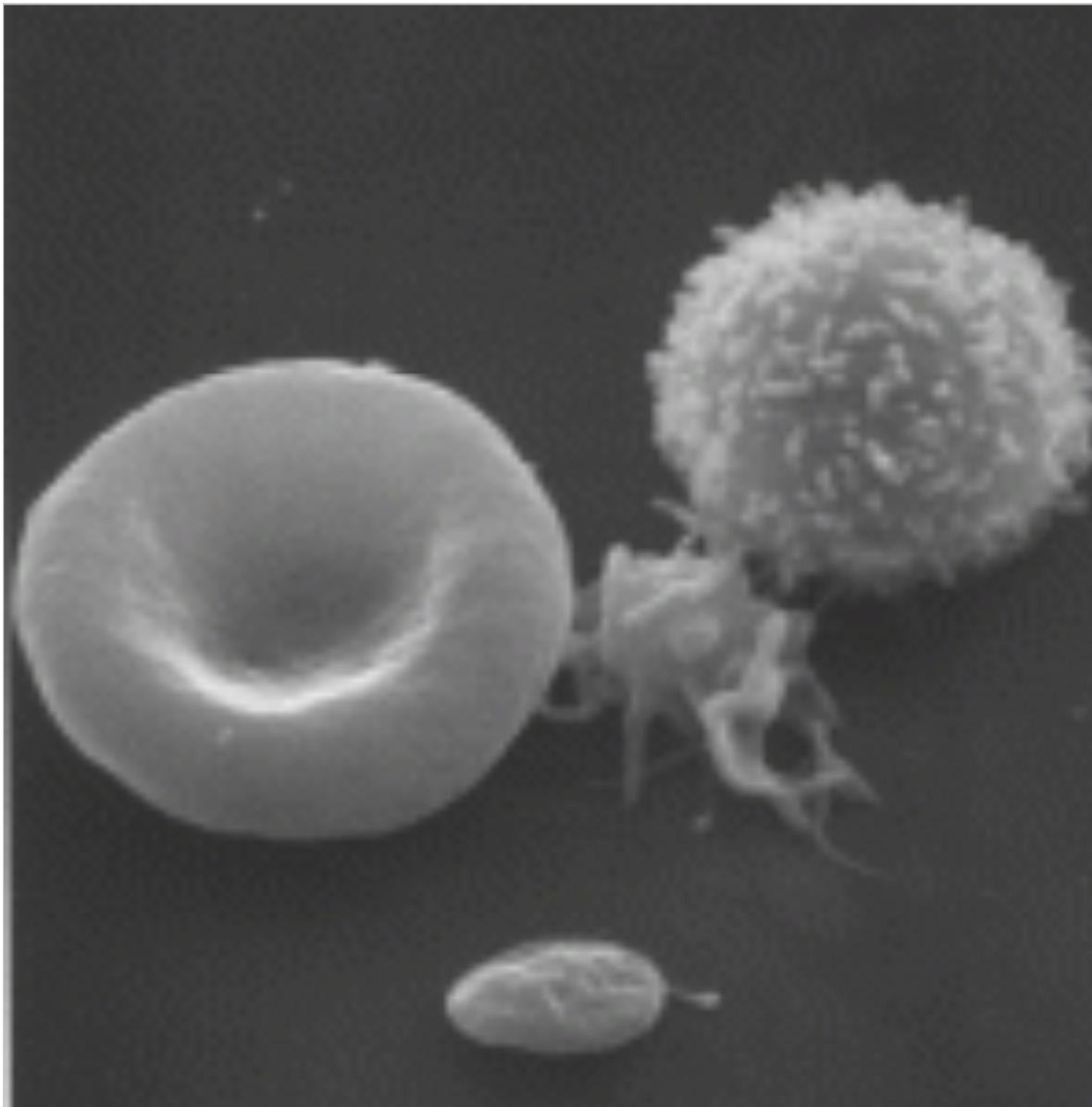


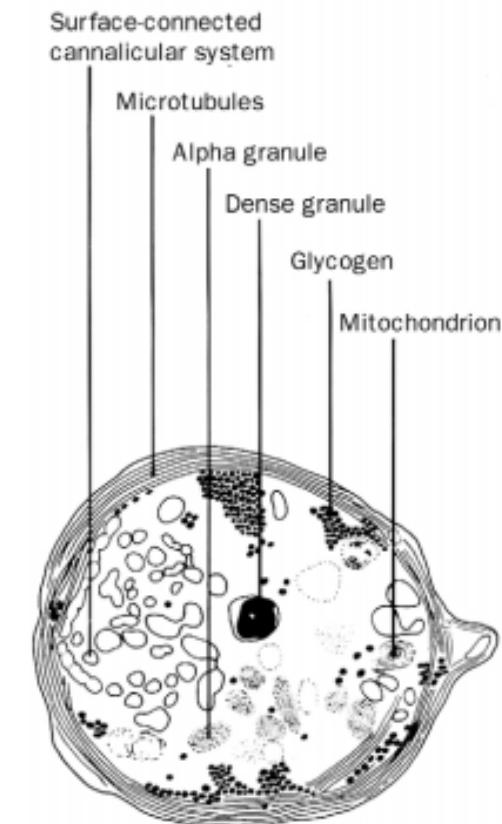
Towards a Greater Understanding of Platelet Metabolism

Rachel LeCover
Biokinetics 2017

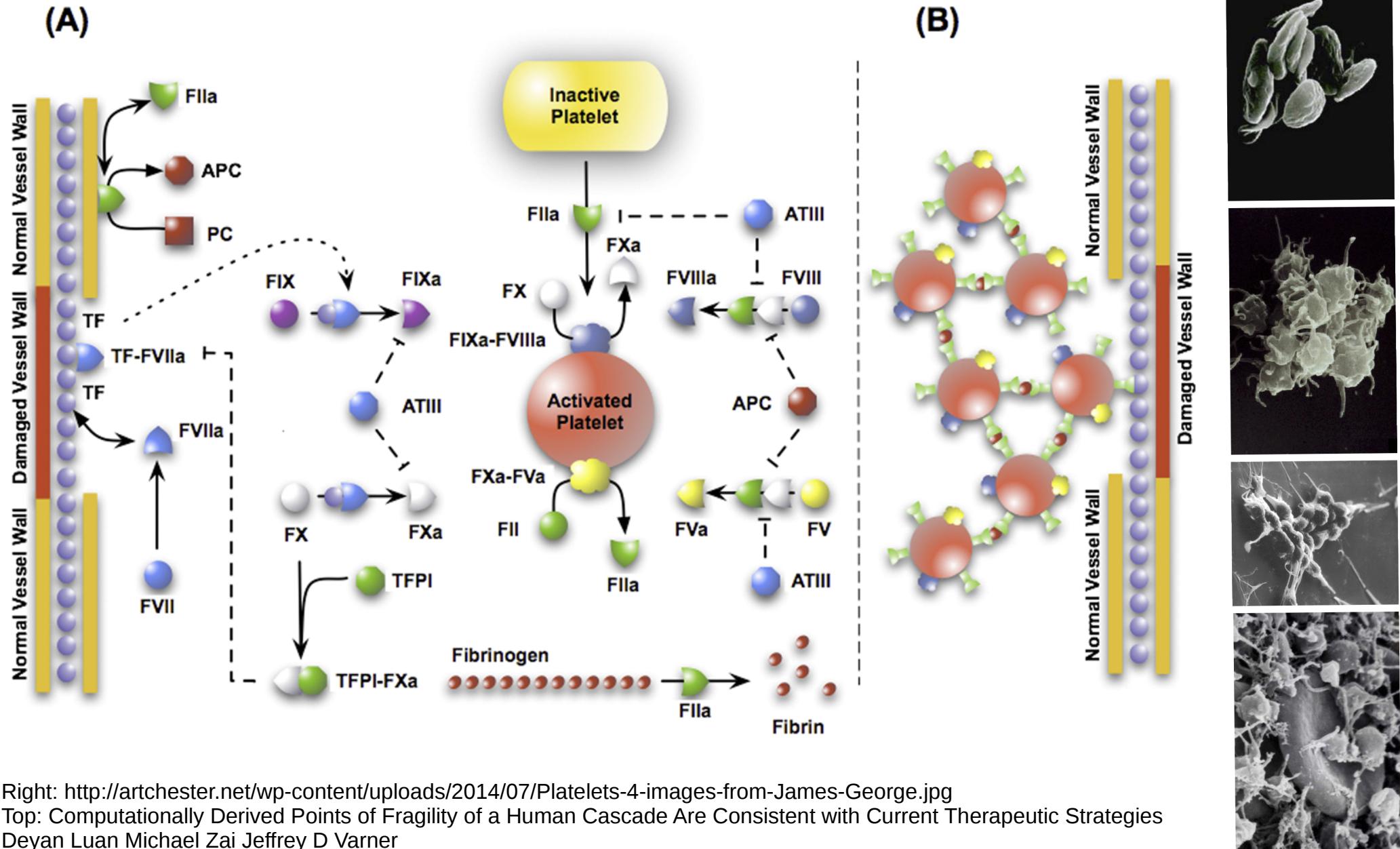
What is a Platelet?



Left: <http://artchester.net/2014/07/platelets-stem-cells-investing-blood/>
Right: Platelets. Haematology James N George



The Importance of Platelets



Right: <http://artchester.net/wp-content/uploads/2014/07/Platelets-4-images-from-James-George.jpg>

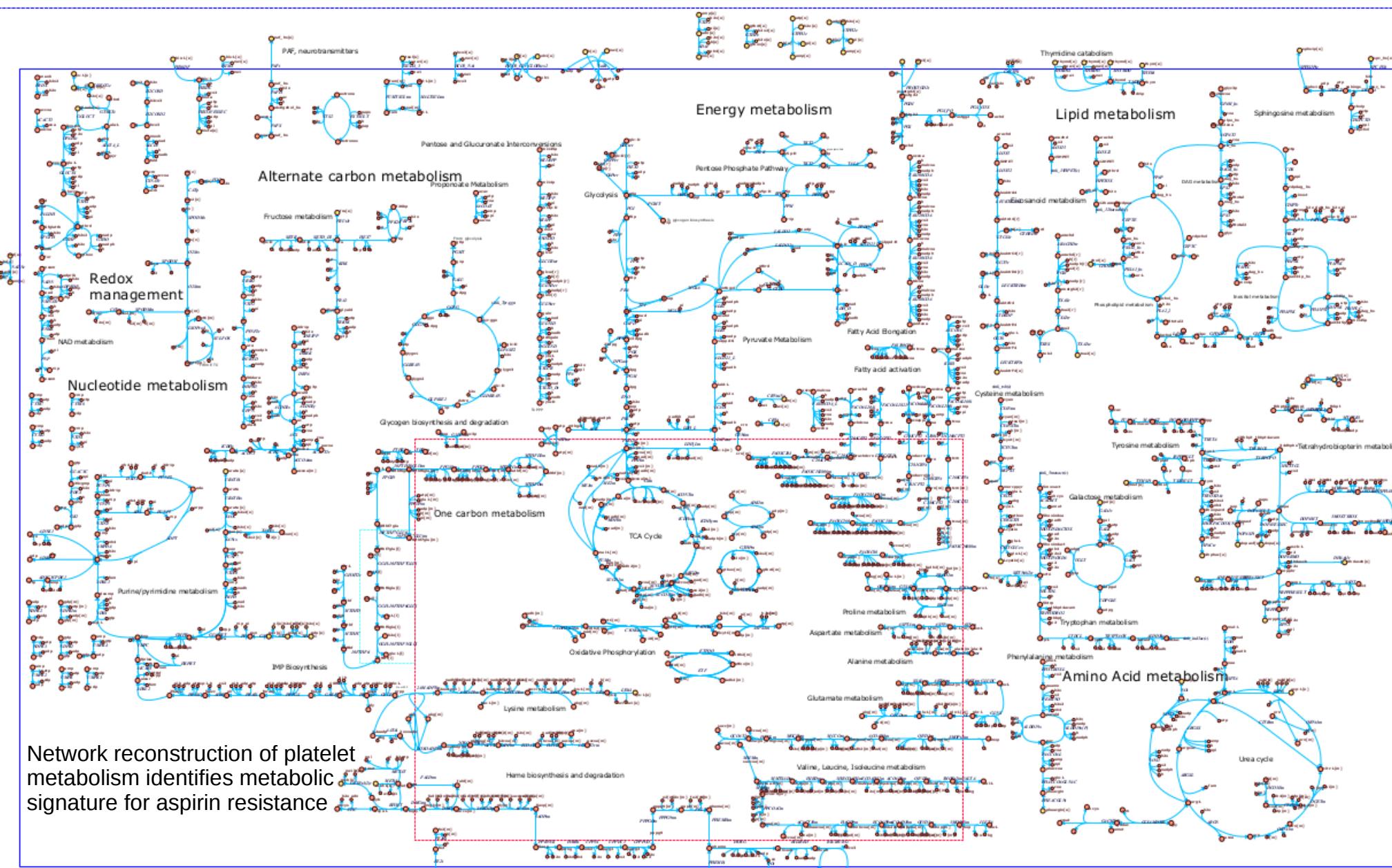
Top: Computationally Derived Points of Fragility of a Human Cascade Are Consistent with Current Therapeutic Strategies

Deyan Luan Michael Zai Jeffrey D Varner

Previous Work

- 1675-Leewenhoeck looks at platelets through his microscope and shares his observations with the Royal Society of London
- 1882-Bizzozzero identifies platelets as being a separate component of blood (not a red or a white cell), that they change shape, and form clots
- 1960-Hellem and Owren discover that ADP causes platelets to activate
- 1989-Asby publishes a model of cAMP metabolism in platelets
- 2008-Diamond publishes a kinetic model of platelet activation
- 2014-Palsson publishes his network reconstruction of platelet metabolism

Palsson's Platelet Model



FBA

Linear objective function

$$\max_{v_1, \dots, v_{\mathcal{R}}} \sum_{i=1}^{\mathcal{R}} c_i v_i$$

Subject to:

Balanced metabolites ($b=0$)

$$\sum_{j=1}^{\mathcal{R}} \sigma_{ij} v_j = b_i$$

Constraints

Unbalanced metabolites

$$\mathcal{L}_i \leq \sum_{j=1}^{\mathcal{R}} \sigma_{ij} v_j \leq \mathcal{U}_i$$

Flux constraints

$$\alpha_j \leq v_j \leq \beta_j$$

dFBA

$$\max_{v_1, \dots v_R} \sum_{i=1}^R c_i v_i$$

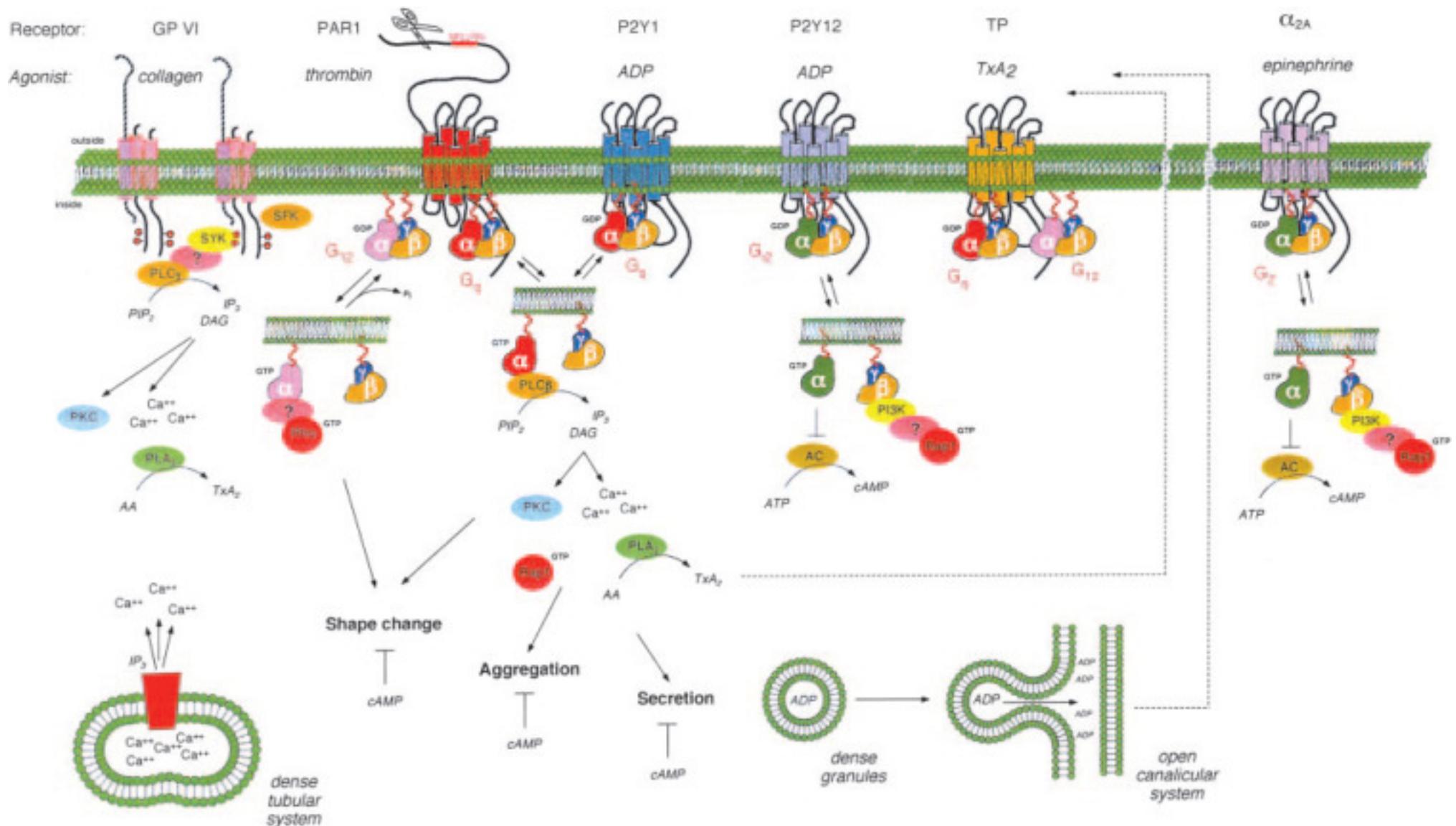
Subject to:

$$Sv = \frac{dy}{dt}$$

$$L_i \leq \sum_{j=1}^R \sigma_{ij} v_j \leq U_i$$

$$\alpha_j(t) \leq v_j(t) \leq \beta_j(t)$$

Platelet Activation



Adding Logical Rules

If (external concentration of calcium is high)

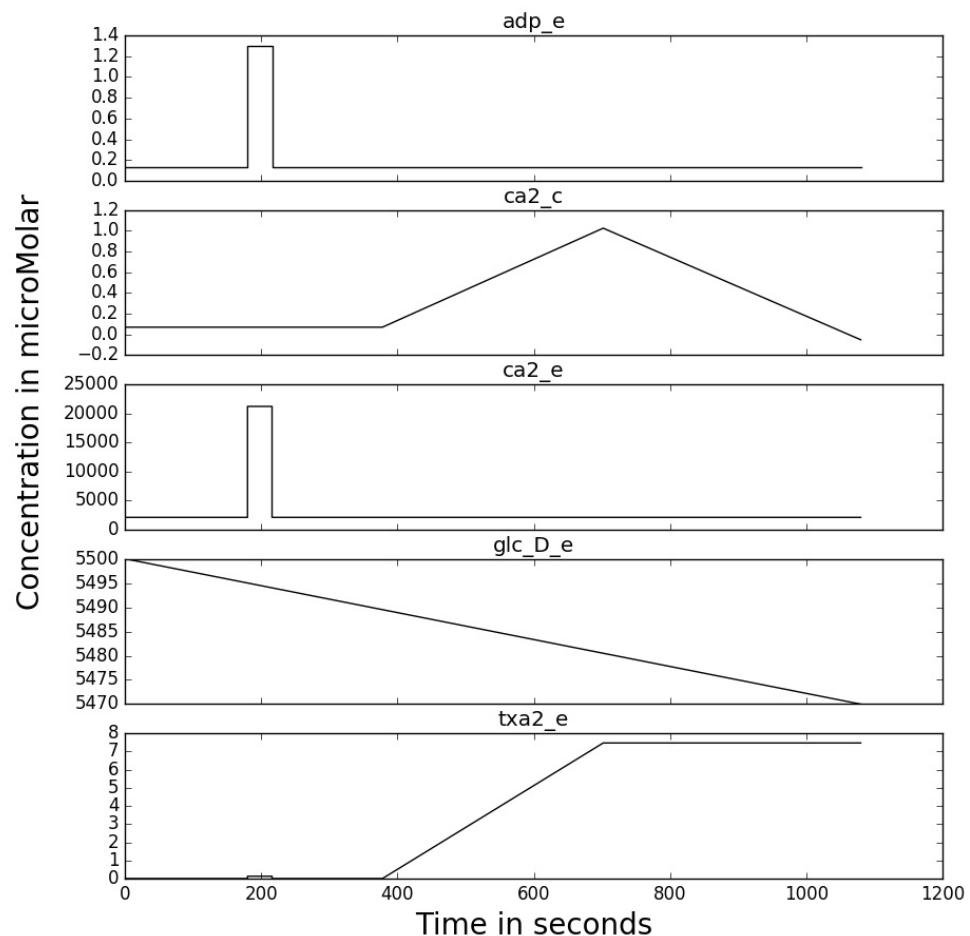
- Import calcium (until it reaches 1 microMolar, then export)

If (external concentration of ADP is high)

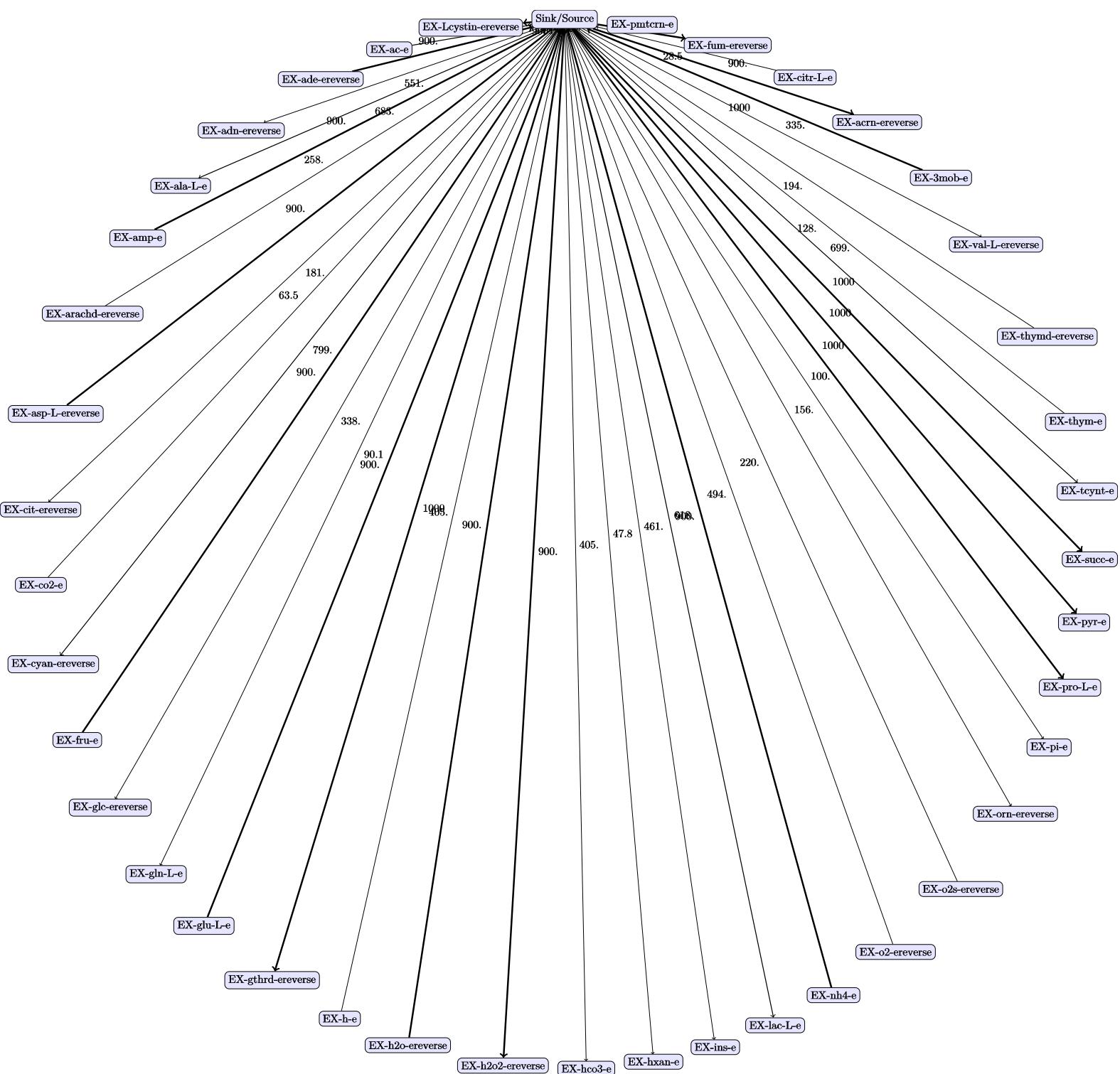
- Increase flux through reactions producing IP₃
- Decrease flux through reactions producing cAMP though adenylyl cyclase
- Increase flux through reactions producing TXA₂

Activating the Platelet

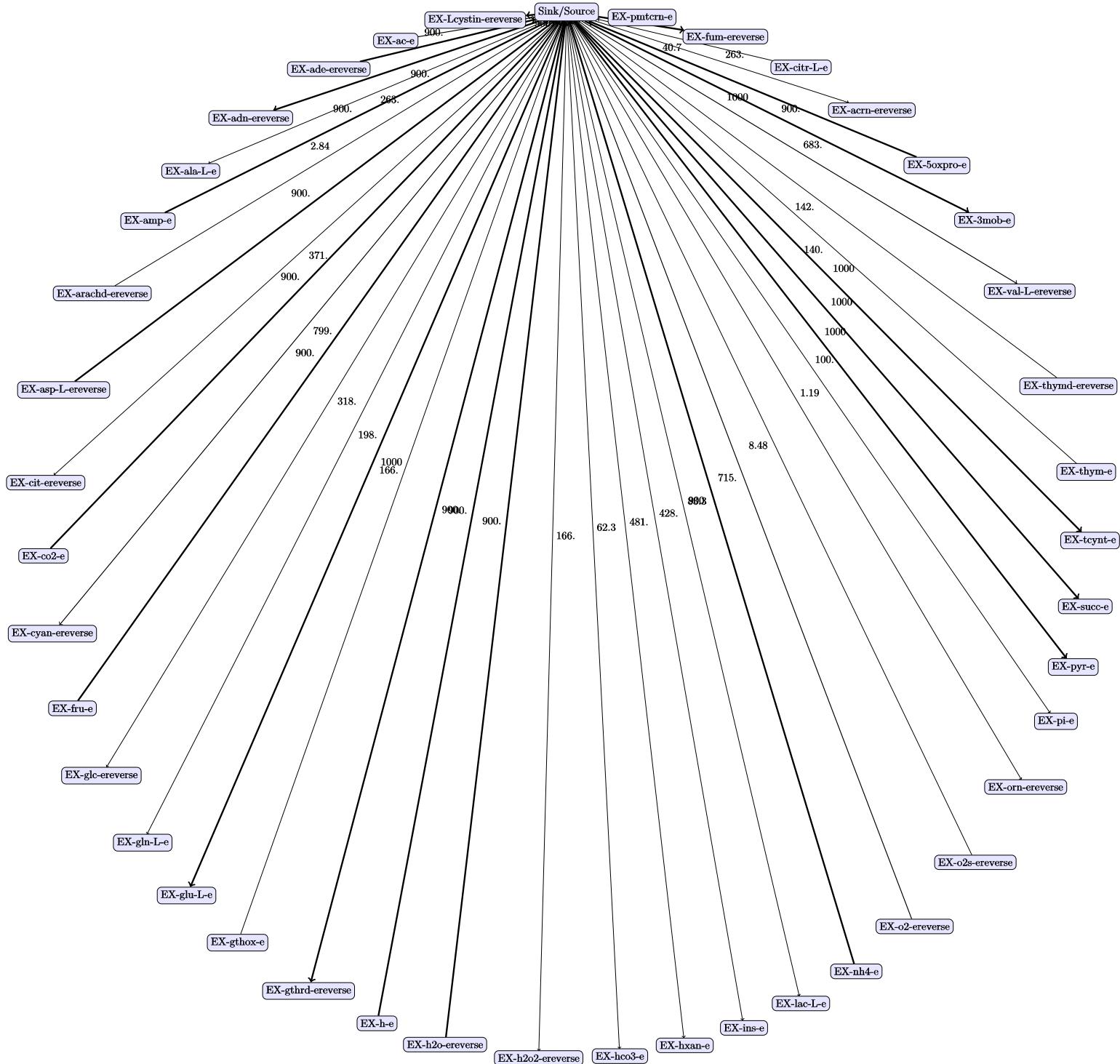
- To simulate activating the platelet, I spiked the external calcium, ADP, and TXA₂ levels



Steady State Extracellular Exchange Fluxes

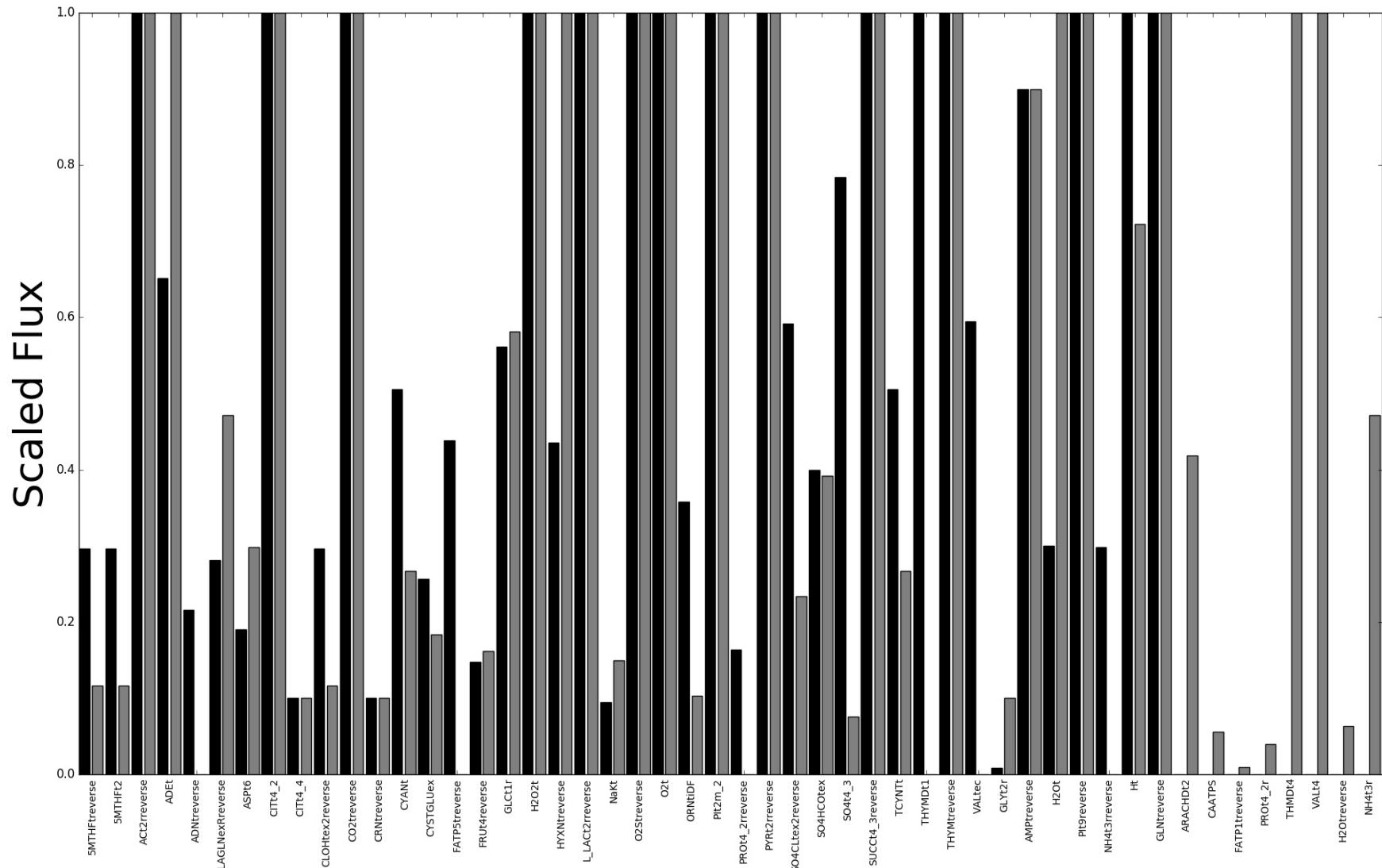


Post Activation Extracellular Exchange Fluxes



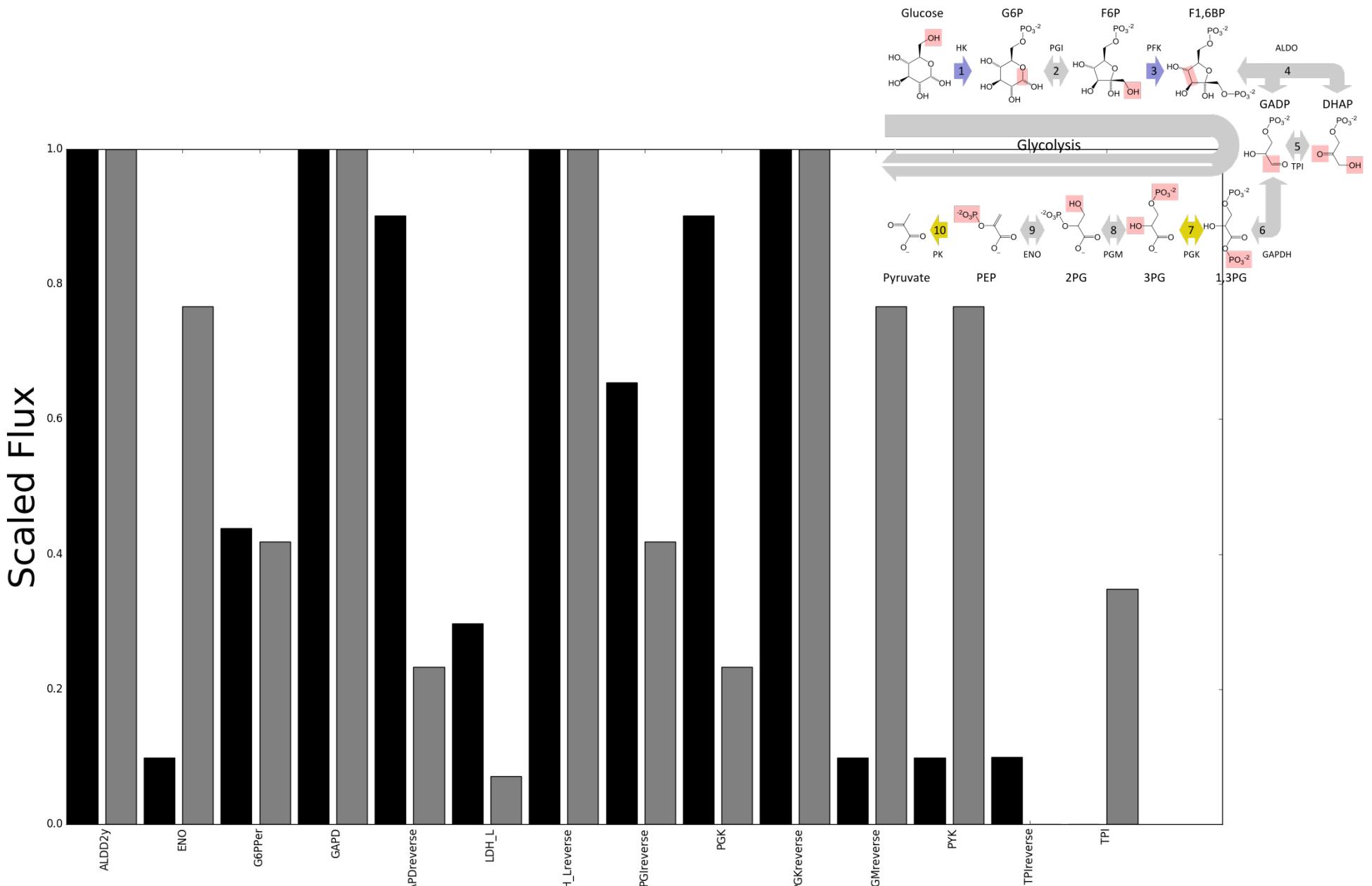
Intracellular Transport

Black-SS
Grey-After
Activation



Glycolysis and Gluconeogenesis

Black-SS
Grey-After Activation



Genetics of Platelet Activation

Candidate gene SNPs used in association studies

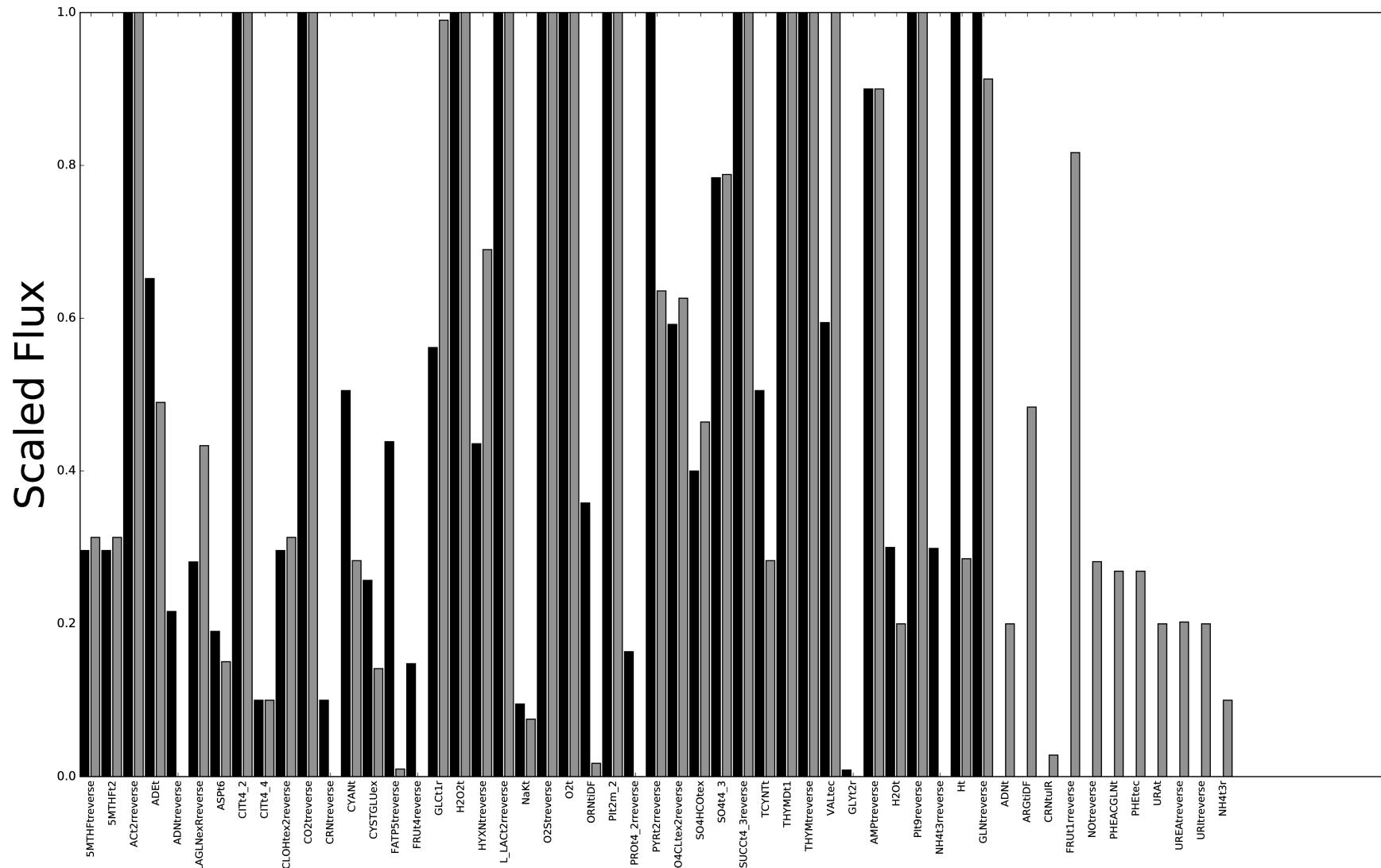
Gene	Protein identification	dbSNP or chromosomal location*	Cytoband	MAF
<i>ADRA2A</i>	α-2A-adrenergic receptor	chr10: 112 839 580†	10q24-q26	0.41
<i>F2R</i>	Coagulation factor II (thrombin) receptor; proteinase-activated receptor 1 (PAR-1)	rs168753	5q13	0.14
<i>FCGR2A</i>	IgG Fc receptor type IIa	chr1: 161 479 745‡	1q23	0.44
<i>GNB3</i>	Guanine nucleotide-binding protein beta-3 subunit variant	rs5443	12p13	0.45
<i>GP1BA</i>	Platelet glycoprotein Ib, α subunit	rs6065	17pter-p12	0.10
<i>GP6</i>	Platelet glycoprotein VI	rs1613662	19q13.4	0.16
<i>ITGA2</i>	Integrin subunit-α2	rs1126643 rs28095 rs1801106	5q11.2	0.38 0.36 0.08
<i>ITGA2B</i>	Integrin subunit-αIIb	rs5911	17q21.32	0.41
<i>ITGB3</i>	Integrin subunit-β3	rs5918	17q21.32	0.17
<i>P2RY1</i>	Purinergic receptor P2Y1	rs1065776	3q25.2	0.05
<i>P2RY12</i>	Purinergic receptor P2Y12	rs6809699	3q24-q25	0.14
<i>PTGS1</i>	Prostaglandin-endoperoxide synthase 1; cyclooxygenase-1 (COX-1)	rs3842787	9q32-q33.3	0.06
<i>TBXA2R</i>	Thromboxane A2 receptor	rs1131882 rs4523 rs5758	19p13.3	0.13 0.30 0.45

The genetics of normal platelet reactivity

Thomas J. Kunicki and Diane J. Nugent

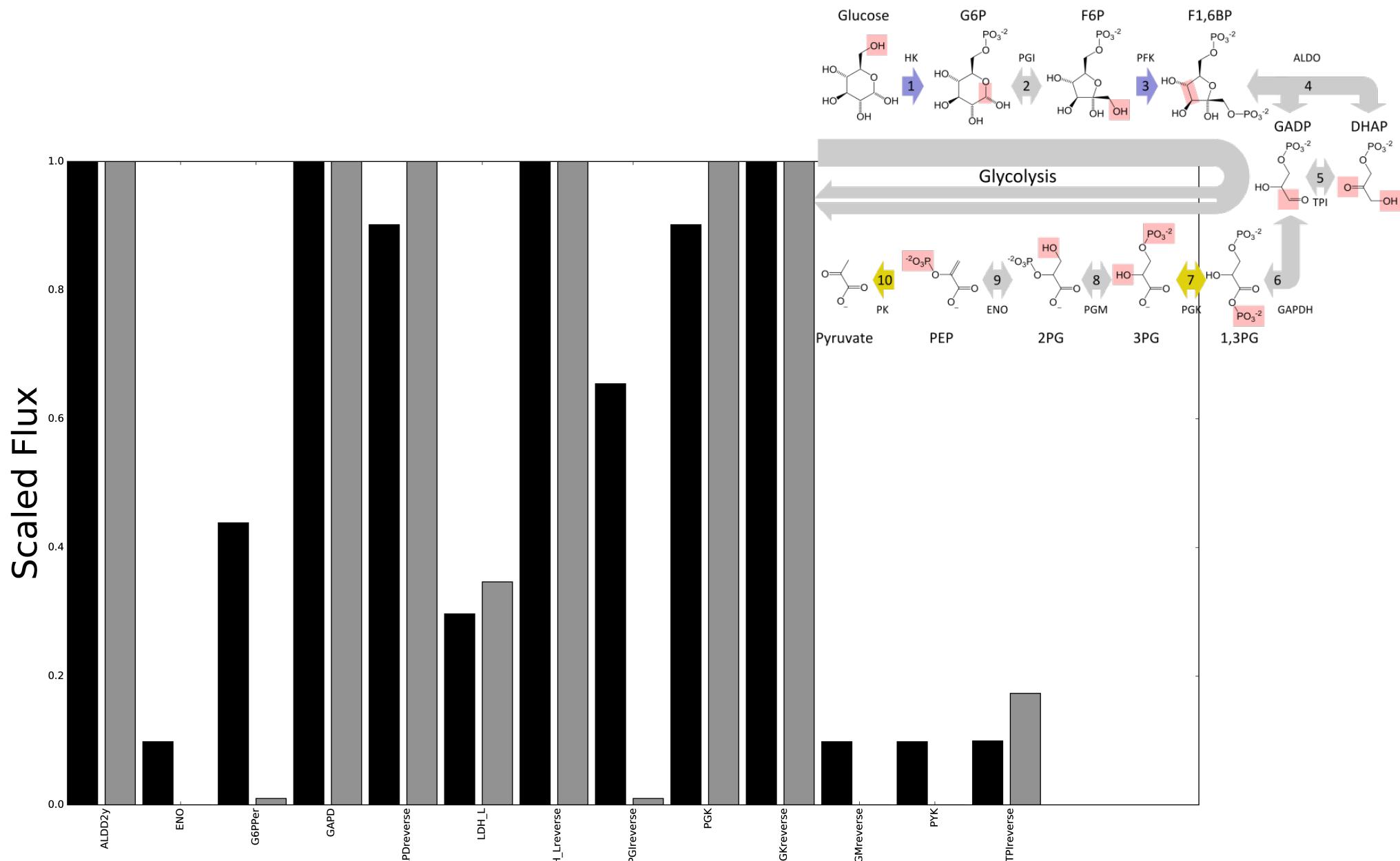
Intracellular Transport

Black-SS
Grey-SS
knockout

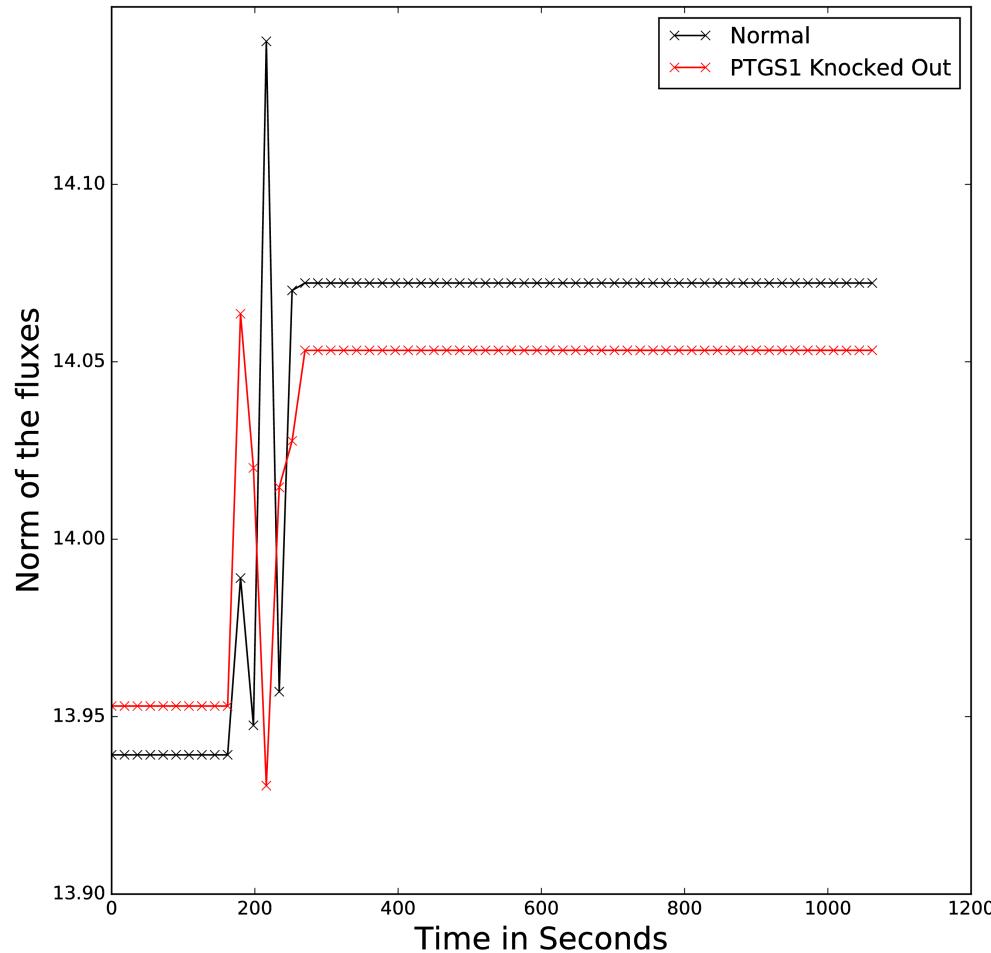


Glycolysis and Gluconeogenesis

Black-SS wild
Grey-SS knockout



Comparison of Wild Type and Knockout



Limitations of the model

- This model doesn't contain reactions for the synthesis of any structural proteins (like actin) which play key roles in platelet shape change post activation
- It's missing granules, the organelles that release the agents of platelet activation
- Most of the signaling mechanisms are absent
- No translation relations

So Far

- Taken a model from literature, parsed it into Varner Flat File format
- Used JuNQC to generate an FBA problem
- Parsed the gene rules and from the literature model into a form that can be used to “knock out” genes (and therefore reactions in the model)
- Converted the experimental bounds in literature to flux bounds for the model
- Converted the FBA problem into a dFBA problem via Euler’s Method
- Simulated platelet activation by changing external concentrations of selected metabolites and developing logical rules to mimic the results of signaling cascades
- Developed tools to visualize fluxes in the network
- Started to compare the differences between “normal” and altered platelet metabolism

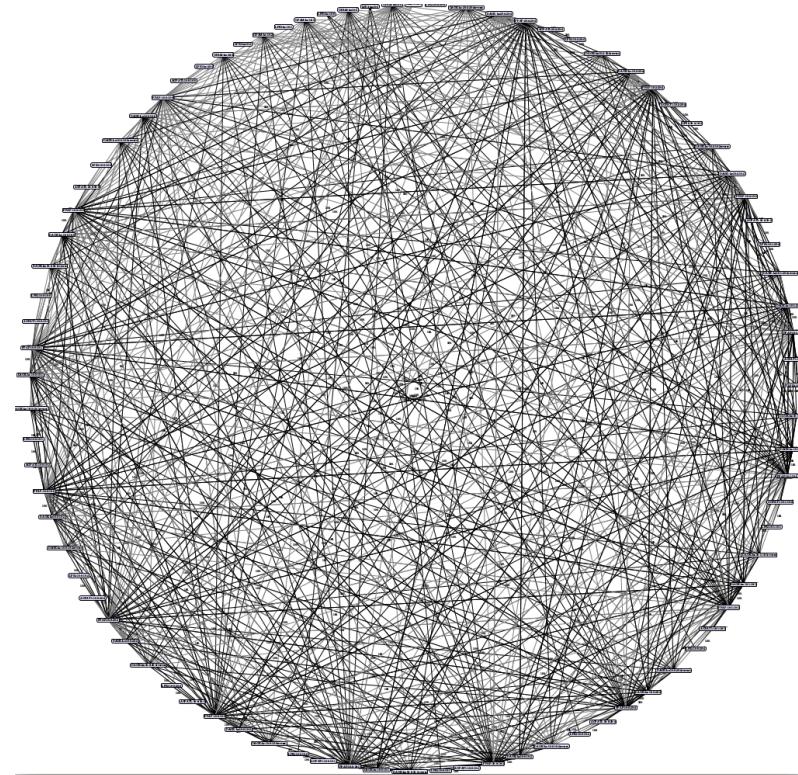
Future Directions

- Add a more comprehensive regulatory layer to capture platelet signaling
- Incorporate cytoskeleton metabolism
- Include granules as separate compartments
- More accurately capture calcium dynamics
- Include a “membrane” compartment, in which accumulation is permitted and include phosphatidylserine export to membrane upon activation
- Figure out a better technique to describe differences in flux profiles

Any questions?

All code used is available at

https://github.com/rlecover007/CHEME7700_FinalProject*



*And a lot more graphs that didn't make it into this presentation!