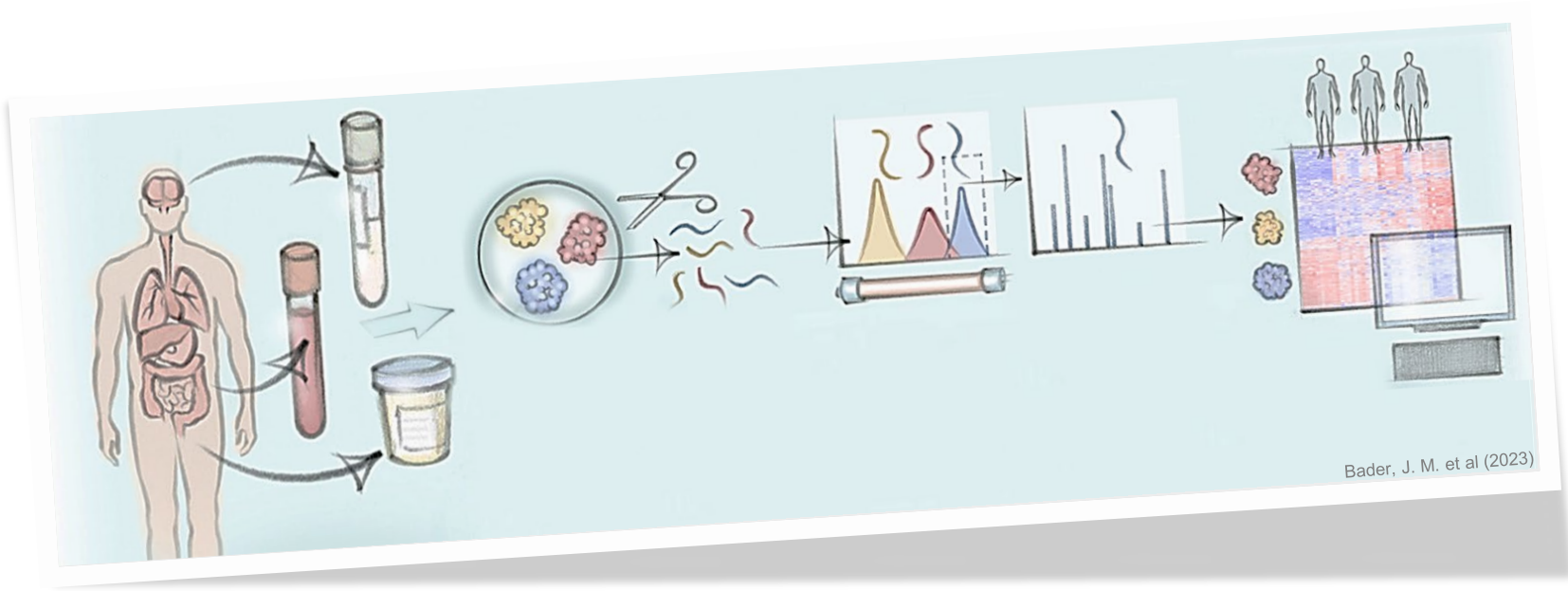
Biomarkers research by proteomics: are we there yet?

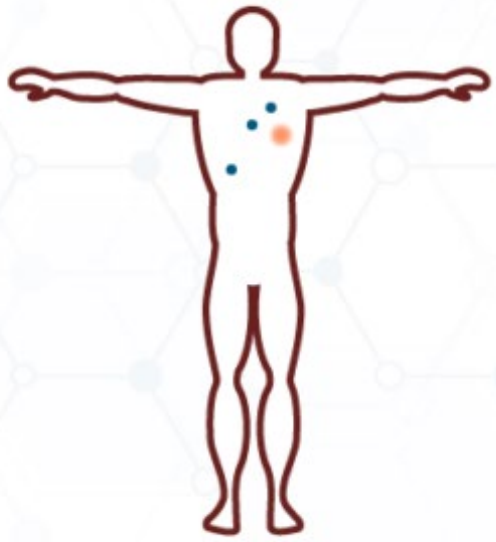


Dr Chiara GUERRERA
SFR Necker
Necker Proteomics,
Université Paris Cité
INSERM US24
Paris

www/sfr-necker/proteomics

Protein **Biomarkers**, not just abundance.

Individual with only normal biomarker proteins indicative of good health.



Individual with normal and abnormal biomarker proteins indicative of disease.

Proteins whose
PRESENCE, ABSENCE, MODIFICATION (PTM, Mutation, variants: proteoforms)
indicate an abnormal biological process.

Protein **Biomarkers** research relies on **cohorts** analysis

Proteomics is the large-scale* study of proteins *and proteoforms*

COVERAGE

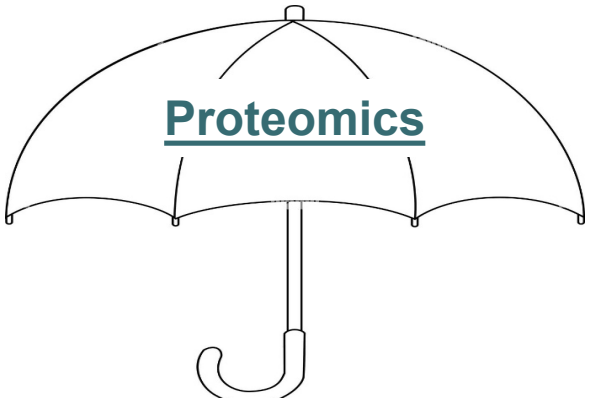
Clinical Proteomics is the large-scale study of proteins in a large** cohort

SCALE

***Proteome as comprehensive as needed (depending on disease)**

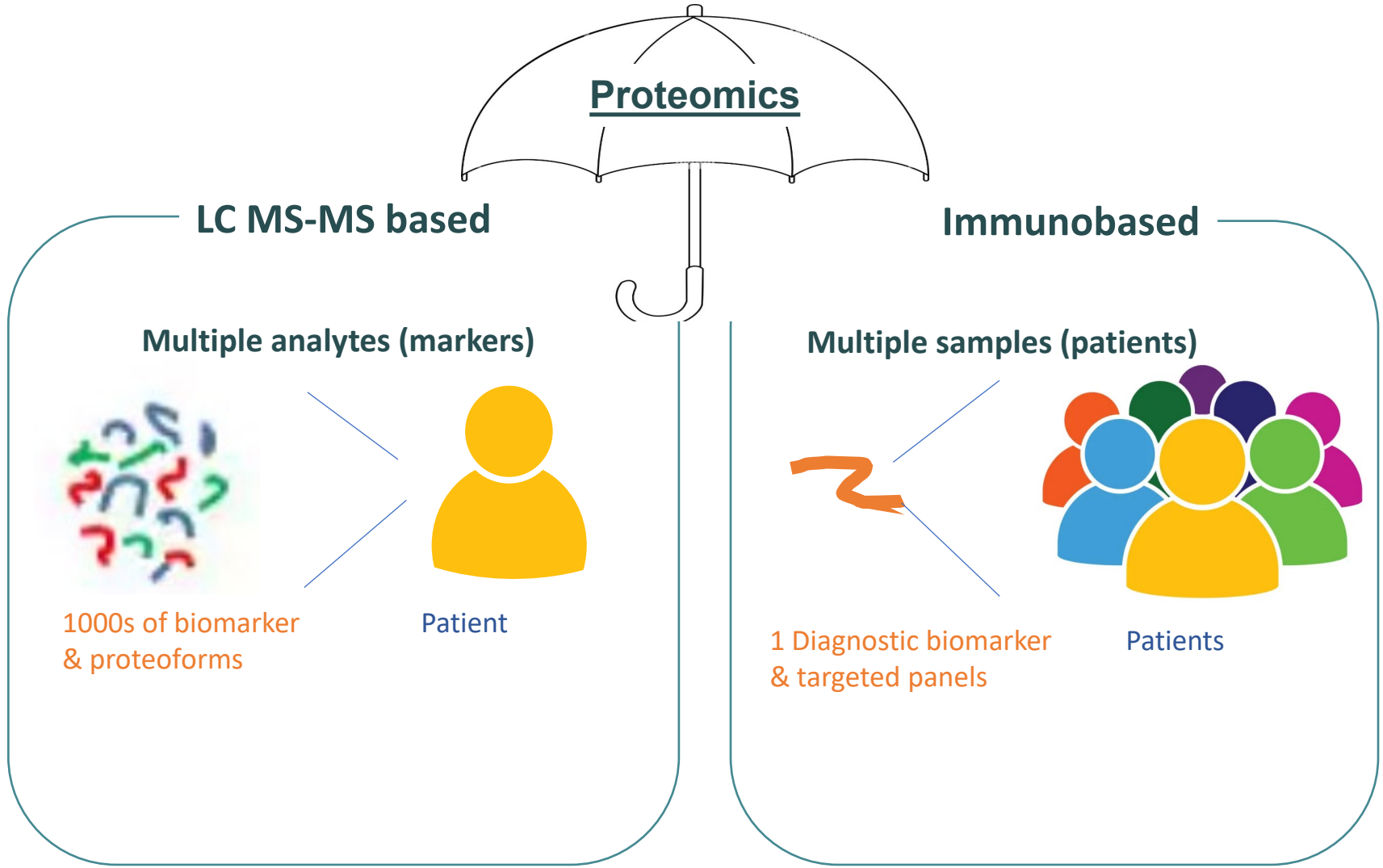
Cohort as large as needed to be **representative & allow stratification

Proteomics is an **umbrella** term

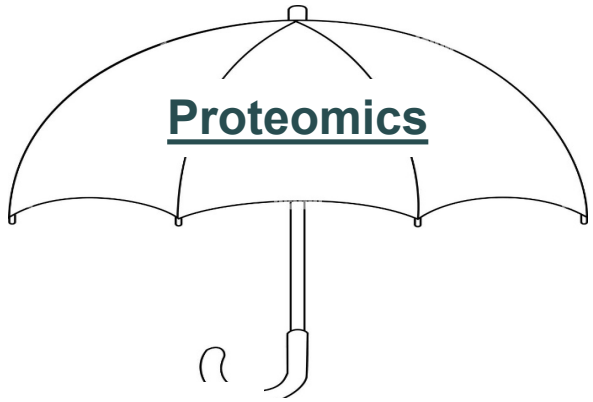


COVERAGE
&
SCALE

Pros & cons of **proteomics approaches**



Combine unbiased vs targeted for **discovery** and **validation**



LC MS-MS based
unbiased

Immunobased
targeted

↑ **COVERAGE**

↓ **SCALE**

DISCOVERY **VALIDATION**

COVERAGE ↓

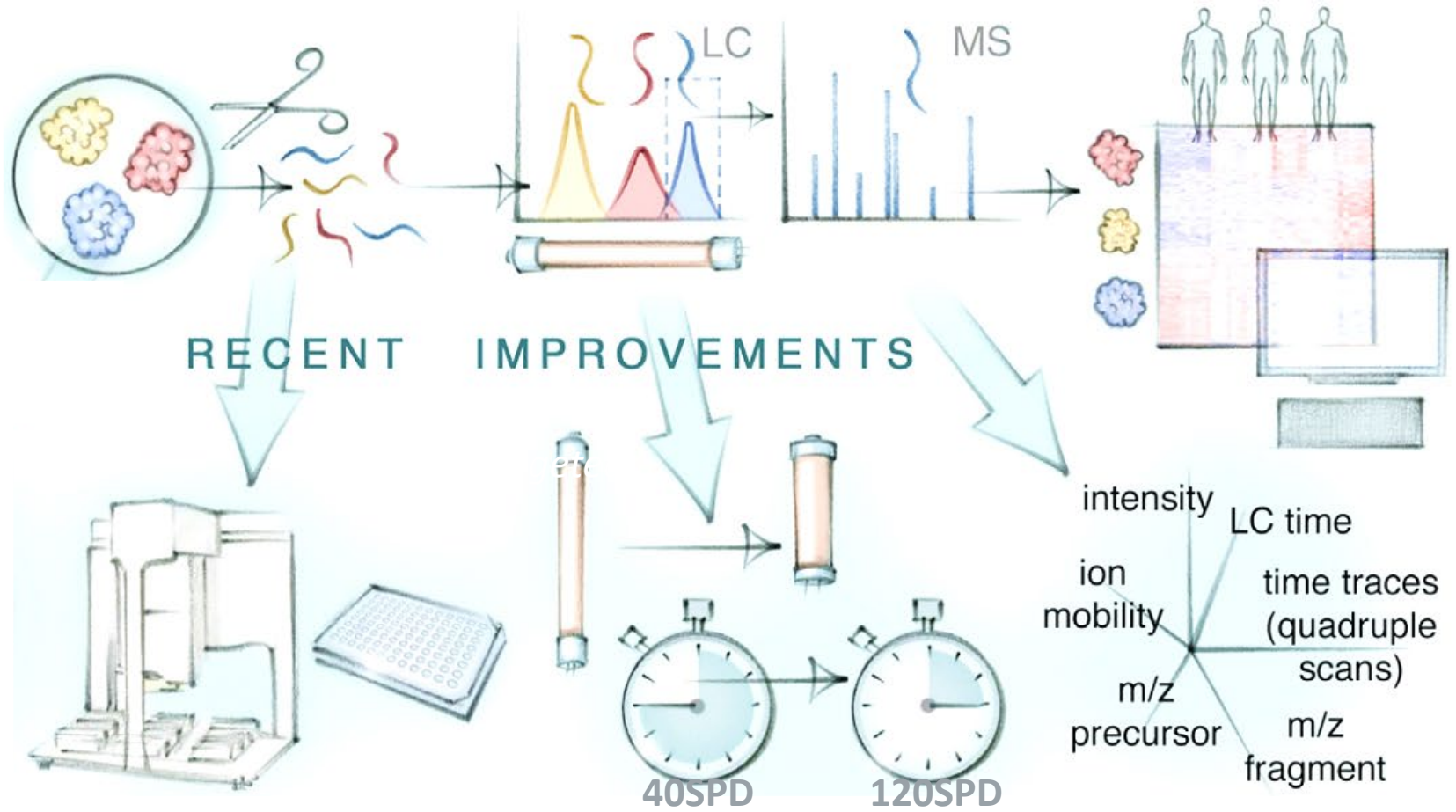
SCALE ↑

Recent improvements of MS-based proteomics

MCP RESEARCH Special Issue: Clinical Proteomics

MS-Based Proteomics of Body Fluids: The End of the Beginning

Authors
Jakob M. Bader, Vincent Albrecht, and Matthias Mann



COVERAGE & SCALE →

Automation
=>from 12-24
to 96 & 384 plates per day

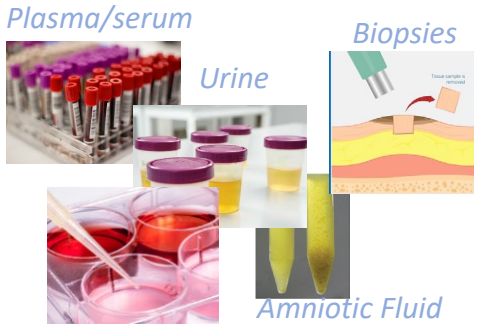
LC: Fast gradients
=>from 12SPD to 40&120 SPD

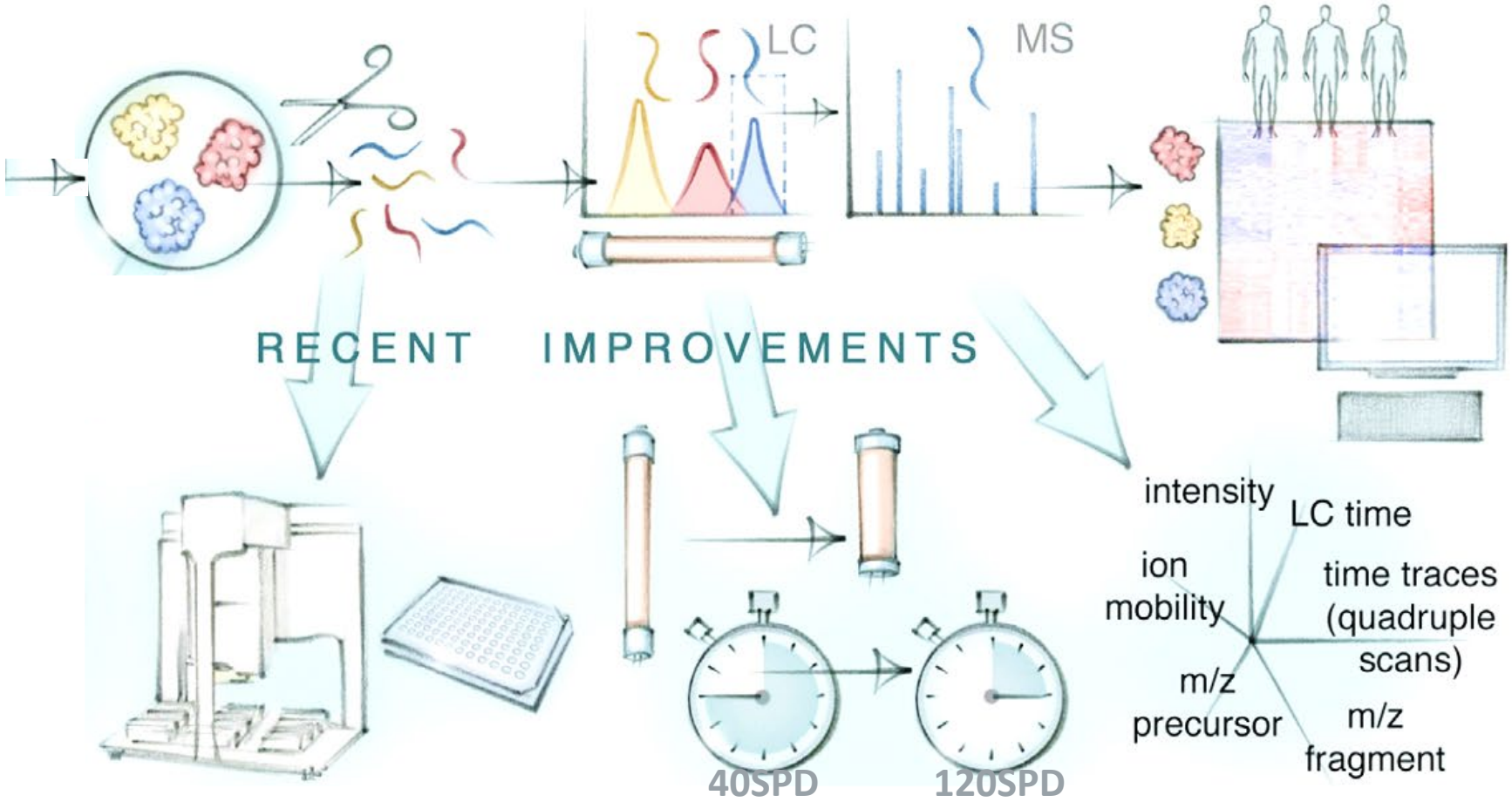
MS: DIA!
x2.5 coverage
++ repeatability

Data analysis:
IA for DIA

Clinical samples present additional **challenges** in LC-MSMS

Clinical samples





Pre-analytical

COVERAGE & SCALE

Automation
=>from 12-24
to 96 & 384 plates per day

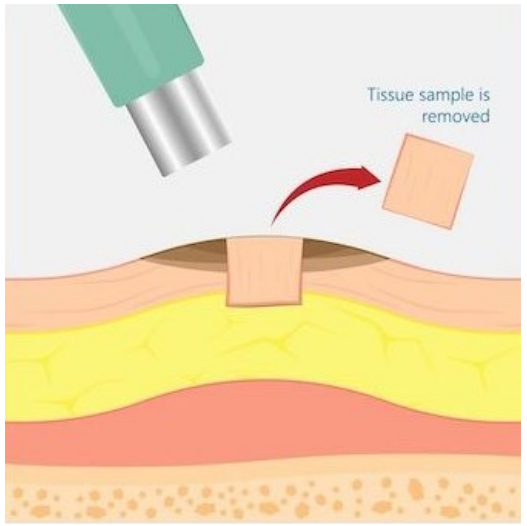
LC: Fast gradients
=>from 12SPD to 40&120 SPD

MS: DIA!
x2.5 coverage
++ repeatability

Data analysis:
IA for DIA

Case studies : what can LC MSMS proteomics achieve?

SKIN BIOPSY



Pachyonychia congenita (PC)

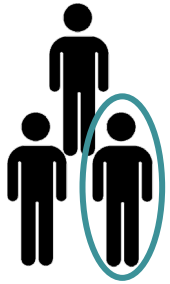
- ✓ *The challenges*
- ✓ *The optimization*
- ✓ *The results*

PLASMA &
deepPLASMA

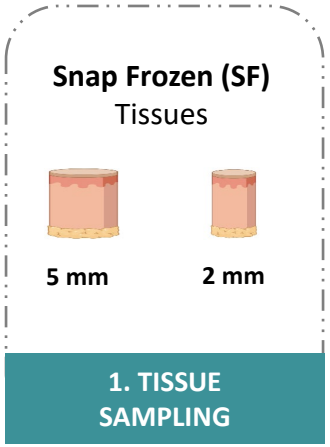
Epidermide Bullosa-Infection (EBRD) &
Becker Myodistrophy (BMD)

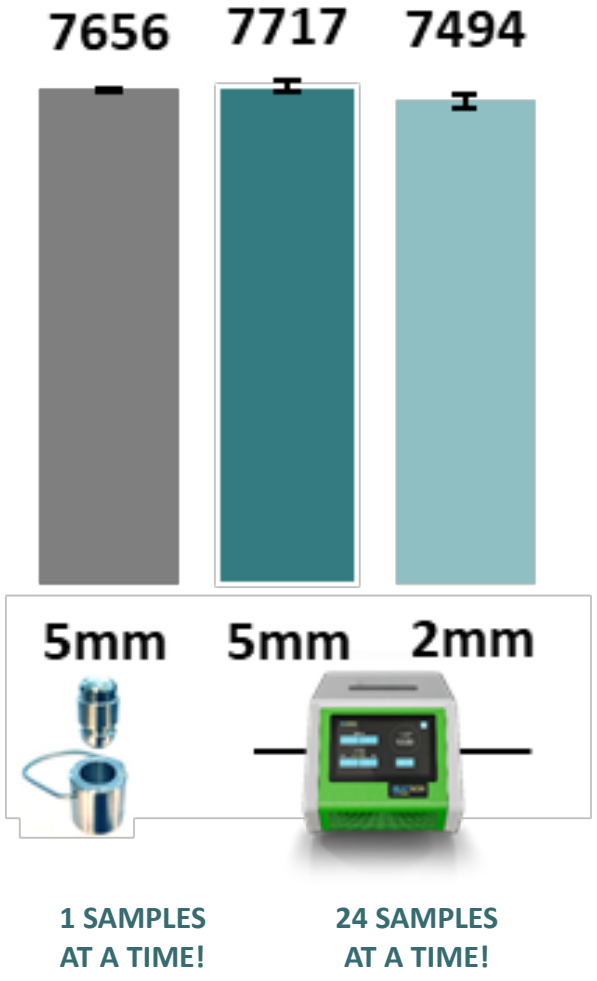
The challenge. Protein extraction from skin.

Sara Ceccacci



3 healthy donors





COVERAGE

SCALE

reduce biopsy size

MOST CHALLENGING STEP!

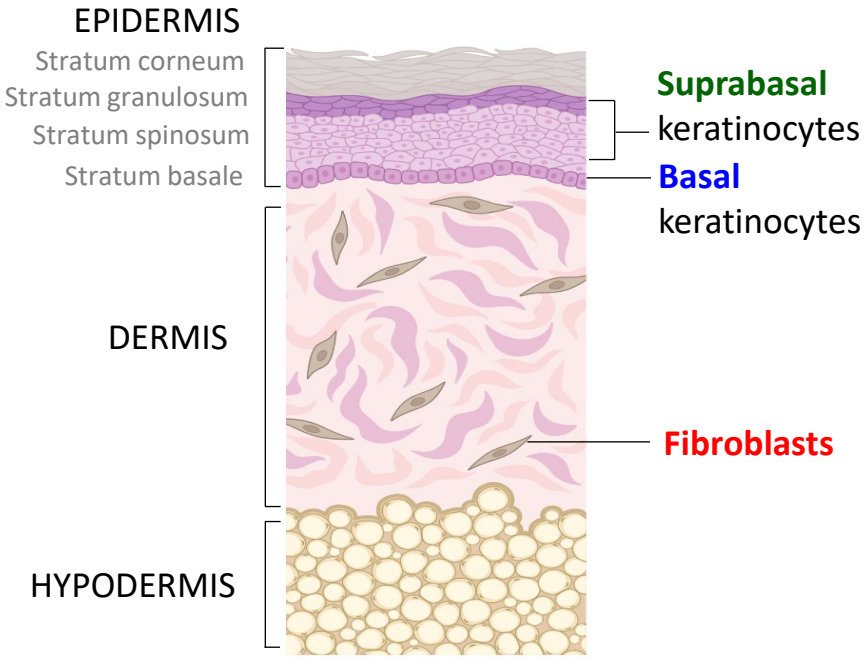
Challenge in **tissue lysis** and **protein extraction** due to the abundance of structural proteins (keratins, collagens)

Created with Biorender.com

Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

The optimization. Skin Proteomics Workflow

Sara Ceccacci



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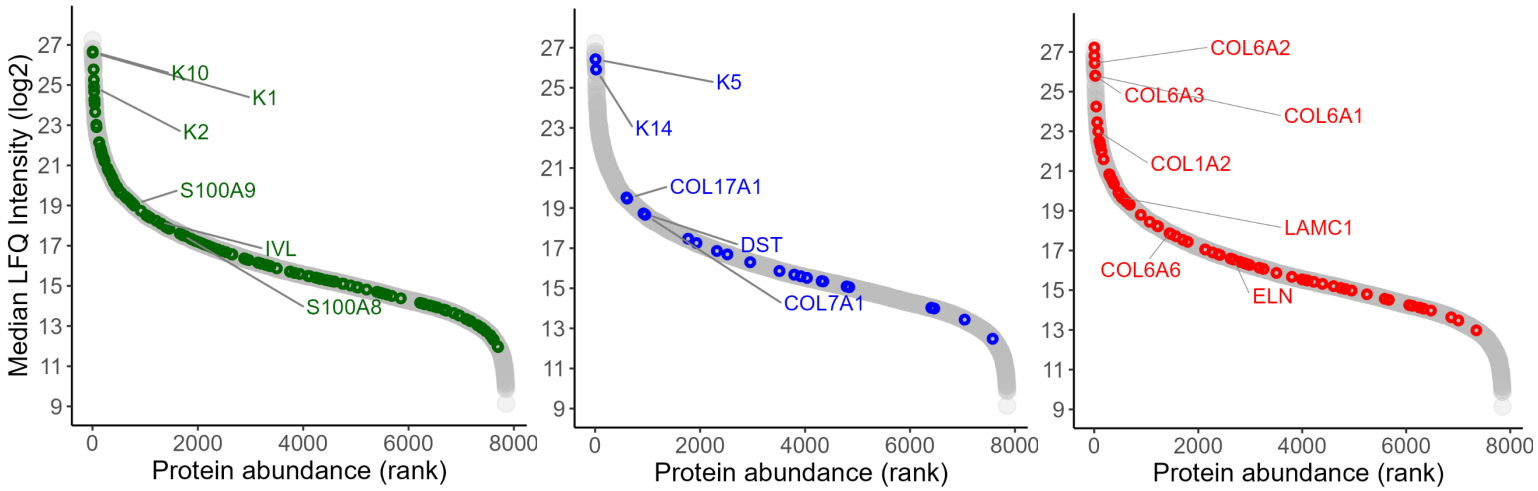


Suprabasal keratinocytes

Basal keratinocytes

Fibroblasts

Our **protein extraction** method is **not biased** and efficiently recovered **proteins from all skin layers**.

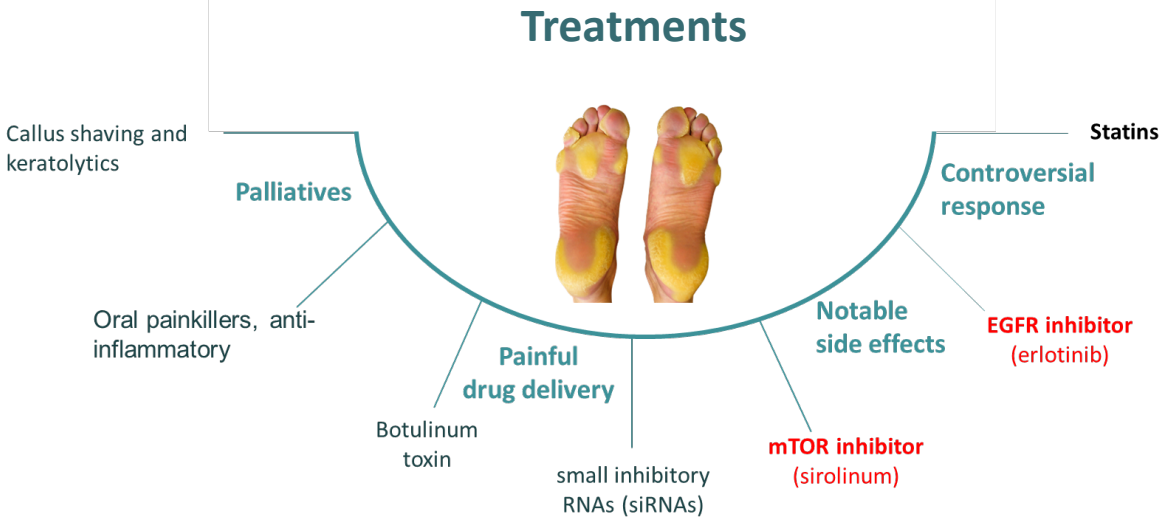
Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

Pachyonychia Congenita:
pushing the coverage to understand and cure


Sara Ceccacci

Pachyonychia congenita

Inheritance	Autosomal recessive, prevalence 1/1000 000
Genotype	mutations on one of 5 Keratins
Phenotype	Painful palmoplantar keratoderma



AIM

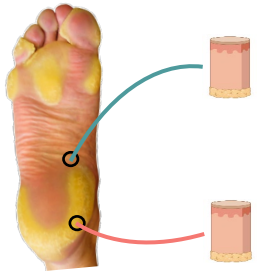
Elucidate pathogenic mechanisms –> refine treatment

Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

Global proteomics of skin tissues from 10 PC patients

10 PC patients

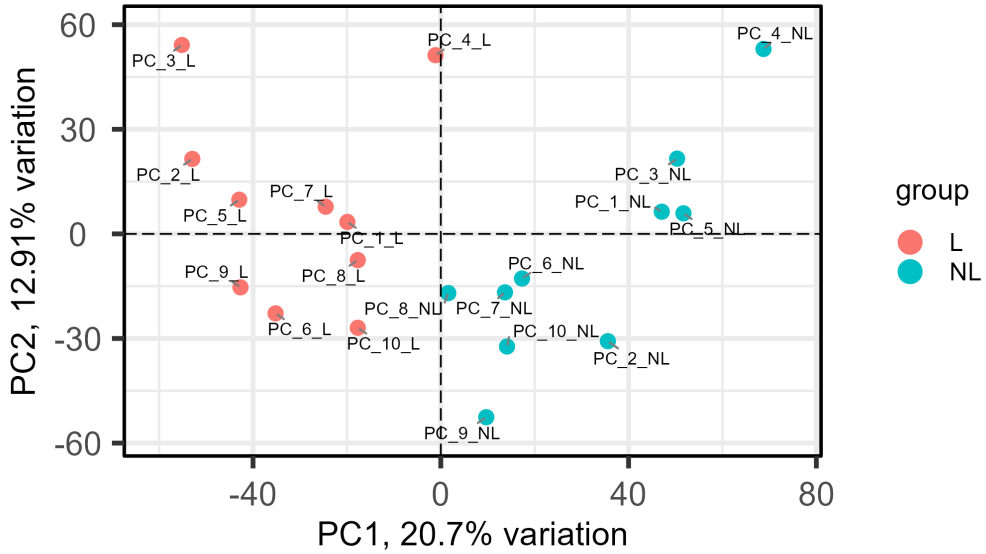
Non-lesional (NL)
2 mm SF skin biopsy



Lesional (L)
2 mm SF skin biopsy

7200 Identified Protein Groups

In average per sample



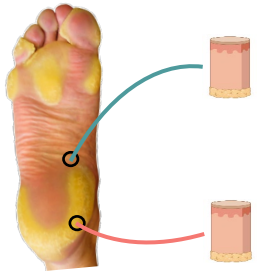
Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

Global proteomics of skin tissues from 10 PC patients


Sara Ceccacci


10 PC patients

Non-lesional (NL)
2 mm SF skin biopsy



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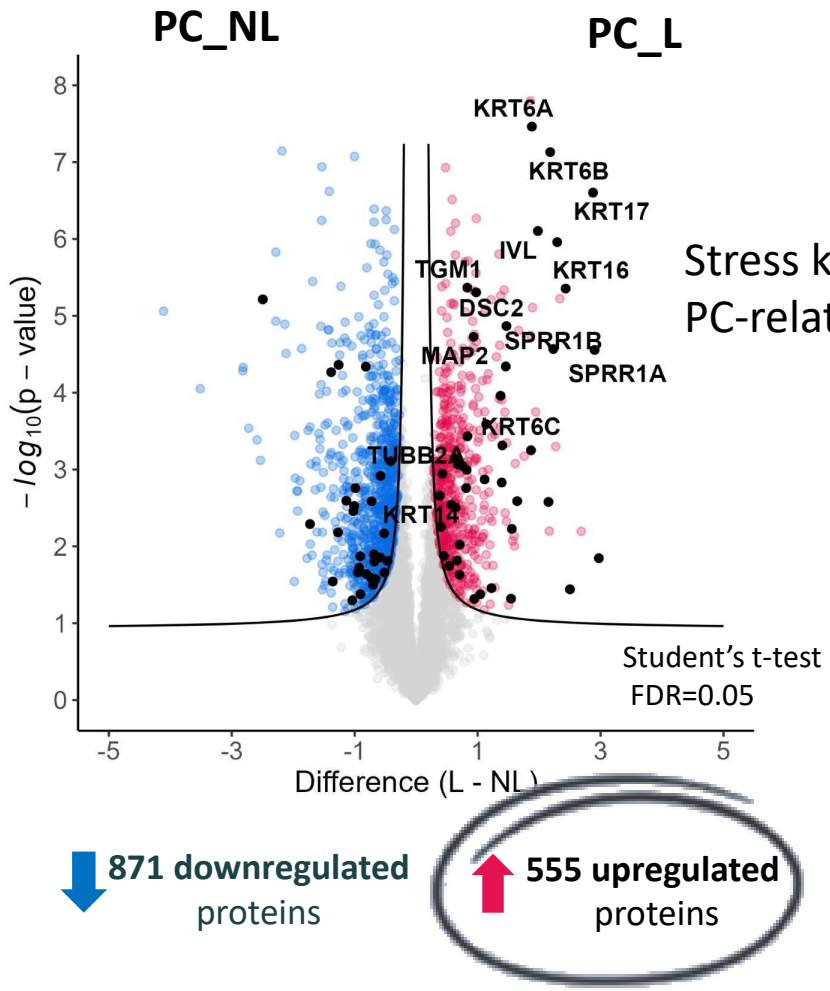


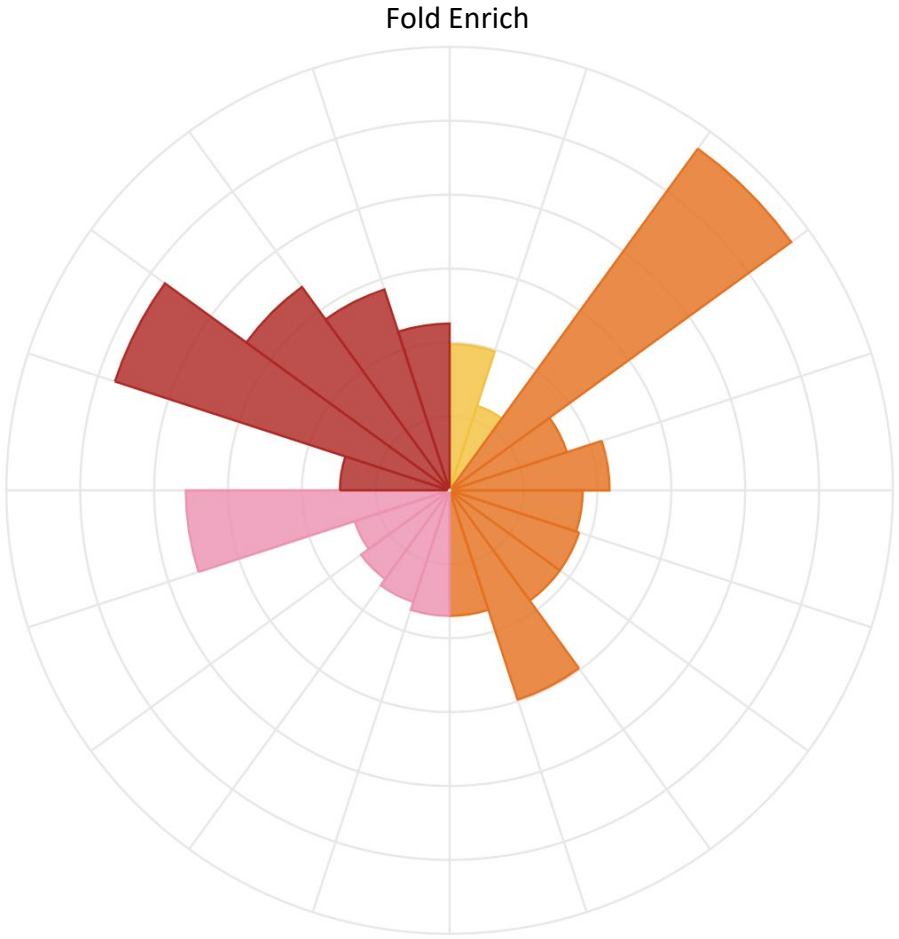
■ **70 candidates** in common with a previous **transcriptomics** study *

*Cao Y-A et al. *J Dermatol Sci.* **2015**

Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

Upregulated proteins in PC lesional samples

Sara Ceccacci



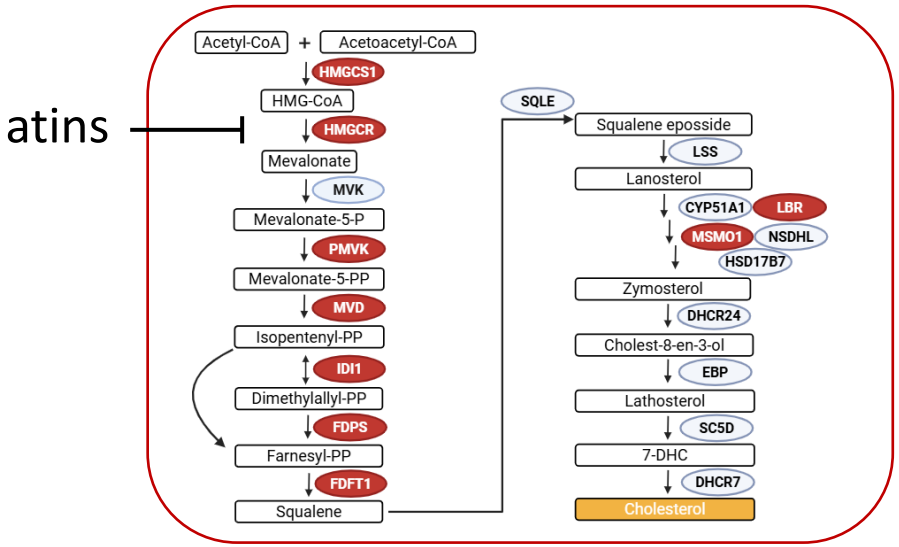
REACTOME Over Representation Analysis (ORA)

Hyperkeratosis

Hyperproliferation and protein synthesis

Immune Response

Lipid biosynthesis

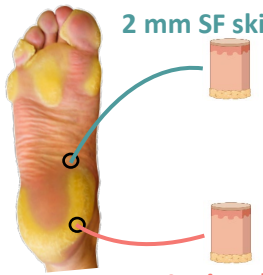


Cholesterol biosynthesis

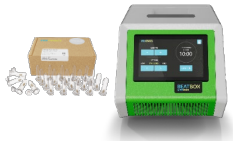
Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

Optimization of deepPhosphoproteomics of the skin biopsy

1. Tissue sampling



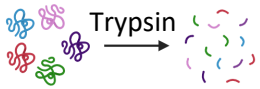
Bead-based Homogenization



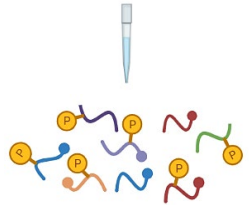
24 SAMPLES AT A TIME!

2. Tissue homogenization

Strap



3. Protein digestion



(TiO_2 and carbon graphite tips)

4. Phospho-enrichment

nanoElute 2-timsTOF Pro2

5. LC-MS/MS analysis

Kevin Roger

PhosVIEW App

Shiny

Phosphodata visualization and integration

RStudio

v19

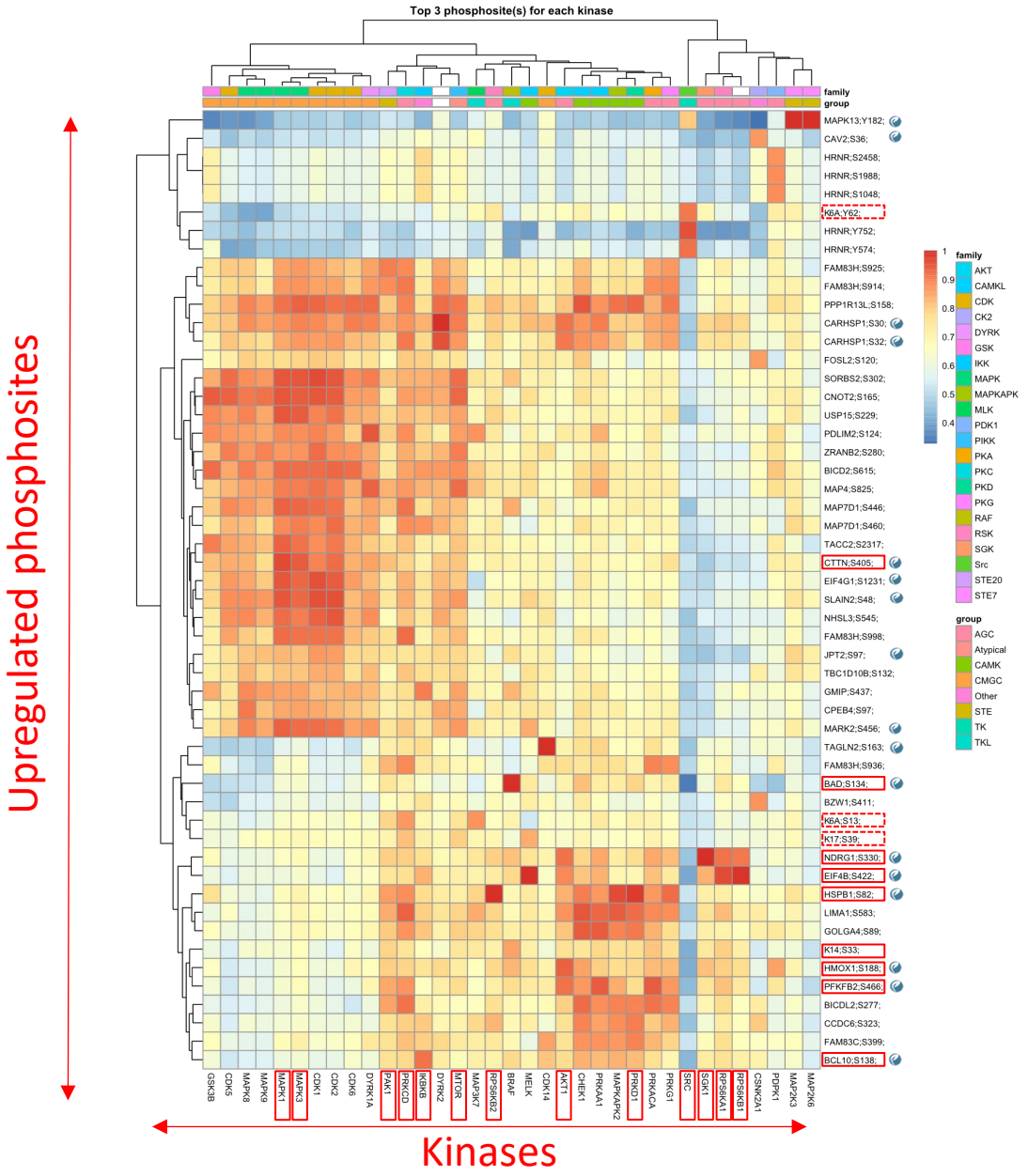
Spectronaut®

6. Data analysis

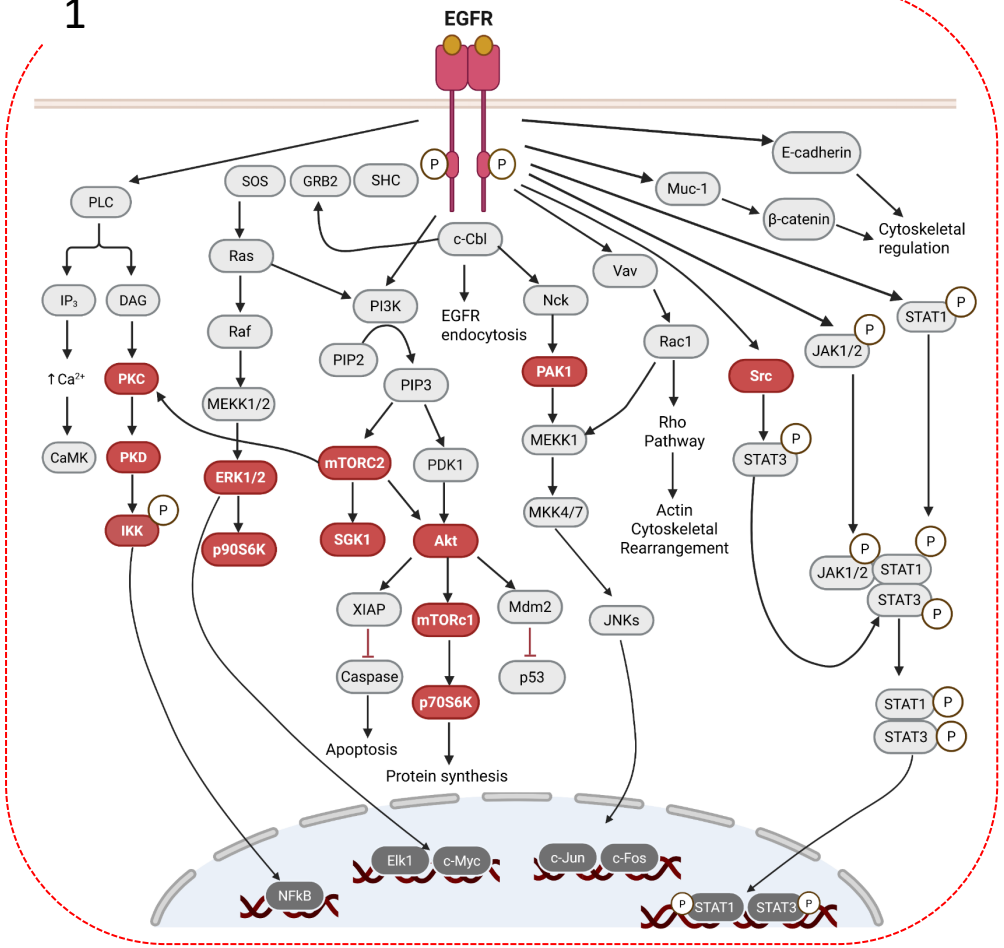
Created with Biorender.com

EGFR pathway overactivation & keratins hyperphosphorylation

Sara Ceccacci



1



2

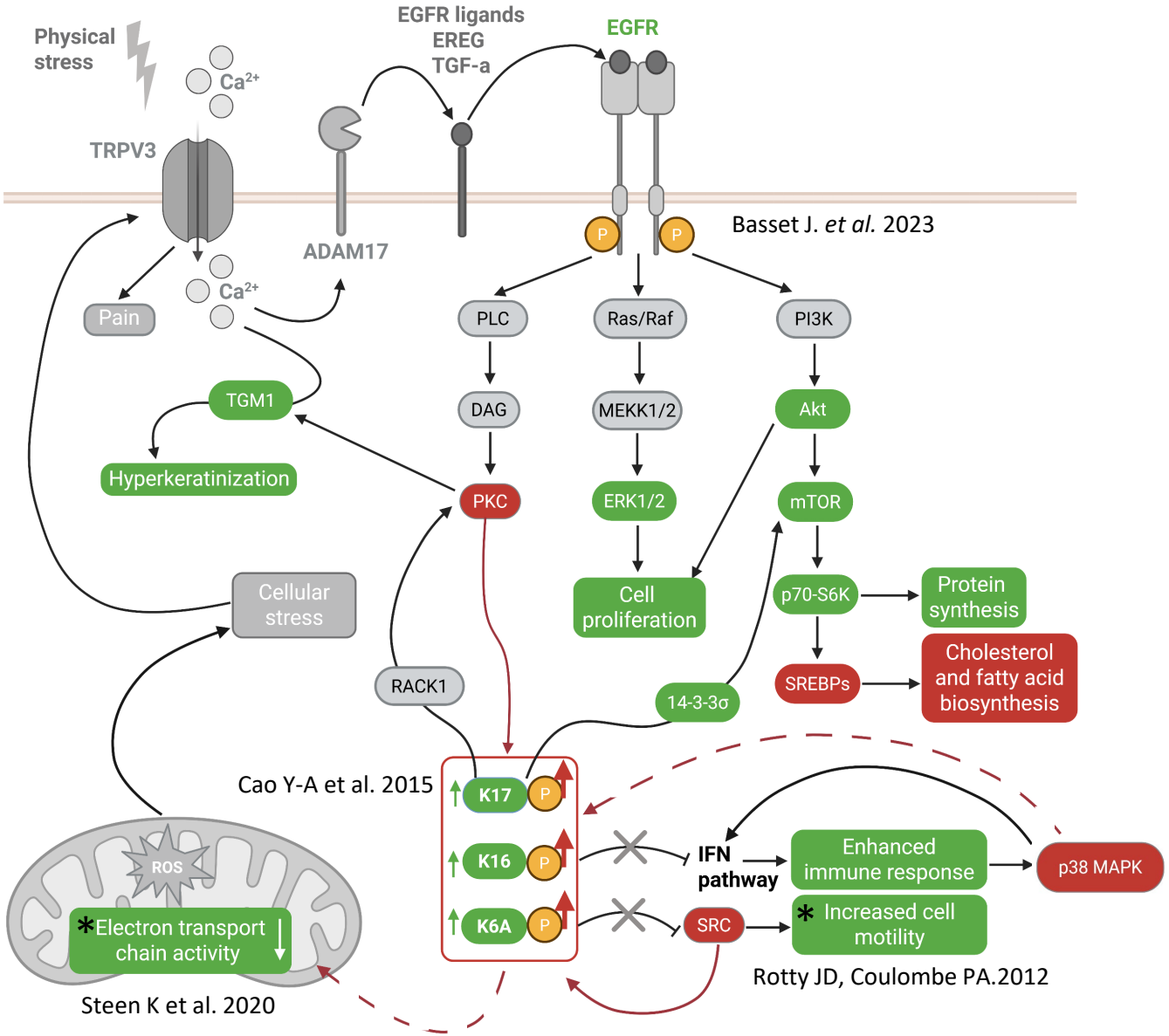
Stress keratins hyperphosphorylation

Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

Refined PC pathogenic mechanism

Sara Ceccacci

- Confirmed in patients
- Novel insights
- * PC murine models



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Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

Conclusions on part I

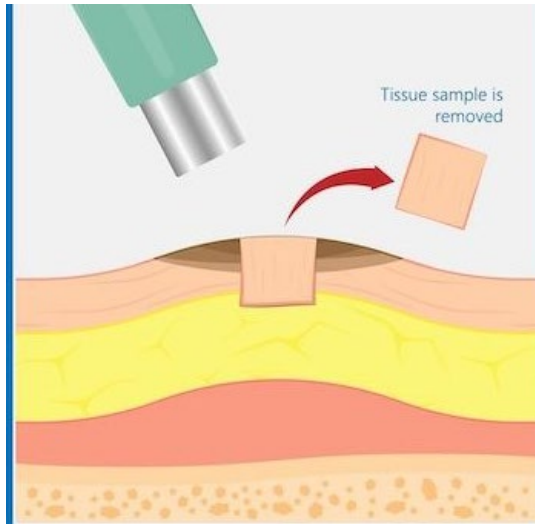
- **SCALE (24 SPD)** and **COVERAGE (+7000 protein +4000 phosphosites)** from full-thickness miniaturized 2 mm **skin biopsies**;

- **UNBIASED LC MSMS** : We **confirmed pathway alterations** reported **in murine models** of the disease **in PC patients** ;

- **New potential targets identified :** informed drug repurposing

Case studies : what can LC MSMS proteomics achieve?

SKIN BIOPSY



Pachyonychia congenita (PC)

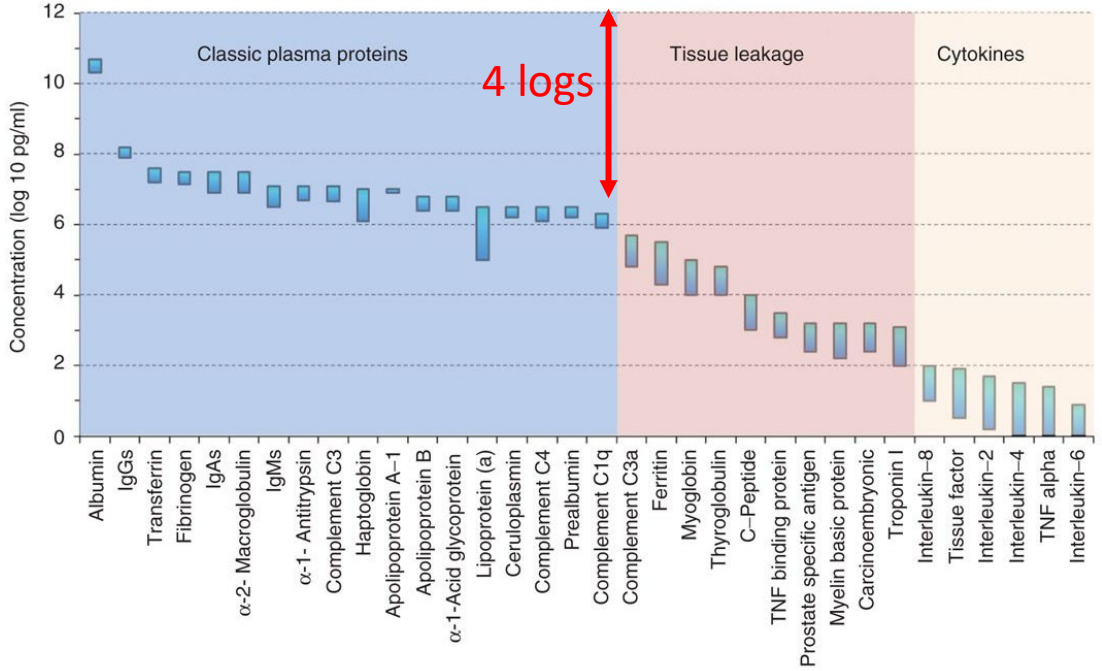
PLASMA &
deepPLASMA

***Epidermolysis Bullosa*-Infection (EBRD)** &
Becker Myodistrophy (BMD)

Very challenging samples. WHY??

Challenge 1. Analytical: proteins dynamic range **in biofluids**

Plasma protein concentration ranking plot



HIGH DYNAMIC RANGE (11-12 logs in plasma)

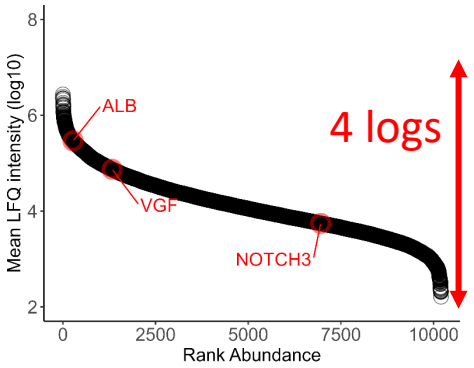
Patients biofluids

✓ In LC-MSMS we see the top 4 -5 logs (most abundant proteins)

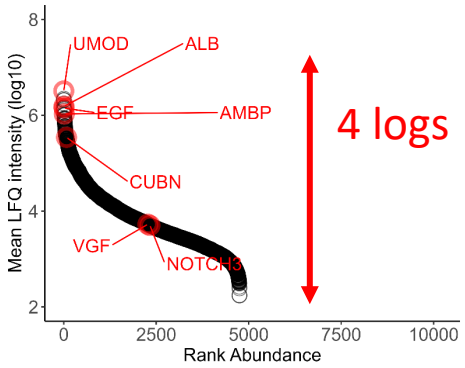
=> Limited **coverage**

Challenge 1. Analytical: proteins dynamic range **in biofluids**

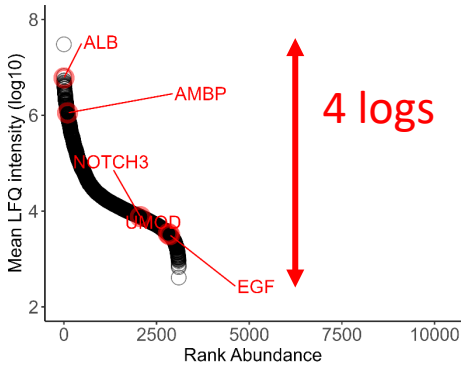
Biopsy



Urine



Neat plasma

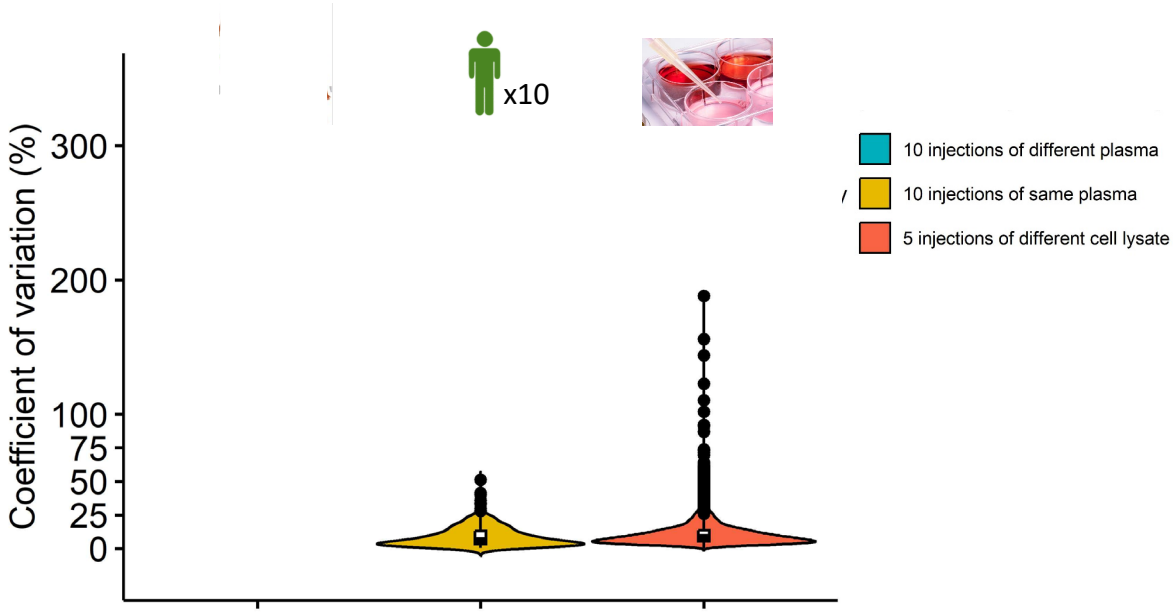


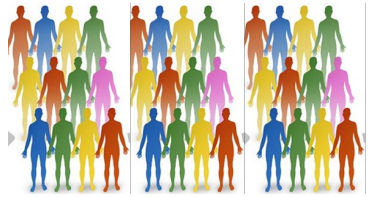
Patients biofluids

✓ In LC-MSMS we see the top 4 -5 logs (most abundant proteins)

=> very limited **coverage in plasma**

Challenge 2. Biological: interpersonal variability





Patients / individuals

✓ **Interpersonal variability is huge**

⇒ More **variability** requires more **samples (especially for small differences!)**

⇒ It adds on pre-analytical, induced **variability**

Metatla et al, Clinical proteomics 2024

Our optimizations for plasma analysis

Neat plasma: limited COVERAGE can be enough

Plasma derived EVs: specific subset of plasma proteins

Plasma enrichment: when higher COVERAGE is necessary

Our optimizations for plasma analysis

Neat plasma: limited COVERAGE can be enough

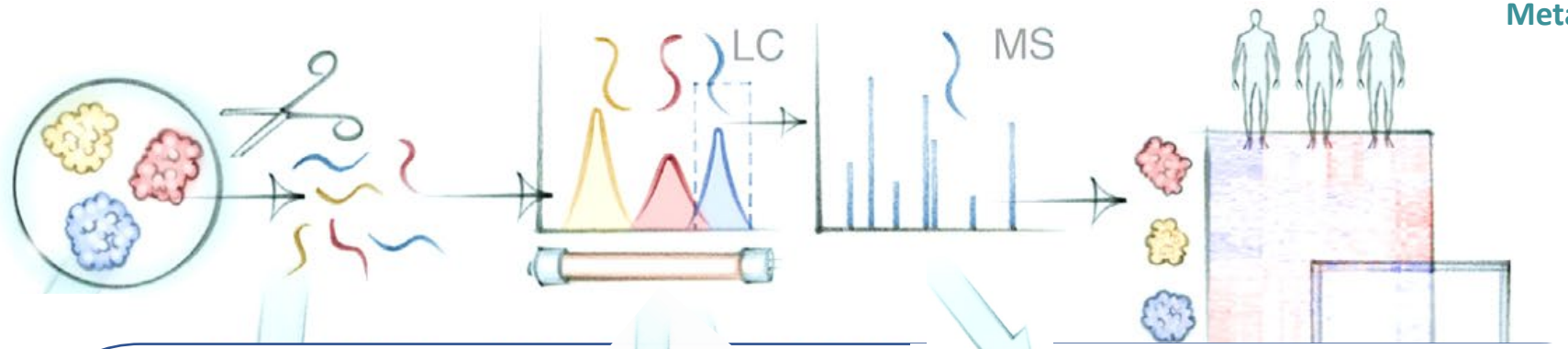
Plasma derived EVs: specific subset of plasma proteins

Plasma enrichment: when higher COVERAGE is necessary

Our optimization for **higher coverage** in neat plasma


Ines Metatla


Kevin Roger

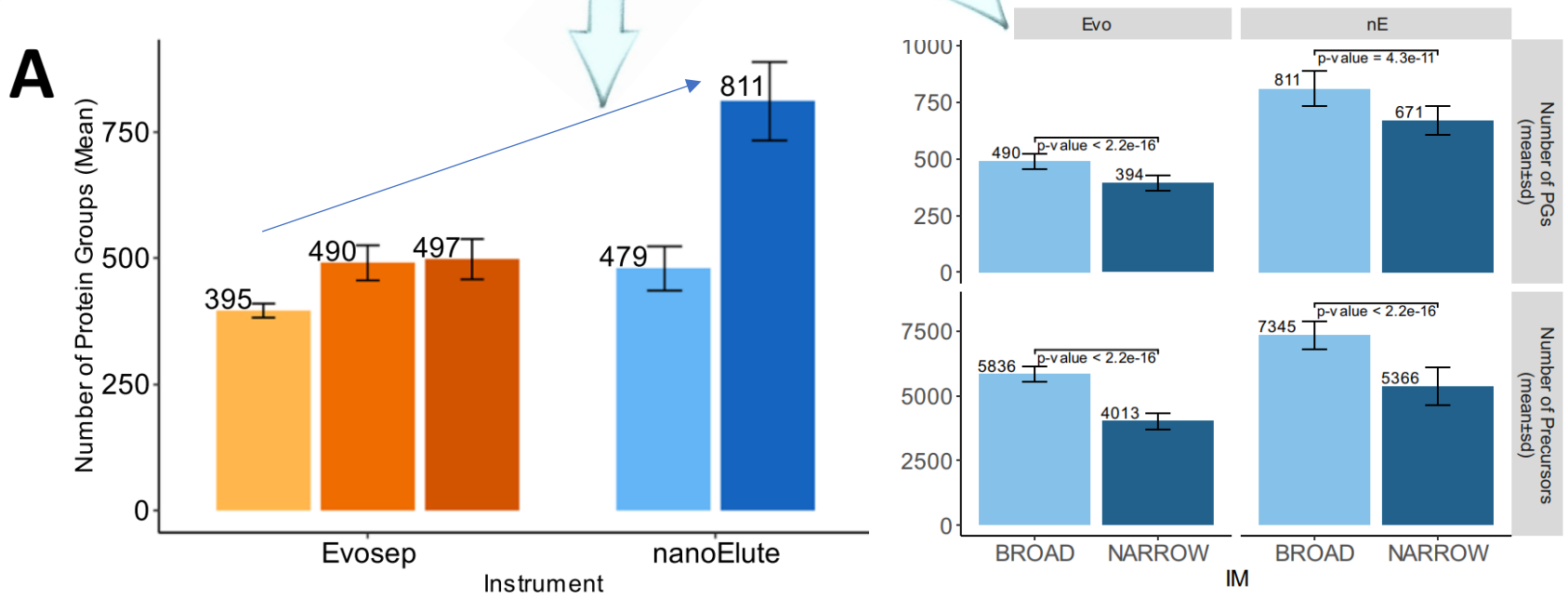


COVERAGE x2

395→ 811 IDs

SCALE x3

12 SPD→ 24SPD, 40SPD



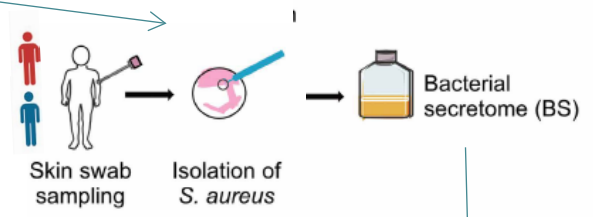
Metatla, Roger…Guerrera, Bruker App Note LCMS214
Metatla, Roger…Guerrera, Proteomics 2024

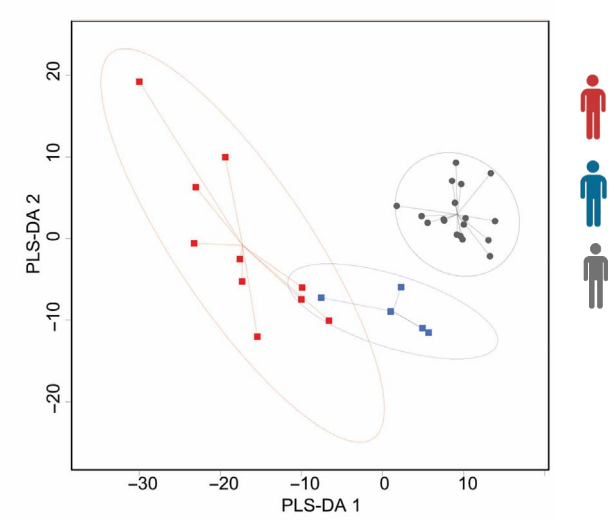
Severity driven by *S. aureus* strain : **precision microbiology**

Severe RDEB
n = 10

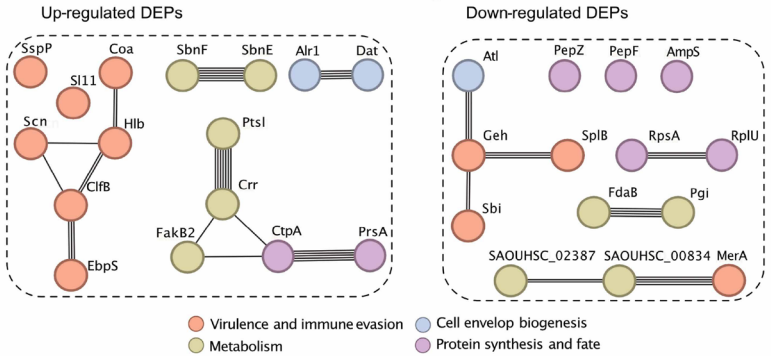
Moderate RDEB
n = 5

Control
n = 18





LC MSMS

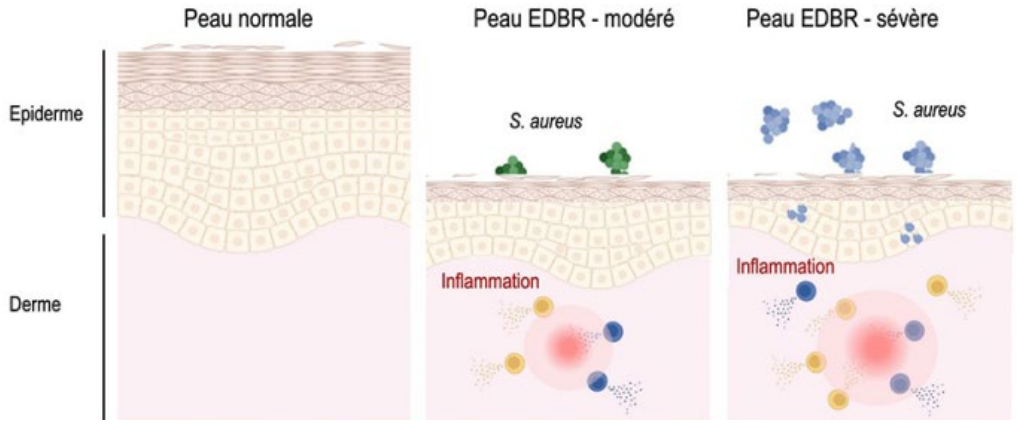


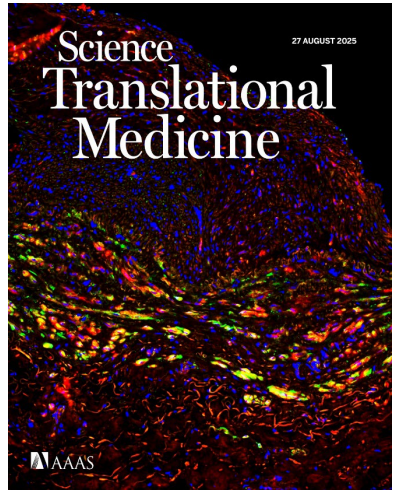
Inflammation

ECM

nt strains,
muno response

Severity driven by *S. aureus* strain : **precision micriobiology**





SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

INFECTIOUS DISEASE

Immune response and clinical severity are shaped by skin-adapted *Staphylococcus aureus* in chronically infected patients

Anne Jamet[1,2*†], Xiali Fu[1‡], Céline Dietrich[3‡], Nathalia Bellon[4,5‡], Messaouda Attailia[3], Elif Uyar[1], Mélanie Montabord[1], Iharilalao Dubail[1], Khanyisile Kunene[3], Agnès Ferroni[2], Laura Polivka[4,5], Marion Dupuis[1], Daniel Euphrasie[1], Stéphanie Leclerc-Mercier[6], Nathalie Four[3], Ines Metatla[7], Kevin Roger[7], Joanna Lipecka[7], Ida Chiara Guerrera[7], Nicolas Mirouze[8], Alain Charbit[1], Mathieu Coureuil[1], Fabienne Charbit-Henrion[9], Smail Hadj-Rabia[4,5], Julie Steffann[10], Guillaume Lezmi[3,11], Christine Bodemer[4,5*], Maria Leite-de-Moraes[3*†]

Inflammation **ECM**

Different strains,
different immuno response

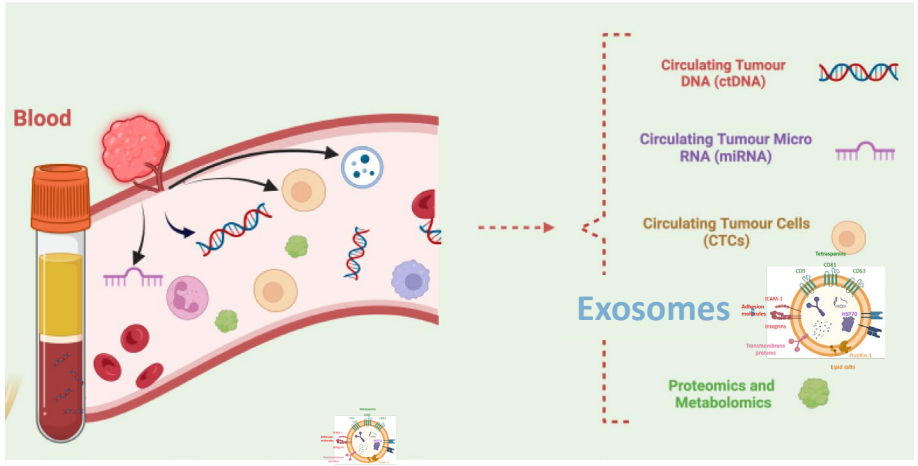
Our optimizations for plasma analysis

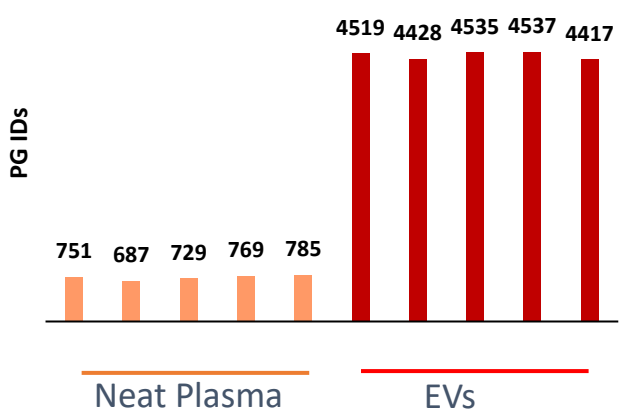
- Neat plasma: limited COVERAGE can be enough
- **Plasma derived EVs:** specific subset of plasma proteins
- **Plasma enrichment**: when higher COVERAGE is necessary

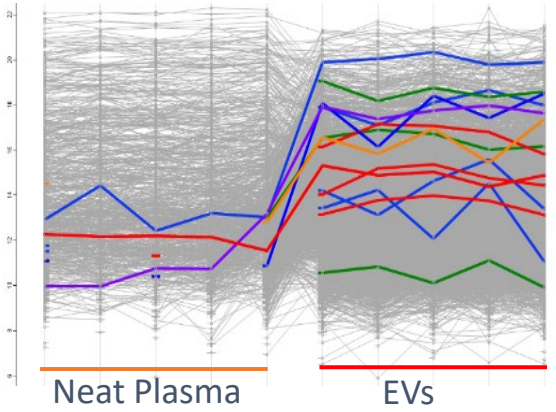
Trick to get higher coverage: **exosomes coprecipitation**


Ines Metatla

Kevin Roger







MHC class I (HLA-A/B/C/G);
MHC class II (HLADRA)

FLOT1
HSP70 (HSP1A1)

Integrins (ITGAM, ITGAX, ITGB2);
ICAM1

CD9; C63;
CD81

EVs markers

500ul plasma → Centrifugation* → **STRAP trypsin digestion** → **nanoLC-MS/MS analysis**

Adapted from New Perspectives on the Role of Liquid Biopsy in Bladder Cancer: Applicability to Precision Medicine, Cancers 2024

Metatla, Roger…Guerrera et al, Clinical proteomics 2024

Our optimizations for plasma analysis

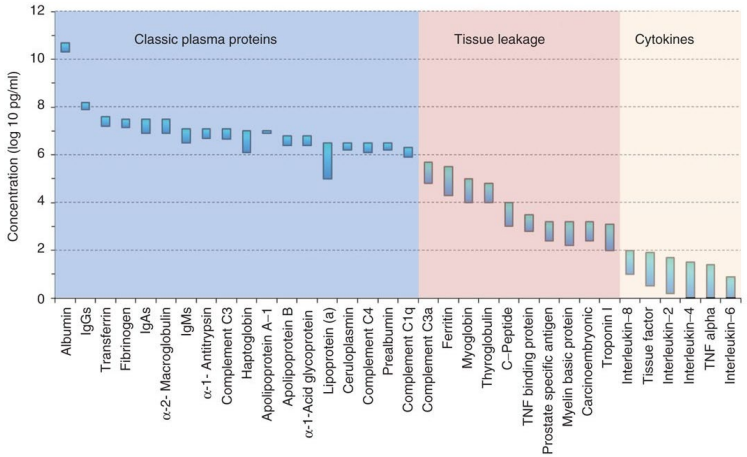
Neat plasma: limited COVERAGE can be enough

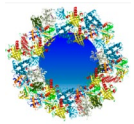
Plasma derived EVs: specific subset of plasma proteins

Plasma enrichment: when higher COVERAGE is necessary

Enrichment methods to **compress the dynamic range**

12 logs





Corona nanoparticles

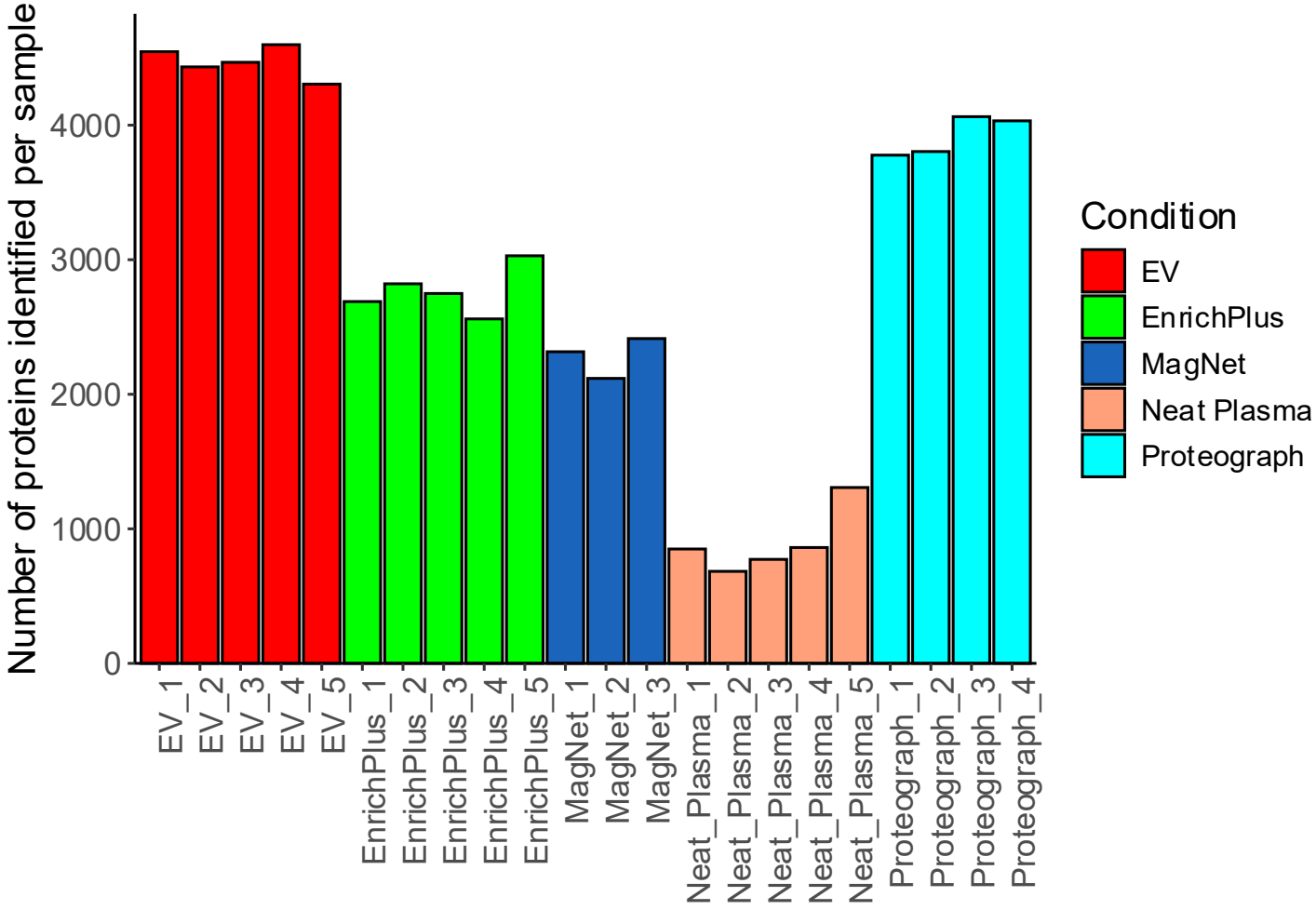
enrichment methods

6-7 logs

..and leads to higher **COVERAGE**


Ines Metatla

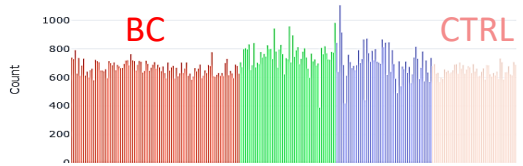

Kevin Roger



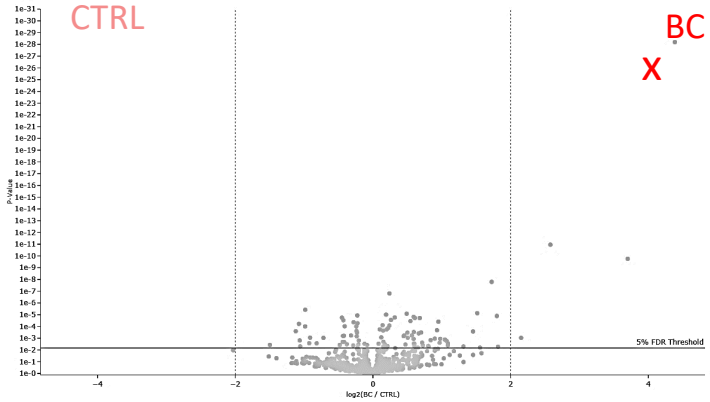
Roger, Metatla… Guerrera, Journal of Proteomics 2025

From 700 to 3350 proteins quantified on average per sample

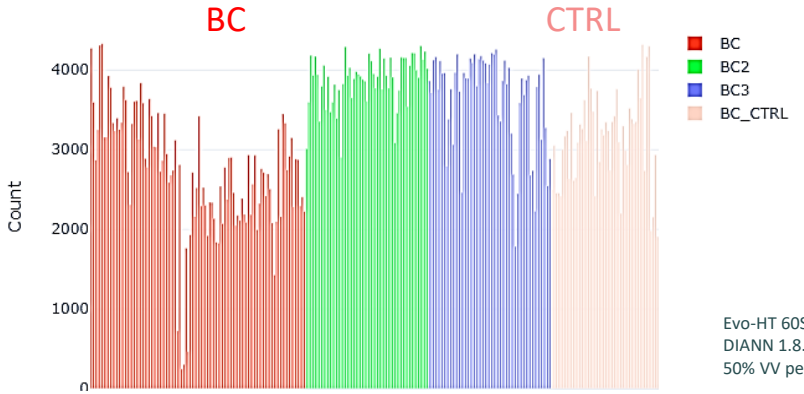
Neat plasma



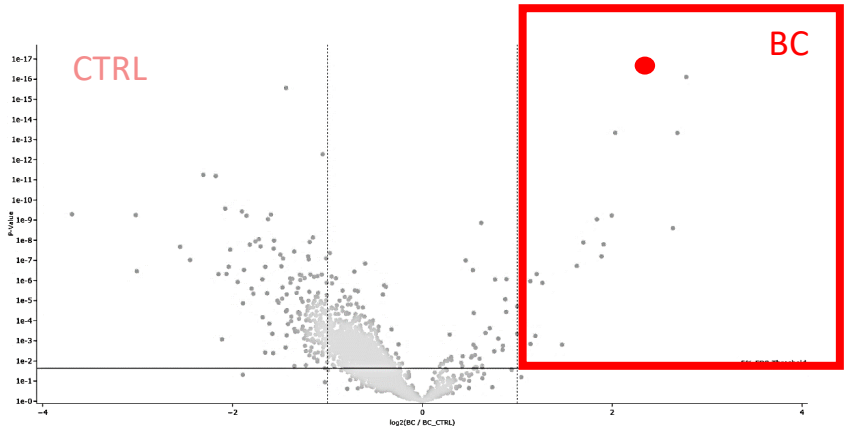
Evo-TTP Pro 60SPD,
DIANN 1.8.2 beta27, EV-boost,
50% VV per group



Proteograph Assay



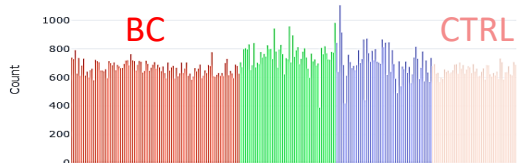
Evo-HT 60SPD,
DIANN 1.8.1,
50% VV per group



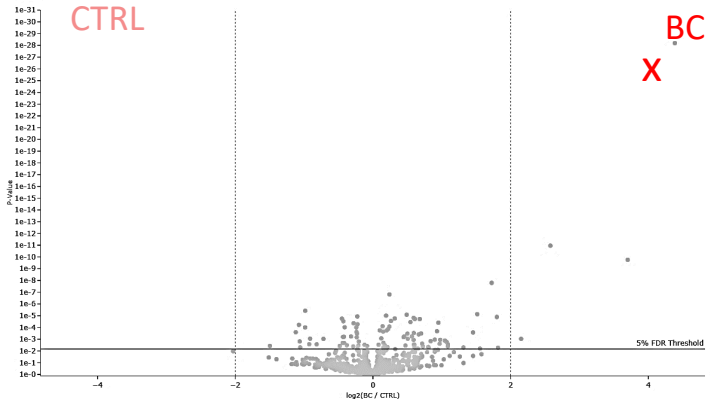
Becker Myodystrophy_ Cohort of 264 samples

Tissue leakage proteins identified after enrichment

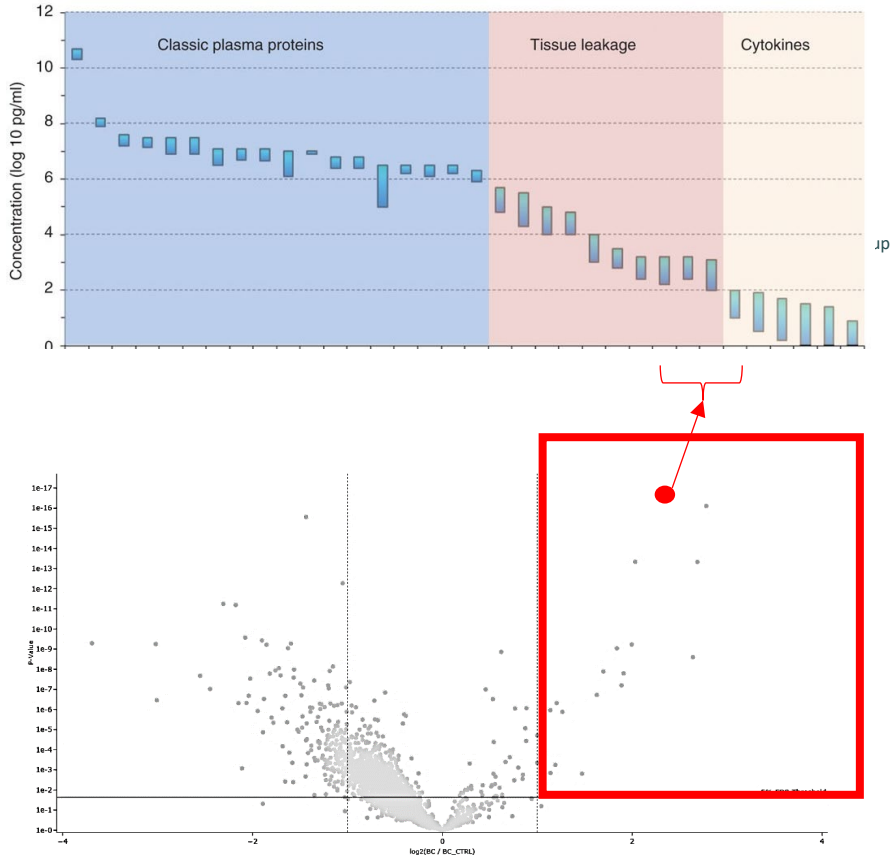
Neat plasma



Evo-TTP Pro 60SPD,
DIANN 1.8.2 beta27, EV-boost,
50% VV per group



Proteograph Assay



Becker Myodystrophy Cohort of 264 samples

Conclusions on plasma

- **COVERAGE** is achievable today, although the proteoforms are yet to be explored on a “large scale”
- **SCALE** is improved fro neat plasma, but not yet for enrichment and EVs prep
- **UNBIASED LC MSMS** allows to see a lot, but can be complemented by immunobased targeted methods

It is the END OF THE BEGINNING in clinical proteomics for biomarkers research.

Necker Proteomics Team

institut imagine
GUÉRIR LES MALADIES GÉNÉTIQUES

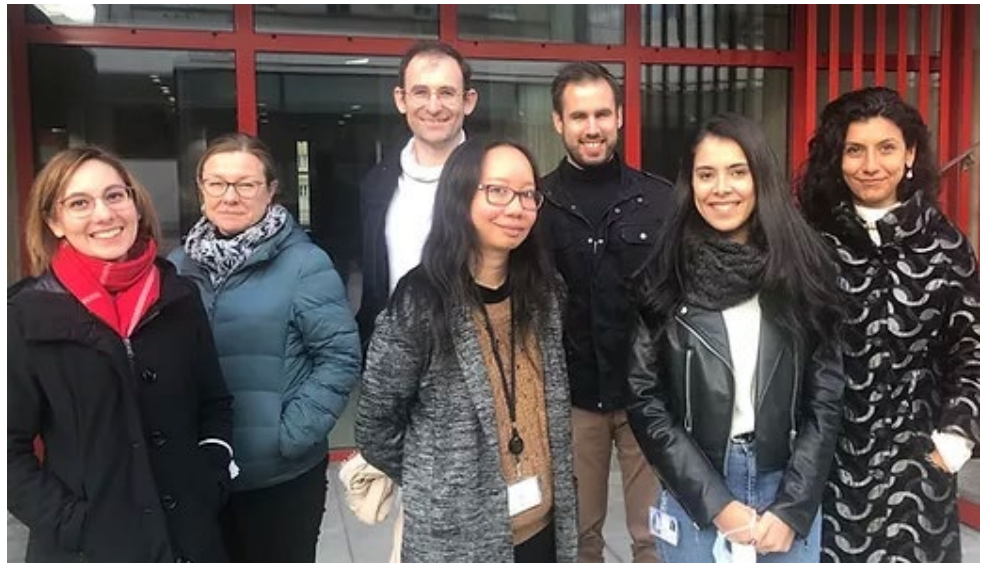
Pr. Alain Hovnanian

INEM Institut Necker Enfants Malades

Anne Jamet
Maria Leite de Moraes

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

Pr. Karim Wahbi
Service Cerdiologie
Hopital Cochin



Sara Ceccacci **Kevin Roger**
Joanna lipecka **Ines Metatla**
Vincent Jung
Cerina Chhuon

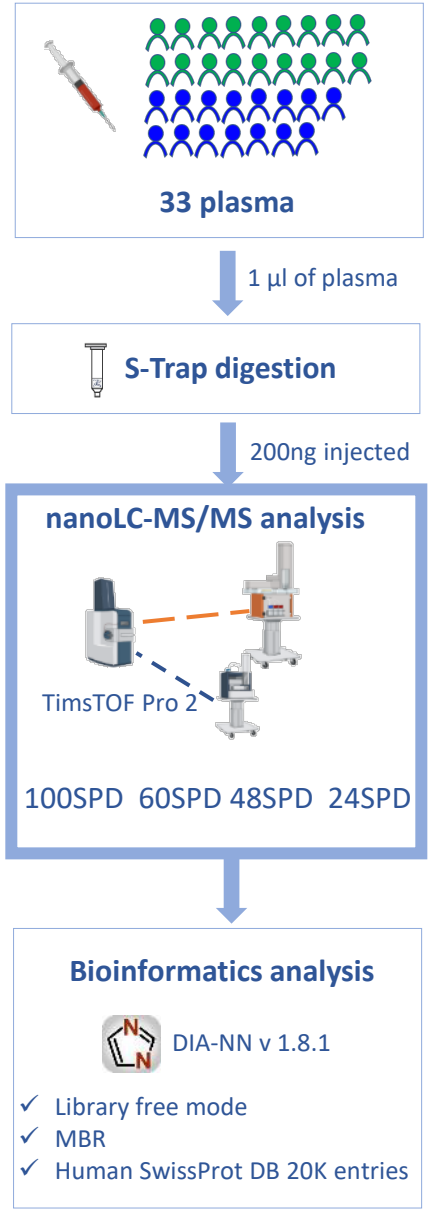
FUNDING

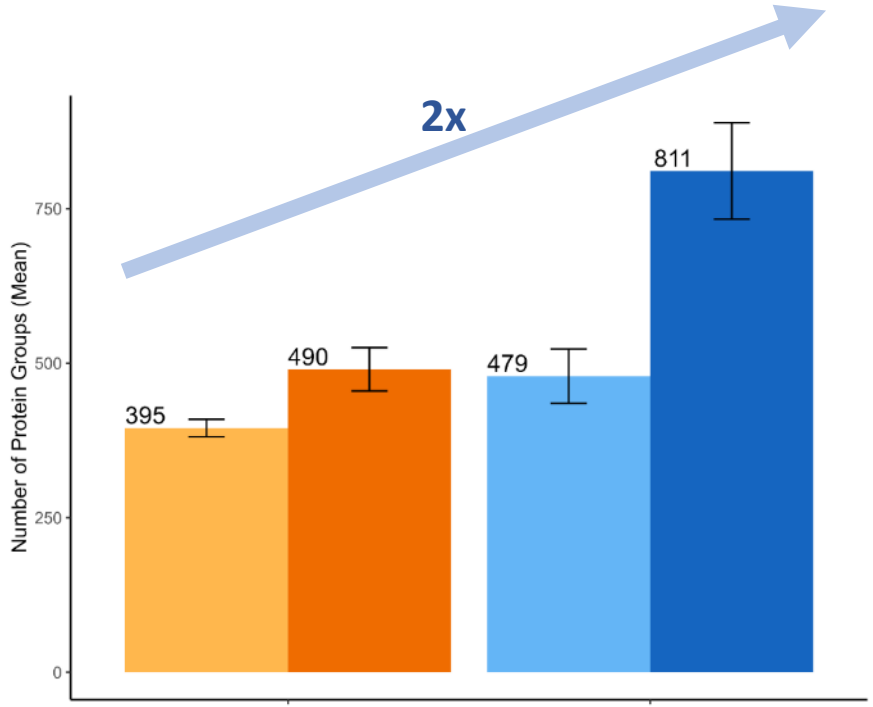
IBiSA Infrastructures en Biologie Santé et Agronomie

PC Project™
Pachyonychia Congenita Research & Patient Support Project

Inserm Université de Paris cnrs ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

2. LC separation time has a high impact on PG IDs

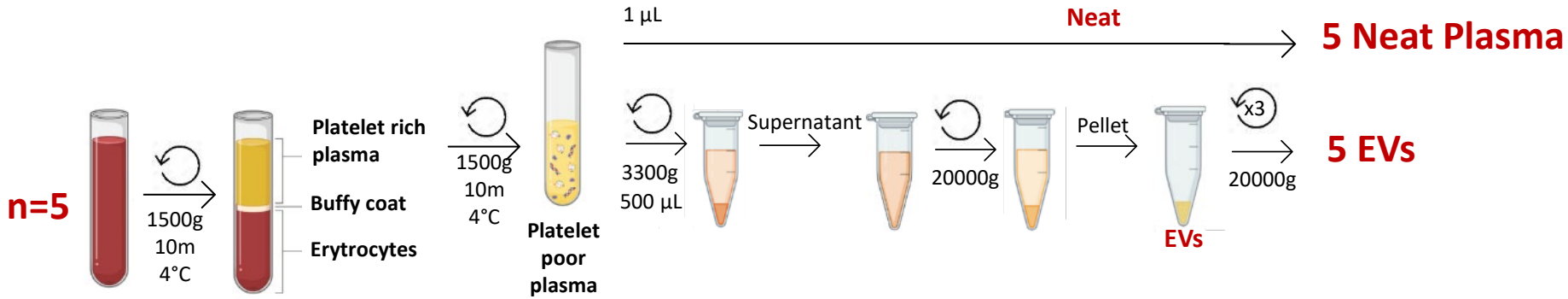


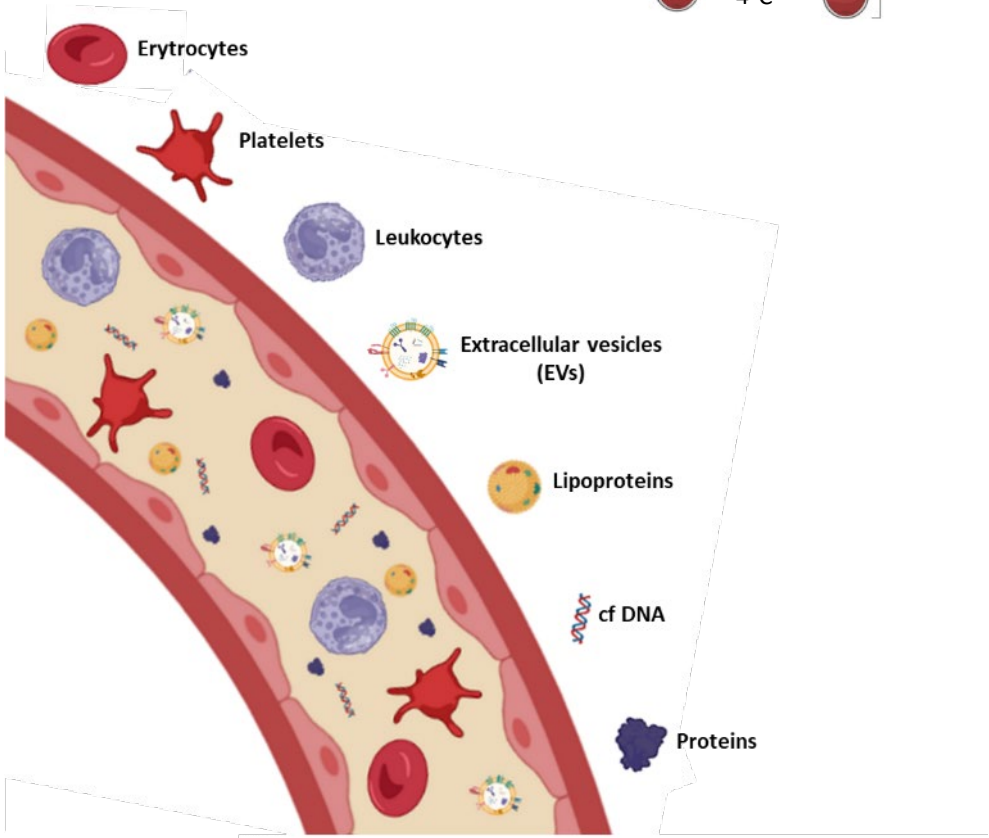


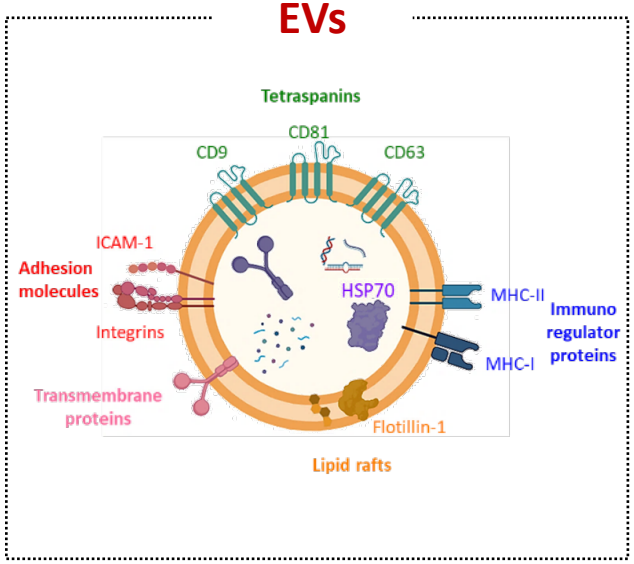
Analytic Column	8cm Performance (Evosep)		25cm Aurora (IonOptiks)	
Separation gradient	11,5min	21min	15min	30min
Actual sample cycle time	14,4min	24min	30min	60 min
SPD	**100 SPD**	**60 SPD**	**48 SPD**	**24 SPD**
LC system	EVOSEP		nanoElute	

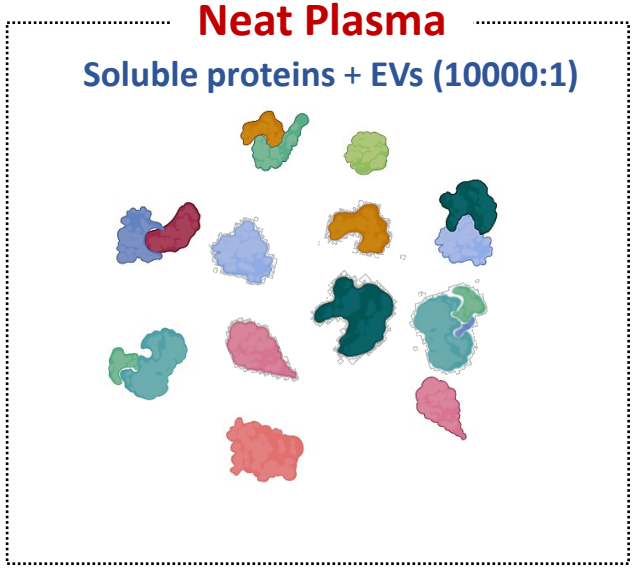
3b. DIA Data analysis: EV-boost

Cerina
CHHUON



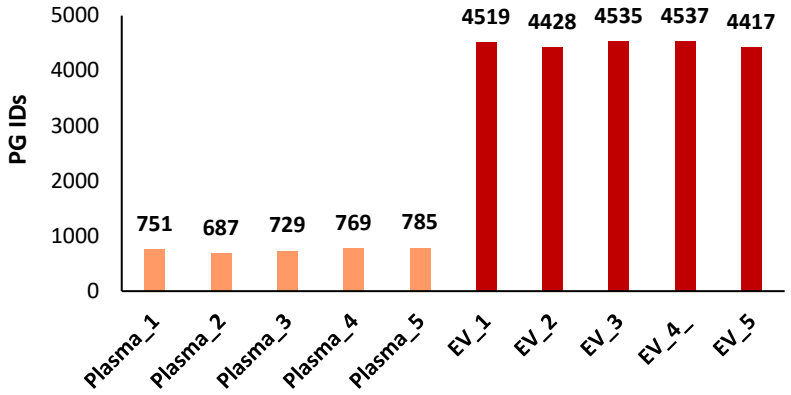


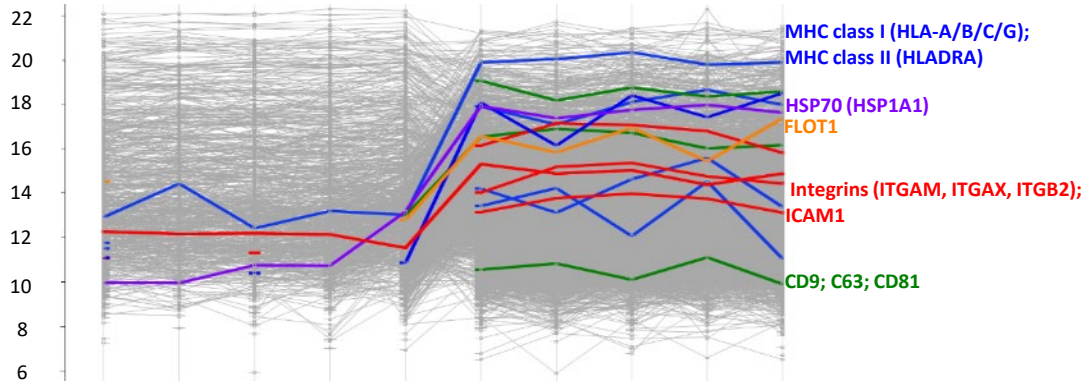




EV protocol from Chhuon, C. et al. *Int. J. Mol. Sci*. **2023**, *24*, 12124

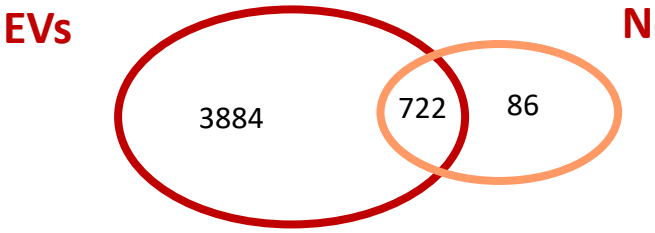
3b. Plasma proteome contains soluble proteins and EVs



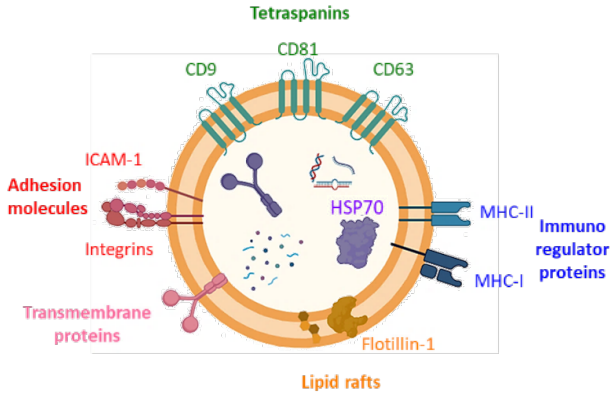


Most EV markers were found in EVs

89% of « Neat Plasma » proteins are also found in « Evs »



EVs

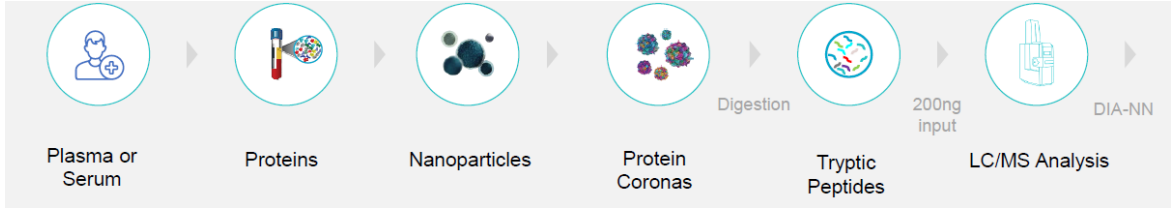


Neat Plasma

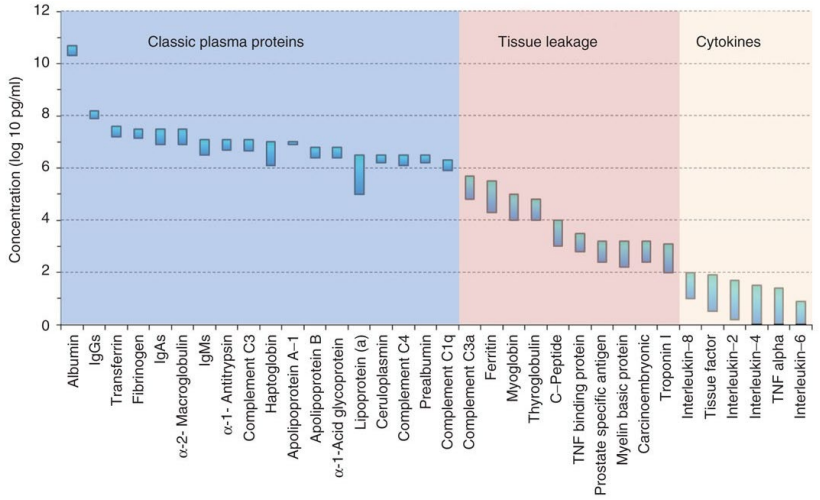
Soluble proteins + EVs (10000:1)

EV fraction is a repertoire of all plasma proteins!

Proteograph: functionalised nanoparticles based assay



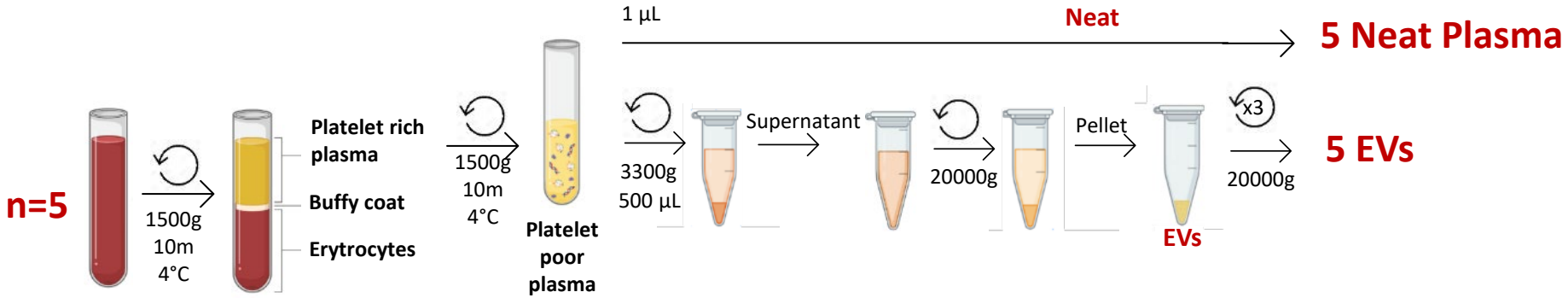
IBiSA Infrastructures en Biologie Santé et Agronomie

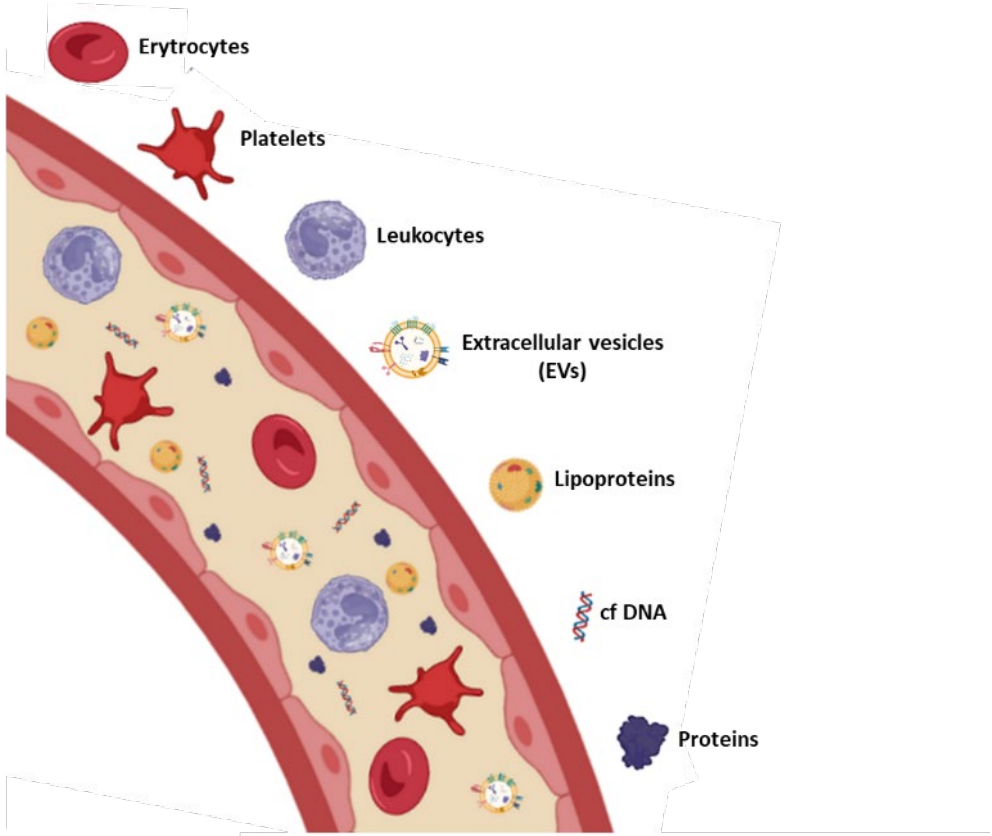


11-12 logs

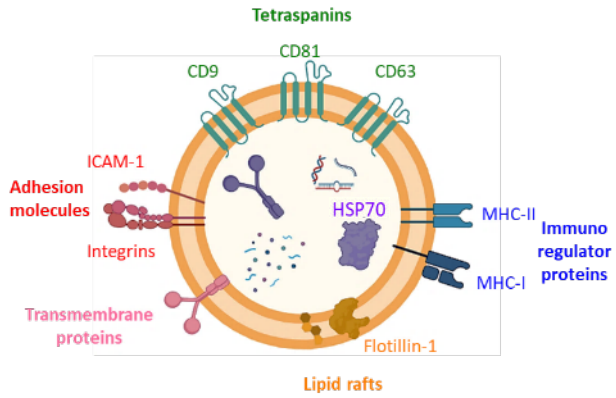
6-7 logs

What is inside « neat plasma »?





EVs

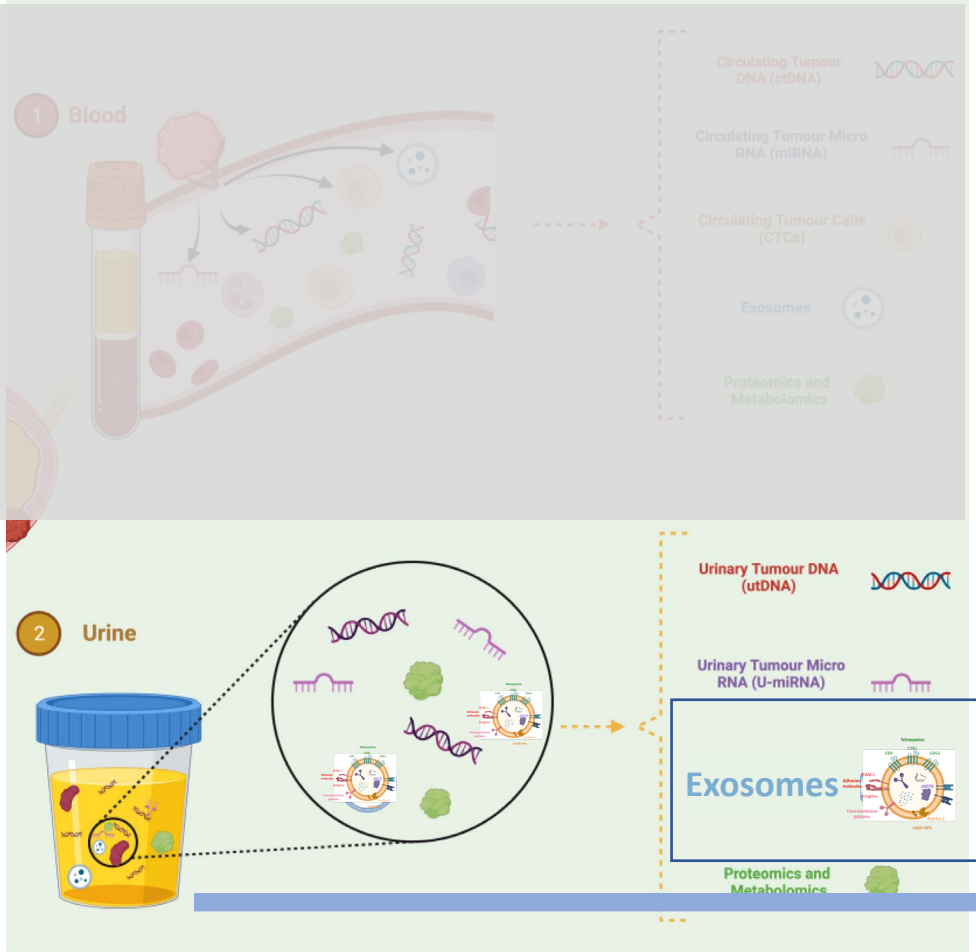


Neat Plasma

Soluble proteins + EVs (10000:1)

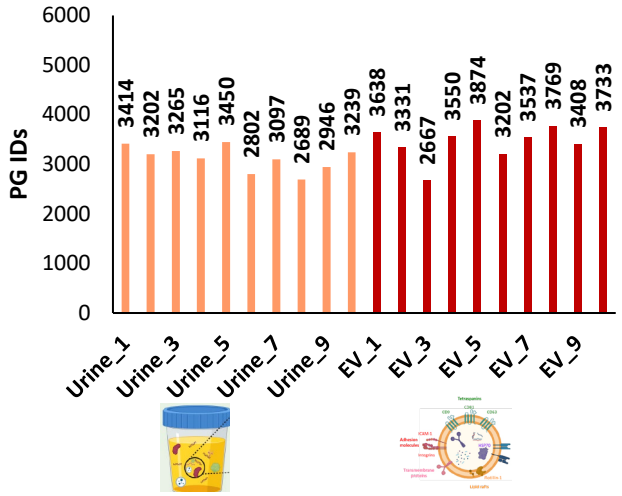
EV protocol adapted from **Harel M et al. Mol Cell Proteomics. 2015**

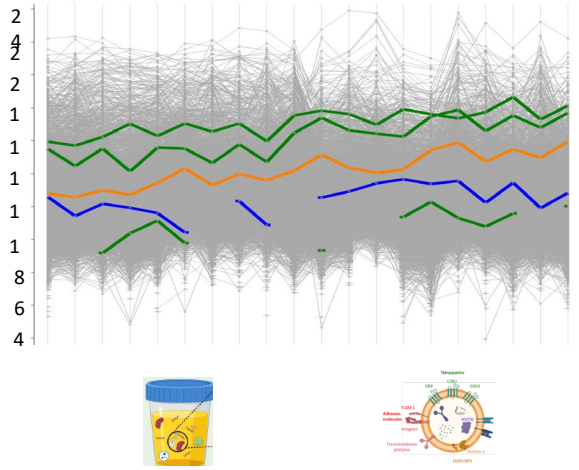
Clever trick to get closer to a **liquid biopsy: exosomes**



Adapted from New Perspectives on the Role of Liquid Biopsy in Bladder Cancer: Applicability to Precision Medicine, Cancers 2024

Exosomes markers & Renal **Intracellular proteins** directly in urines





~~20-50ml of urine~~
~~Ultracentrifugation*~~

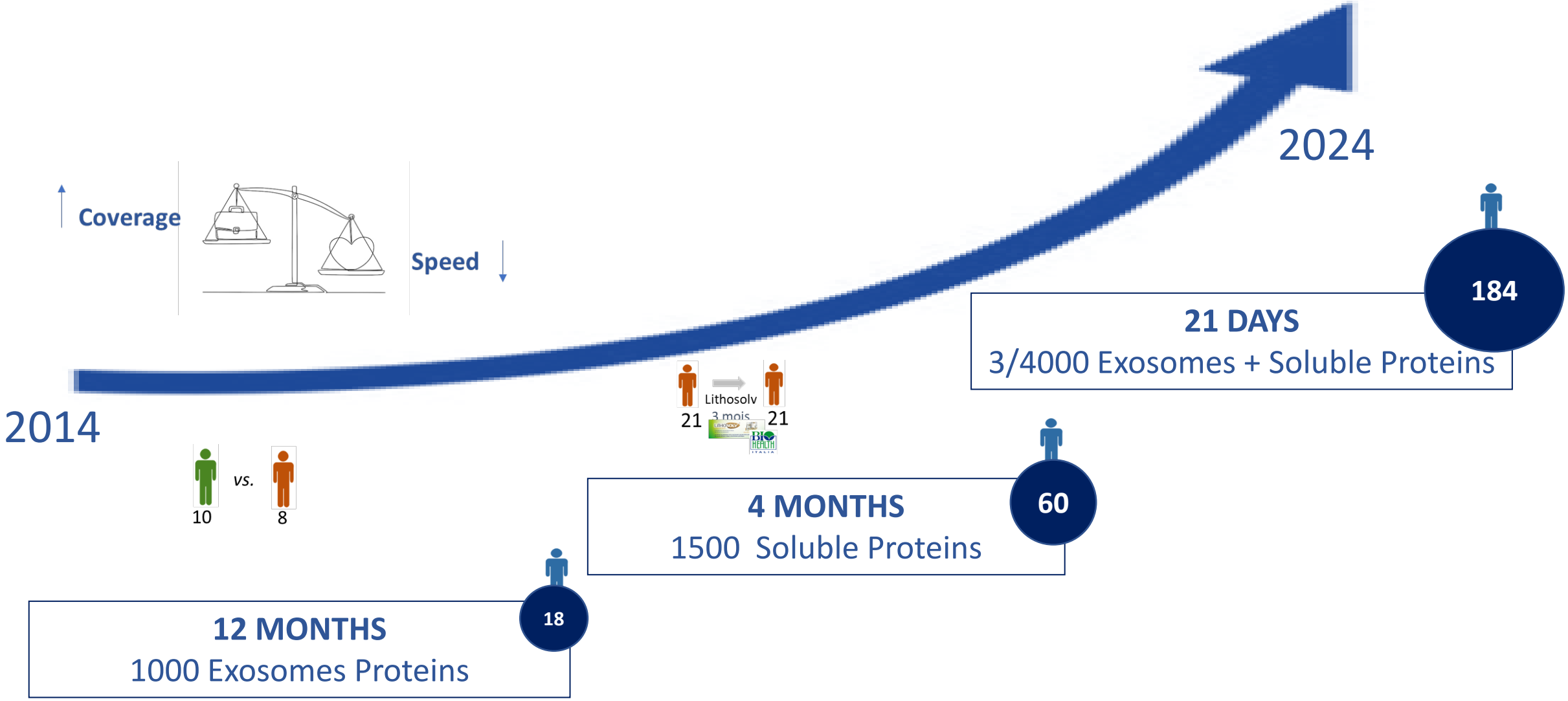
100 ul of urine

trypsin Digestion → **nanoLC-MS/MS analysis**

Total urine is directly a liquid biopsy!

Jung et al; In preparation

Evolution of **speed** and **coverage** of urinary proteomics



Ongoing study on cohort of 184 samples: **rare ciliopathies**

184 Urine

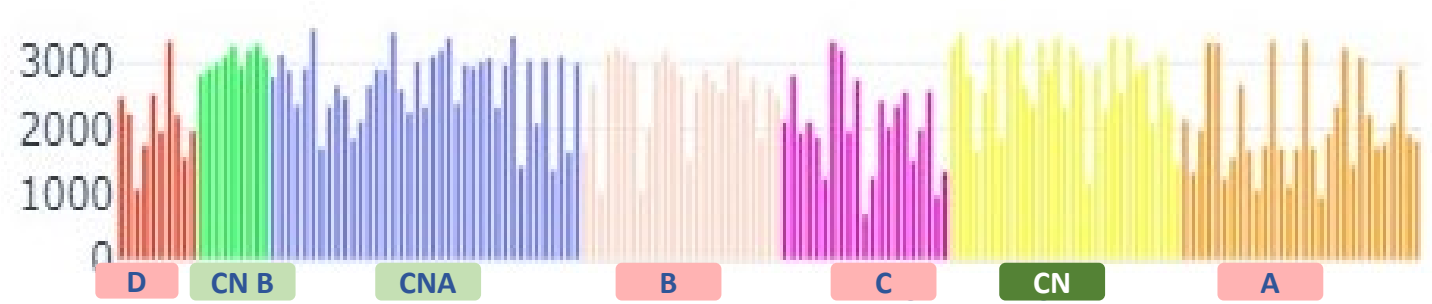
Collection over 4 years

Rare nephropathies
A*,B*,C

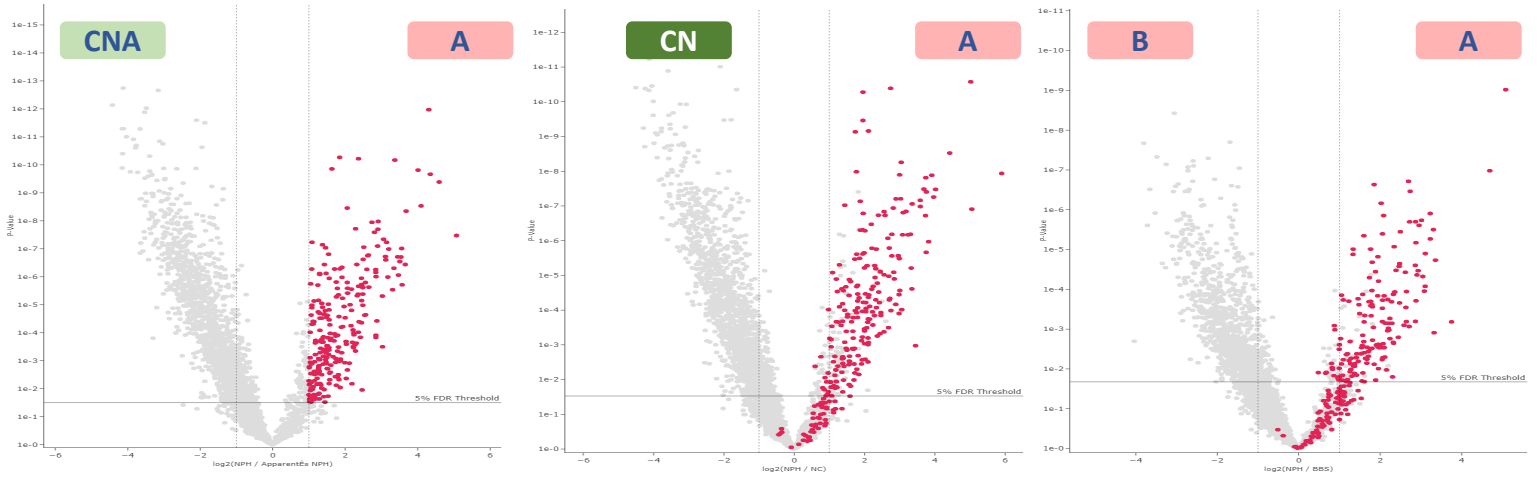
***ciliopathies**

Controls
CNA, CNB, CN HEALTHY

Proteins quantified in each urine sample



Differential proteins quantified in « disease A » vs controls



Strong signature for A
(compared to any control !)

Proteomics recent technlogical advances:

Metatla *et al. Clinical Proteomics* (2024) 21:22
https://doi.org/10.1186/s12014-024-09477-6

Clinical Proteomics

2024

RESEARCH Open Access

Neat plasma proteomics: getting the best out of the worst

Ines Metatla[1†], Kevin Roger[1†], Cerina Chhuon[1], Sara Ceccacci[1], Manuel Chapelle[2], Pierre-Olivier Schmit[2], Vadim Demichev[3] and Ida Chiara Guerrera[1*]

Acta Neuropathol. 2024 Mar 11;147(1):52. doi: 10.1007/s00401-024-02706-0.

2024

Comprehensive proteomics of CSF, plasma, and urine identify DDC and other biomarkers of early Parkinson's disease

5000 CSF, Plasma, Urine

Jarod Rutledge [1 2], Benoit Lehallier [3], Pardis Zarifkar [3 4], Patricia Moran Losada [3 5], Marian Shahid-Besanti [3], Dan Western [6 7], Priyanka Gorijala [6 7], Sephira Ryman [3 8], lizabeth Mormino [3], Alexandra Trelle [9], Anthony D Wagner [5 9], [1], Carlos Cruchaga [6 7], Victor W Henderson [3 12], mer [14], Tony Wyss-Coray [15 16 17], Kathleen L Poston [18 19 20 21]

2024

SCIENCE ADVANCES | RESEARCH ARTICLE

CORONAVIRUS

Longitudinal plasma proteomic analysis of 1117 hospitalized patients with COVID-19 identifies features associated with severity and outcomes

Arthur Viode[1,2]†, Kinga K. Smolen[2,3]†, Patrick van Zalm[1,2,4]†, David Stevenson[1], Meenakshi Jha[1], Kenneth Parker[1], IMPACC Network‡, Ofer Levy[2,3,5], Judith A. Steen[2,6,7], Hanno Steen[1,2,3,7]*

ELSEVIER

Journal of Proteomics
Volume 321, 30 October 2025, 105519

2025

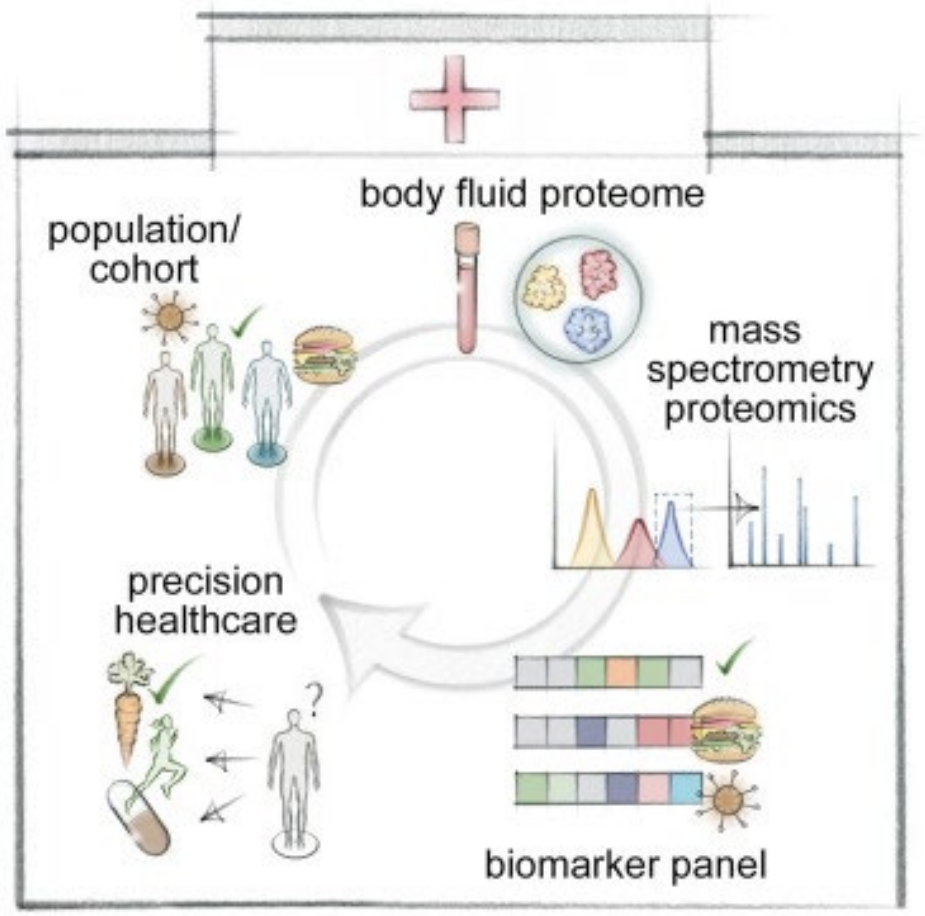
Mining the plasma proteome: Evaluation of enrichment methods for depth and reproducibility

K. Roger [a 1], I. Metatla [a 1], S. Ceccacci [a], K. Wahbi [b c d], L. Motté [b c d], C. Chhuon [a], I.C. Guerrera [a]

MCP RESEARCH Special Issue: Clinical Proteomics

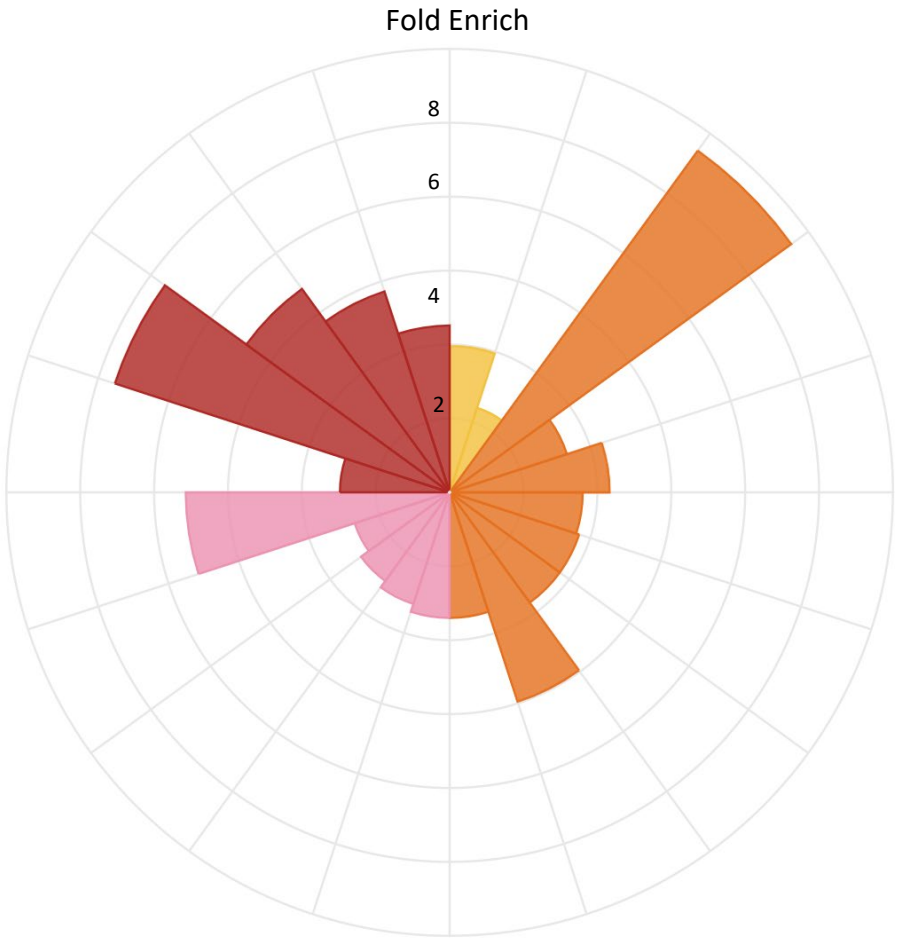
MS-Based Proteomics of Body Fluids: The End of the Beginning

Authors
Jakob M. Bader, Vincent Albrecht, and Matthias Mann



Upregulated proteins in PC lesional samples

Sara Ceccacci



Hyperkeratosis

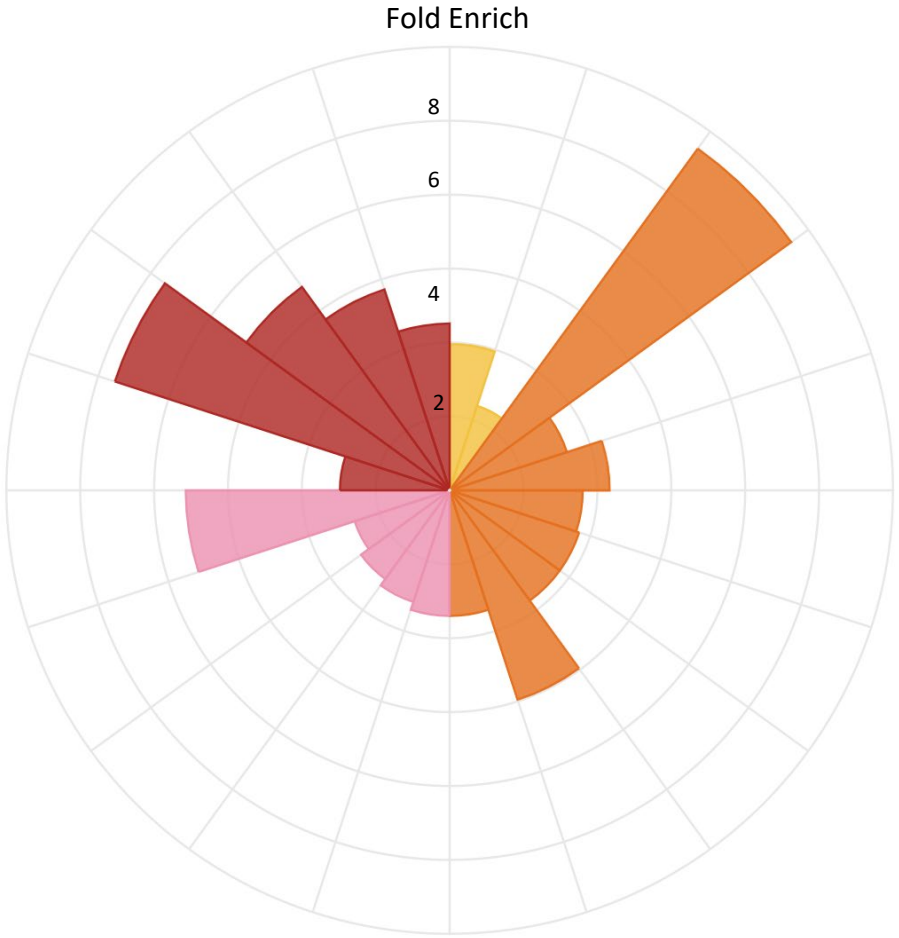
Hyperproliferation and protein synthesis

Immune Response

Lipid biosynthesis

Upregulated proteins in PC lesional samples


Sara Ceccacci



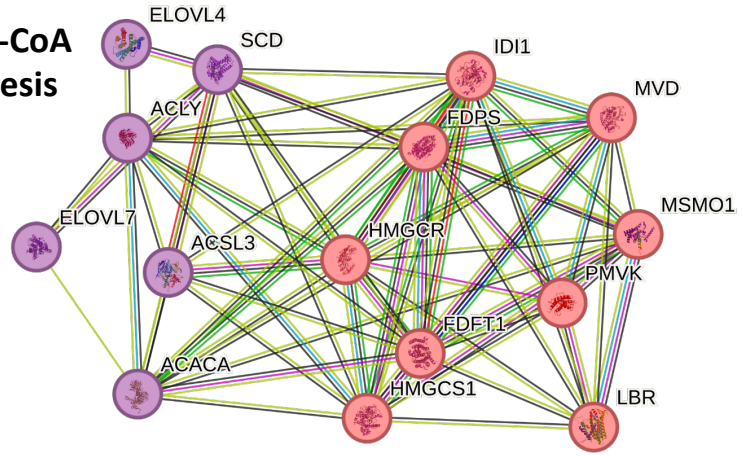
Hyperkeratosis

Hyperproliferation and protein synthesis

Immune Response

Lipid biosynthesis

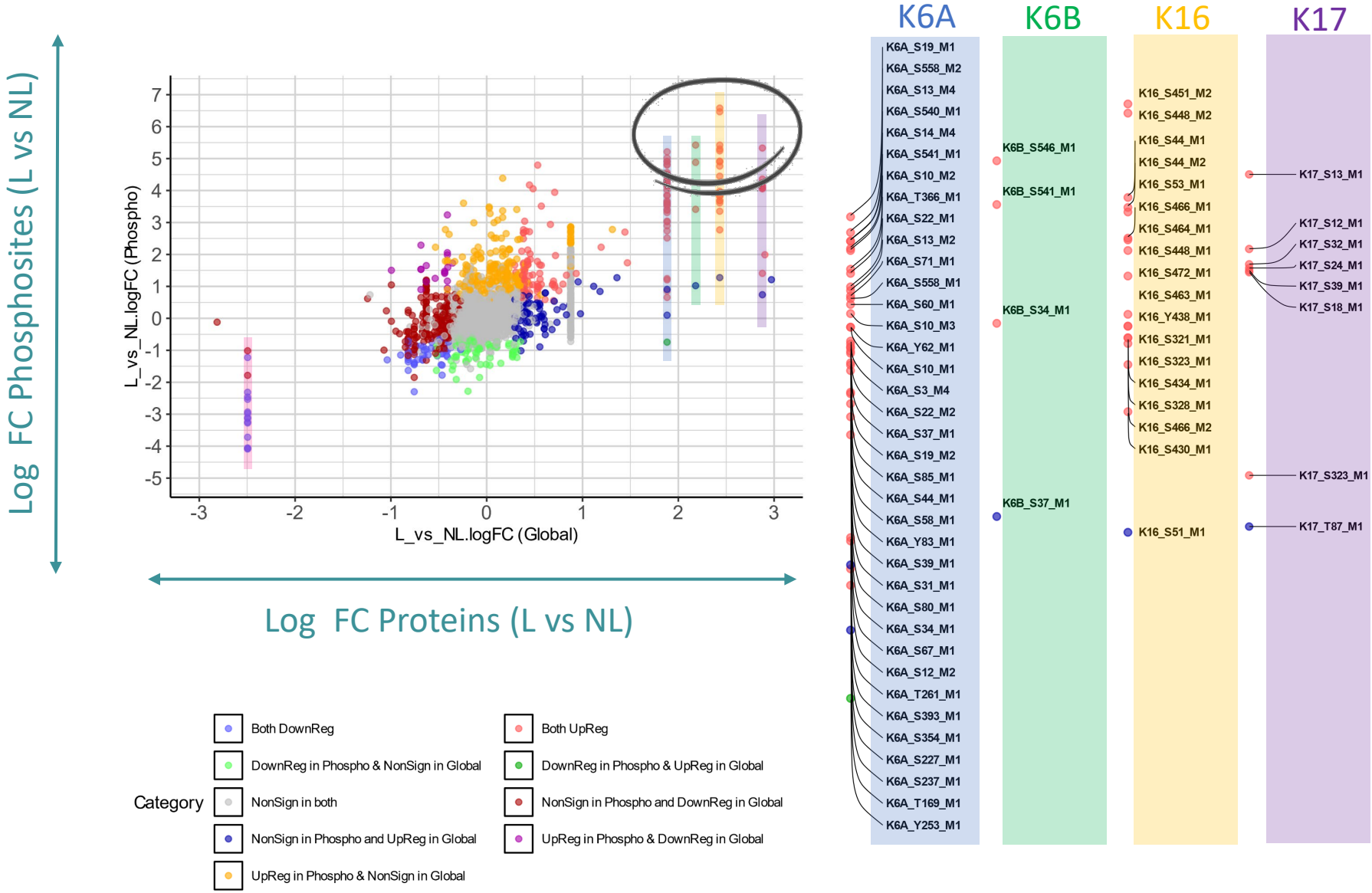
Fatty acyl-CoA biosynthesis



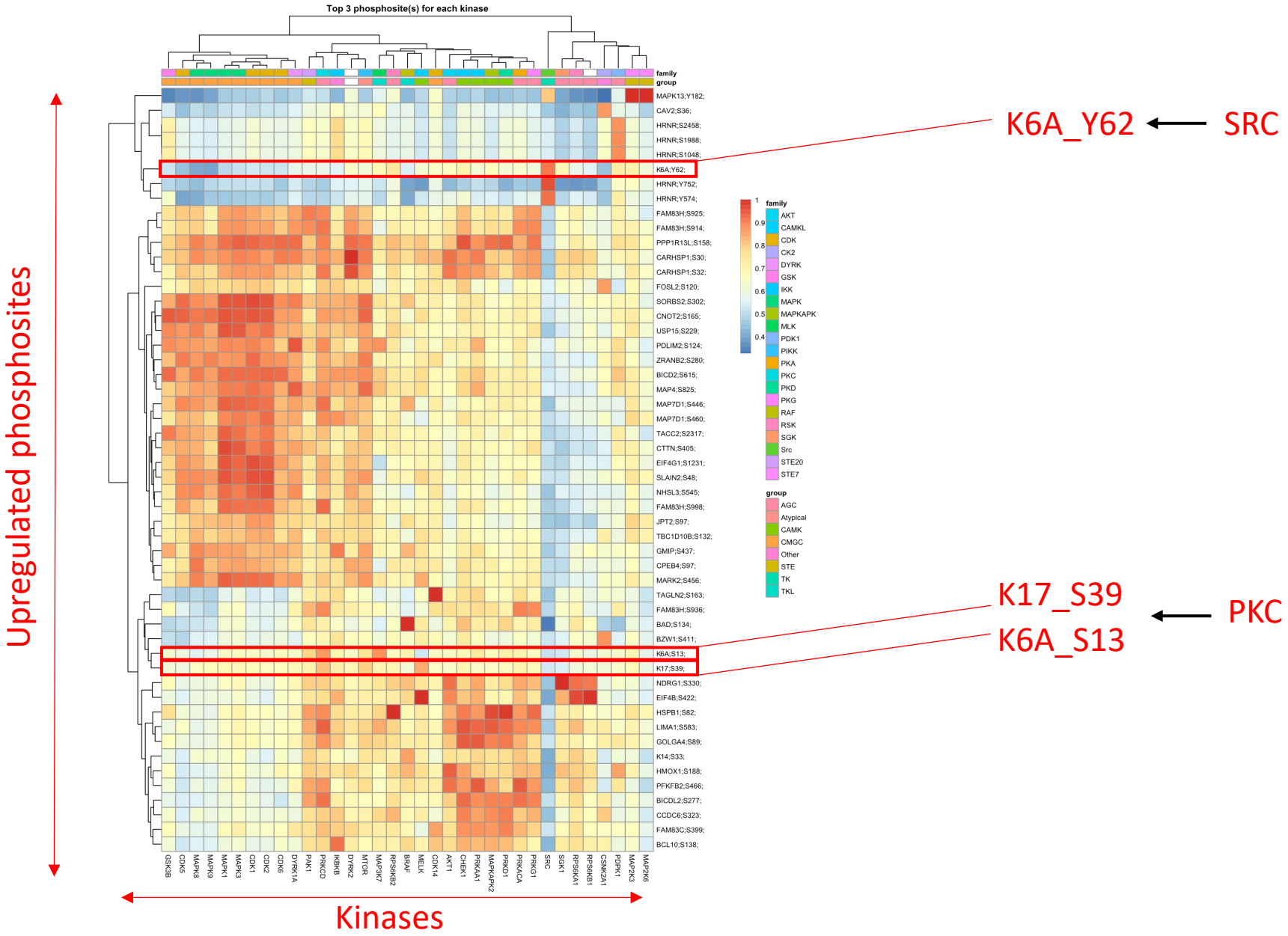
Cholesterol biosynthesis

PC Phosphoproteomics Analysis

PhosVIEW App



PhosR analysis of upregulated phosphosites



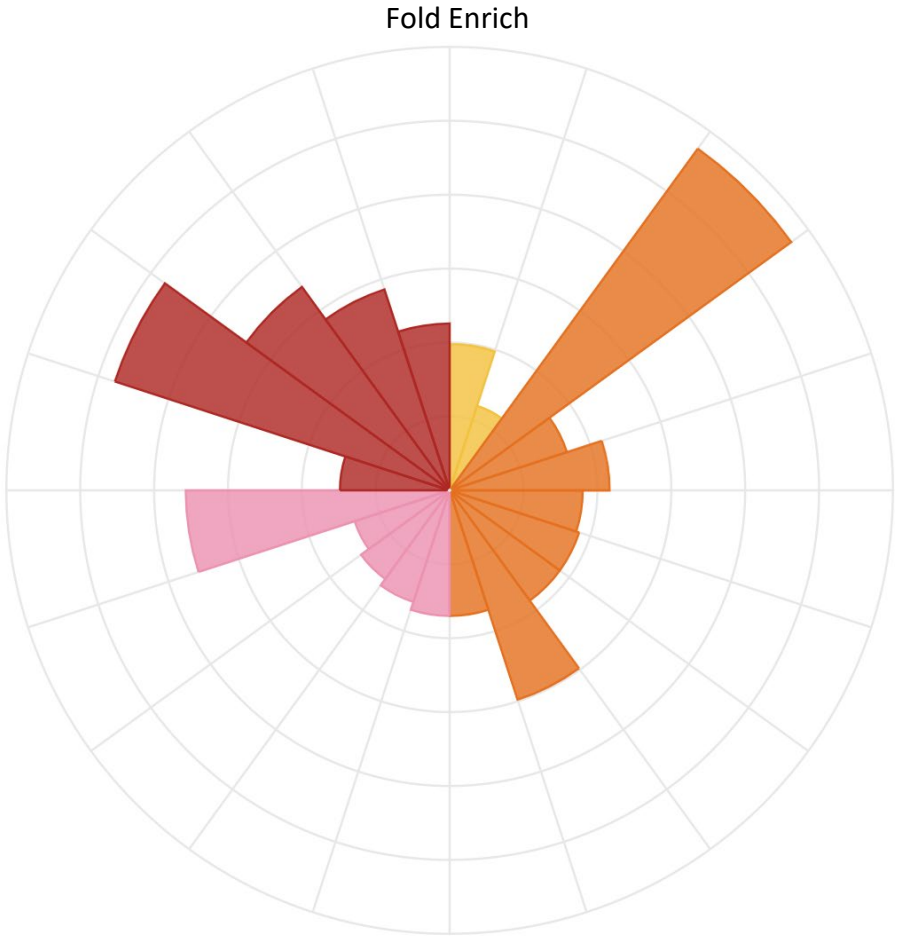
K6A_Y62 ← SRC

K17_S39 ← PKC

K6A_S13

Upregulated proteins in PC lesional samples

Sara Ceccacci



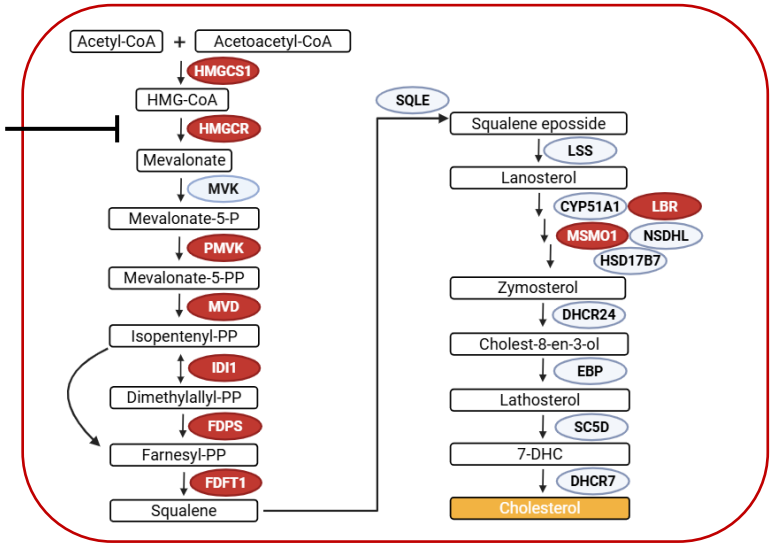
Hyperkeratosis

Hyperproliferation and protein synthesis

Immune Response

Lipid biosynthesis

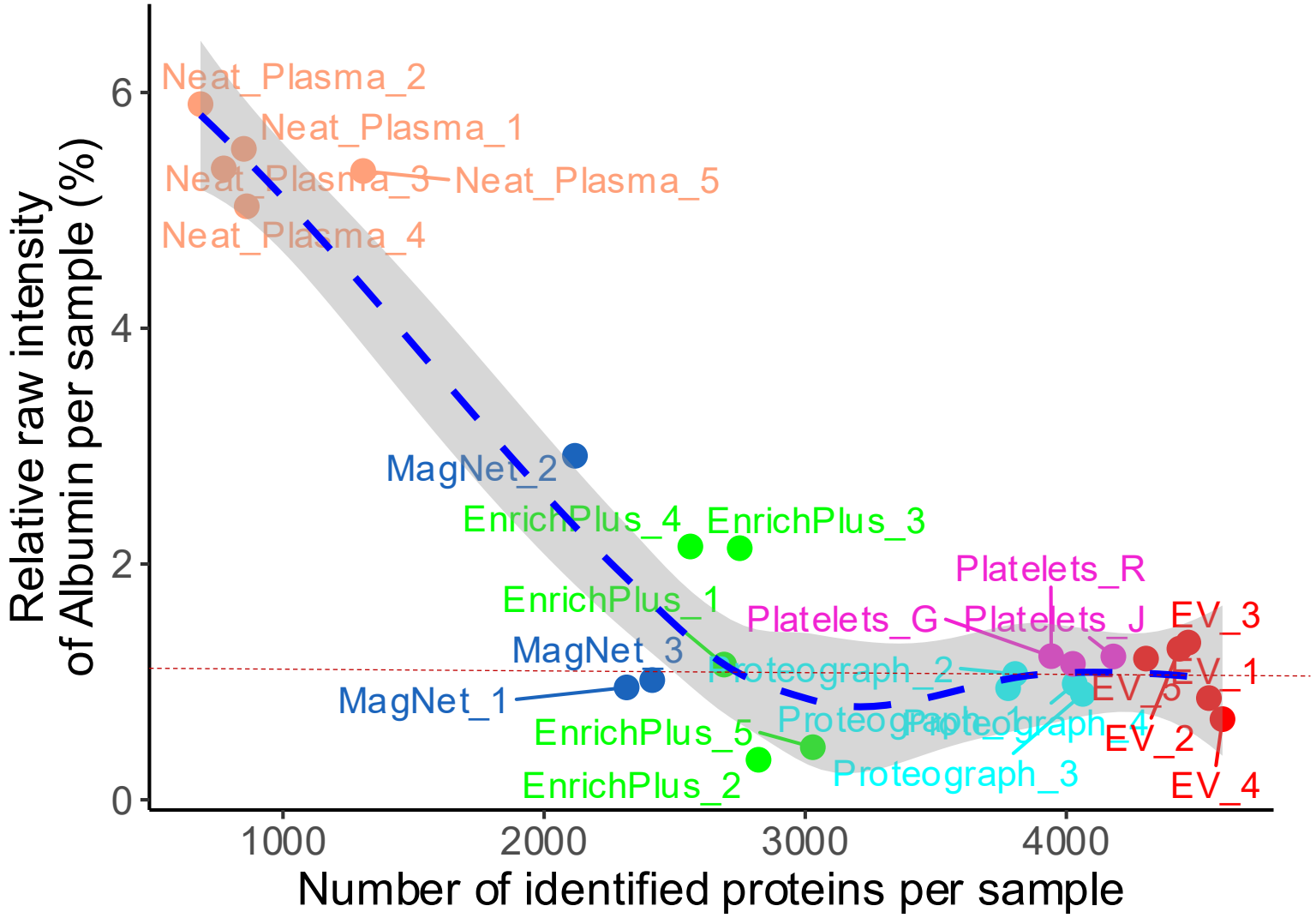
atins



Cholesterol biosynthesis

Ceccacci, Roger… Hovnanian, Guerrera, SUBMITTED

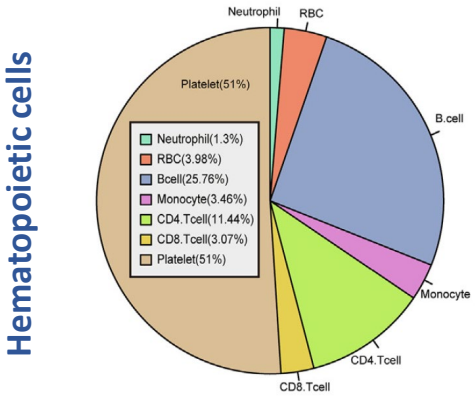
It’s more about enrichment than depletion



- Albumin is *de facto* depleted
- Lower Albumin → Higher PG
- BUT, at same residual albumin level,
  → higher PG for Proteograph
- The residual abundance of Albumin is VARIABLE

Plasma EV come mostly from platelets

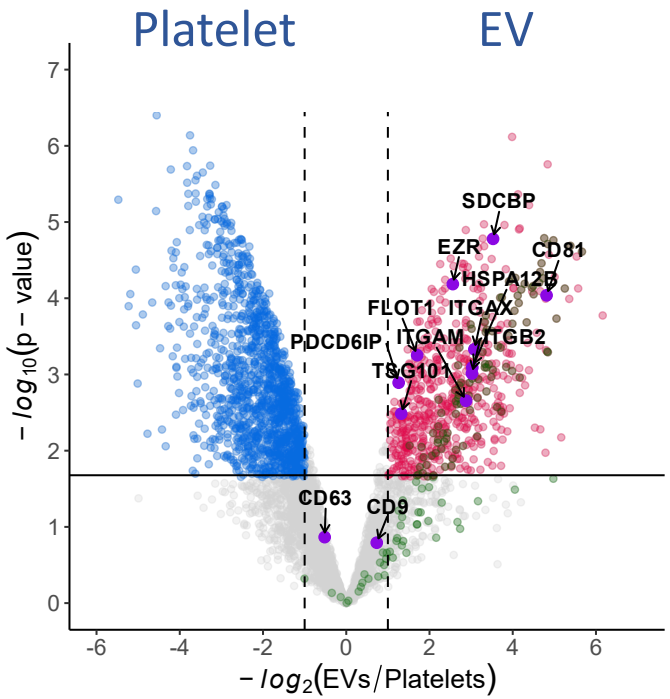
EV origin in plasma



99.8%

from **hematopoietic cells**
(of which 50% platelets!)

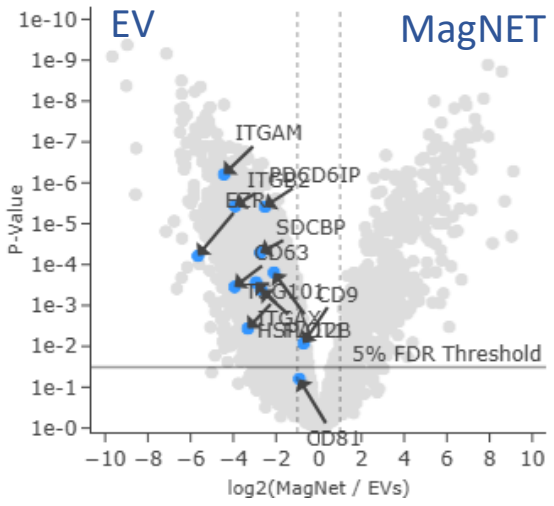
EV-origin: Enumerating the tissue-cellular origin of circulating extracellular vesicles using exLR profile.
Computational and Structural Biotechnology Journal 18, 2020

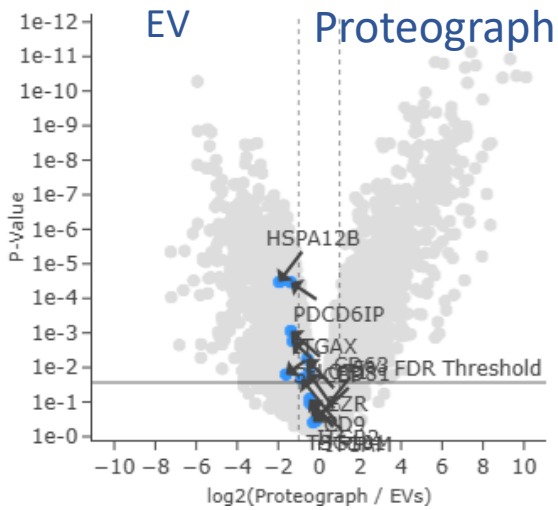


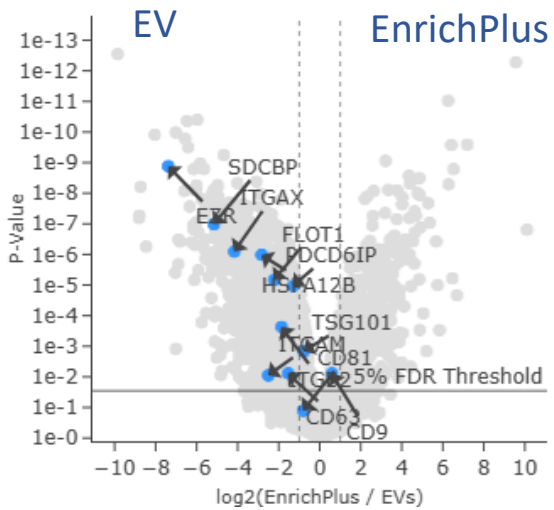
UP in Evs (626 proteins)
UP in Platelets (1329 proteins)

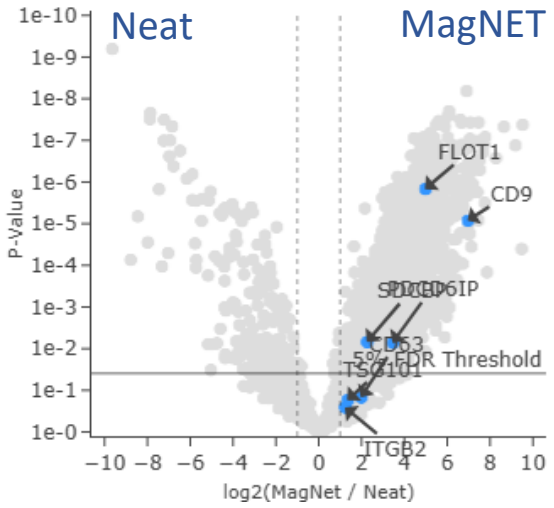
- Purified EV are not (just) Platelets
- Purifed EV are not (just) FROM Platelets

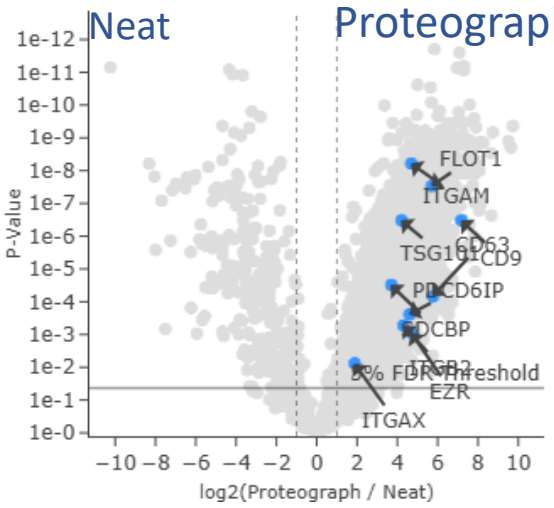
EV assessement in all the enrichments

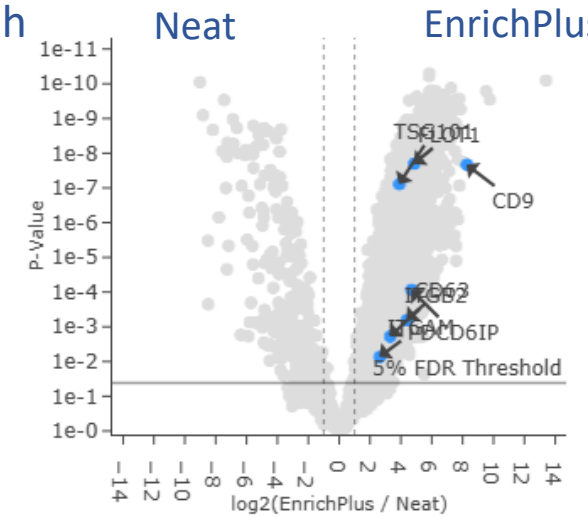




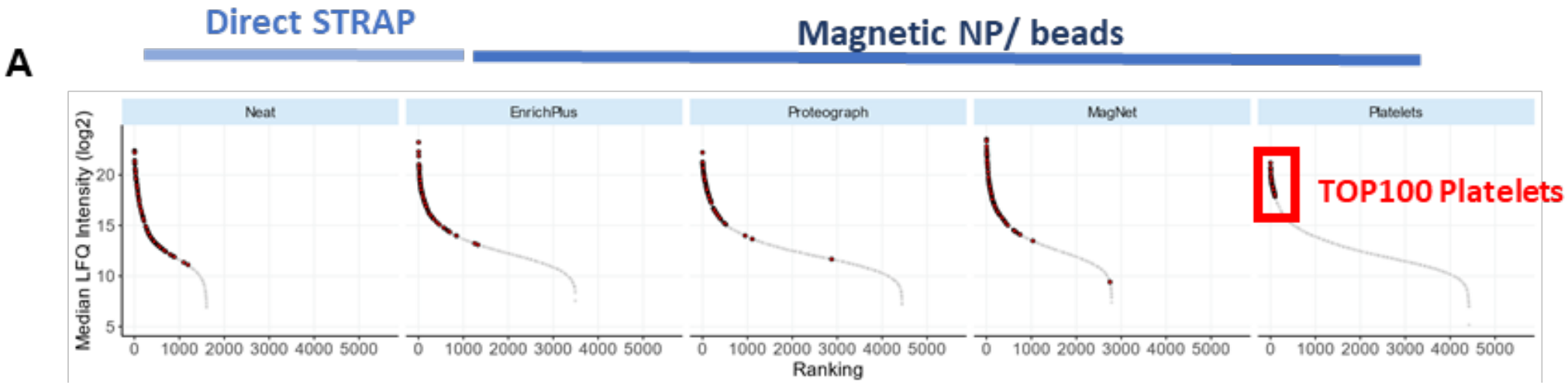








Platelet derived proteins intensity ion the deep plasma



- Abundant platelets proteins (TOP100) are still high ranking after enrichment
- Enrichment methods also enrich low abundance platelet proteins (BOTTOM 100)

I do not have any conflict of interest to disclose.

Necker is a University Hospital in Paris

Necker proteomics focuses on clinical and translational research

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