ALPIC2012
Advanced Learning on Platelets & Thrombosis International Course

The University of Nottingham

Modulation of platelet function by prostanoid receptors

Stan Heptinstall

University of Nottingham, UK


Nottingham


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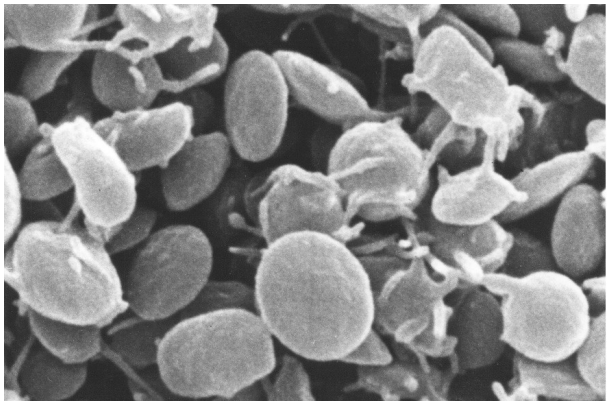
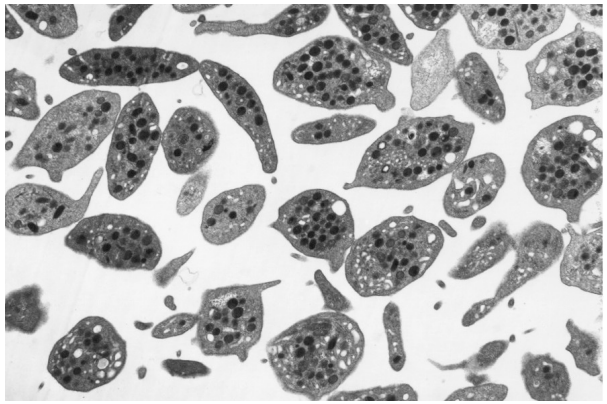
NOTTINGHAM PLATELET CONFERENCE
Platelets – Past, Present and Future, July 2010

Modulation of platelet function by prostanoid receptors

- Platelets contribute to thrombosis
- Overview of some natural agents involved in promotion and inhibition of platelet function
- Overview of platelet receptors involved in promotion and inhibition of platelet function
- Current pharmacological approaches to antithrombotic therapy
- Potential new approaches based on new insights

Unstimulated platelets





Thrombus

Aspects of platelet function that contribute to thrombus formation

- Platelet adhesion
- Platelet aggregation
- TXA_2 synthesis
- Granule secretion
- Microparticle formation
- Platelet-leucocyte conjugation
- Thrombin/fibrin generation
- Response to natural inhibitors of platelet function

Some natural agents that influence platelet function

Promoters of platelet function

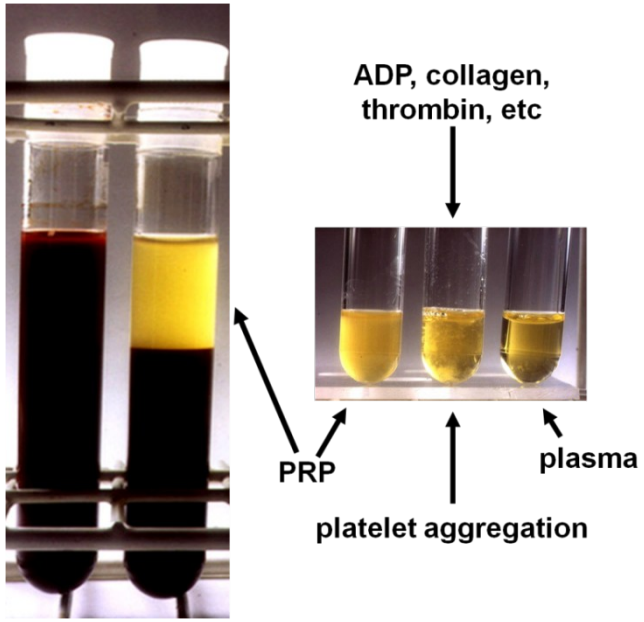
collagen
thrombin
thromboxane A_2
adenosine diphosphate
adenosine triphosphate

Inhibitors of platelet function

prostaglandin I_2
prostaglandin E_1
prostaglandin D_2
nitric oxide

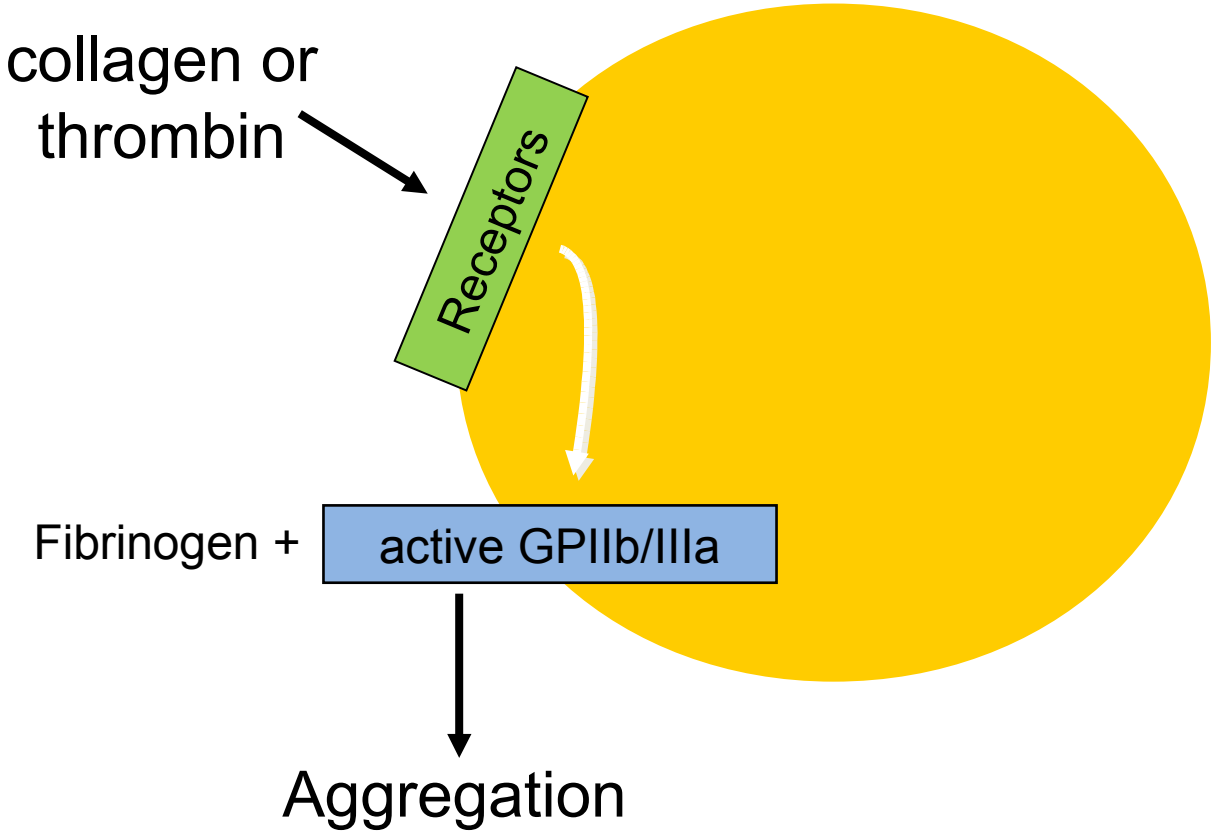
adenosine?
prostaglandin E_2?

Promoters of platelet function



Collagen	GPIb/V/IX, GPVI
Thrombin	PAR1
TXA_2	TP
ADP	$P2Y_1$, $P2Y_{12}$
ATP	CD39

Collagen/thrombin: mechanism of action



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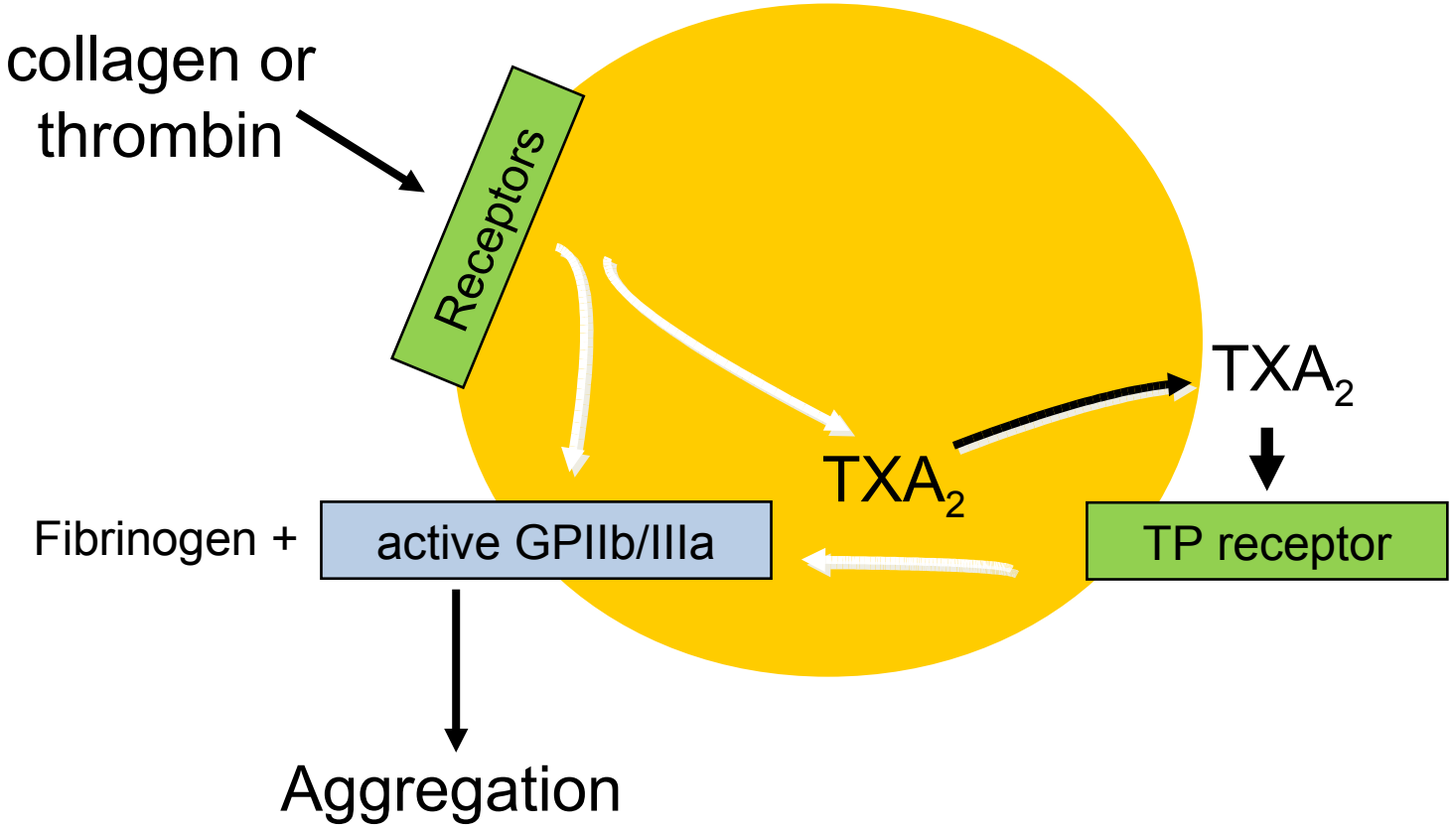

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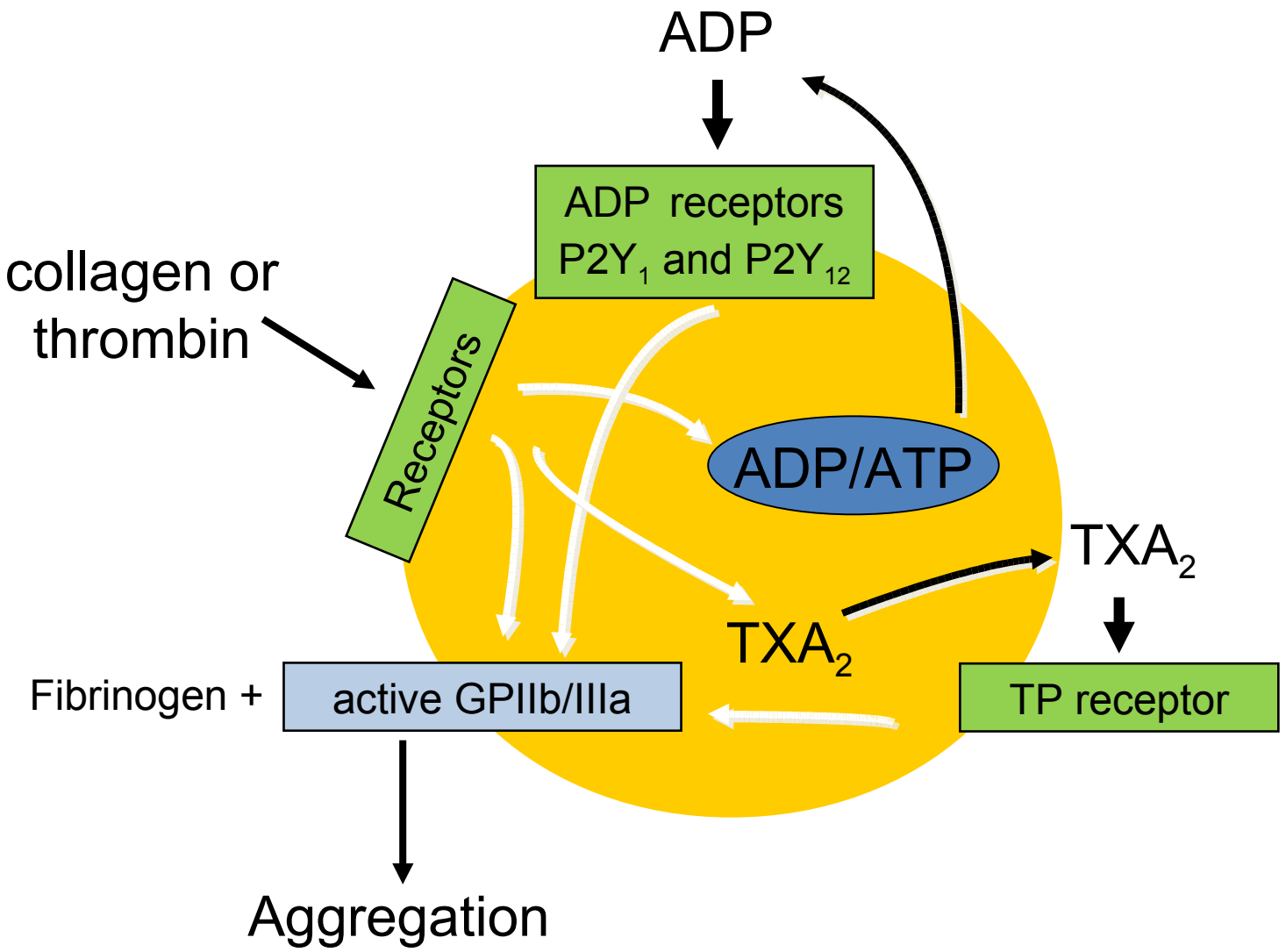
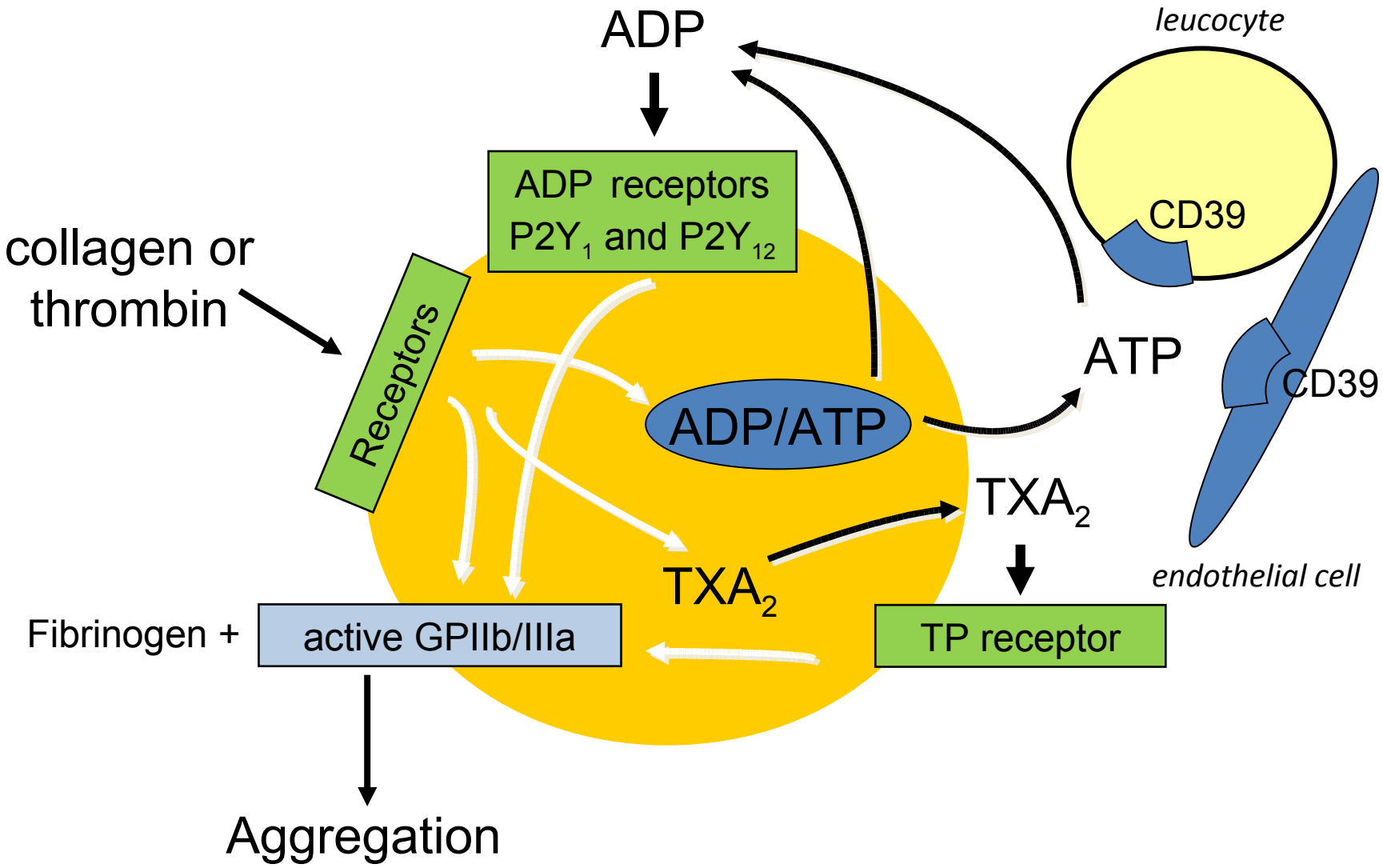
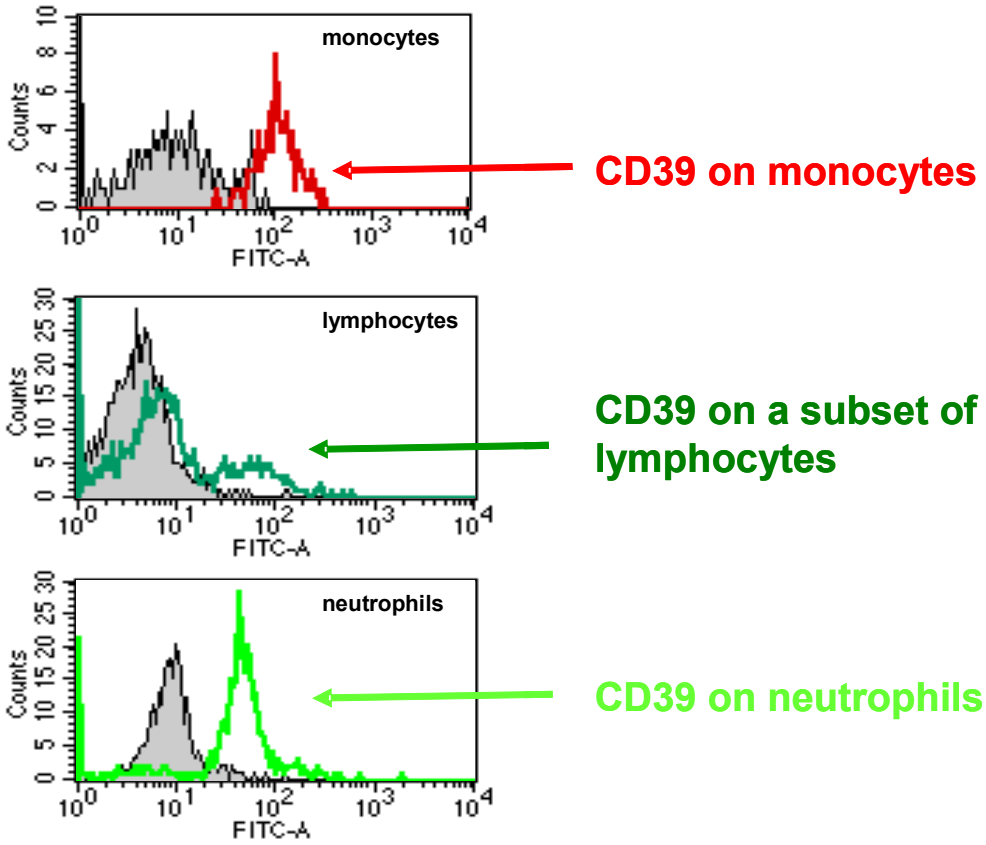


Collagen/thrombin: mechanism of action

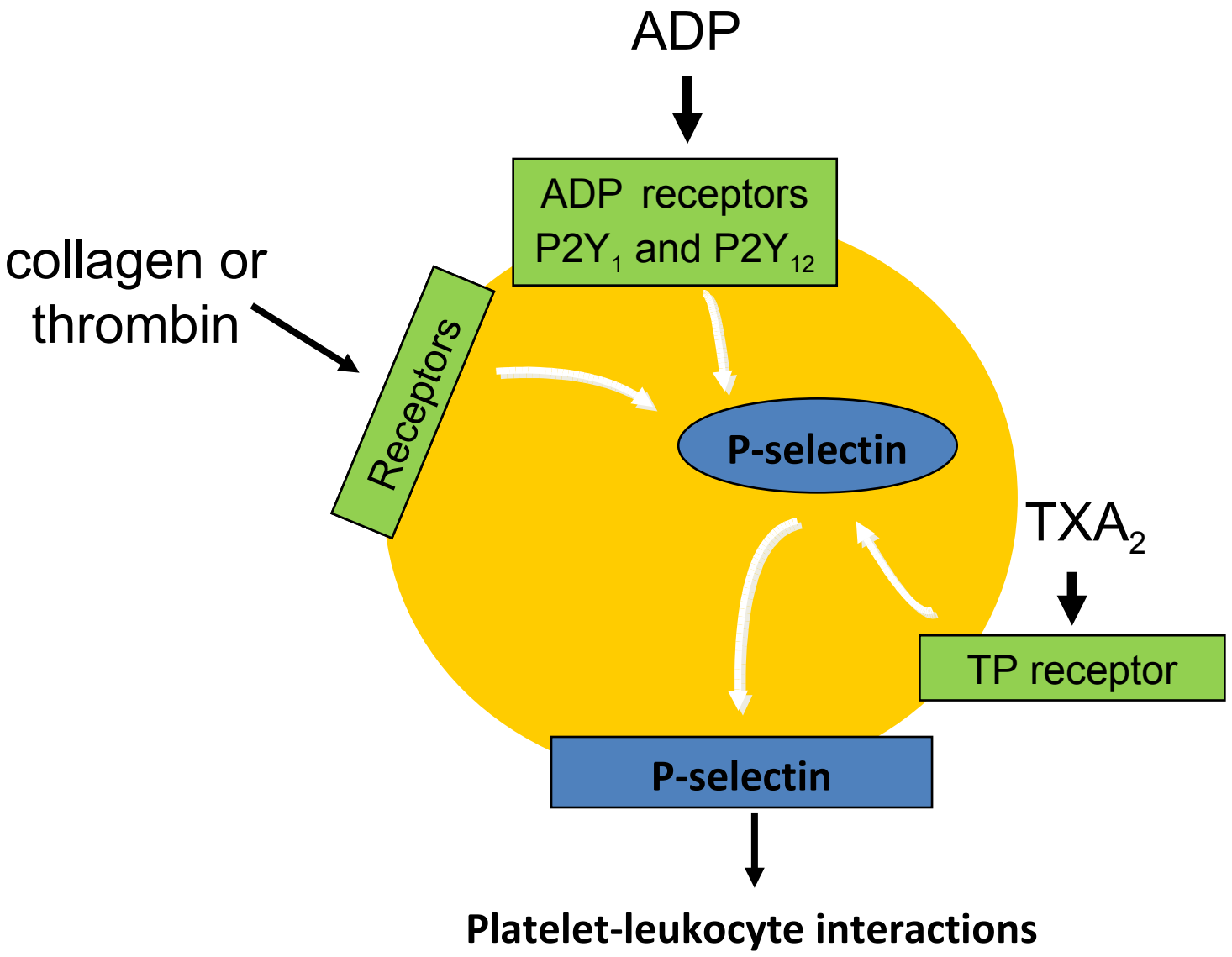


Leucocytes (all neutrophils, all monocytes and some lymphocytes) test positive for CD39



Glenn et al, Platelets 2008;19:59-69

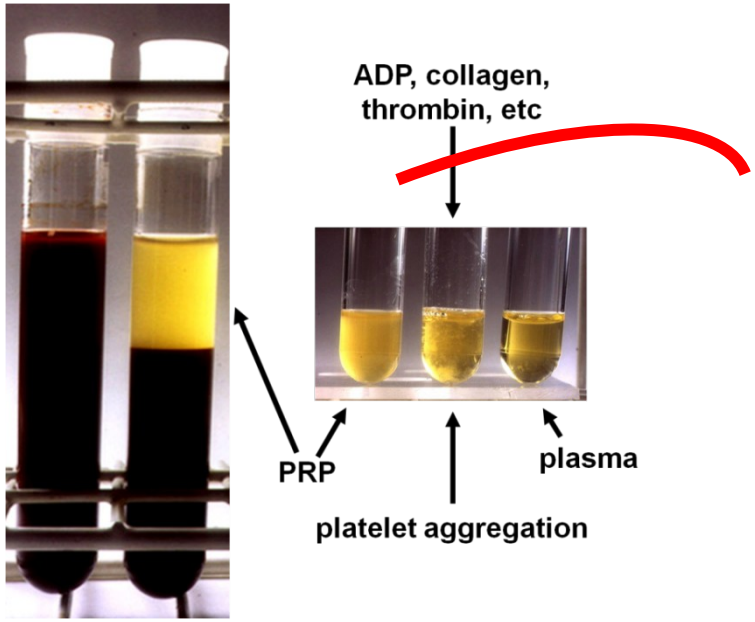
Collagen/thrombin: mechanism of action



Some natural agents that influence platelet function

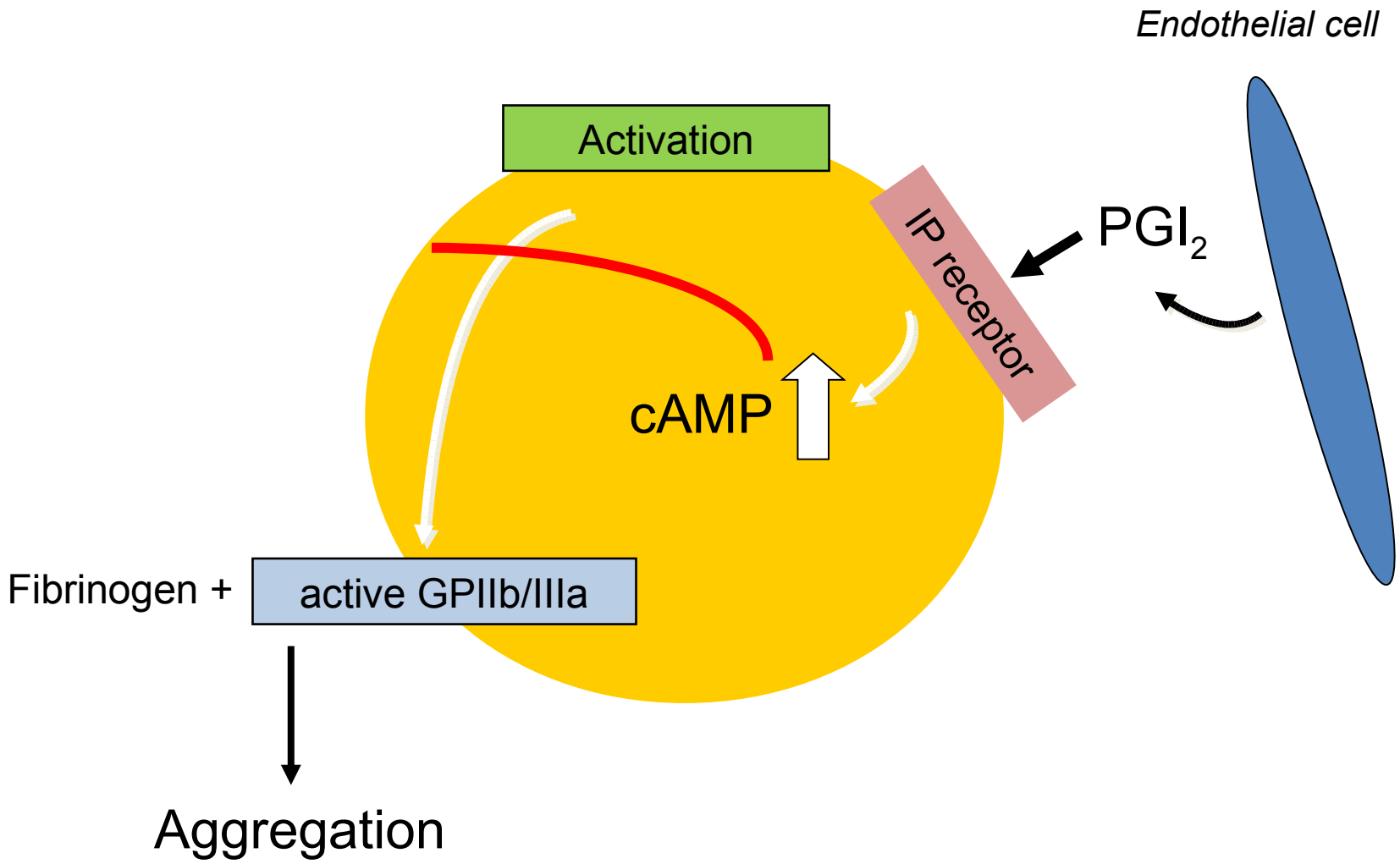
Promoters of platelet function	Inhibitors of platelet function
thrombin	prostaglandin I_2
collagen	prostaglandin E_1
thromboxane A_2	prostaglandin D_2
adenosine diphosphate	nitric oxide
	adenosine?
	prostaglandin E_2?

Inhibitors of platelet function

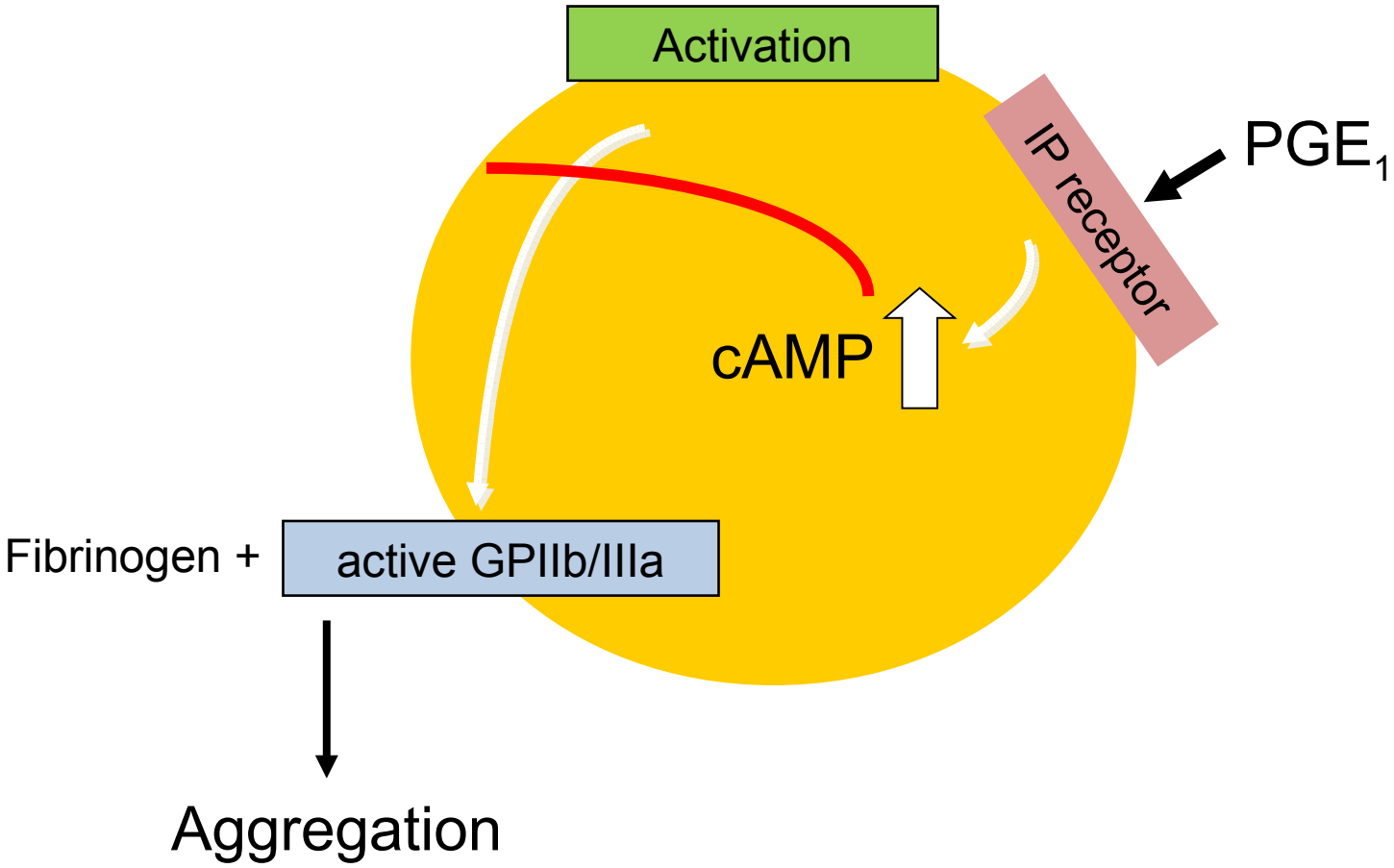


PGI_2	IP	▲cAMP
PGE_1	IP	▲ cAMP
	EP3	▼ cAMP
PGD_2	DP	▲cAMP
NO		▲cGMP
adenosine?	A2A	▲cAMP
	ENT	▼cAMP
PGE_2?	EP4	▲cAMP
	EP3	▼cAMP

PGI_2: mechanism of action



PGE_1: mechanism of action



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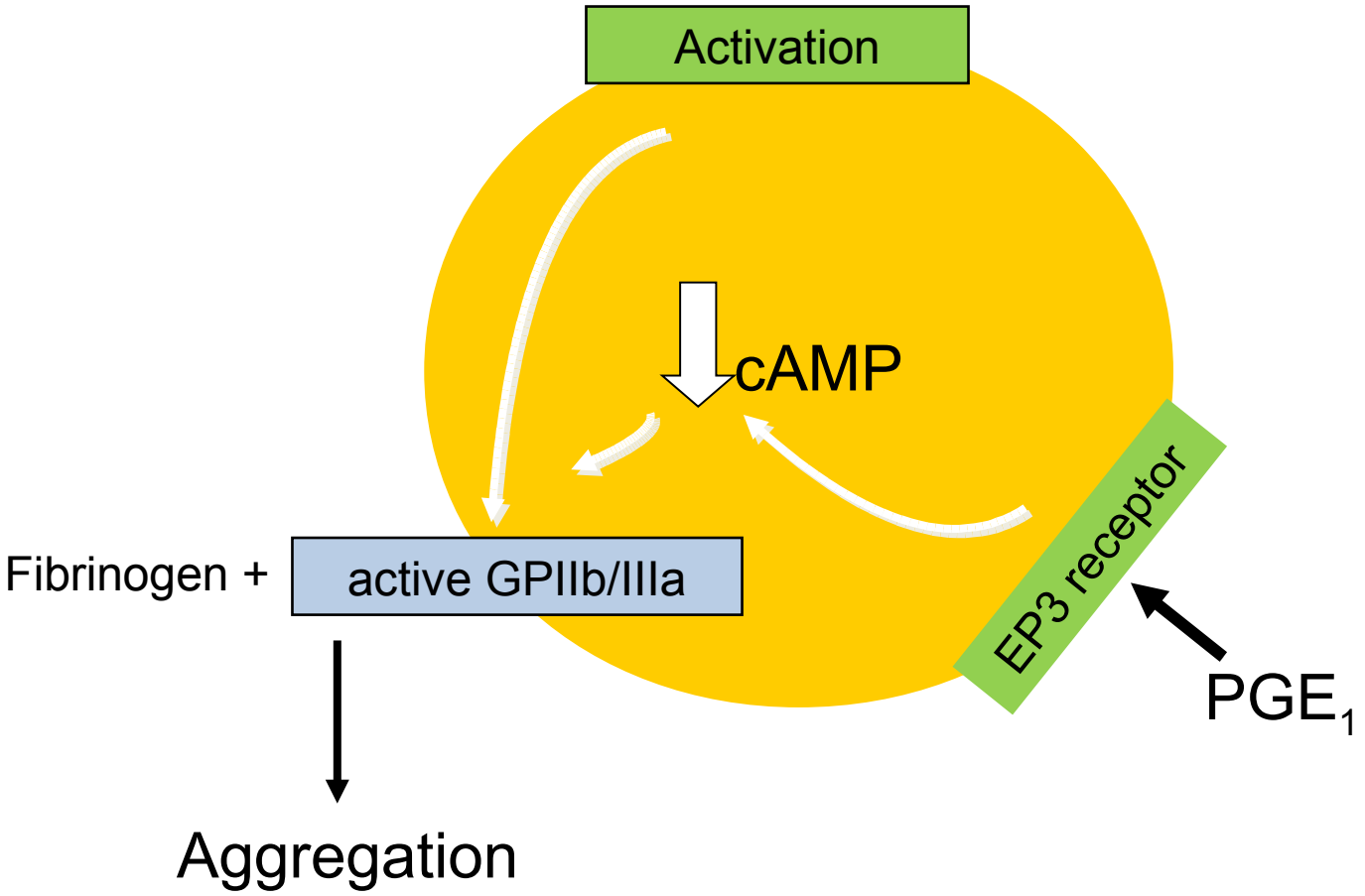

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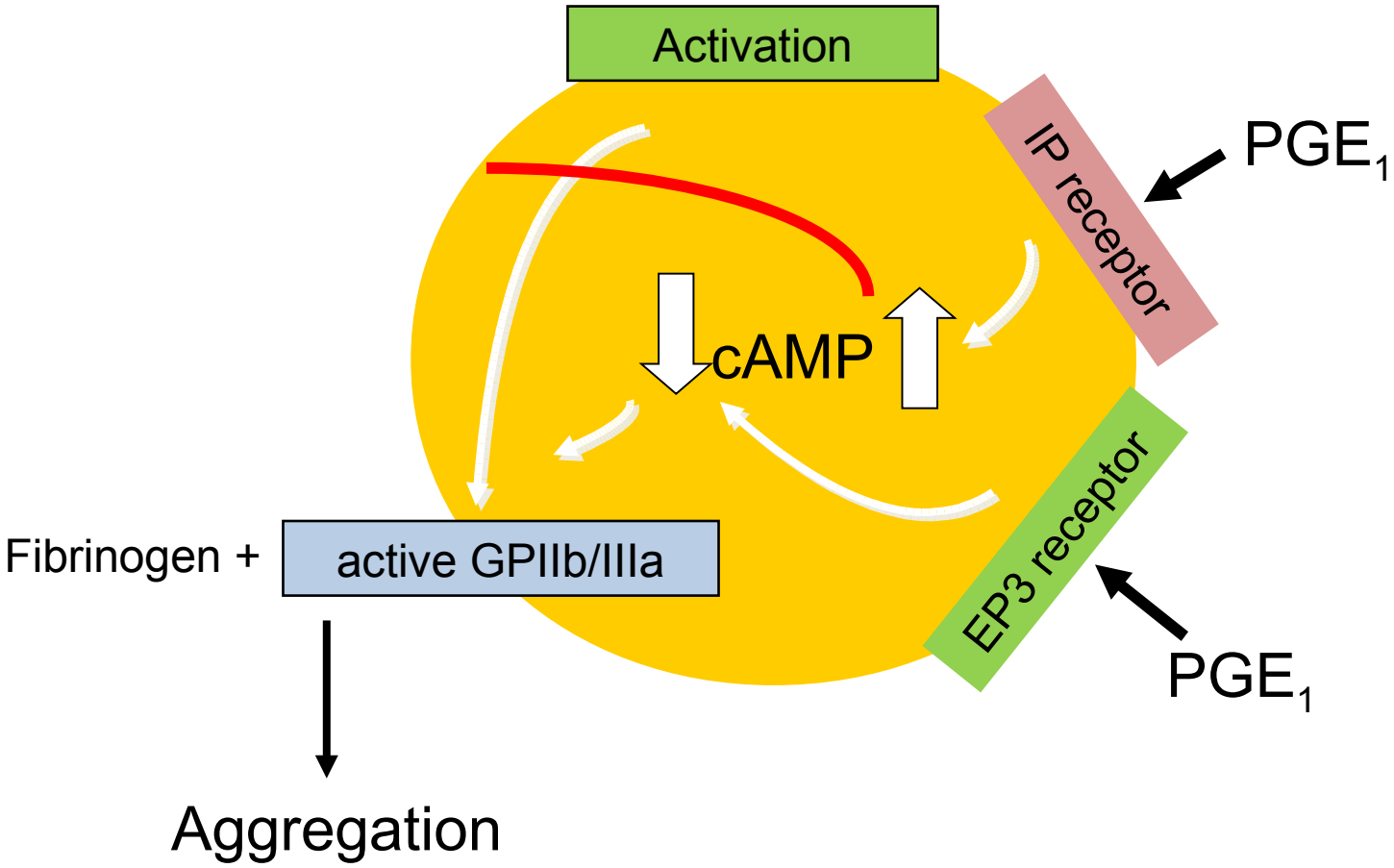
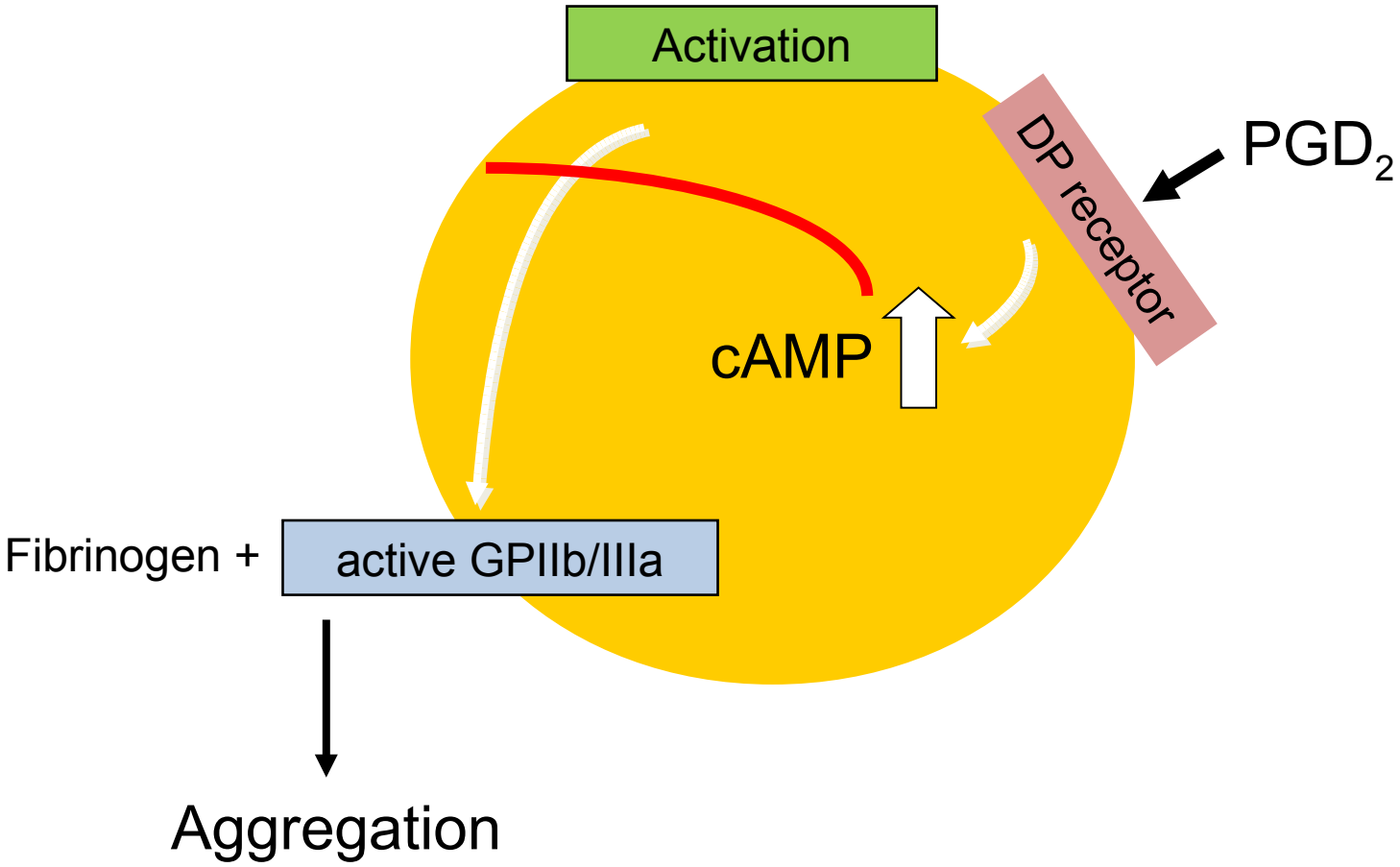
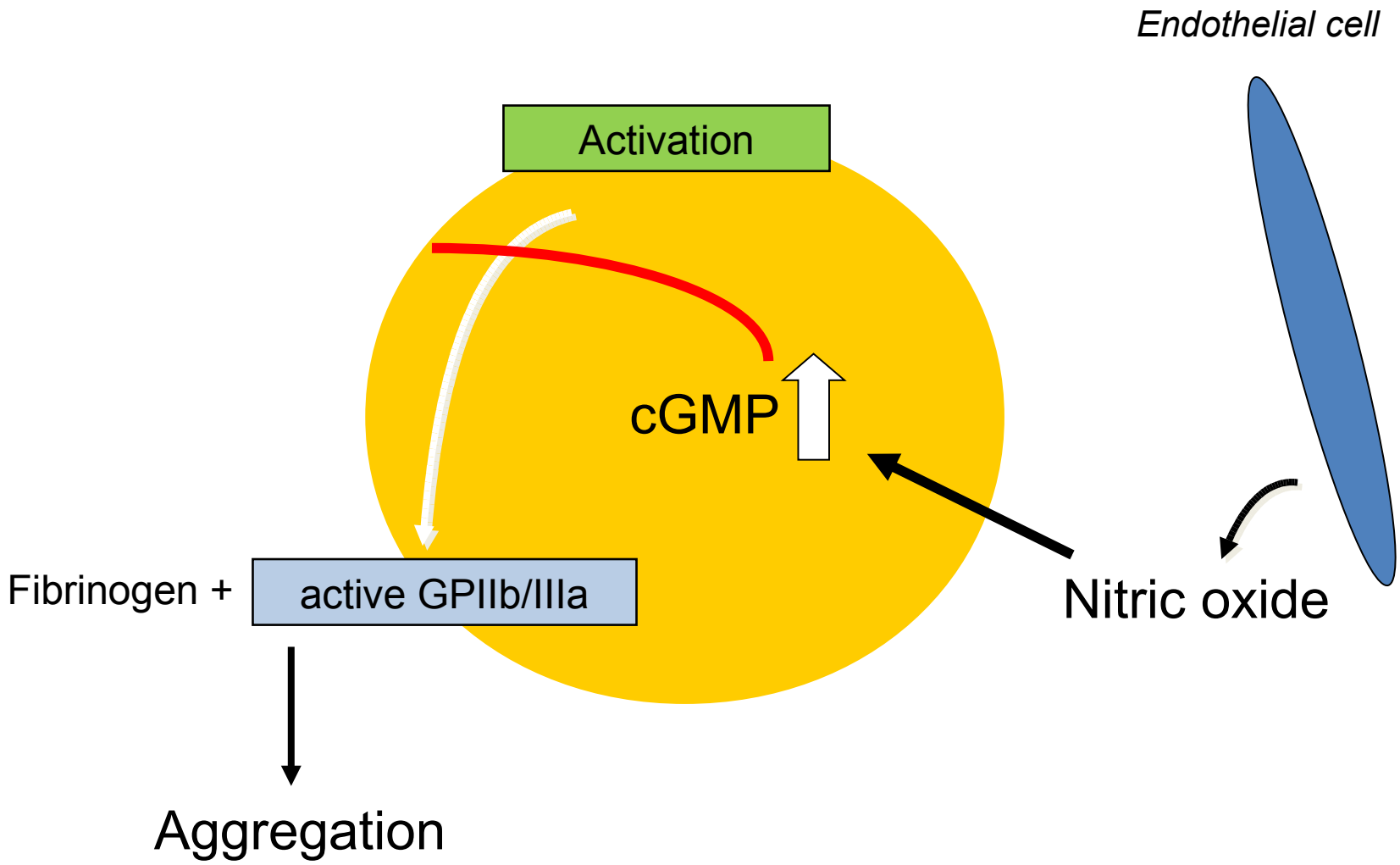


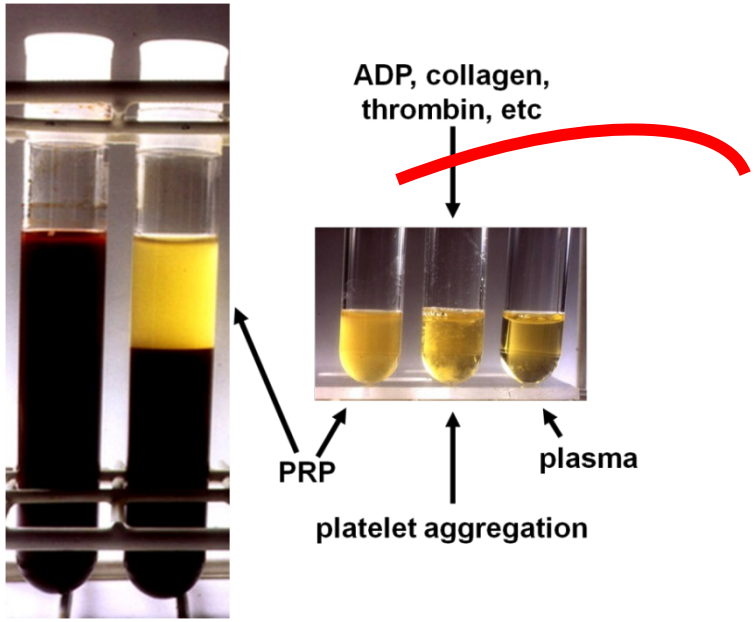
PGD_2: mechanism of action



Nitric oxide: mechanism of action



Inhibitors of platelet function



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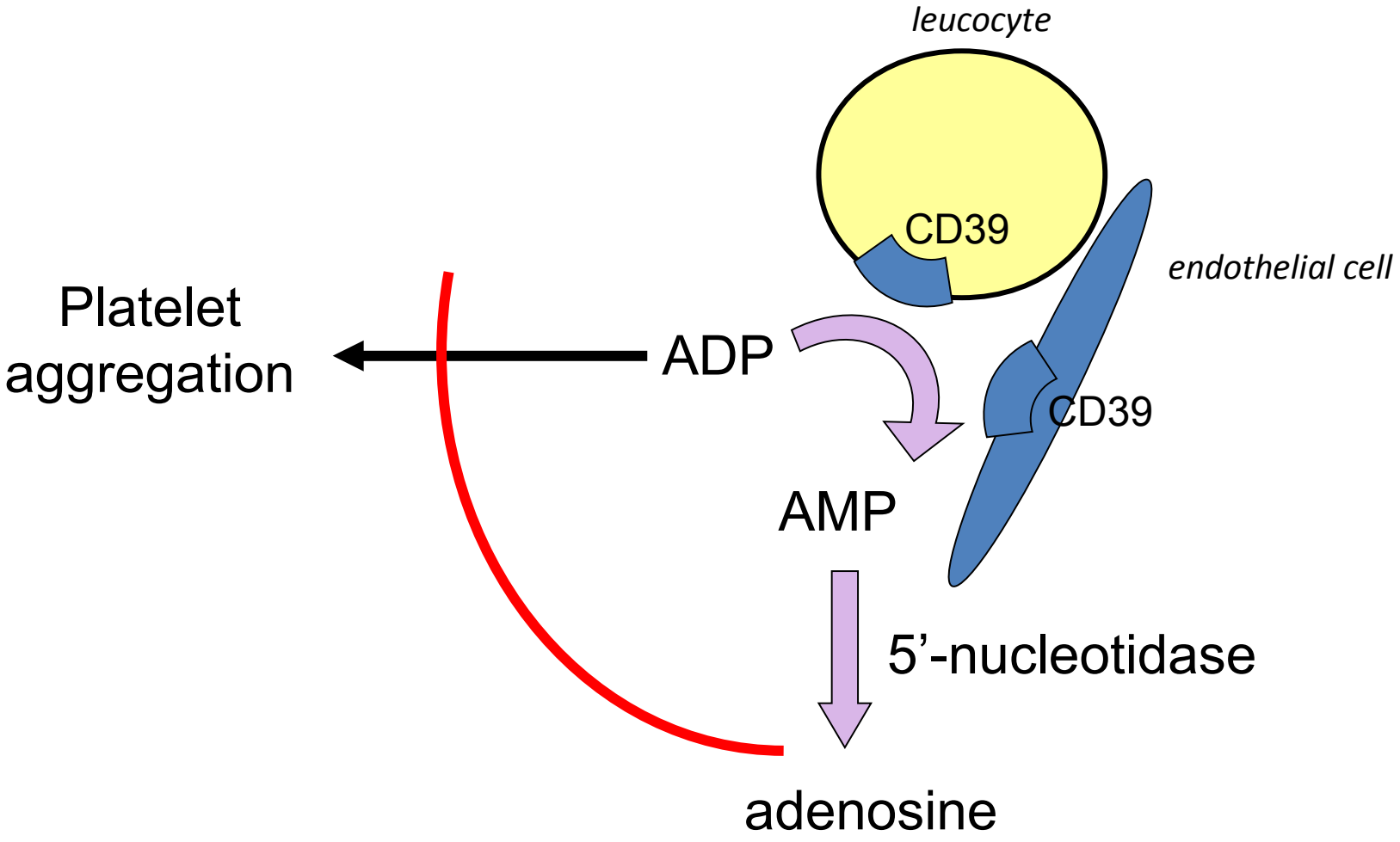
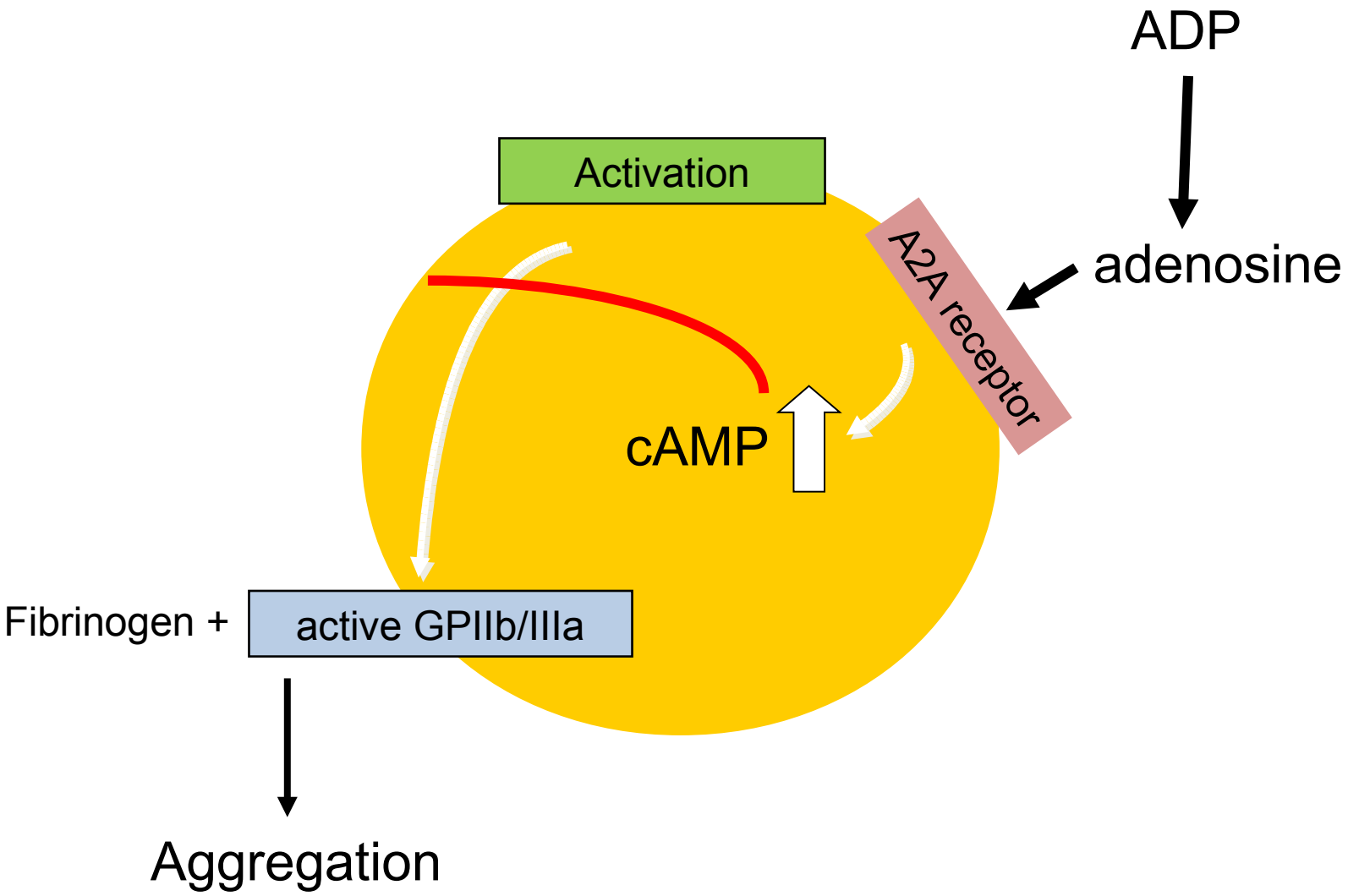
NO		▲cGMP
adenosine?	A2A	▲cAMP
	ENT	▼cAMP
PGE_2?	EP4	▲cAMP
	EP3	▼cAMP

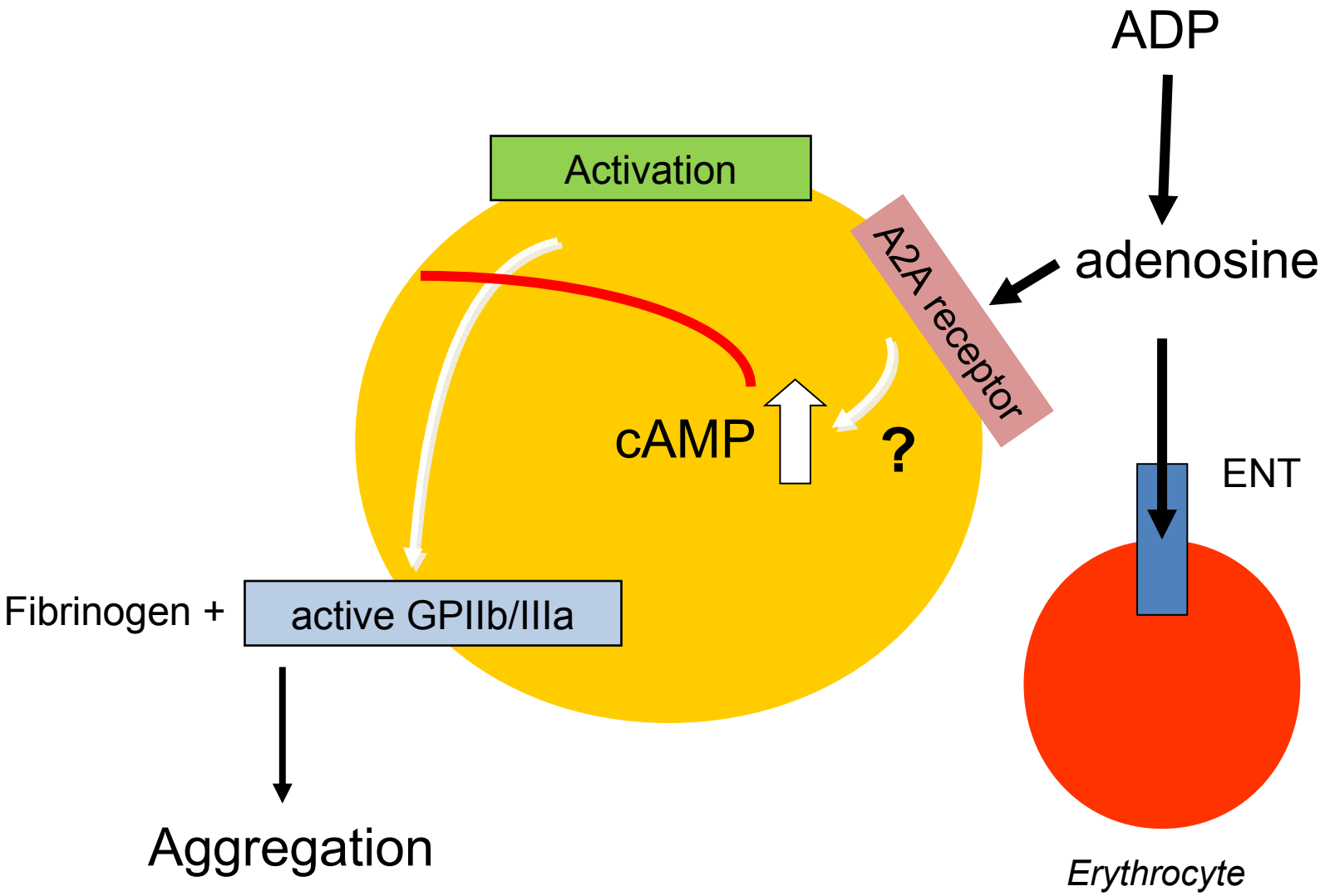
Inhibition of platelet function by adenosine derived from ADP?



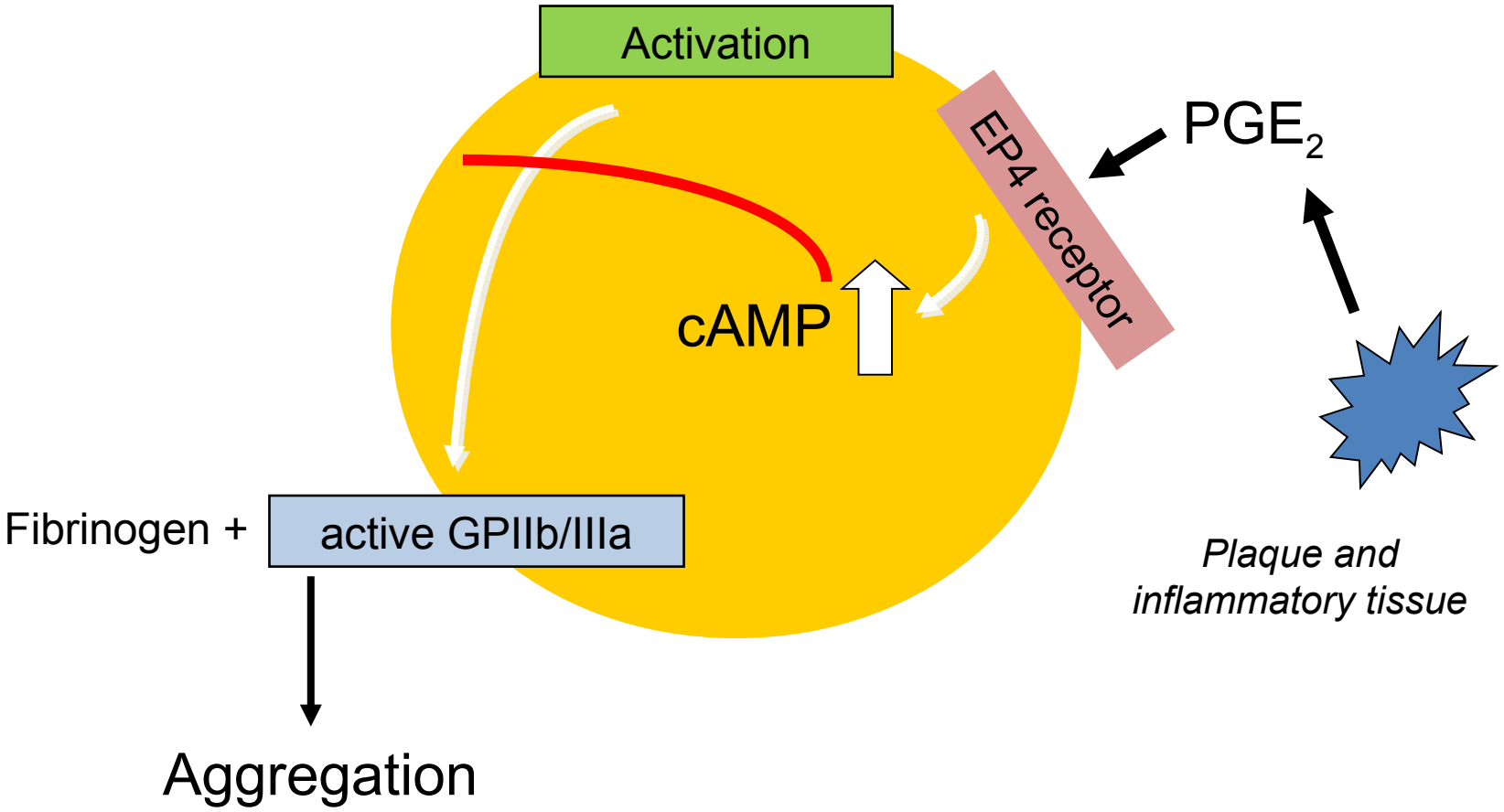
Adenosine: mechanism of action



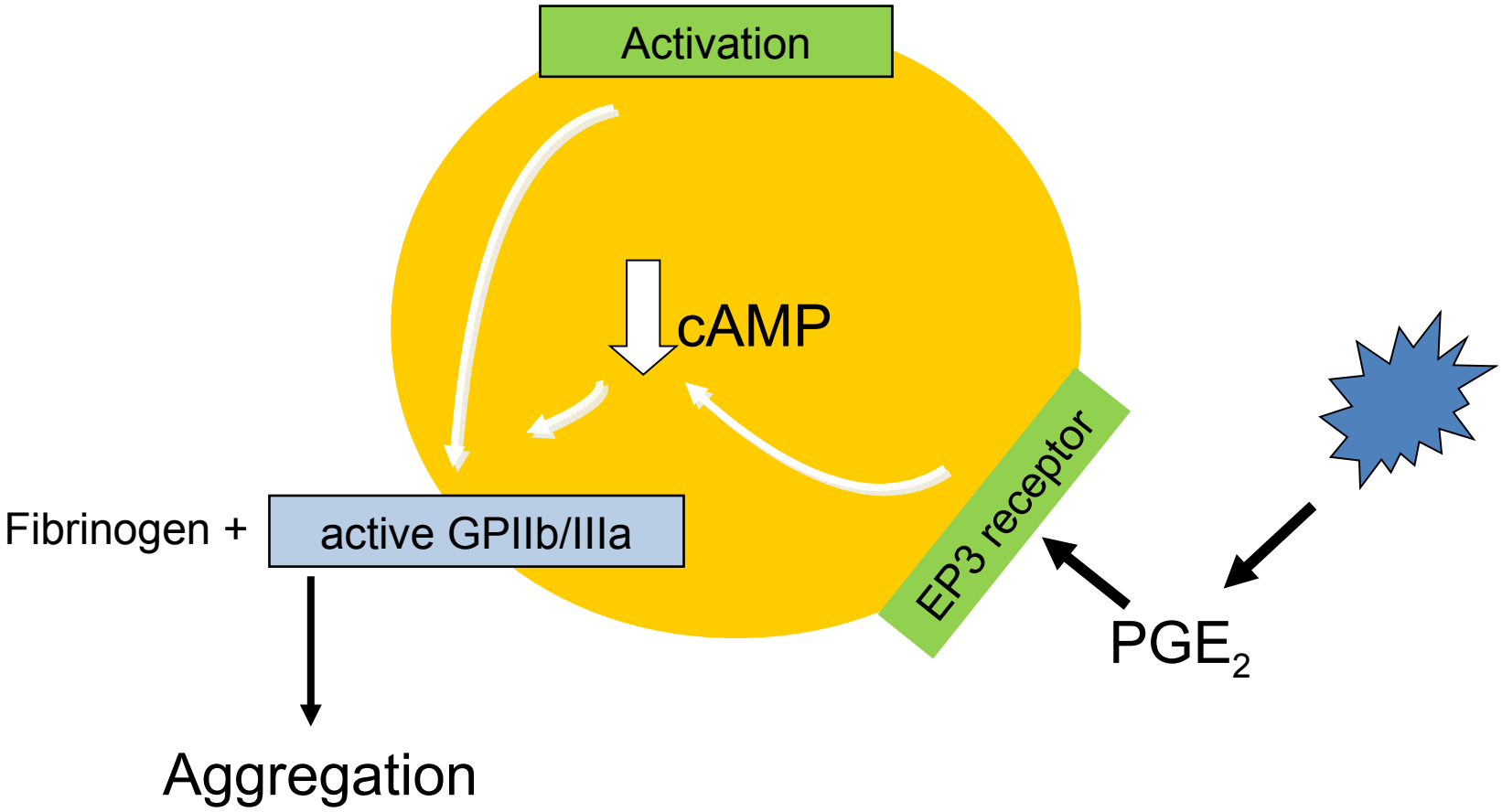
Adenosine: mechanism of action



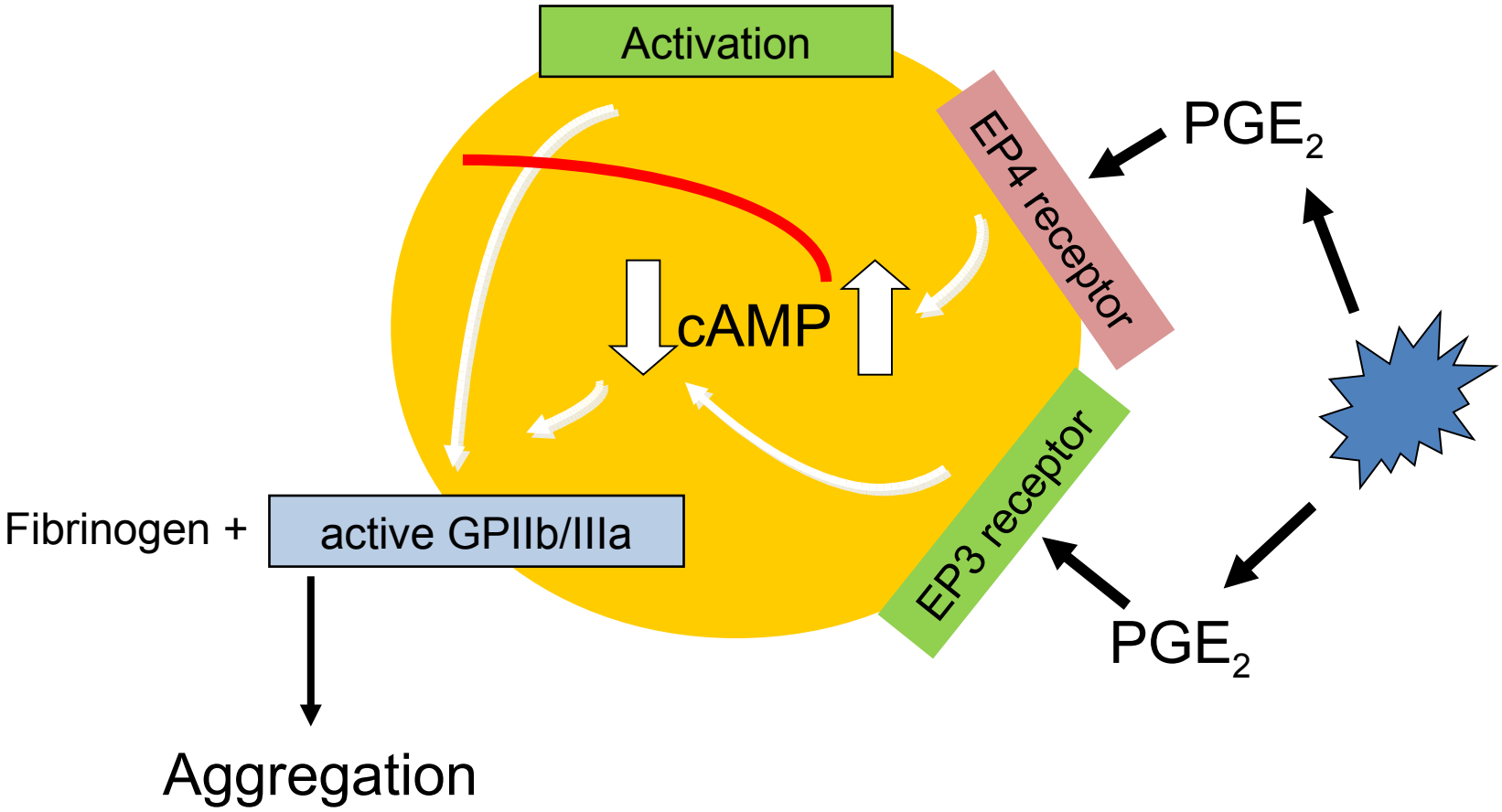
PGE_2: mechanism of action



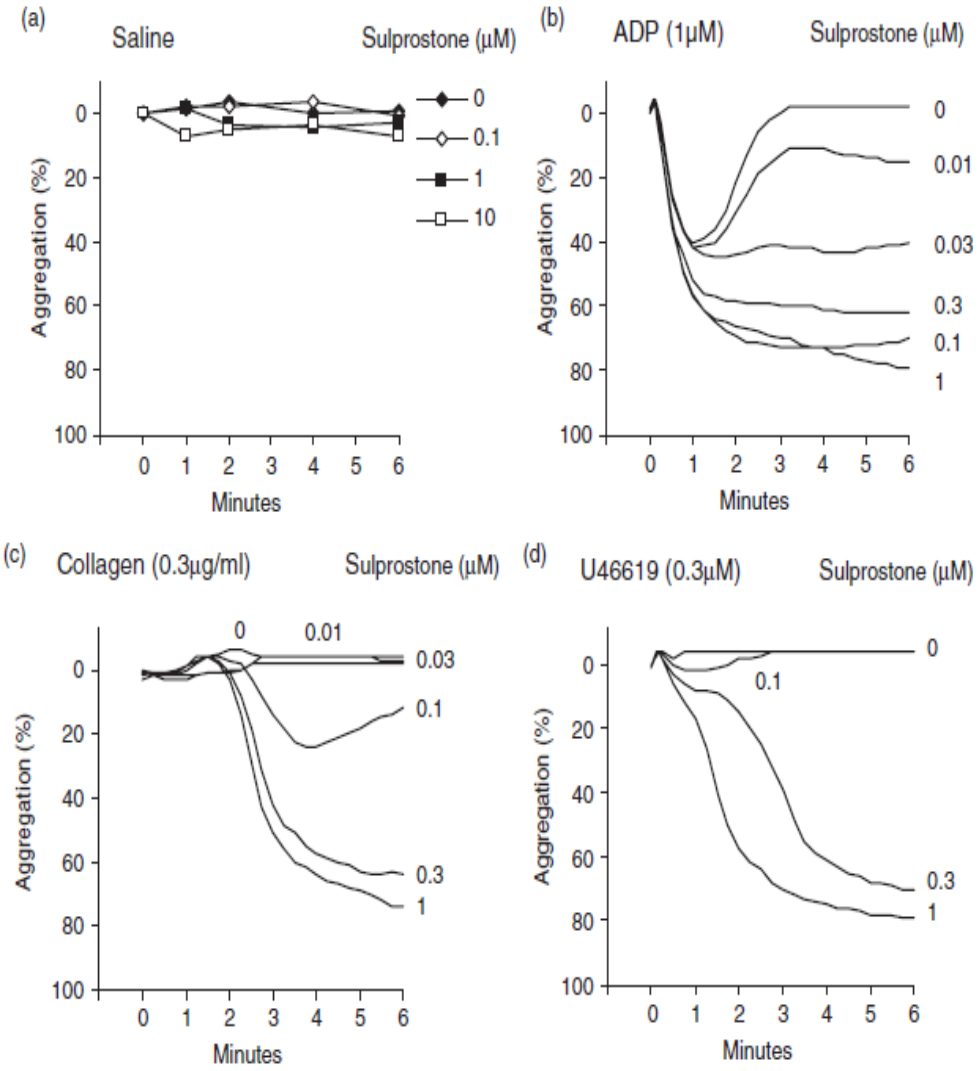
PGE_2: mechanism of action



PGE_2: mechanism of action



EP3 receptors and platelet function

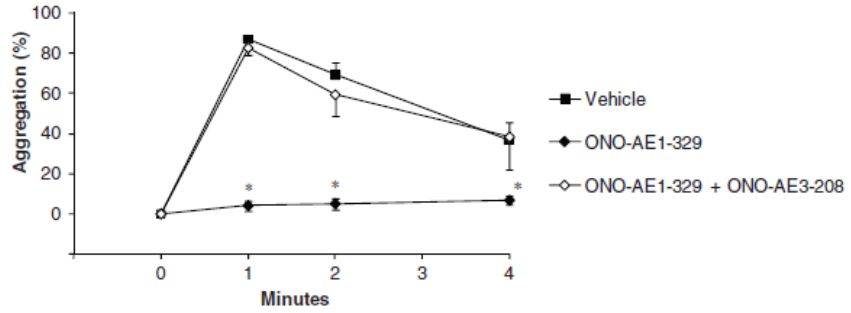


- The EP3 agonist sulprostone promotes platelet function induced by other agonists

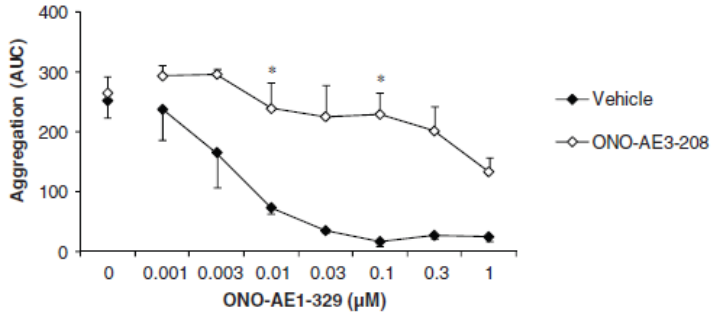
Heptinstall et al, Platelets 2008;19: 605-13

EP4 receptors and platelet function

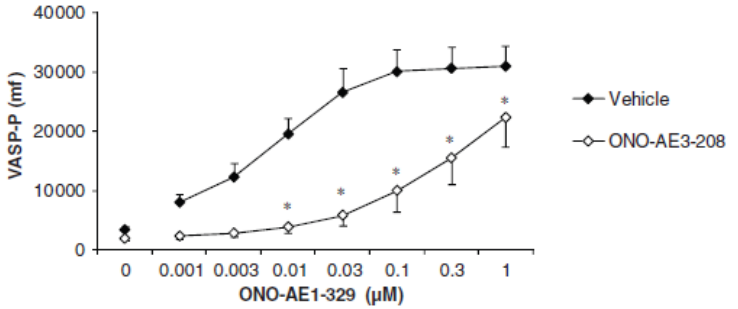
(a) Effects of the EP4 agonist ONO-AE1-329 and the EP4 antagonist ONO-AE3-208 on platelet aggregation (% aggregation)



(b) Effects of the EP4 agonist ONO-AE1-329 and the EP4 antagonist ONO-AE3-208 on platelet aggregation (area under the curve)



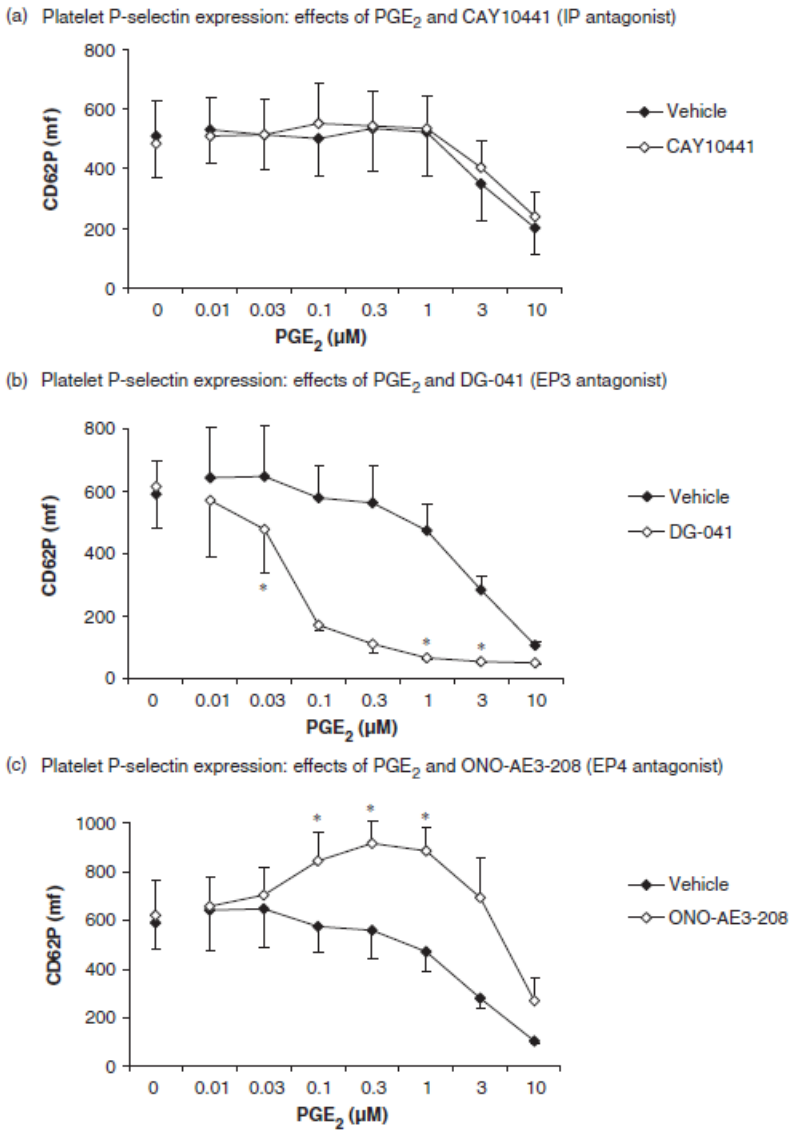
(c) Effects of the EP4 agonist ONO-AE1-329 and the EP4 antagonist ONO-AE3-208 on platelet VASP- phosphorylation



- A selective EP4 agonist inhibits platelet function via increased levels of cAMP

Iyú et al, Platelets 2010;21:329-42

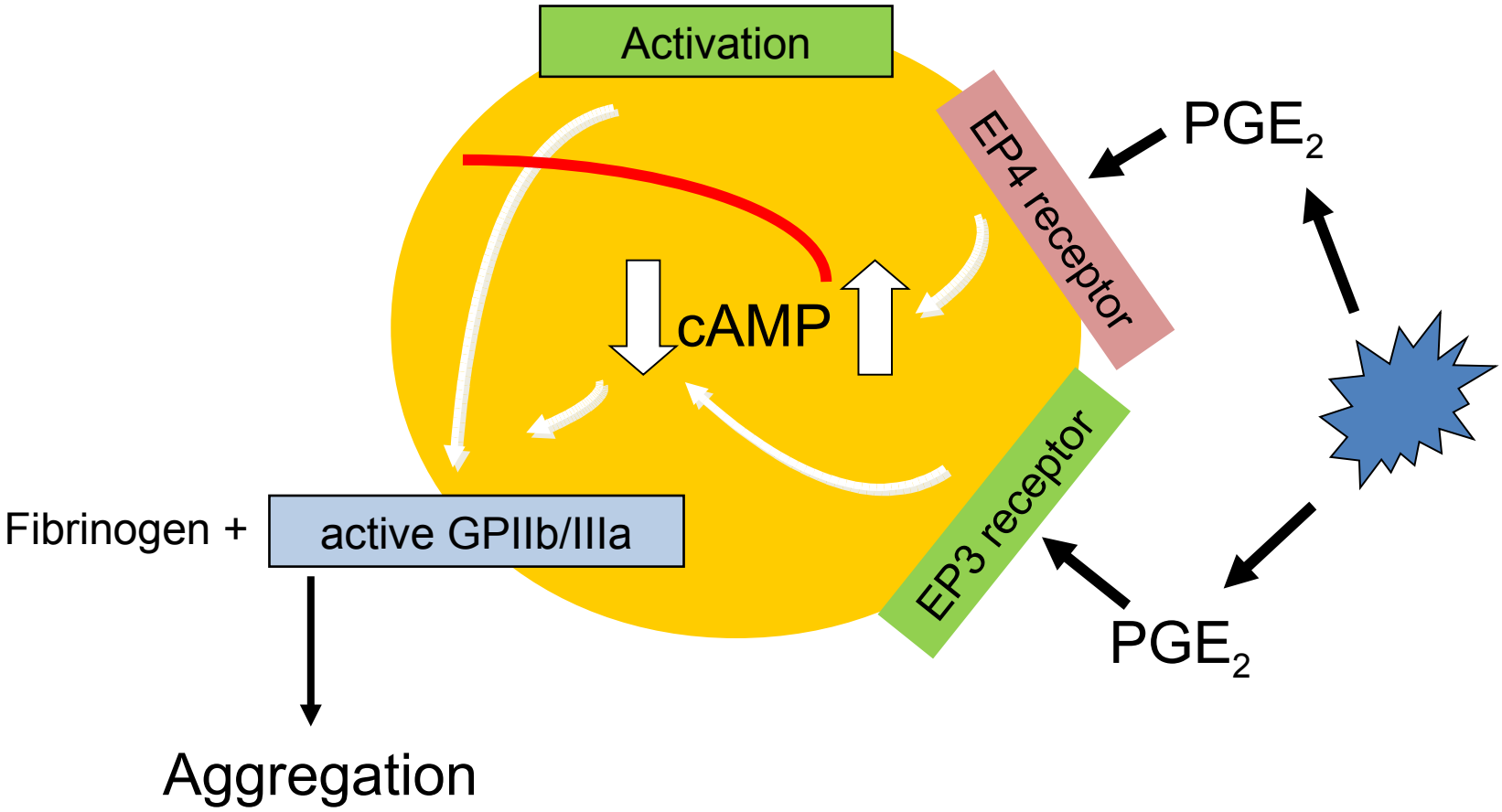
PGE_2: EP4 and EP3 receptors but not the IP receptor



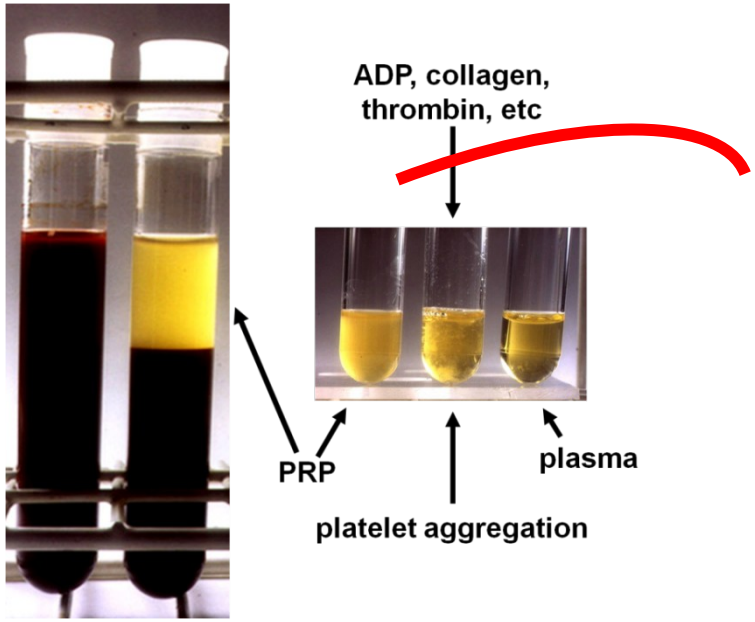
- IP receptor antagonist does not modify the effects of PGE_2 on platelet function
- EP3 receptor antagonist promotes inhibition of platelet function by PGE_2
- EP4 receptor antagonist negates inhibition of platelet function by PGE_2

Iyú et al, Platelets 2010; 21:329-42

PGE_2: mechanism of action



Inhibitors of platelet function



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adenosine?	A2A	▲cAMP
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Some pharmacological agents used as antithrombotic therapy

- Aspirin - inhibition of TXA_2 synthesis via COX-1
- $P2Y_{12}$ antagonists – clopidogrel, prasugrel, ticagrelor, cangrelor
- GPIIb/IIIa antagonists - tirofiban, eptifibatide, abciximab
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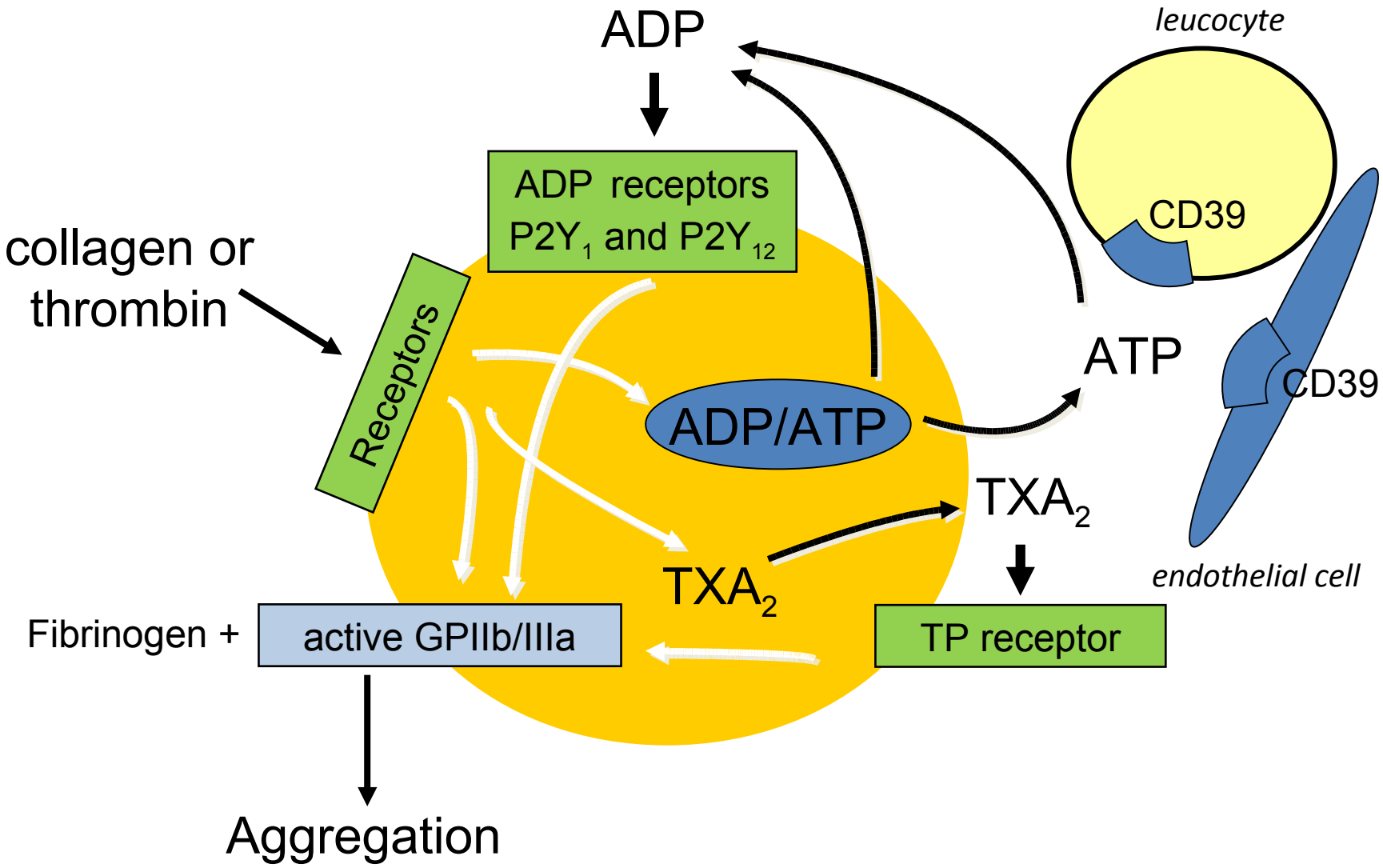
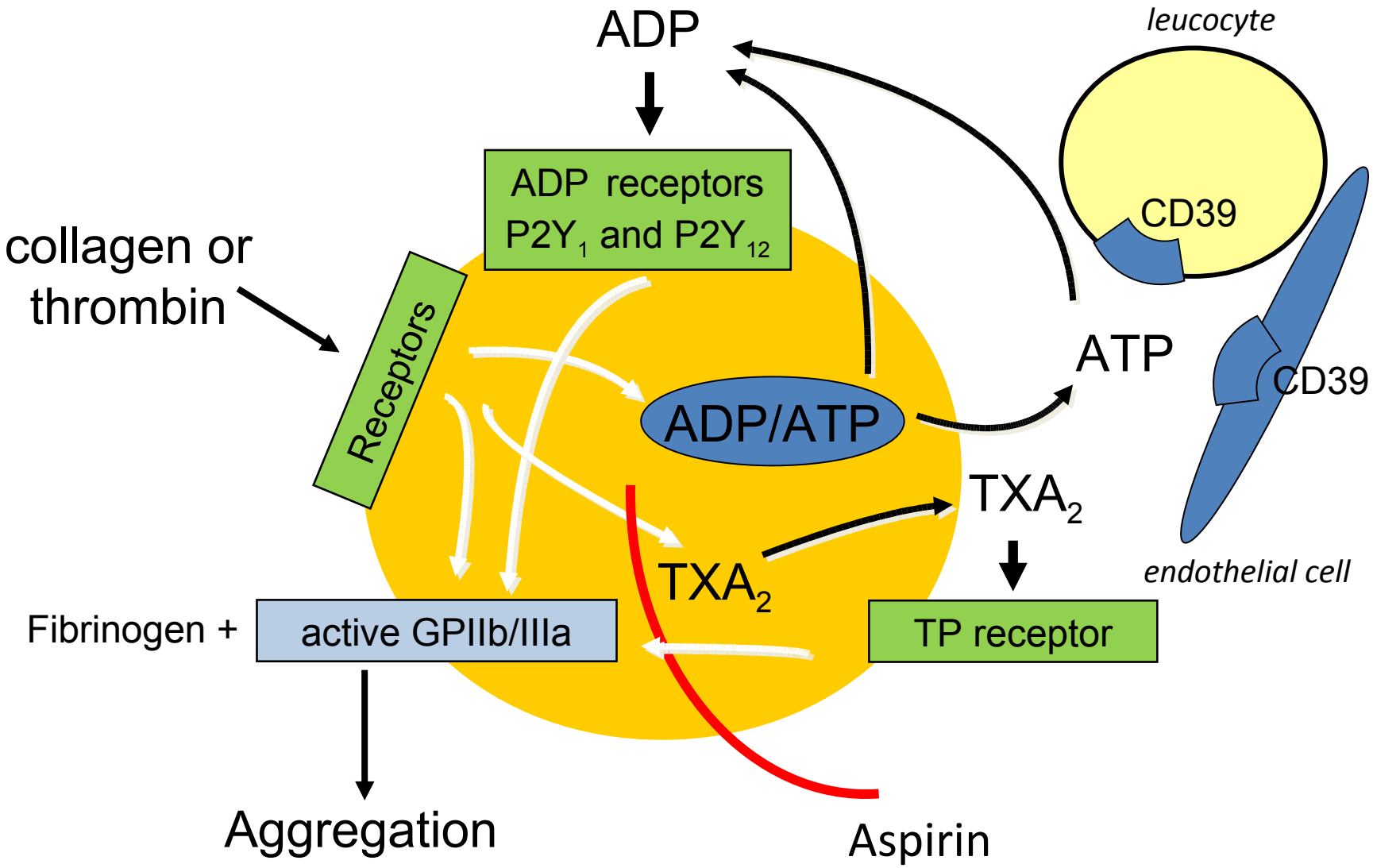
New approaches?

- EP3 antagonists – DG-041?
- Consideration of interactions between $P2Y_{12}$ antagonists and vascular prostaglandins – avoidance of co-administration of high dose aspirin?

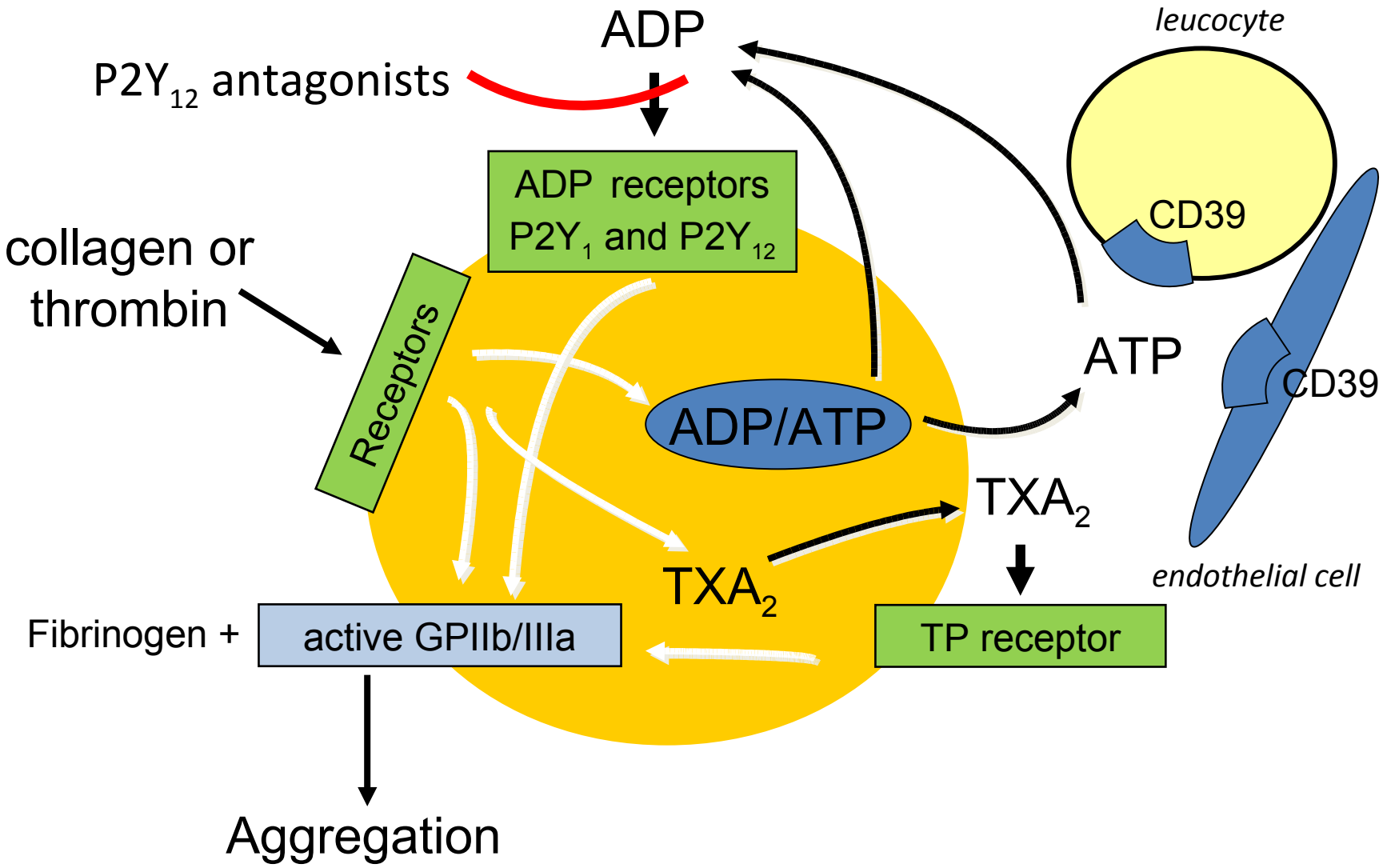
Antiplatelet agents: mechanism of action



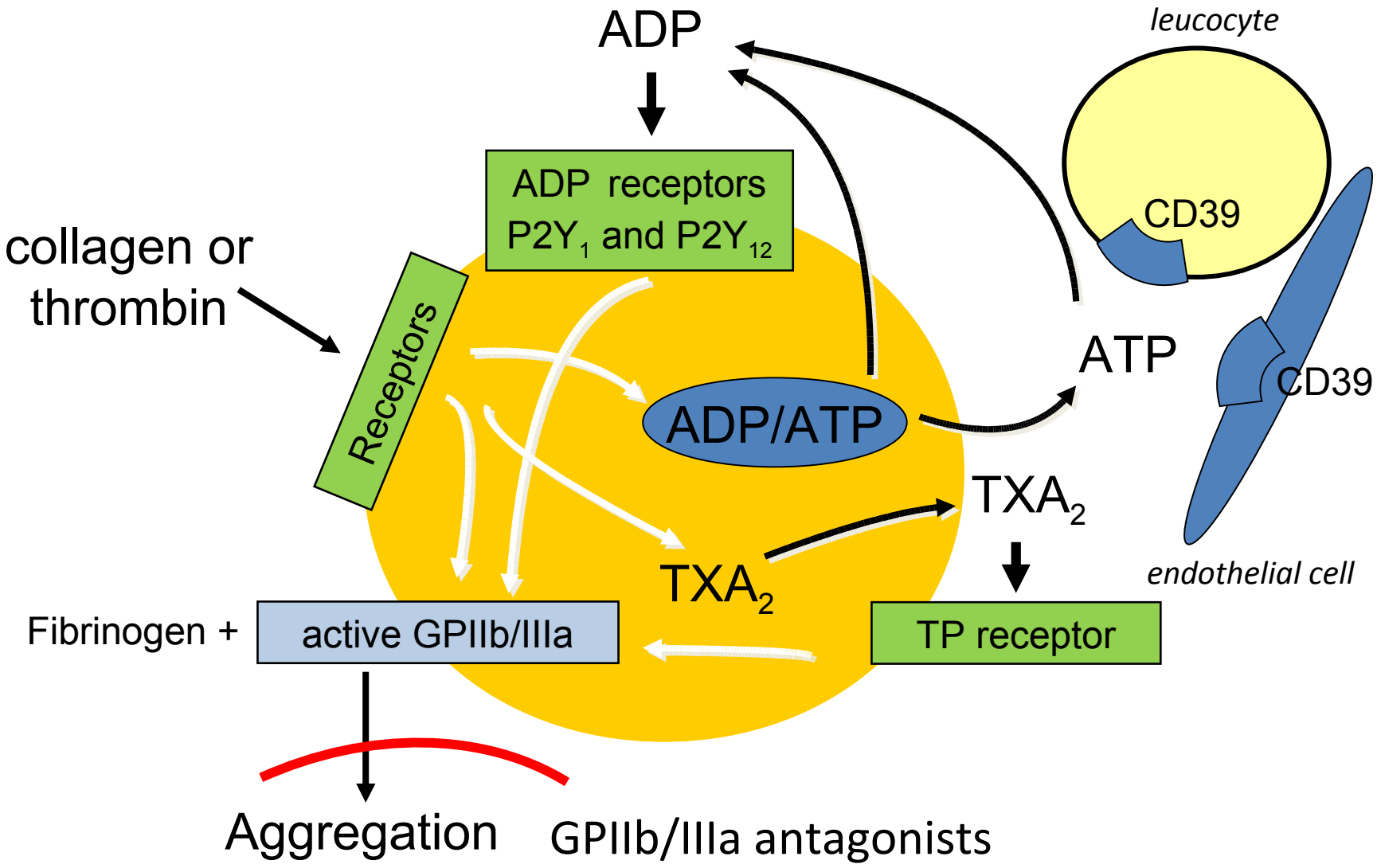
Aspirin: mechanism of action



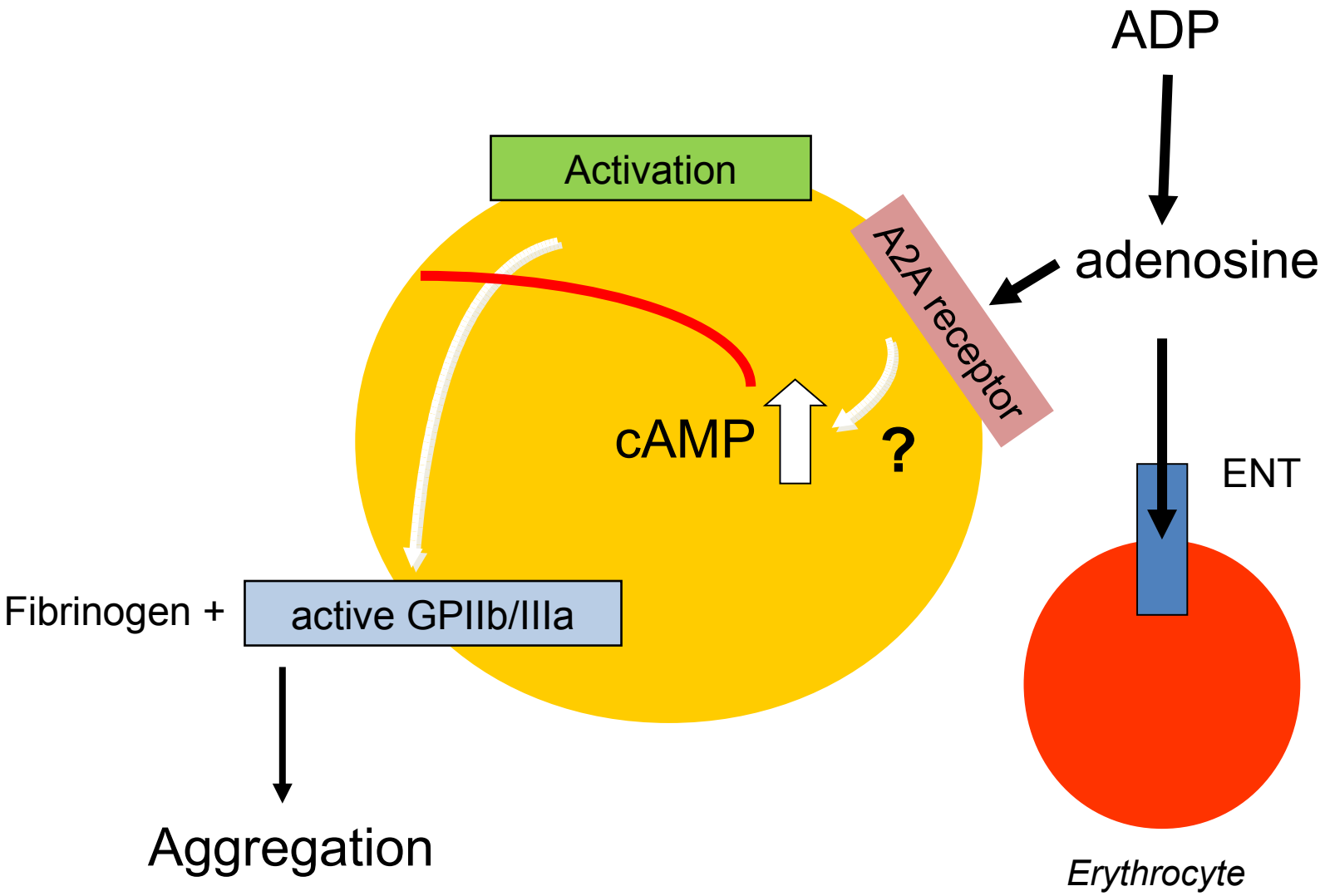
$P2Y_{12}$ antagonists: mechanism of action



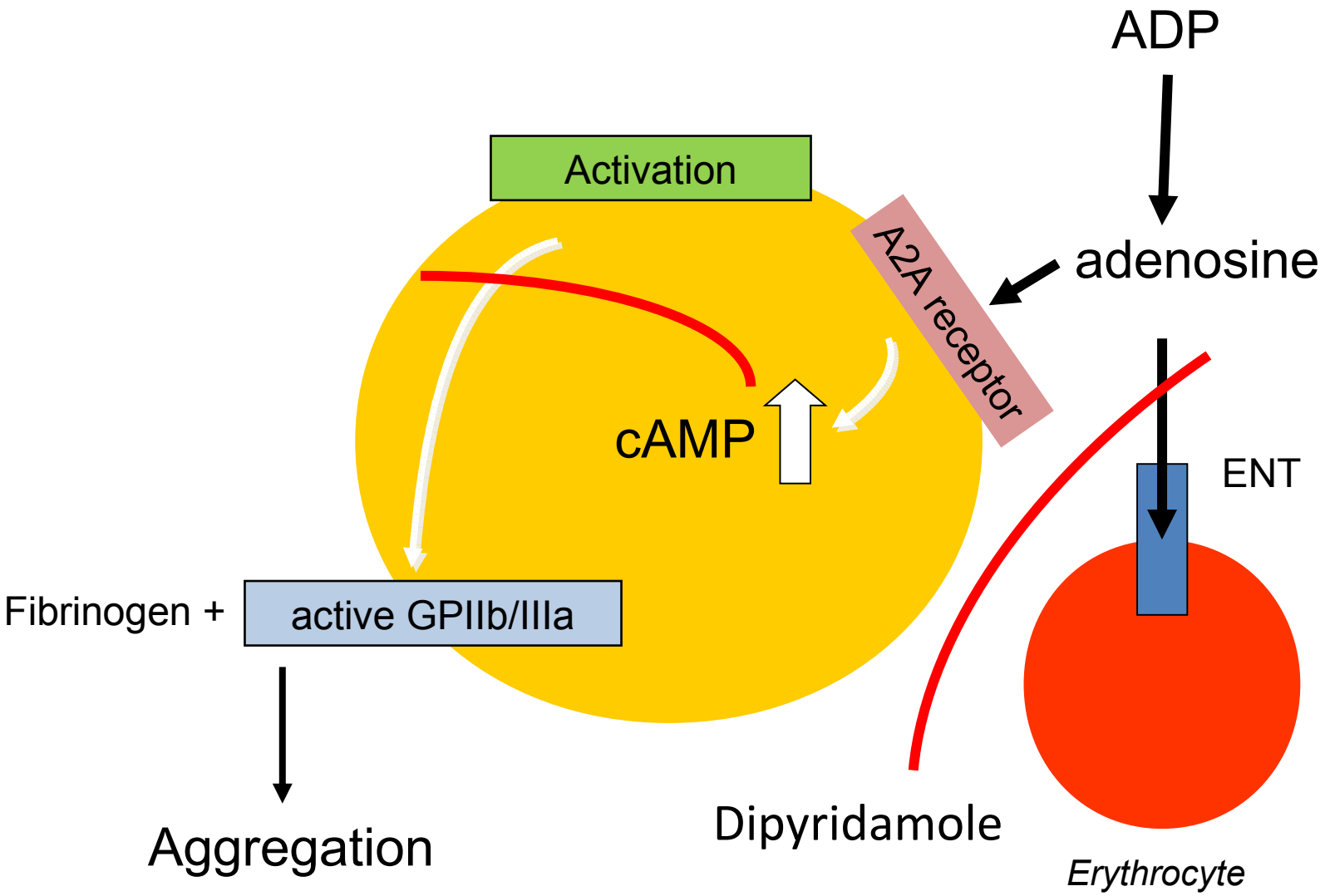
GPIIb/IIIa antagonists: mechanism of action



adenosine: mechanism of action



Dipyridamole: mechanism of action



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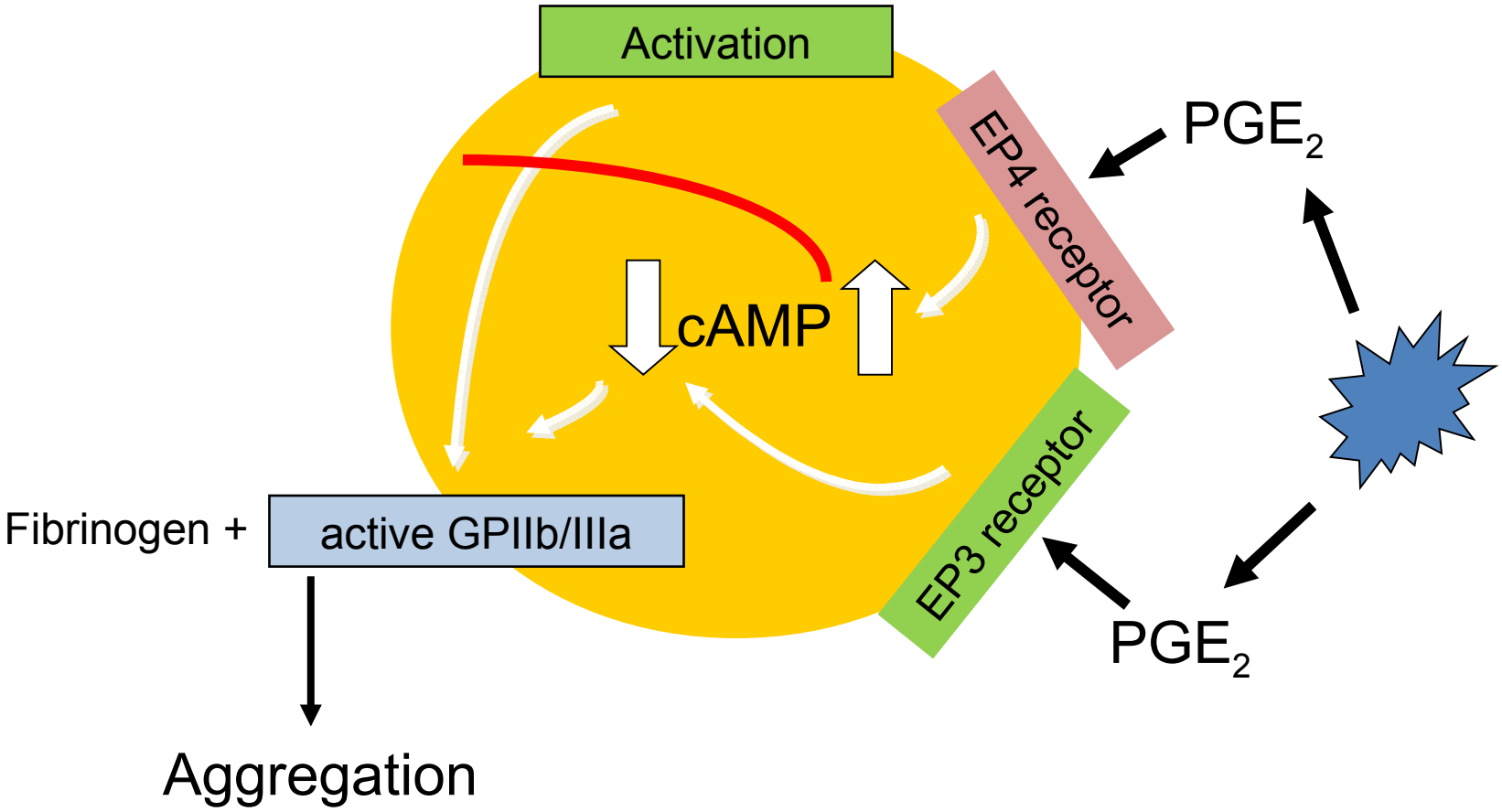
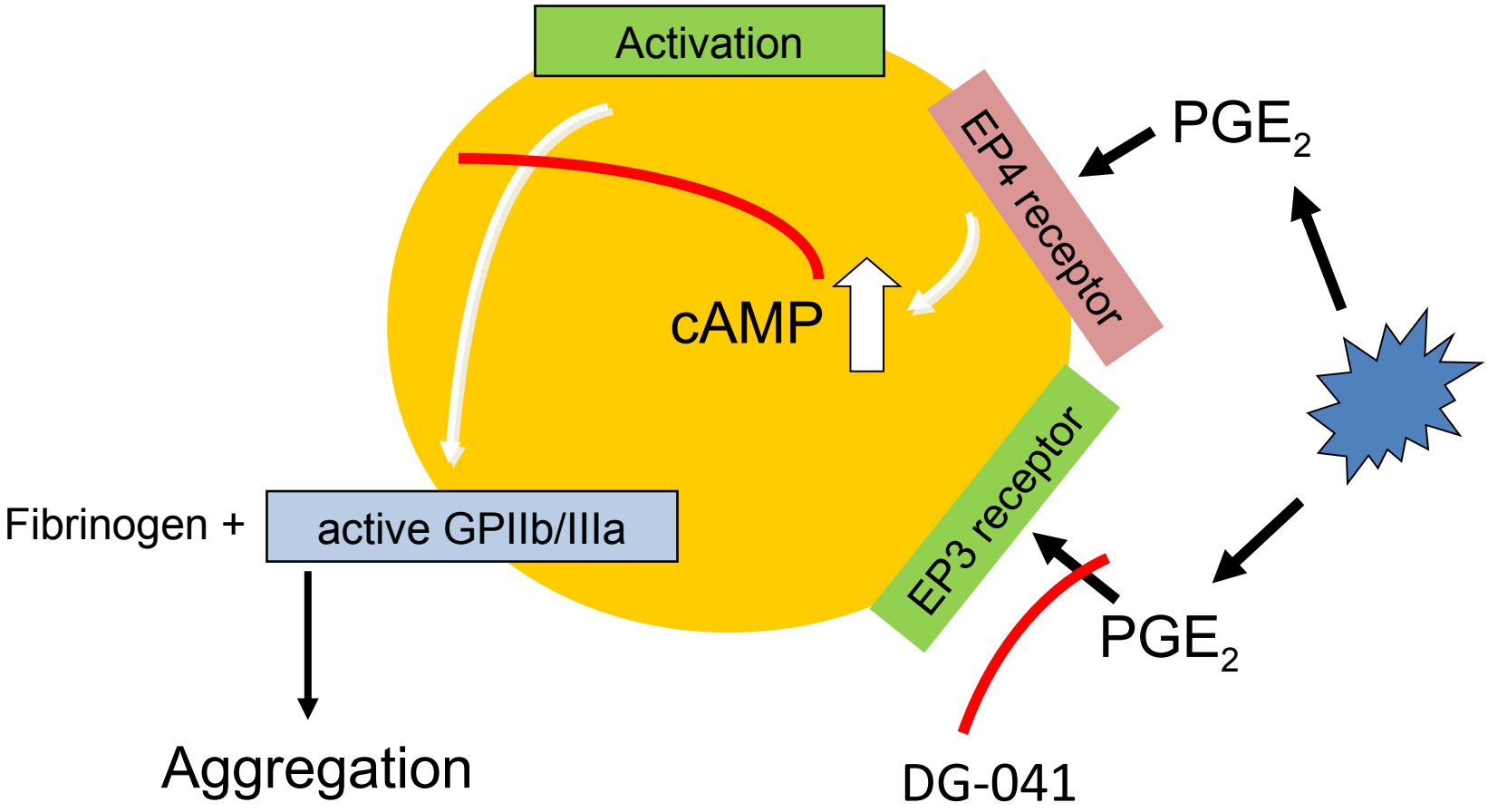
PGE_2: mechanism of action



Inhibition of platelet function by an EP3 antagonist



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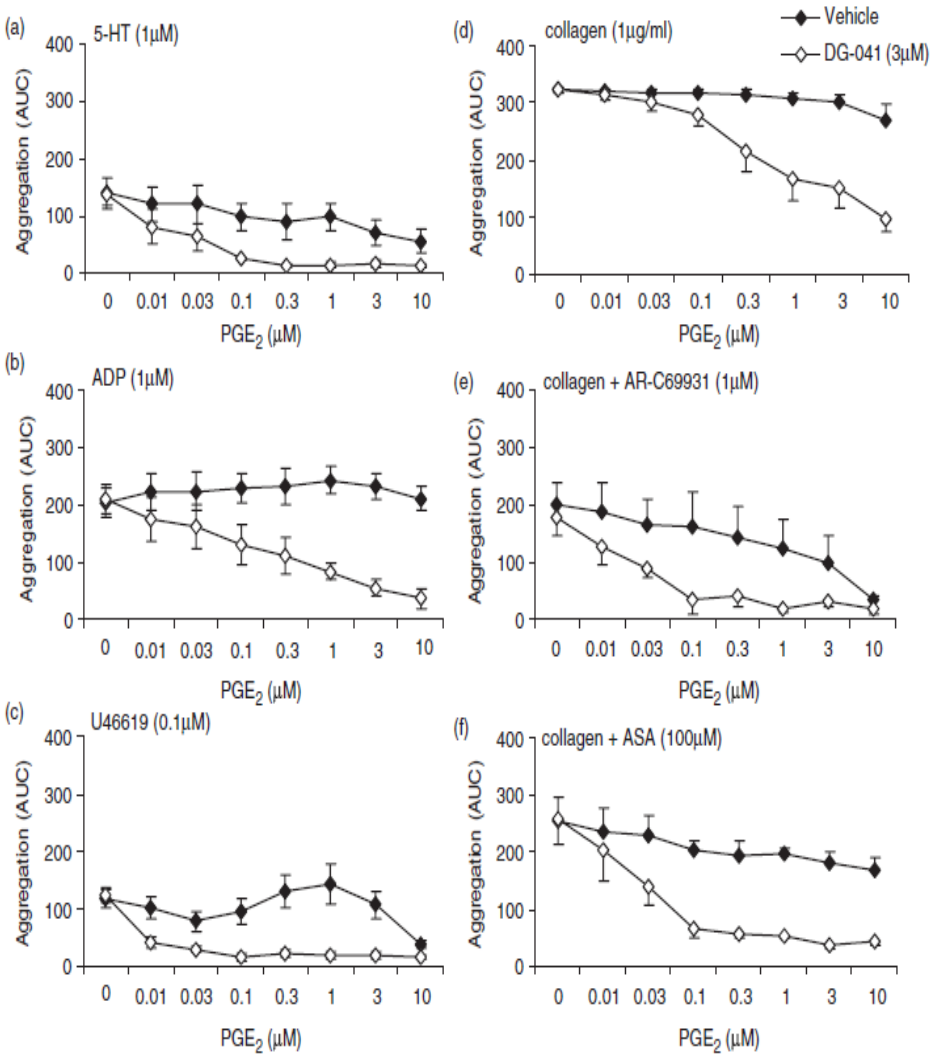
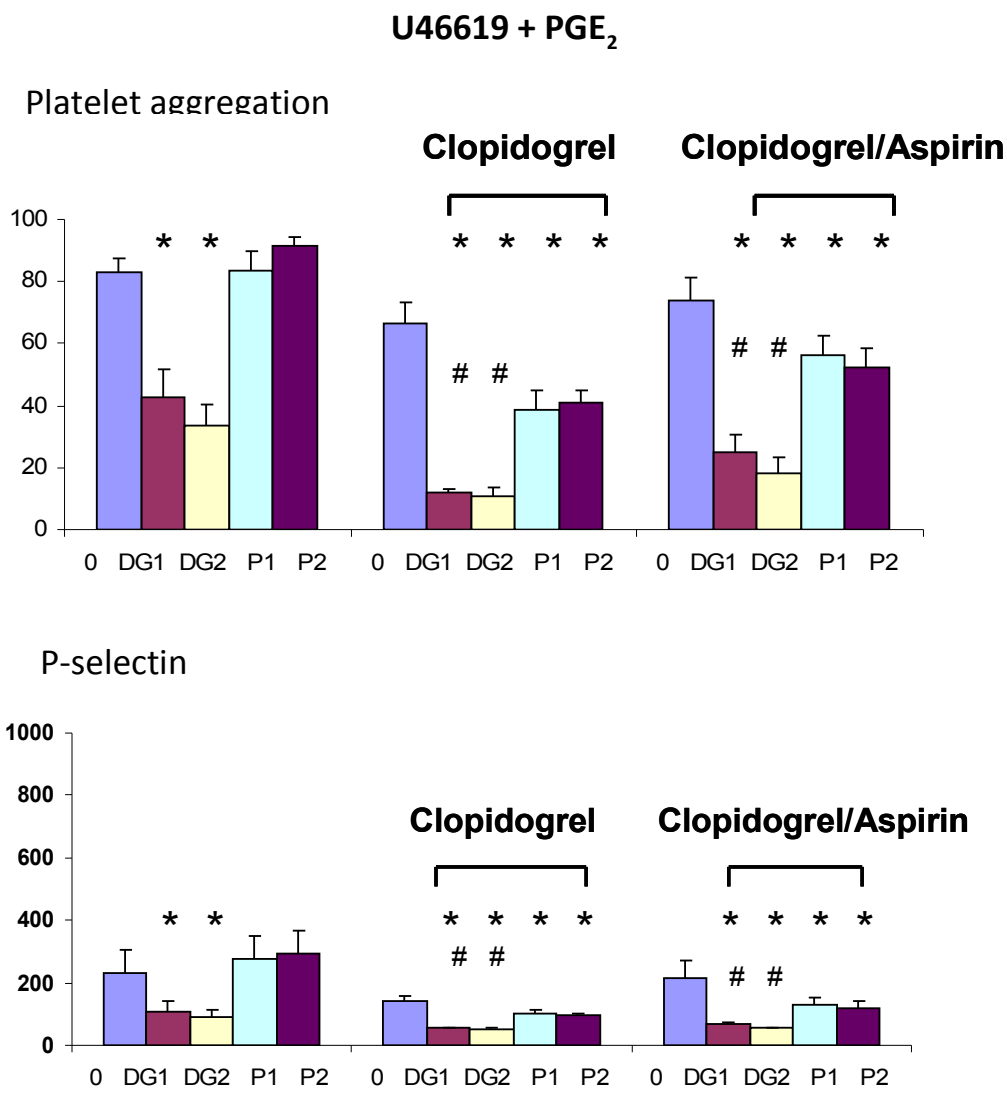


- DG-041 enhances inhibition of platelet function in the presence of PGE_2

Heptinstall et al, Platelets 2008;19:605-13

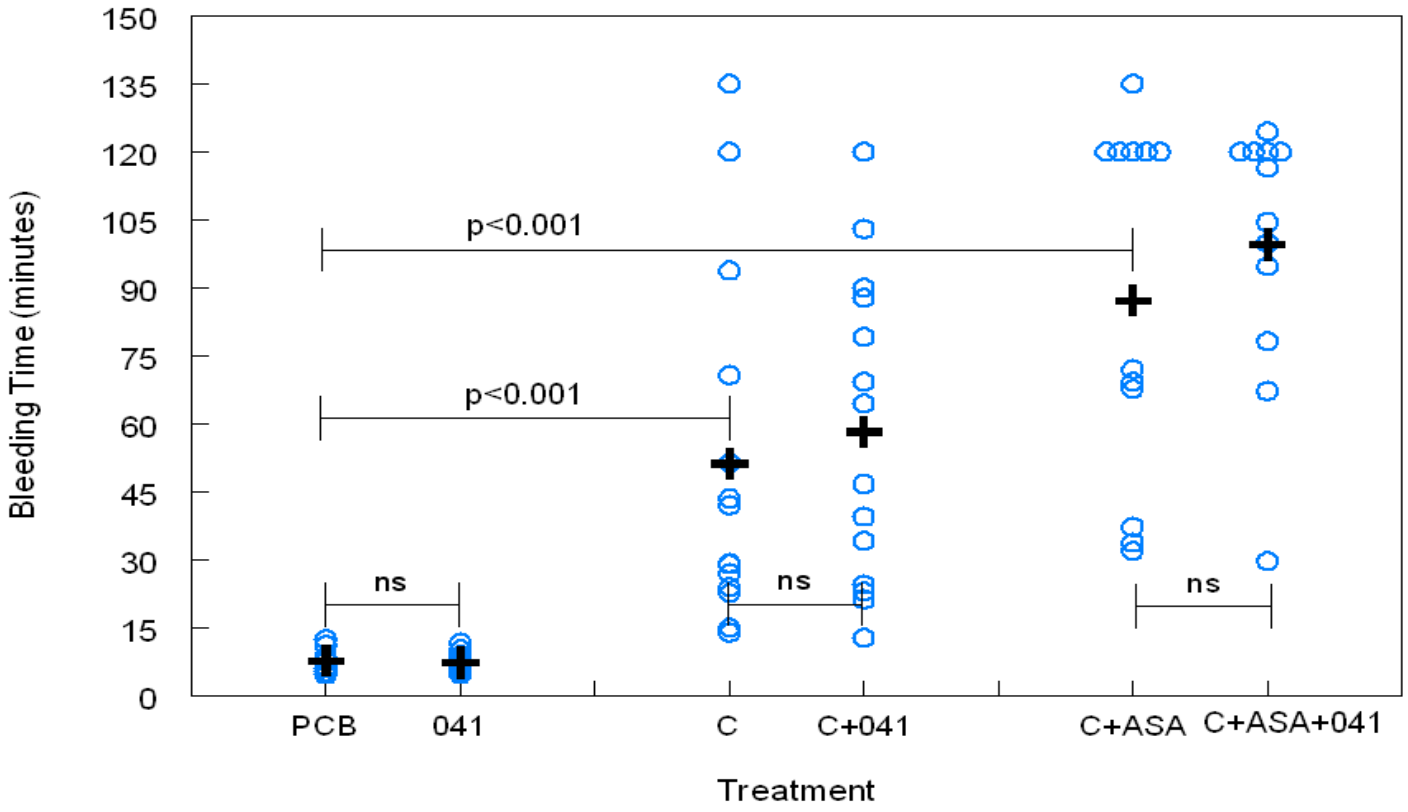
Inhibition of platelet function by an EP3 antagonist



- DG-041 in the presence of PGE_2 inhibits platelet function ex vivo after administration to man.
- DG-041 in the presence of PGE_2 adds to the inhibition brought about by co-administration of other antiplatelet agents.

Fox et al, submitted for publication

Inhibition of platelet function by an EP3 antagonist: no effect on bleeding time



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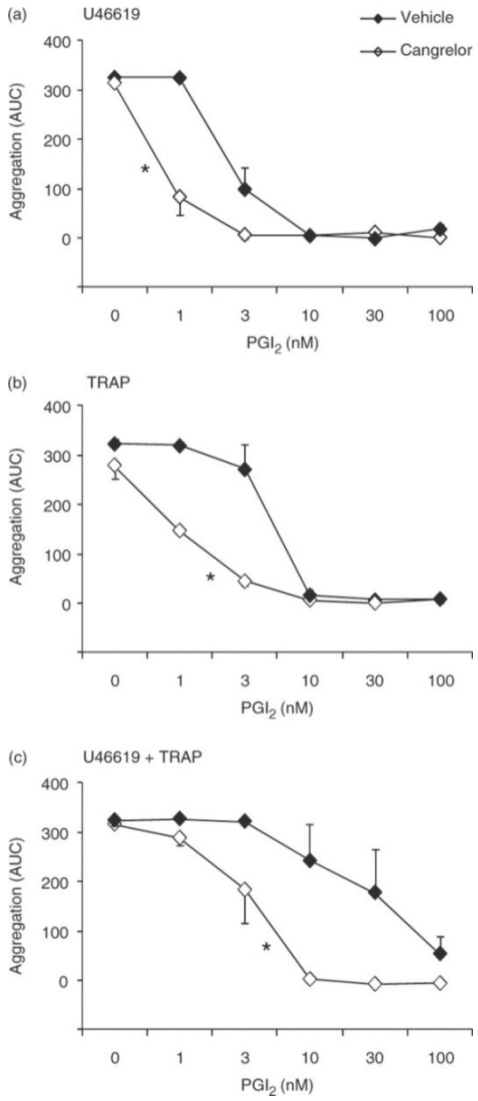
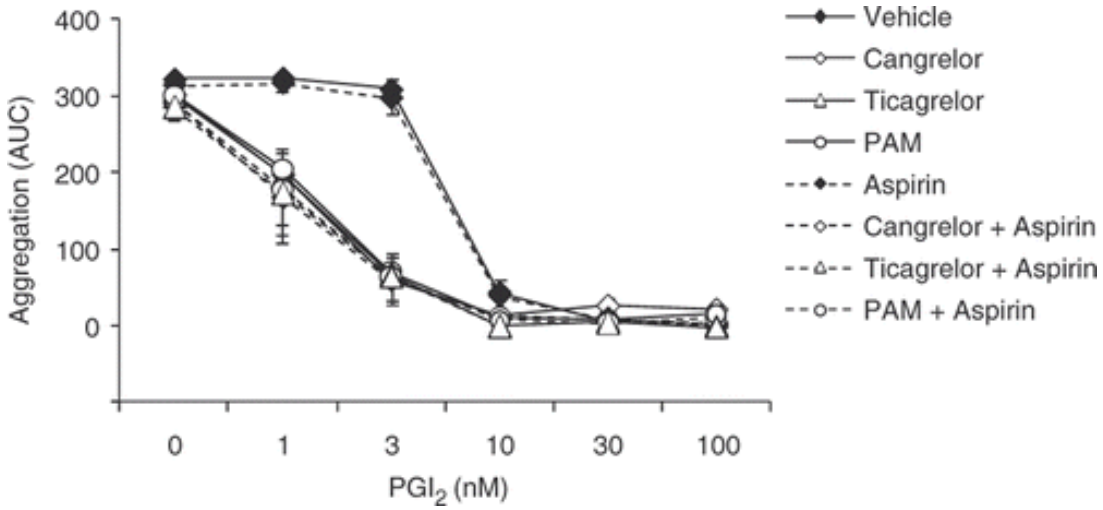
$P2Y_{12}$ antagonists: enhance inhibition of platelet function by PGI_2





- All $P2Y_{12}$ antagonists promote inhibition of platelet aggregation by PGI_2

Iyú et al, Platelets 2011;22:504-15

$P2Y_{12}$ antagonists: enhance inhibition of platelet function by all agents that ▲cAMP

Table I. IC_{50} values for various cAMP-elevating agents in the absence and presence of cangrelor.

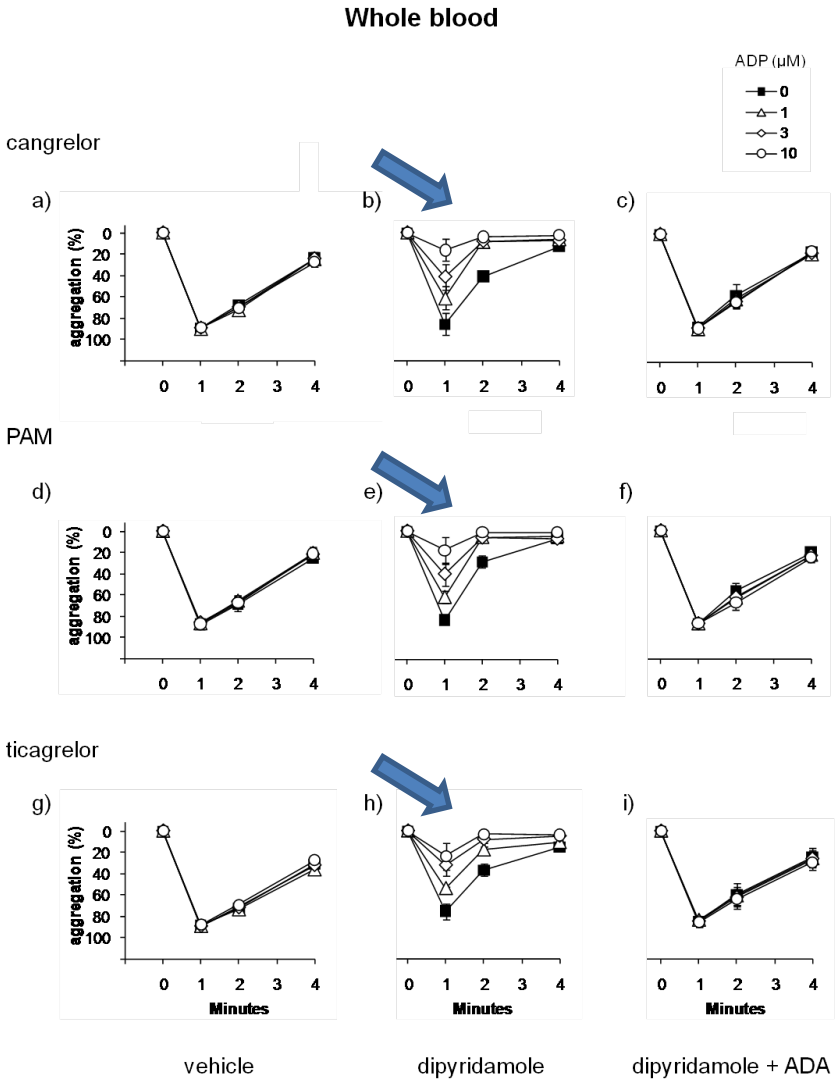
cAMP-elevating agent	Aggregating agent	Vehicle	Cangrelor
PGI_2 (nM)	U46619	2.8 ± 0.6	0.8 ± 0.2
	TRAP	6.0 ± 1.0	1.1 ± 0.4
	U46619 + TRAP	49 ± 22.6	4.3 ± 1.2
Iloprost (nM)	U46619	3.3 ± 1.3	0.9 ± 0.1
	TRAP	4.5 ± 1.1	1.0 ± 0.0
	U46619 + TRAP	19.7 ± 0.3	2.1 ± 0.1
PGD_2 (nM)	U46619	33 ± 13	19 ± 5
	TRAP	117 ± 62	15 ± 8
	U46619 + TRAP	413 ± 213	43 ± 24
Adenosine (μM)	U46619	>10	0.7 ± 0.1
	TRAP	>10	2.3 ± 0.4
	U46619 + TRAP	>10	>10
Forskolin (μM)	U46619	>10	1.9 ± 0.5
	TRAP	>10	3.2 ± 1.3
	U46619 + TRAP	>10	6.4 ± 2

Notes: IC_{50} values were determined in response to aggregation induced by U46619, TRAP or a combination of U46619 and TRAP in whole blood. For determination of the IC_{50} value for adenosine, experiments were performed in the presence of dipyridamole.

Iyú et al, Platelets 2011;22:504-15

- All $P2Y_{12}$ antagonists promote inhibition of platelet aggregation by all agents that raise cAMP in platelets

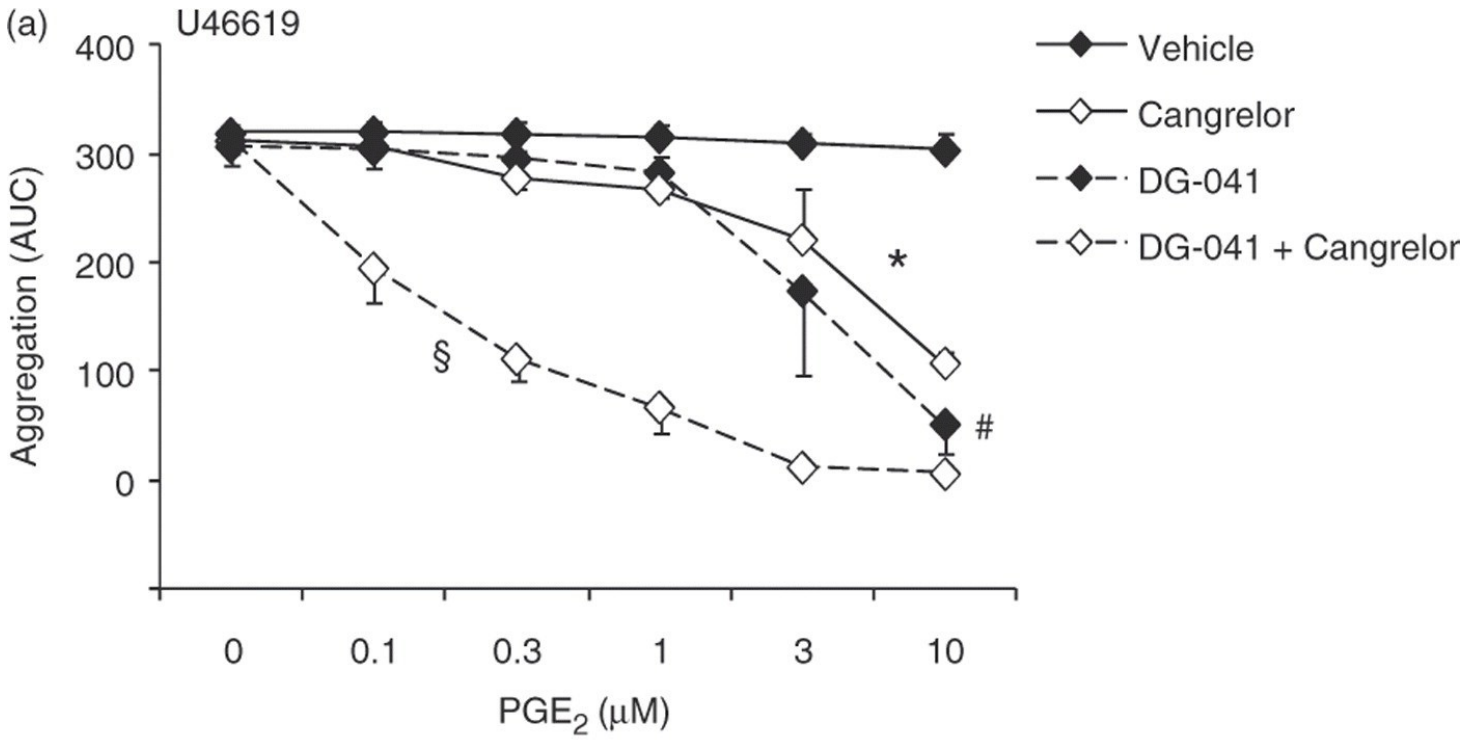
$P2Y_{12}$ antagonists: enhance inhibition of platelet function by all agents that ▲cAMP



ADP is able to markedly inhibit platelet function in whole blood when a $P2Y_{12}$ antagonist has been added provided that uptake of its metabolite adenosine into red cells is prevented using dipyridamole

Iyú et al, ATVB 2011;31:416-22

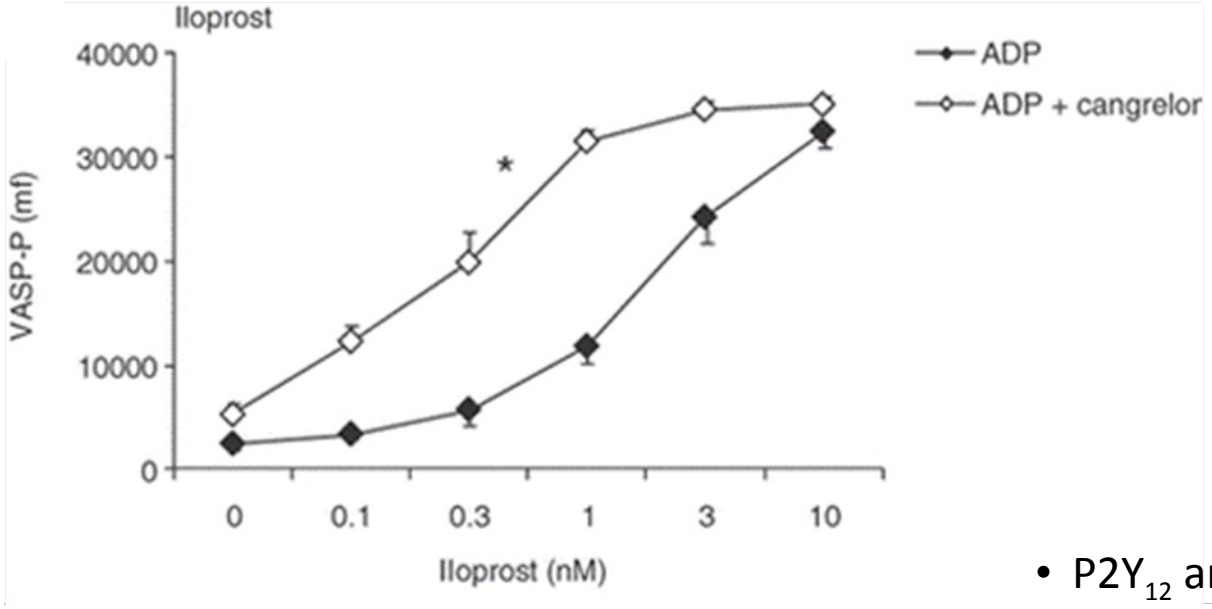
$P2Y_{12}$ antagonists: enhance inhibition of platelet function by all agents that ▲cAMP



- $P2Y_{12}$ antagonists promote inhibition of platelet aggregation by PGE_2 in the presence of an EP3 antagonist

Iyú et al, Platelets 2011;22:504-15

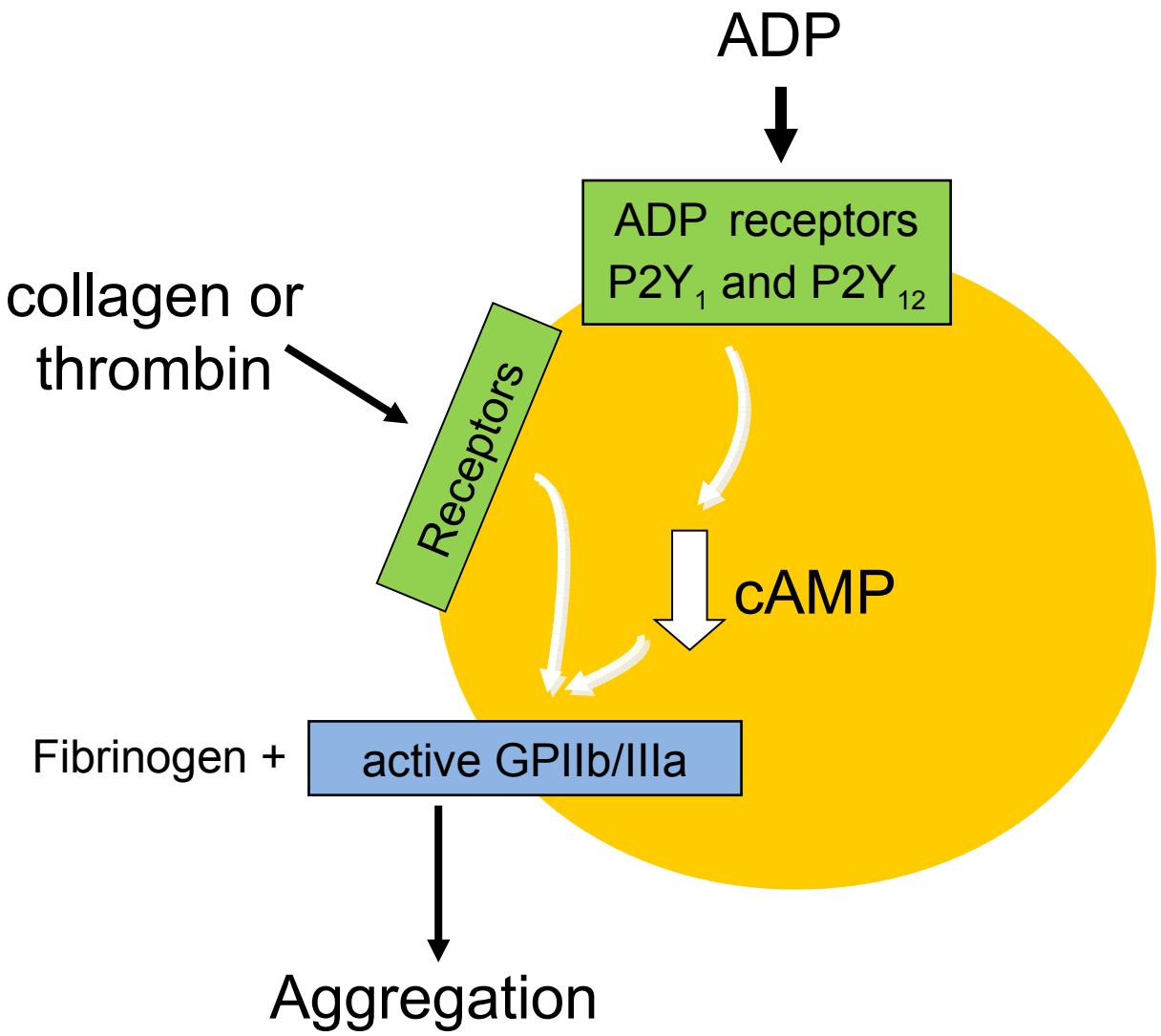
$P2Y_{12}$ antagonists: enhance inhibition of platelet function by all agent that ▲cAMP



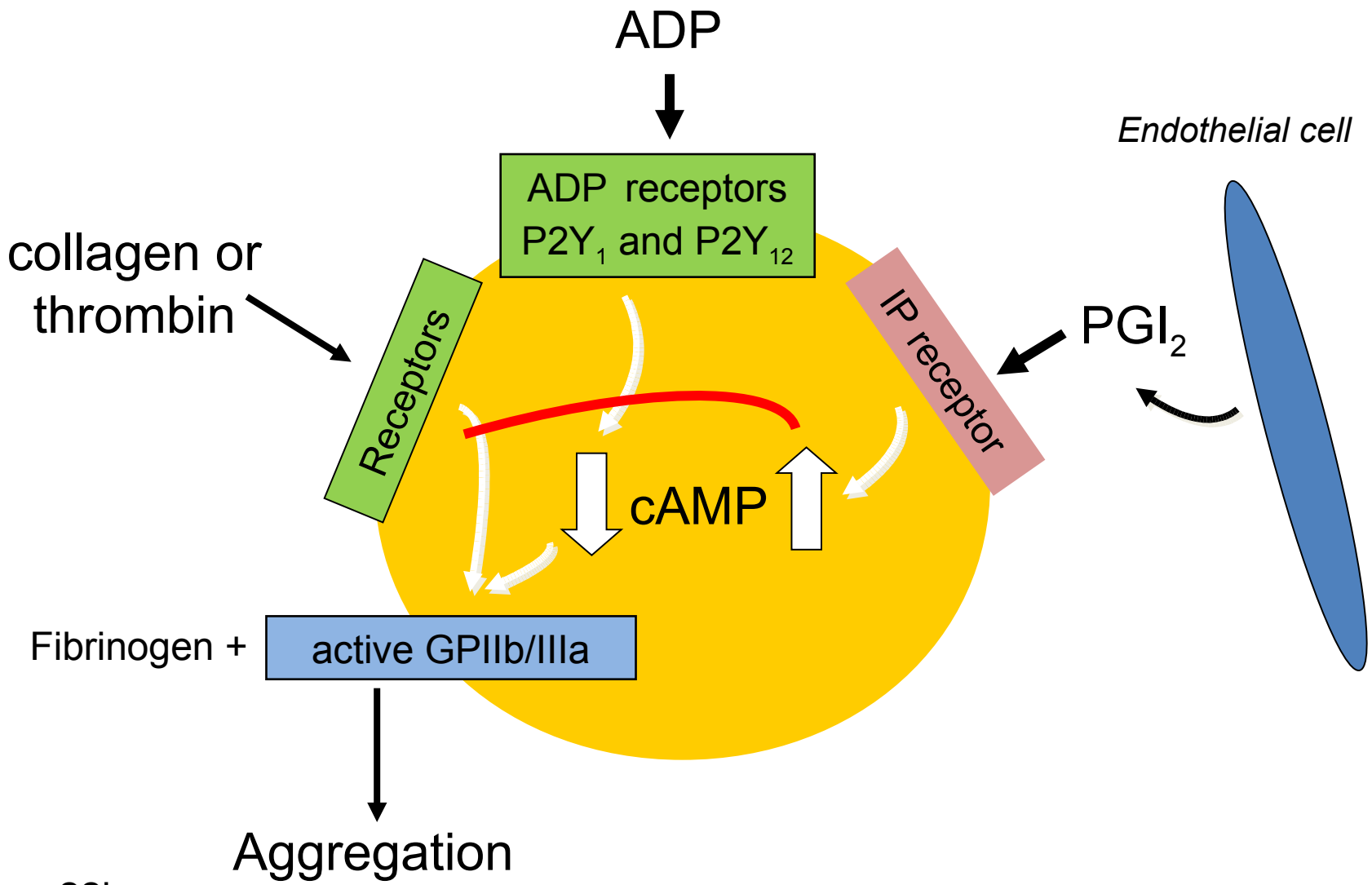
- $P2Y_{12}$ antagonists promote inhibition of platelet aggregation by increasing the levels of cAMP attained

Iyú et al, Platelets 2011;22:504-15

a) ADP lowers cAMP and promotes platelet function

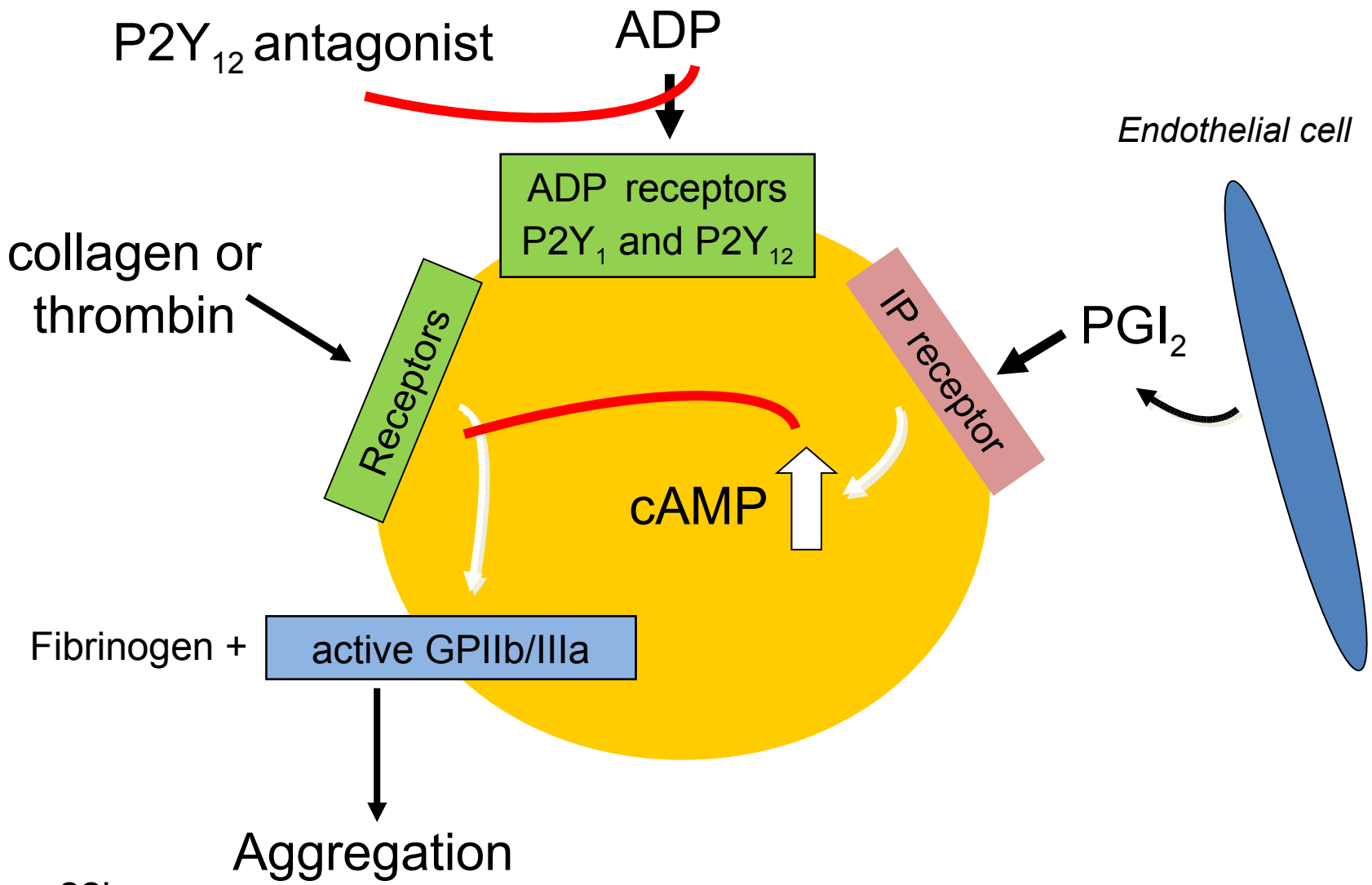


b) PGI_2 counters the effect of ADP on cAMP



Picture 22b

c) A $P2Y_{12}$ antagonist prevents ADP lowering cAMP allowing PGI_2 to provide very effective inhibition of platelet function

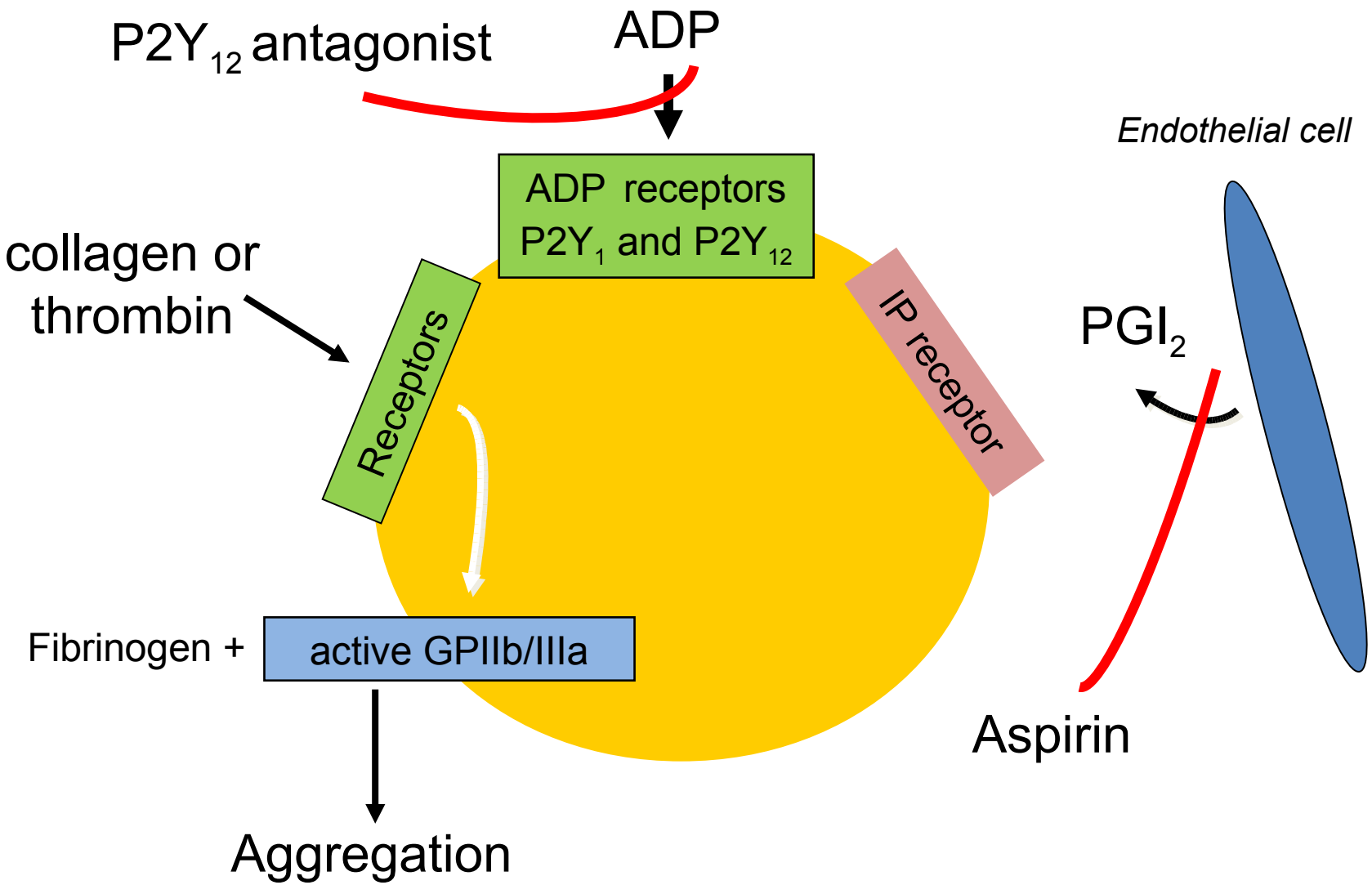


Picture 22b

Consideration of interactions between $P2Y_{12}$ antagonists and vascular prostaglandins

- $P2Y_{12}$ antagonists act by blocking promotion of platelet function by ADP
- $P2Y_{12}$ antagonists also promote the inhibitory effects of natural prostaglandins and other agents that increase cAMP in platelets
- These two actions might contribute to the clinical benefit derived from $P2Y_{12}$ antagonists

d) If aspirin blocks PGI_2 synthesis, cAMP is unable to contribute to inhibition of platelet function



Consideration of interactions between $P2Y_{12}$ antagonists and vascular prostaglandins

- $P2Y_{12}$ antagonists act by blocking promotion of platelet function by ADP
- $P2Y_{12}$ antagonists also promote the inhibitory effects of natural prostaglandins and other agents that increase cAMP in platelets
- These two actions might contribute to the clinical benefit derived from $P2Y_{12}$ antagonists

If so .....

- Additional therapies (e.g. high doses of aspirin) that may reduce vascular prostaglandin synthesis should be avoided