Assessment of hemocompatibility of biomaterials with arterial blood flow by platelet functional tests

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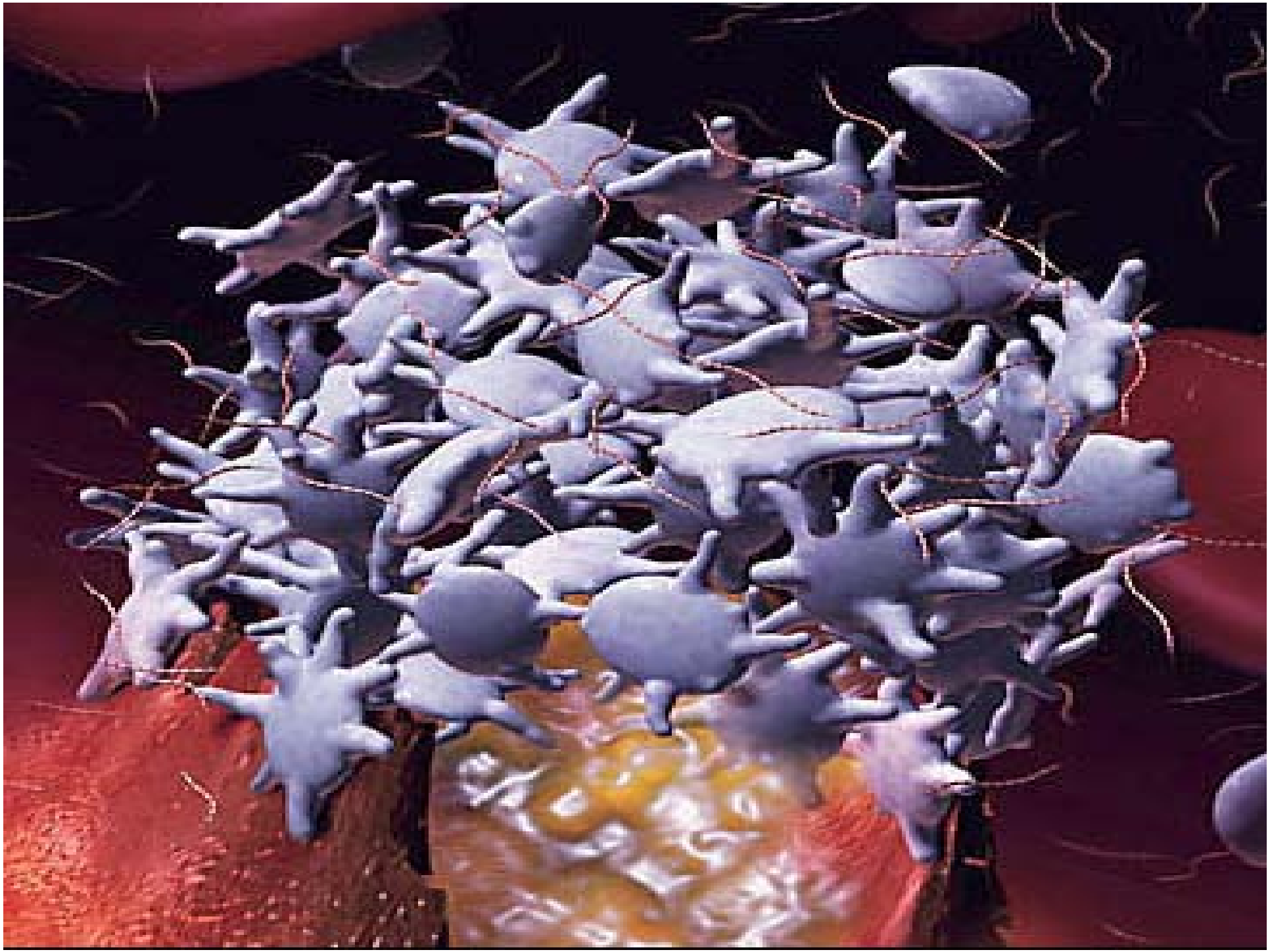
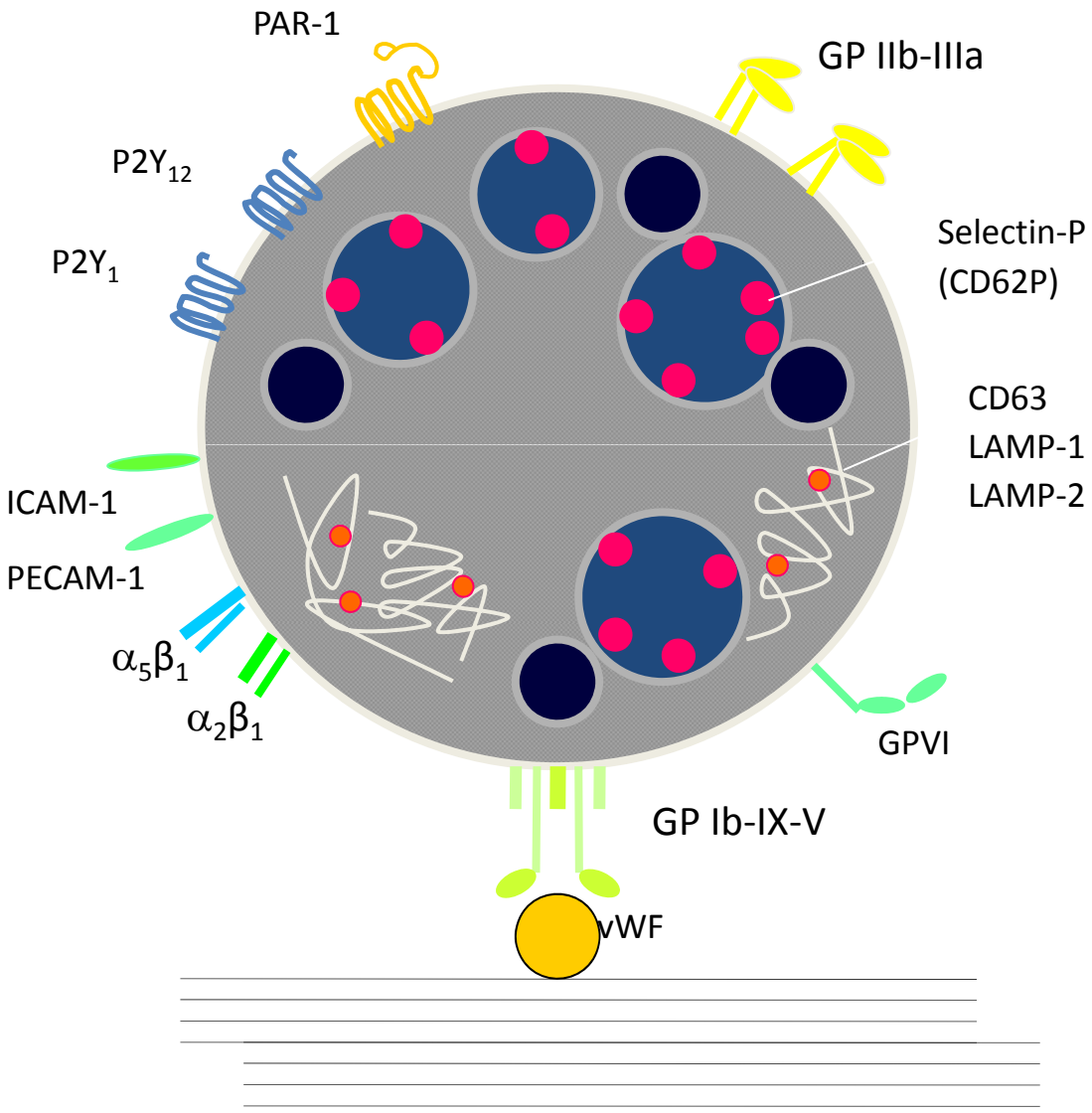
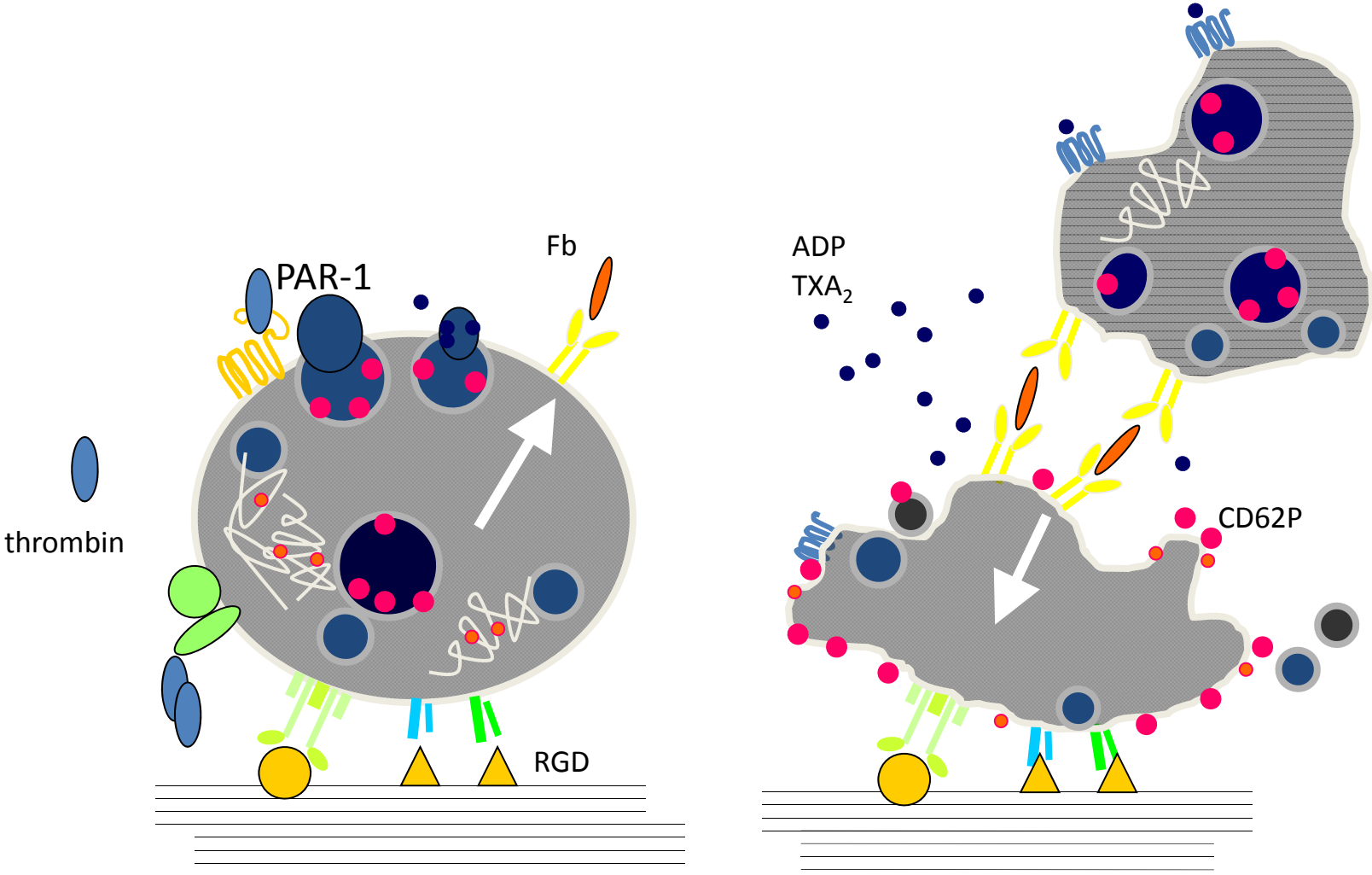


signaling molecules of blood platelet



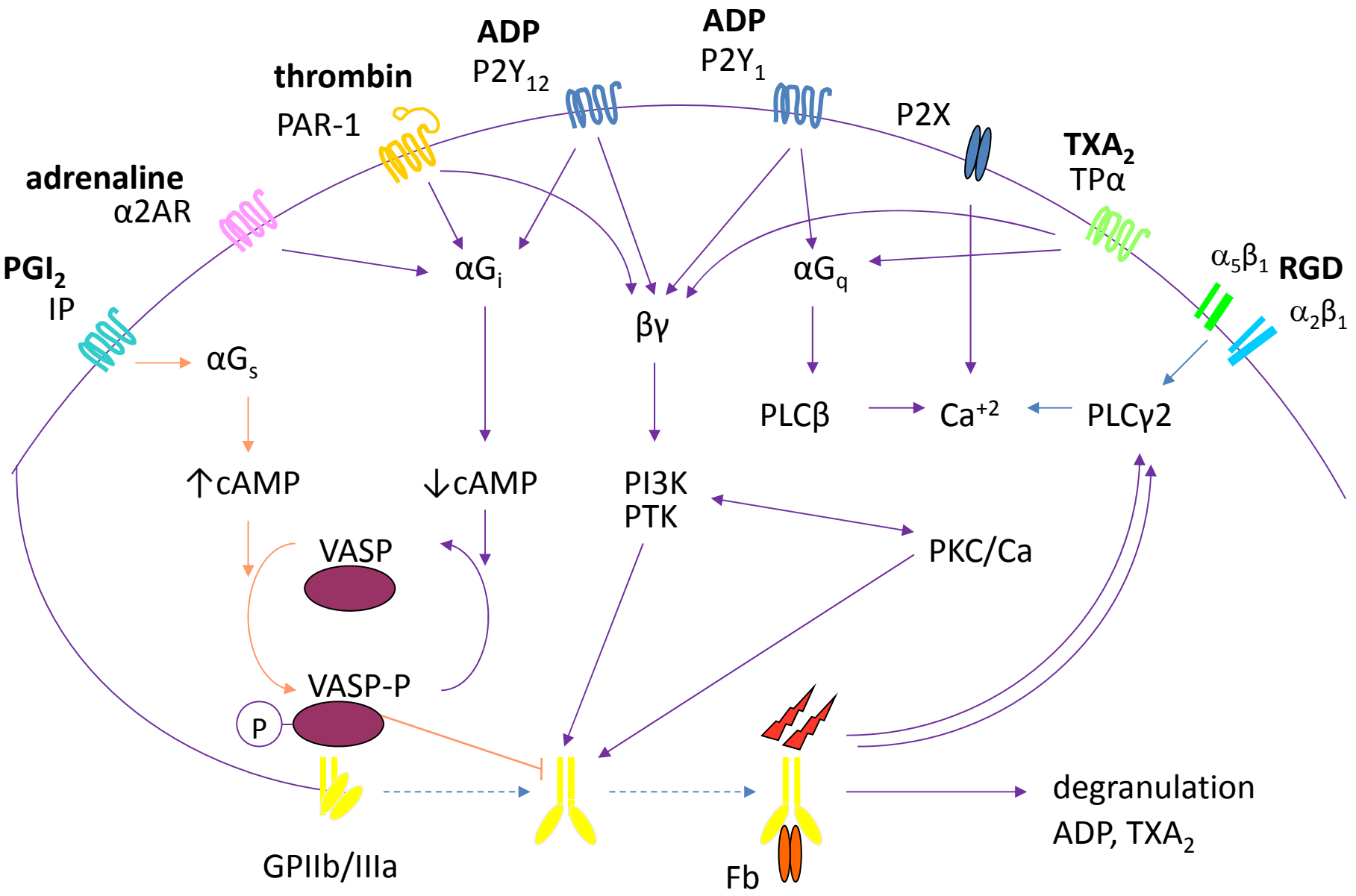
signaling events of activated platelet



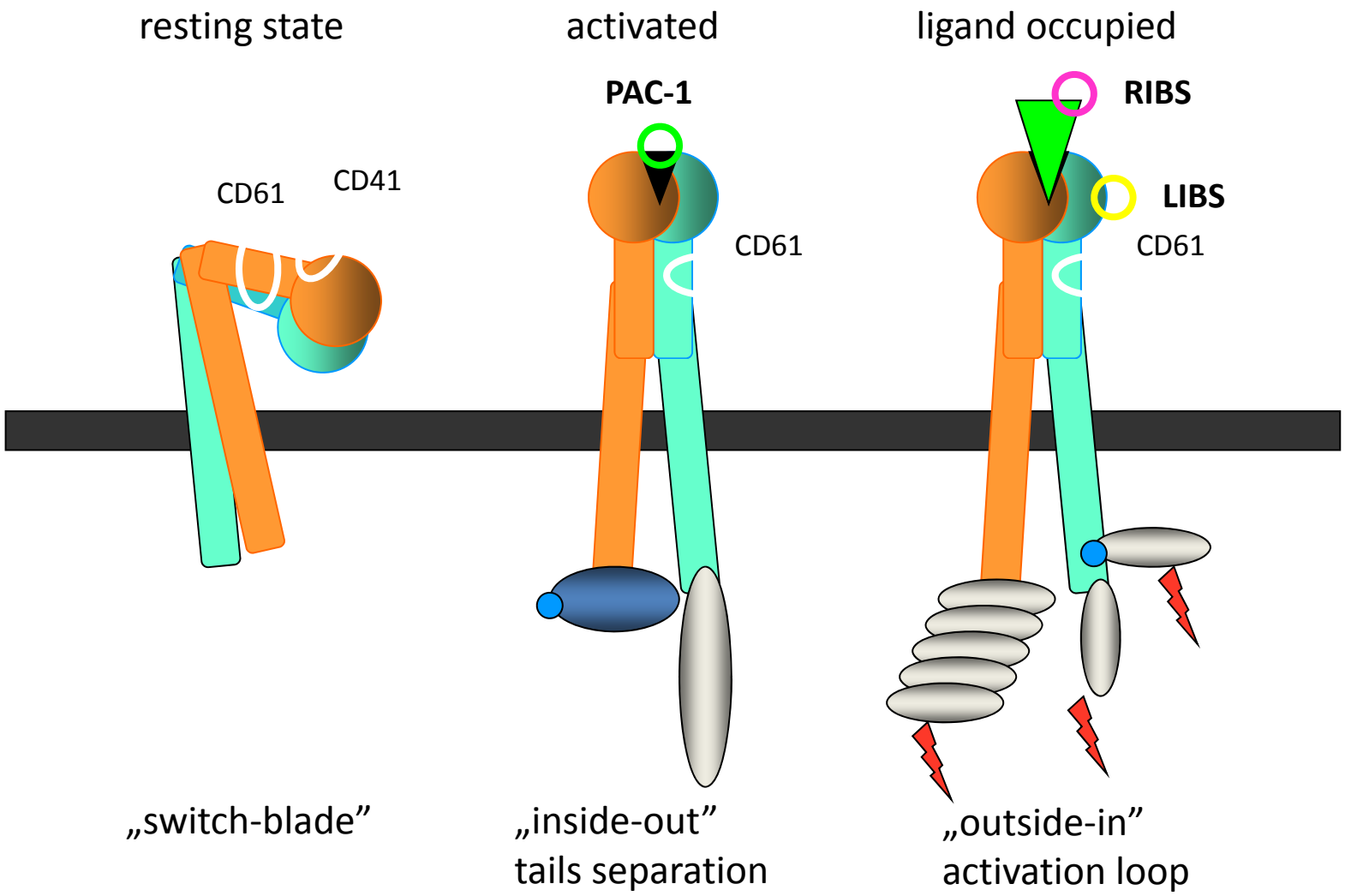
„inside-out"

„outside-in"

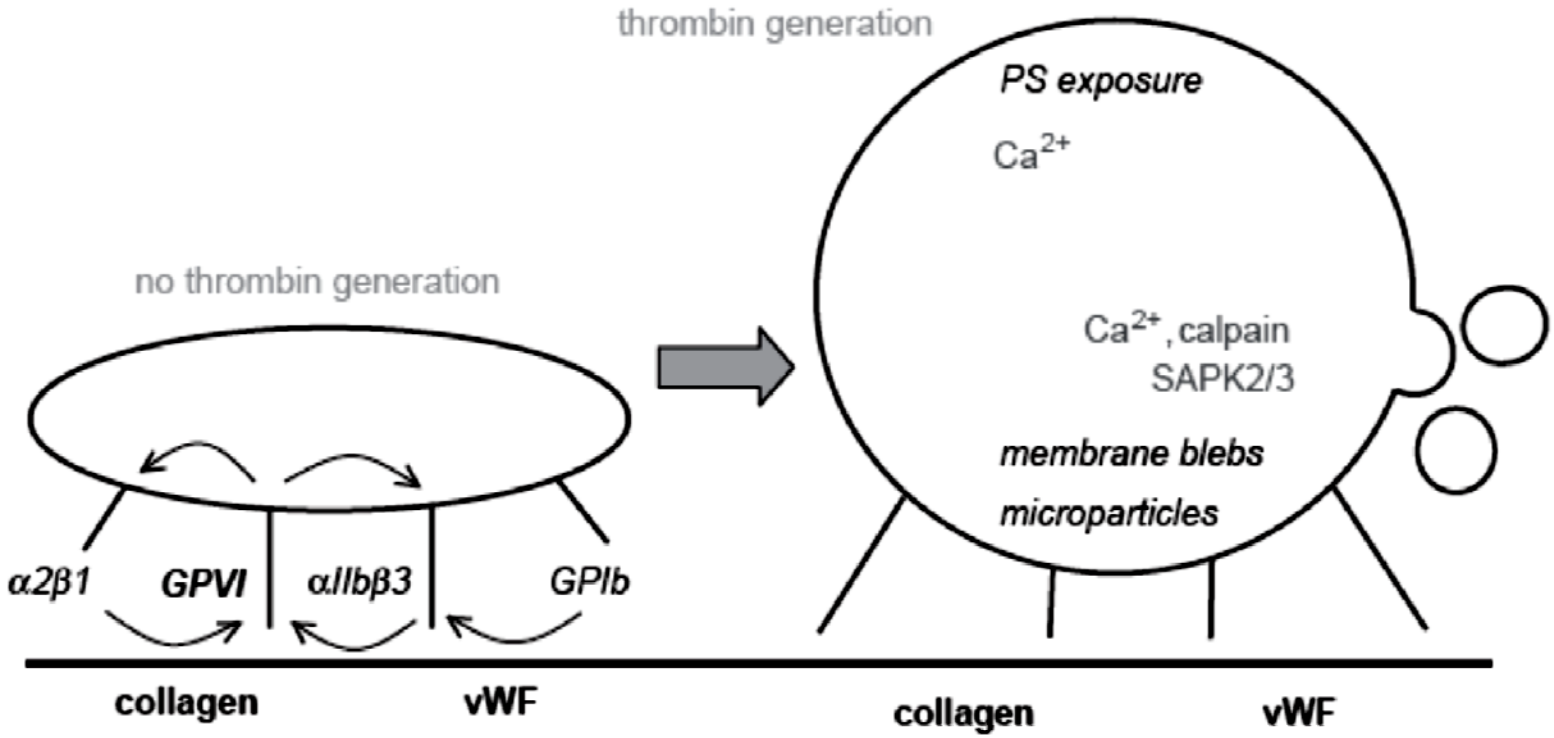
a map of intracellular platelet signaling



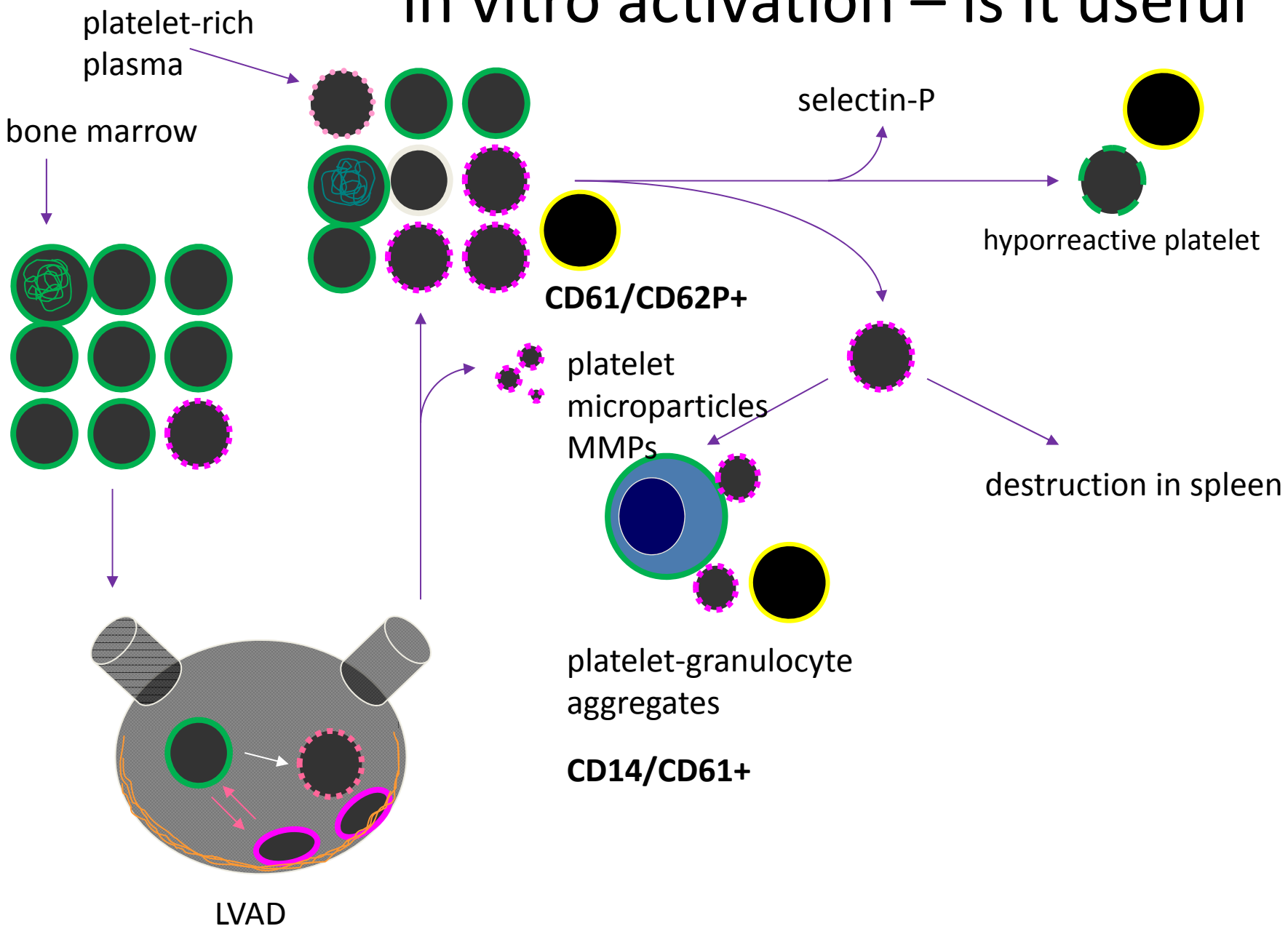
exemplary marker of platelet activation - GPIIb-IIIa (CD41-CD61)



adhesion of platelet could be reversible until no thrombin is generated

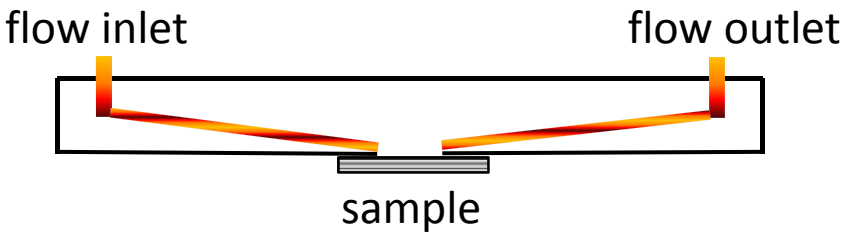


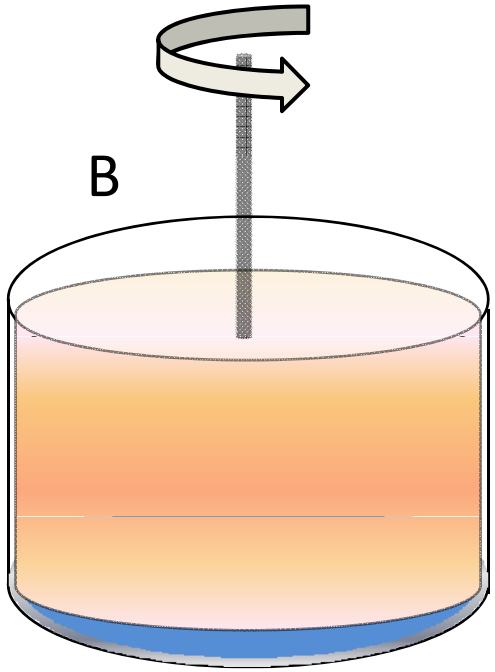
in vitro activation – is it useful



cone and plate chamber

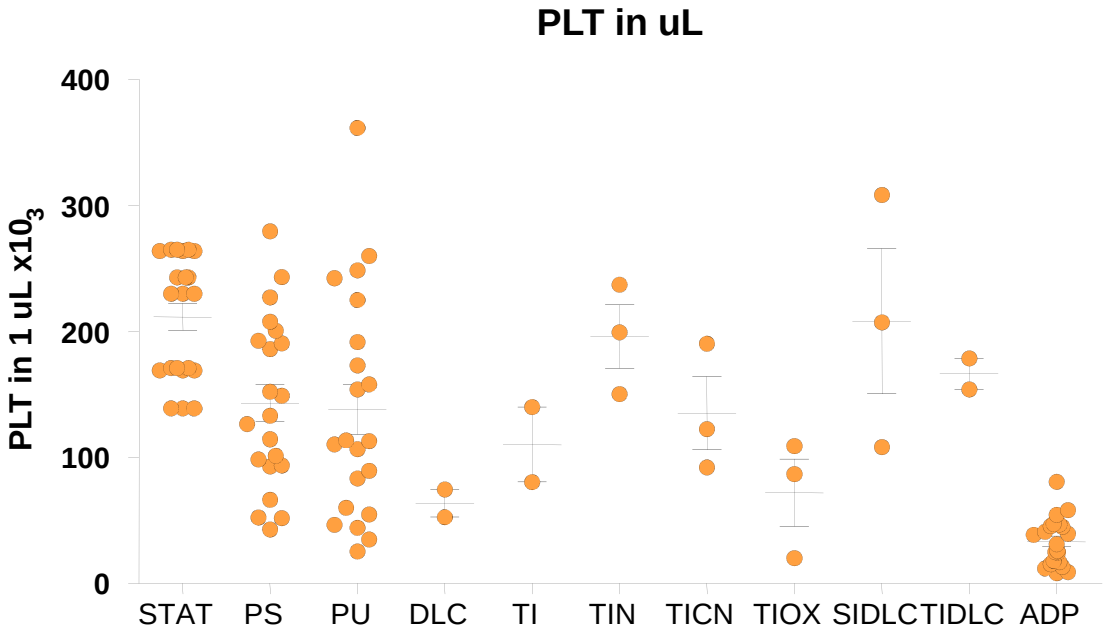
a conventional flow chamber

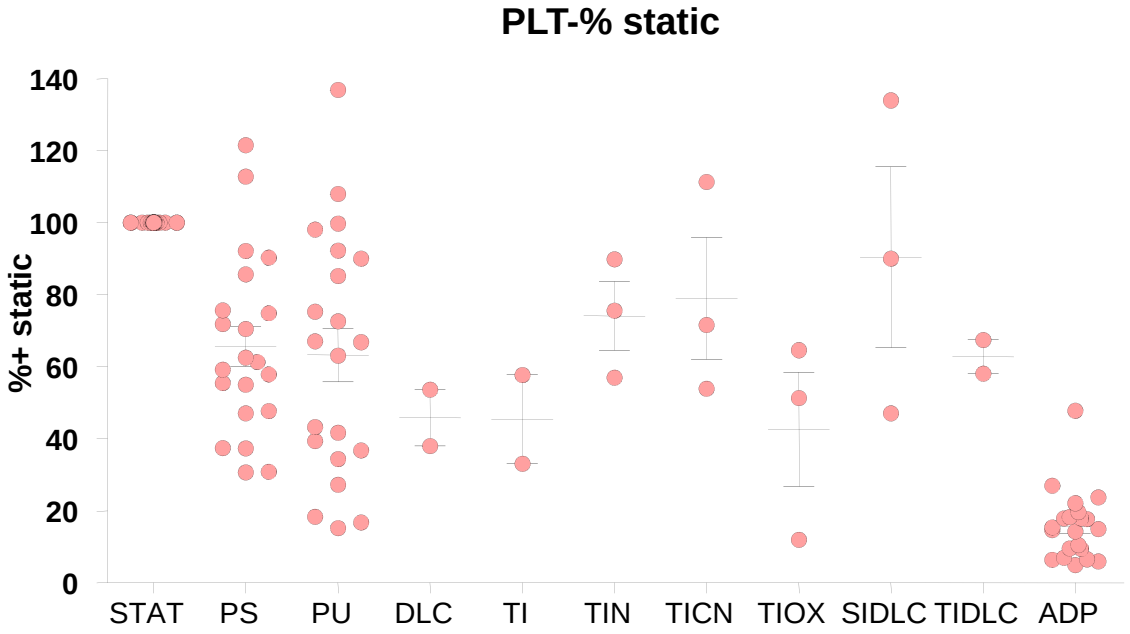




arterial shear forces ≈ 1800 s^{-1}

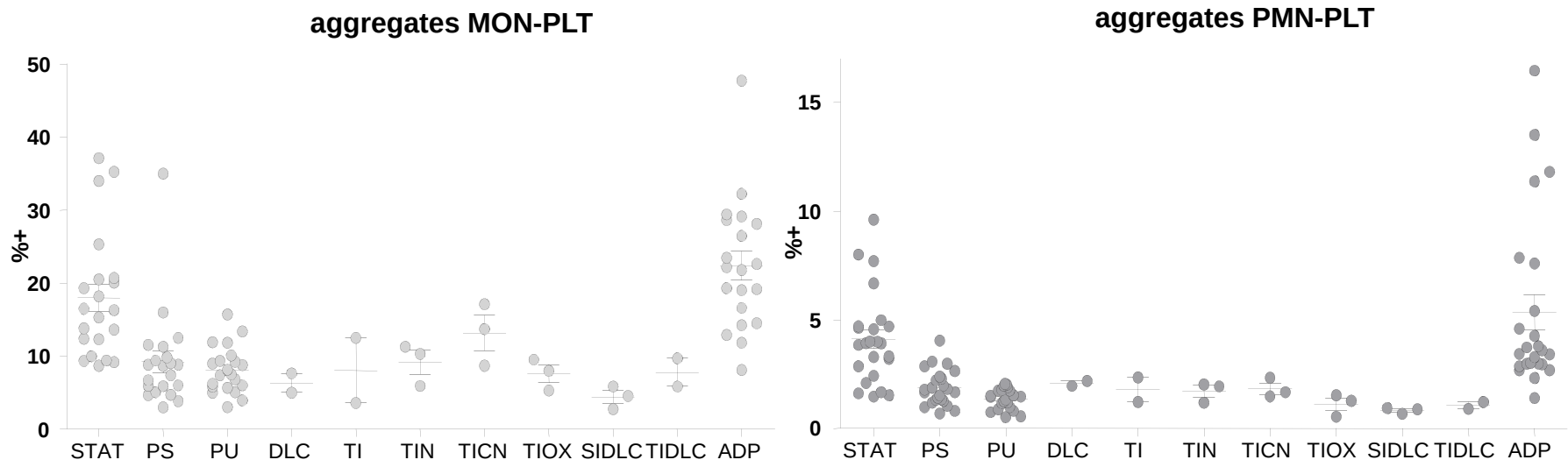
platelets are aggregated and dissappear from the blood undear shear stress

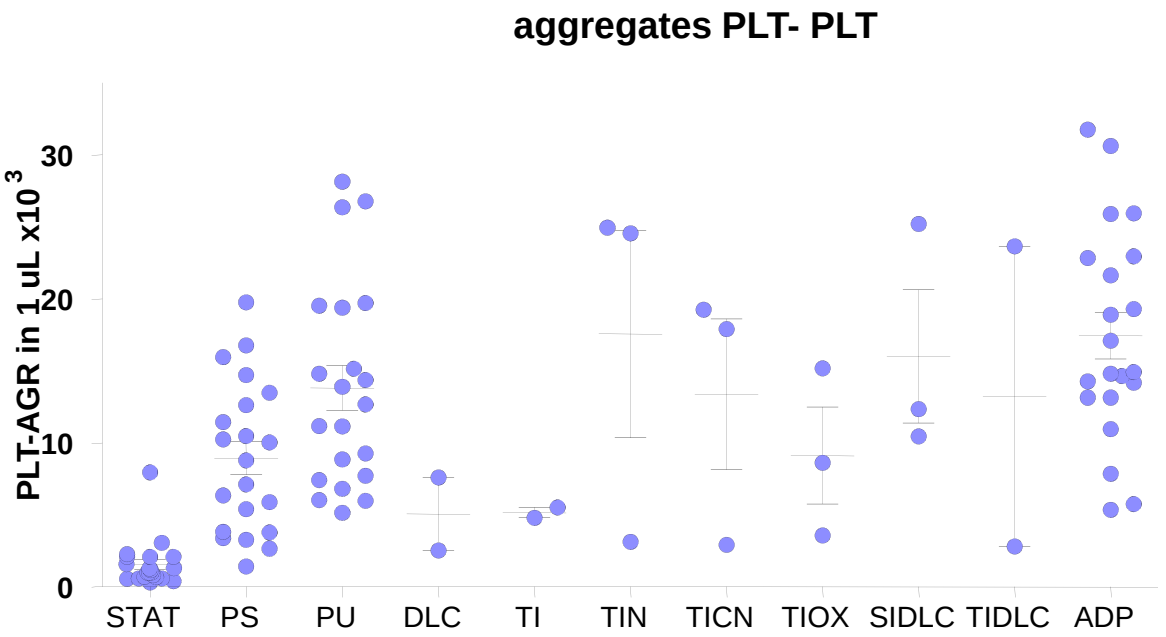


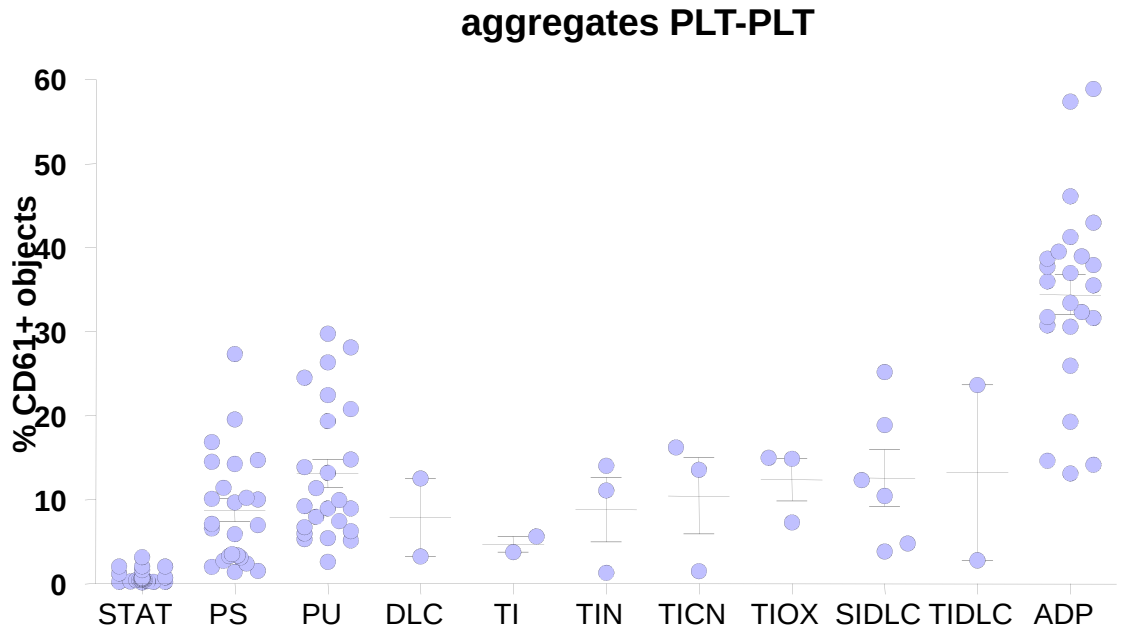


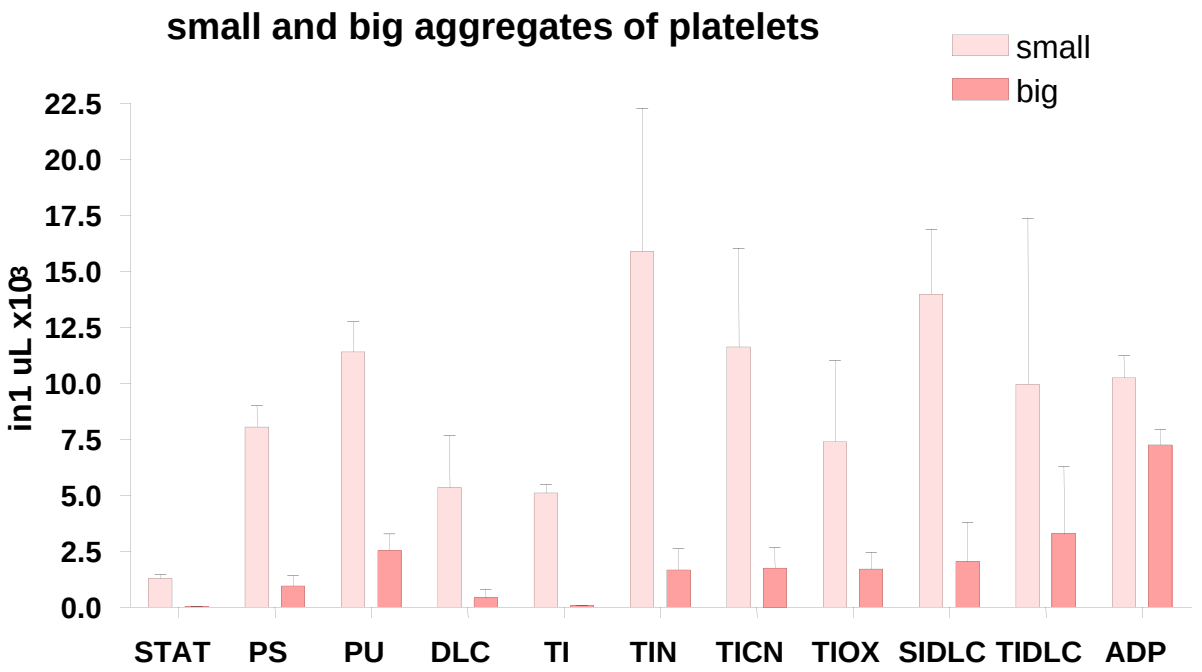
PS – polystyrene reference surface
PU – medical polyurethane
DLC – diamond like carbon layer
Ti – titanium layer
SI – silicon layer
(N, CN, OX) – modifications of the layer by nitride, carbonitride or oxide deposition

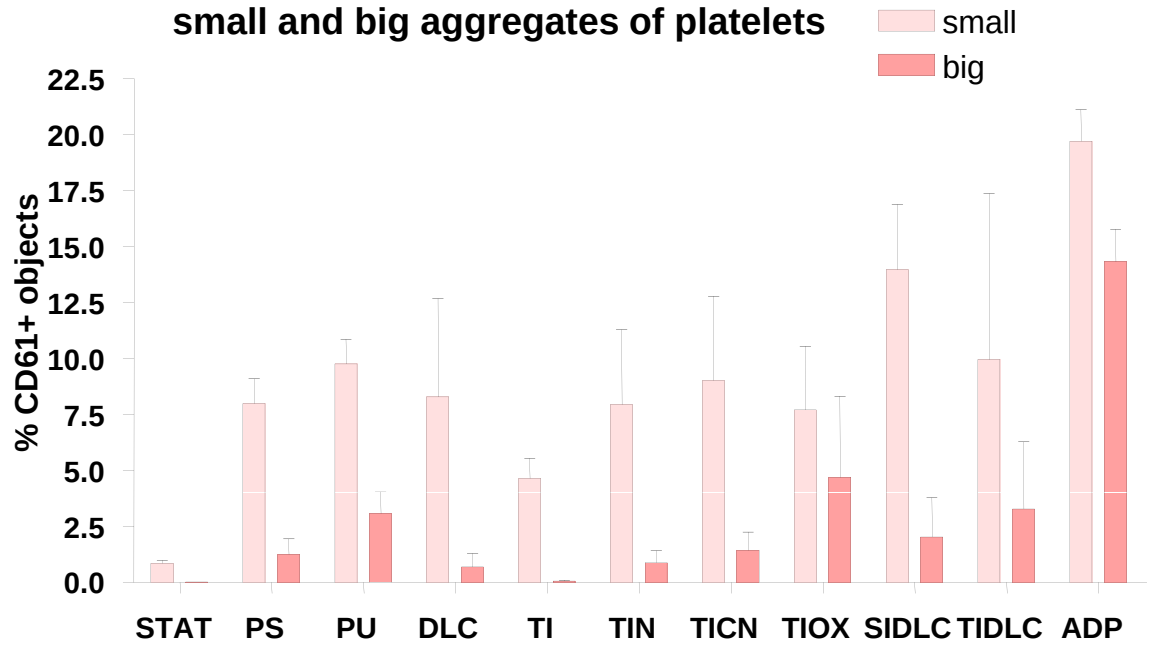
platelets also aggregate with monocytes and polymorphonuclear granuloctes and deposit on the surface during shear stress




aggregates PLT- PLT
PLT-AGR in 1 uL x10 3
30
20
10
0
STAT
PS
PU
DLC
TI
TIN
TICN
TIOX
SIDLC
TIDLC
ADP


aggregates PLT-PLT
% CD61+ objects
60
50
40
30
20
10
0
STAT
PS
PU
DLC
TI
TIN
TICN
TIOX
SIDLC
TIDLC
ADP


small and big aggregates of platelets
small
big
in1 uL x10⁸
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
STAT
PS
PU
DLC
TI
TIN
TICN
TIOX
SIDLC
TIDLC
ADP


small and big aggregates of platelets
small
big
% CD61+ objects
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
STAT
PS
PU
DLC
TI
TIN
TICN
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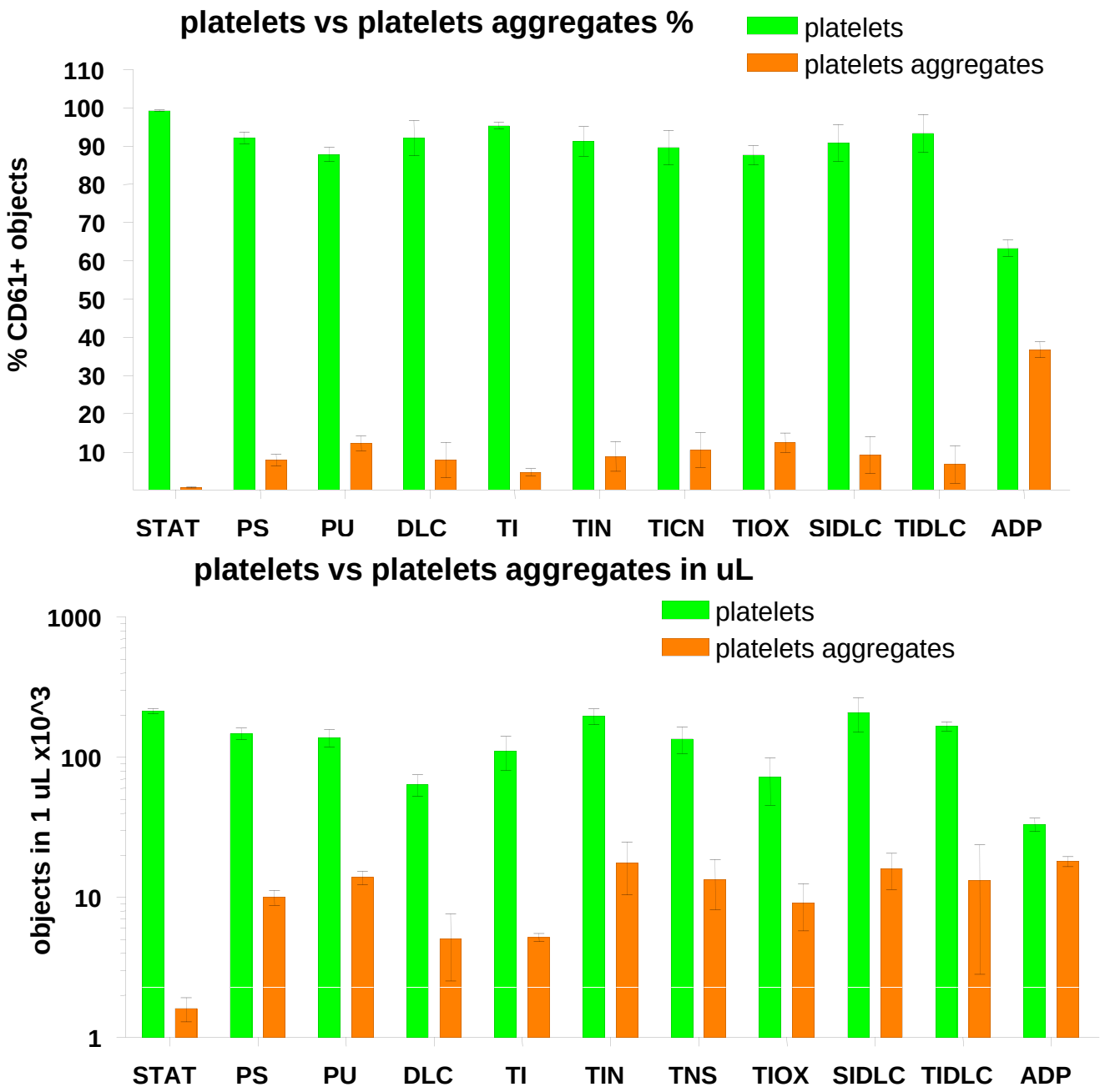
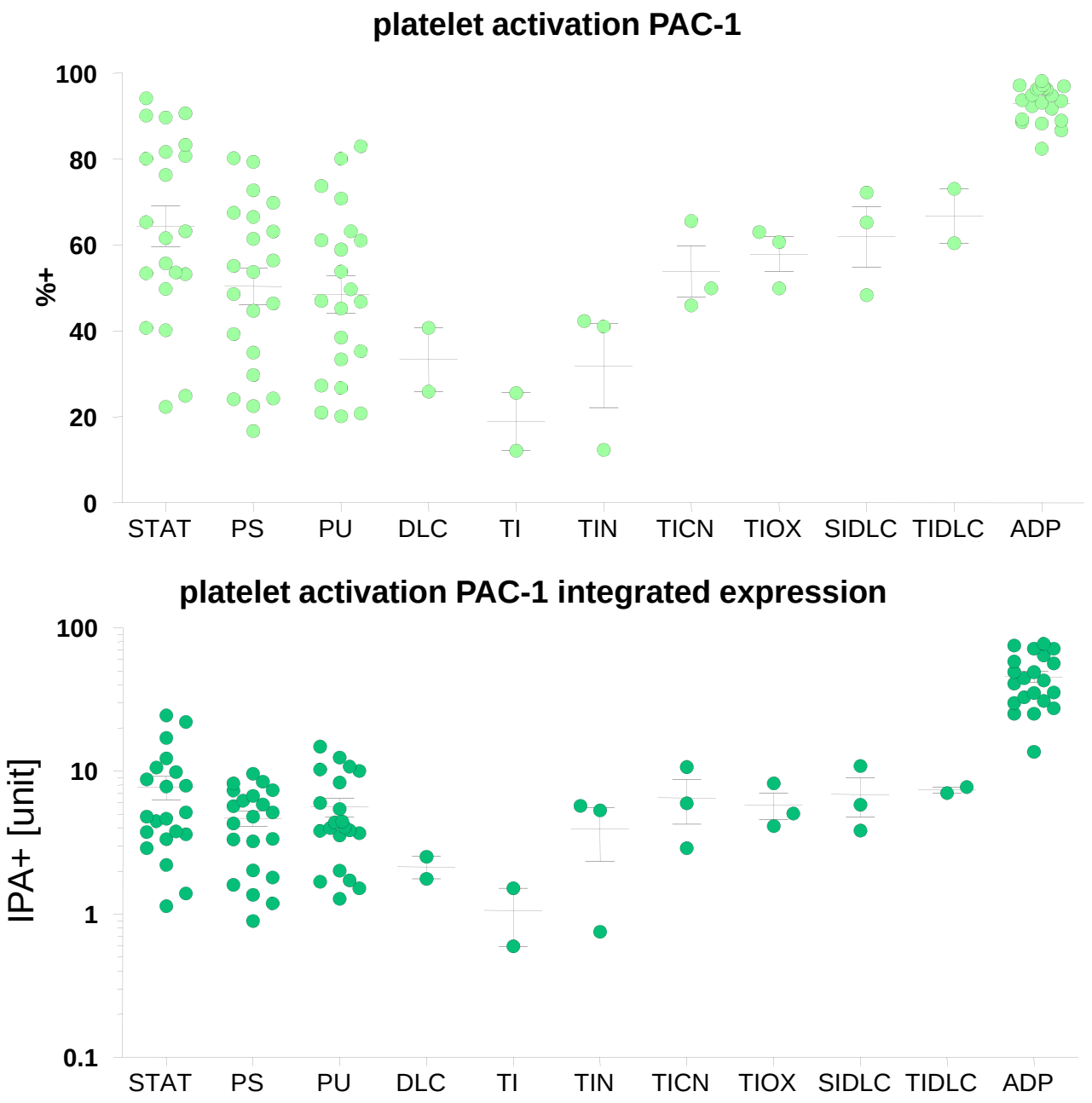
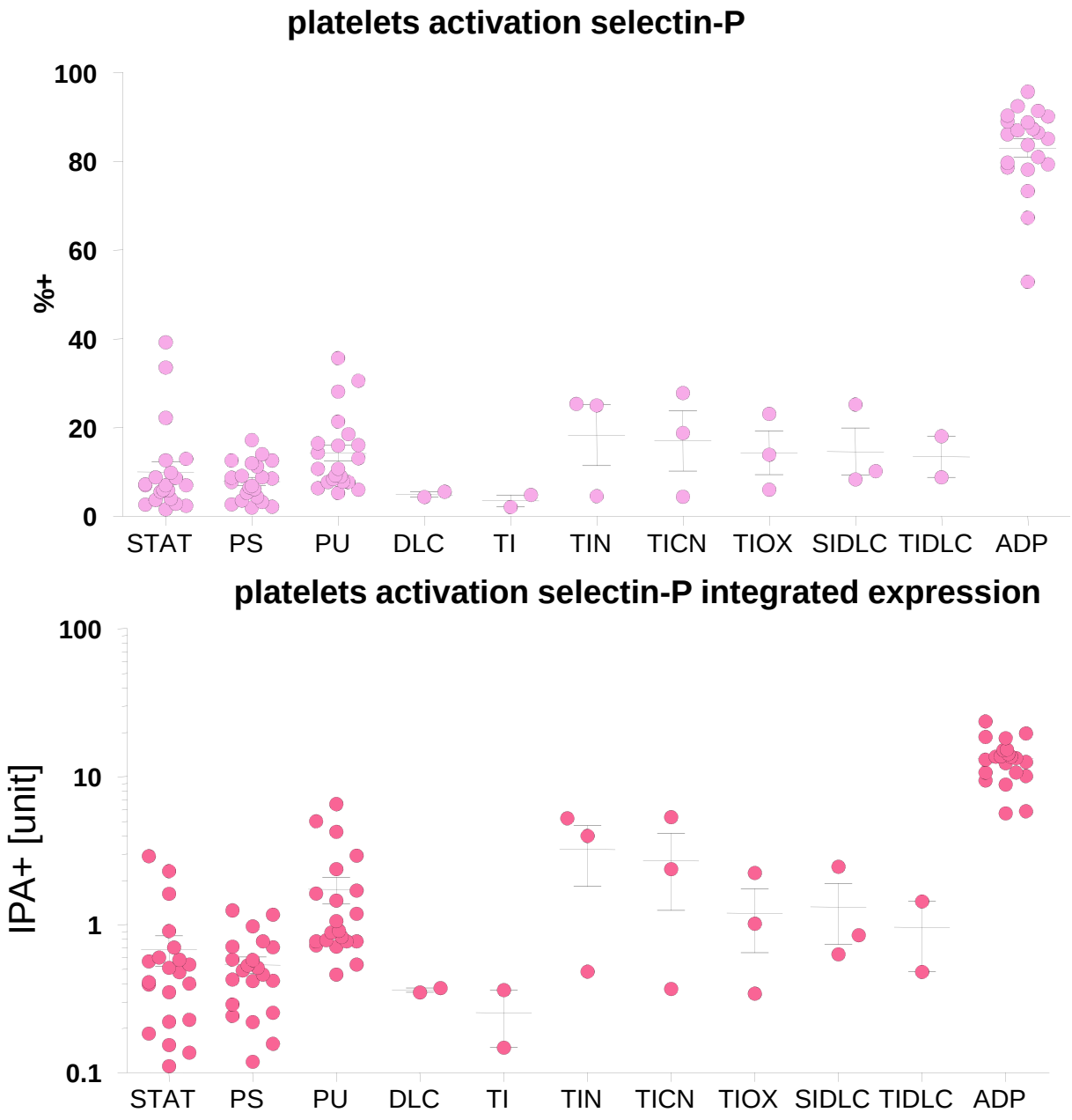
SIDLC
TIDLC
ADP

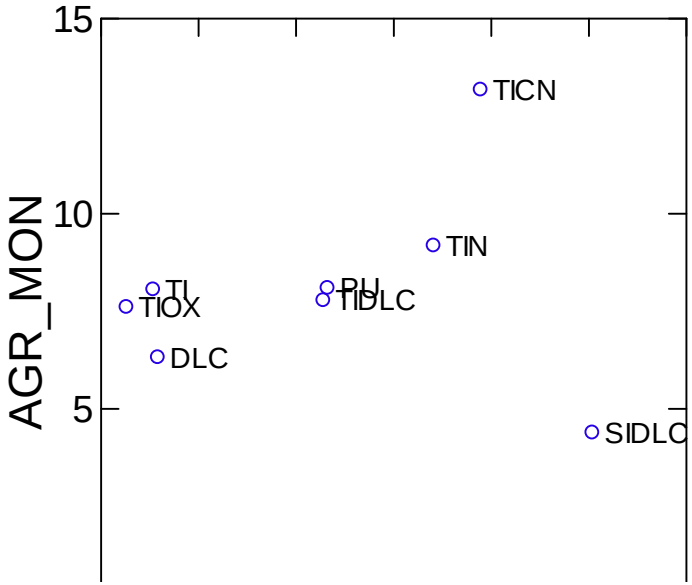

platelets vs platelets aggregates %
platelets
platelets aggregates
% CD61+ objects
110
100
90
80
70
60
50
40
30
20
10
STAT
PS
PU
DLC
TI
TIN
TICN
TIOX
SIDLC
TIDLC
ADP
platelets vs platelets aggregates in uL
platelets
platelets aggregates
objects in 1 uL x10^3
1000
100
10
1
STAT
PS
PU
DLC
TI
TIN
TNS
TIOX
SIDLC
TIDLC
ADP

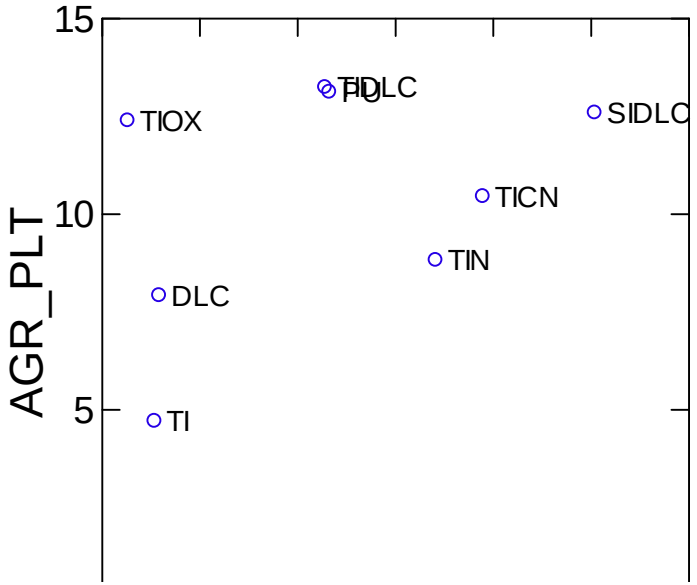
deposition of platelets on the surface affects selectively activated platelets



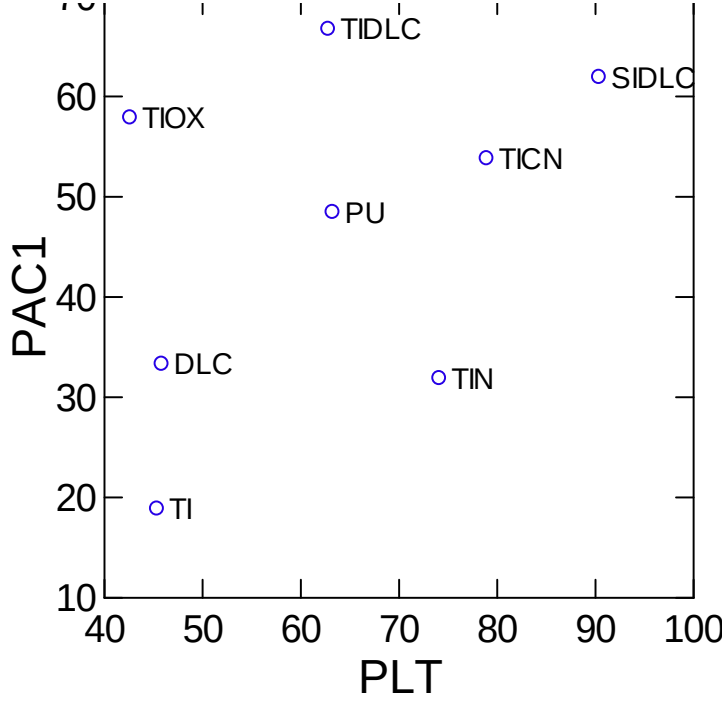
number of platelets activated in static conditions by ADP is very high

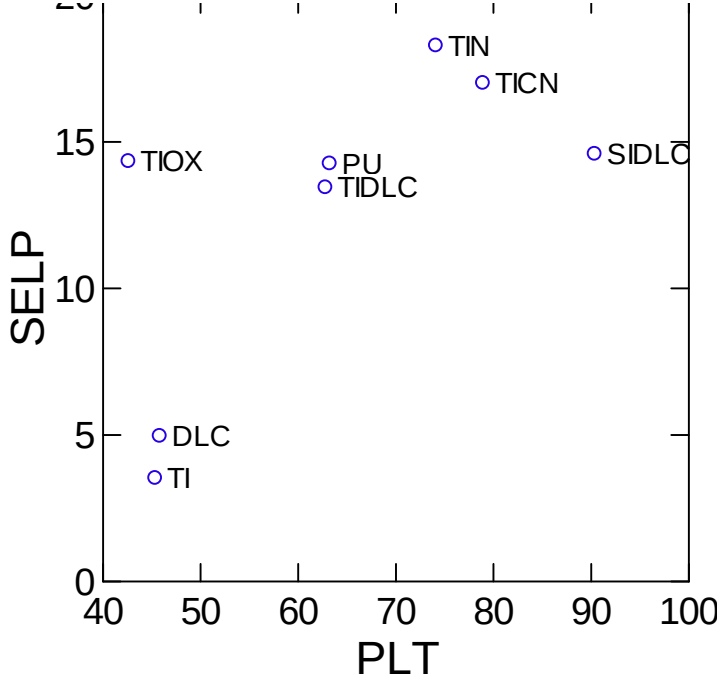






best hemocompatible biomaterials show limited platelets adhesion (PLT % remains high) and moderate activation/aggregation

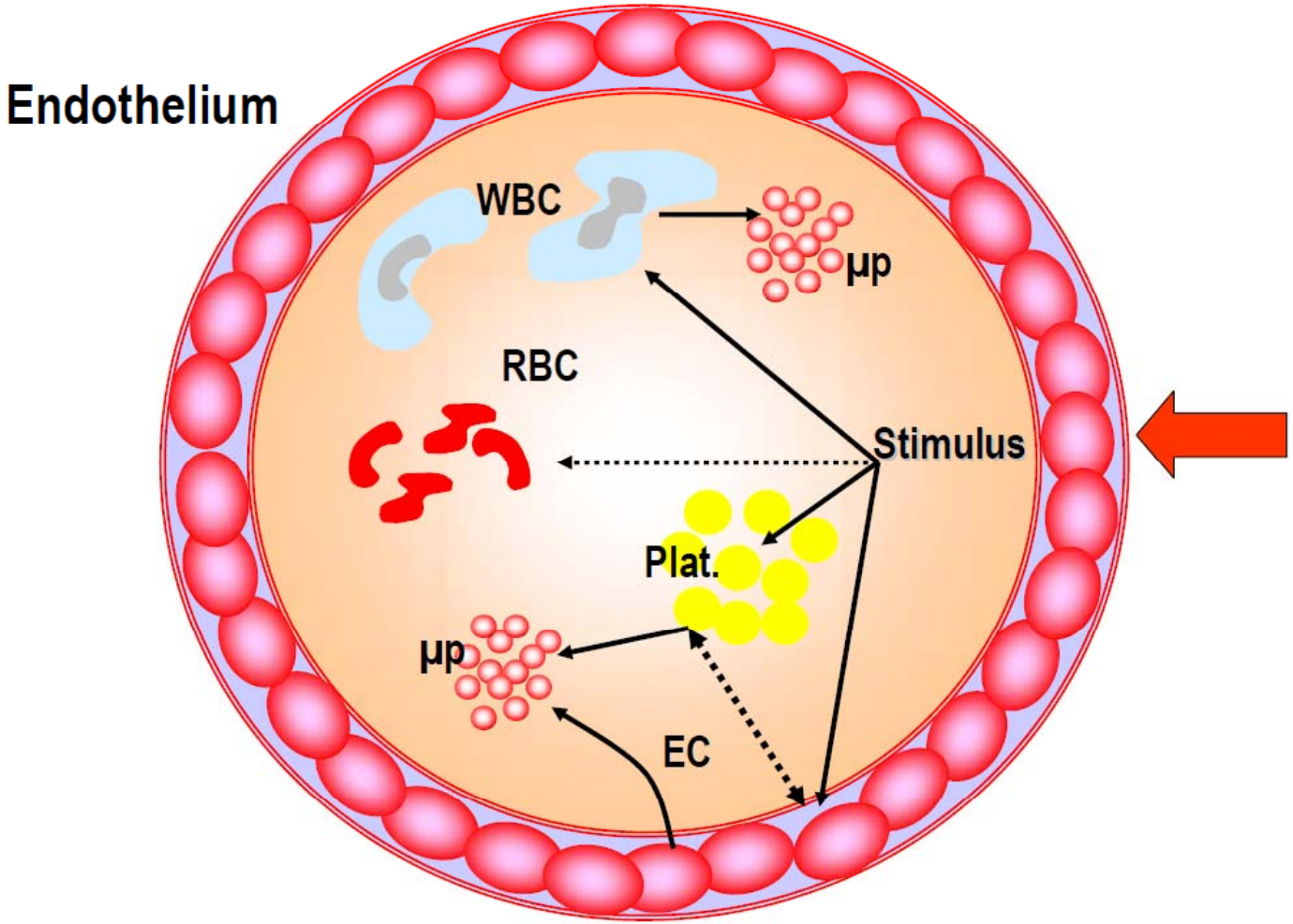




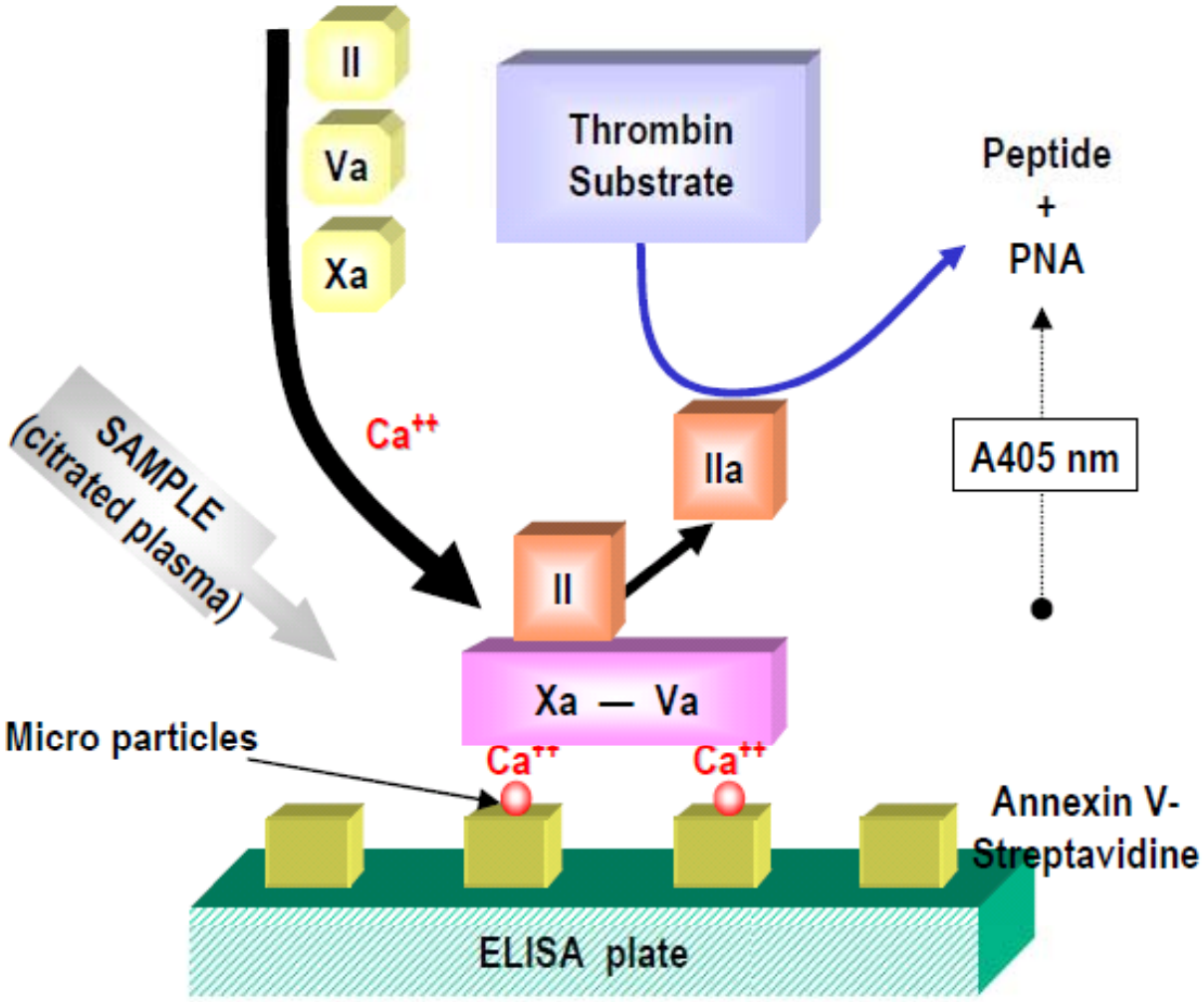
Concentration of platelet microparticles correlates with atheromathosis and risk for stroke or heart infarct

S. Nomura, Y. Ozaki and Y. Ikeda, „Function and role of microparticles in various clinical settings" *Thromb. Res.* 123 (1), 8-23 (2008).

A. Blann, E. Shantsila and A. Shantsila, "Microparticles and arterial disease", *Semin. Thromb. Hemost.* 35 (5), 488-496 (2009).



ZYMUPHEN MP-Activity – a method for measurement of thrombogenic activity of platelet microparticles



Limit of detection – 0.05 nM phosphatidylserine (PS)

Reference values ~ 5 nM PS

Abnormal values > 10 nM PS

Intrasassay CV – 3-8%

Interassay CV – 5-10%

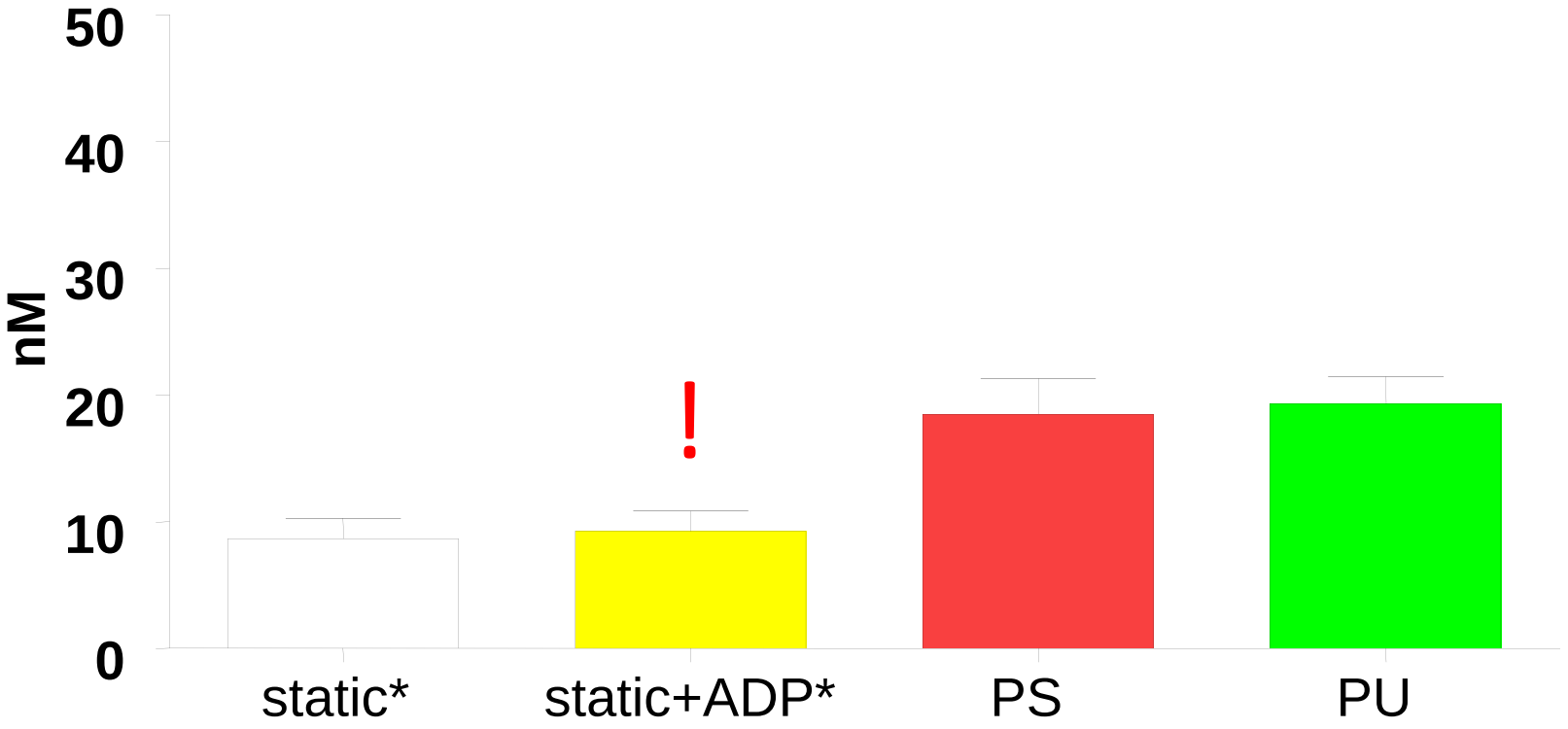
Under the arterial shear stress concentration of platelet microparticles increases threefold in average :

Static cond.:	8.6±7.45 nM
PS:	18.48±12.65 nM
PU:	19.32±9.61 nM

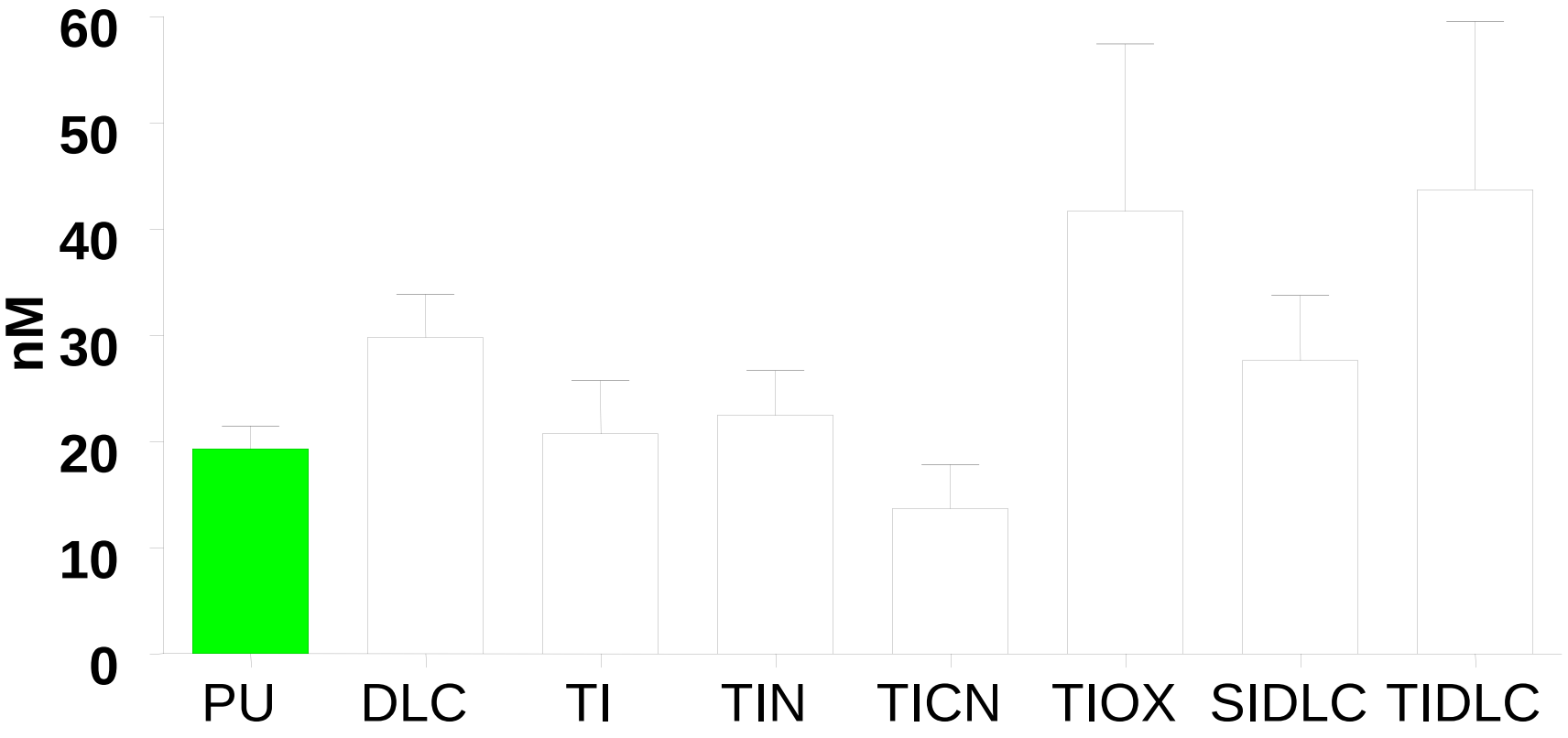
Platelet MP correlate with selectin-P expression following the shear stress

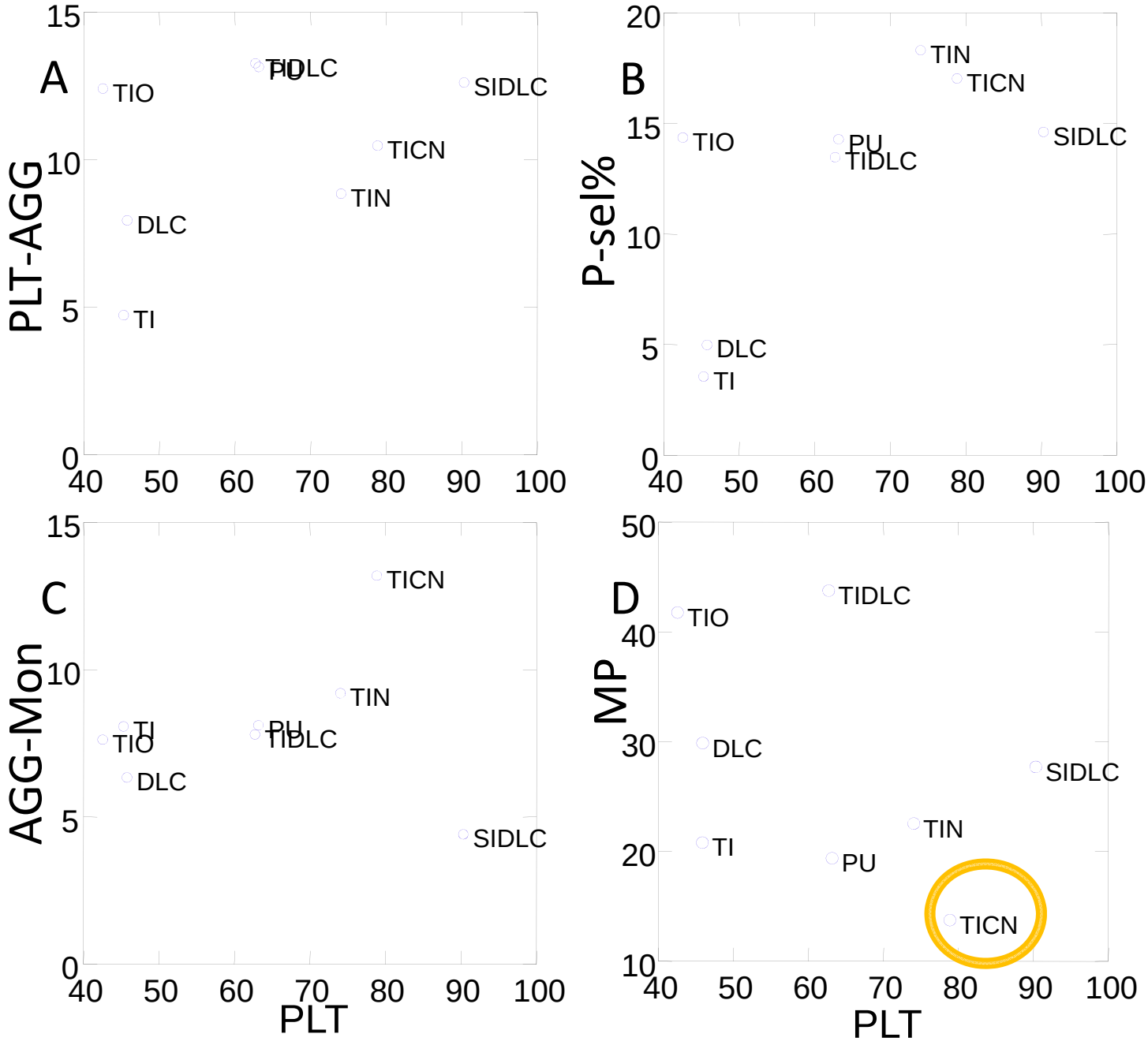
	% platelets	integrated expression
PS:	R^2=0.194; p<0.05	R^2=0.567; p<0.01
PU:	R^2=0.390; p=0.002	R^2=0.374; p=0.003

Platelet microparicles thrombogenic activity in the static conditions and following arterial shear stress using reference materials



Platelet microparicles thrombogenic activity following arterial shear stress




A
PLT-AGG
15
10
5
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90
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TIDLC
PU
SIDLC
TICN
TIN
DLC
TI
B
P-sel%
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TIO
PU
SIDLC
TIDLC
DLC
TI
C
AGG-Mon
TICN
TIN
TI
PU
TIO
TIDLC
DLC
SIDLC
PLT
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MP
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40
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TIDLC
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DLC
SIDLC
TIN
TI
PU
TICN
PLT

Conclusions

- a simple cone and platelet device is a useful analyser of arterial shear forcess interaction between whole blood and biomaterials

- the method coupled to the flow cytometry provides numerous parametrs of platelets activation and consumption

- level of platelet microparticles seems the best proxy linking in vitro models and clinical conditions