Conditions under which platelets release extracellular vesicles in arterial thrombosis and bleeding

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September 5th, 2022

Amsterdam UMC
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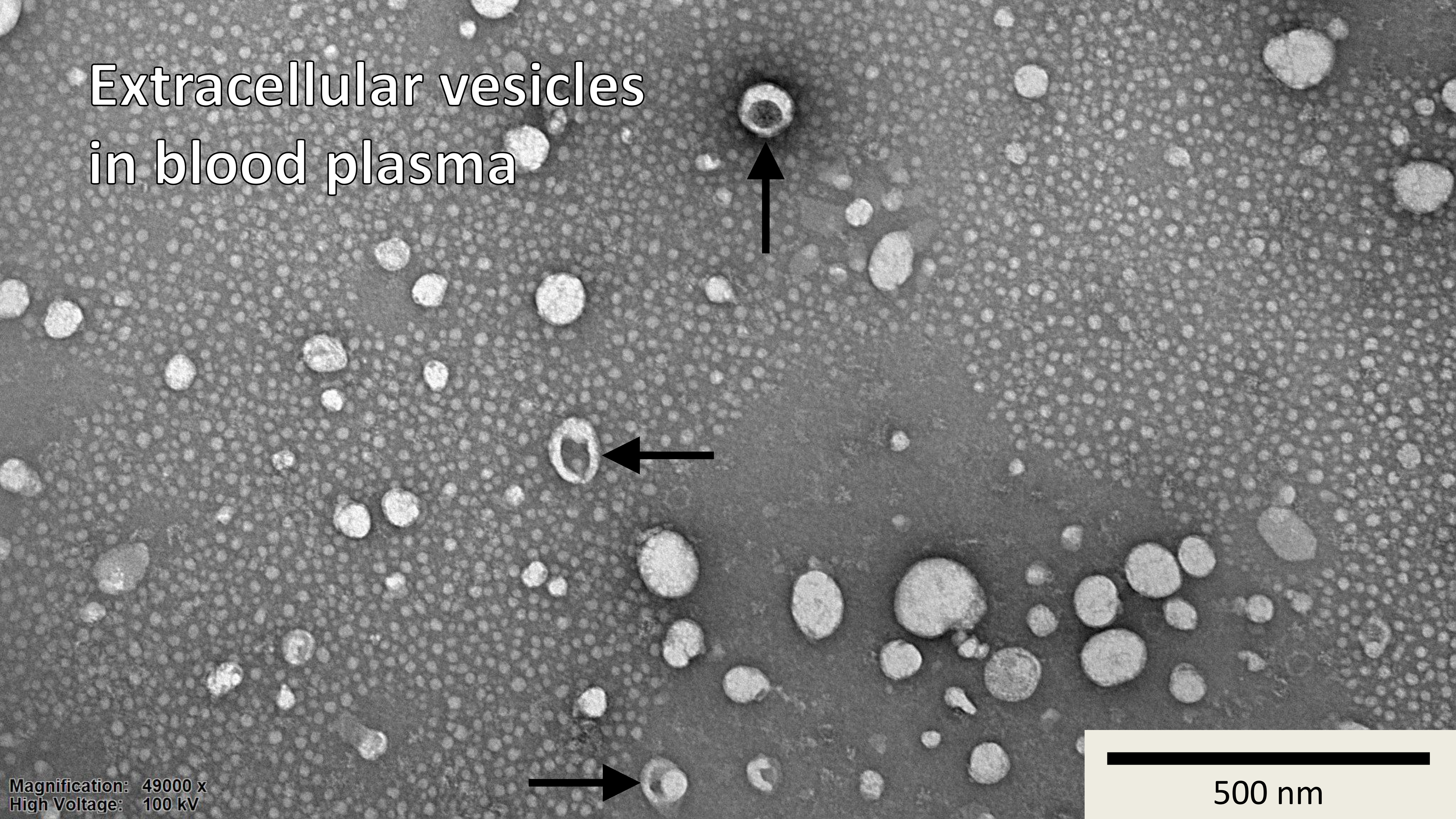
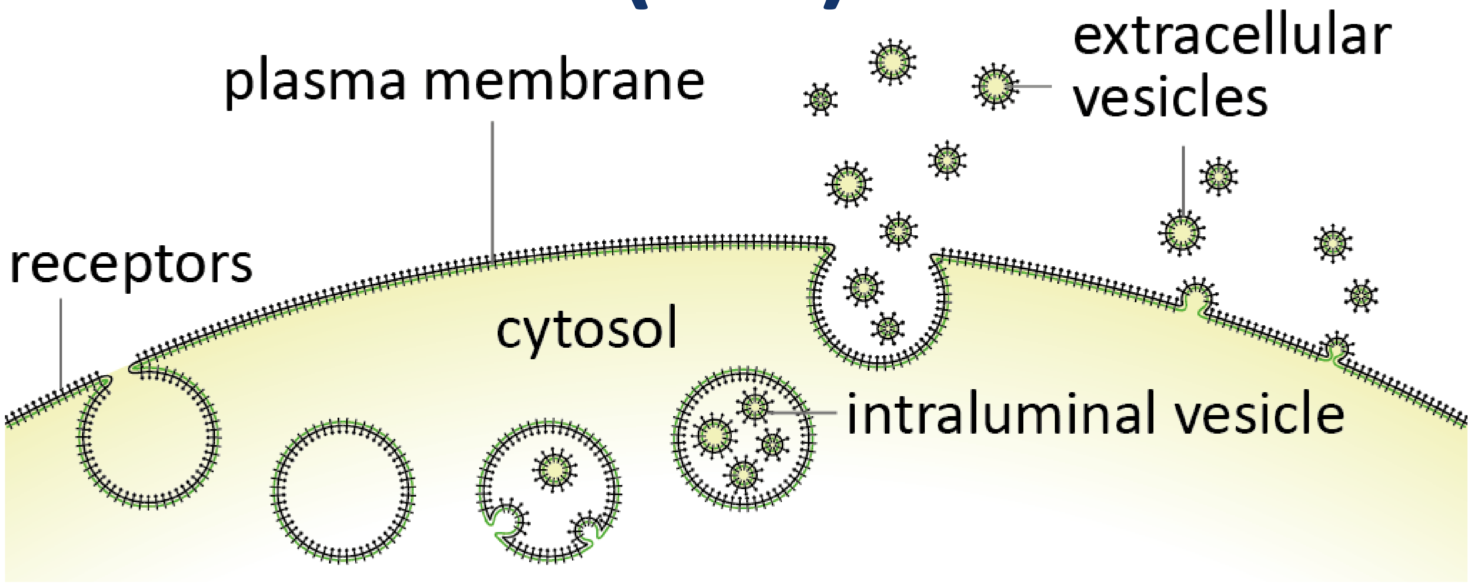
Vesicle Observation Center

Outline

- Extracellular vesicles (EVs)
- Flow cytometry
- Calibration and standardization (*Edwin's PhD*)
- Application: platelet-derived EVs
  - Plasma reference ranges
  - Conditions for platelet-EV release (*Naomi's PhD*)
  - Immune thrombocytopenia
  - Arterial ischemic stroke

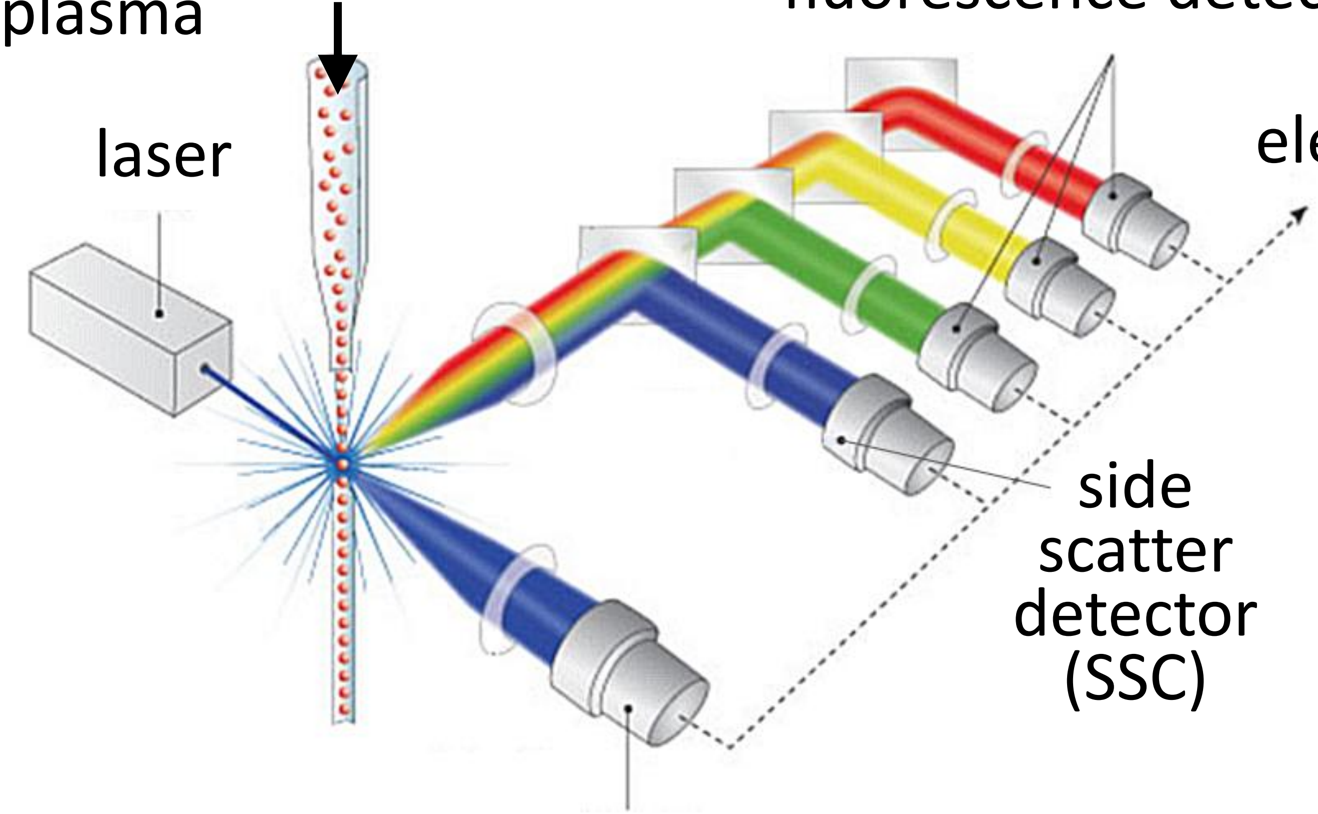

Extracellular vesicles
in blood plasma
Magnification: 49000 x
High Voltage: 100 kV
500 nm

Extracellular vesicles (EVs)



- cells release vesicles:
  biological nanoparticles with receptors, DNA, RNA
- specialized functions
- clinically relevant

Flow cytometry

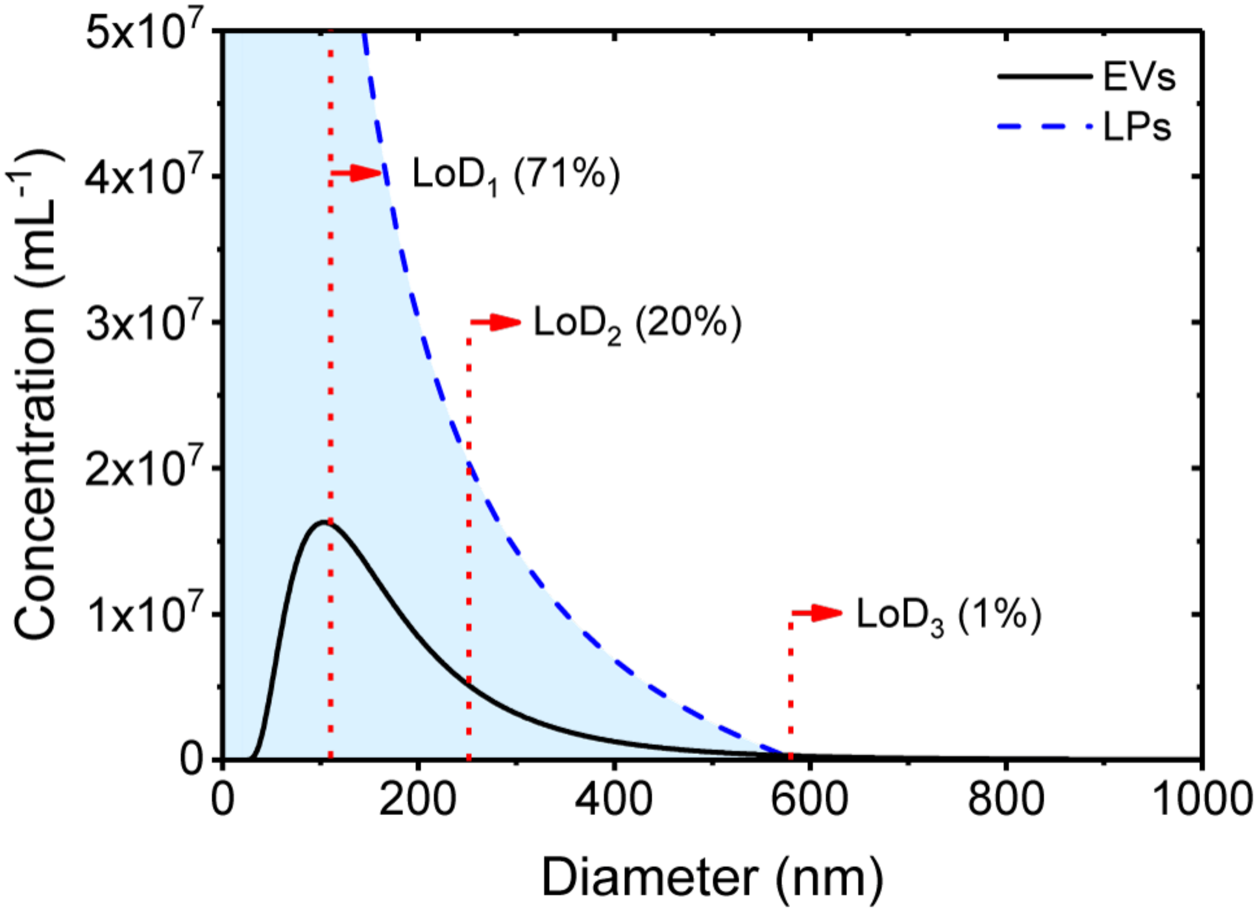


Specialized instrument:
>~50 nm EVs | 200 particles/s

Modern flow cytometer:
>~100 nm EVs | 10.000 particles/s
~100 samples per day

Image: semrock.com

Problem 1: flow cytometers do not measure all EVs

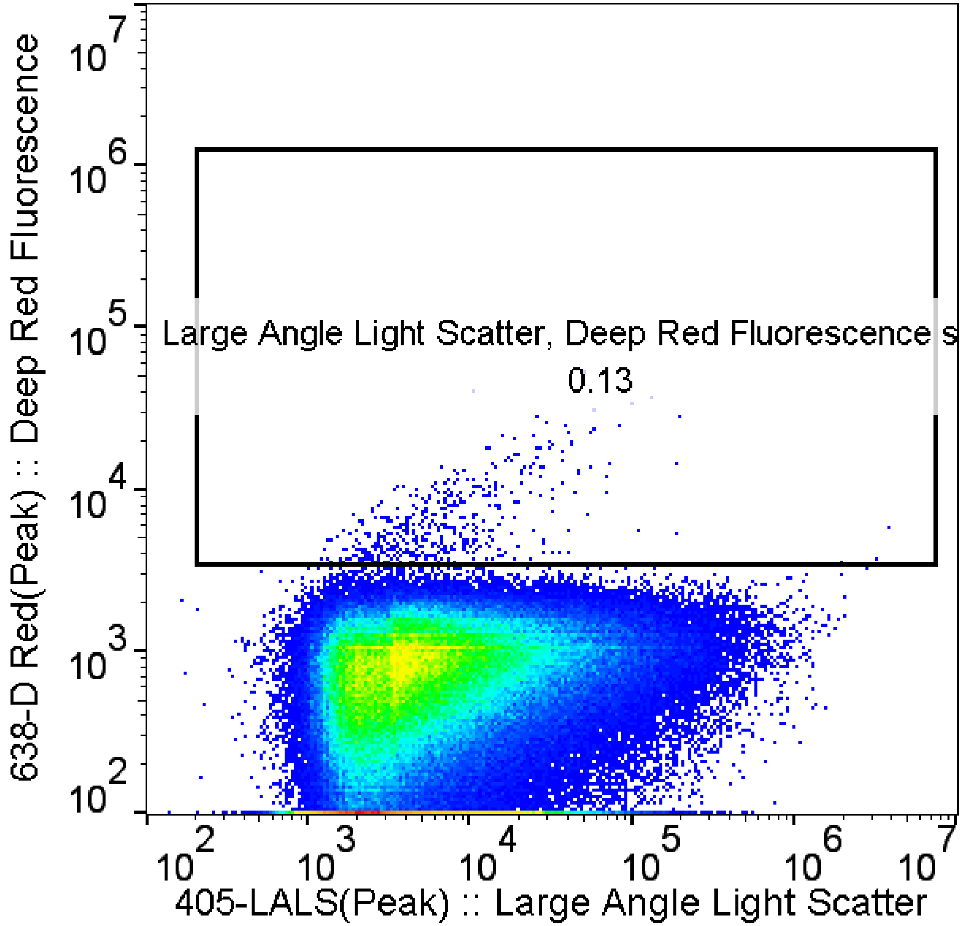


LoD: limit of detection, LPs: lipoprotein particles

Confidential: manuscript in preparation

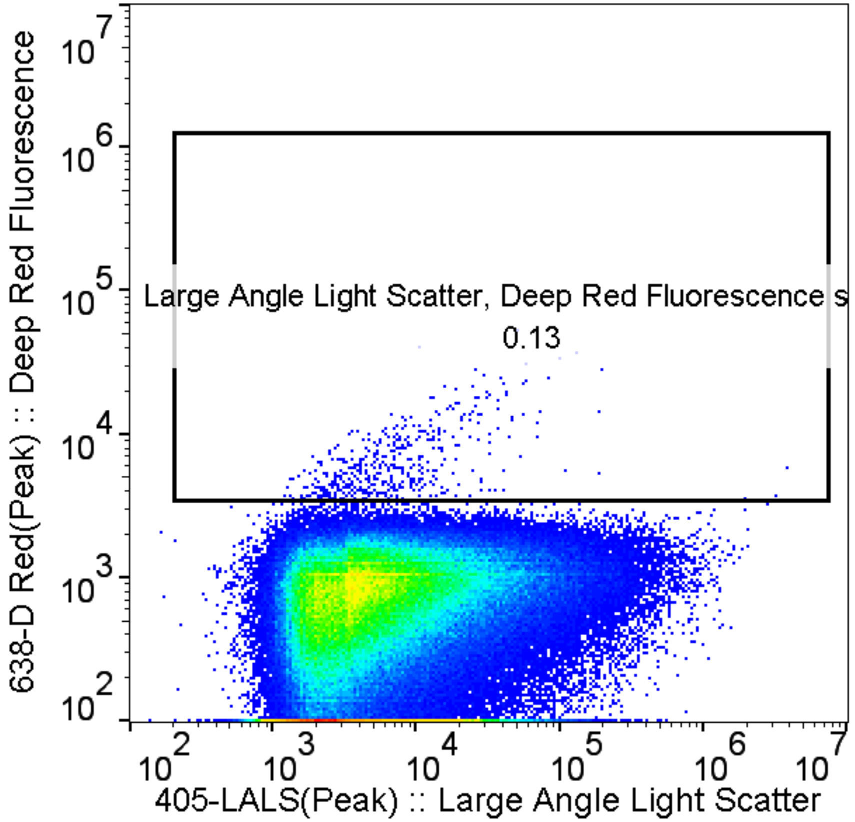
Problem 2: flow cytometry data have arbitrary units

- Size range of measured EVs remains unclear
- Are EVs at all measured?

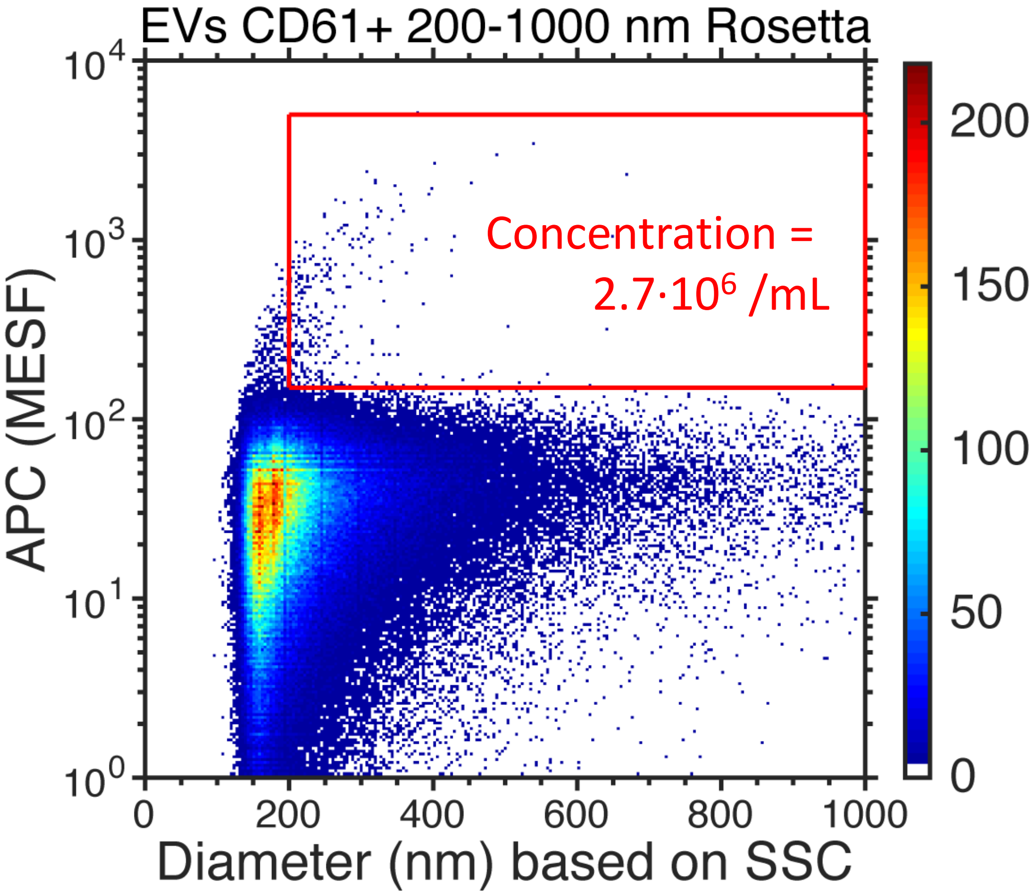


Flow cytometry calibration

Uncalibrated



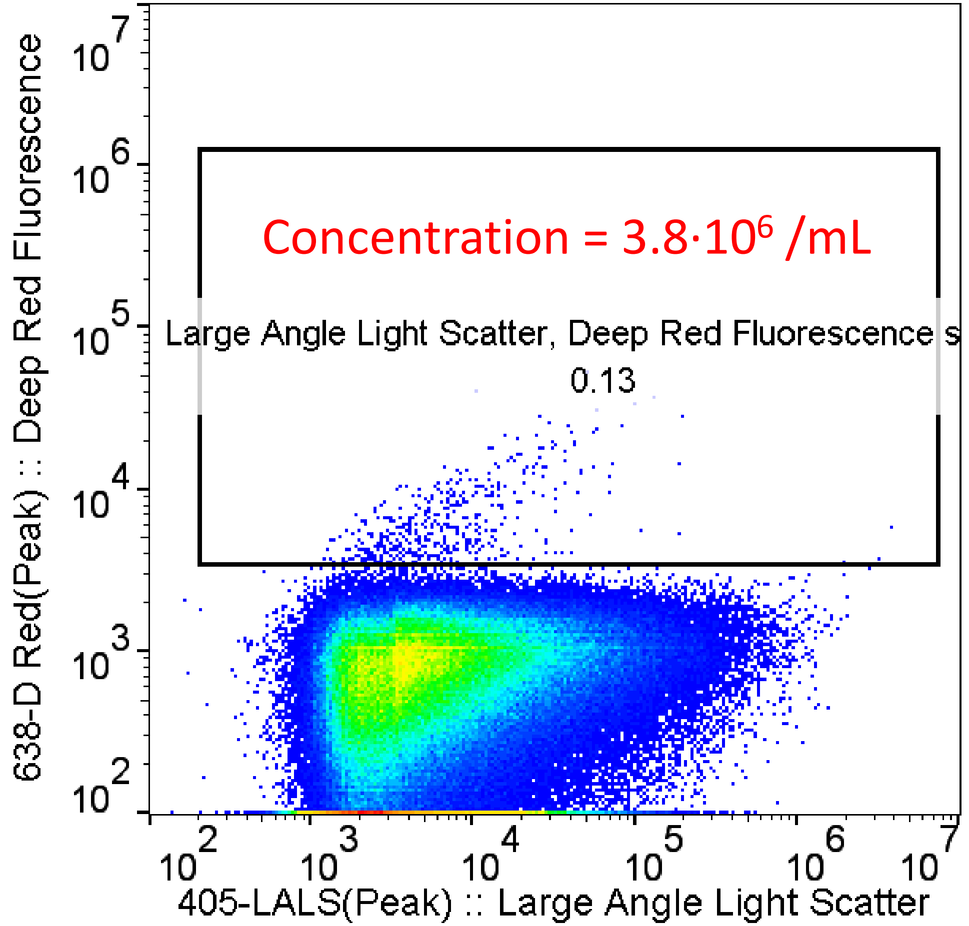
Calibrated



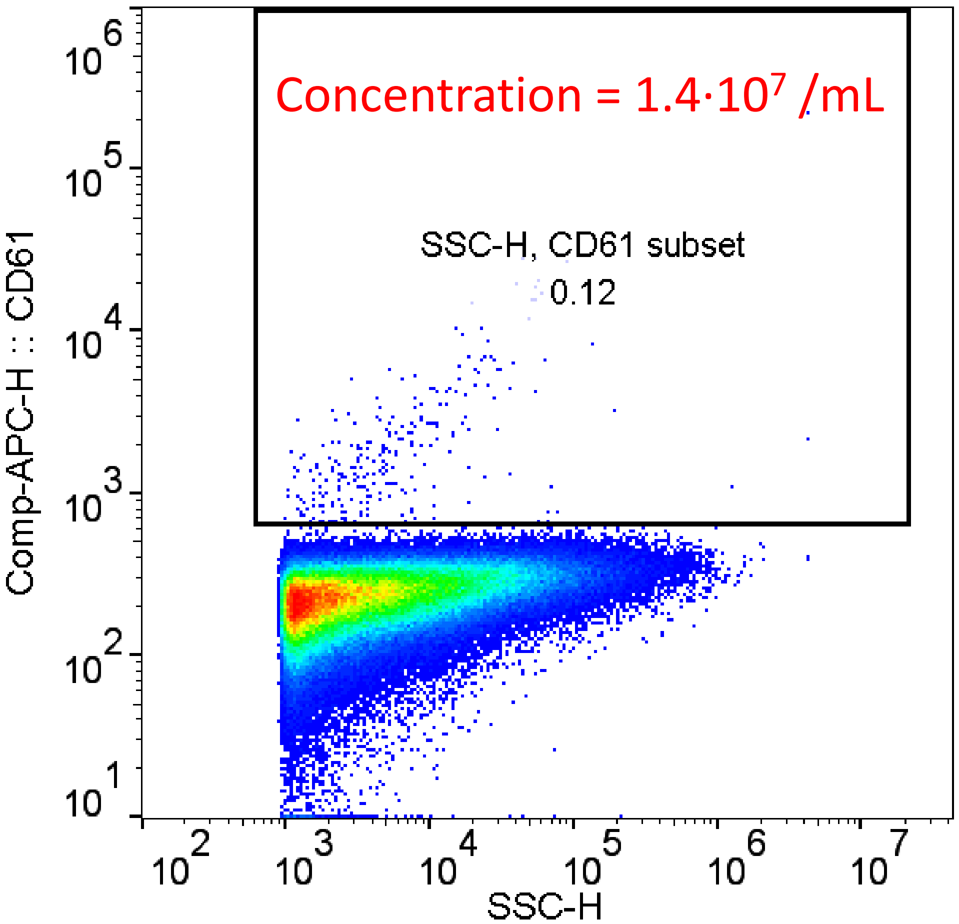
APC: allophycocyanin; SSC: side scattered light
MESF: molecules of equivalent soluble fluorophores

Without calibration, data do not reproduce

Apogee A60-Micro



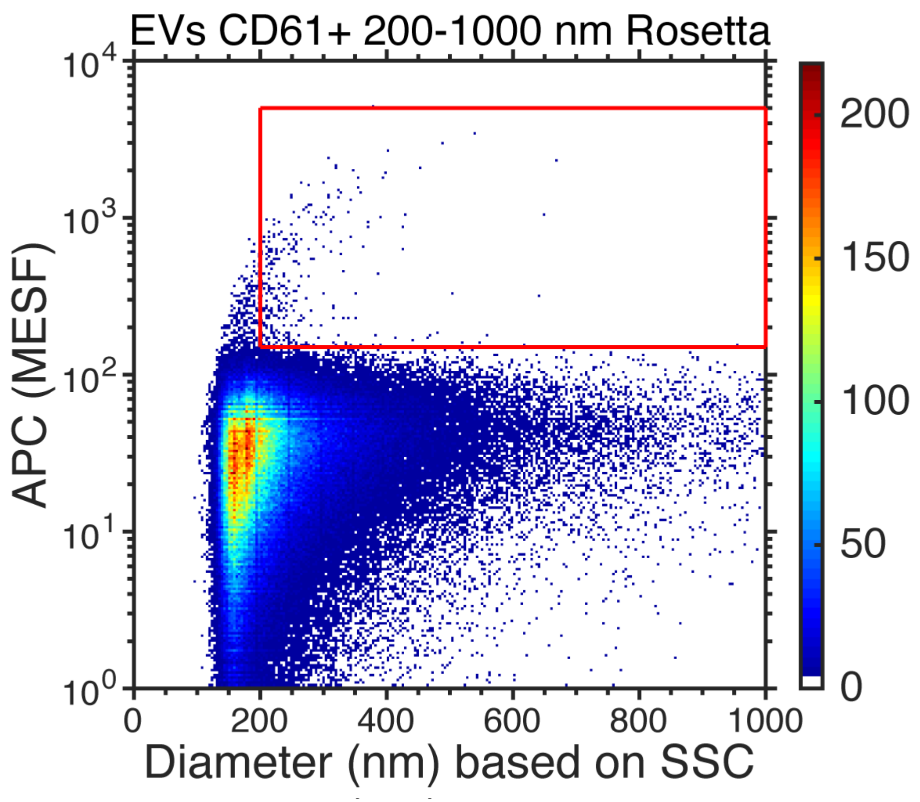
Cytek Aurora



Calibration improves data reproducibility

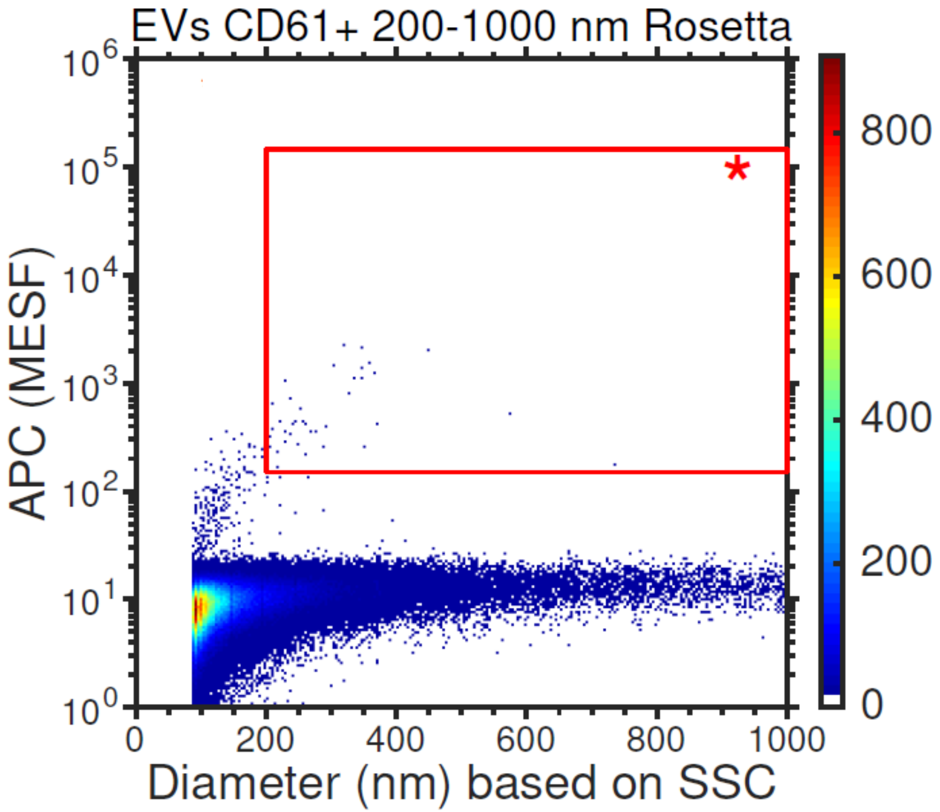
Apogee A60-Micro

Concentration = $2.7 \cdot 10^6$ /mL

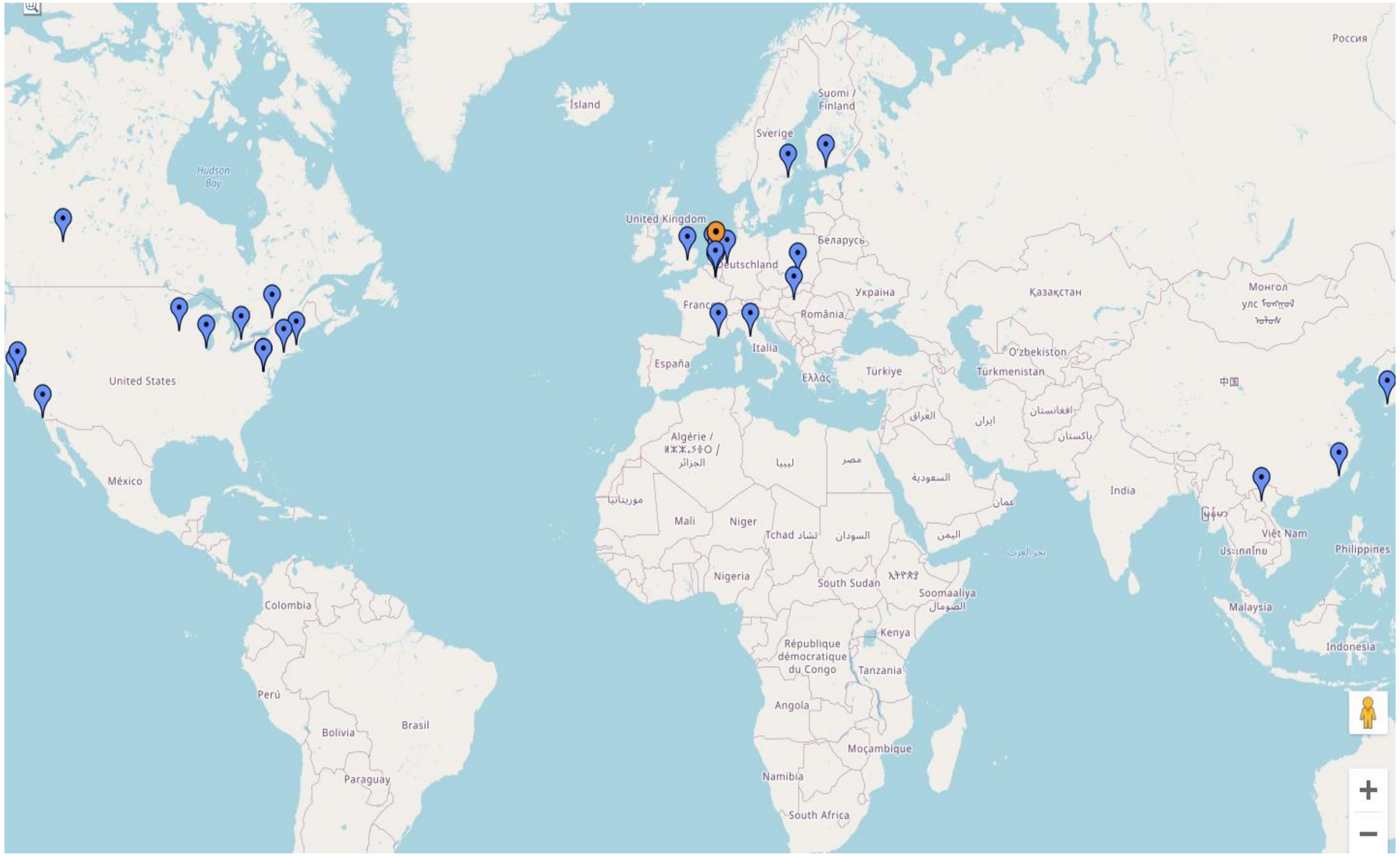


Cytek Aurora

Concentration = $2.2 \cdot 10^6$ /mL



METVES II: 23 participants and 35 flow cytometers



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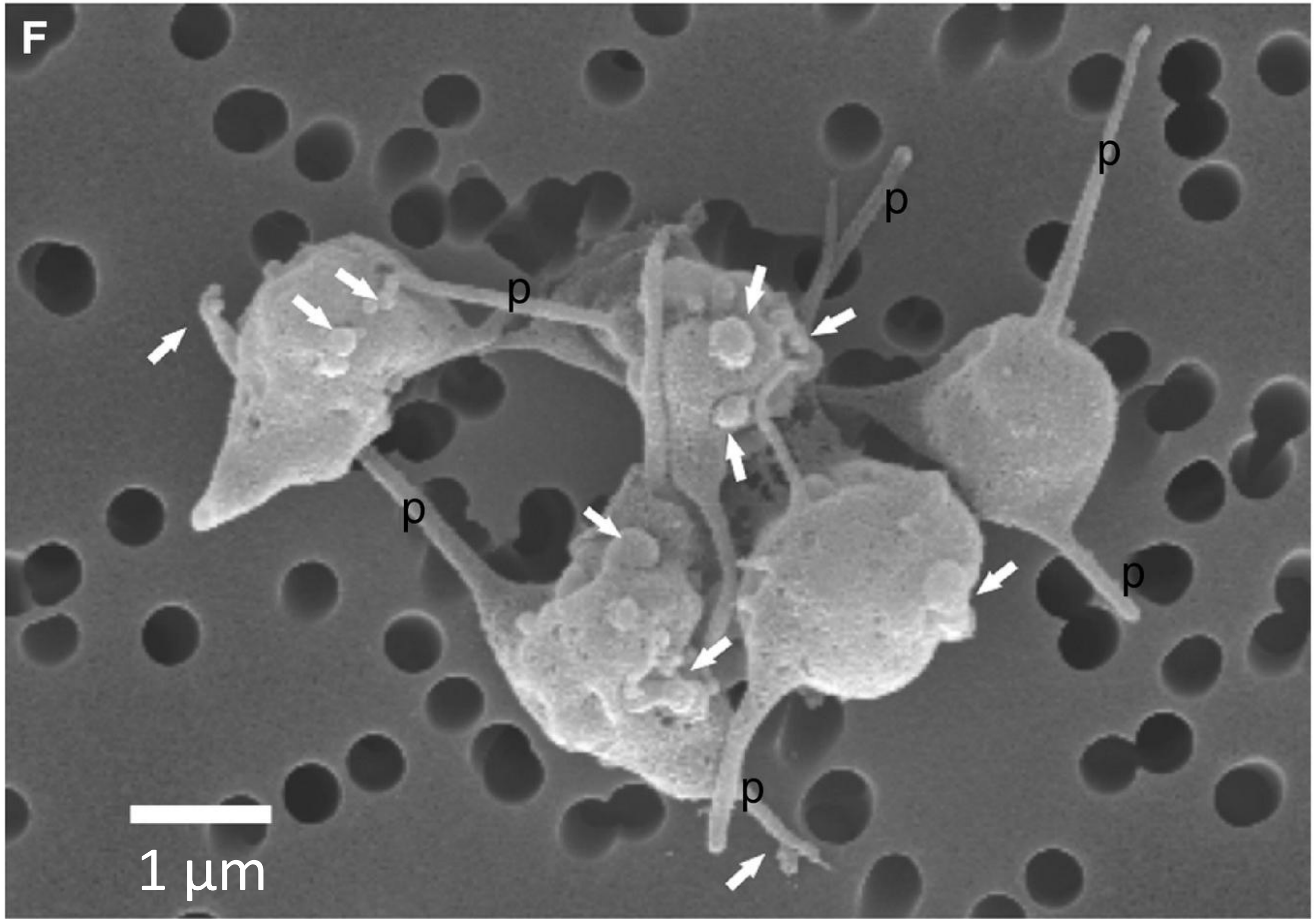
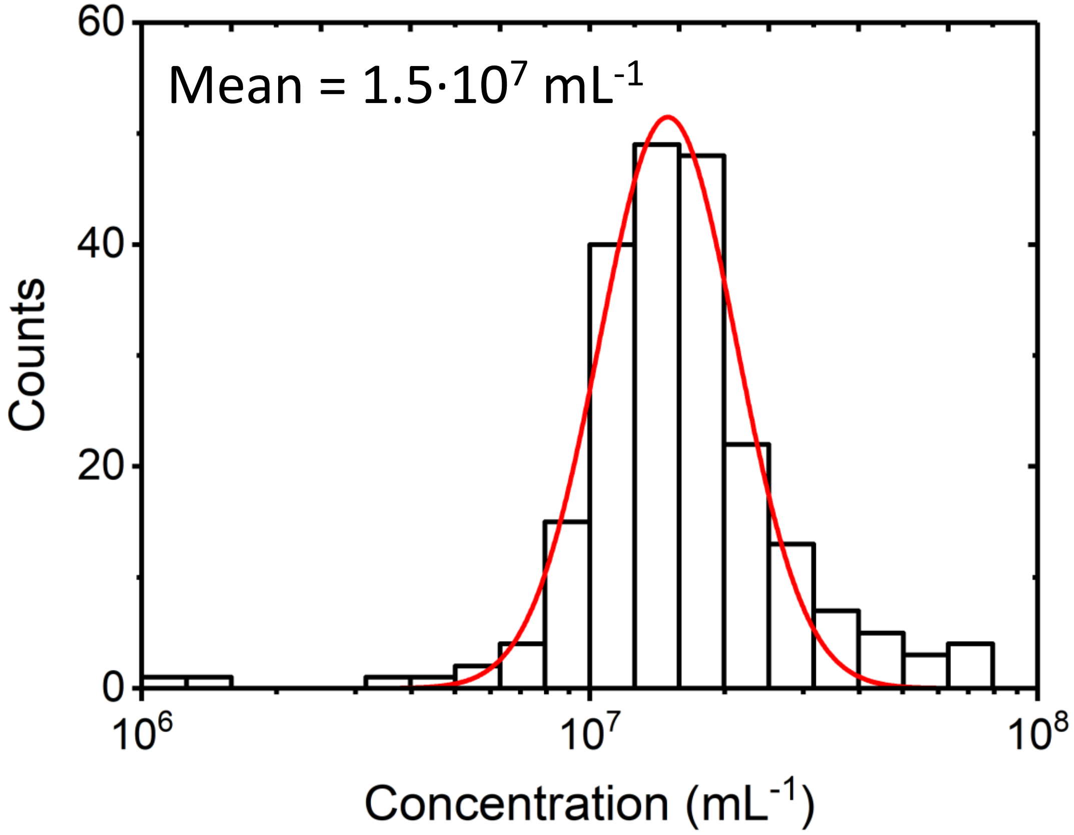
Platelets release EVs upon activation

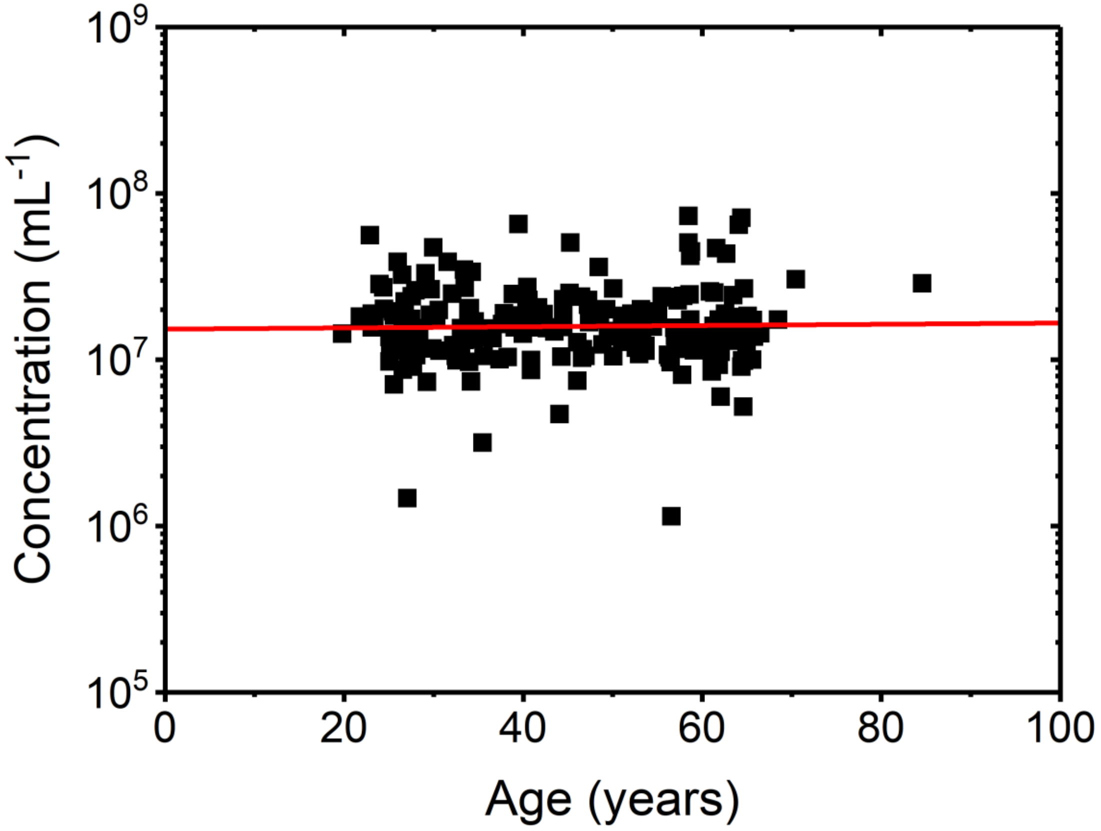


First ever reference ranges of EVs

Reference range of platelet-derived EVs in plasma

CD61+ EVs, Diameter = 230 nm – 1,000 nm, >50 MESF BV-421





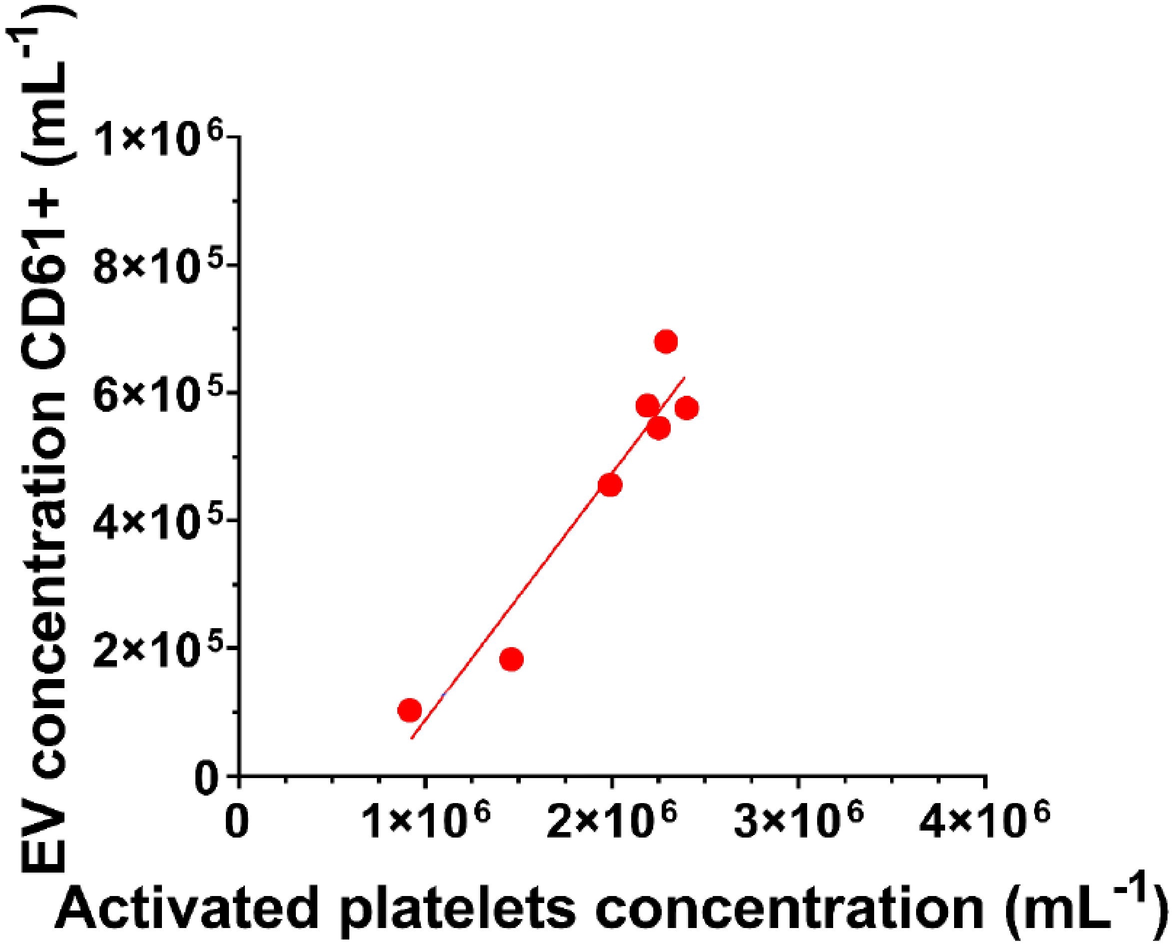
Data by Bo Li

CD61: integrin beta-3 (glycoprotein IIIa)

Research question

- How many platelet-derived EVs (CD61+) do we measure (>160 nm) per activated platelet?

Number of platelet-EVs per activated platelet

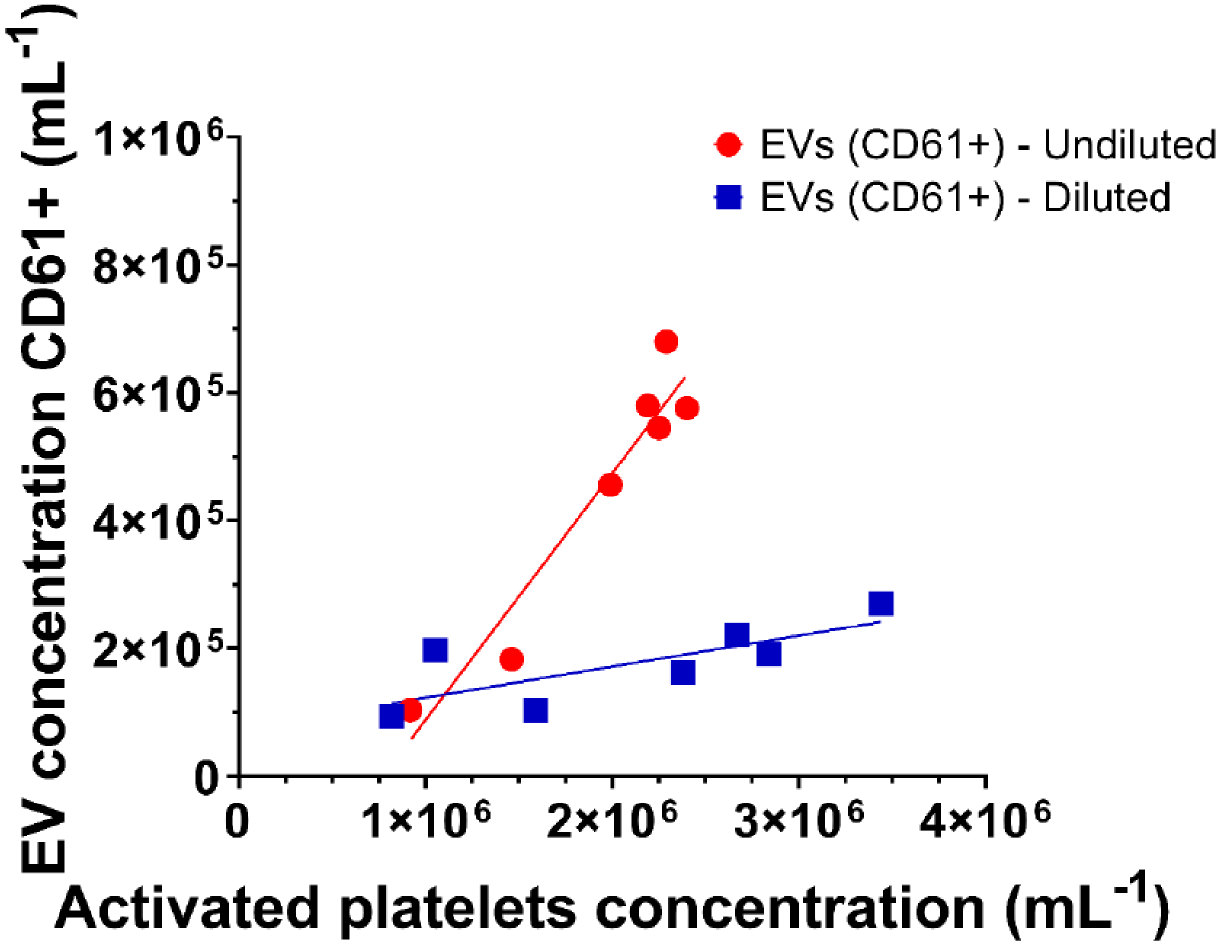


We measured between 0.4 and 2.8 CD61+ EVs* per activated platelet (n=3)

*diameter >160 nm

Data by Naomi Buntsma; TRAP: thrombin receptor activating peptide

Number of platelet-EVs per activated platelet

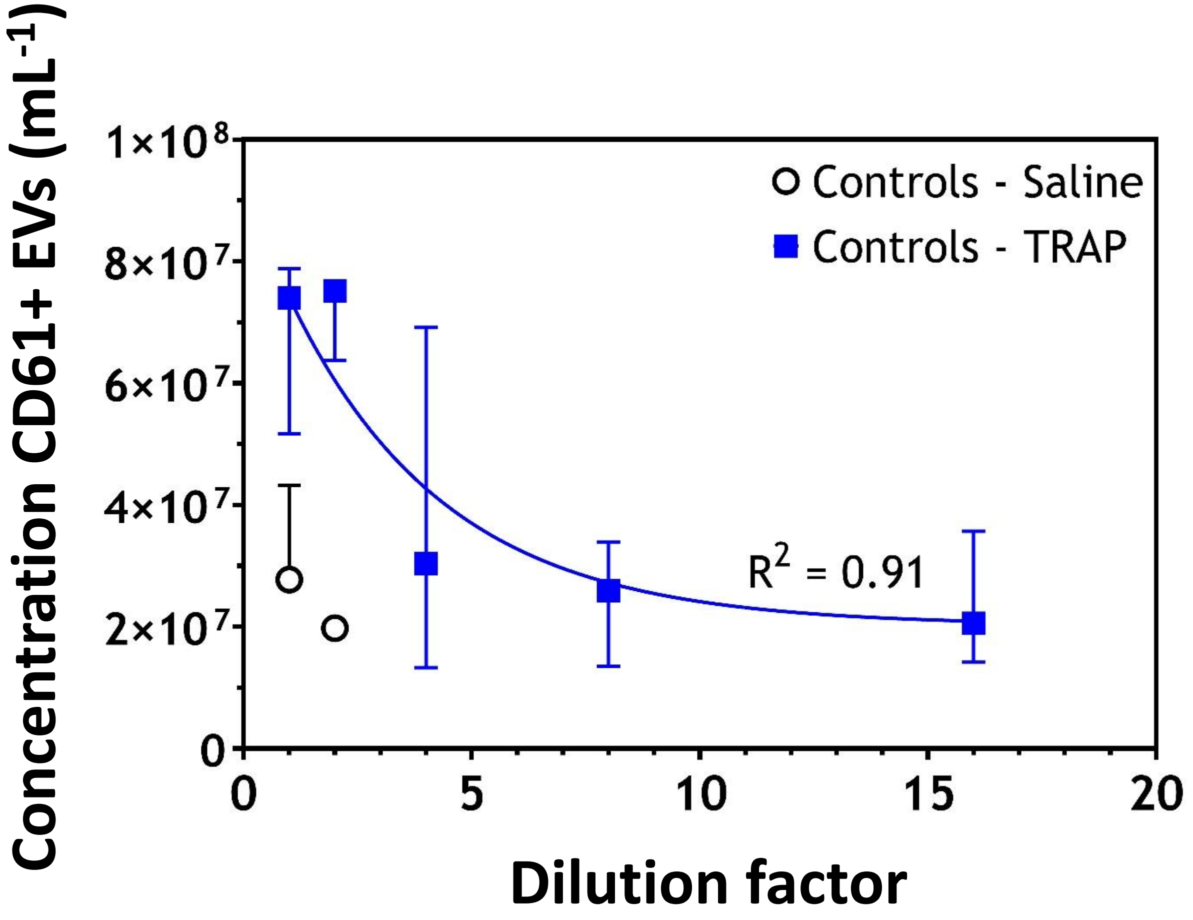


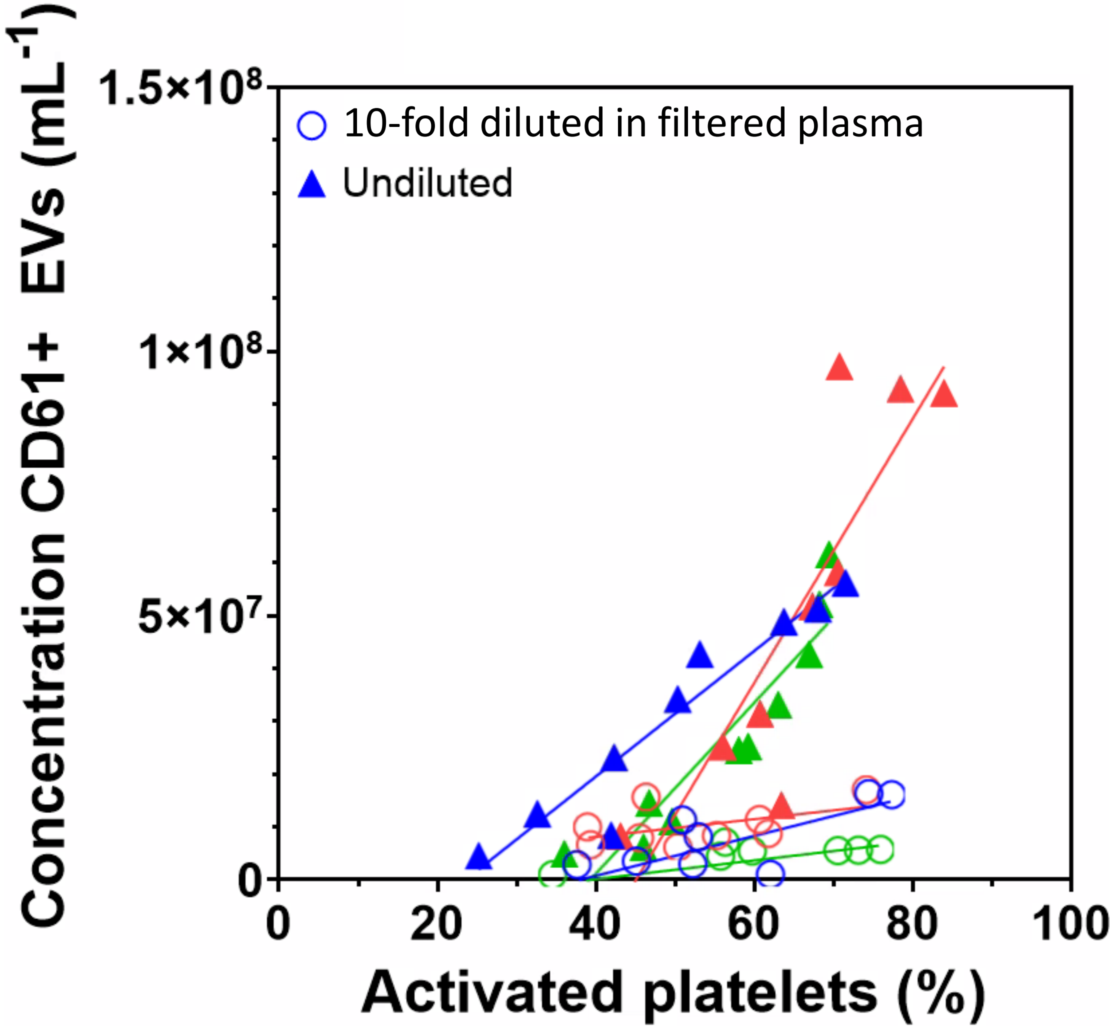
Lower concentration of released CD61+ EVs* per activated platelet in <u>diluted</u> blood

*diameter >160 nm

Data by Naomi Buntsma

Activated platelets do not release EVs* after 10-fold dilution

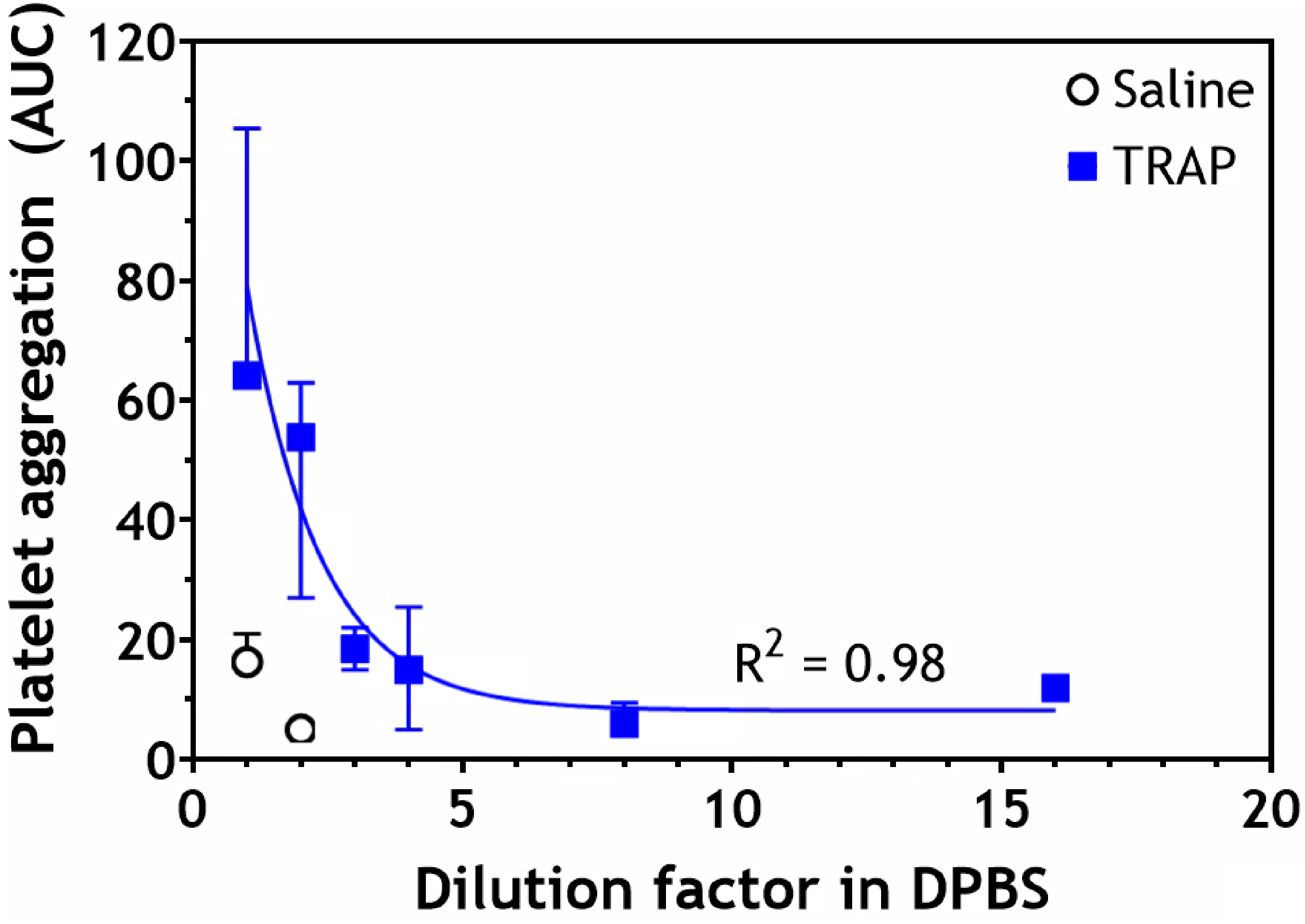




TRAP: thrombin receptor activating peptide

* diameter >160 nm

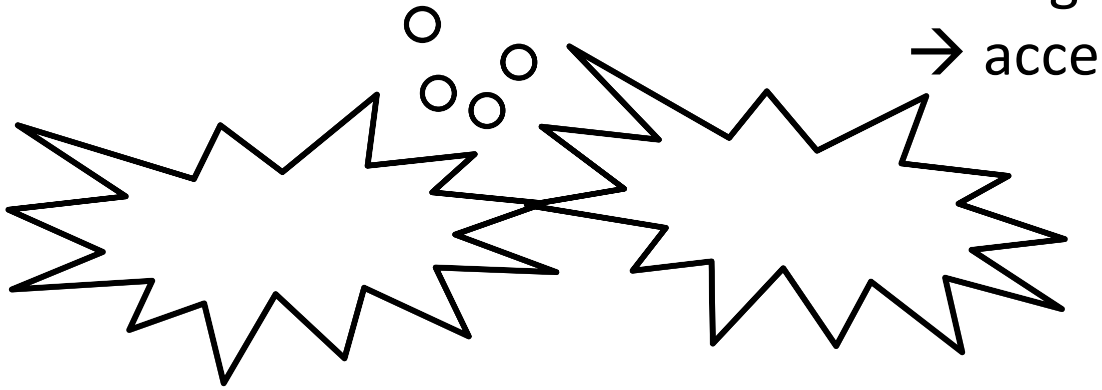
Platelets in 10-fold diluted plasma do not aggregate



Inter-platelet distance and pseudopod length

	[µm]
Inter-platelet distance plasma	14
Inter-platelet distance 10-fold diluted plasma	31
Pseudopod length:	2-10

CD61^{+} EVs → fibrinogen binding surface
→ accelerate coagulation cascade



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Immune thrombocytopenia (ITP)

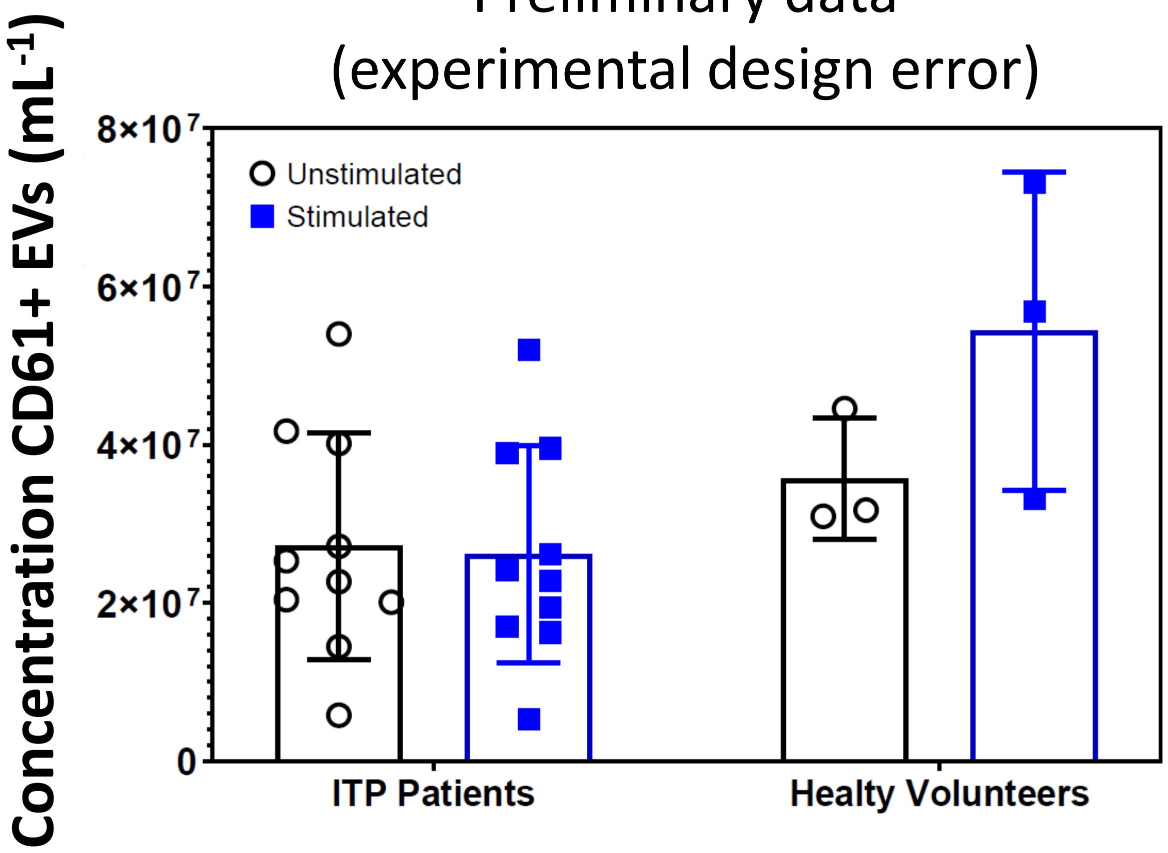
- Bleeding disorder caused by unusually low platelet concentration ($<5.0 \cdot 10^7$ mL^{-1}) compared to normal ($>1.5 \cdot 10^8$ mL^{-1})
- Hypotheses:
  Low platelet concentration leads to inhibition of…
  - $CD61^+$ EV release
  - platelet aggregation

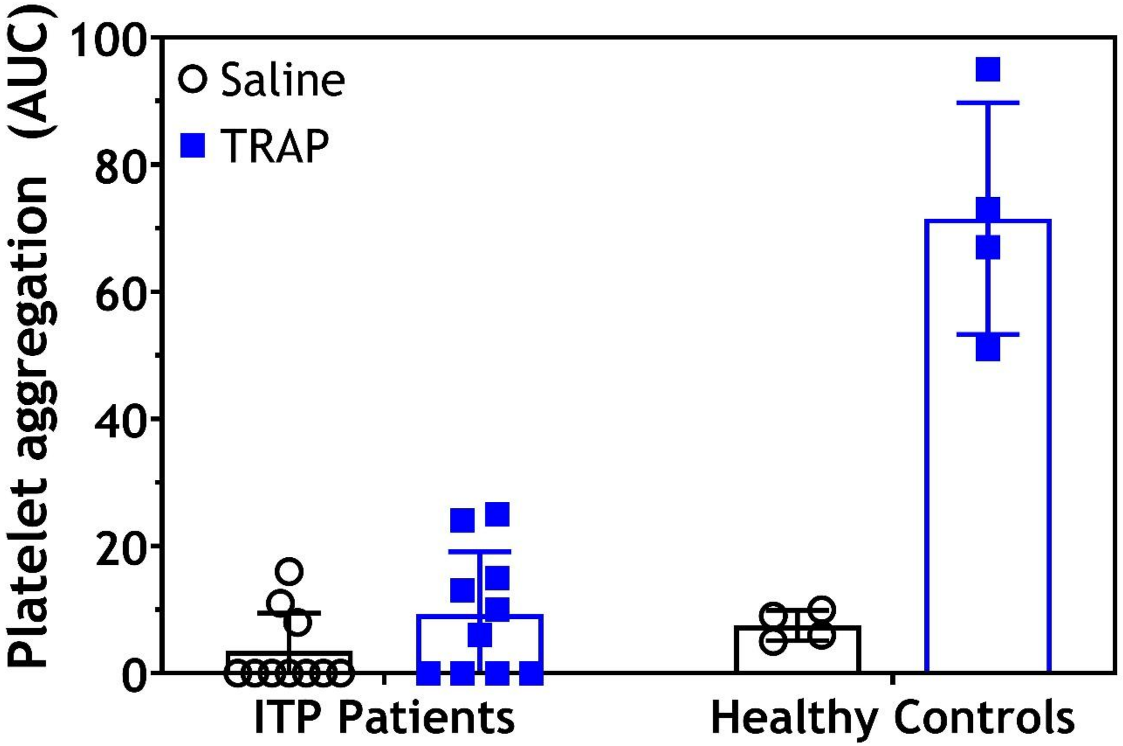
Inter-platelet distance and pseudopod length

	[µm]
Inter-platelet distance plasma	14
Inter-platelet distance 10-fold diluted plasma	31
Pseudopod length:	2-10
Inter-platelet distance “critically low” platelet concentration in blood plasma of immune thrombocytopenia patients	26

Low platelet-derived EV concentration in ITP

Preliminary data
(experimental design error)





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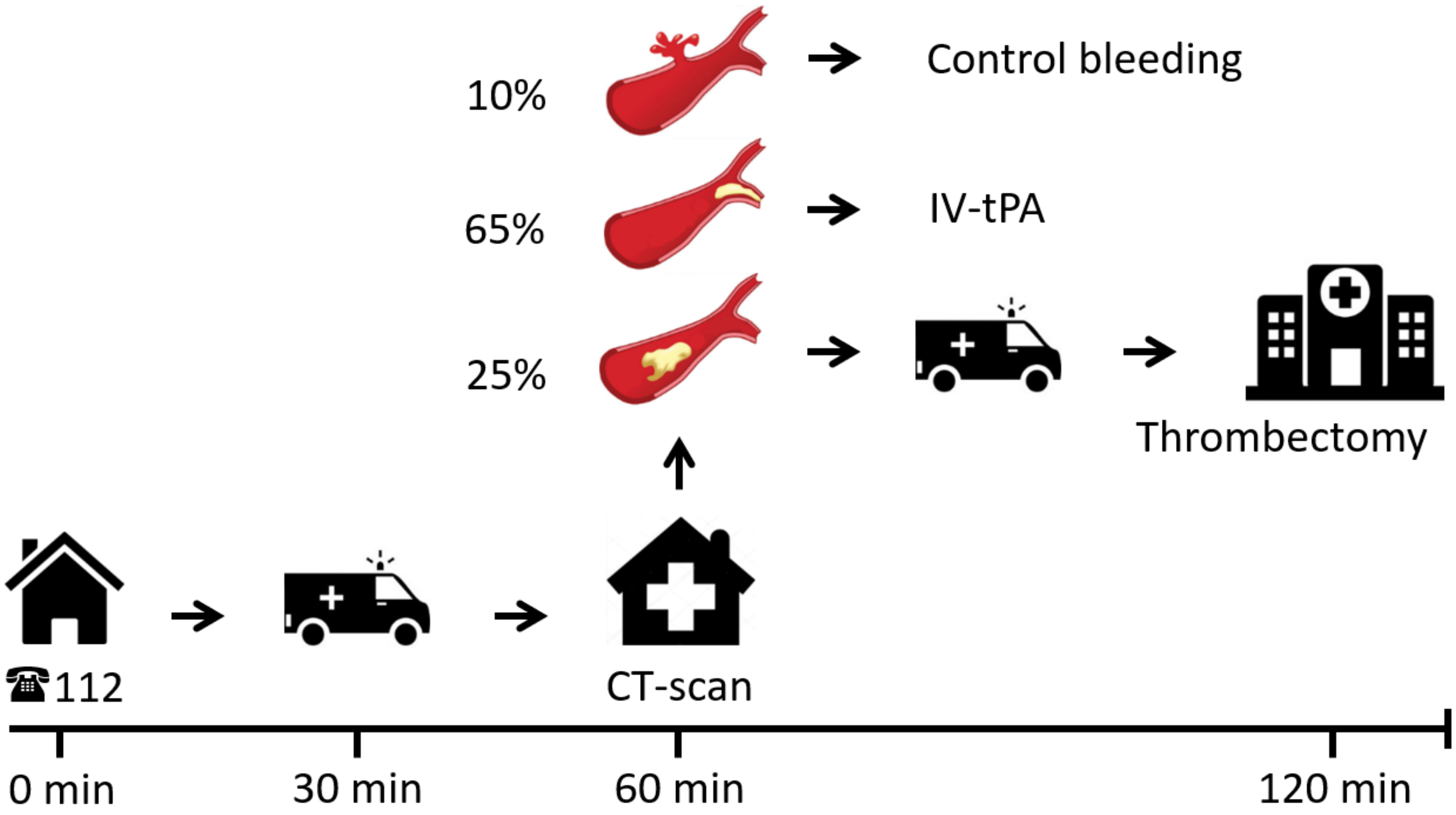
  - ✓ Immune thrombocytopenia
  - ➢ Arterial ischemic stroke (Outlook)

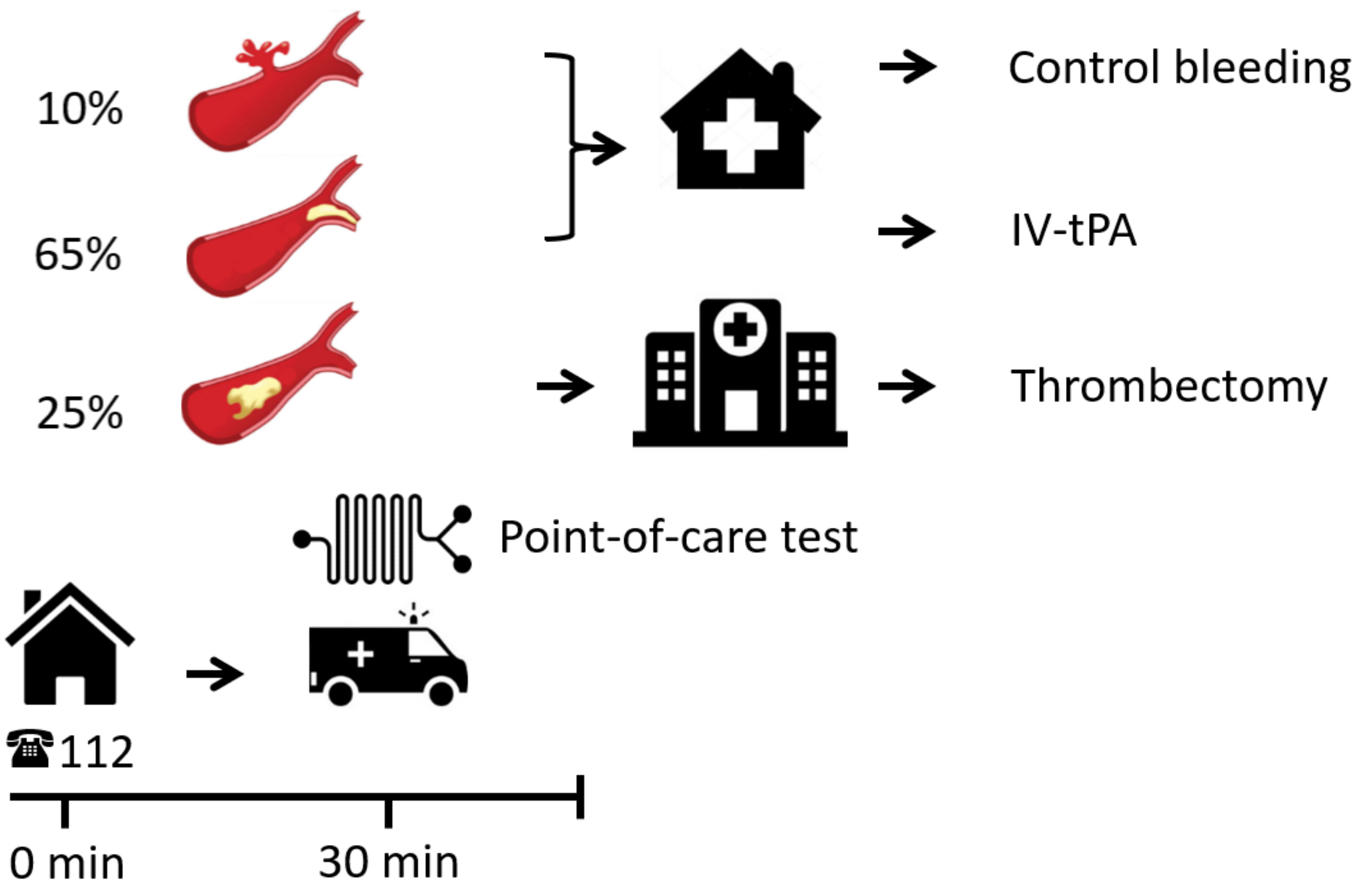
Project CINTICS (part of CONTRAST)

- Circulating nanotraces to identify the cause of stroke

Current scheme for diagnosis and treatment of stroke



CINTICS' scheme for diagnosis and treatment of stroke



Outlook

- Biobank of plasma (pre-treatment) of >500 stroke patients and controls
- Calibrated flow cytometry*
- Sample analysis start this year

* Detection limit: EVs with diameter >100 nm

Summary

- Blood plasma contains extracellular vesicles (EVs): potential disease biomarkers
- Amsterdam UMC: leading in EV flow cytometry
- Platelets release EVs upon activation if inter-platelet distance is within pseudopod length
  - Immune thrombocytopenia
  - Arterial ischemic stroke (outlook)

NLSEV 2022

17-18 November

Crowne Plaza | Maastricht

Abstract submission: 1st Sept - 1st Oct

Registration: 1st Sept - 1st Nov

More info: nlsev.nl loc@nlsev.nl

Keynote speakers

Eva Rohde
Salzburg,
Austria

Jayanta Debnath
San Francisco,
USA

Peter Peters
Maastricht,
The Netherlands

Acknowledgements

- Amsterdam University Medical Centers
  - Biomedical Engineering & Physics
  - Laboratory of Experimental Clinical Chemistry
    - Aleksandra Gąsecka
    - Bo Li
    - Chi Hau
    - **Naomi Buntsma**
    - Rienk Nieuwland
    - Sytske Talsma
  - Vesicle Observation Center
  - Neurology
    - Yvo Roos

Vesicle Observation Center

- More information
  - edwinvanderpol.com
  - evflowcytometry.org
  - isev.org
  - metves.eu
  - nlsev.nl

- Funding
  - EURAMET project 18HLT01 (METVES II)