Jagiellonian University Medical College
Department of Medicine, Krakow, Poland

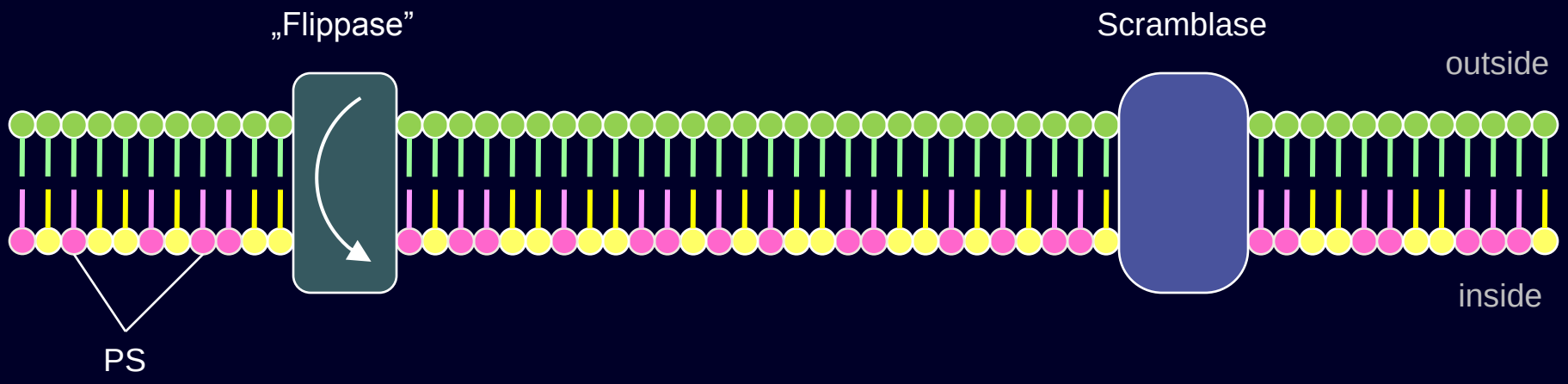
Detection and characteristics of platelet derived microparticles

Bogdan Jakiela, Marek Sanak

1. Origin and function of platelet-derived microparticles
2. How to detect MPs in biological samples?
3. Measuring of MPs in *in vitro* model of biocompatibility

Microparticles (MPs) are small membrane vesicles that are released by activated or apoptotic cells.

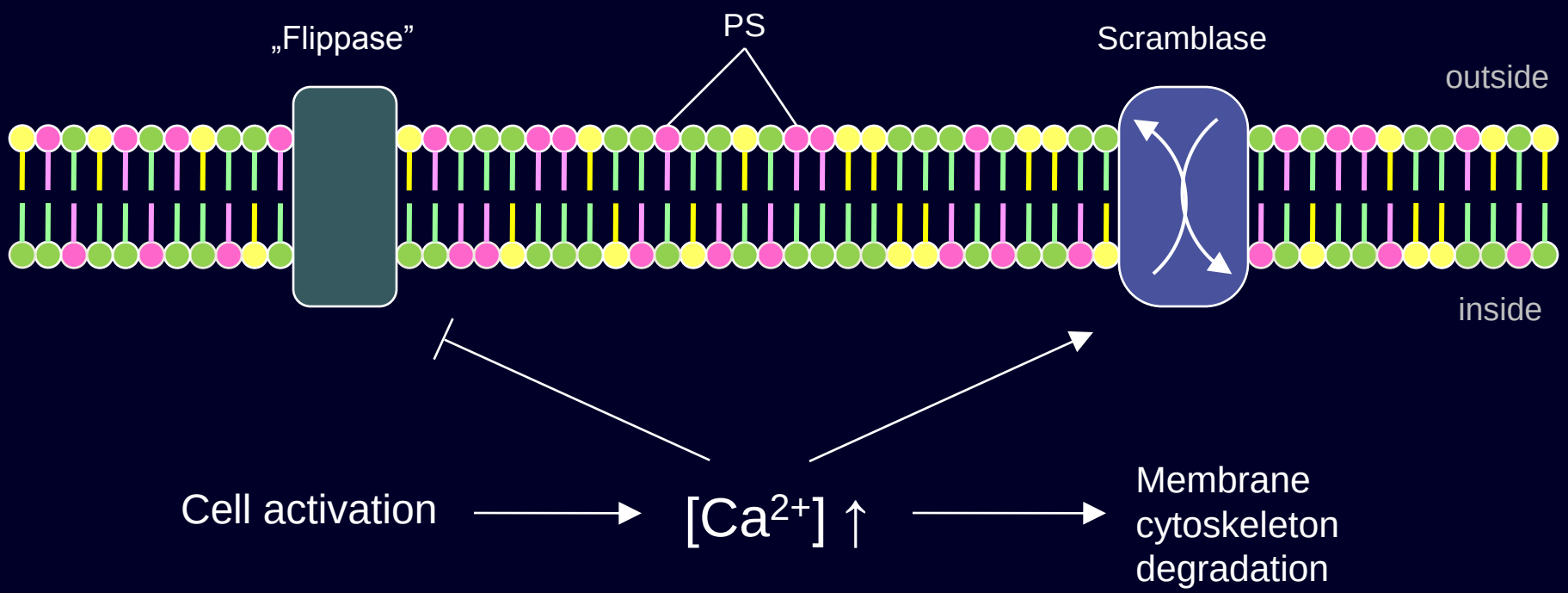
Origin of cell-derived MPs



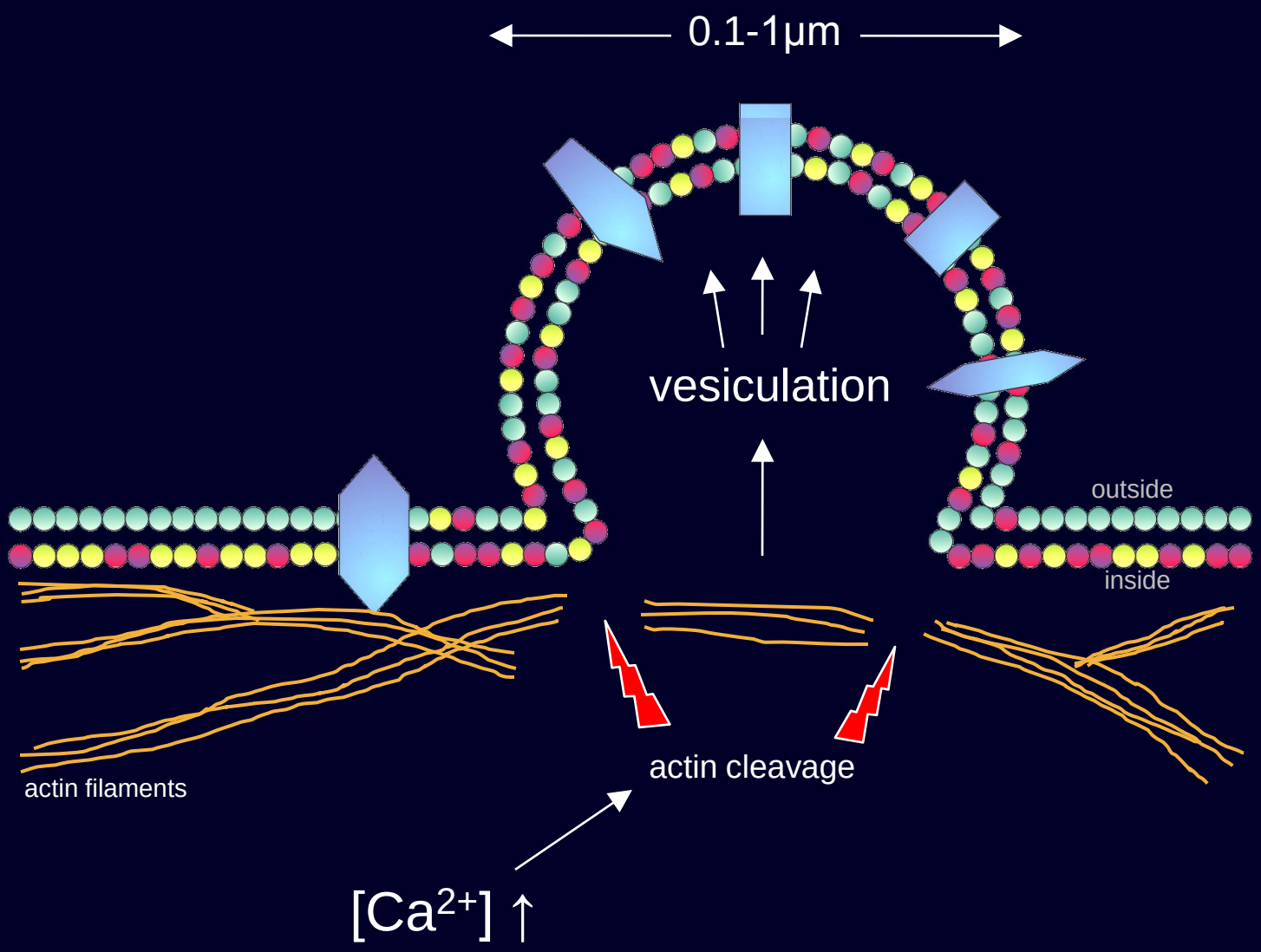
$[Ca^{2+}] \downarrow$

- Phospholipids of outer layer
- Phospholipids of inner layer
- Phosphatidylserine (PS)

Origin of cell-derived MPs



Origin of cell-derived MPs

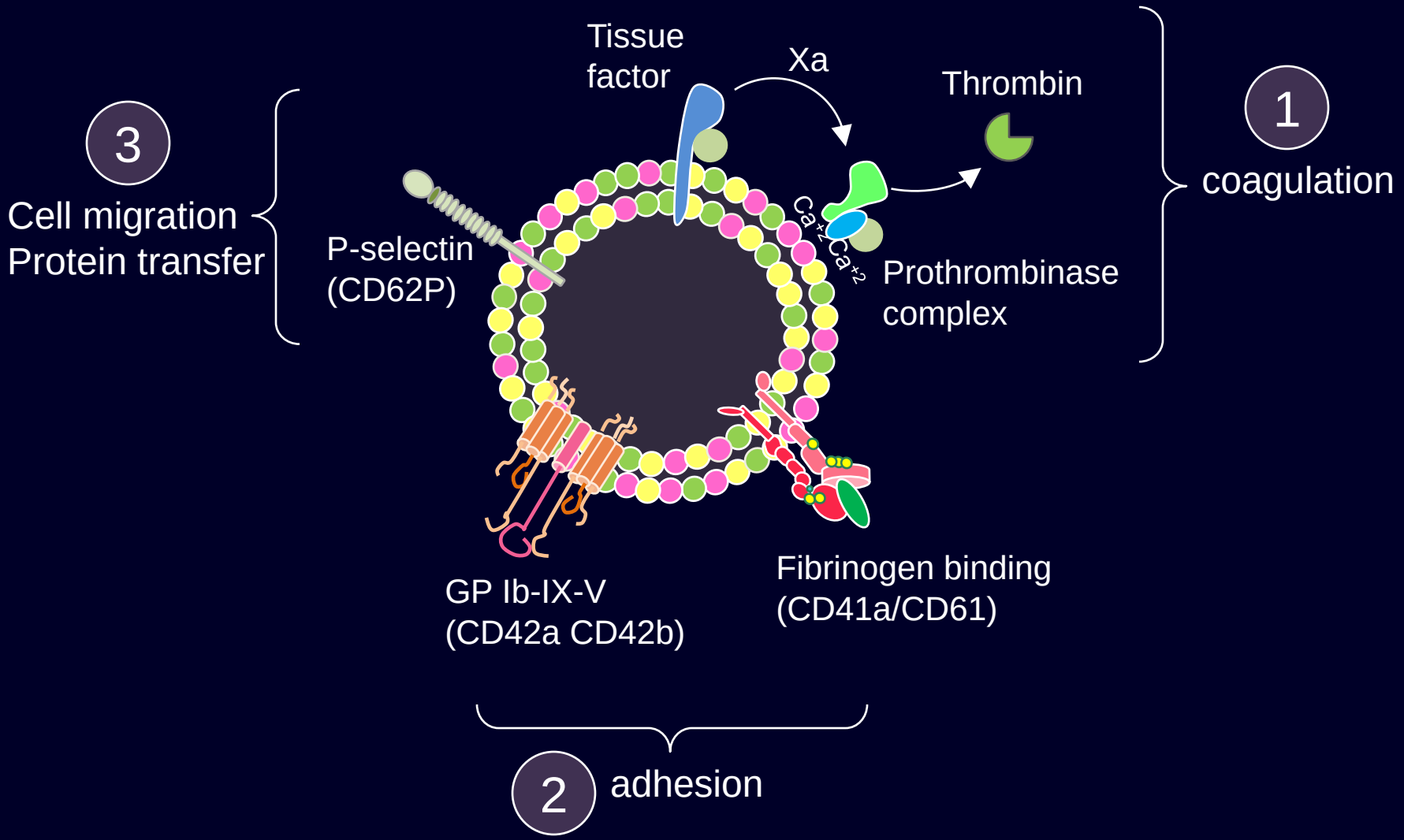


Origin of cell-derived MPs

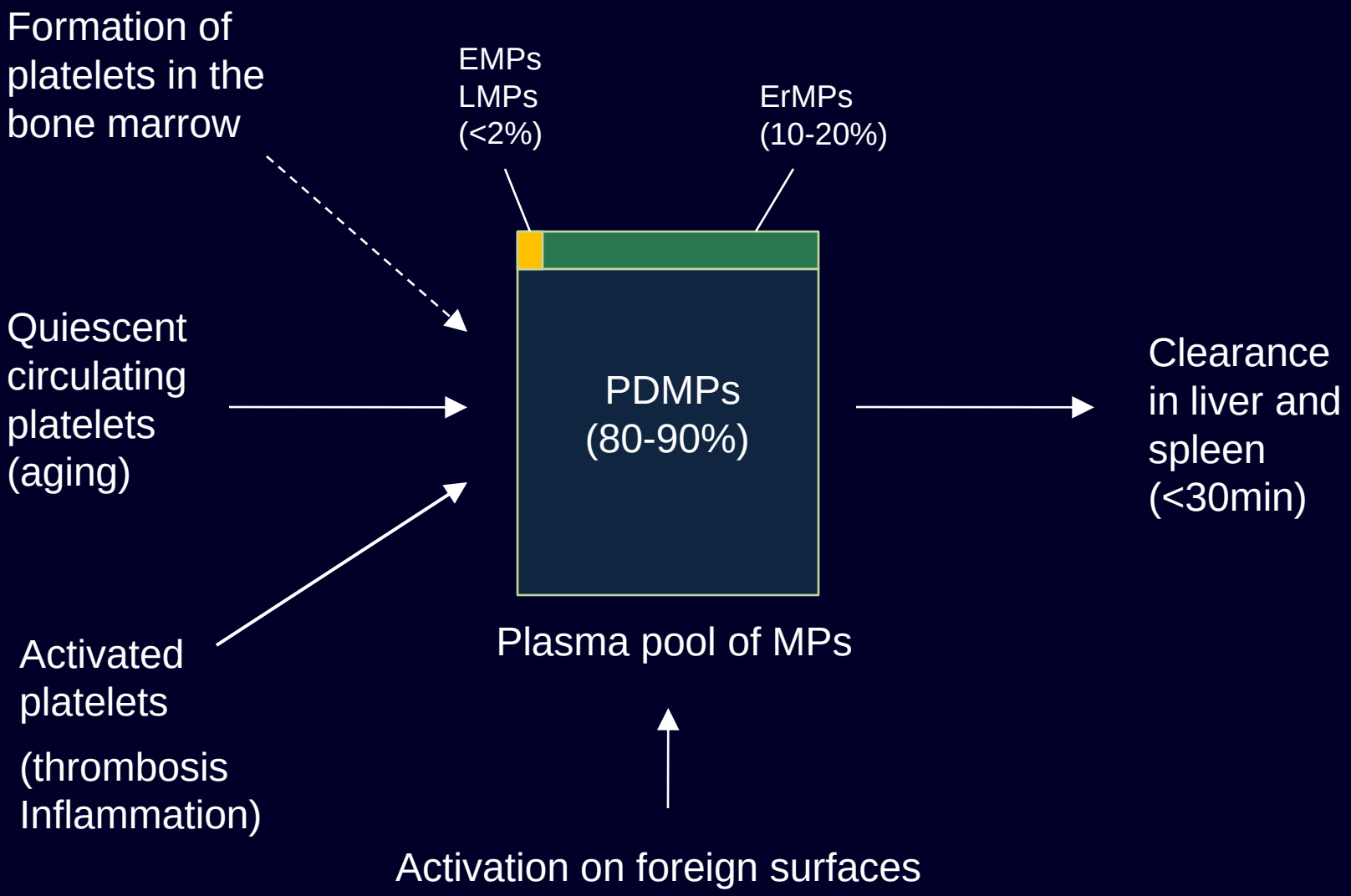
The origin of MPs can be identified by the presence of cell-specific surface antigens

Platelets	PDMPs	CD61, CD41a, CD42a, CD42b, CD31, CD62P
Endothelial cells	EMPs	CD31, CD62E, CD34
Leukocytes	LMPs	CD45
Monocytes	MMPs	CD14
Granulocytes	GMPs	CD66b
Lymphocytes	LMPs	CD4, CD8
Erythrocytes	ErMPs	CD235a

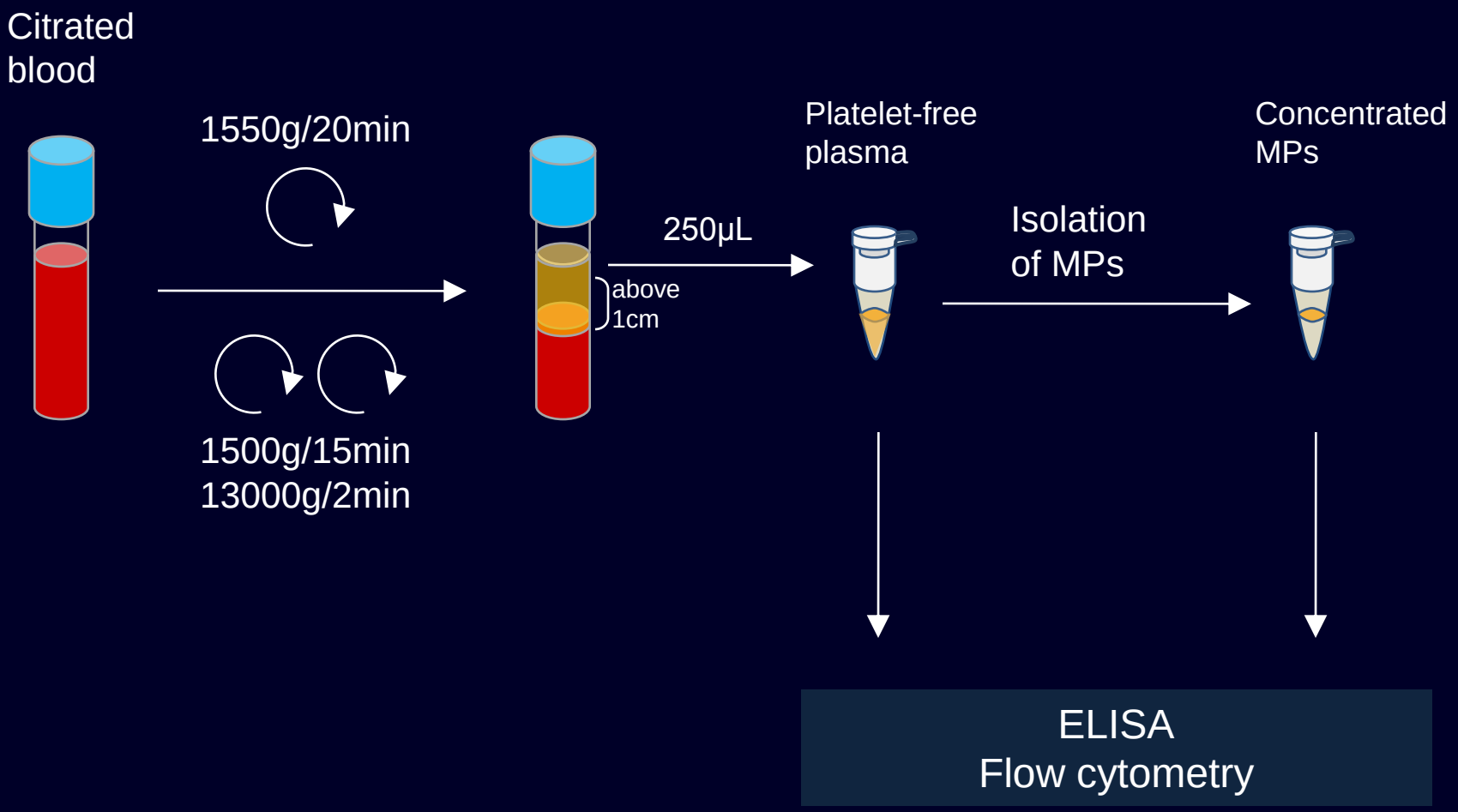
Function of PDMPs



Origin of cell-derived MPs



Methods of the detection of MPs in vivo

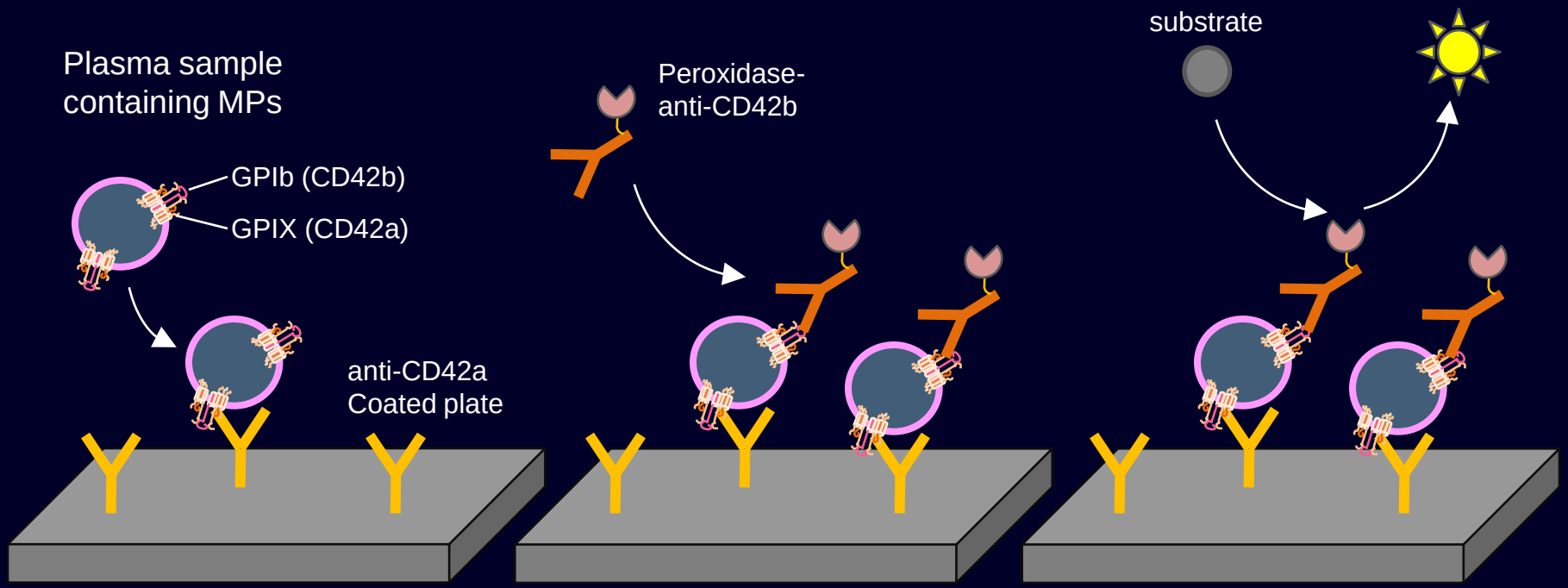


Biro E, Nieuwland et al. J Thromb Haemost 2004;2:1843

Detecting of PDMPs by ELISA

ELISA are designed to detect PDMP-specific antigens (immunological methods) or their procoagulant activity (functional methods) using plates coated with MP specific reagents.

1 Immunological methods

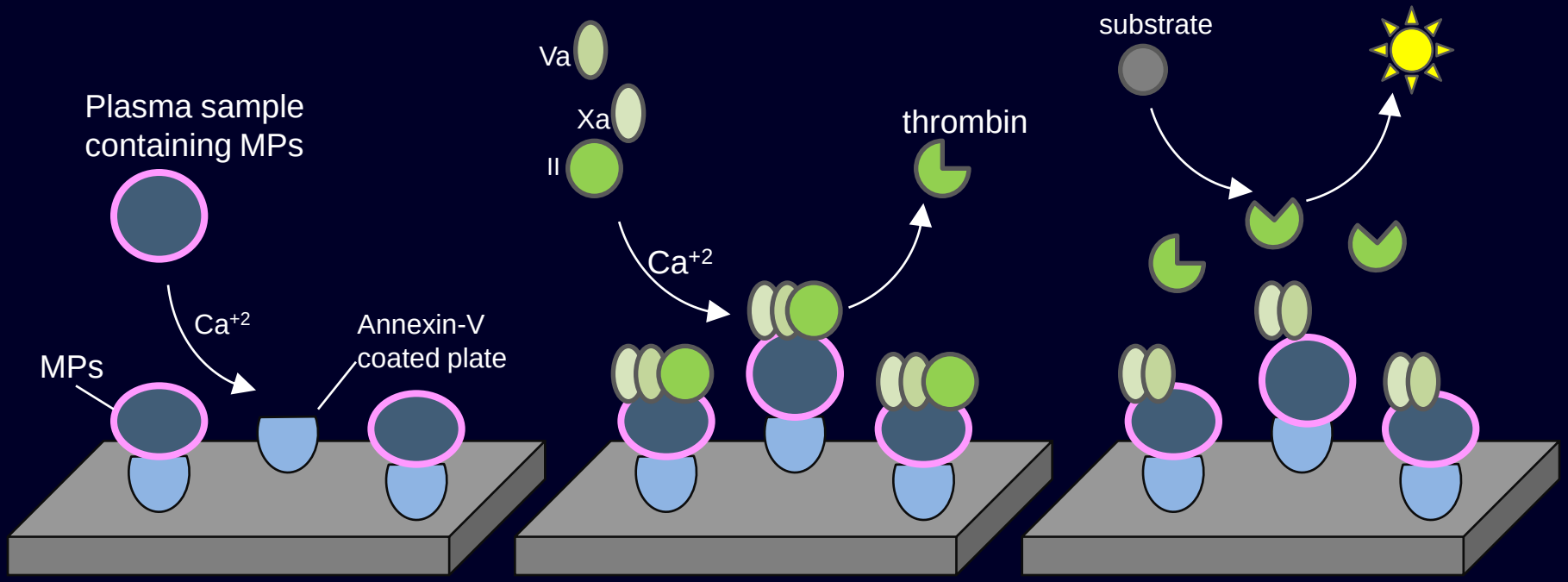


Nomura et al. J Atheroscler Thromb. 2009;16:878

Detecting of PDMPs by ELISA

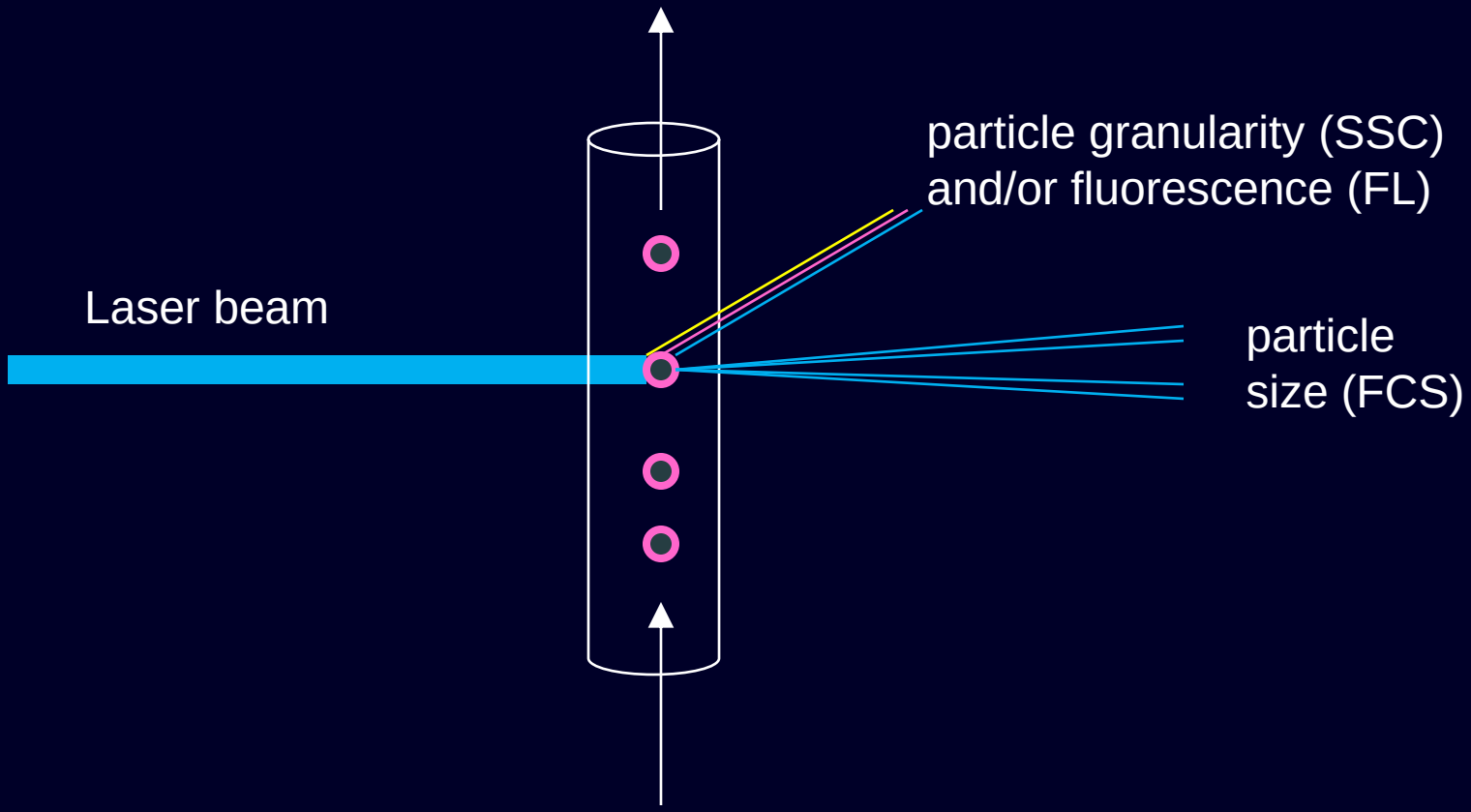
2 Functional methods

Functional ELISA measures the procoagulant activity of MPs



Detecting of PDMPs by flow cytometry

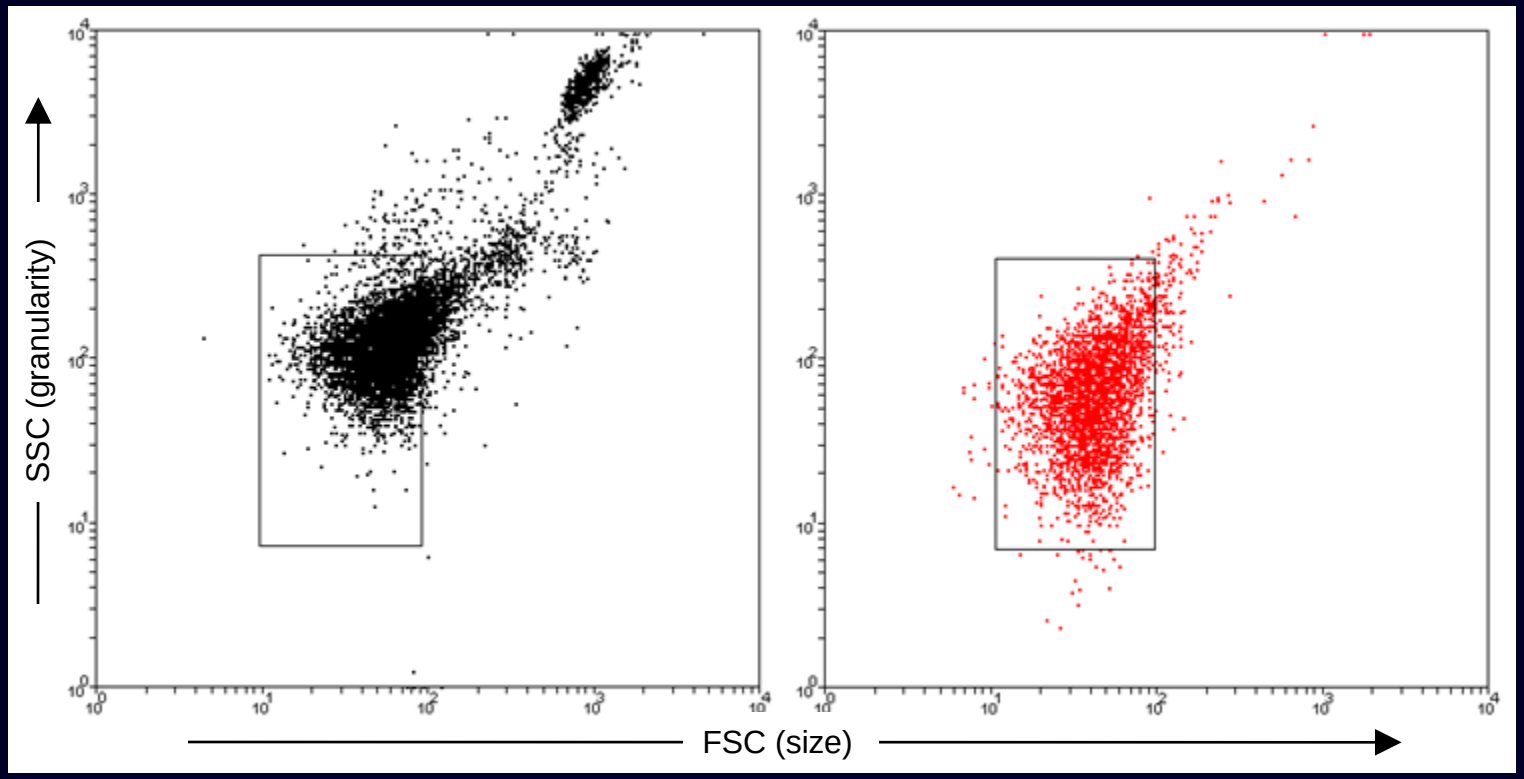
Flow cytometry measures different physical and biochemical properties of single particles (e.g.cells), during their controlled flow through the laser beam.



Detecting of PDMPs by flow cytometry

Whole blood (erythrocytes are lysed)

Platelet free plasma (PFP)



Detecting of PDMPs by flow cytometry

1 Direct detection of PFP

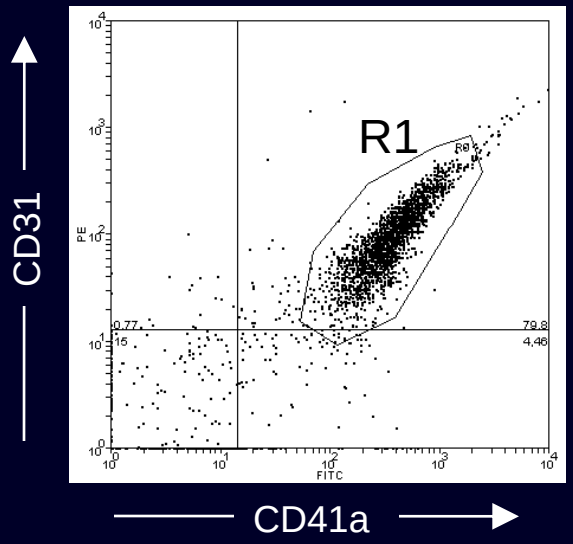
PFP

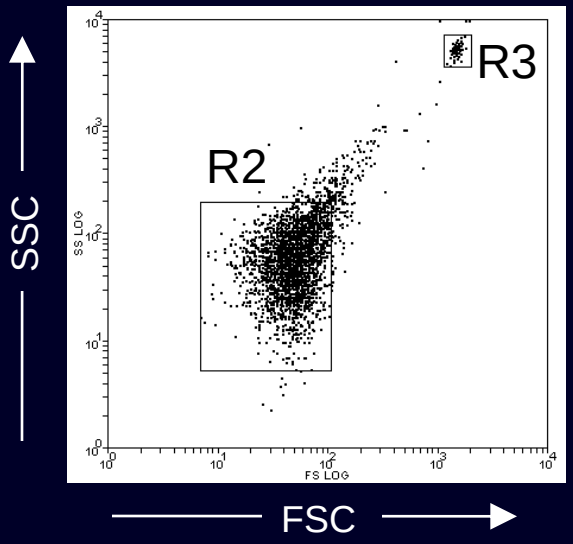
fixed volume
(e.g. 20 µL)

Stained for 20 minutes with
monoclonal antibodies
(e.g. CD41a and CD31)

Fixed amount of fluorescent
beads added (6 µm in size)

Diluted with PBS-buffer
and analyzed in FC





1 µL of PFP is equivalent
to „n" added beads

PDMPs
in 1 µL of
plasma

$$= \frac{(R1_{and}R2)\bullet n}{R3}$$

Detecting of PDMPs by flow cytometry

Direct detection of PDMPs

Advantages

1. Good reproductibility (intraassay variation ~10%)
2. Multiple antigens can be detected in a single sample

Disadventages

1. Problems in detection of PS-bearing MPs in citrated plasma samples
2. Problem in detecting objects smaller than 0.5 μm in diameter.

Detecting of PDMPs by flow cytometry

2 Detection of PDMPs in concentrated PFP samples

PFP

PBS-w/o-Ca

18000g/40min

MPs in PBS

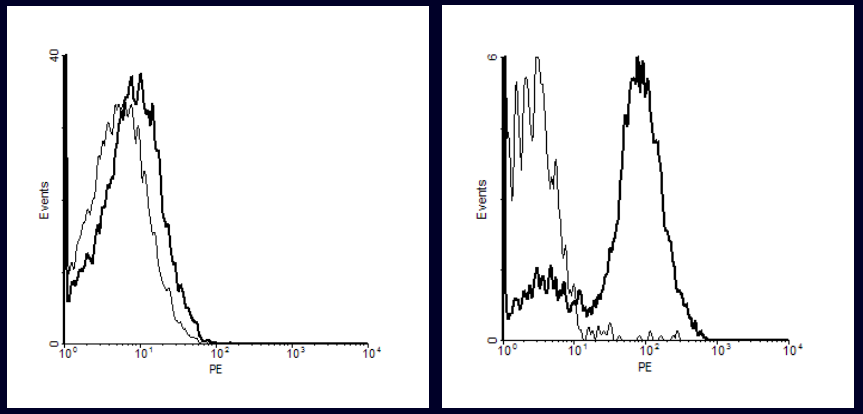
fixed volume (e.g. 10 µL)

Stained for 20 minutes in 2.5 mM calcium buffer with PE-Annexin-V and platelet specific mAb (e.g. CD41a)

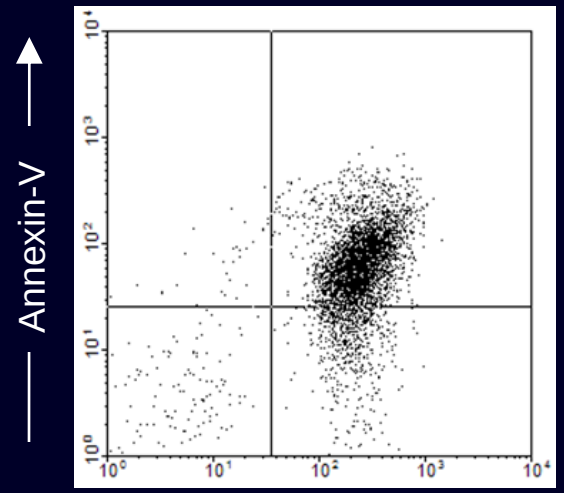
Dilution with fixative buffer and microbeads

1.0 mM Ca

2.5 mM Ca



Annexin-V



Detecting of PDMPs by flow cytometry

Detection of PDMPs isolated from plasma

Advantages

1. PDMPs expressing phosphatidylserine can be identified and counted

Disadventages

1. Methodological differences between laboratories
2. Problems with reproductibility (intraassay variation ~15-25%)
3. Problems with interpretation of results (e.g. not all CD41a+ MPs bind annexin-V) and quantification

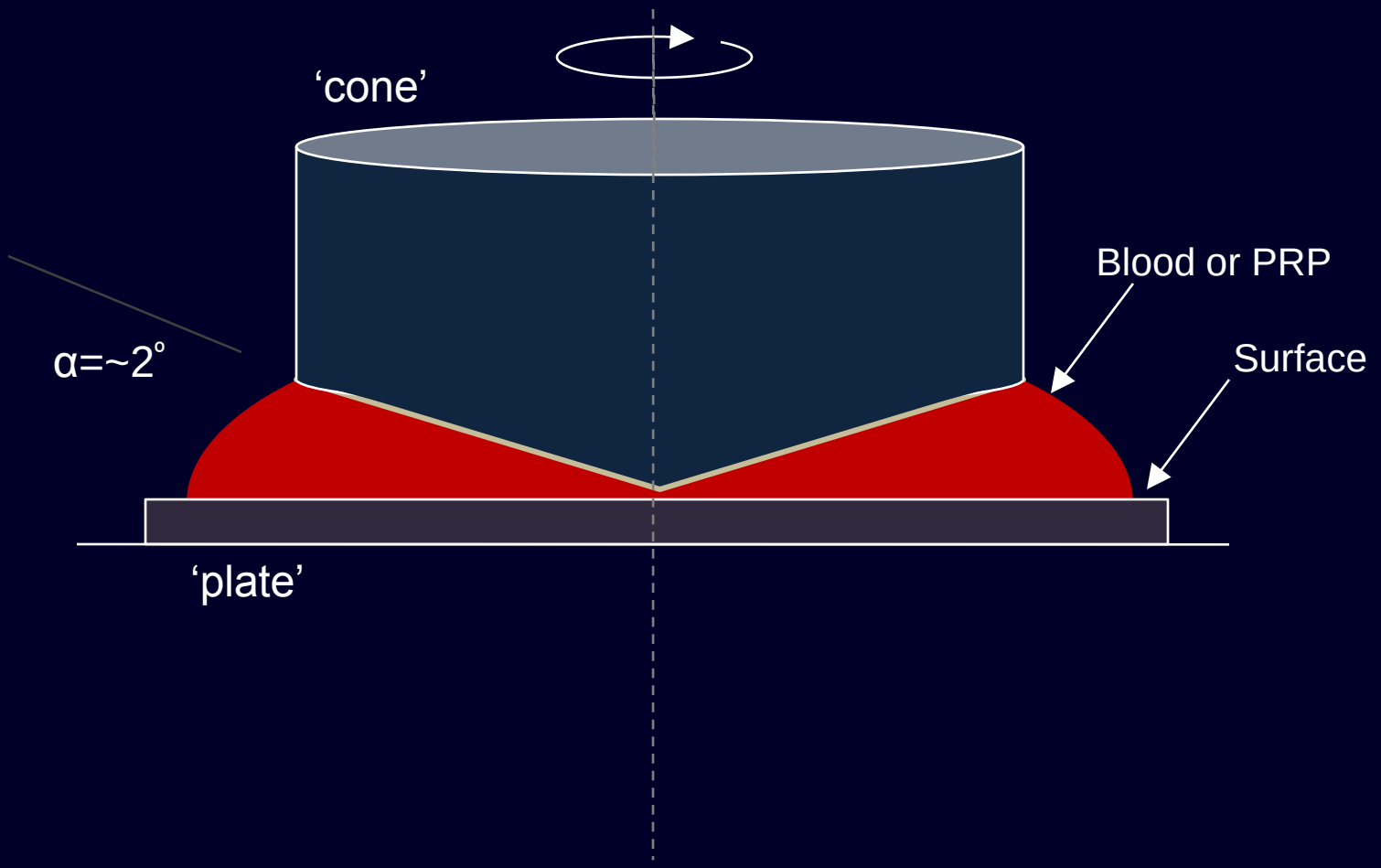
MPs generated *in vitro*

1. PDMPs can be generated in static in vitro conditions by stimulating platelets with classical agonists

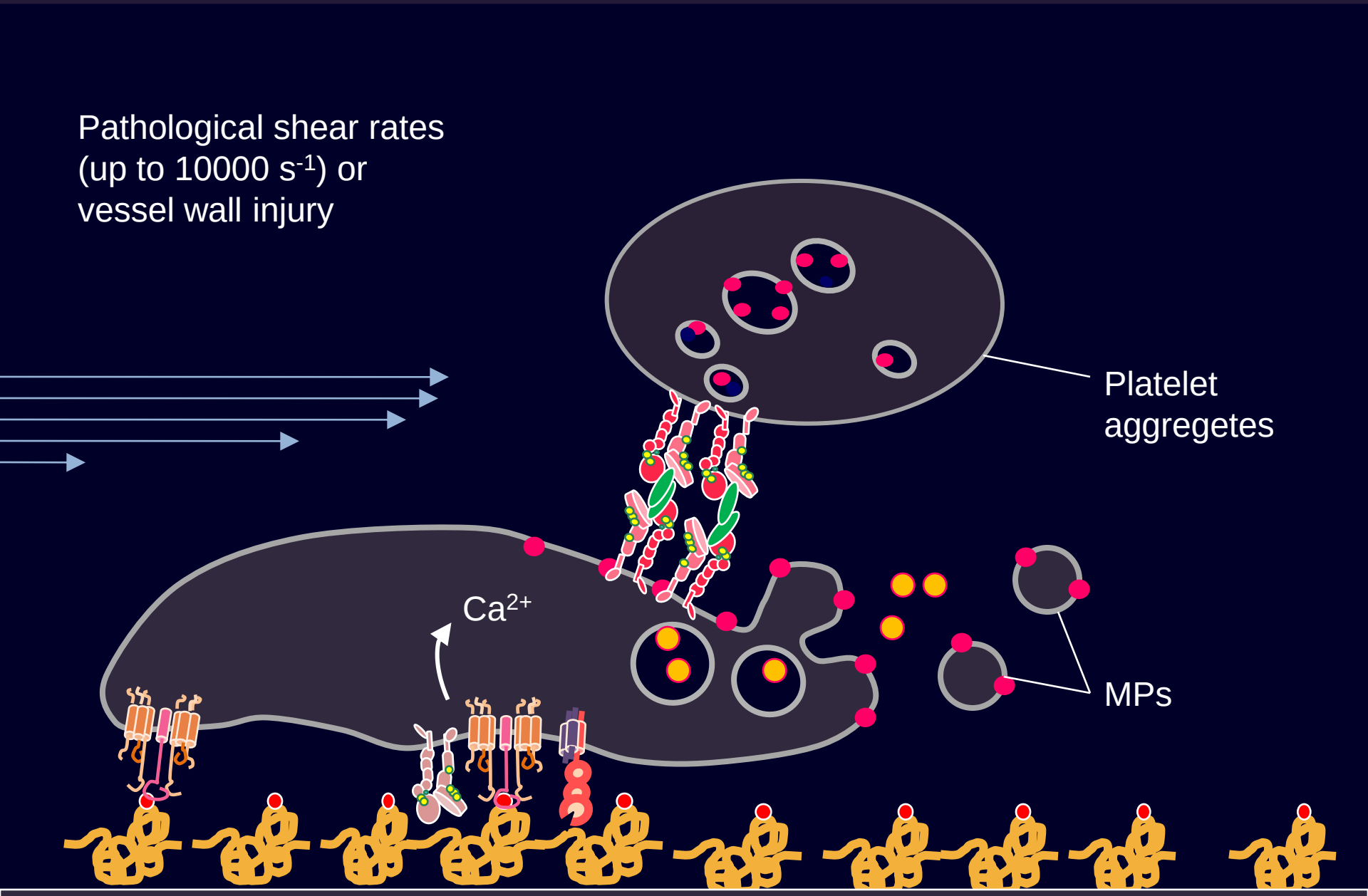
Epinephrin
ADP
< Thrombin
< Collagen
< Calcium ionophore
PMA

2

Generation of MPs under shear-stress conditions.
‘Cone-and-plate’ viscometer model



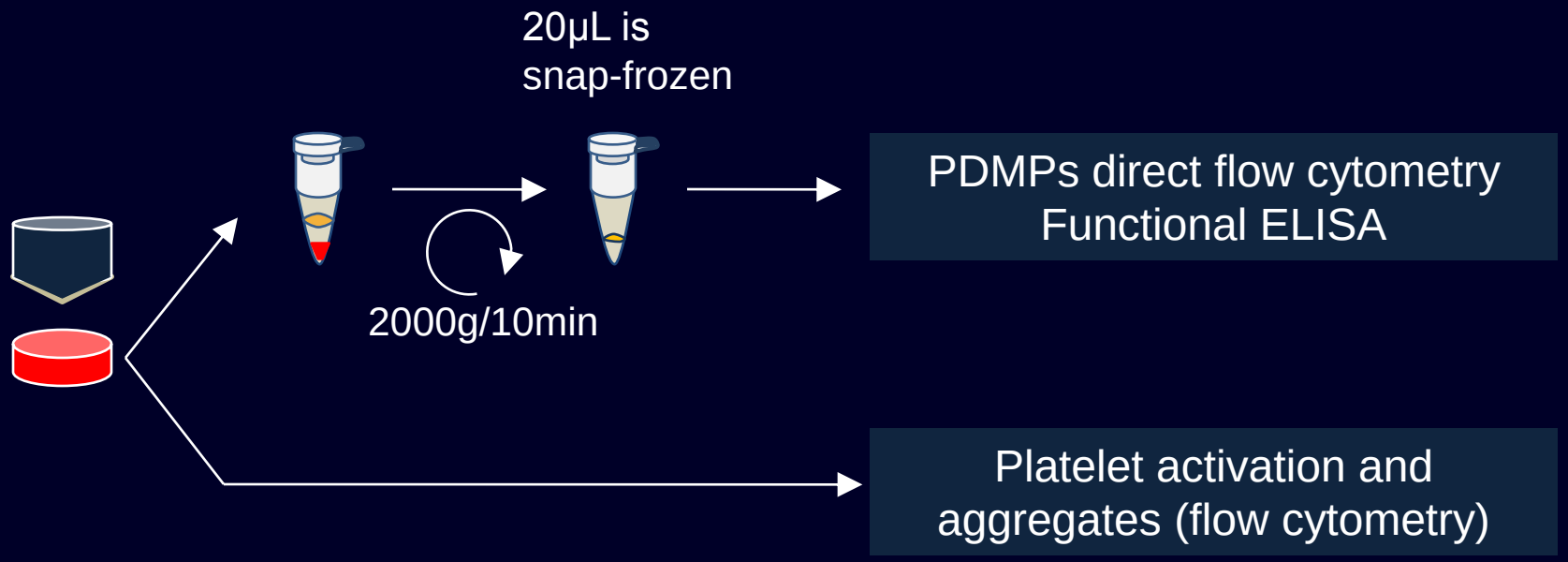
Shear stress dependent platelet activation



MPs generated *in vitro*

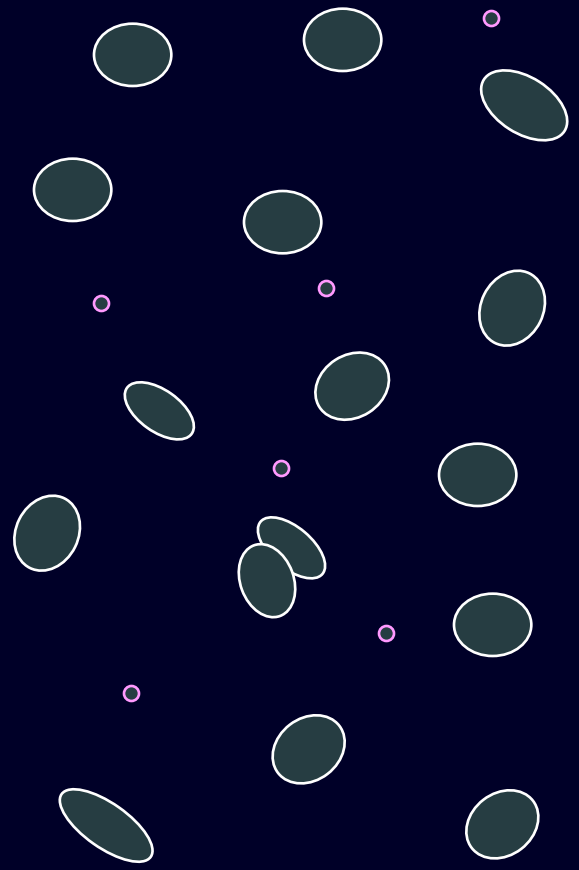
Shear-force exposure to blood samples

1. Citrate anticoagulated blood or PRP (200g/10min)
2. 130µL of blood (or PRP) is added to a plastic polystyrene well (or any other artificial surface)
3. Tested wells are covered with a plastic cone and blood sample is subjected to a shear rate 1800 s^{-1} for 300 sec at room temperature.
4. When rotation ends, plastic cone is discarded and ~100 µL of sample could be collected

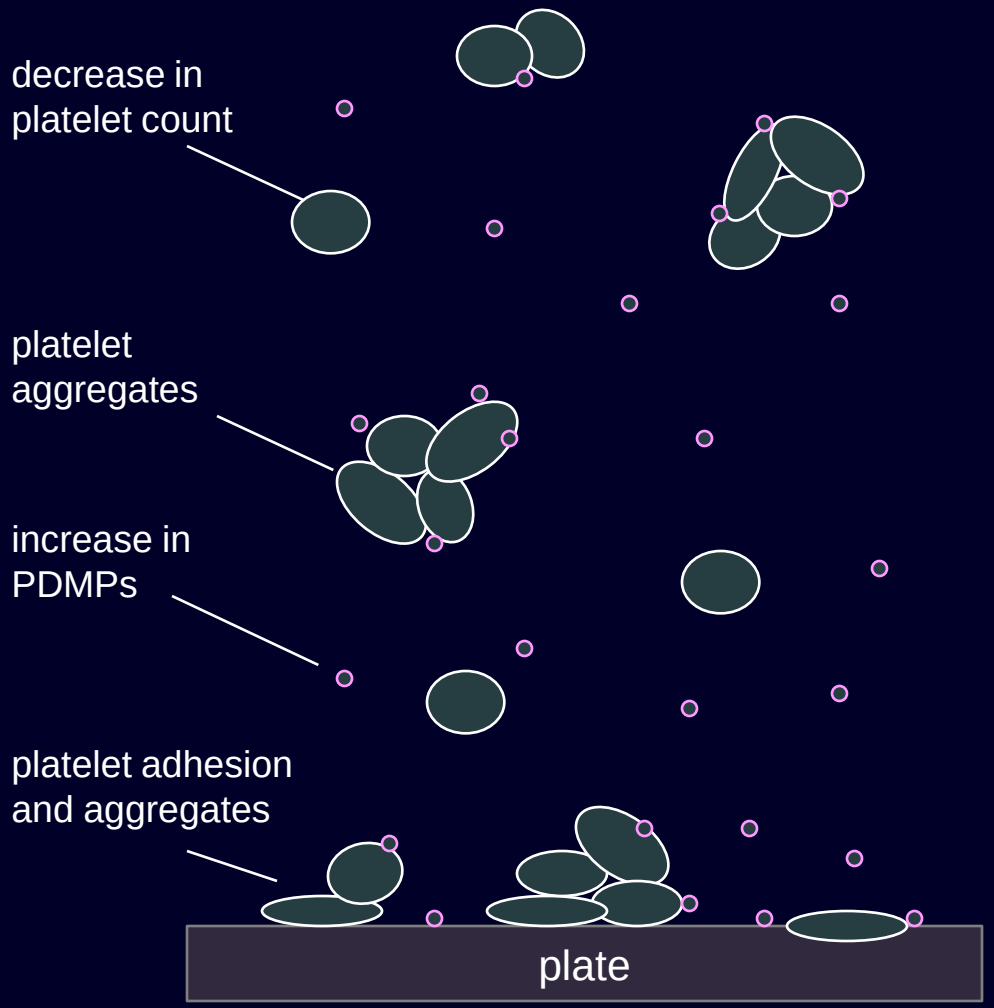


MPs generated *in vitro*

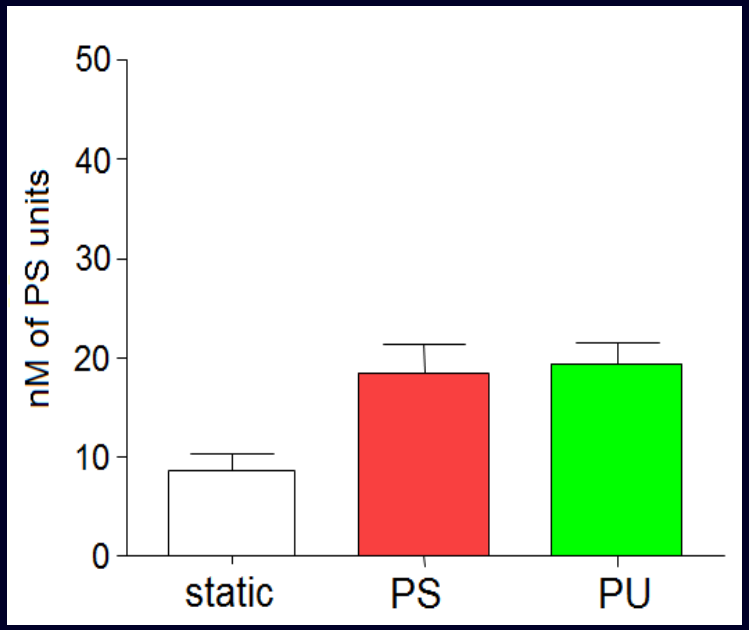
1 Static conditions (tube)



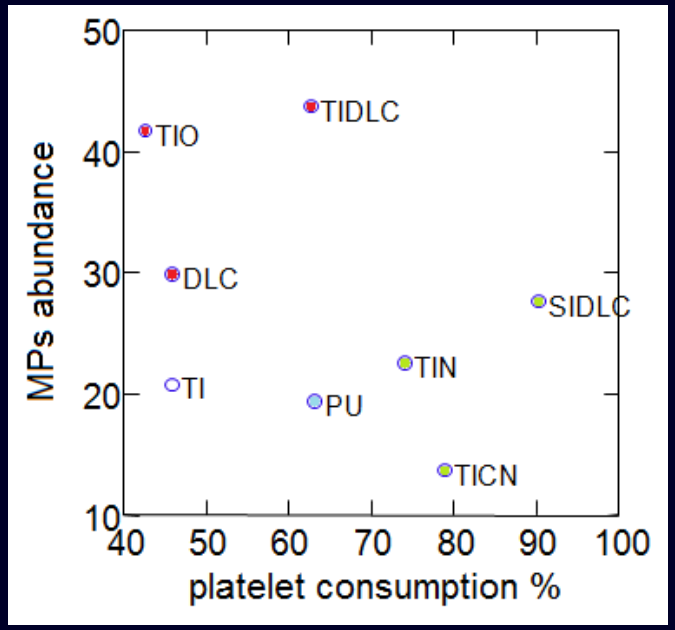
2 Shear stress (cone-and-plate)



MPs generated *in vitro*



~3 fold increase in the procoagulant activity of MPs upon shear stress



Both the relative decrease in platelet count and MPs activity help to identify surfaces with best biocompatibility

Shear stress model of platelet activation on different surfaces

Problems

1. The quality of material (any scratches can disrupt platelets mechanically)
2. The volume of PFP that can be used for laboratory studies is very small
3. Better standarization for relative decrease in the platelet count
4. The correlation between direct-flow cytometry methods and procoagulant activity of shear-stress induced platelet microparticles need to be determined.
5. A reliable method of quantification of surface bound aggregetes need to be developed.

Jagiellonian University Medical College
Department of Medicine, Krakow, Poland

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correspondence: nfsanak@cyf-kr.edu.pl