.*, 2023. Nature. DOI: 10.1038/s41375-021-01339-6

Arancio *et al.*, 2017. Liquid biopsy in cancer patients. DOI: 10.1007/978-3-319-55661-1_4

Geyer *et al.*, 2017. DOI: 10.15252/msb.20156297

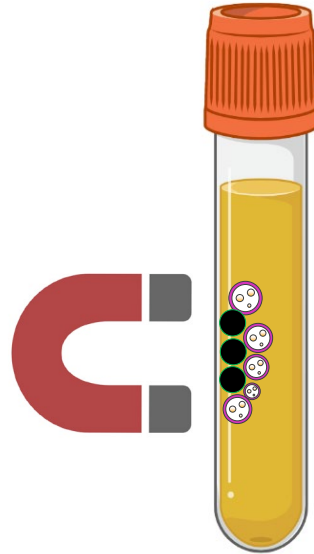


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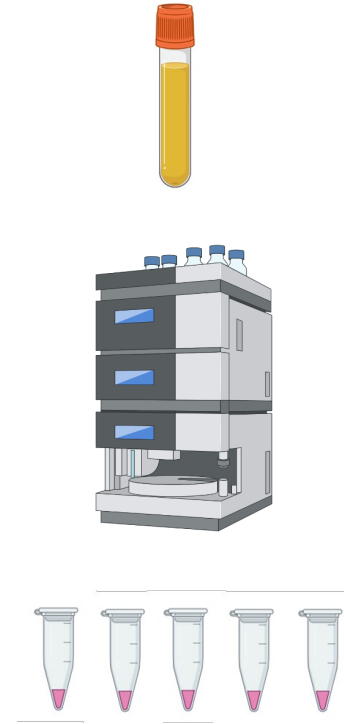
Plasma Proteome Profiling: Divide and Conquer



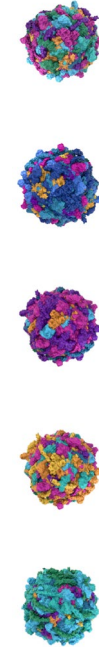
DEplete



ENRICH



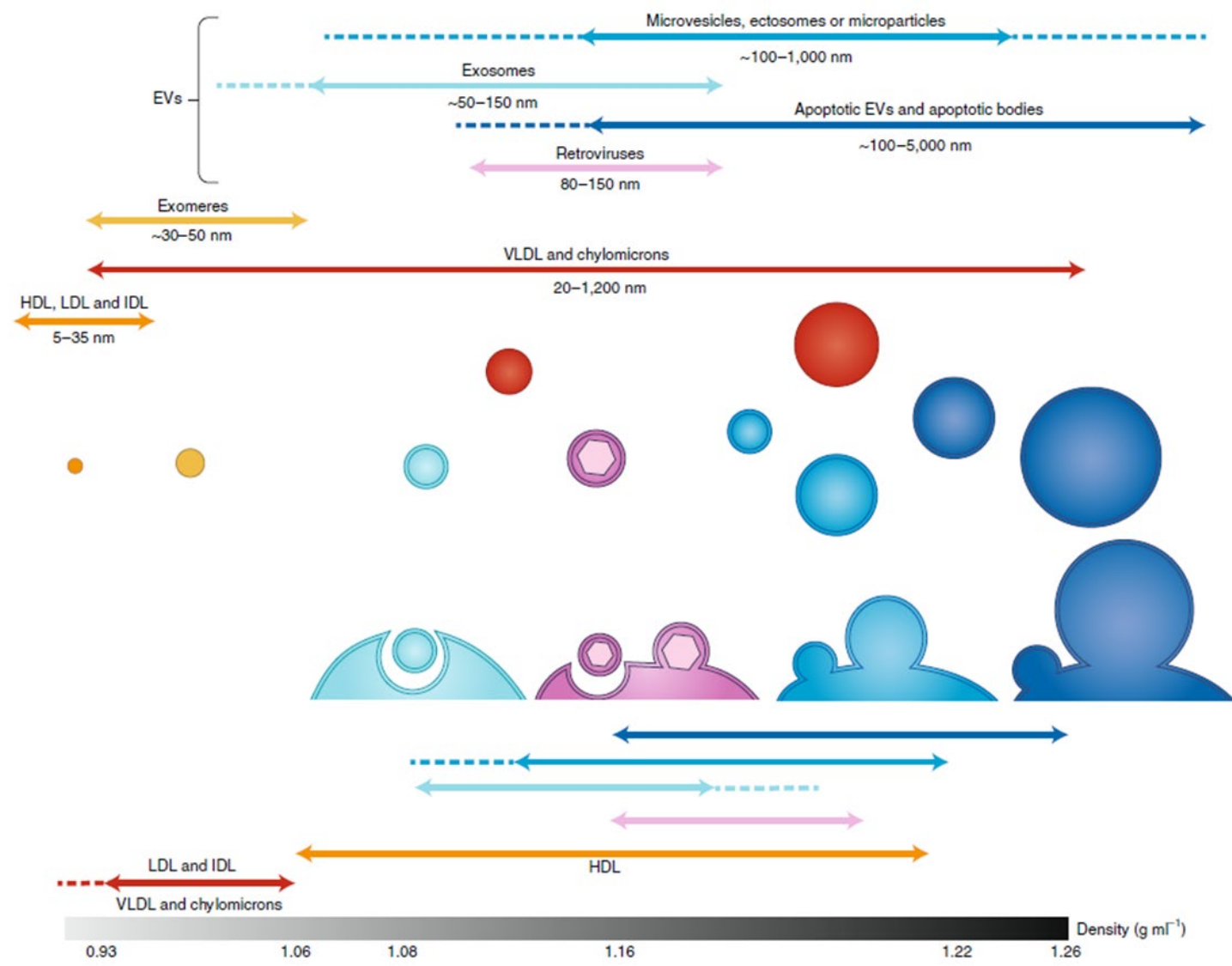
FRACTIONATE



PROTEIN CORONA

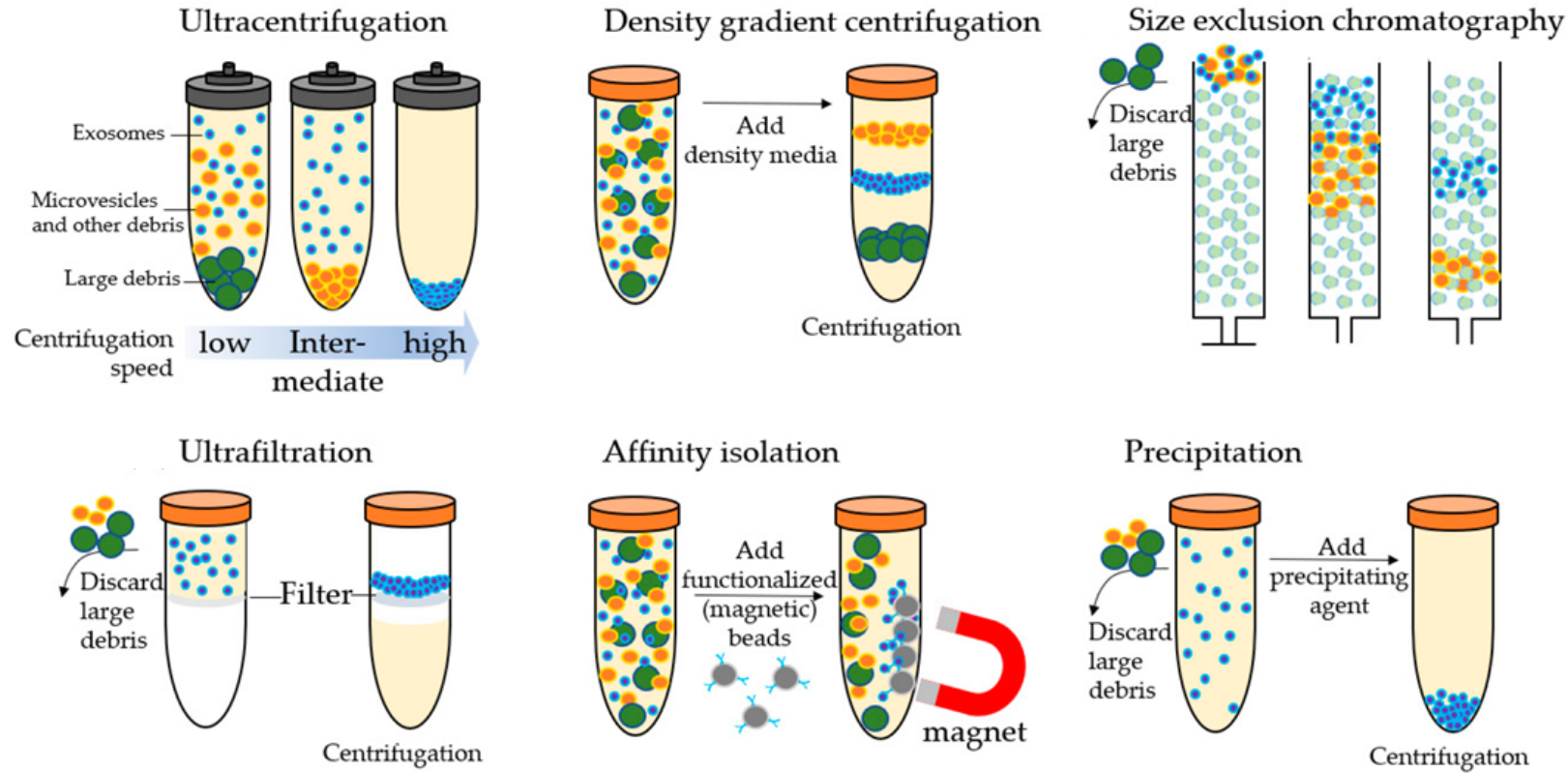


Extracellular vesicles (EVs)



Mathieu, et al. 2019. DOI:10.1038/s41556-018-0250-9

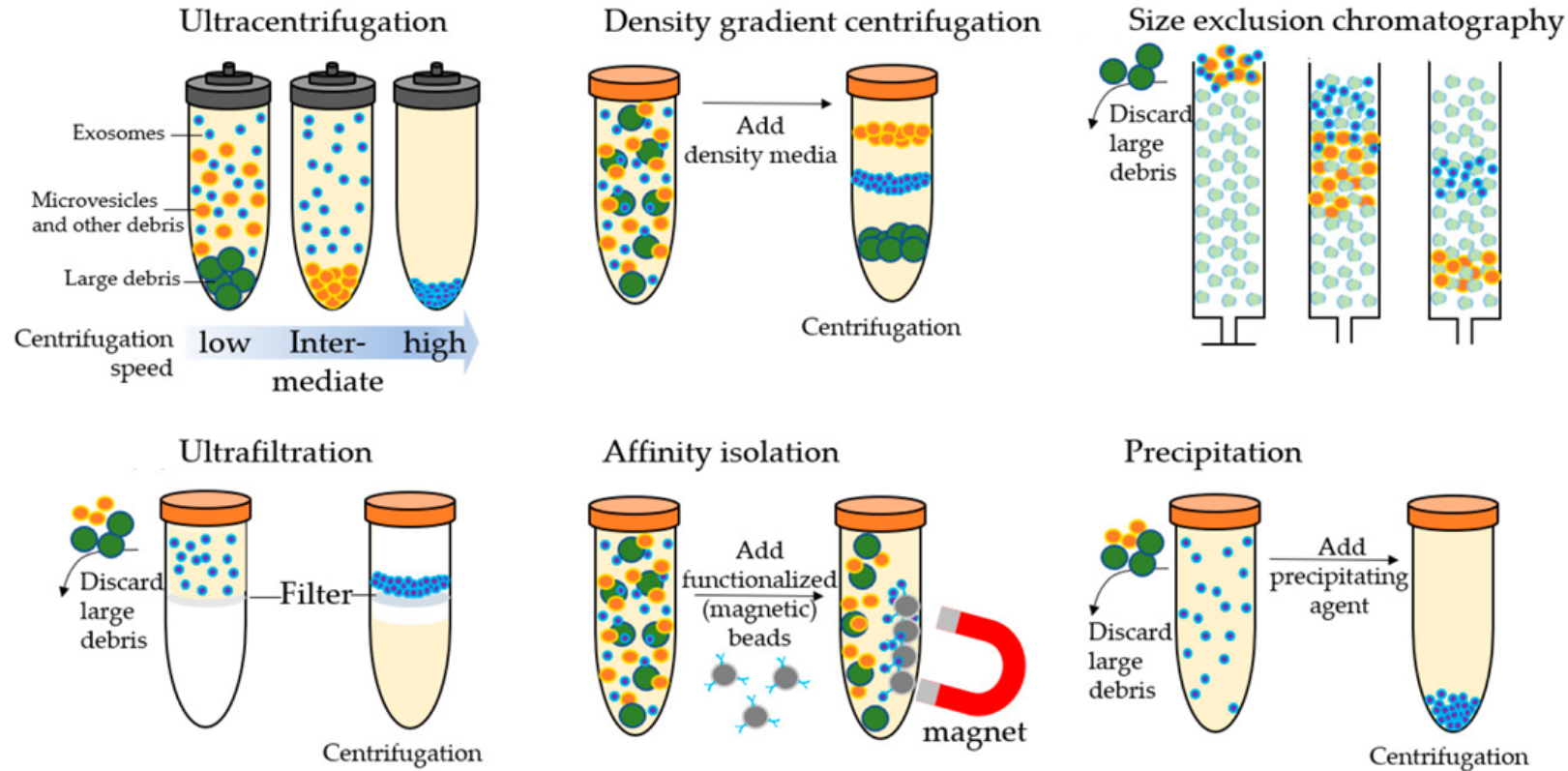
Current Methods to Isolate EVs



Wang *et al.*, 2020. *Cancers*: DOI:10.3390/cancers12092335



Current Methods to Isolate EVs



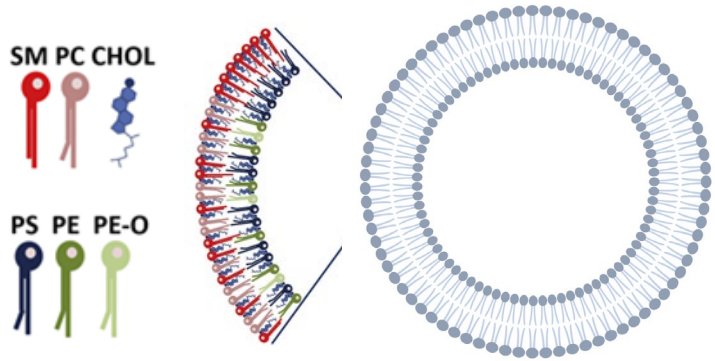
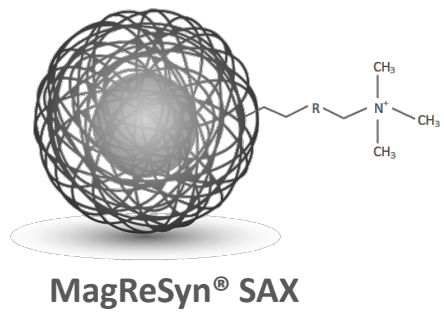
- Large volume of plasma
- Difficult to scale
- Can be costly especially immunoaffinity

Wang *et al.*, 2020. *Cancers*: DOI:10.3390/cancers12092335

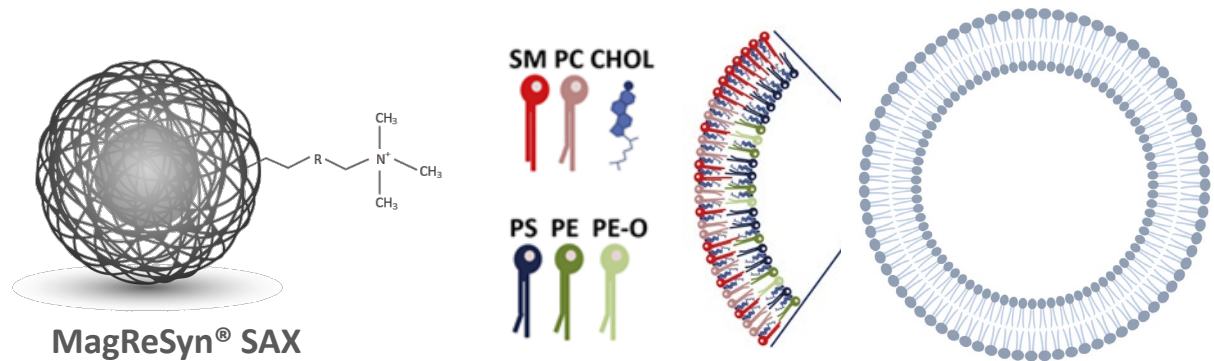


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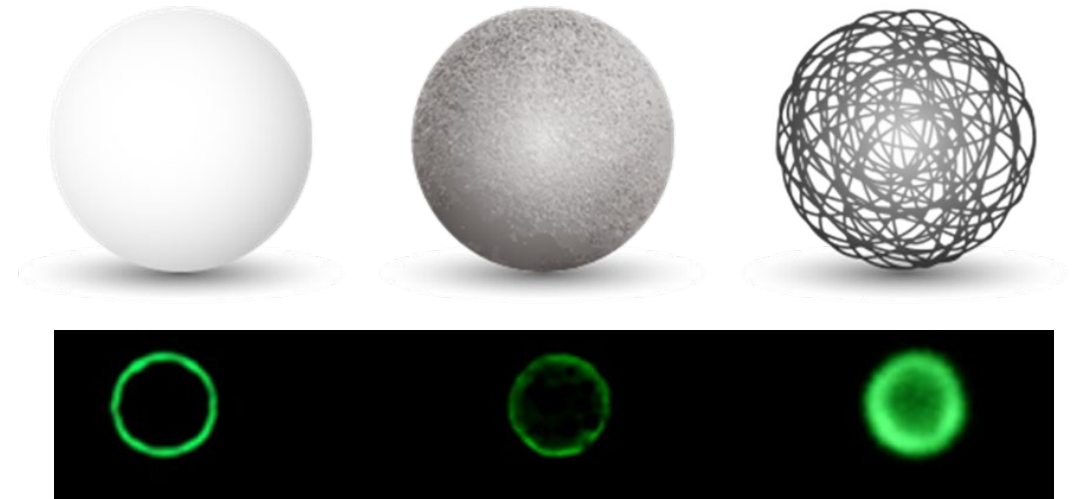
Mag-Net: Capture of EVs Using MagReSyn® Technology



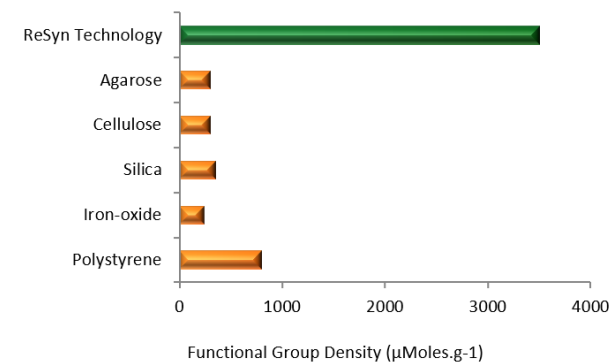
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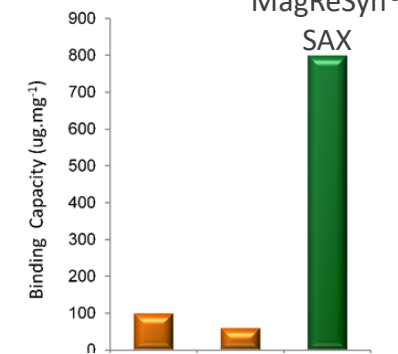
Hyper-porous polymer bead technology



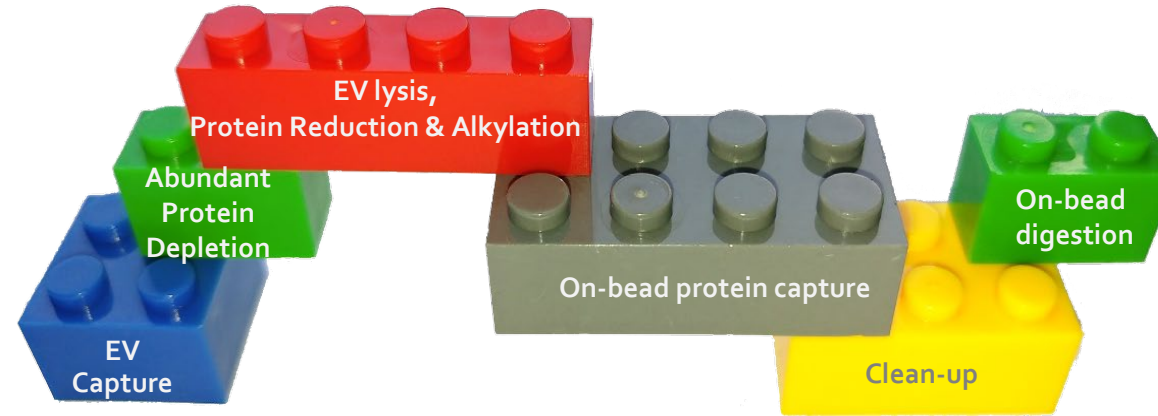
High functional group density



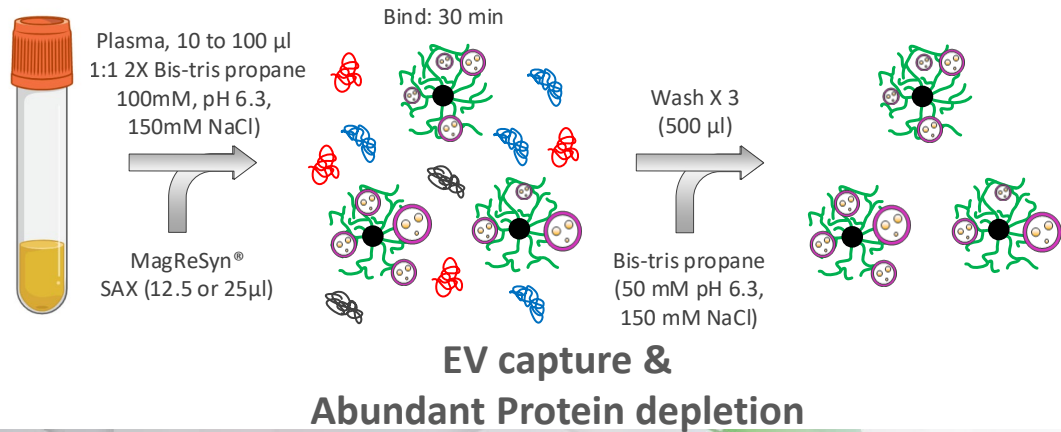
Increased capacity MagReSyn®



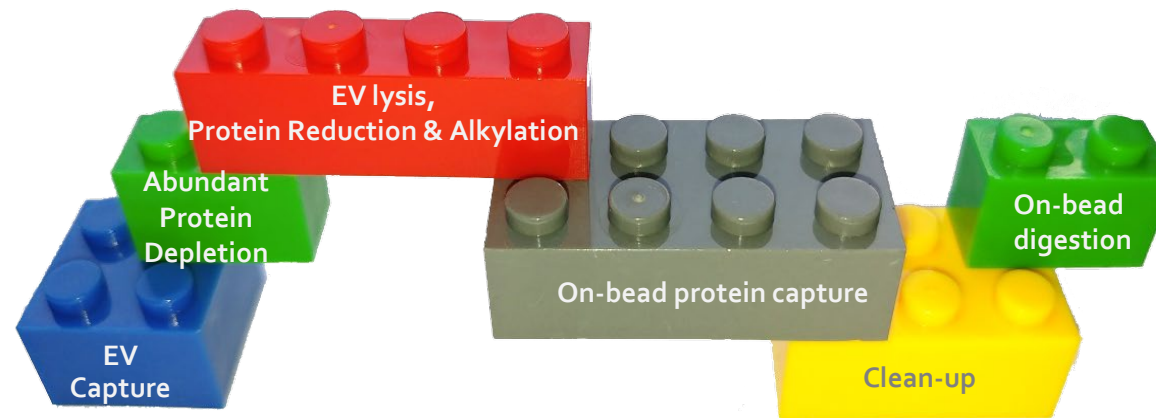
Mag-Net: End-to-End Plasma to Mass Spec Workflow



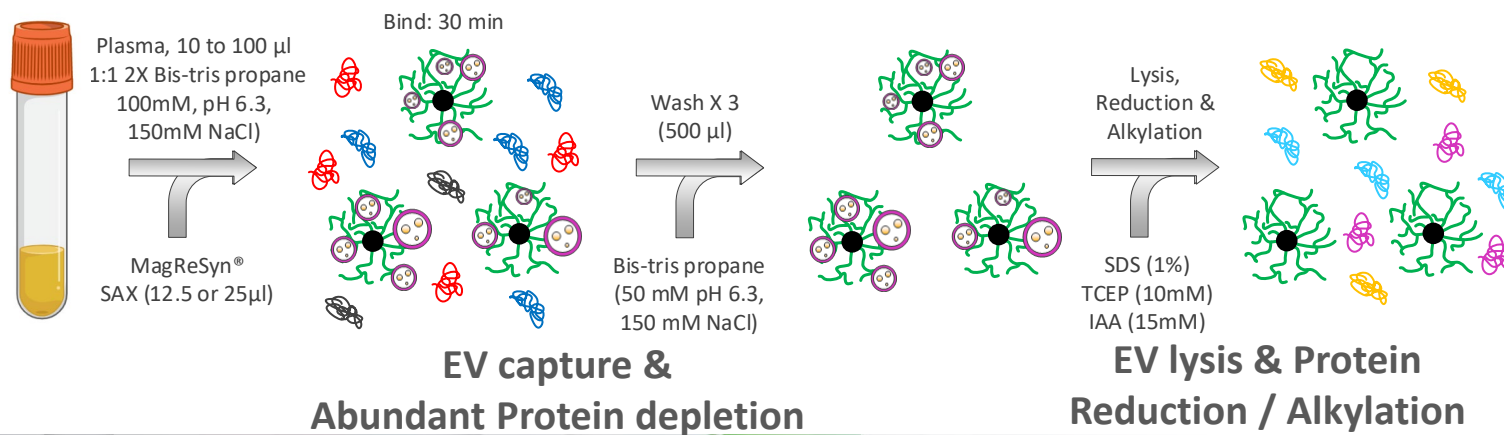
Seamless, High-Throughput, Easy to Automate



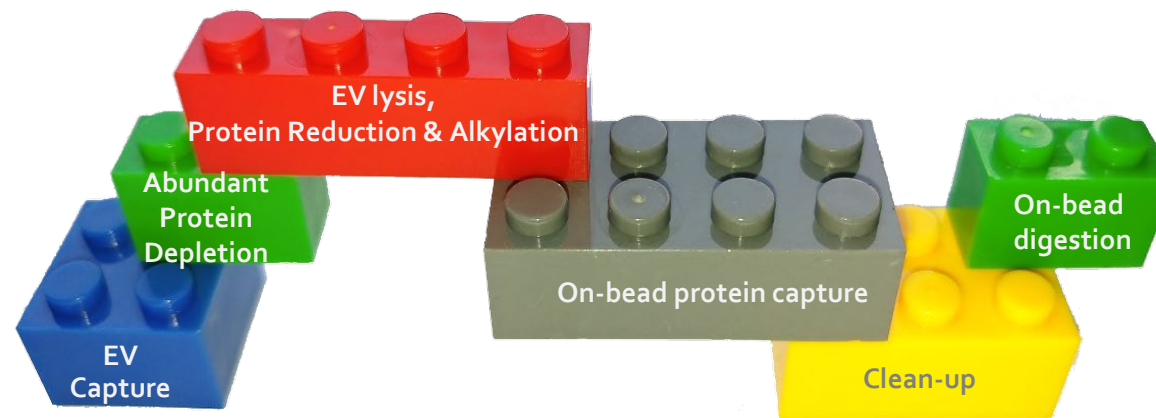
Mag-Net: End-to-End Plasma to Mass Spec Workflow



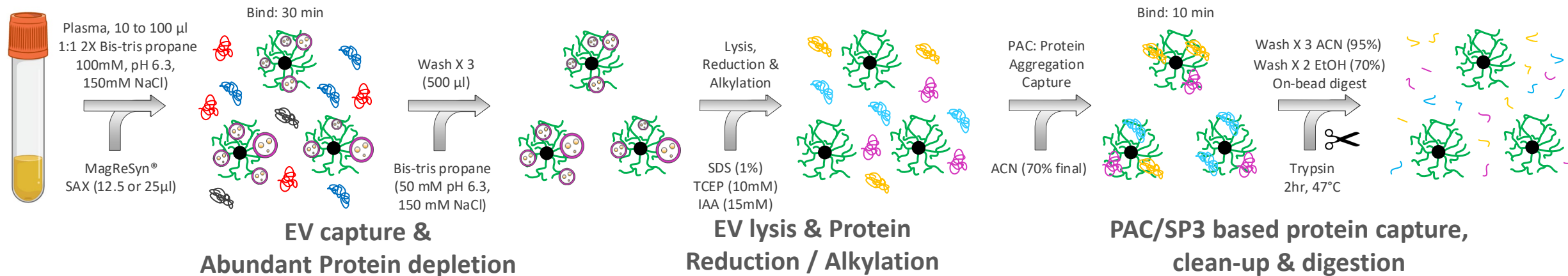
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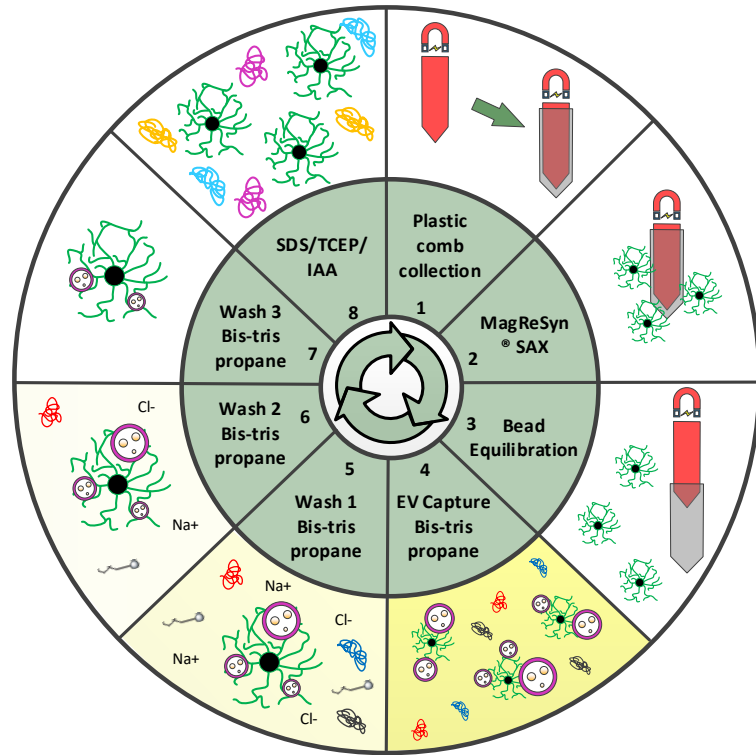
Mag-Net: End-to-End Plasma to Mass Spec Workflow



No bead change & No transfers – Minimal sample loss & Cost-effective

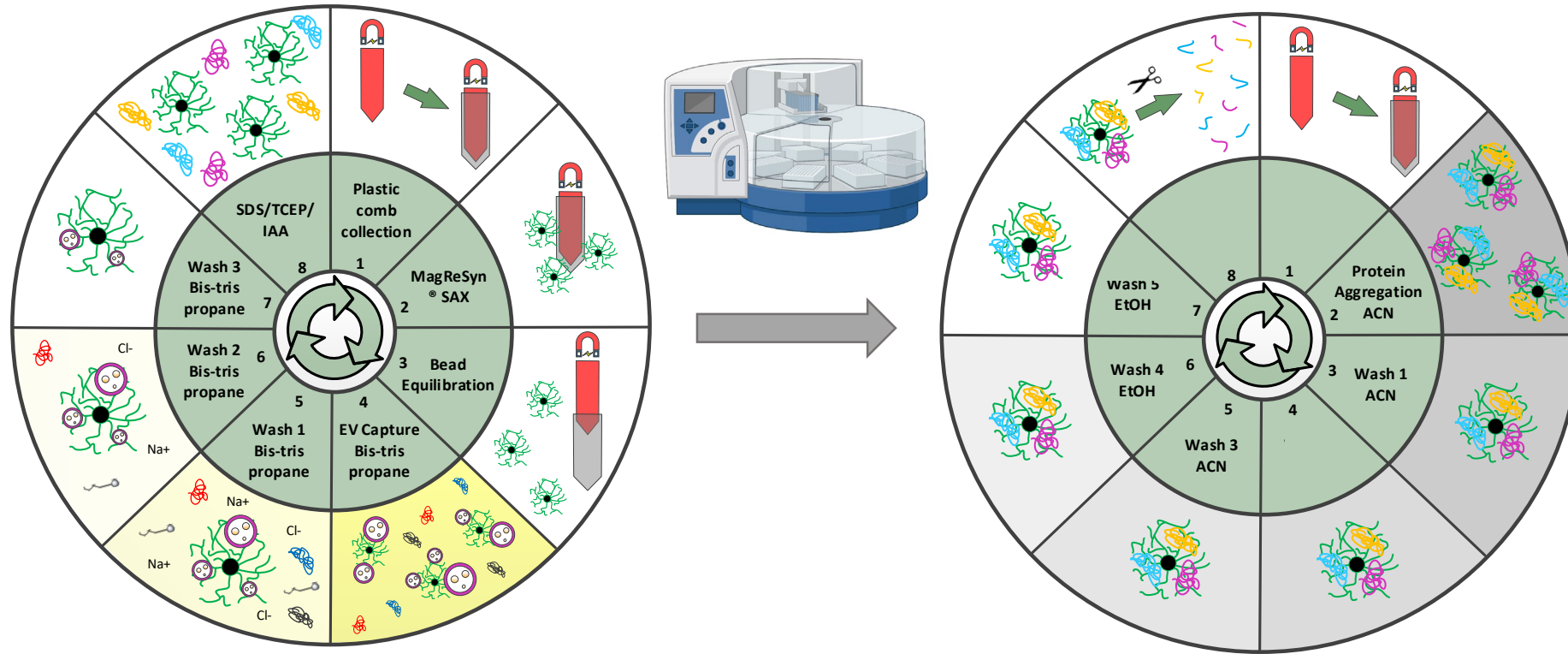


Mag-Net: Plasma to Mass Spec Automation



- EV Capture
- Abundant protein depletion
- EV lysis + protein reduction & alkylation

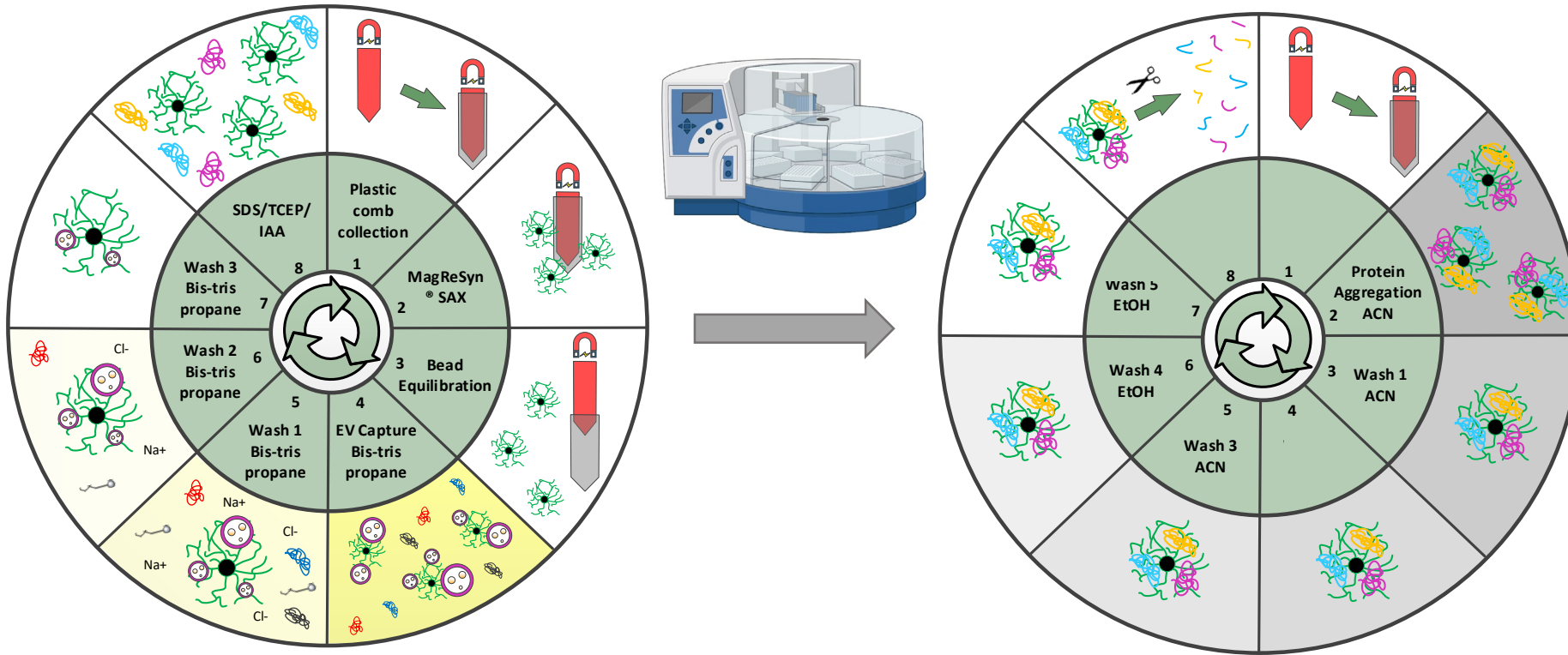
Mag-Net: Plasma to Mass Spec Automation



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- EV lysis + protein reduction & alkylation

- PAC/SP3 based protein capture, clean-up and digestion

Mag-Net: Plasma to Mass Spec Automation

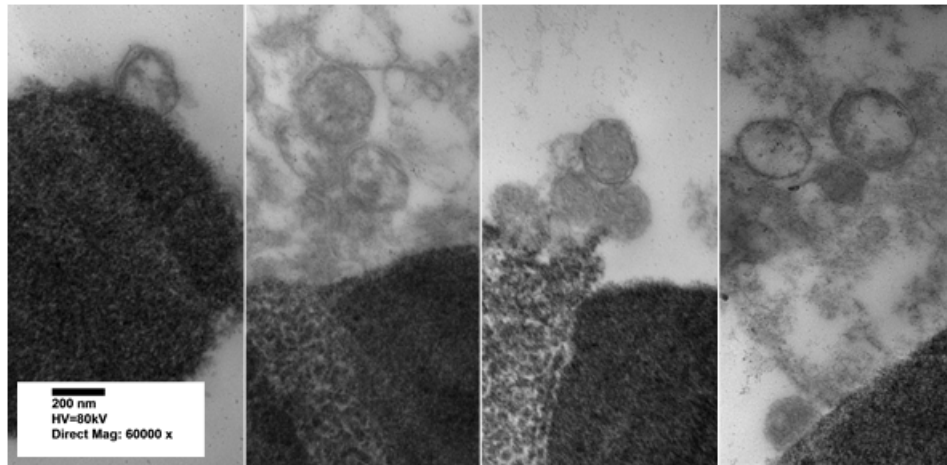
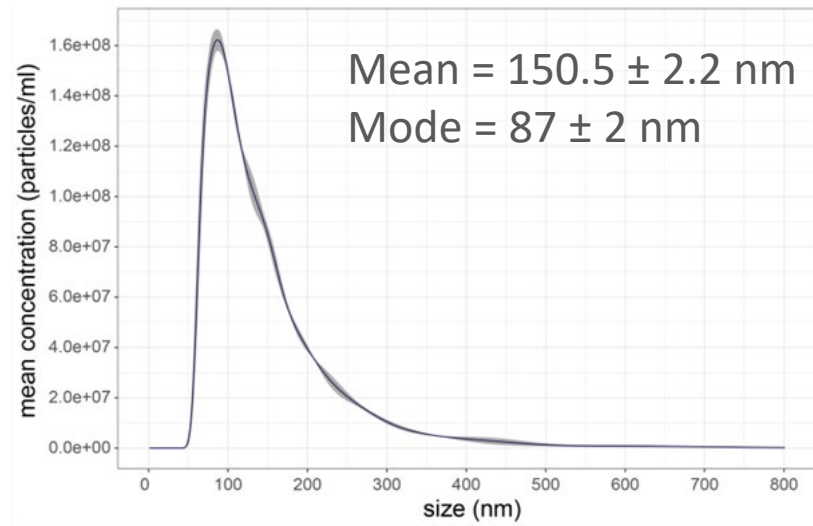


- EV Capture
- Abundant protein depletion
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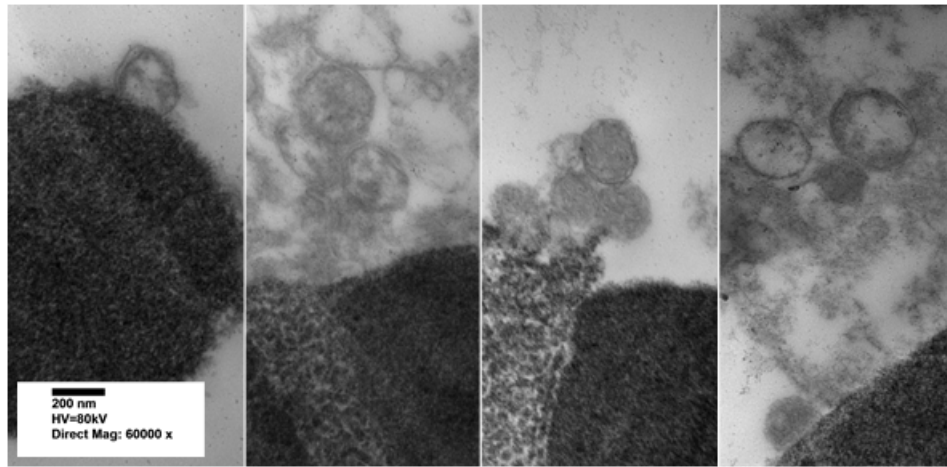
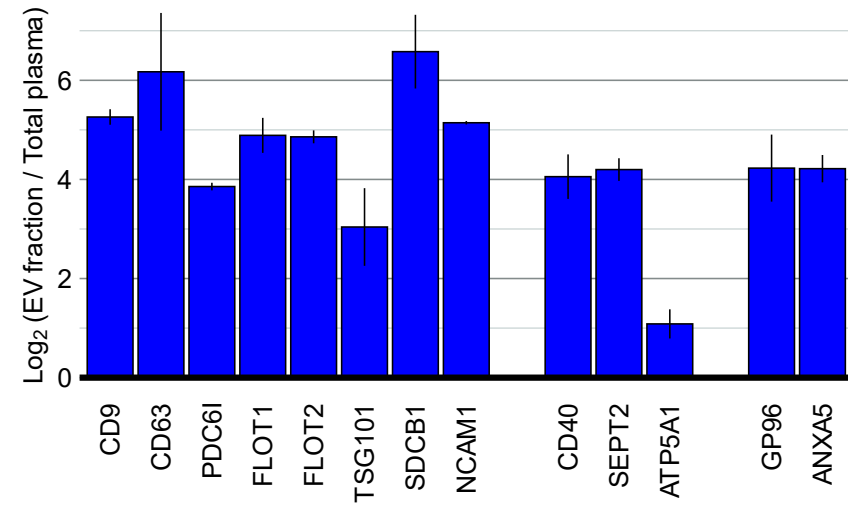
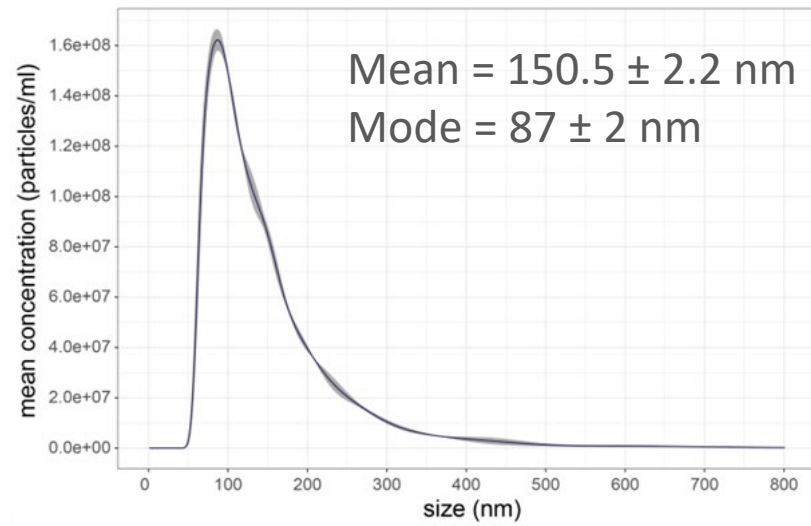
- PAC/SP3 based protein capture, clean-up and digestion

- 96 samples in parallel
- No bottleneck steps
 - Centrifugation
 - Filtration
 - Buffer exchange
- ~4hrs (incl. digest): plasma to mass spec ready peptides

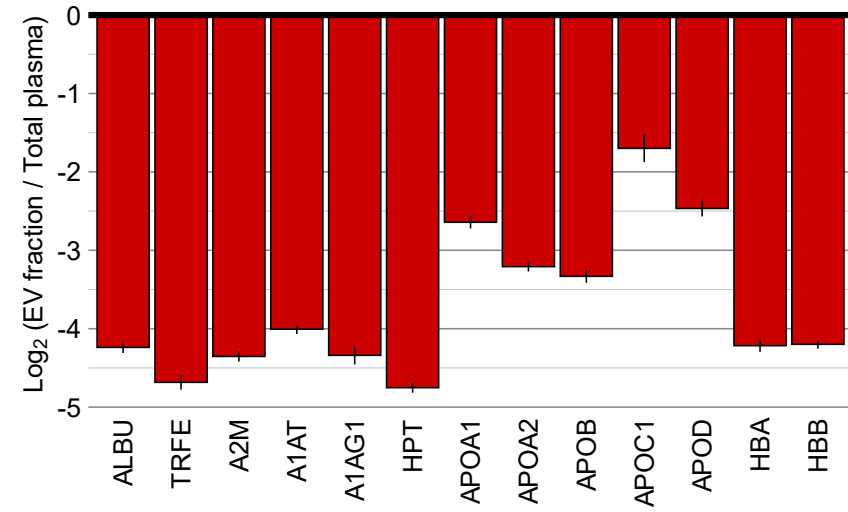
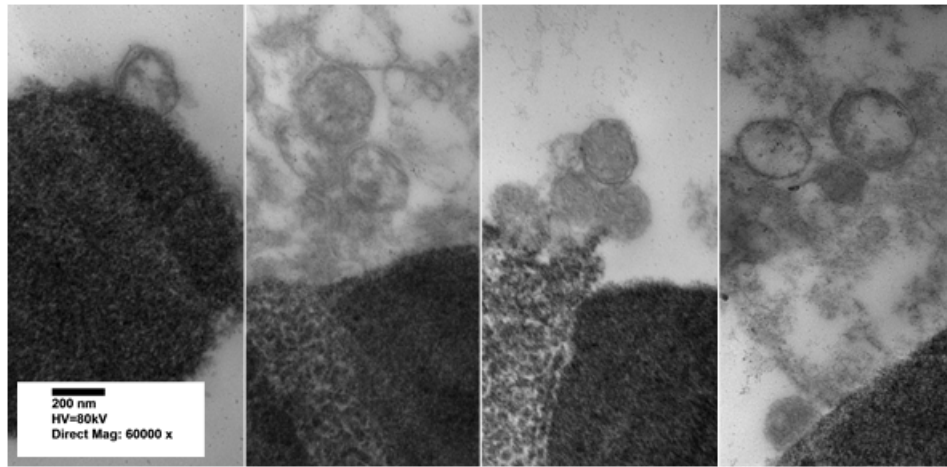
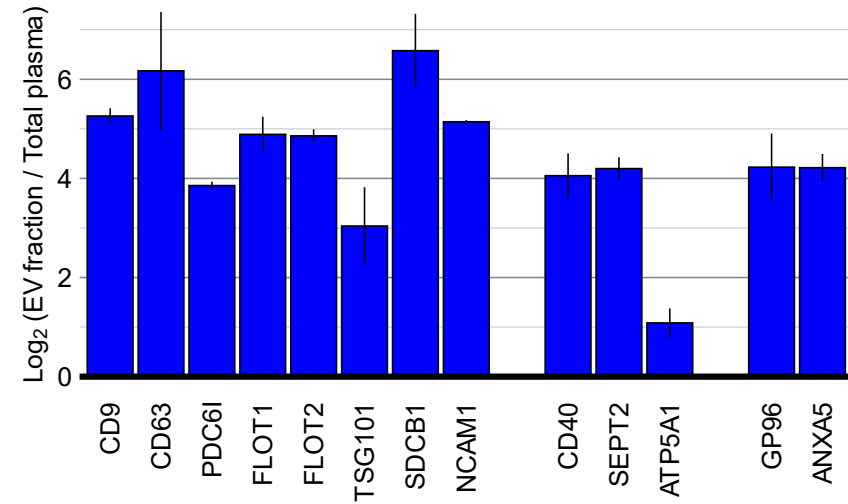
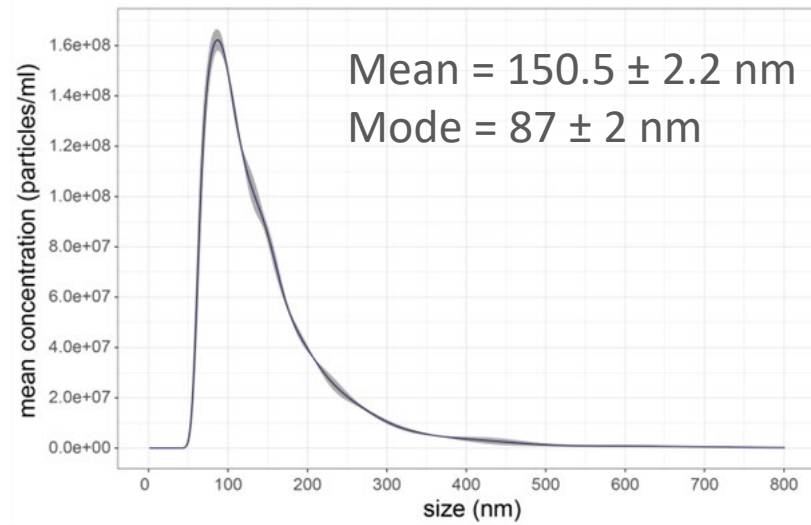
Mag-Net: EV Capture



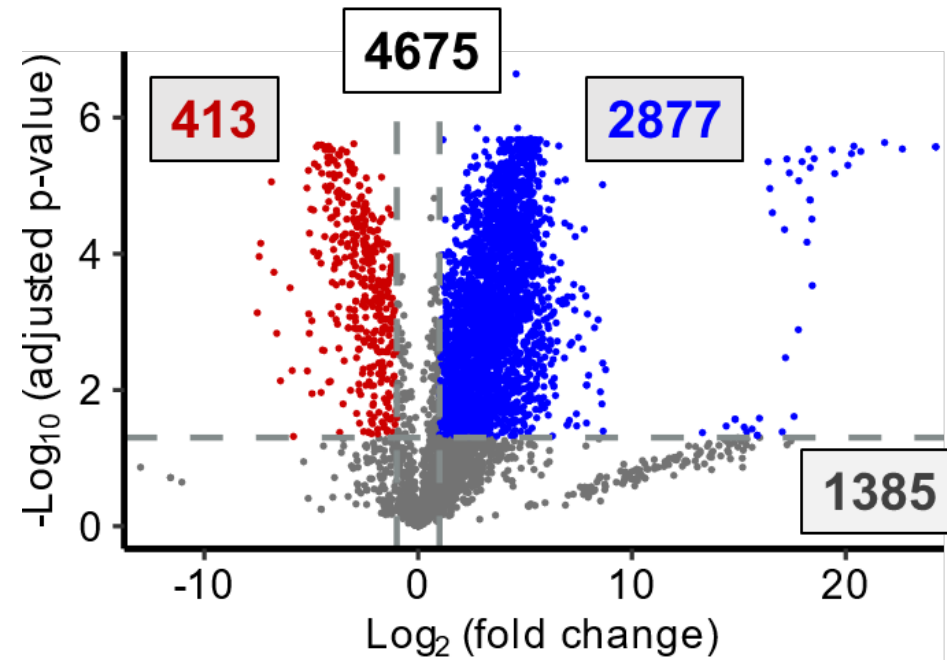
Mag-Net: EV Capture



Mag-Net: EV Capture & Abundant Protein Depletion

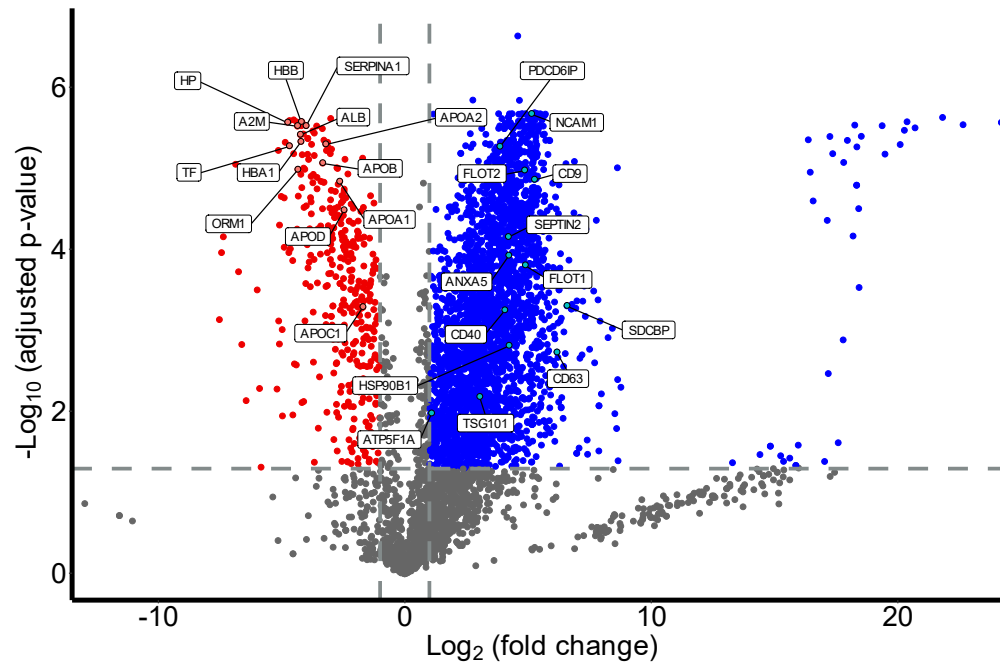


Mag-Net: Improves dynamic range for deeper plasma profiling



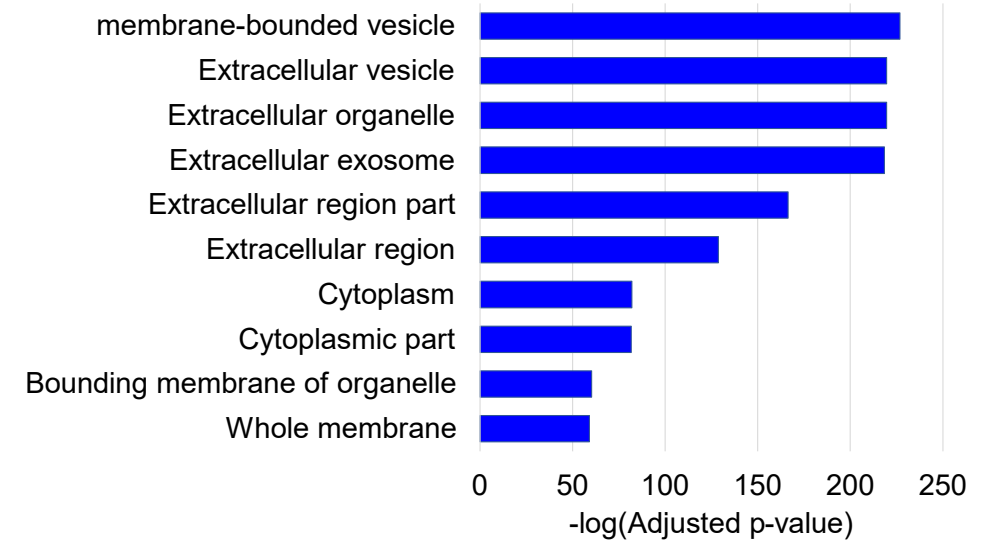
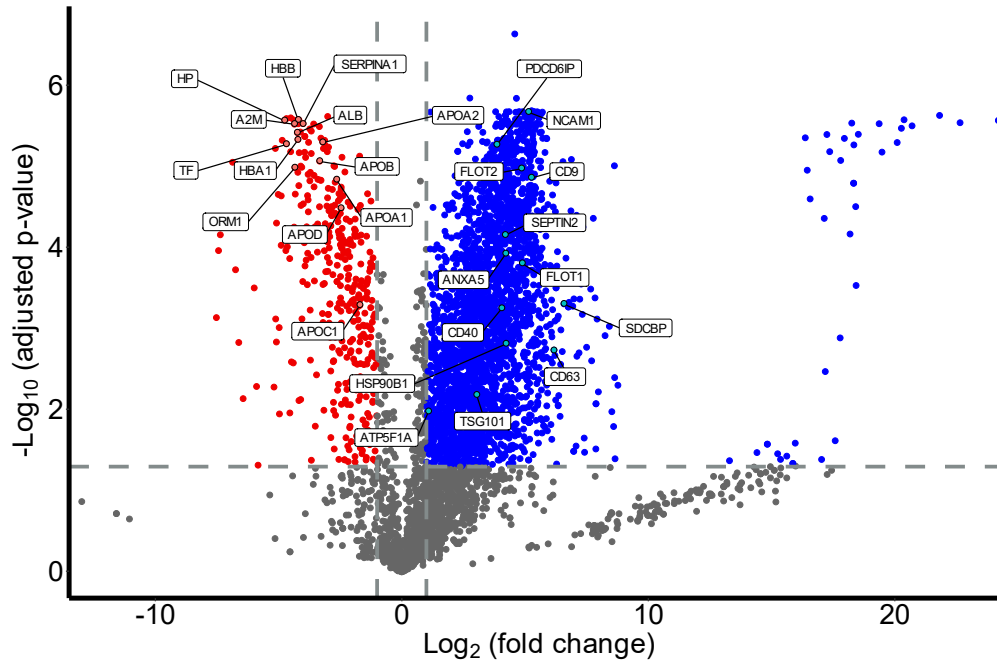
- 100 μl plasma
- 75 μm x 25cm, 90min Gradient
- Orbitrap Eclipse
 - 4mz staggered window GPF library
 - 12mz staggered window individual injections
- EncyclopeDIA + GPF library

Mag-Net: Improves dynamic range for deeper plasma profiling



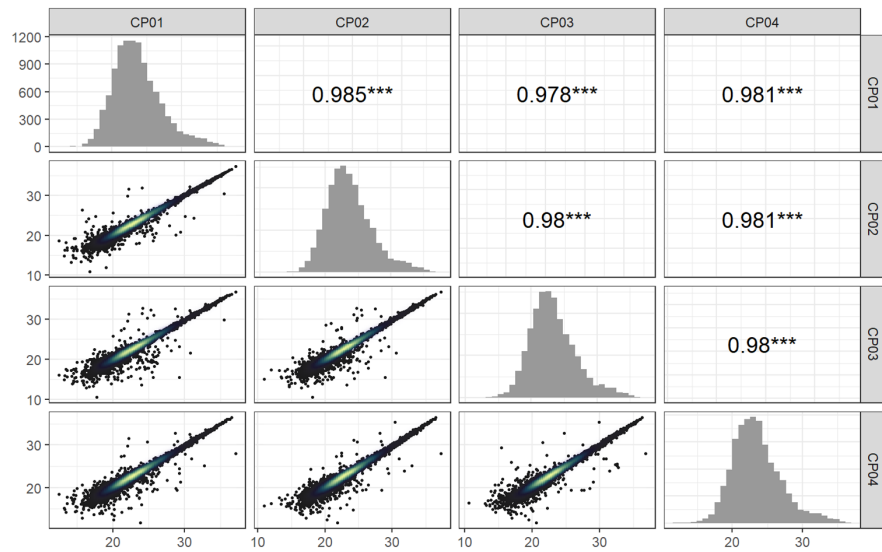
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Mag-Net: Robustness

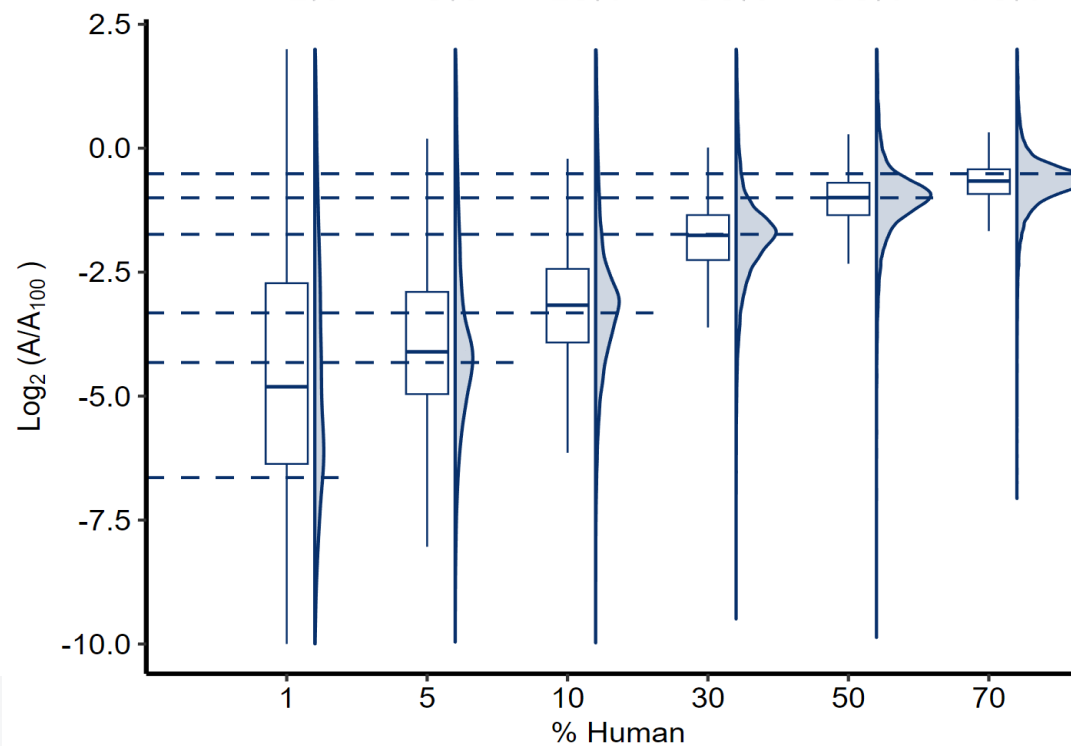
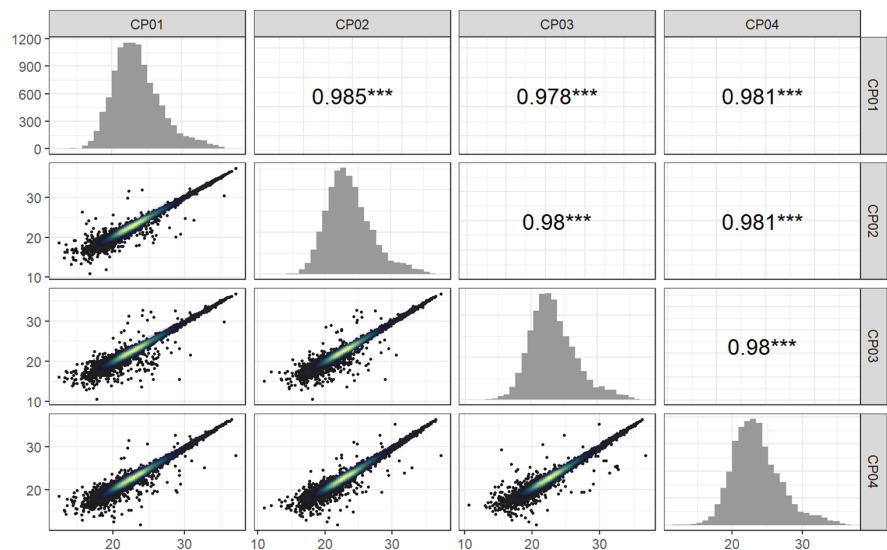
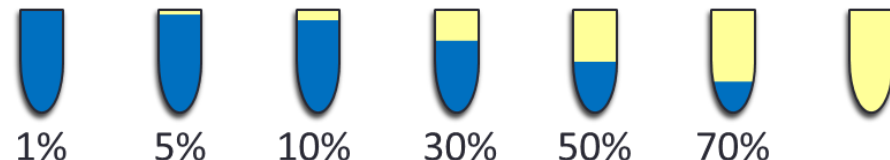


Mag-Net: Robustness & Quantitative accuracy

Pino et al., 2020. JPR. DOI:10.1021/acs.jproteome.9b00666

Chicken Plasma

Human Plasma







Skyline

Ecosystem: Enabling Inter-Lab Method Comparison



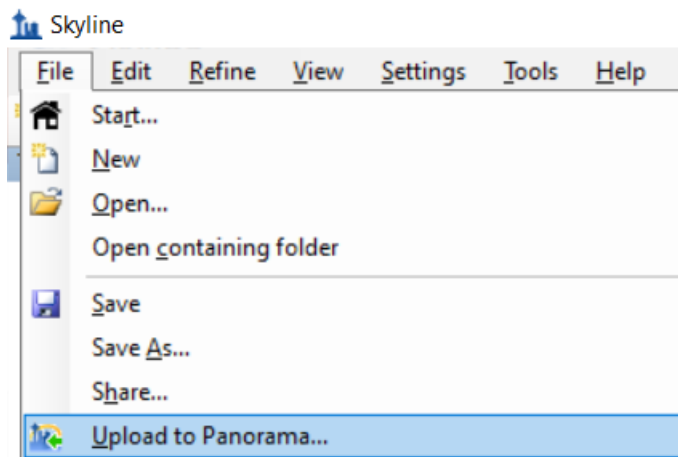
- 75 μ m x 25cm, 90min Gradient
- Orbitrap Eclipse
- EncylopeDIA



- 75 μ m x 25cm, 30min Gradient
- Sciex 6600 TToF
- Spectronaut v17



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MacCoss ▾

MacCoss / ChrisWu / Extracellular Vesicle Method Development / Mag-Net Method

ReSyn Lab

ReSyn Biosciences


EVs QC Targeted assays

MacCoss Lab Bead Comparisons (Exp 13)

MacCoss Lab Bead Exp 2

ReSyn Experiments



MacCoss ▾

Mag-Net Method

Mass Spec Search

Protein Search Peptide Search Modification Search

Protein name *?

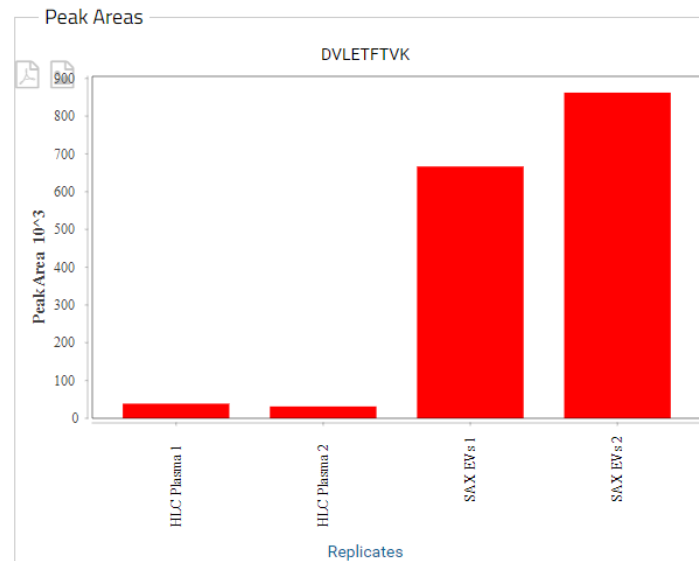
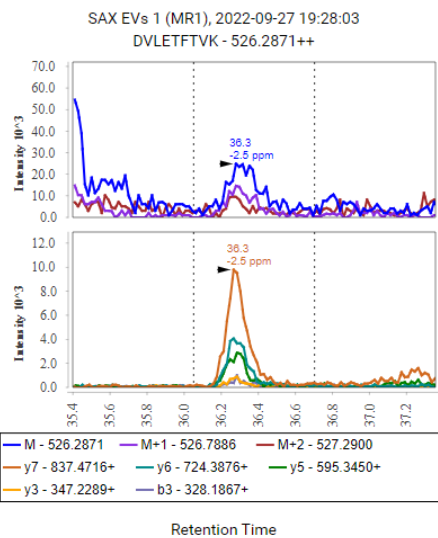
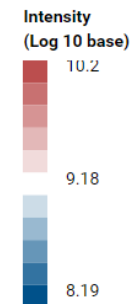
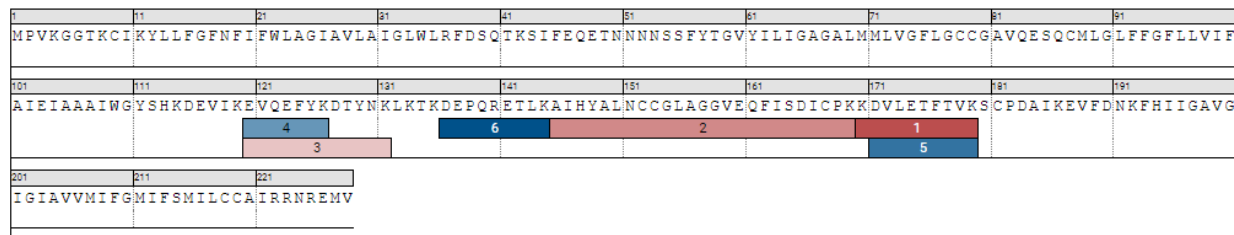
Exact matches only?

Search in subfolders?

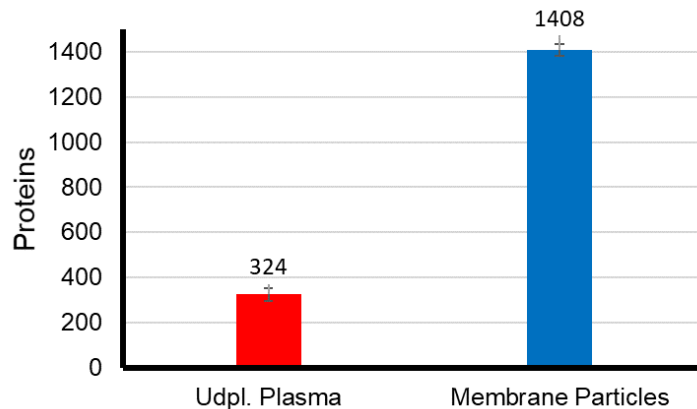
Sequence Coverage

View Settings

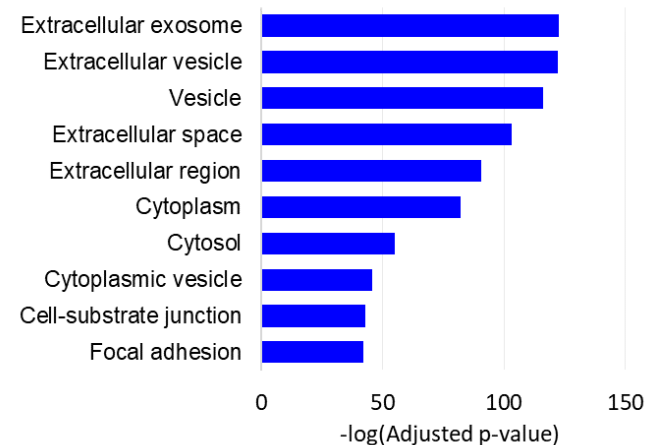
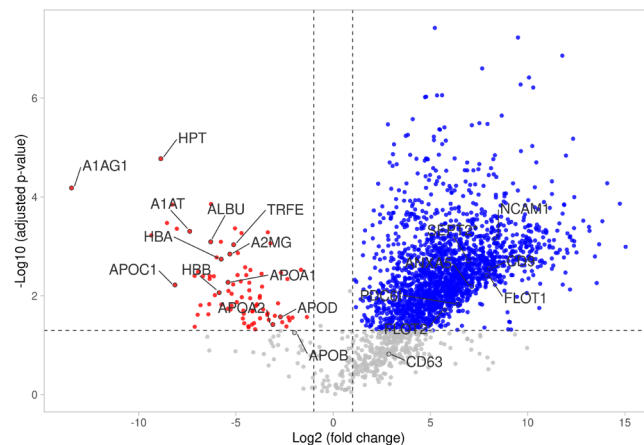
By: Replicate: Modified forms: Combined Stacked



Inter-Lab Methods: Proteome Coverage



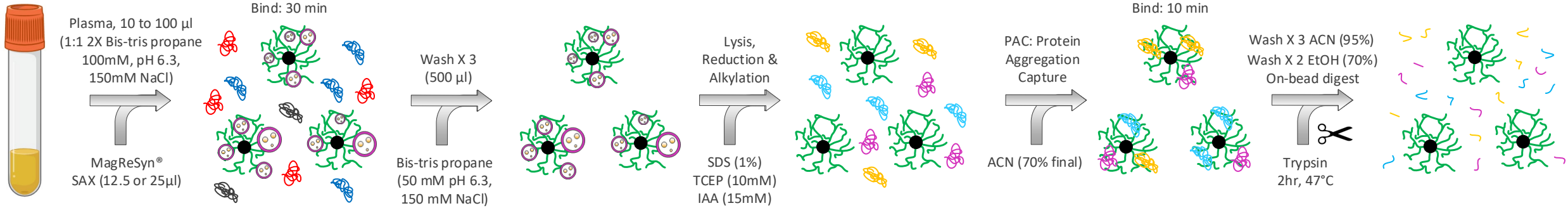
- 100 μ l plasma input
- 75 μ m x 25cm, 30min Gradient
- 48VW SWATH Sciex 5600 TToF





Skyline

Monitoring performance

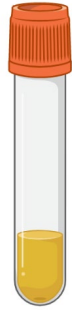


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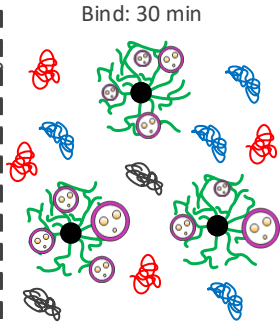
EV capture & Abundant protein removal

EV protein digestion



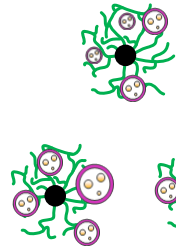
Plasma, 10 to 100 μ l
1:1 2X Bis-tris propane
100mM, pH 6.3,
150mM NaCl

MagReSyn[®]
SAX (12.5 or 25 μ l)



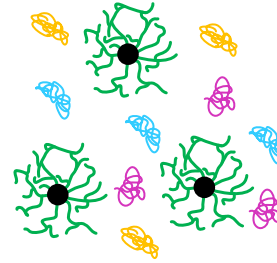
Wash X 3
(500 μ l)

Bis-tris propane
(50 mM pH 6.3,
150 mM NaCl)



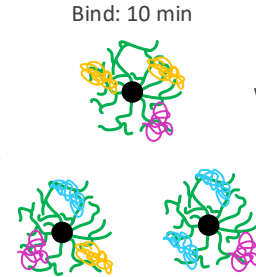
Lysis,
Reduction &
Alkylation

SDS (1%)
TCEP (10mM)
IAA (15mM)



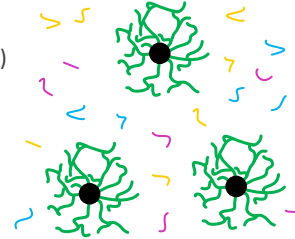
PAC: Protein
Aggregation
Capture

ACN (70% final)



Wash X 3 ACN (95%)
Wash X 2 EtOH (70%)
On-bead digest

Trypsin
2hr, 47°C



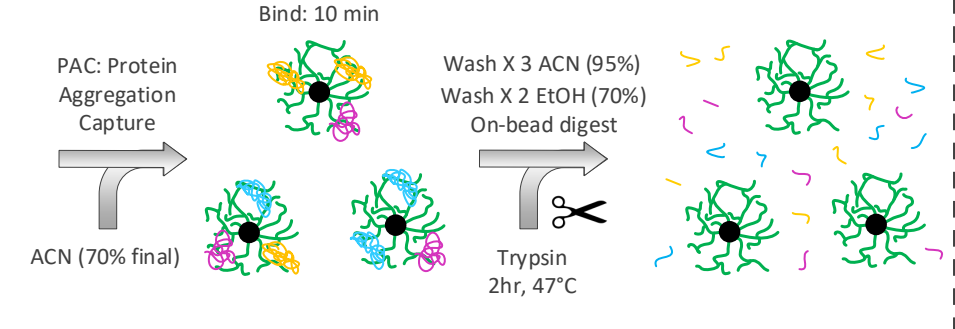
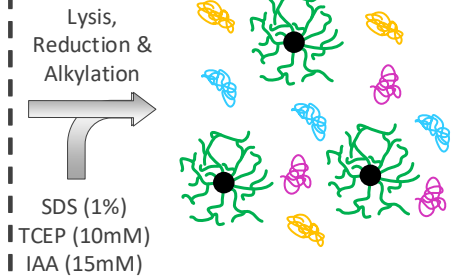
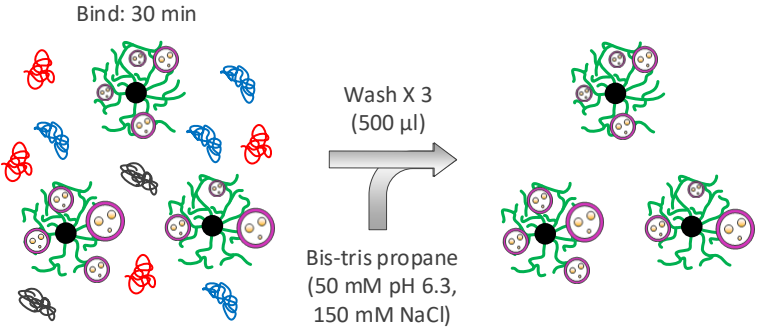
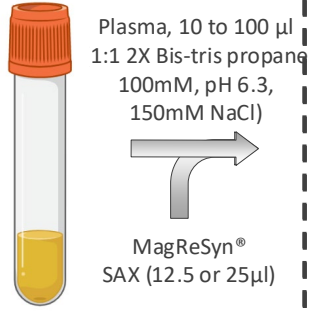


Skyline

Workflow QC: Transition to Targeted Analysis

EV capture & Abundant protein removal

EV protein digestion



Yeast Enolase



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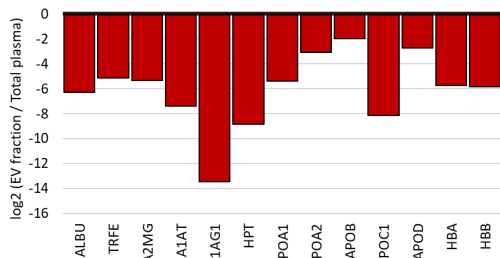
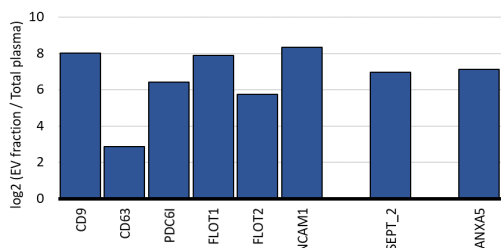
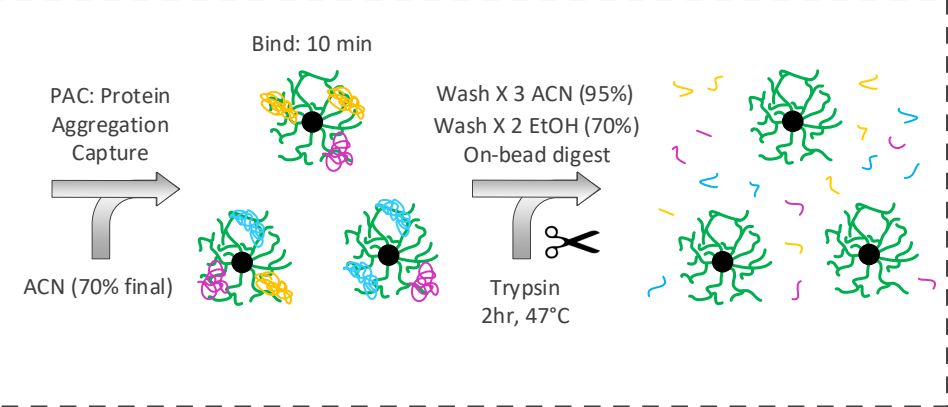
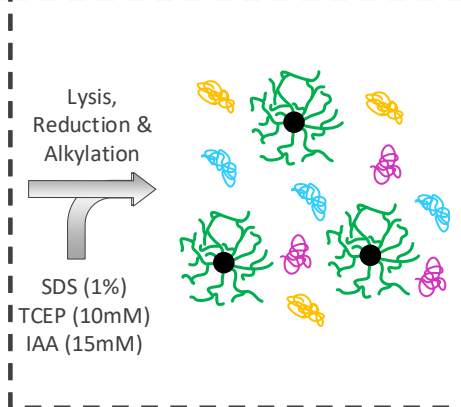
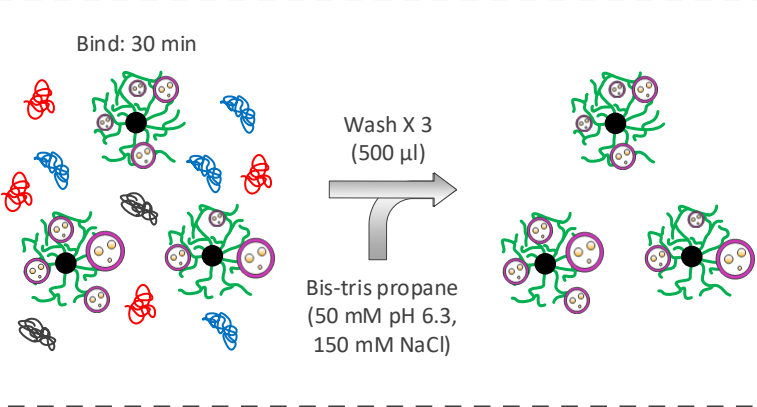
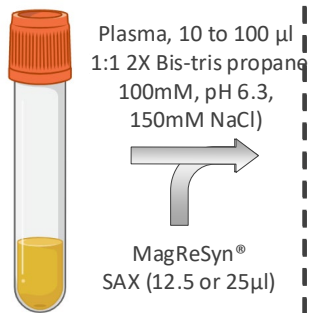


Skyline

QC: Transition to Targeted Analysis

EV capture & Abundant protein removal

EV protein digestion



Yeast Enolase



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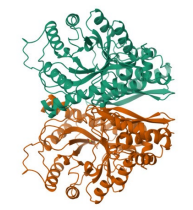
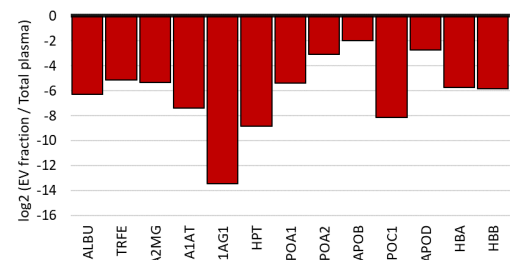
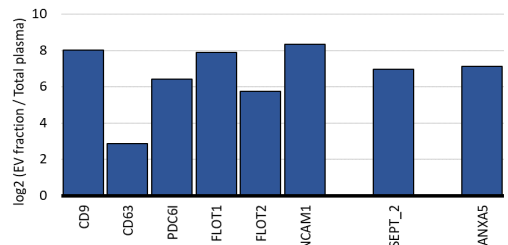
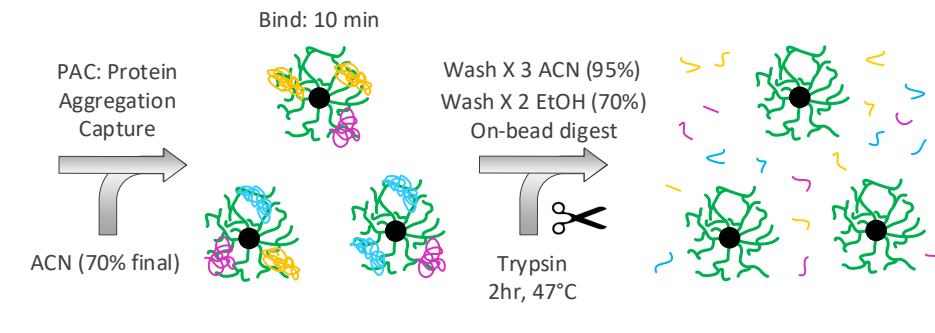
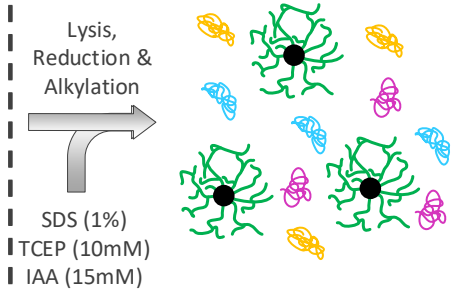
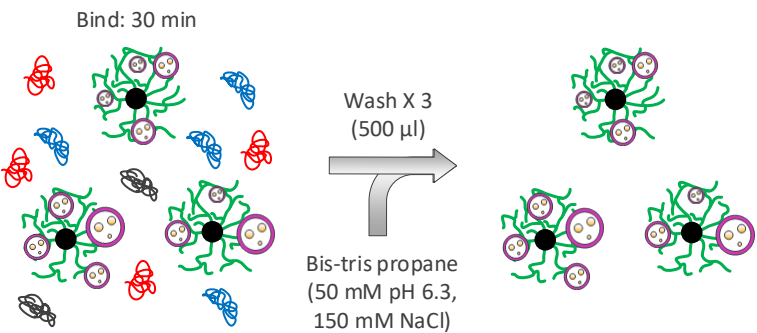
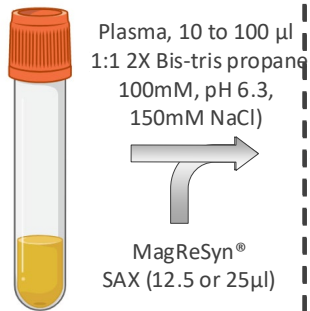


Skyline

QC: Transition to Targeted Analysis

EV capture & Abundant protein removal

EV protein digestion



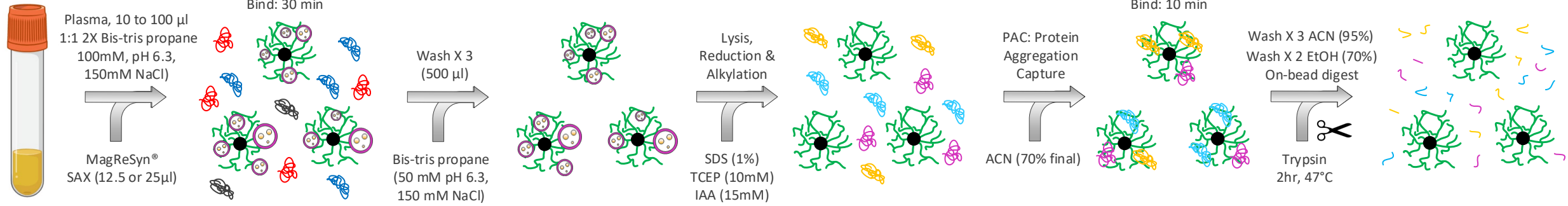
Yeast Enolase

Targeted assay ~60 proteins monitored



Skyline

QC Example: Probing Plasma Storage



**Fresh vs 6 month
Plasma**

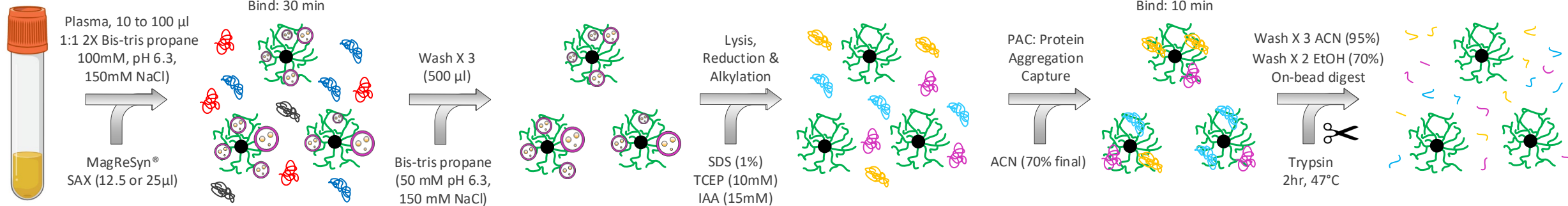


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Skyline

QC Example: Probing Plasma Storage



Fresh vs 6 month Plasma

	EVs Plasma New Batch 1	EVs Plasma New Batch 2	EVs Plasma New Batch 3	EVs Plasma Old Batch 1	EVs Plasma Old Batch 2	EVs Plasma Old Batch 3	Undpl Plasma	Undpl Plasma	Undpl Plasma
EVs Plasma New Batch 1	1.00	1.00	1.00	0.95	0.94	0.96	0.06	0.06	0.07
EVs Plasma New Batch 2	1.00	1.00	1.00	0.94	0.94	0.95	0.05	0.05	0.05
EVs Plasma New Batch 3	1.00	1.00	1.00	0.95	0.94	0.96	0.05	0.05	0.05
EVs Plasma Old Batch 1	0.95	0.94	0.95	1.00	0.99	0.99	0.07	0.07	0.07
EVs Plasma Old Batch 2	0.94	0.94	0.94	0.99	1.00	1.00	0.07	0.07	0.08
EVs Plasma Old Batch 3	0.96	0.95	0.96	0.99	1.00	1.00	0.07	0.07	0.08
Undpl Plasma	0.06	0.05	0.05	0.07	0.07	0.07	1.00	1.00	0.99
Undpl Plasma	0.06	0.05	0.05	0.07	0.07	0.07	1.00	1.00	1.00
Undpl Plasma	0.07	0.05	0.05	0.07	0.08	0.08	0.99	1.00	1.00

Protein Pearson r

	EVs Plasma New Batch 1	EVs Plasma New Batch 2	EVs Plasma New Batch 3	EVs Plasma Old Batch 1	EVs Plasma Old Batch 2	EVs Plasma Old Batch 3	Undpl Plasma	Undpl Plasma	Undpl Plasma
EVs Plasma New Batch 1	1.00	0.99	0.99	0.96	0.95	0.96	0.09	0.10	0.11
EVs Plasma New Batch 2	0.99	1.00	0.99	0.95	0.95	0.96	0.08	0.08	0.09
EVs Plasma New Batch 3	0.99	0.99	1.00	0.96	0.95	0.96	0.08	0.09	0.10
EVs Plasma Old Batch 1	0.96	0.95	0.96	1.00	0.98	0.99	0.10	0.11	0.12
EVs Plasma Old Batch 2	0.95	0.95	0.95	0.98	1.00	0.99	0.09	0.10	0.10
EVs Plasma Old Batch 3	0.96	0.96	0.96	0.99	0.99	1.00	0.10	0.11	0.11
Undpl Plasma	0.09	0.08	0.08	0.10	0.09	0.10	1.00	0.97	0.96
Undpl Plasma	0.10	0.08	0.09	0.11	0.10	0.11	0.97	1.00	0.98
Undpl Plasma	0.11	0.09	0.10	0.12	0.10	0.11	0.96	0.98	1.00

Peptide Pearson r

- 100µl plasma input
- 75µm x 25cm CSH C18, 30min
- 48VW SWATH Sciex 6600 TToF
- Spectronaut v17



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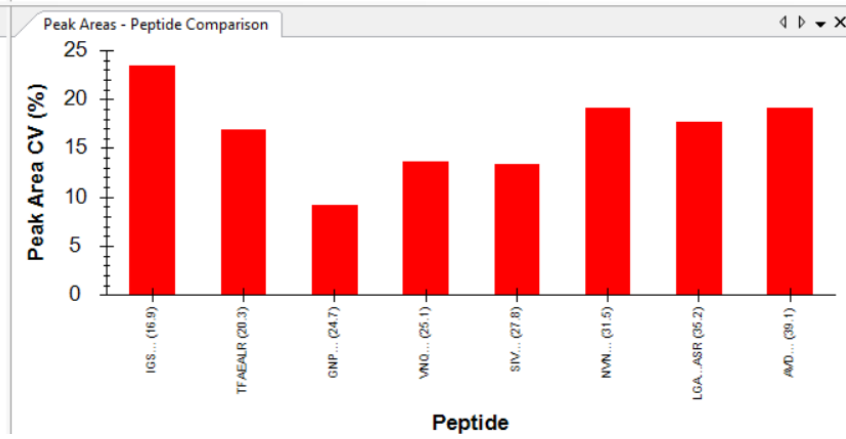
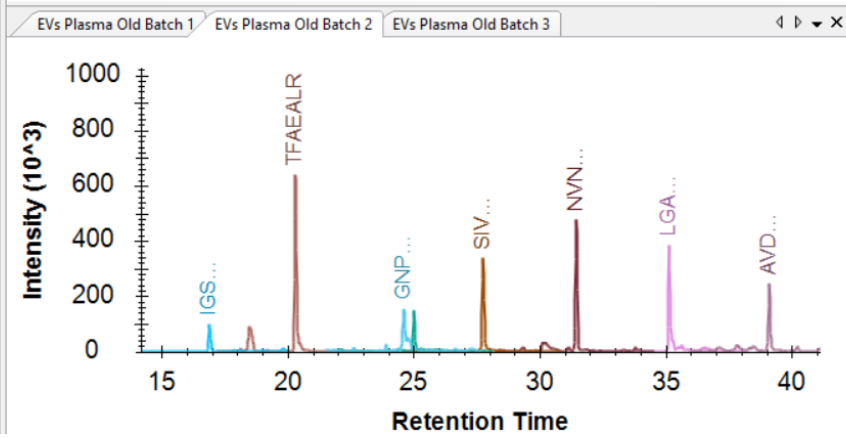
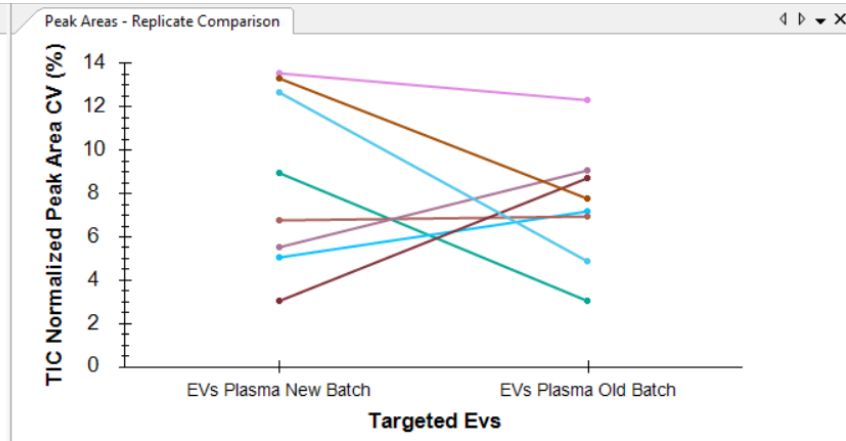
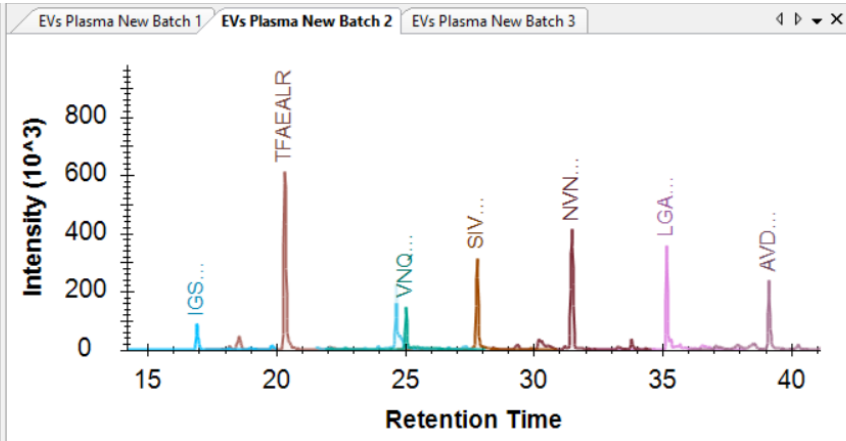
Skyline

Evaluation of EV Digestion (PAC) Performance

Targets

Replicates: EVs Plasma New Batch

sp P02787 TRFE_HUMAN	●
sp P02790 HEMO_HUMAN	●
sp P04004 VTNC_HUMAN	●
sp P04114 APOB_HUMAN	●
sp P05155 IC1_HUMAN	●
sp P08519 APOA_HUMAN	●
sp P27169 PON1_HUMAN	●
sp P08758 ANXA5_HUMAN	●
sp Q8NG11 ITSN14_HUMAN	●
sp P37840 SYUA_HUMAN	●
sp Q99816 TS101_HUMAN	●
sp O75954 ITSN9_HUMAN	●
sp Q96QS1 ITSN32_HUMAN	●
sp P08962 CD63_HUMAN	●
sp Q08722 CD47_HUMAN	●
sp P16671 CD36_HUMAN	●
sp Q15762 CD226_HUMAN	●
sp Q9HCU0 CD248_HUMAN	●
sp O14672 ADA10_HUMAN	●
sp P21926 CD9_HUMAN	●
sp P48509 CD151_HUMAN	●
sp O75955 FLOT1_HUMAN	●
sp Q14254 FLOT2_HUMAN	●
sp P11279 LAMP1_HUMAN	●
sp P13591 NCAM1_HUMAN	●
sp Q99497 PARK7_HUMAN	●
sp P02654 APOC1_HUMAN	●
sp P05090 APOD_HUMAN	●
sp P68871 HBB_HUMAN	●
sp P69905 HBA_HUMAN	●
sp P00924 ENO1_YEAST	●
R.GNPTVEVELTT	●
R.SIVPSGASTGVI	●
K.NVNDVIAPAFVH	●
K.AVDDFLISLDG1	●
K.LGANAILGVSLA	●
K.TFAEALR.I [179]	●
R.IGSEVYHNLK.S	●
K.VNQIGTLESSEIK	●

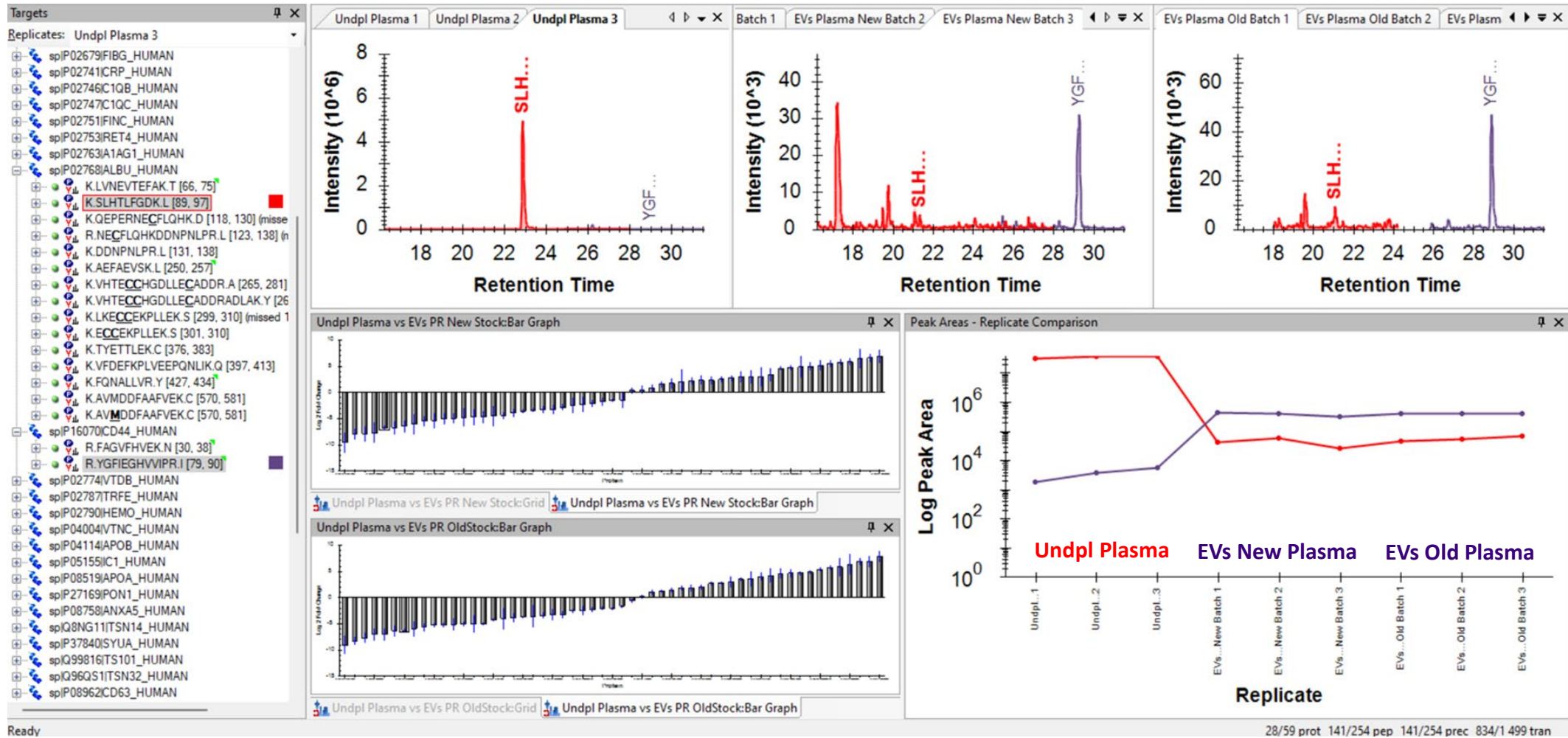


- Intra Batch CV

- Inter Batch CV

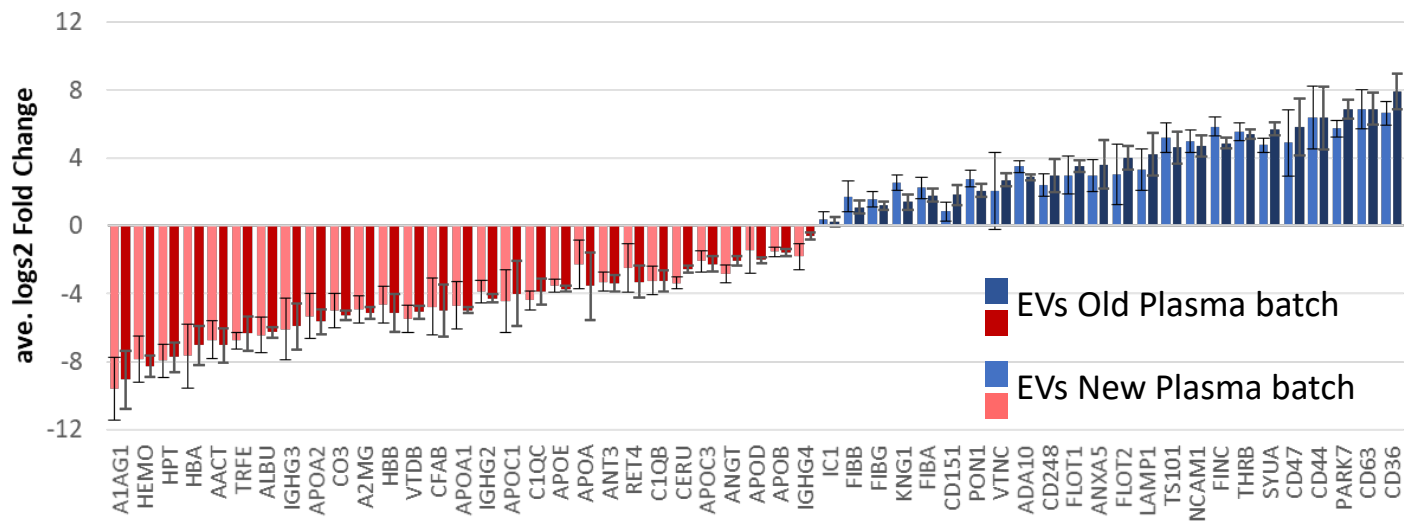


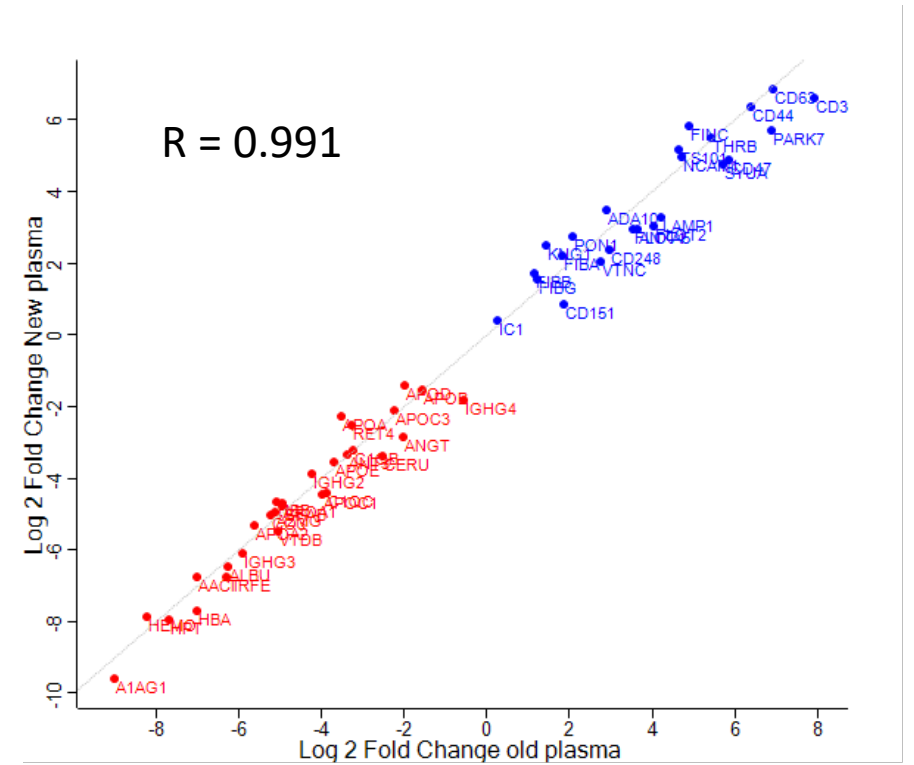
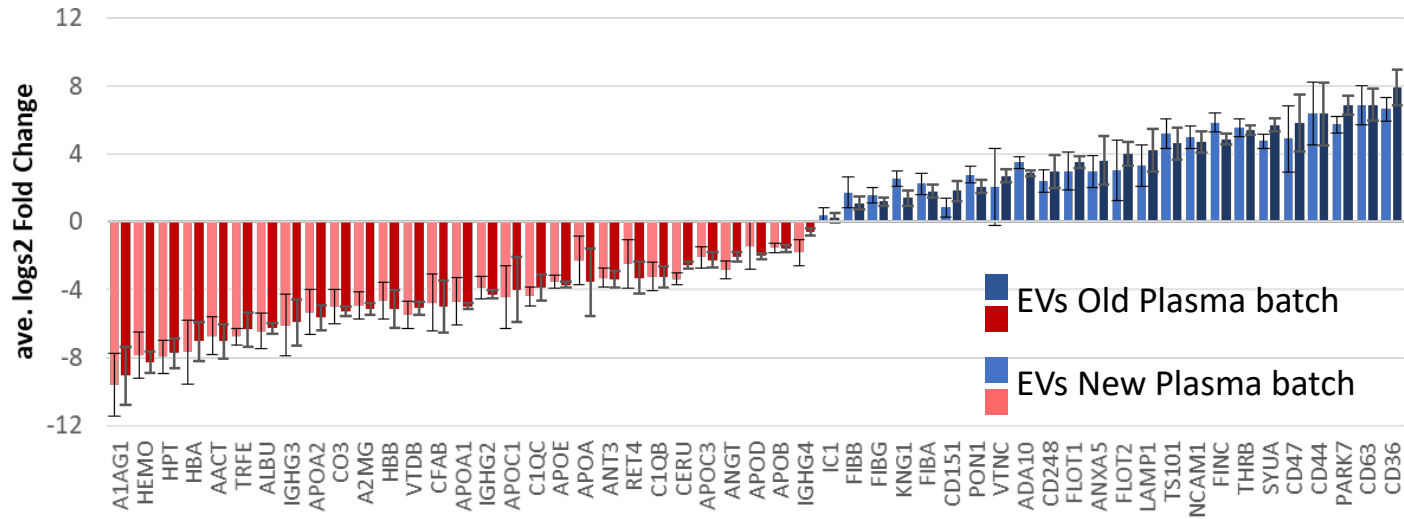
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28/59 prot 141/254 pep 141/254 prec 834/1 499 tran









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BiodiversityPlugin
Cross-link Transition
DeepMRM
LipidCreator
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MS1Probe
MSInspector
MSstats
Population Variatio
Prego
Proteotypic Peptide
Protter
QuaSAR
Skyline Gadget for
SProCoP
TFExport
TurnoverR

Organization: IMSB (ETH Zurich)
Authors: Ulrich Omasits
Languages:

PROTTER

More Information:
<http://wlab.ethz.ch/protter/>

Status:
Not currently installed. Version: 1.0.1 is available

Description:
Protter visualizes your peptides on the protein s
transmembrane topology and other features as
etc.

Export Report

Report:

- Main
 - MJM-Peptide Matrix
 - MJM-Protein Matrix
 - Peak Boundaries
 - Peptide Quantification
 - Peptide Ratio Results
 - Peptide RT Results
 - Peptide Transition List
 - Transition Results
- External Tools
 - Protter
 - SRM Collider Input

Tool Store...

Preview: Protter

1 of 42489

	Protein Name	Protein Sequence	Peptide Sequence	Peptide Modified Sequence
▶	sp A6NIH7 IU119...	MSGSNPKAAA...	YQFTPAPFLR	YQFTPAPFLR
	sp O15194 CTDS...	MDGPAITQVTN...	EDEGRLPGAGEK	EDEGRLPGAGEK
	sp O15194 CTDS...	MDGPAITQVTN...	ASQCNVSLKK	ASQC[+57]NVSL...
	sp O15194 CTDS...	MDGPAITQVTN...	YLLPEVTVLDYG...	YLLPEVTVLDYG...
	sp O15194 CTDS...	MDGPAITQVTN...	RPHVDEFLQR	RPHVDEFLQR
	sp O15194 CTDS...	MDGPAITQVTN...	YADPVADLLDR	YADPVADLLDR
	sp O15194 CTDS...	MDGPAITQVTN...	YADPVADLLDR...	YADPVADLLDR...
	sp O15194 CTDS...	MDGPAITQVTN...	ESCVFHR	ESC[+57]VFHR
	sp O95445 APO...	MFHQIWAALLY...	MKDGLCVPR	MKDGLC[+57]VPR
	sp O95445 APO...	MFHQIWAALLY...	KWIYHLEGST...	KWIYHLEGST...
	sp O95445 APO...	MFHQIWAALLY...	WIYHLEGSTD...	WIYHLEGSTD...
	sp O95445 APO...	MFHQIWAALLY...	WIYHLEGSTD...	WIYHLEGSTD...
	sp O95445 APO...	MFHQIWAALLY...	TEGRPDMK	TEGRPDMK
	sp O95445 APO...	MFHQIWAALLY...	TELFSSSCPGGI...	TELFSSSC[+57]...





PROTTER 1 protein 2 topology 3 styles 4 misc. ? help manual questions, feedback, suggestions **ETH zürich**

by accession { please enter a UniProt protein accession:
e.g: BST2_HUMAN
submit load example

by sequence { or, alternatively you can:
enter a list of proteins
load a proteomics result file

refresh export share open in UniProt

sequence input

Please select one or more proteomics result files:
Choose Files No file chosen

Supported proteomics files are:

- Protein XML (.prot.xml)
ProtXML file from TPP or from ProteomeDiscoverer
- TPP ProtXLS (.prot.xls)
exported and optionally filtered ProteinProphet file
- TPP PepXLS (.pep.xls)
exported and optionally filtered PeptideProphet file
- Mascot (.csv)
exported and optionally filtered Mascot file
- MaxQuant (evidence.txt)
Andromeda search result file
- Skyline (.csv)
exported Skyline file using the "Protter" report

Example files can be downloaded on the [help page](#).

submit cancel





PROTTER 1 protein 2 topology 3 styles 4 misc. ? help manual questions, feedback, suggestions **ETH zürich**

4675 proteins:

▼ sp|P08962|CD63_HUMAN

- DKVMSEFNNNFR
- VMSEFNNNFR
- QQMENYPK
- NRVPDSC₄₅₇C₄₅₇INVTVGC₄₅₇GIN
- AIHKEGC₄₅₇VEK
- IGGWLRK

by accession { please enter a UniProt protein accession:

by sequence

or, alternatively you can:

-
-

Legend:

- exp. mods
- exp. pep.s
- PTMs
- variants
- disulfide bonds
- signal peptide
- exp. pep.s
- Tryps
- N-term: UniProt
- TMRs: UniProt



Mag-Net: Geared to tackle large clinical cohorts

- EVs capture via electrostatic interactions & hyper-porous magnetic beads
- **Efficient:** Improved dynamic range enables deep plasma profiling
- **Scalable:** Low input plasma – High throughput – Cost effective



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Skyline Ecosystem instrumental for method development

- Monitor data generated & processed from different LCMS platforms
- Protter to characterize transmembrane topology
- Group comparisons function to evaluate EV capture mechanism of action
- Key to targeted assay development to QC beads & method components





Mag-Net at ASMS 2023

Presentation (TOA B3 – 2:50 PM)

Thermo Bfast Seminar (WED 5 – 7 AM)

Poster TP589

ReSynBio Booth #709

Thank you!

Christine Wu
Kristine Tsantilas
Jea Park
Deanna Plubell
Gennifer Marrihew
Michael MacCoss

Isak Gerber
Ireshyn Govender
Justin Jordaan

Sindisiwe Buthelezi
Previn Naicker

