XXX Symposium on Bioinformatics
and
Computer-Aided Drug Discovery (BCADD-2024)

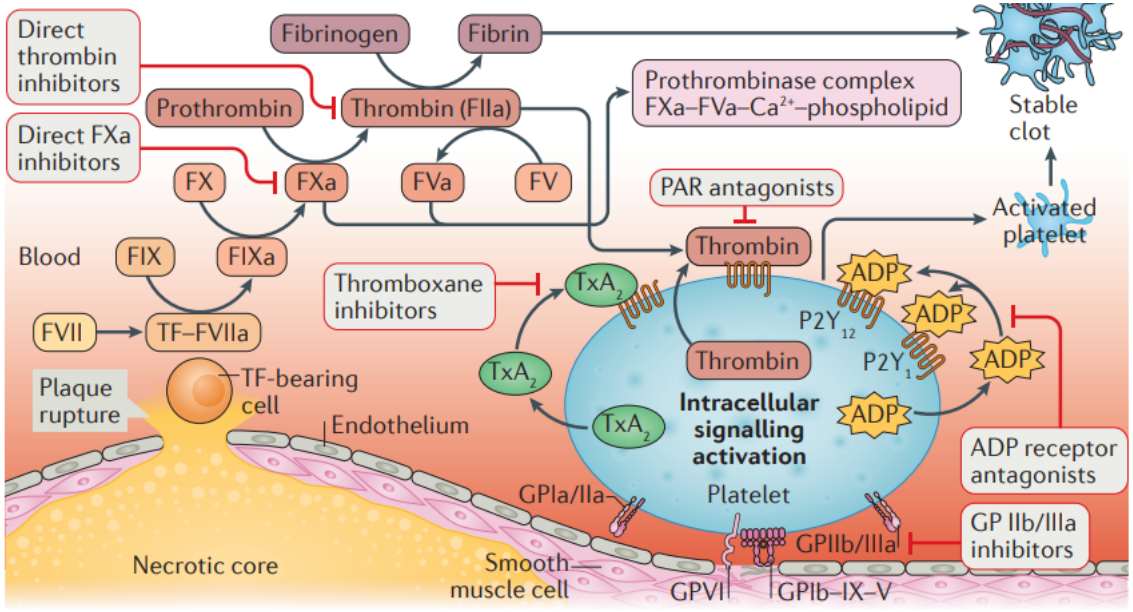
COMPREHENSIVE COMPUTATIONAL SYSTEMS BIOLOGY MODEL OF BLOOD PLATELET SIGNALLING: A TOOL FOR BASIC RESEARCH, DIAGNOSTICS AND PHARMACOLOGY

Anastasia Sveshnikova, Mikhail Panteleev

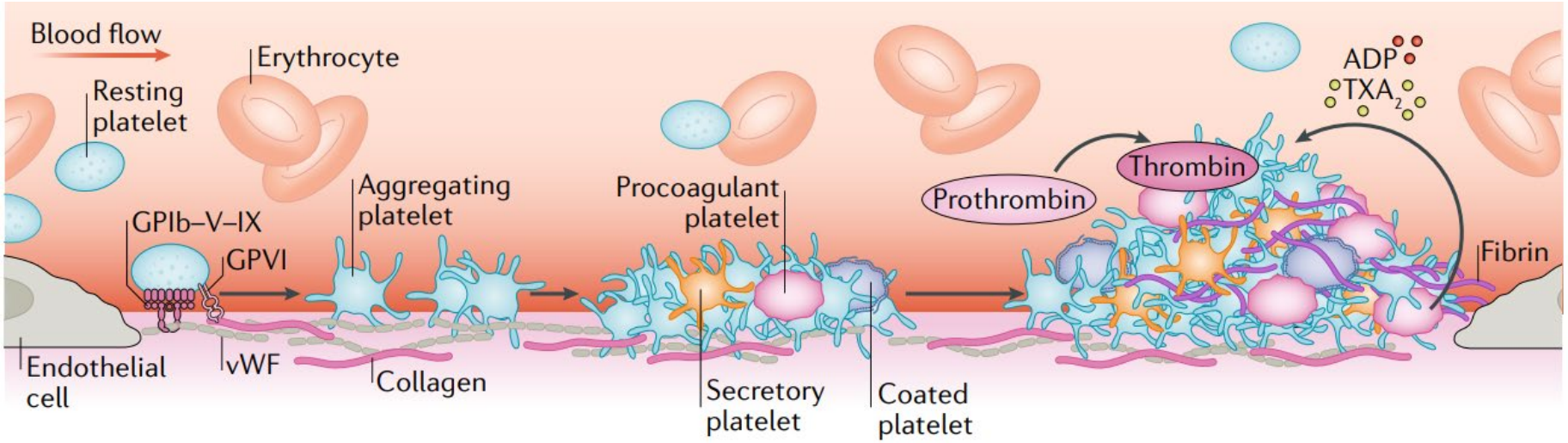
Moscow 2024

Blood coagulation and thrombus formation

During thrombus formation platelets have **multiple activators** and platelets perform **multiple functions**

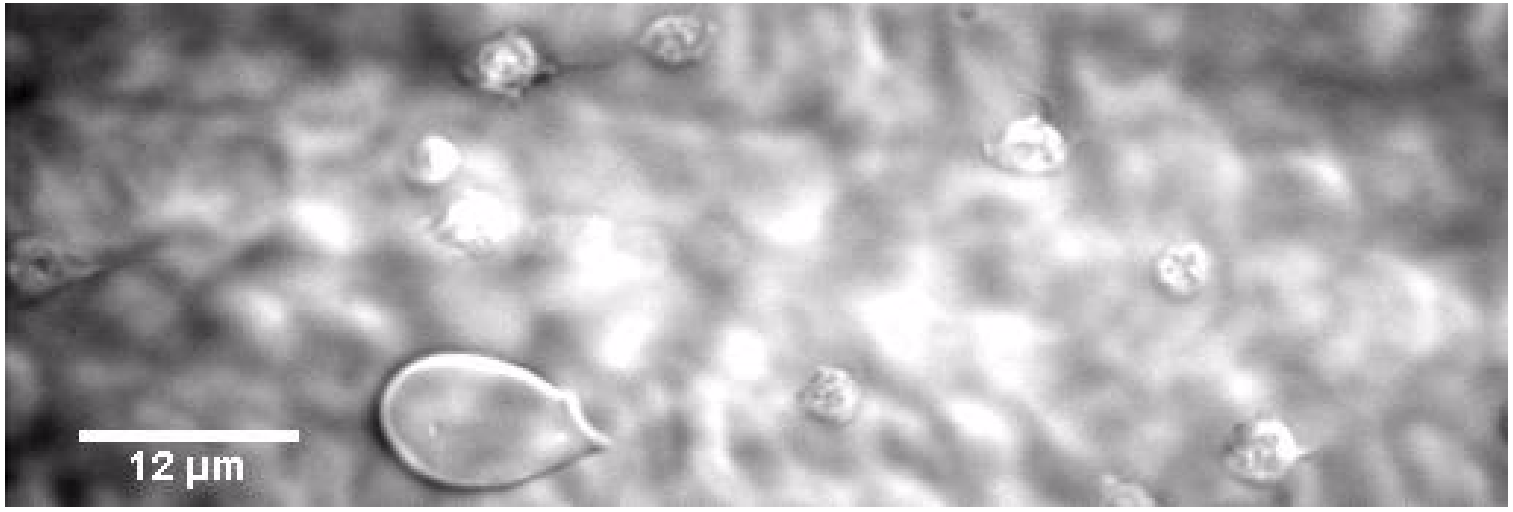


D.L. Bhatt Nature Reviews Cardiology 15:71–72(2018)



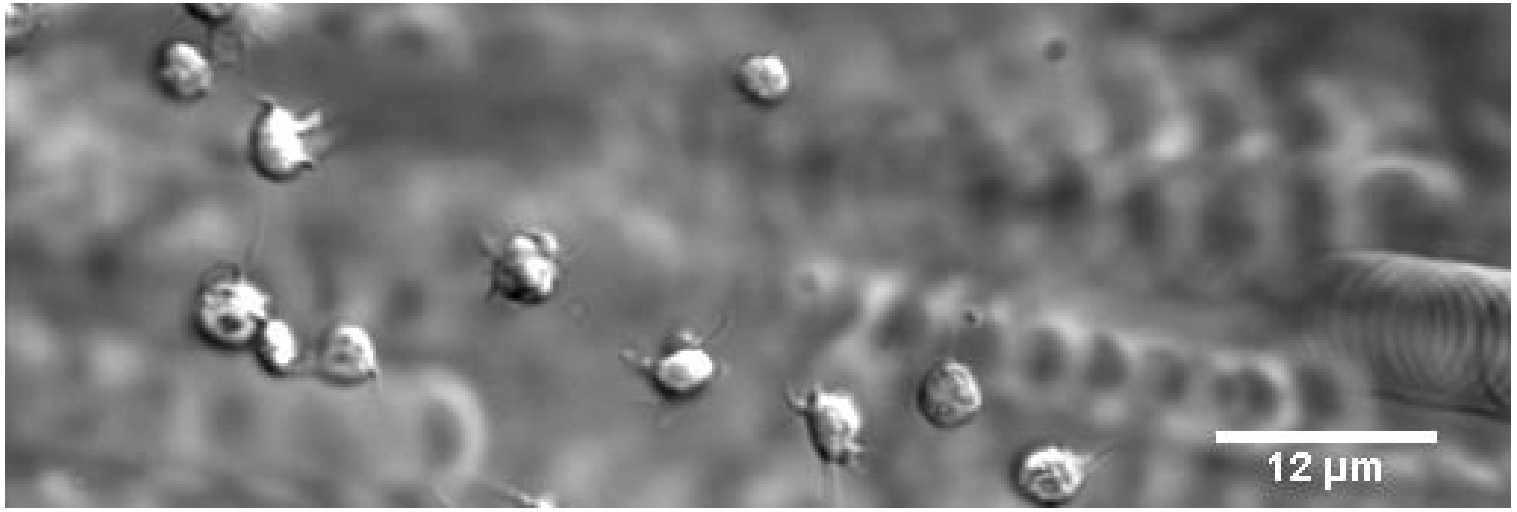
P.E.J. van der Meijden & J.W. M. Heemskerk Nature Reviews Cardiology 16:166–179(2019)

Platelet adhesion to collagen



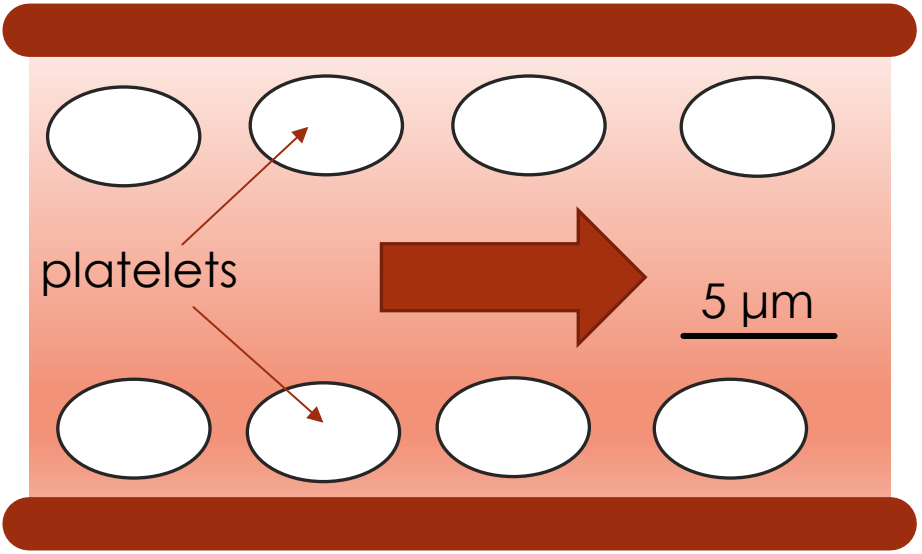
12x speed

Platelet activation on collagen

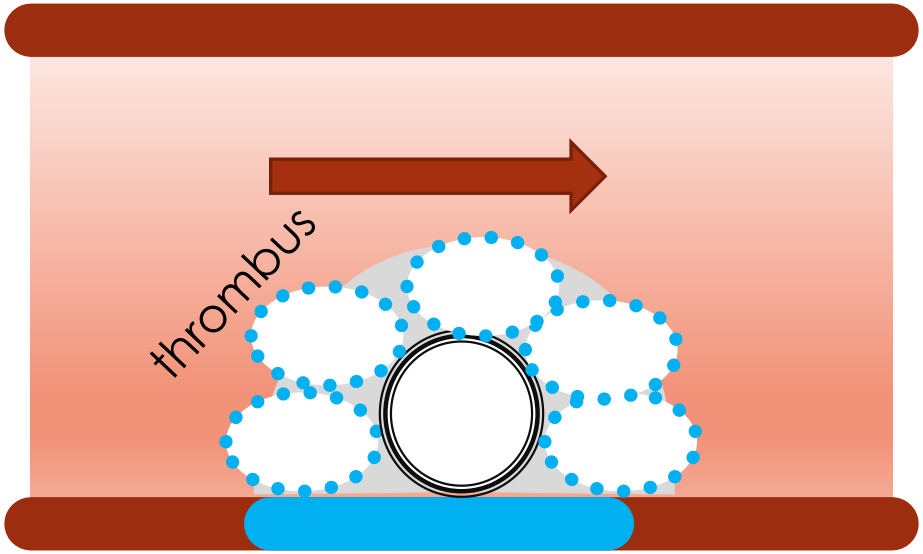


24x speed

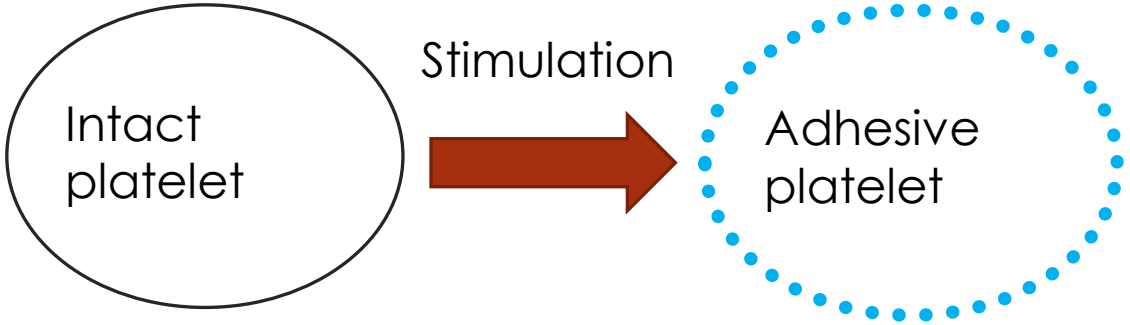
Aims: to decipher platelet 'coding' and 'decoding' mechanisms for **integrin activation** (pro-aggregation response) and **phosphatidylserine exposure** (procoagulant response)



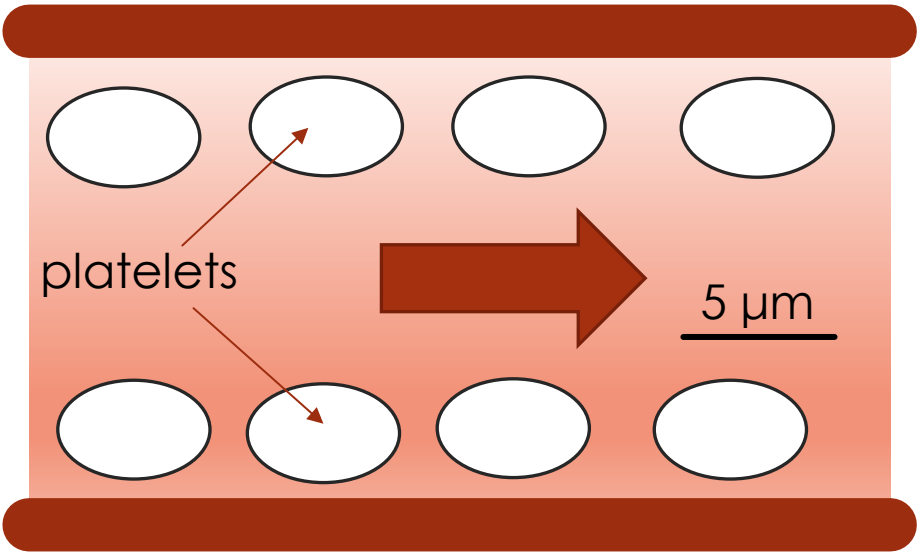
Healthy blood vessel



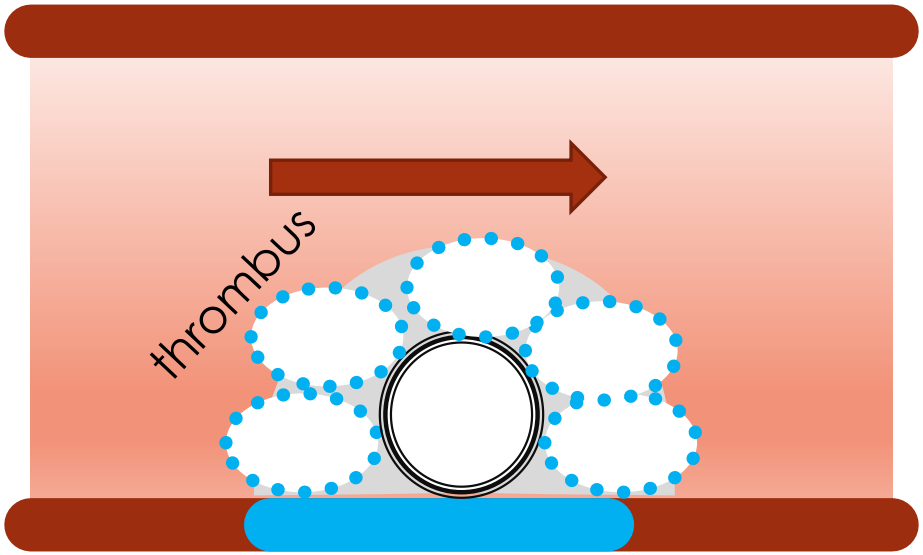
Vessel injury



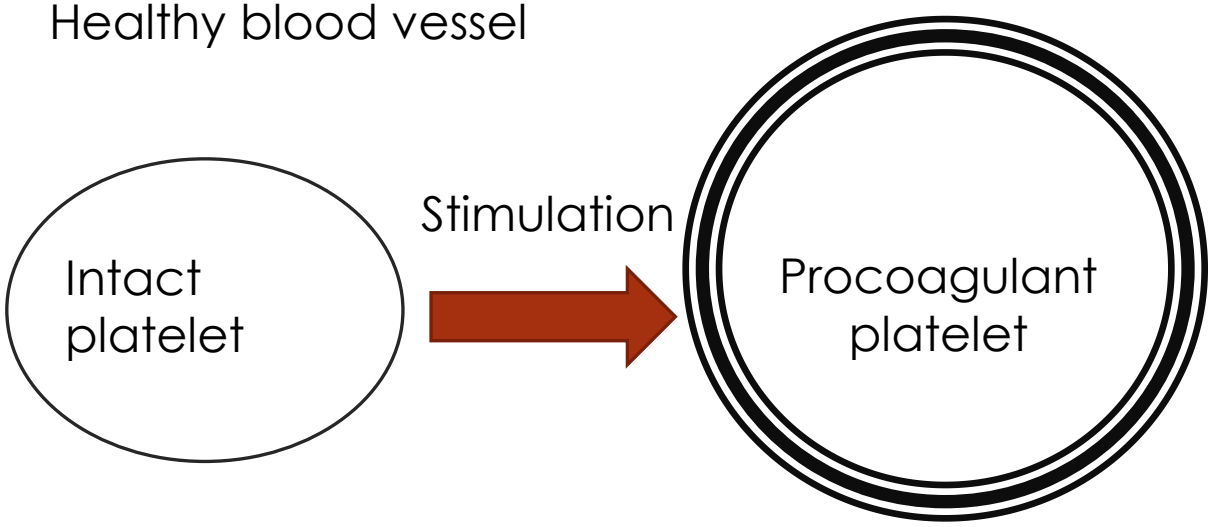
Aims: to decipher platelet 'coding' and 'decoding' mechanisms for **integrin activation** (pro-aggregation response) and **phosphatidylserine exposure** (procoagulant response)

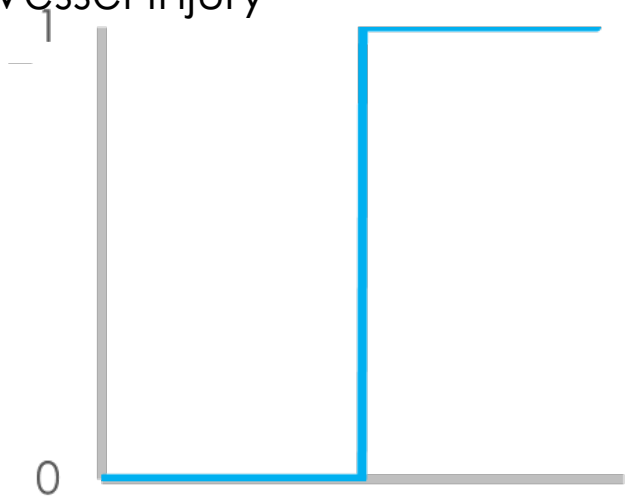


Healthy blood vessel

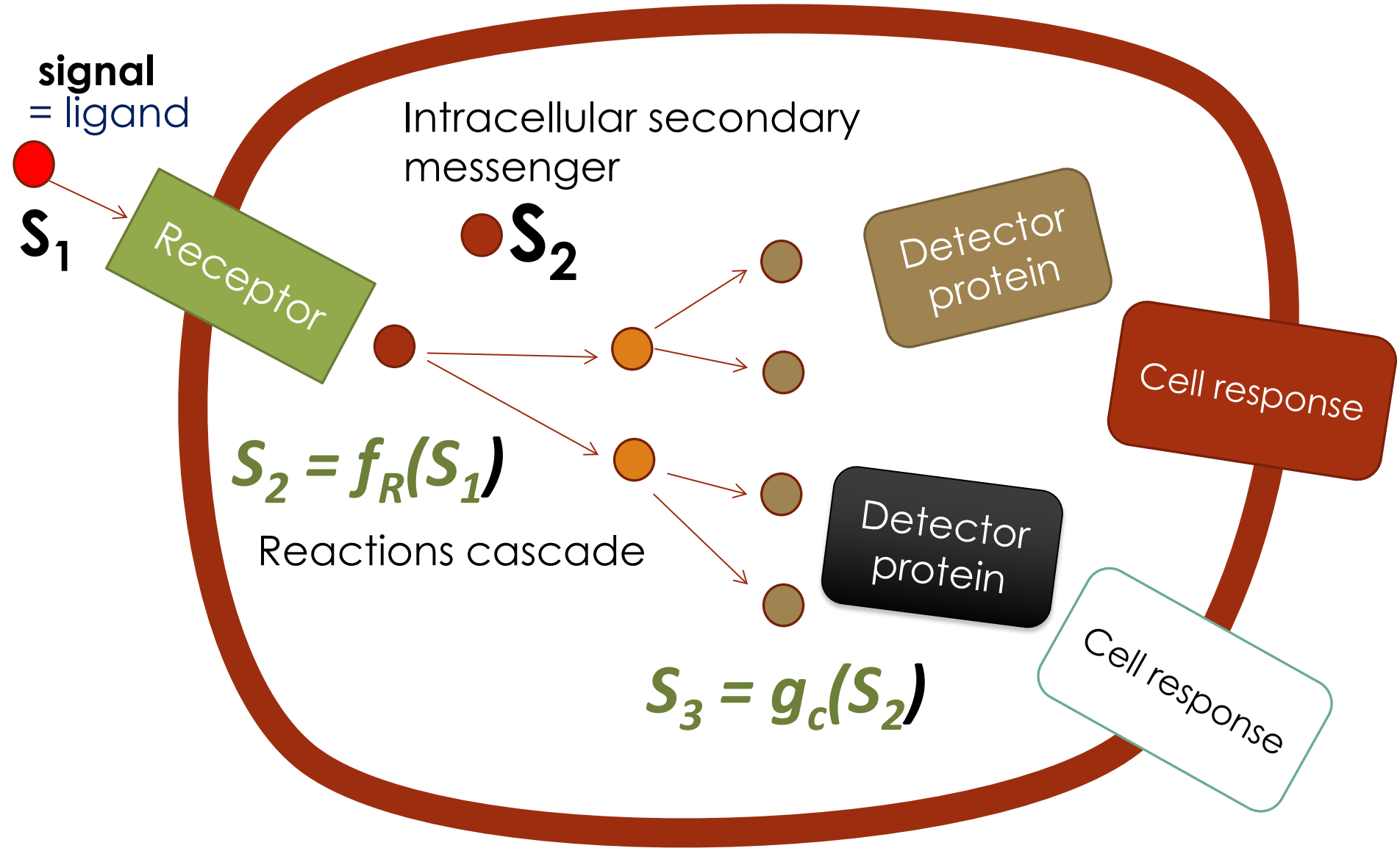


Vessel injury

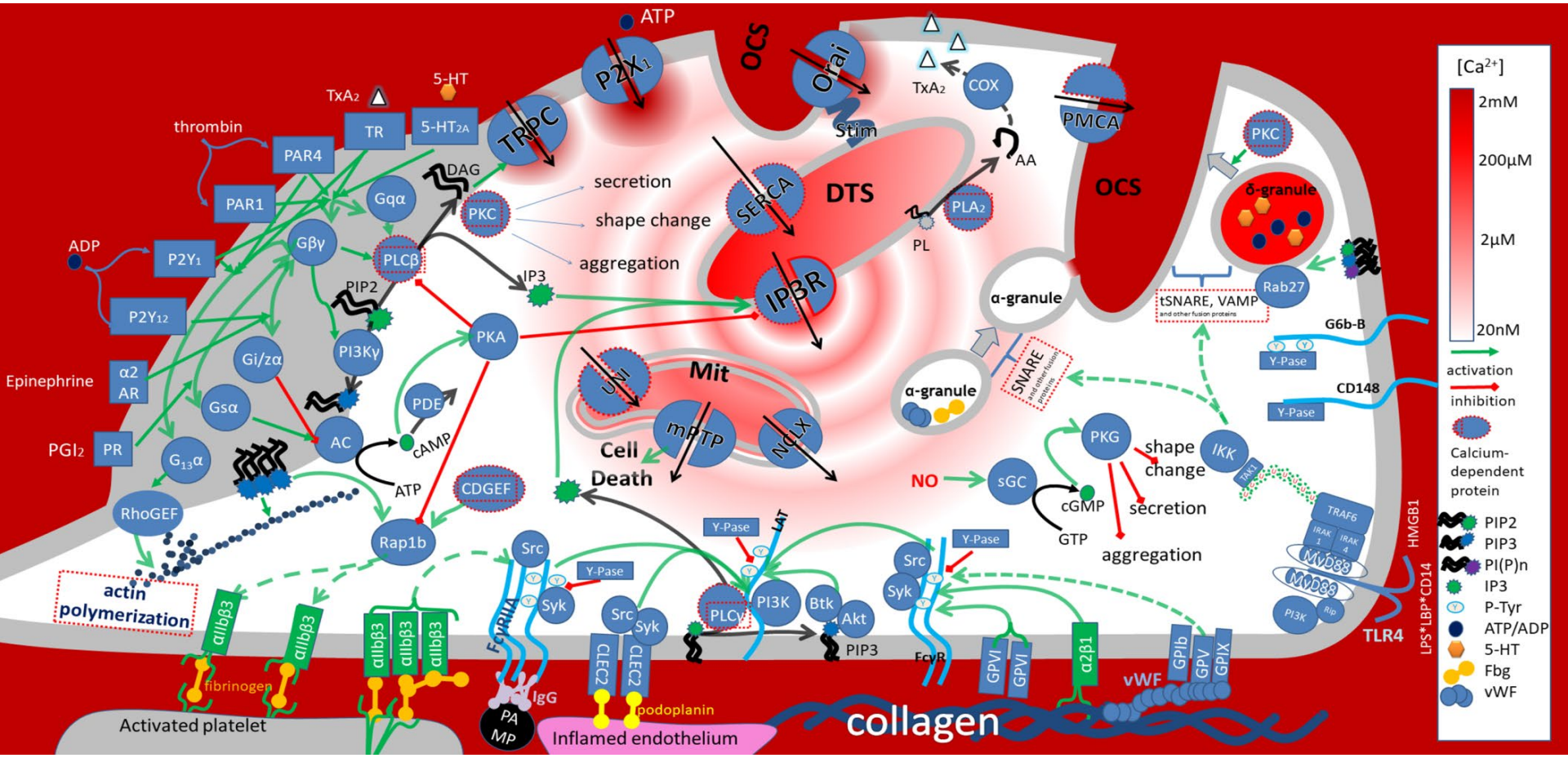




Principles of signal transduction

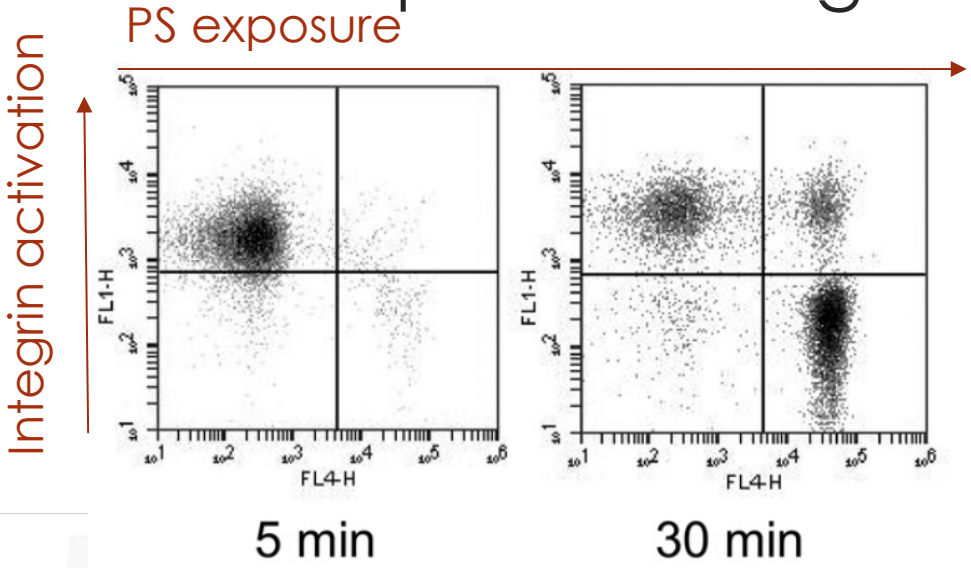


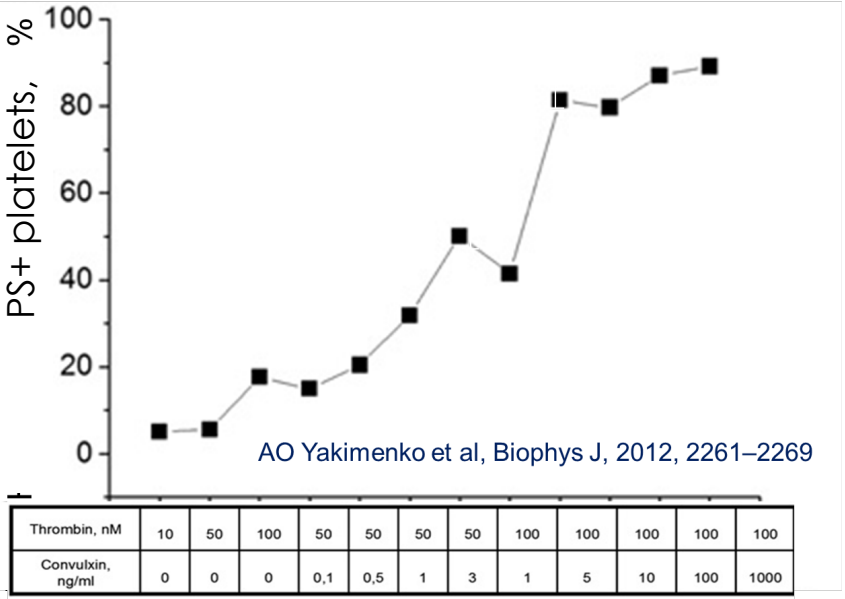
Scheme of platelet intracellular signalling



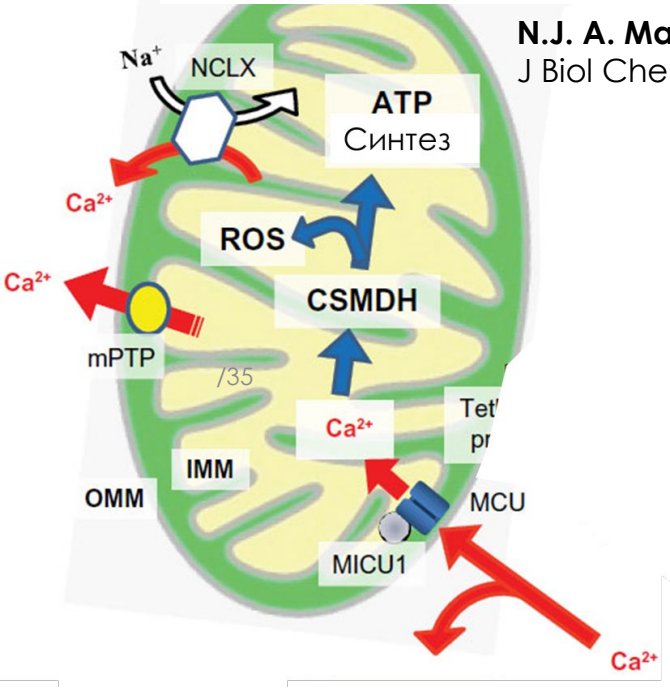
Biomedical Chemistry, 60(2):182–200, 2014; BBA - General Subjects, 1850(12):2518–2529, 2015; Molec. BioSyst, 11:1052–1060, 2015; J Thromb Haemost, 14(10):2045–2057, 2016; Math Mod Nat Phenom, 11(6):91–101, 2016; Math Biosci, 276:67–74, 2016; ; J Thromb Haemost, 14(9):1867–1881, 2016; Биофизика, 63(3):475–483, 2018; Биол. мембраны, 35(4):1–13, 2018; ВМУ Серия 3, 2018(5):63–70, 2018; Онкогематология, 13(3):83–90, 2018; Meth Mol Biol, 1661:255–279, 2018; Биол. мембраны, 36(1):15–31, 2019; УФН, 189(07):703–719, 2019; Биохимия, 85(10):1267–1276, 2020; Int J Mol Sci, 21(9):3035, 2020; Biophys J, 118(11):2641–2655, 2020; Sci Reports, 10(1):12296, 2020; Биол. мембраны, 37(6):442–452, 2020; Cells, 10(3):584–584, 2021; SBP Reports, 1(1):4, 1(3):3, 2021

Example: Procoagulant response





Thrombin, nM	10	50	100	50	50	50	50	100	100	100	100	100
Convulxin, ng/ml	0	0	0	0,1	0,5	1	3	1	5	10	100	1000

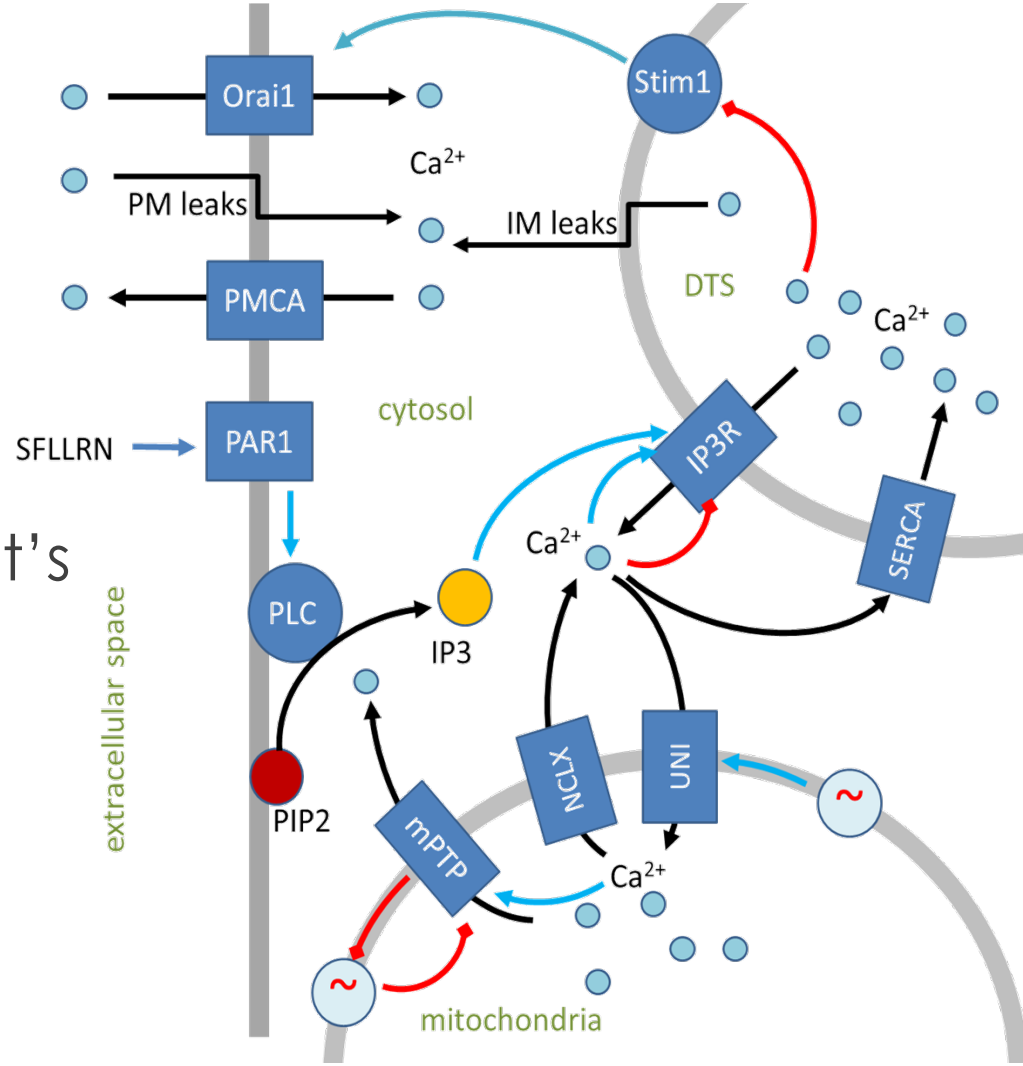


N.J. A. Mattheij et al.
J Biol Chem 288(19):13325–13336, 201

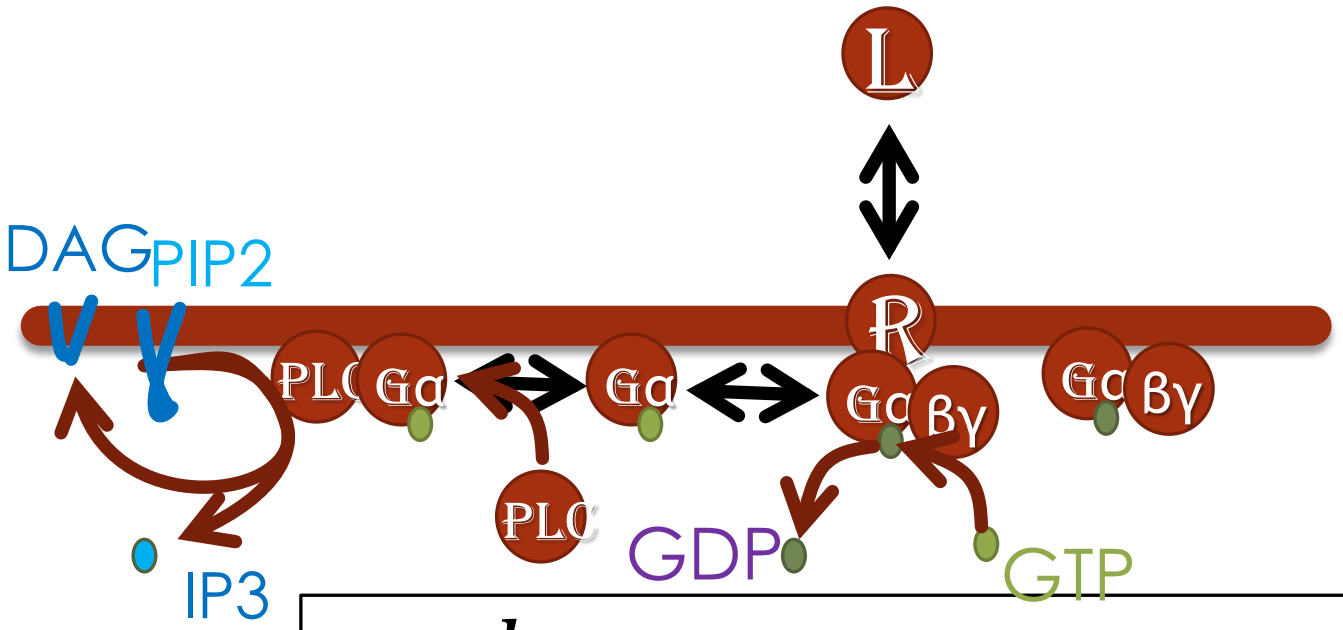
Hypothesis: mitochondria calcium overload leads to platelet cell death

Model construction in modular fashion

- Stimulus -> IP3
- IP3 -> Cytosolic calcium
- Cytosolic calcium –> Mitochondria collapse
- Plasma membrane channels support platelet's steady state in the absence of activation



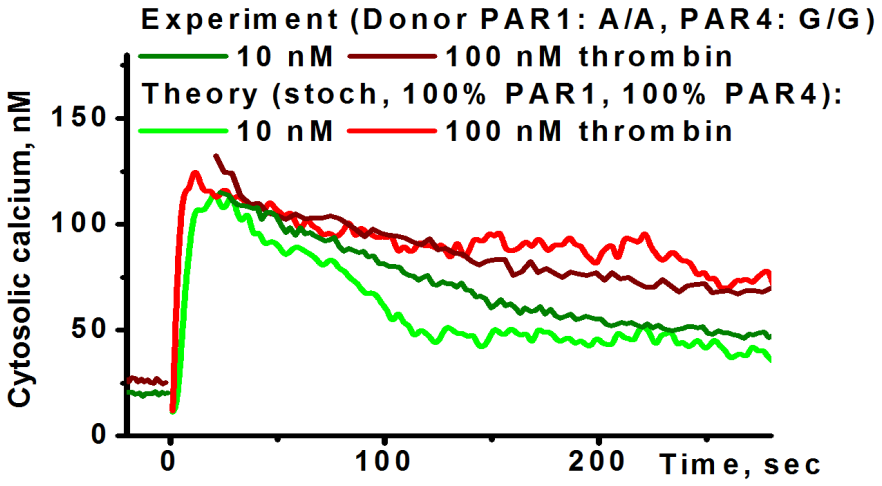
Mechanistic model for each module

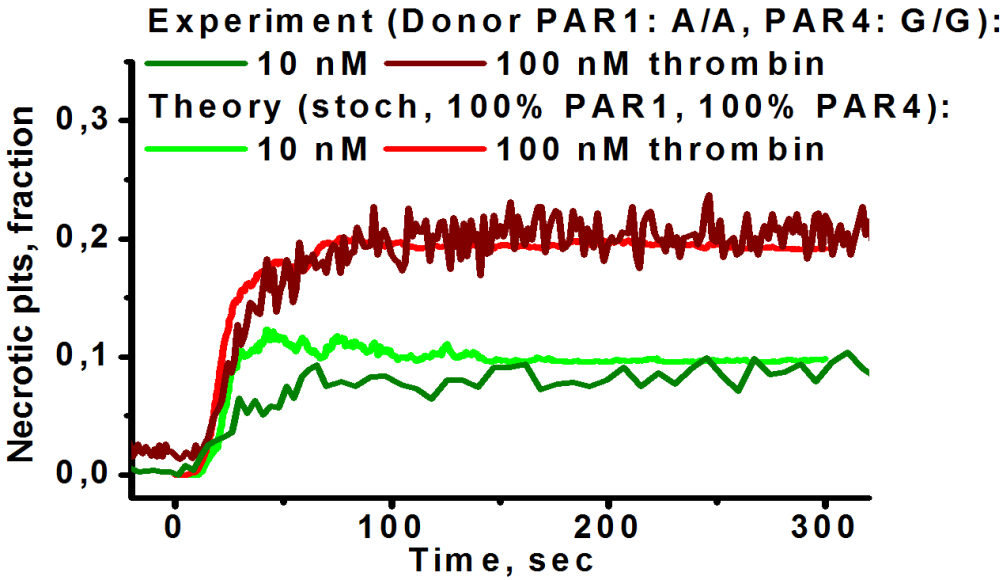


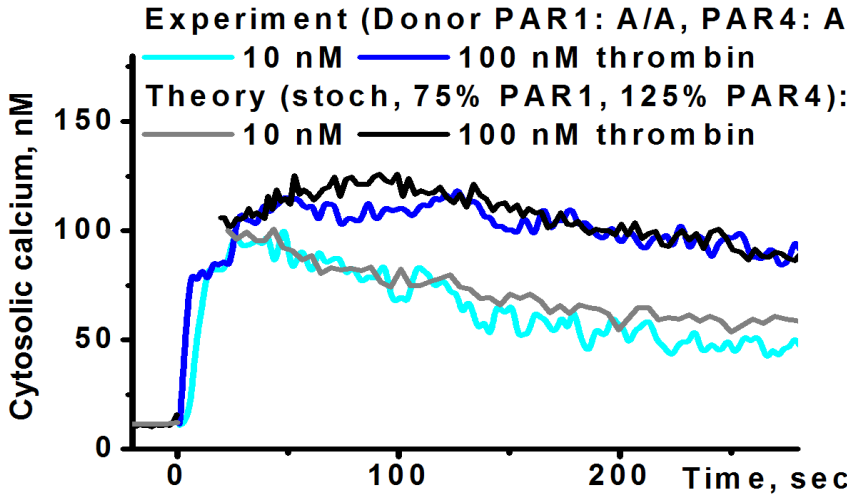
$$\frac{d}{dt}[R] = -k_1[R][L] + k_{m1}[RL]$$

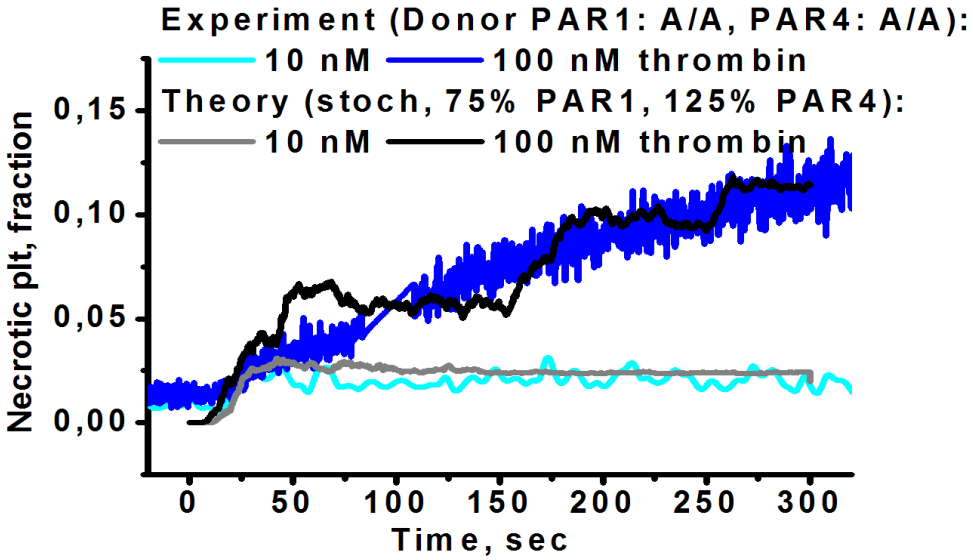
Reaction	Flux, compartment	Parameters
$PAR1 + \mathbf{SFLLRN} \leftrightarrow PAR1^*$	$k[PAR1][\mathrm{SF}] - k_m[PAR1^*]$, PM	$k = 0.03(nM \cdot s)^{-1}$ [Lenoci 2011], $k_m = 0.001s^{-1}$[Lenoci 2011], $[\mathrm{PAR1}]_0$=11 mol./um^2 [Zahedi 2013]
$PAR1^* \rightarrow$	$k[PAR1^*]$, PM	$k = 20s^{-1}$ (tuning)

Model validation



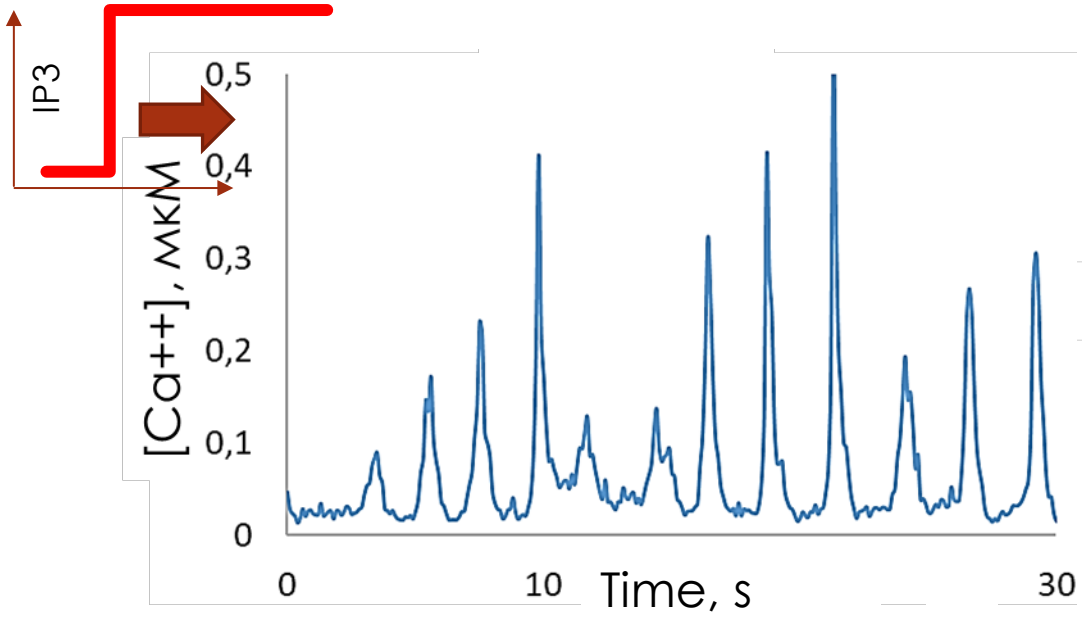


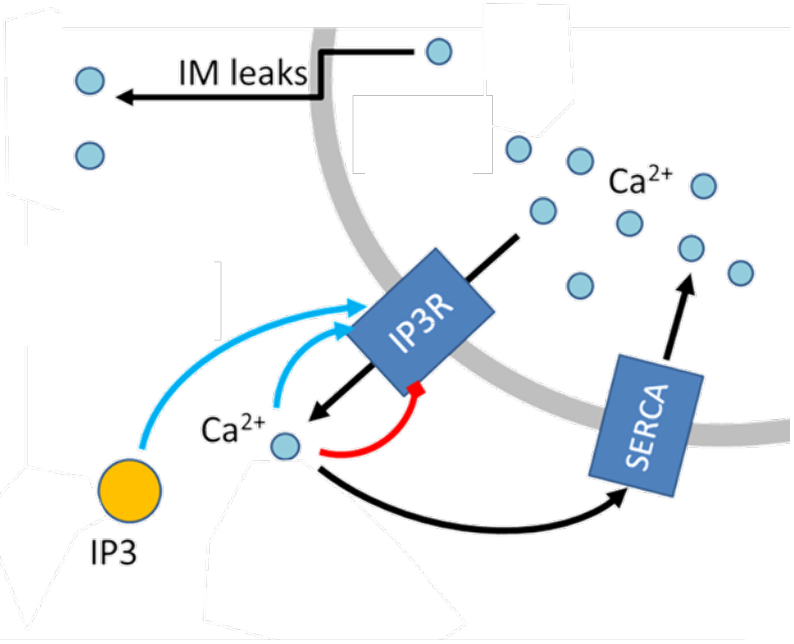


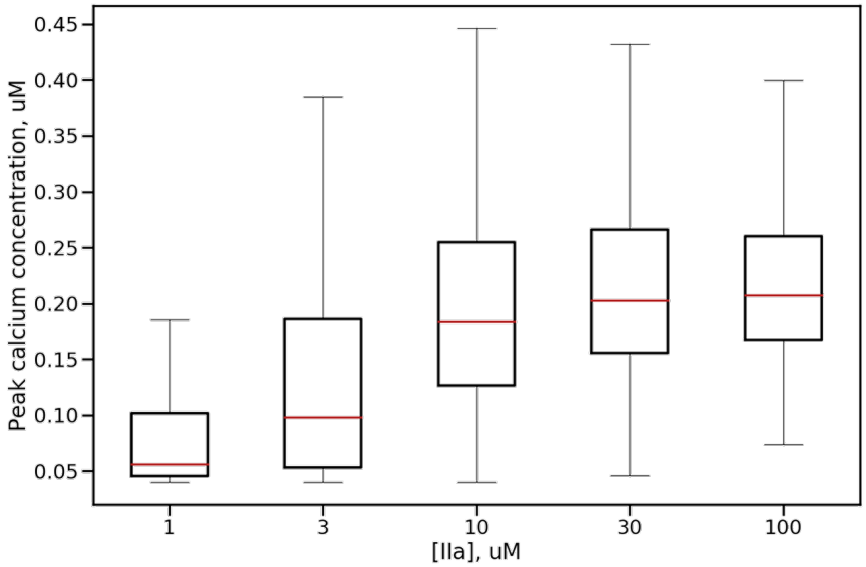


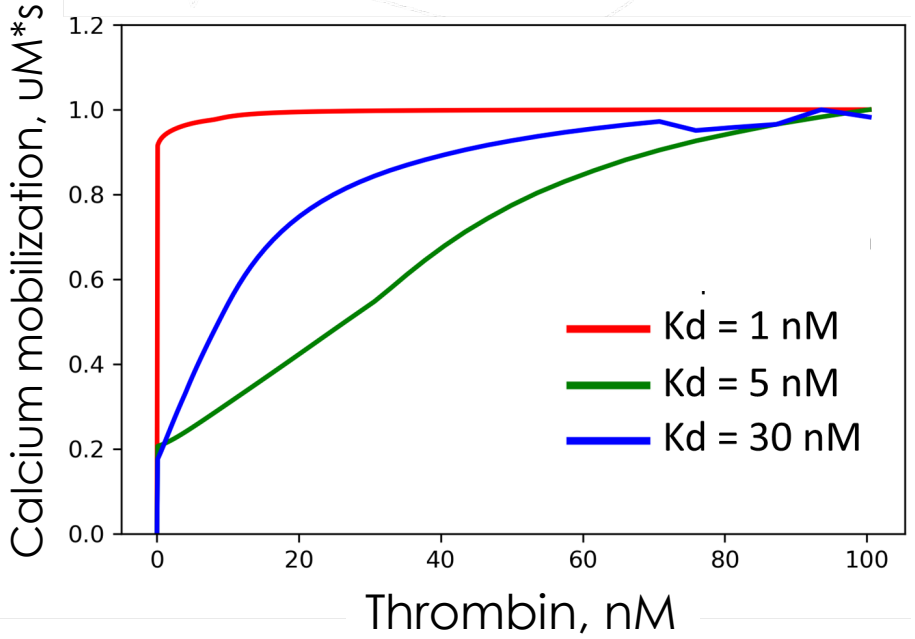
J Thromb Haemost, 2016

‘Coding’ at the level of calcium signaling

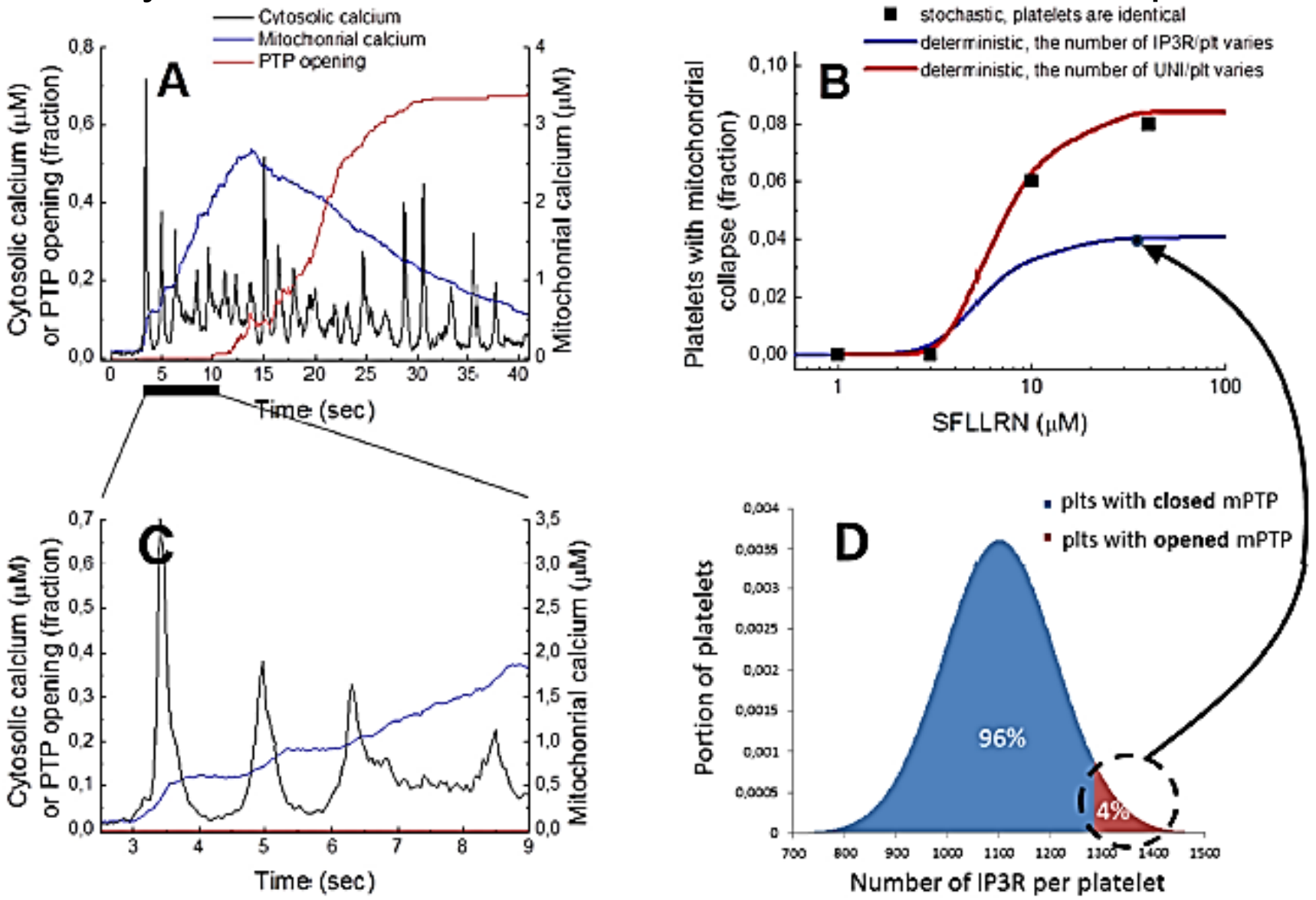








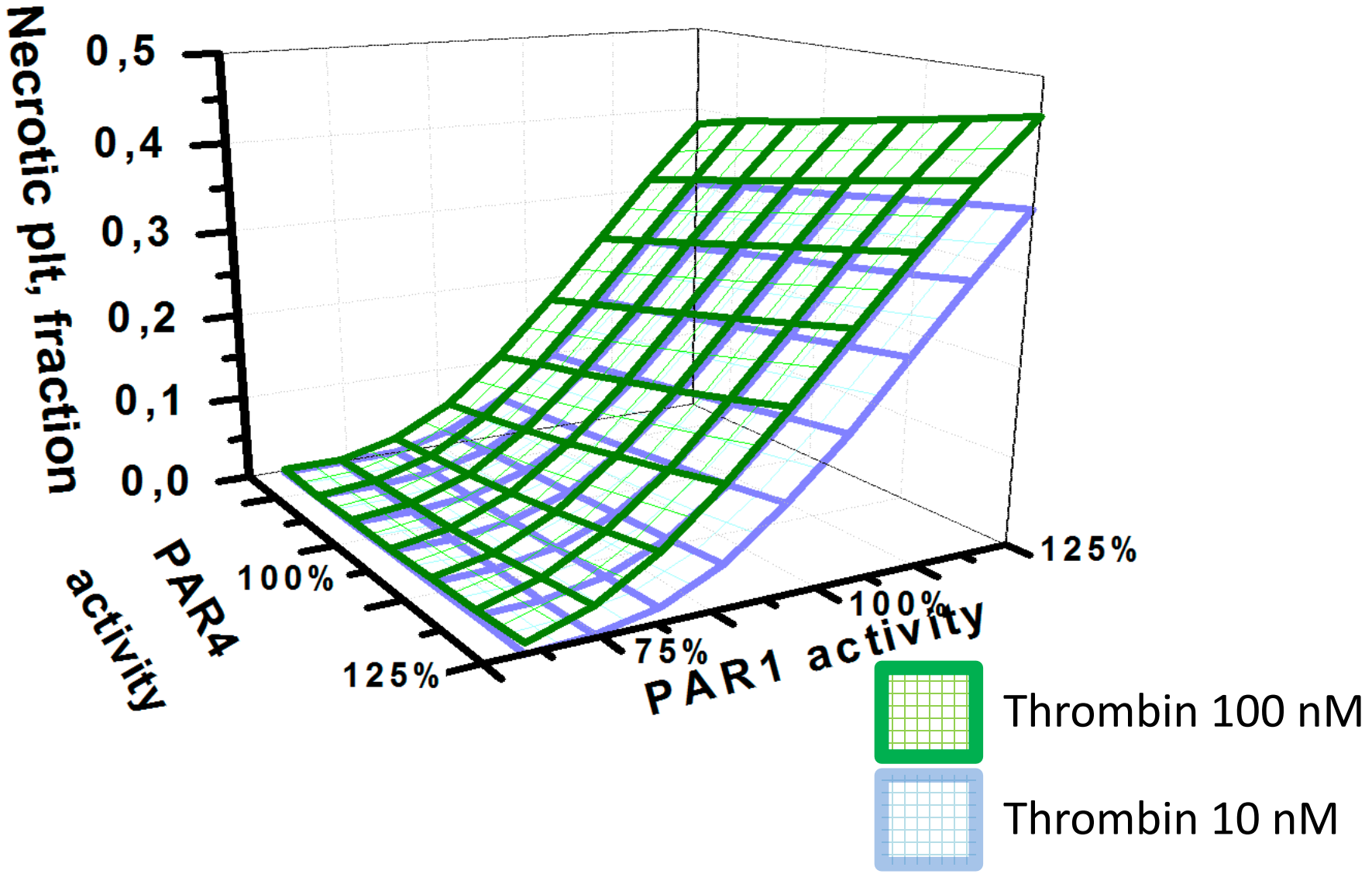
Stochastic calcium spiking in the model leads to accumulation of cytosolic calcium in mitochondria and their collapse



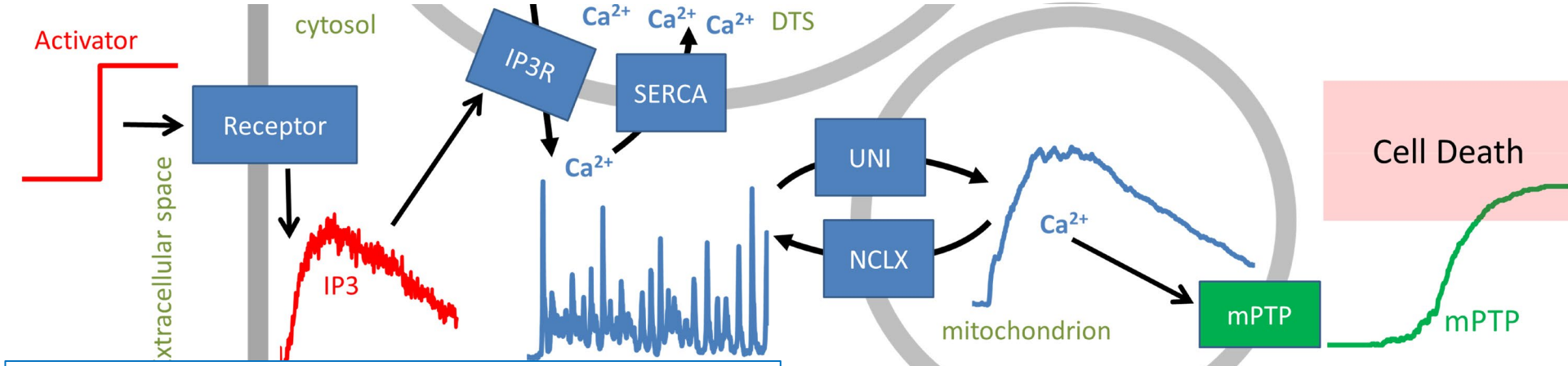
Sveshnikova et al. Mol Biosyst 2015

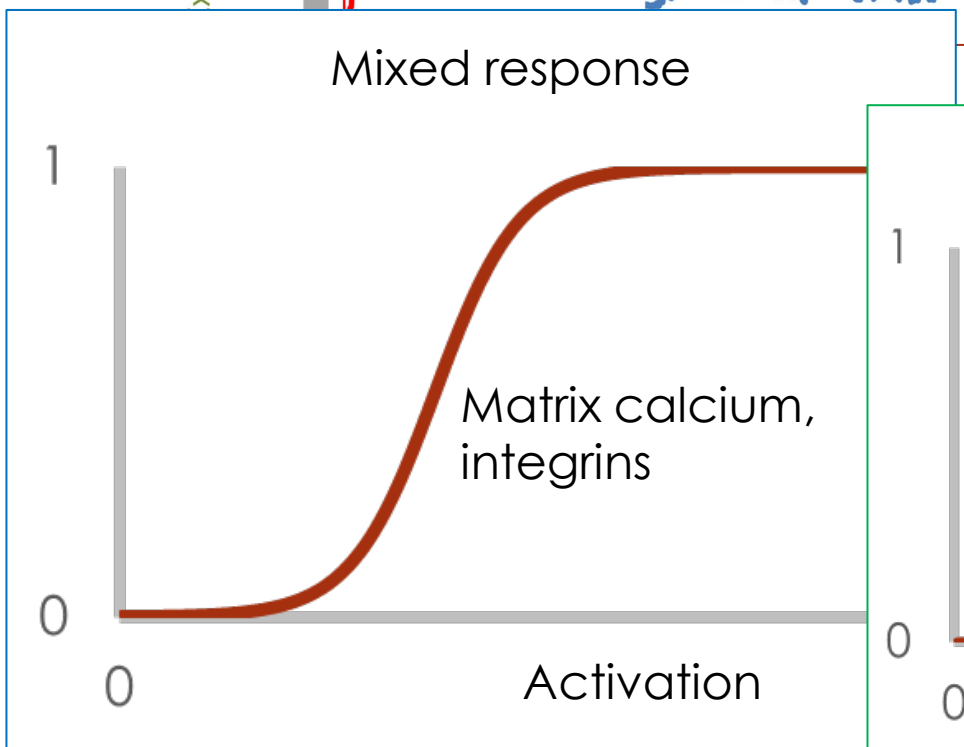
The dual receptor combination is critical to produce pronounced procoagulant respons

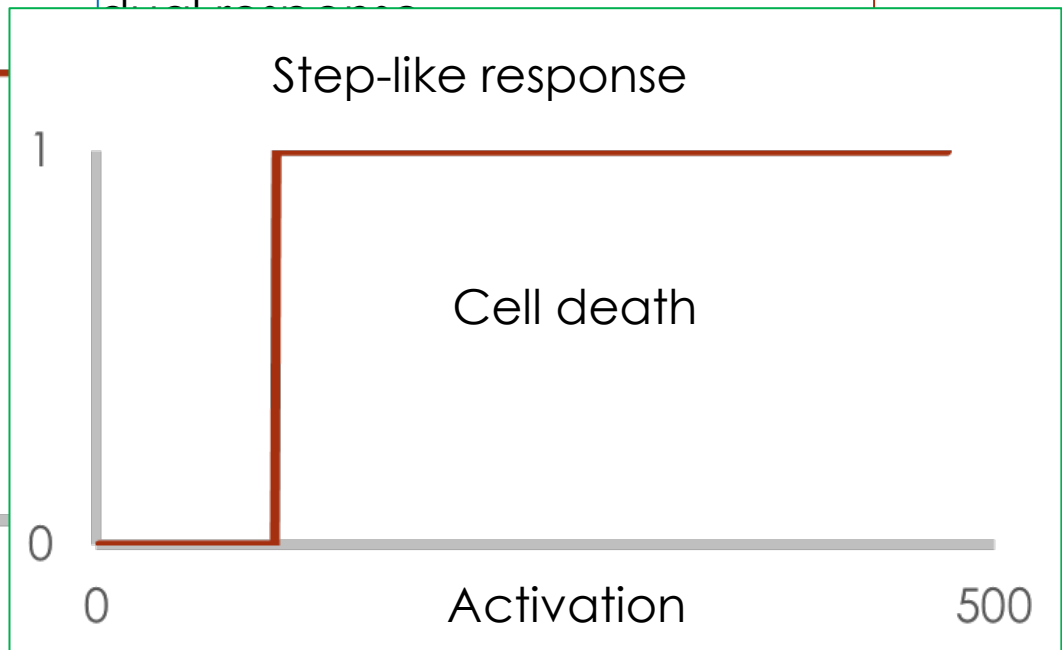
- **PAR1 activity determine the level of the procoagulant response**
- **PAR4 activity determine the dynamic s of procoagulant response**



Regulation mechanisms of platelet responses





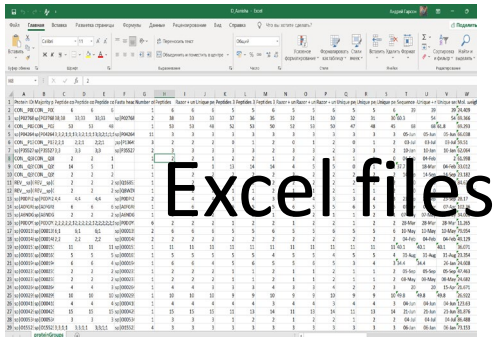


Interim conclusion

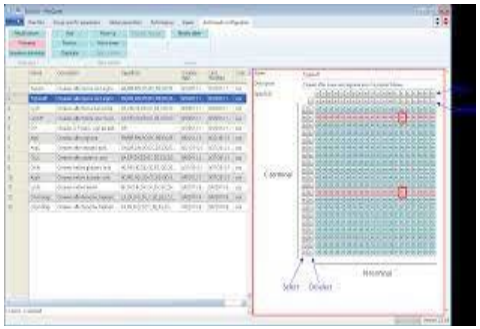
Personalization could be achieved by assessing the number of proteins per platelet

Workflow

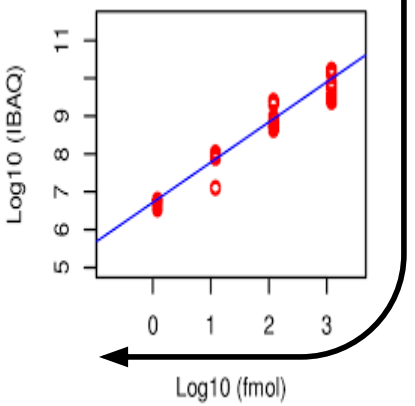
Raw LC-MS/MS files → MaxQuant Software



Excel files



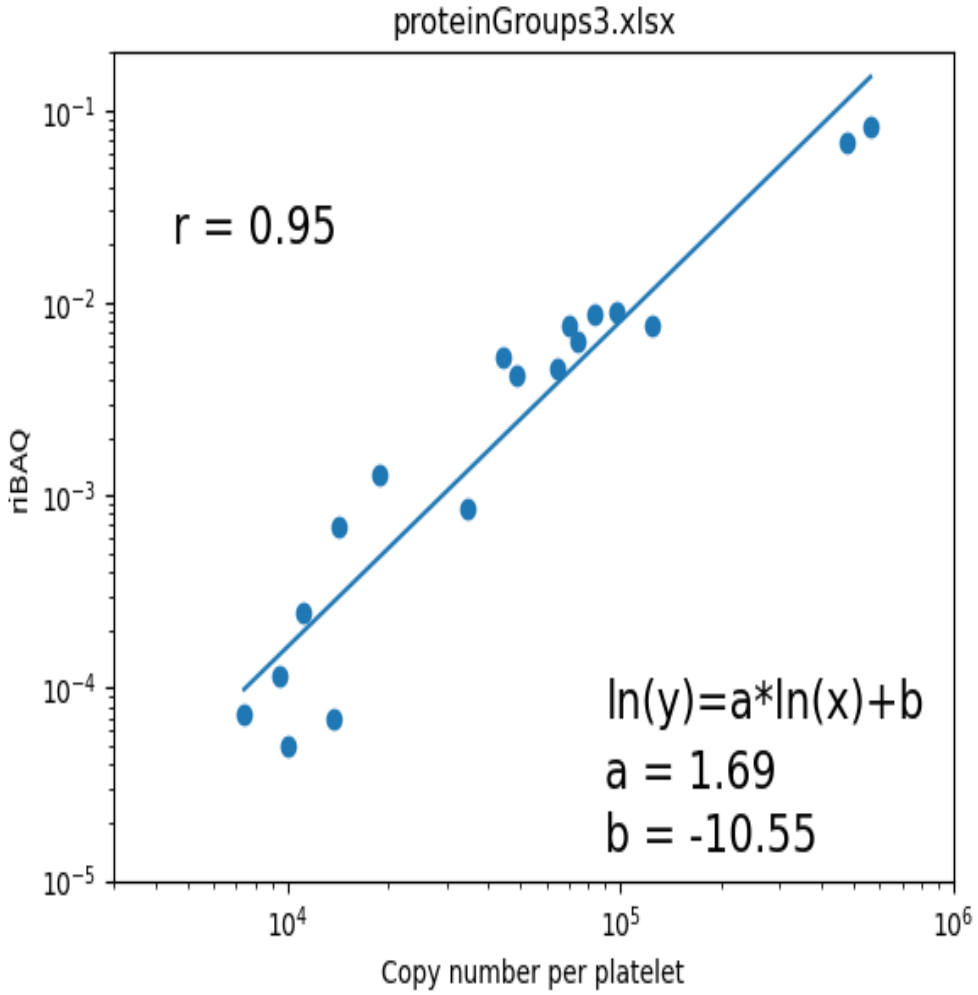
iBAQ
to
copy number per platelet



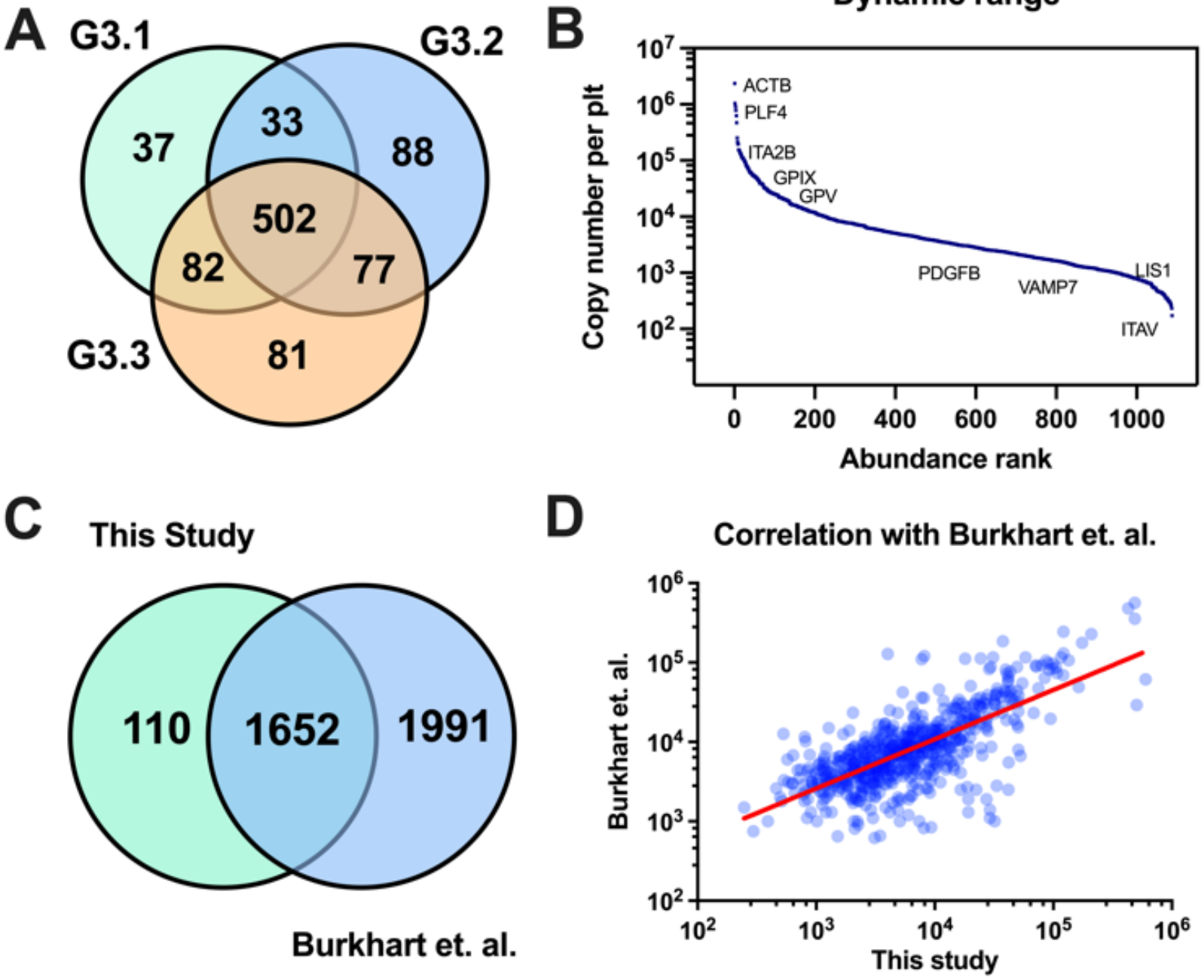
Combine
data per
group

Analyze

Calculation of protein copy numbers

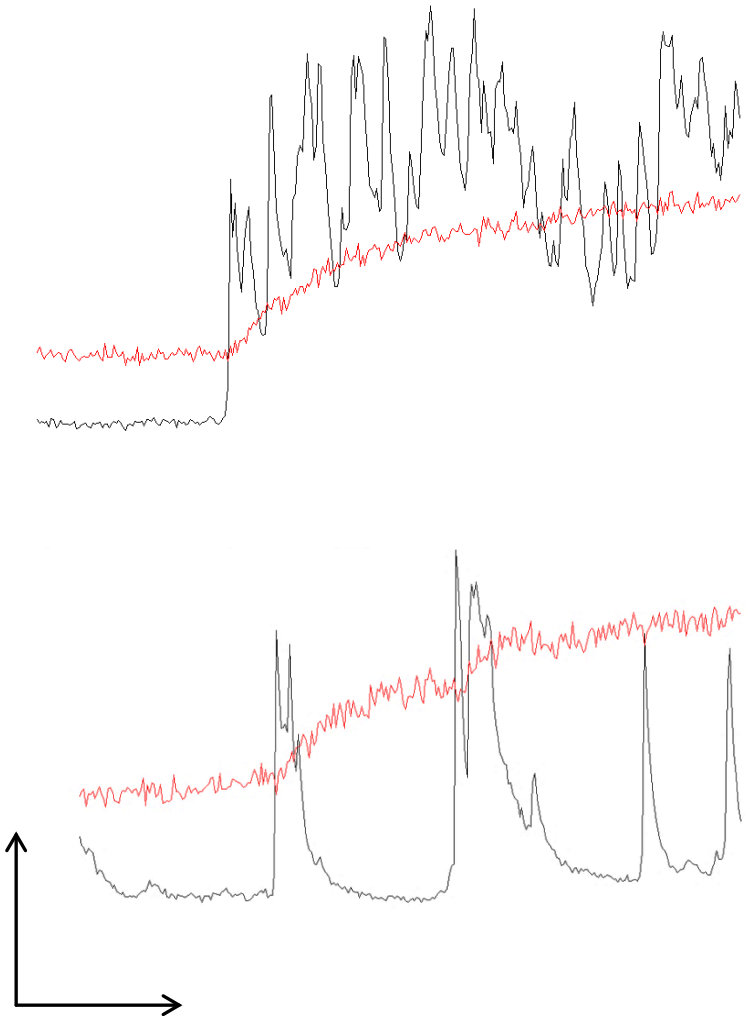


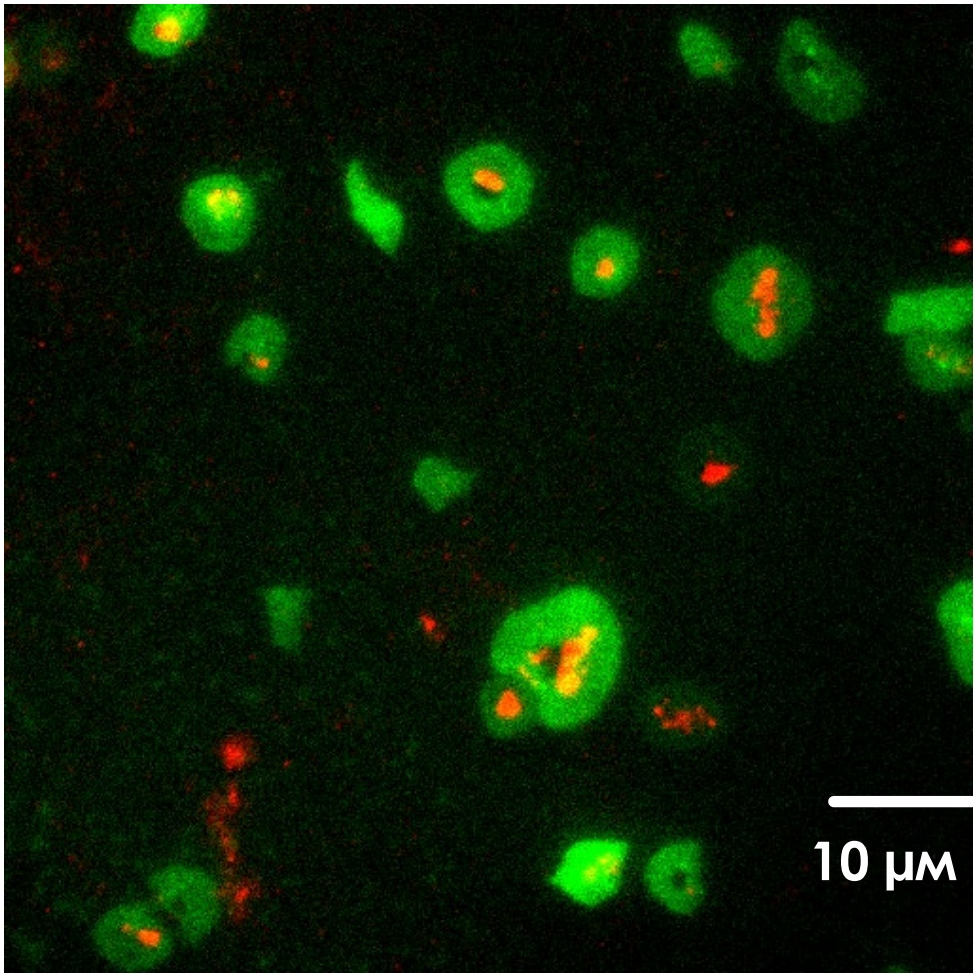
Julia M. Burkhart et. al. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. *Blood* 2012; 120 (15): e73–e82. https://doi.org/10.1182/blood-2012-04-416594


A
G3.1
G3.2
G3.3
37
33
88
502
82
77
81
B
Dynamic range
Copy number per plt
Abundance rank
ACTB
PLF4
ITA2B
GPIX
GPV
PDGFB
VAMP7
LIS1
ITAV
C
This Study
Burkhart et. al.
110
1652
1991
D
Correlation with Burkhart et. al.
Burkhart et. al.
This study

Single cell responses

Typical platelets



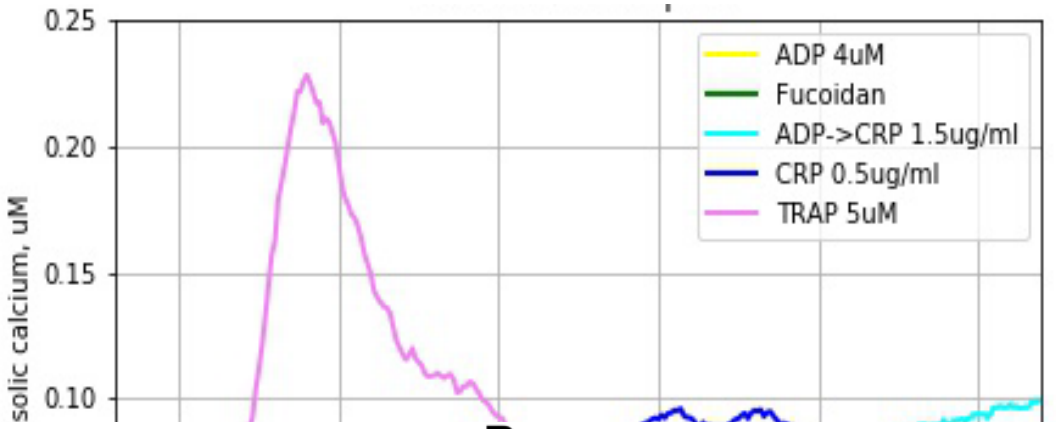


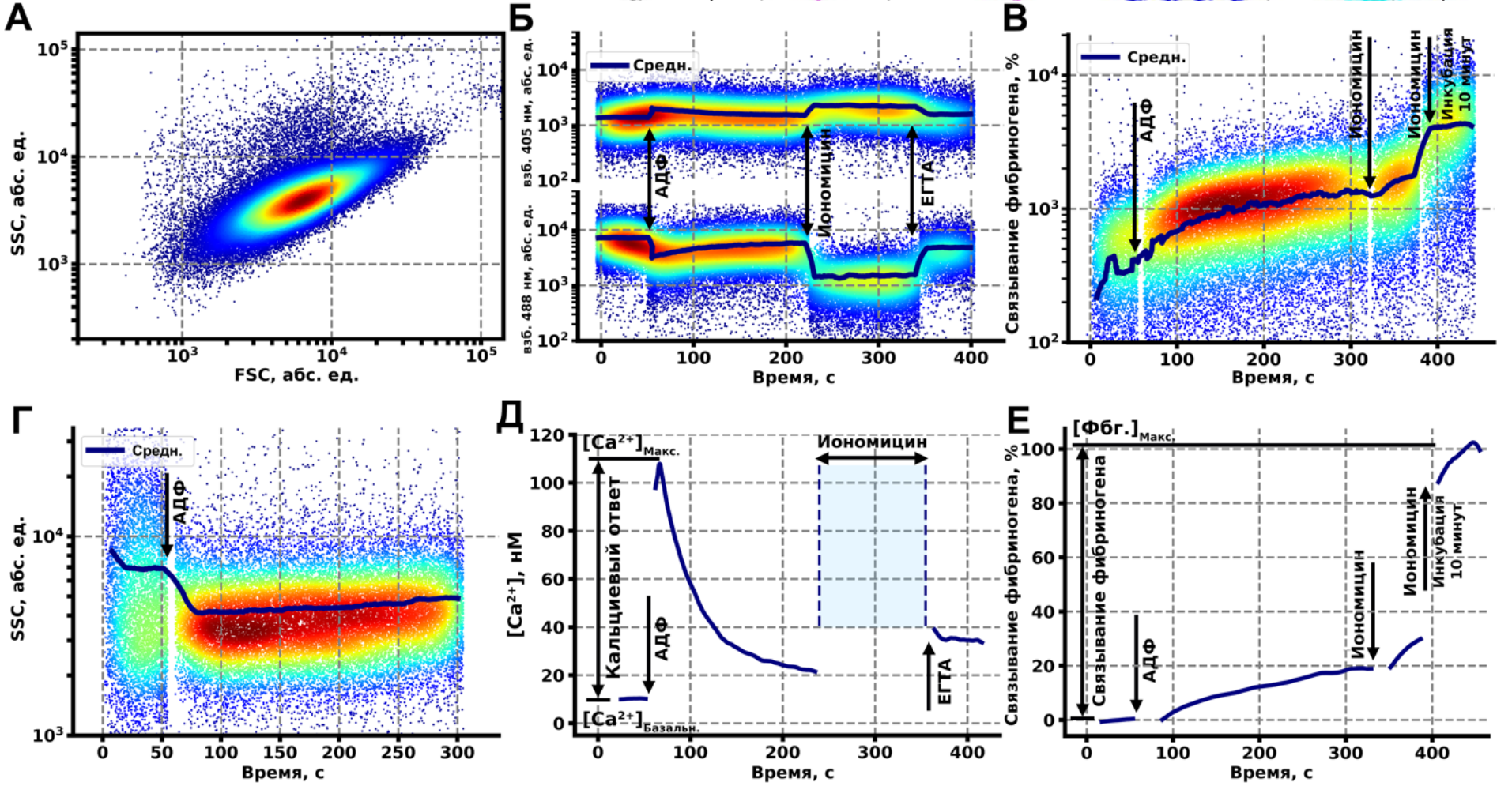
Inverted calcium

Fibrinogen

J Thromb Haemost, 14(9):1867–1881, 2016; Биол. мембраны, 35(4):1–13, 2018; ВМУ Серия 3, 2018(5):63–70; RPTH, 3(supp. 1), 2019; Biophys J, 118(11):2641–2655, 2020; BMC Biol, 20: 32, 2022.

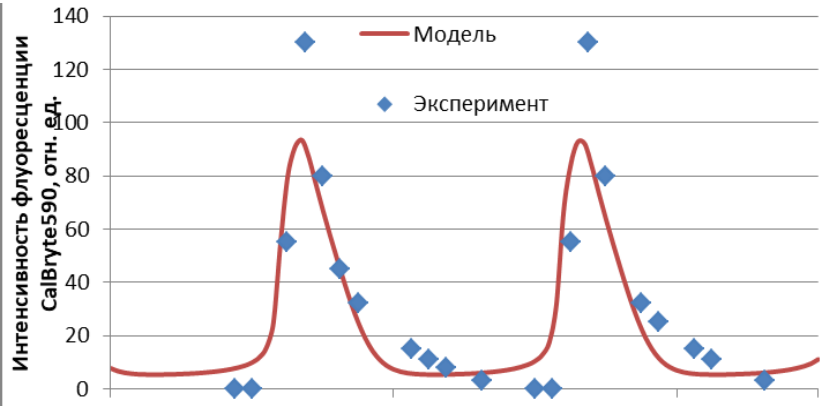
Flow cytometry





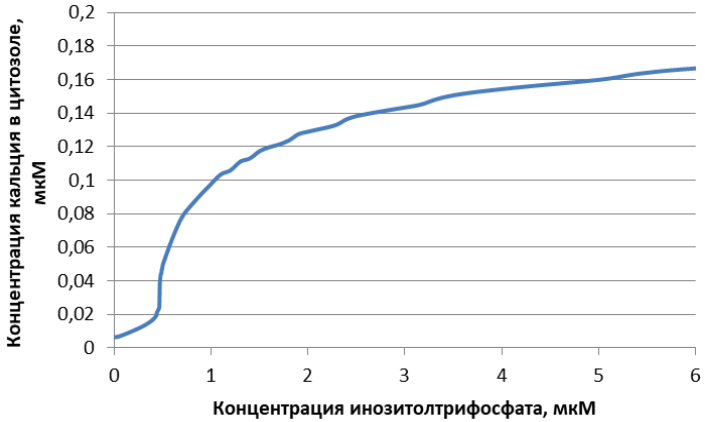
Scheme of model personalization

Single platelet calcium



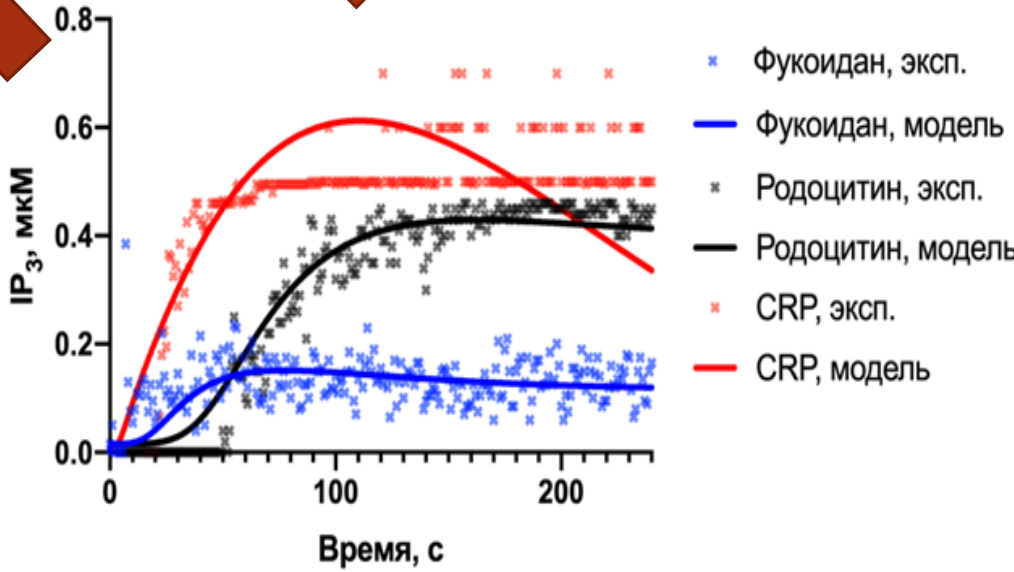
$[Ca^{2+}]$

Transformation: $[IP_3] = f([Ca^{2+}])$



$[IP_3]$

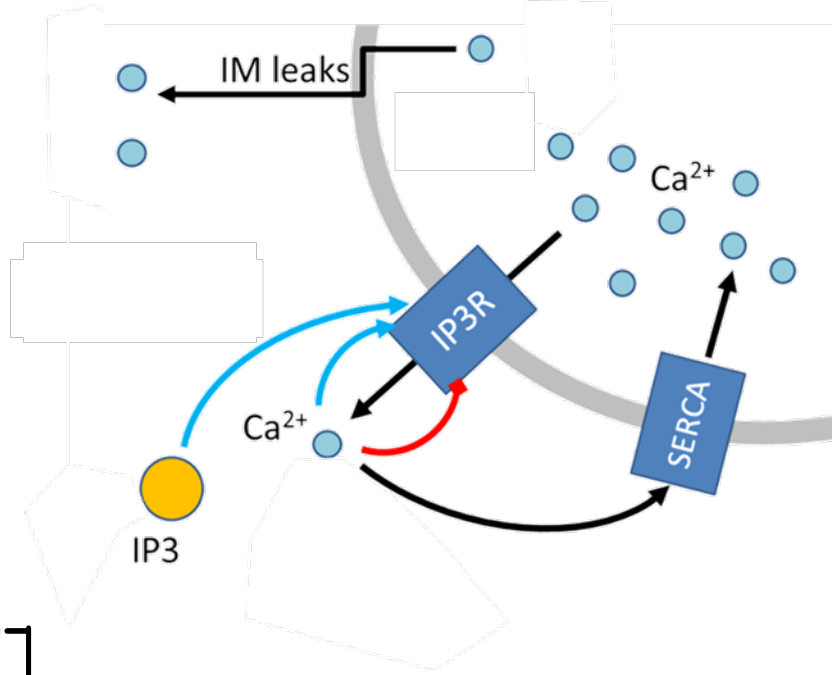
Recalculation of suspension data

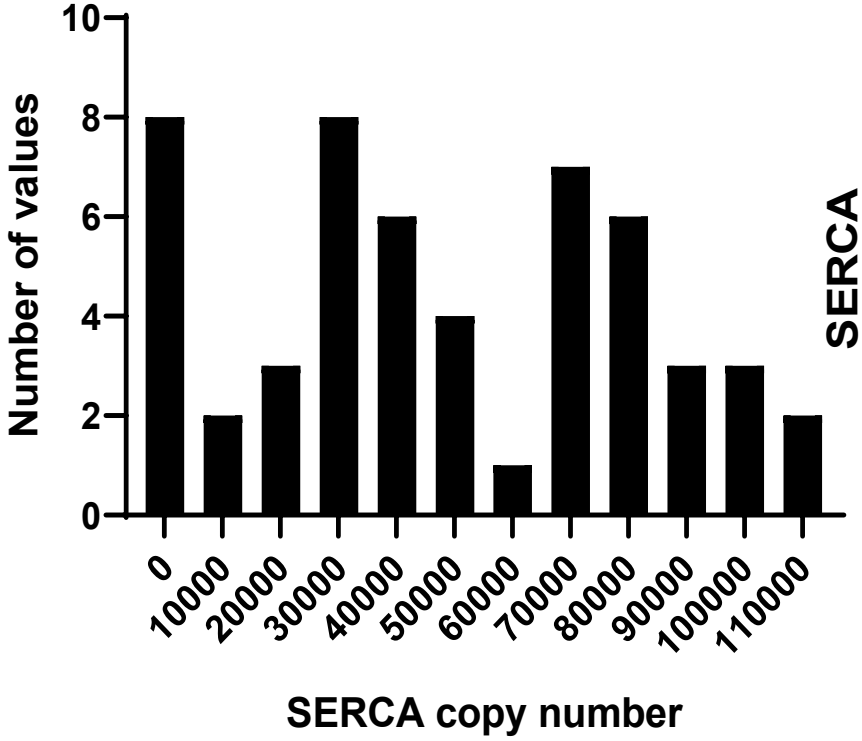


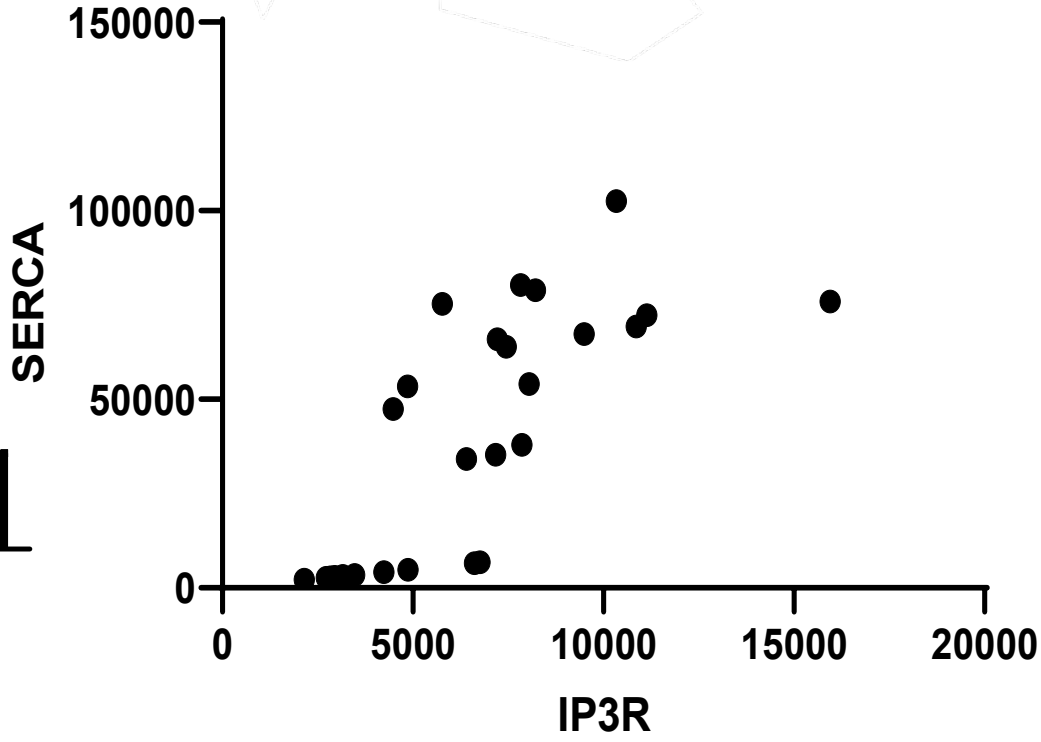
$[PIP_3]$

Determination of personal enzyme turnover rates

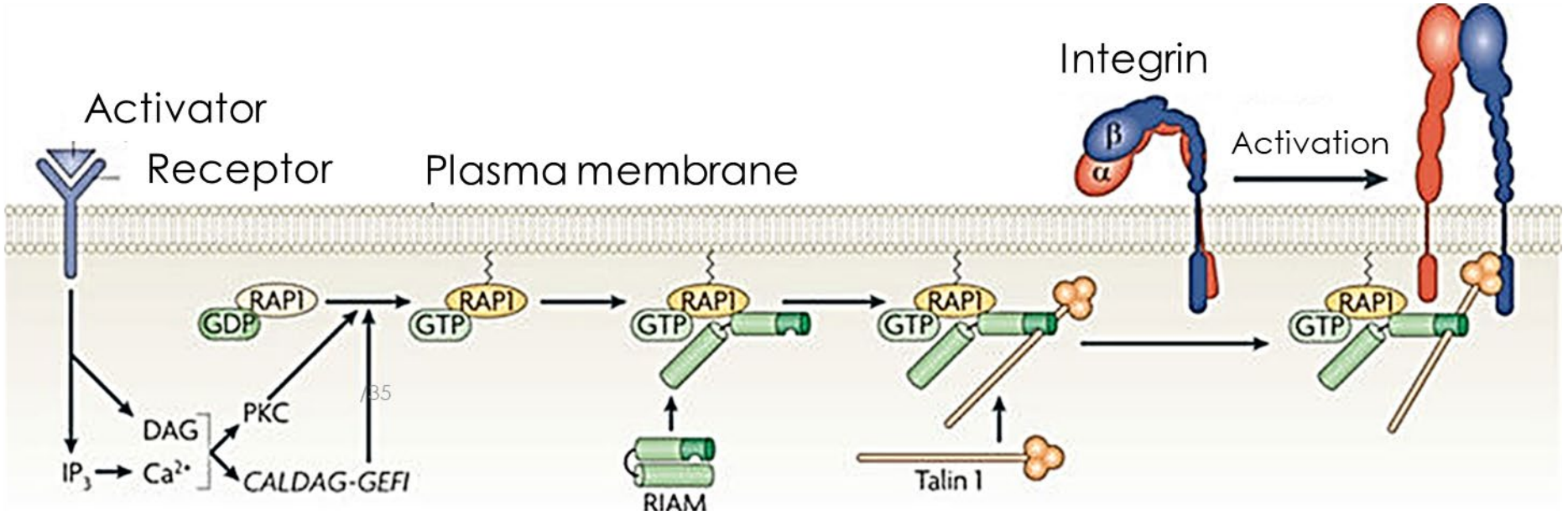
Balance in calcium signaling

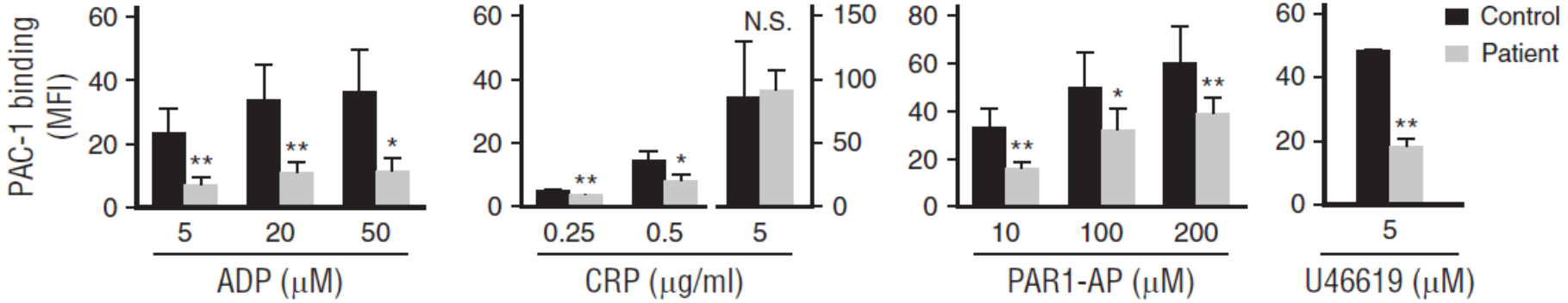






Decoding: integrin activation

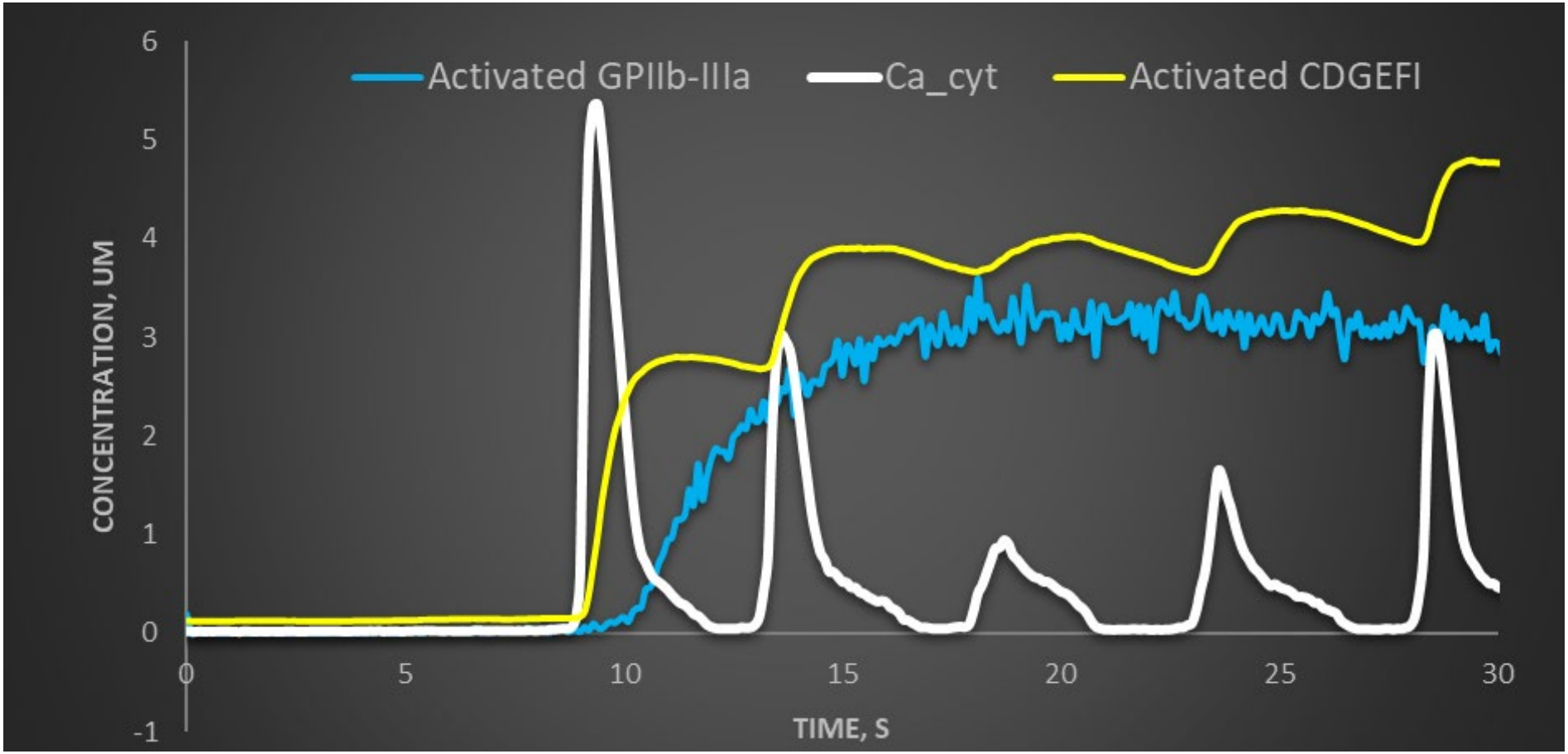




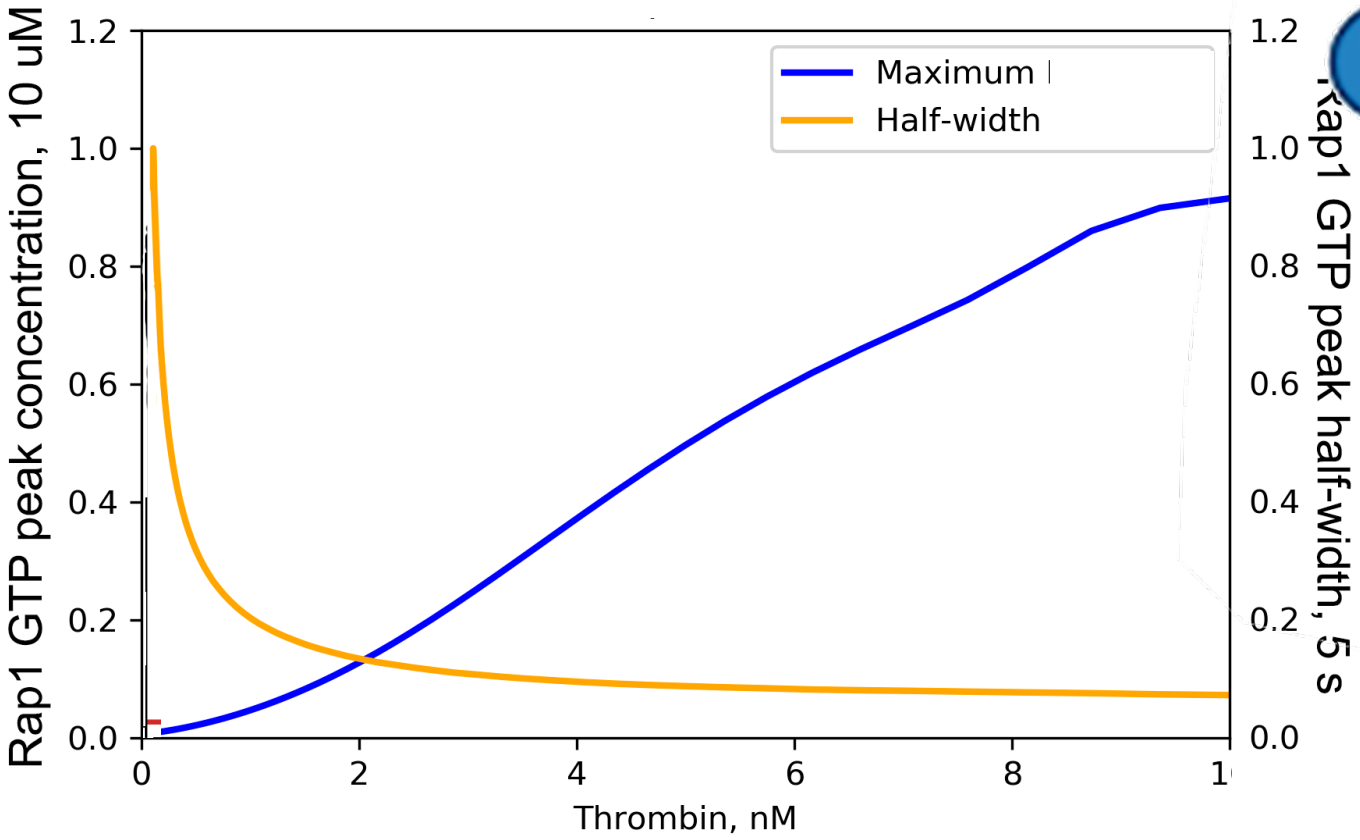
Franke et al The EMBO Journal Vol.16 No.2 pp.252–259, 1997
Hisashi Kato, et all 2017 BLOOD

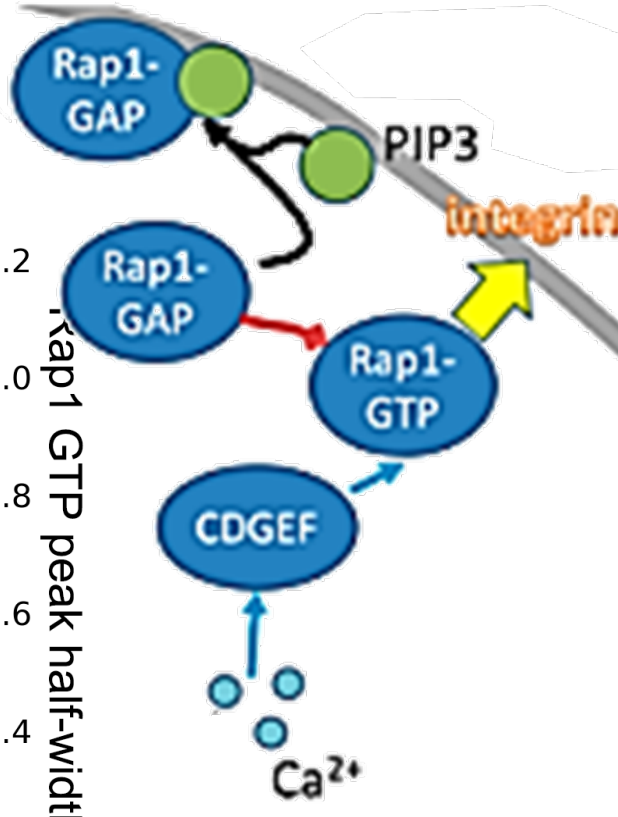
Simulation of integrin activation in single platelets

1 μM PAR1AP

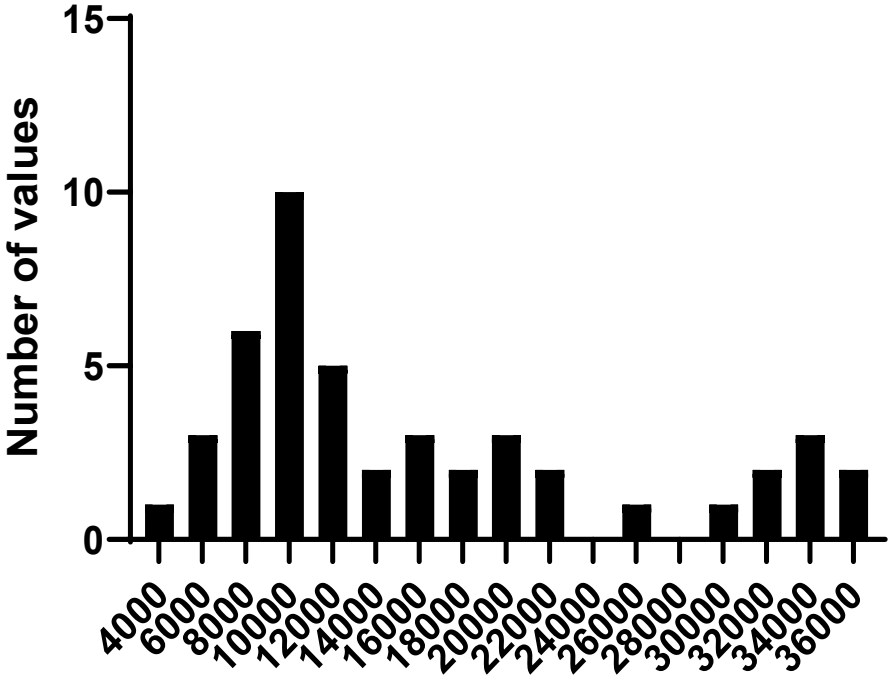


‘Decoding’ by proteins





$$\frac{d}{dt}[Rap1GTP] = \gamma \frac{[Rap1GDP]\ \left[Ca^{2+}_{cyt}\right]^2}{K^2 + \left[Ca^{2+}_{cyt}\right]^2} - k[Rap1GTP]$$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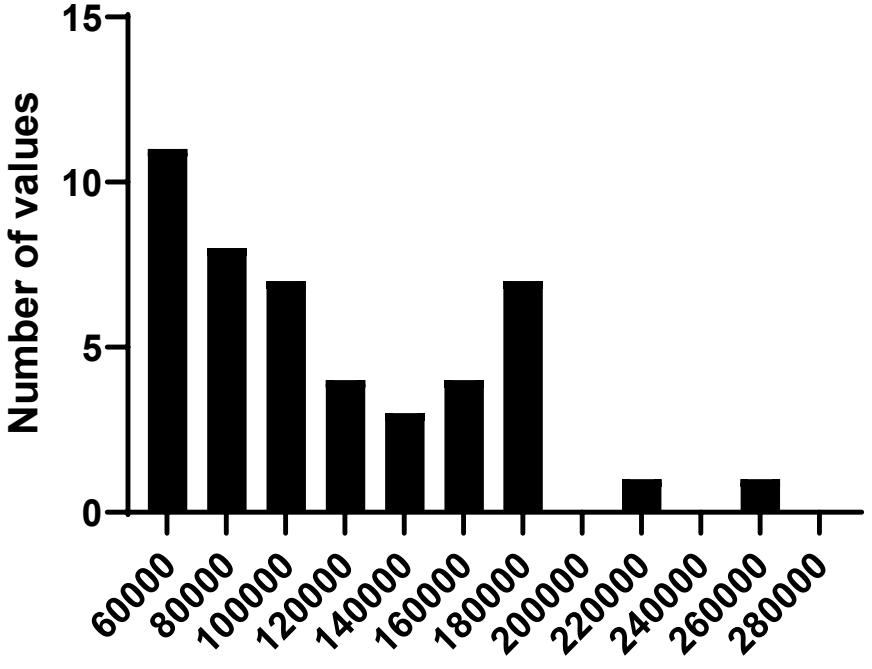
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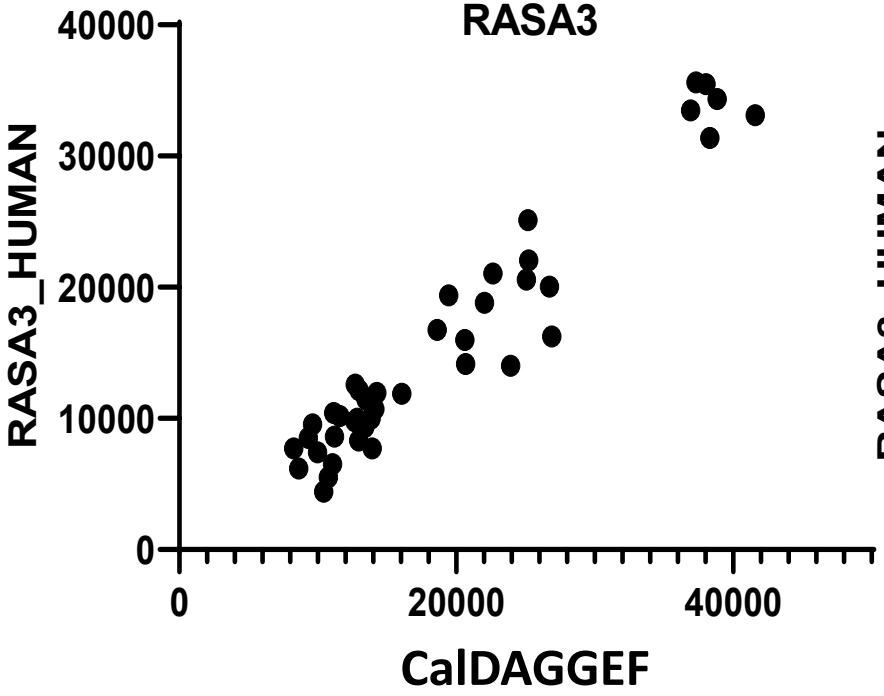
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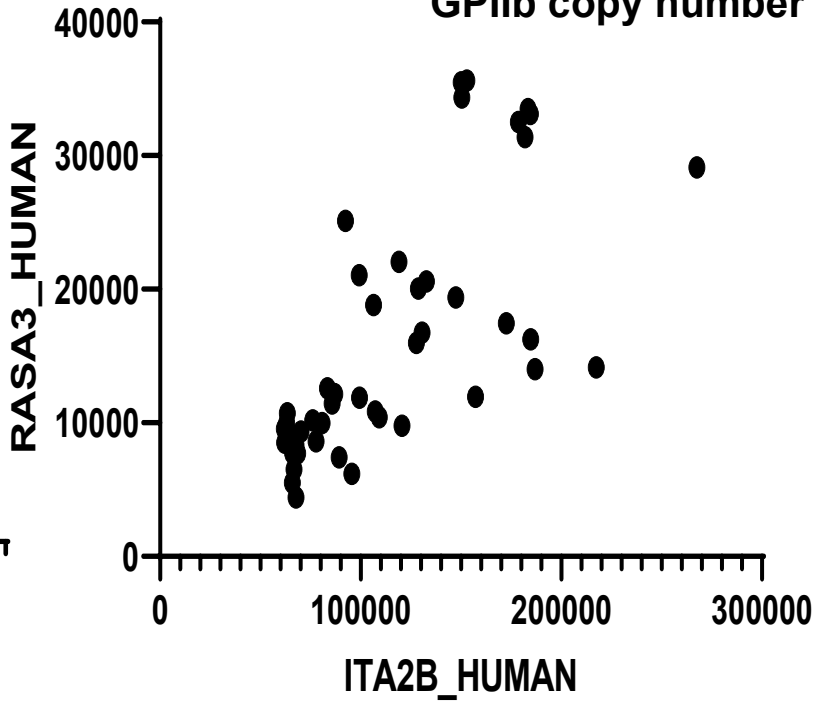
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Conclusions

- Platelet proteomics allows personalization of the Virtual Platelet computational model
- Copy numbers of each enzyme varies greatly in healthy population
- In each biochemical module the “drivers” and “leaks” appear to be balanced

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Acknowledgements

Experiments& calculations

Andrey Garzon
Fedor Balabin
Alexey Martyanov
Sergey Obydennyi
Soslan Shakhidzhanov

Ideas& Discussions

Mikhail Panteleev
Fazly Ataullakhanov

Dmitry Rogachev
Children's Hematology Center