Intracellular Cyclic Nucleotide (cAMP, cGMP) signaling

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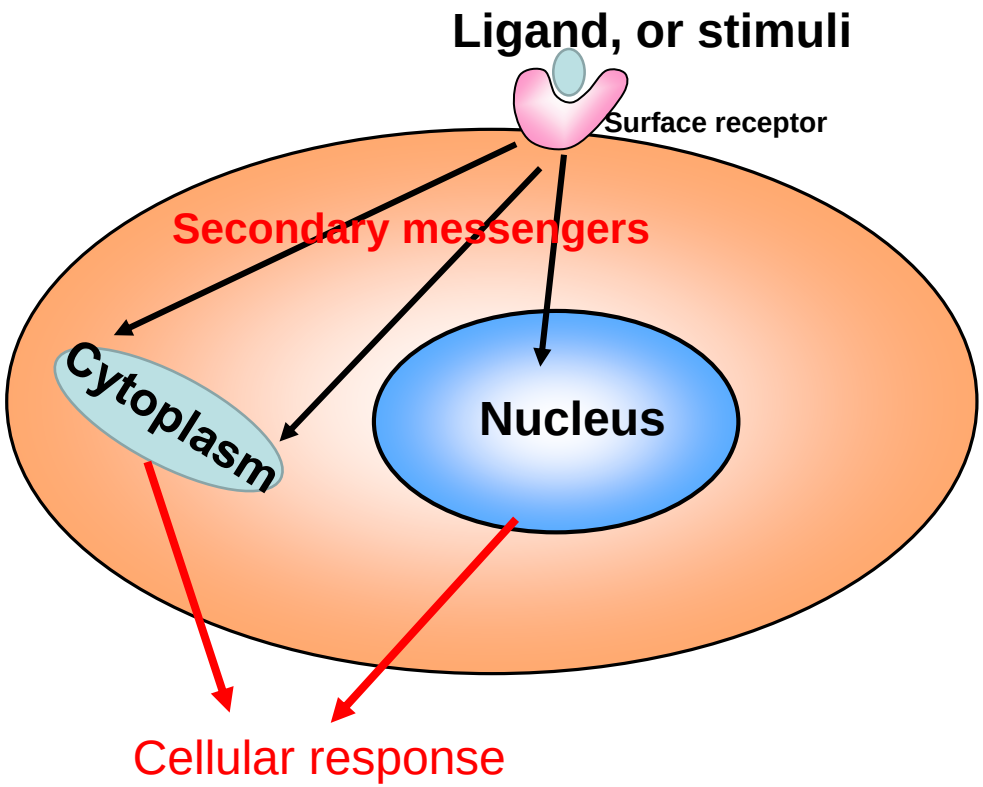
Roschino, March 15, 2017

Main Topics

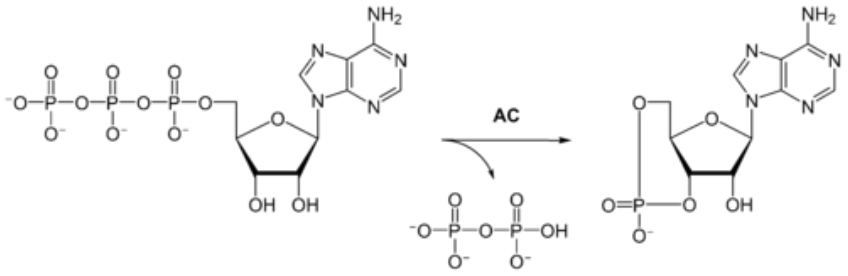
- General overview on cAMP and cGMP signaling
- Regulation of renin-angiotensin system
- Aldosterone production
- Platelet functions

Simplified scheme of cellular signaling



Part 1 cAMP cGMP signaling

cAMP synthesis



ATP

cAMP

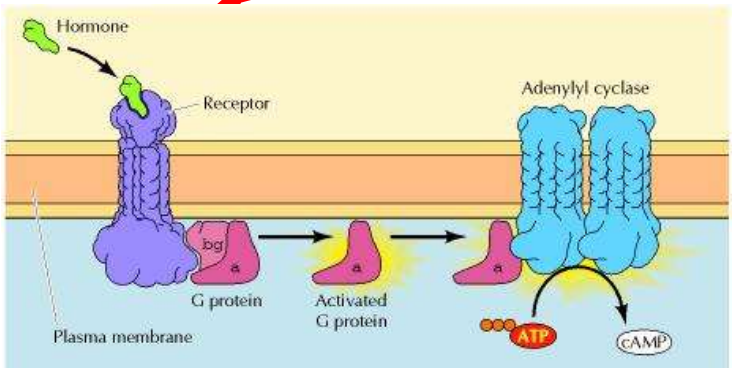
Discovery of cAMP

SUTHERLAND EW, RALL TW.
Formation of adenosine-3,5-phosphate (cyclic adenylate) and its relation to the action of several neurohormones or hormones.
Acta Endocrinol Suppl (Copenh).
1960;34(Suppl 50):171-4.

Earl Sutherland of Case Western Reserve University won a Nobel Prize in Physiology or Medicine in 1971 "for his discoveries concerning the mechanisms of the action of hormones", especially epinephrine, via second messengers (such as cyclic adenosine monophosphate, **cyclic AMP**).

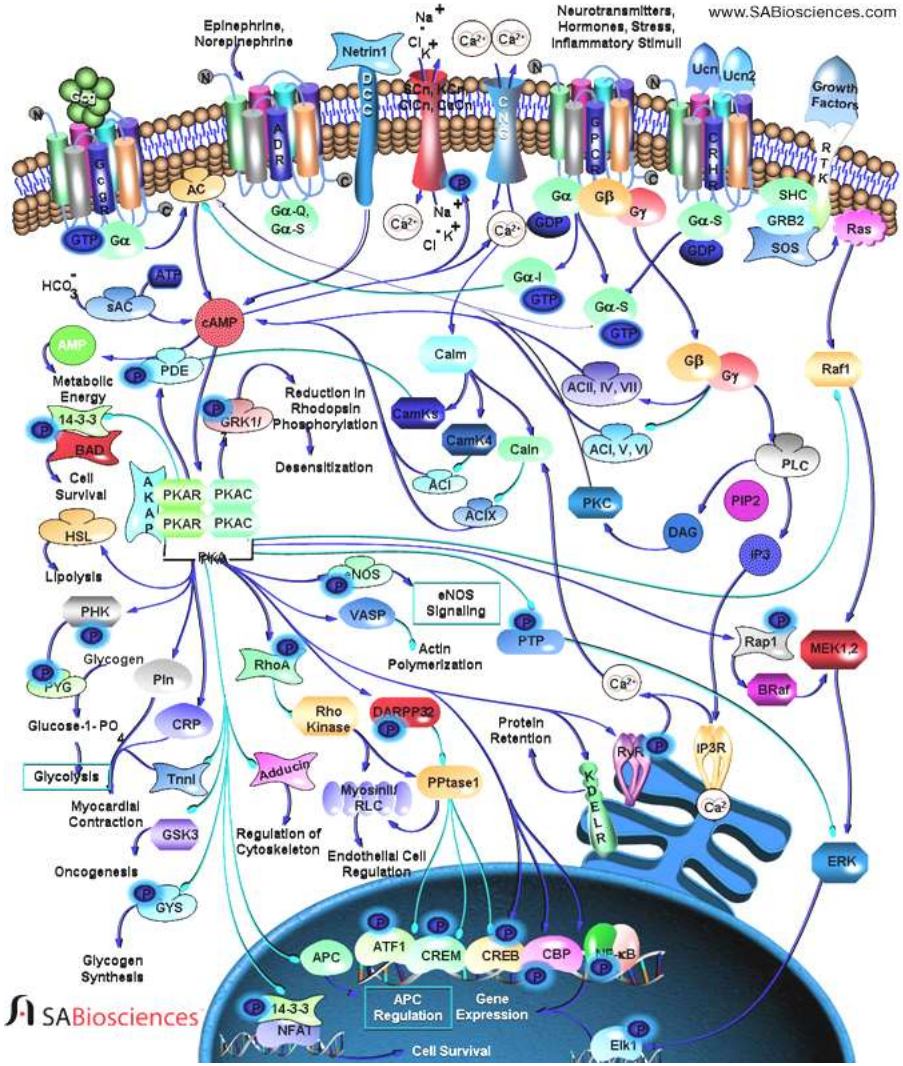
How cAMP was discovered

Epinephrine, glucagon

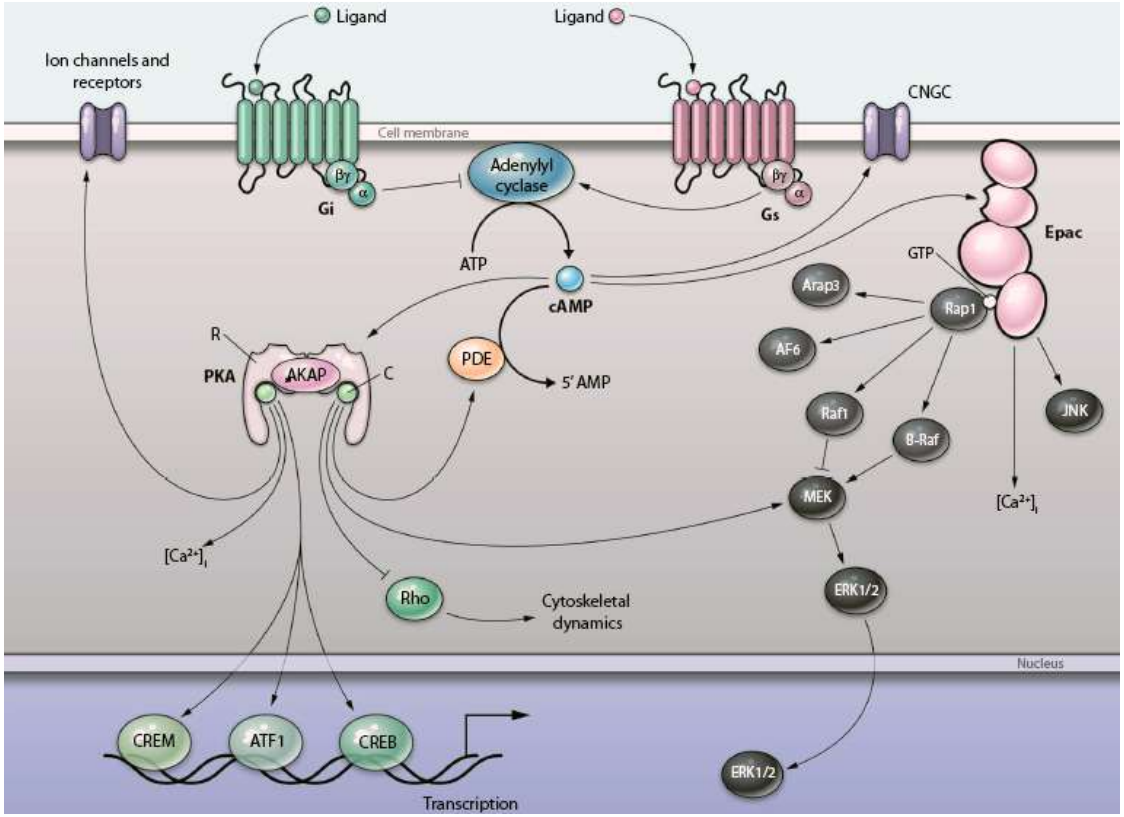


Cyclic AMP was discovered in the course of investigations into the mechanism of the hyperglycemic action of epinephrine and glucagon

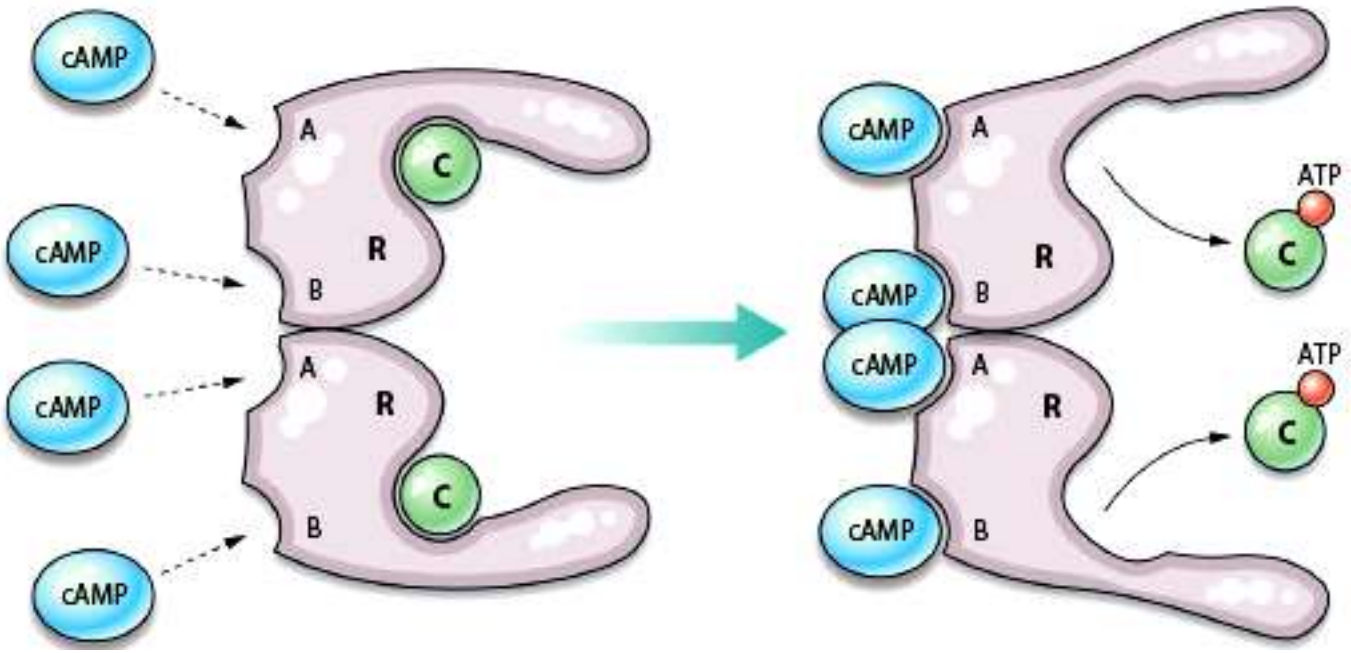
cAMP signaling pathways



cAMP signaling pathways



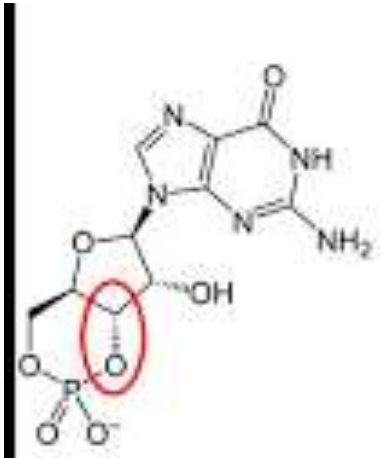
cAMP/protein kinase A (PKA) signaling



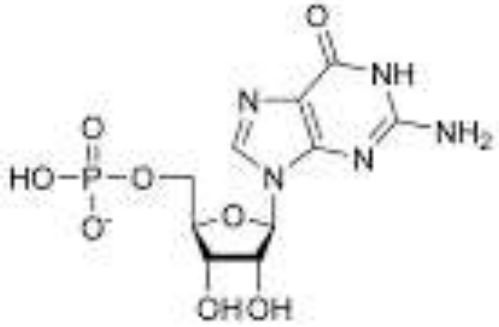
cGMP

cGMP was first synthesized in 1960 (Smith, Drummond et al. 1961) and shortly after this endogenously produced cGMP was identified from rabbit urine (Ashman, Lipton et al. 1963).

cGMP synthesis

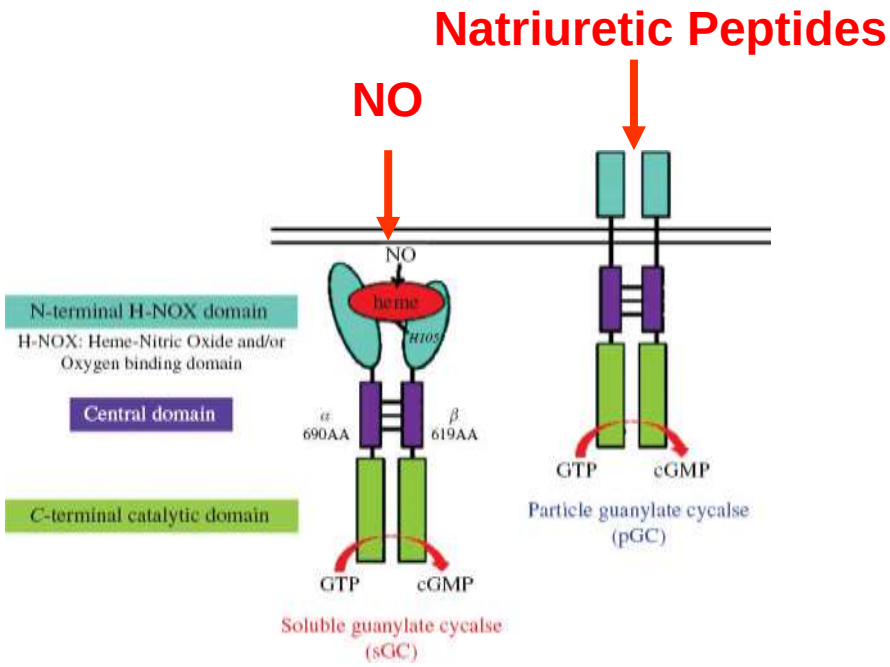


Cyclic GMP



GMP

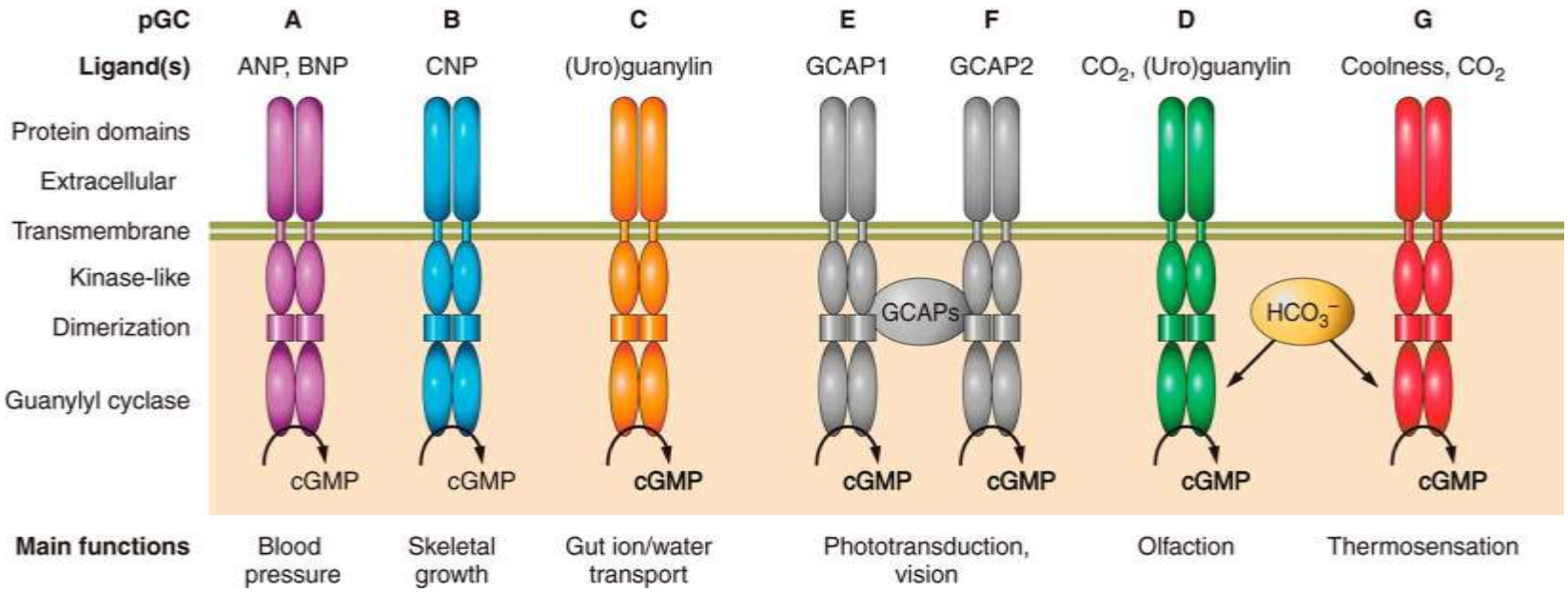
cGMP is synthesized by two independent enzymes, particulate and soluble guanylate cyclases.



Natriuretic Peptides

ANP was discovered in the early 1980s. de Bold and colleagues in Kingston, Canada found that rat atrial extracts contained a substance that increased salt and urine output in the kidney. Later, the substance was purified from the heart by several groups and named ANF or ANP

Endogenous activators, general structure, and main regulatory functions of particulate guanylyl cyclases.

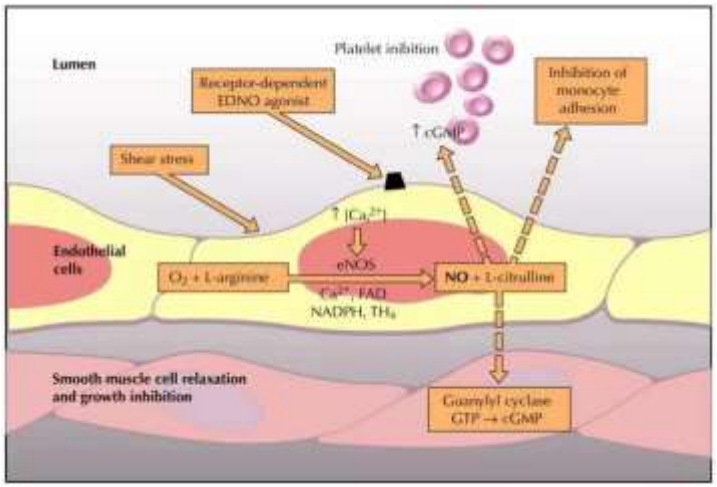


Michaela Kuhn Physiol Rev 2016;96:751-804

Physiological Reviews

History of NO

Furchgott RF, Zawadzki JV.
The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine.
Nature. 1980



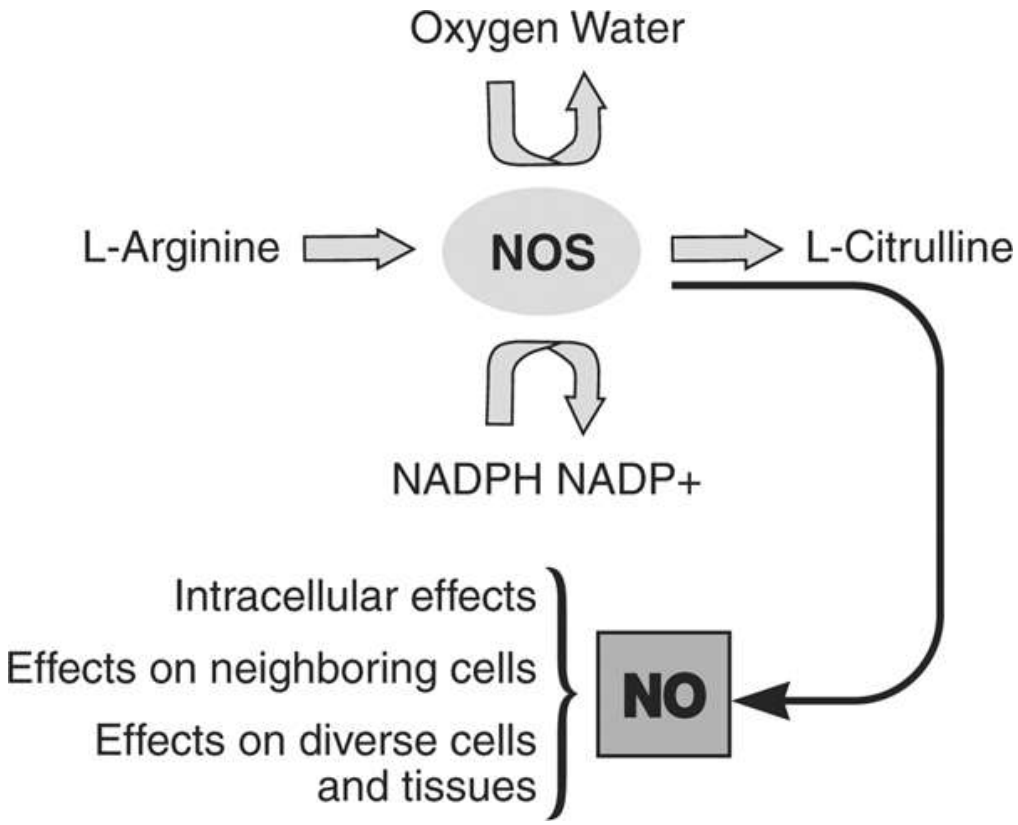
The Nobel Prize in Physiology or Medicine 1998 was awarded jointly to Robert F. Furchgott, Louis J. Ignarro and Ferid Murad *"for their discoveries concerning nitric oxide as a signalling molecule in the cardiovascular system"*.

Robert F. Furchgott

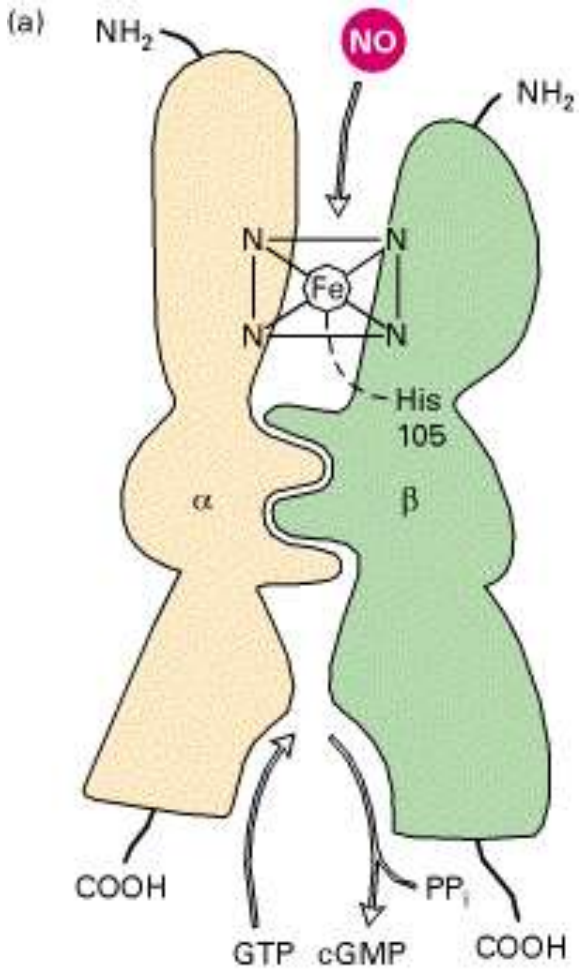
Louis J. Ignarro

Ferid Murad

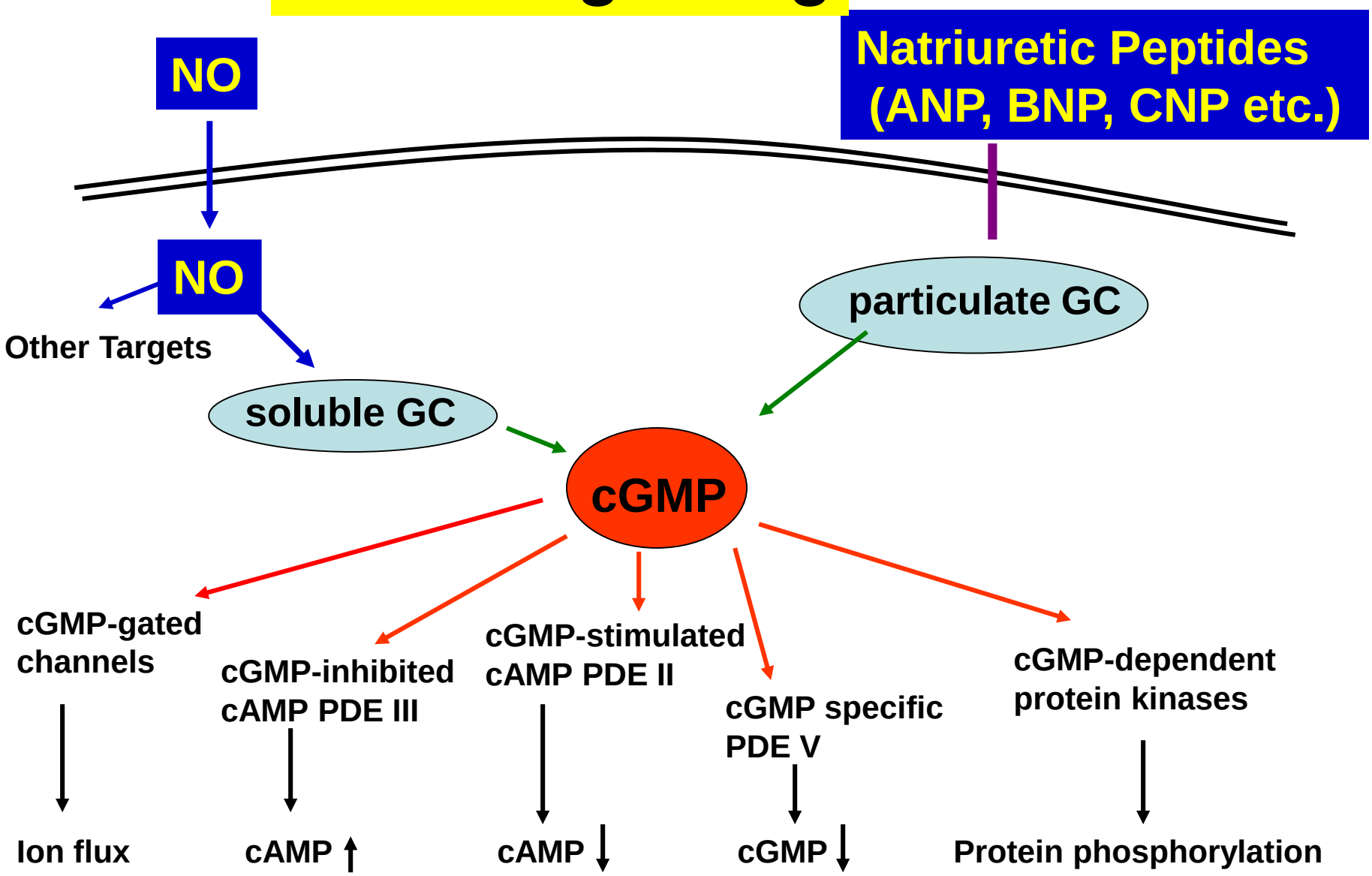
NO production by NOS proteins



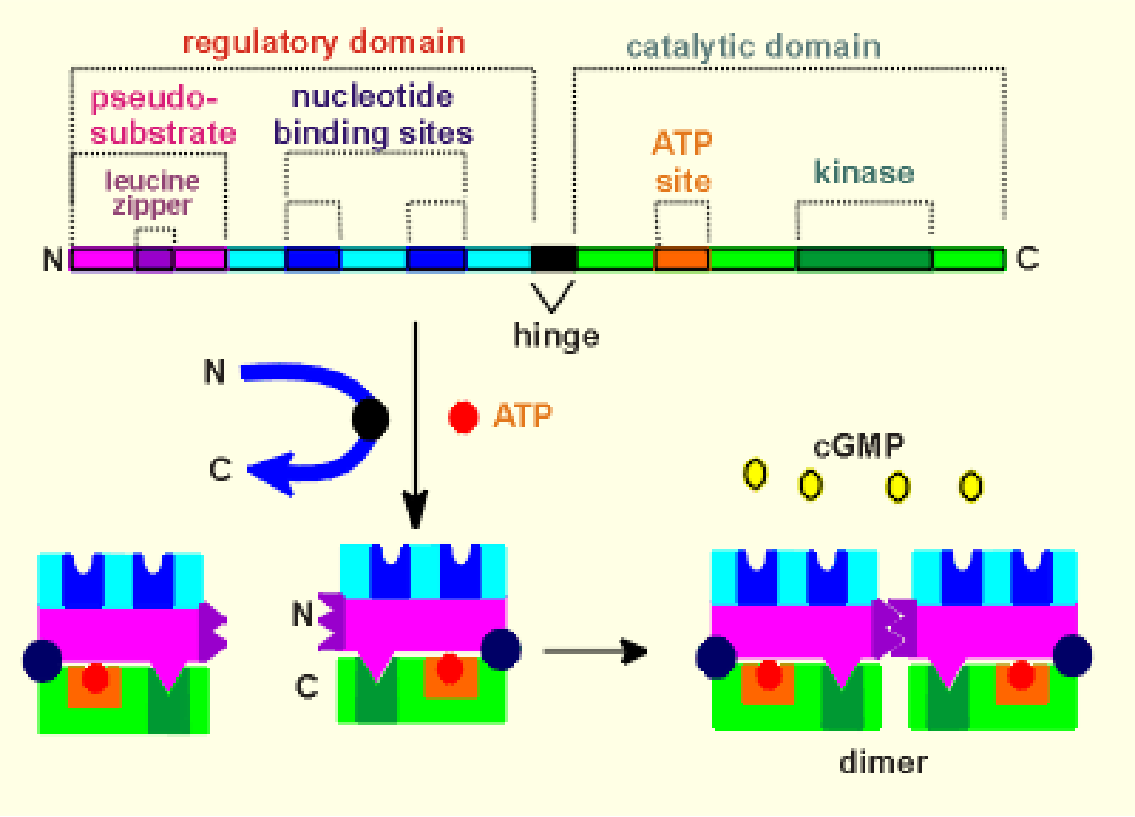
Mechanism of sGC activation



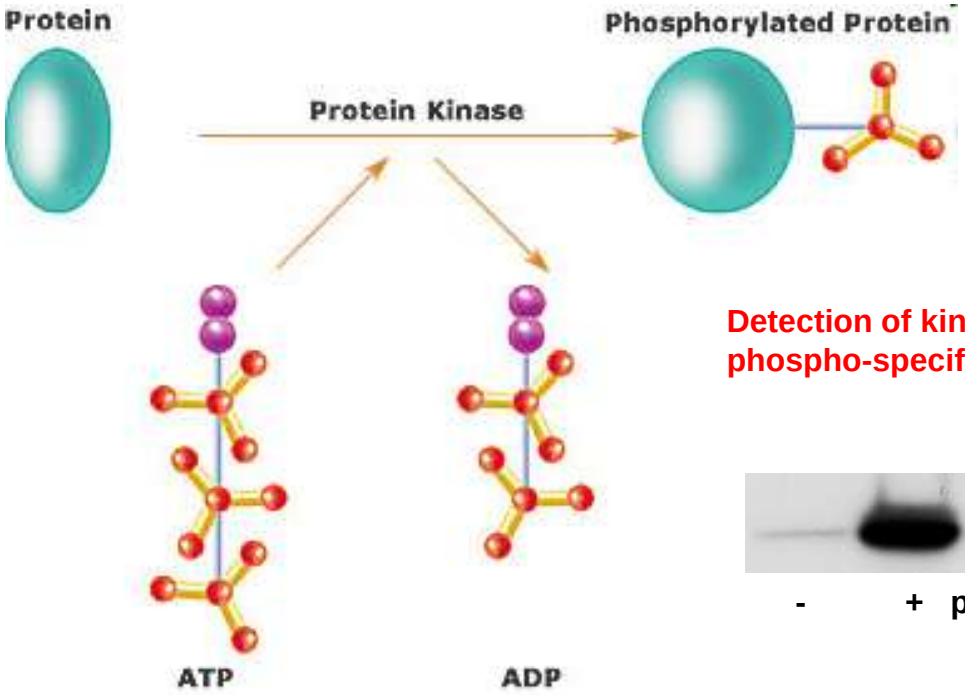
cGMP Signaling



cGMP/ protein kinase G (PKG) signaling



Protein kinases



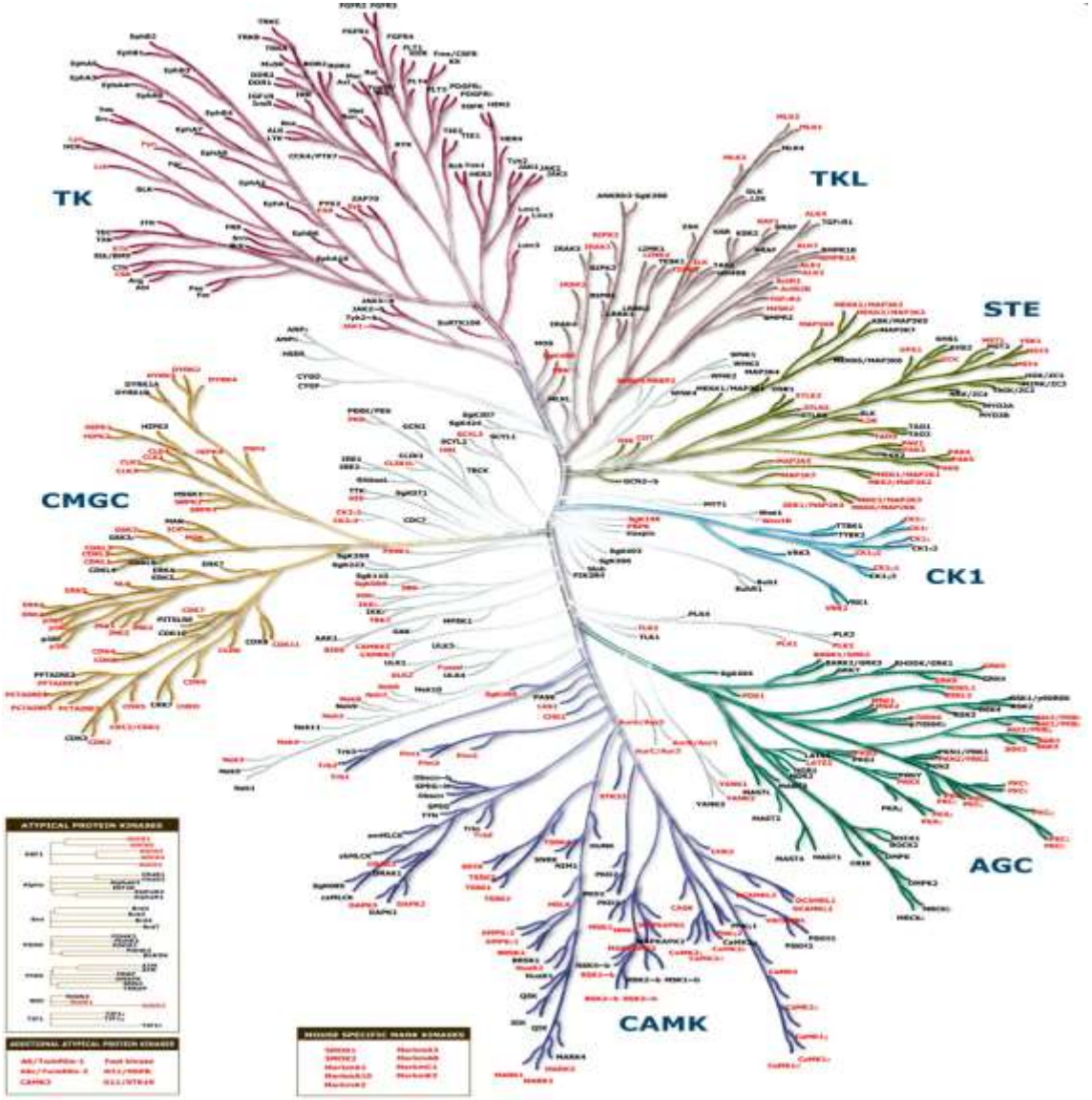
Detection of kinase activity by phospho-specific antibodies

Kinase substrate protein

- + protein kinase

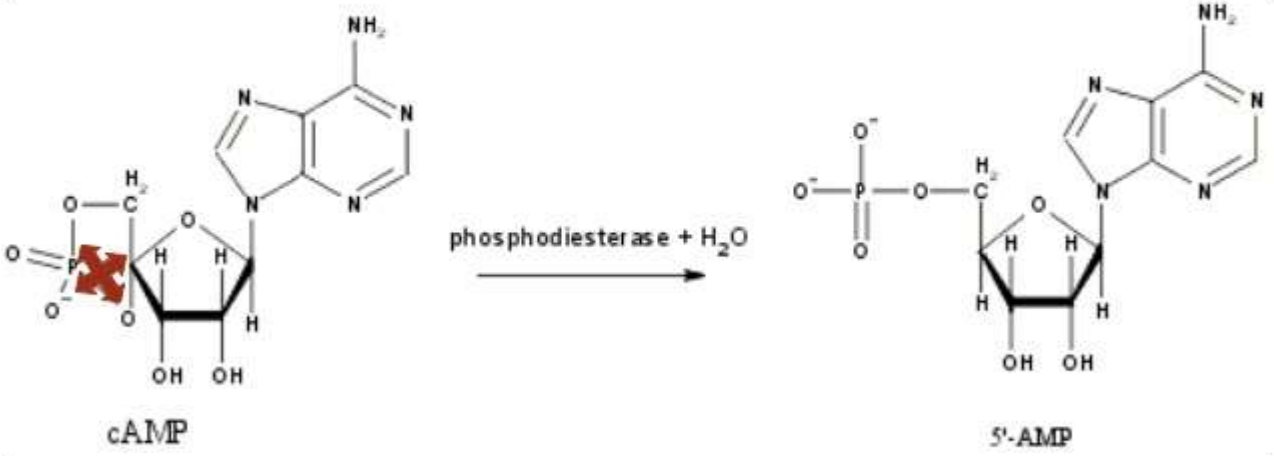
The human genome contains about 500 protein kinase genes and they constitute about 2% of all human genes.

Protein kinase phylogenetic tree



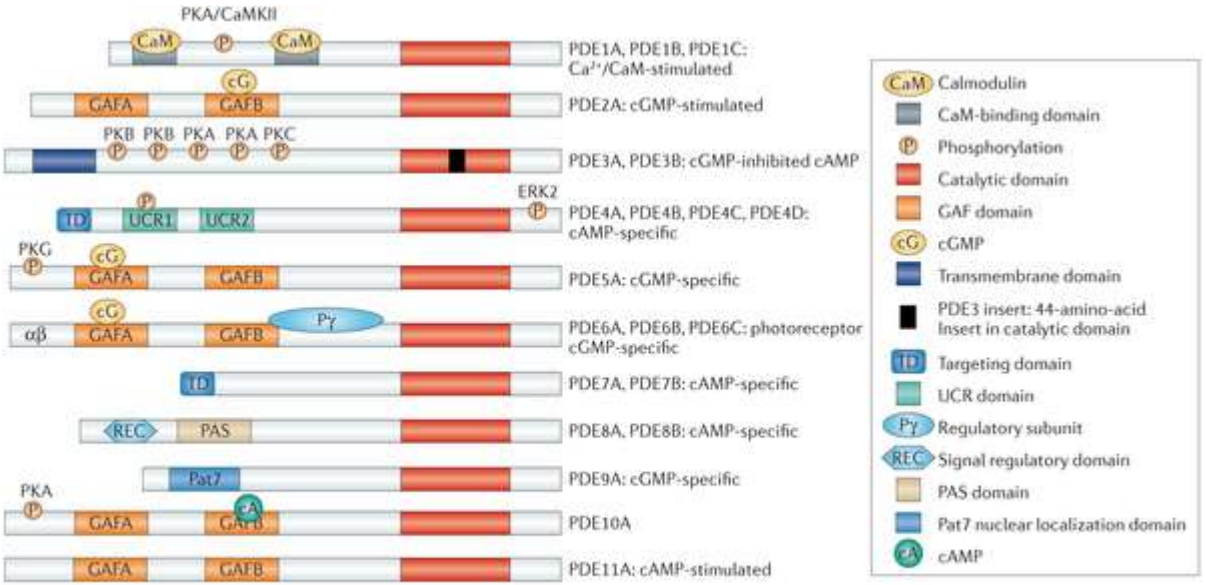
PHOSPHODIESTERASES

- Breaks phosphodiester bond in the second messenger molecules cAMP and cGMP



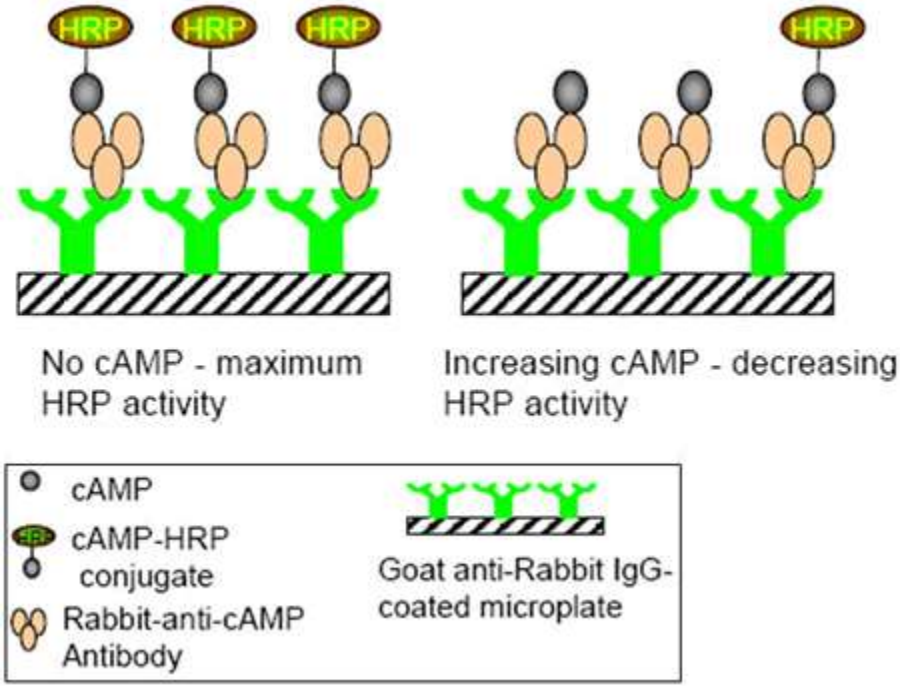
cGMP —PDE→ 5'GMP

Phosphodiesterase (PDE) Family

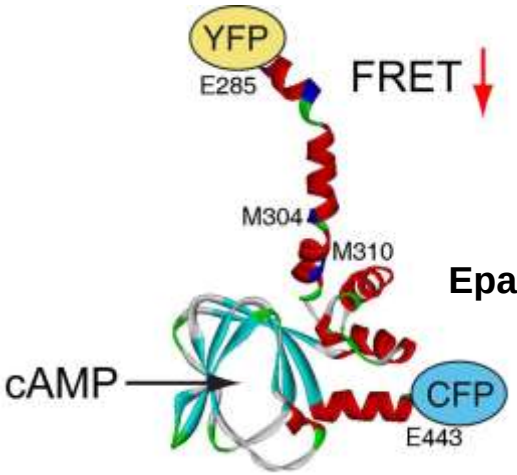


Nature Reviews | Drug Discovery

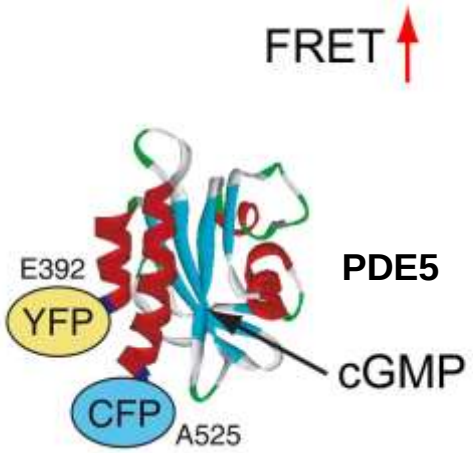
ELISA method for cyclic nucleotides



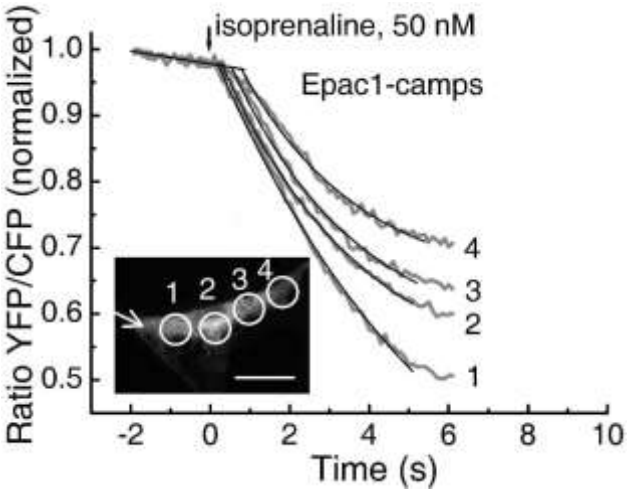
FRET-based sensors for in vivo registration of cAMP and cGMP in cells



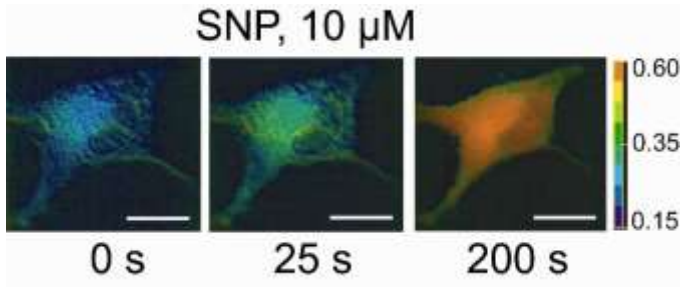
Epac1



PDE5



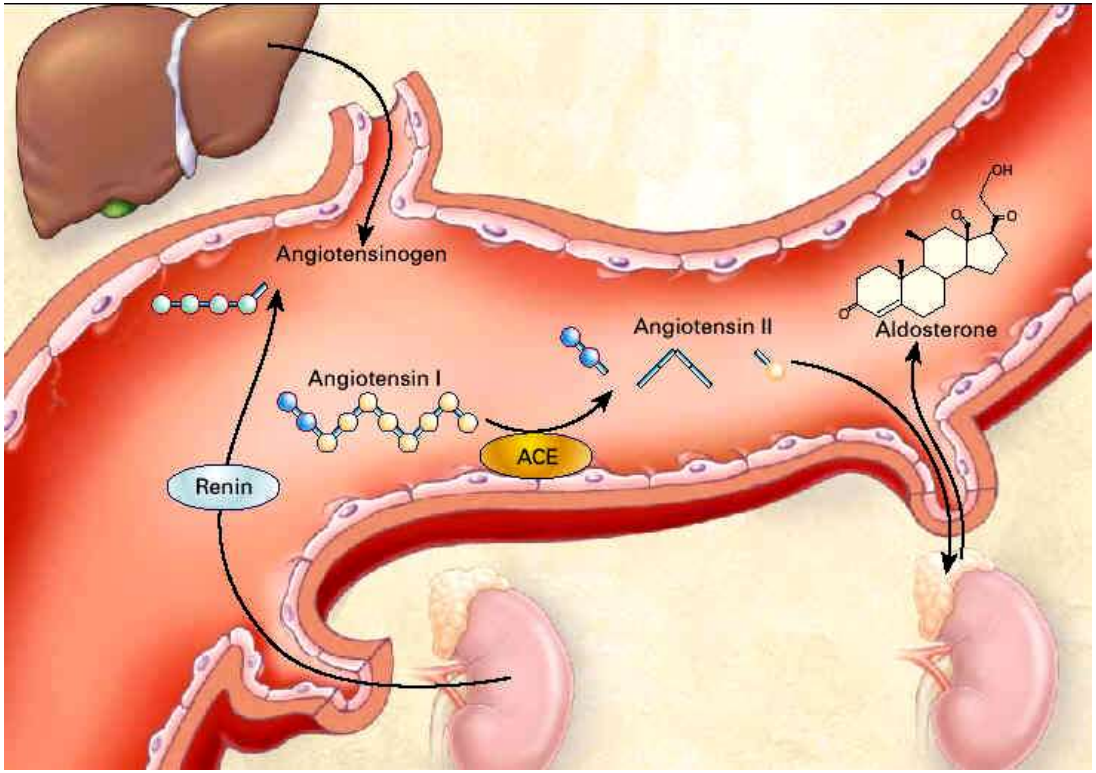
Nikolaev, Gambaryan et al. JBC 2004



Nikolaev, Gambaryan, Lohse et al. Nat. Met. 2006

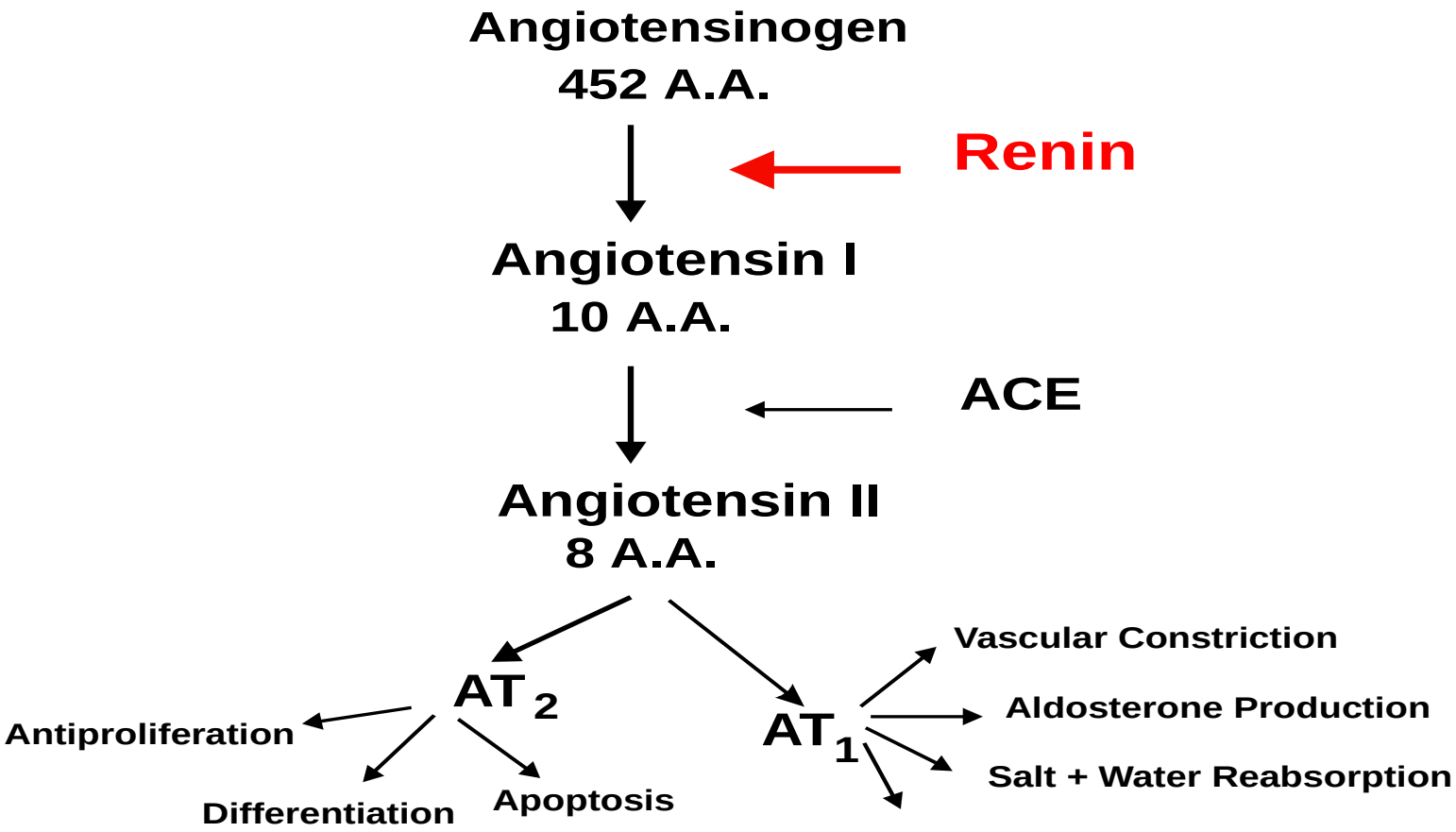
Part 2 Renin-Angiotensin system

Renin-Angiotensin- Aldosterone system (RAAS)

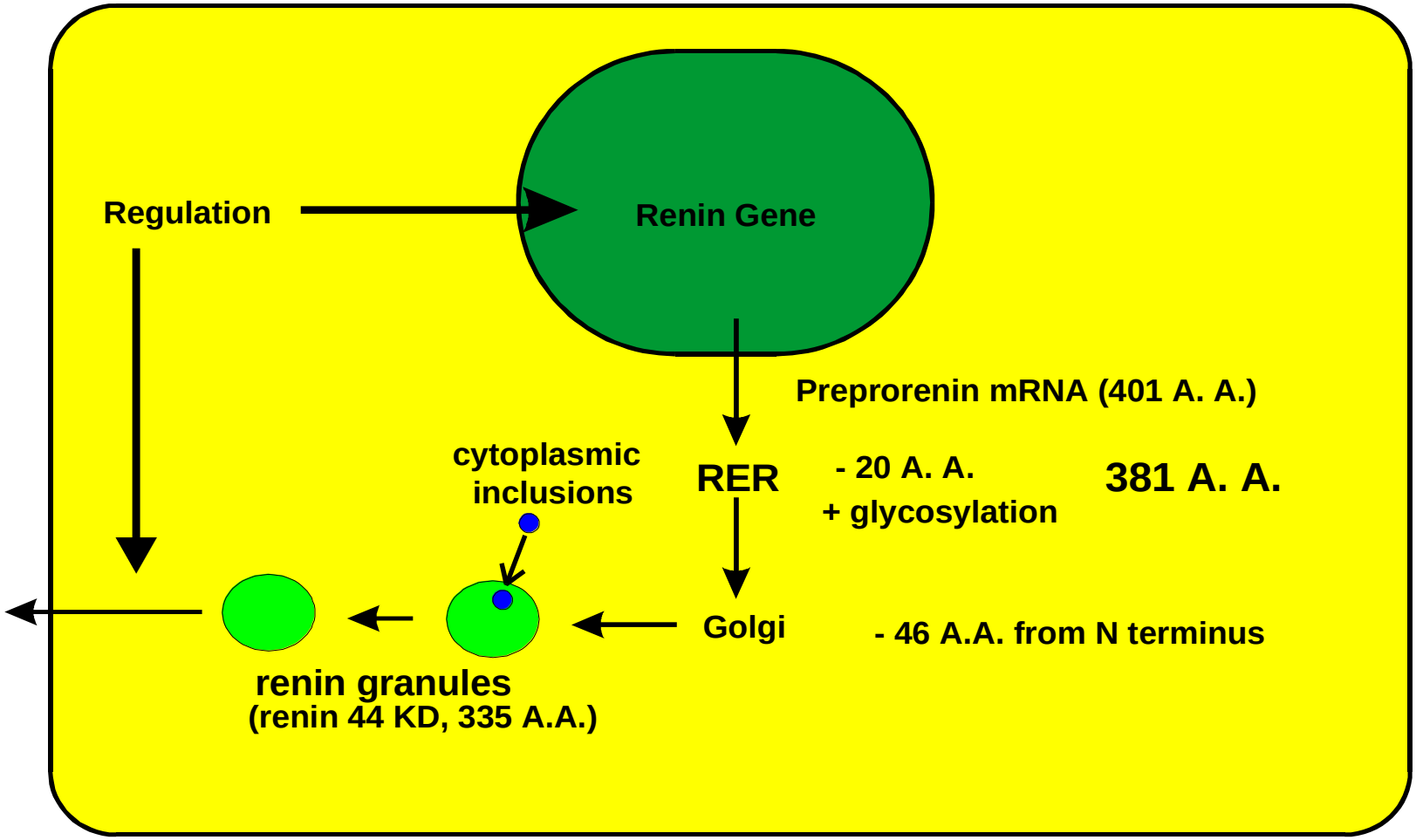


from Weber K., N Engl J Med 2001

Renin-Angiotensin system

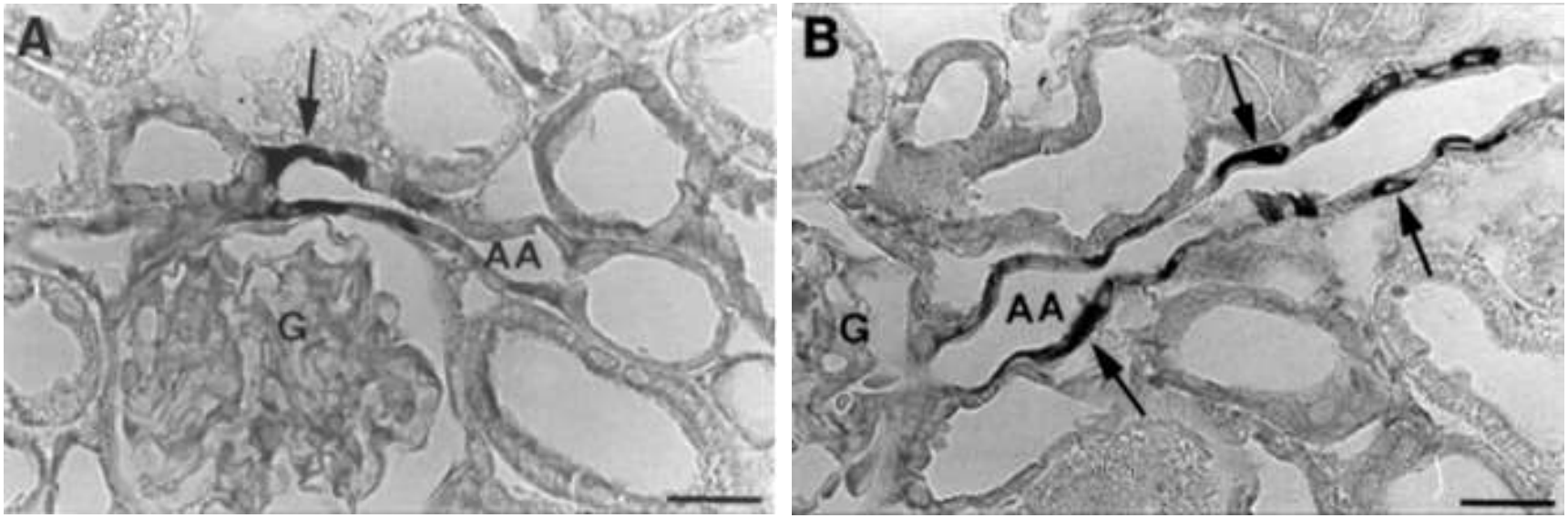


Renin synthesis and secretion in JG cells



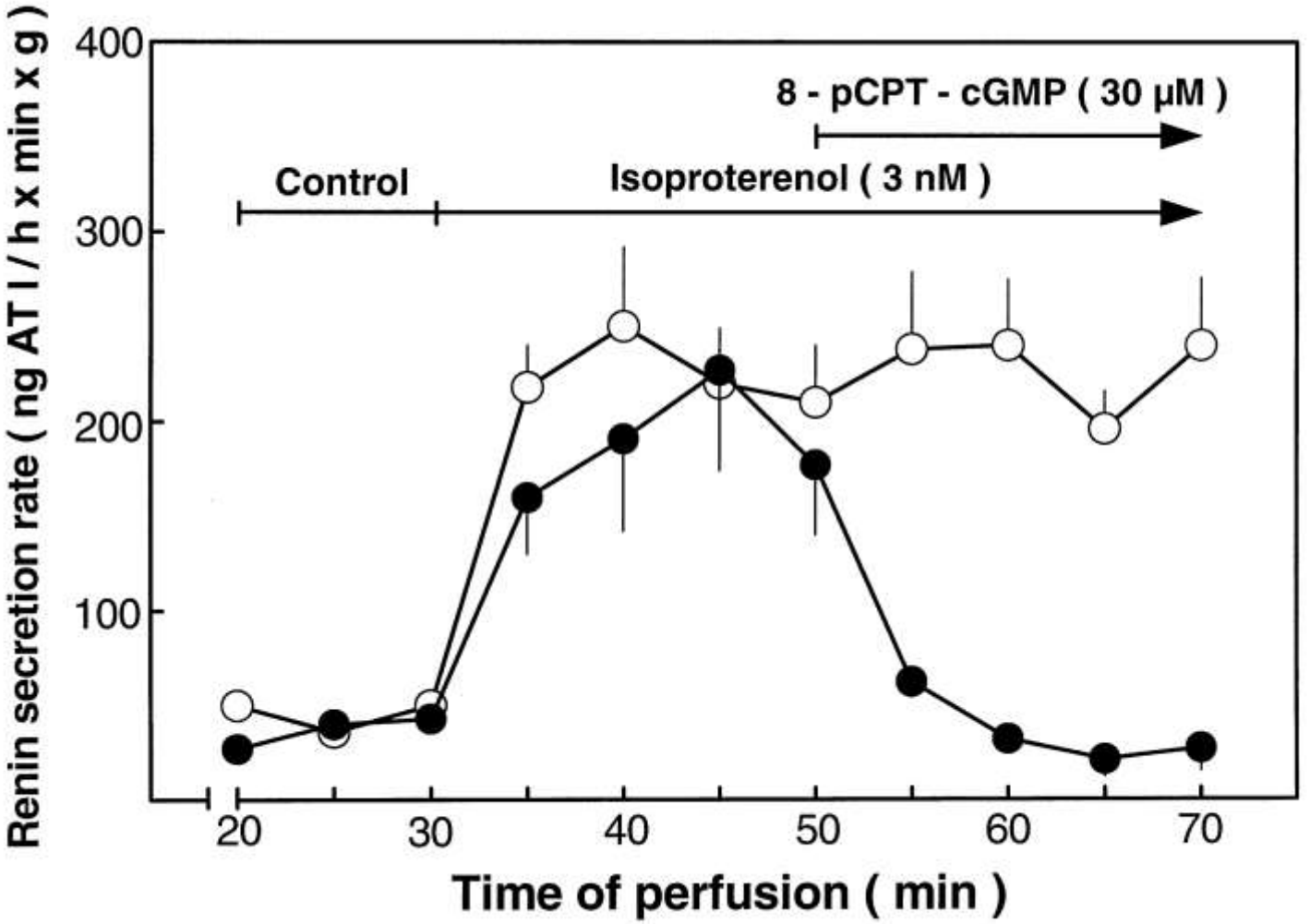
Losartan treatment activates cGK II expression in JG cells

control **losartan**



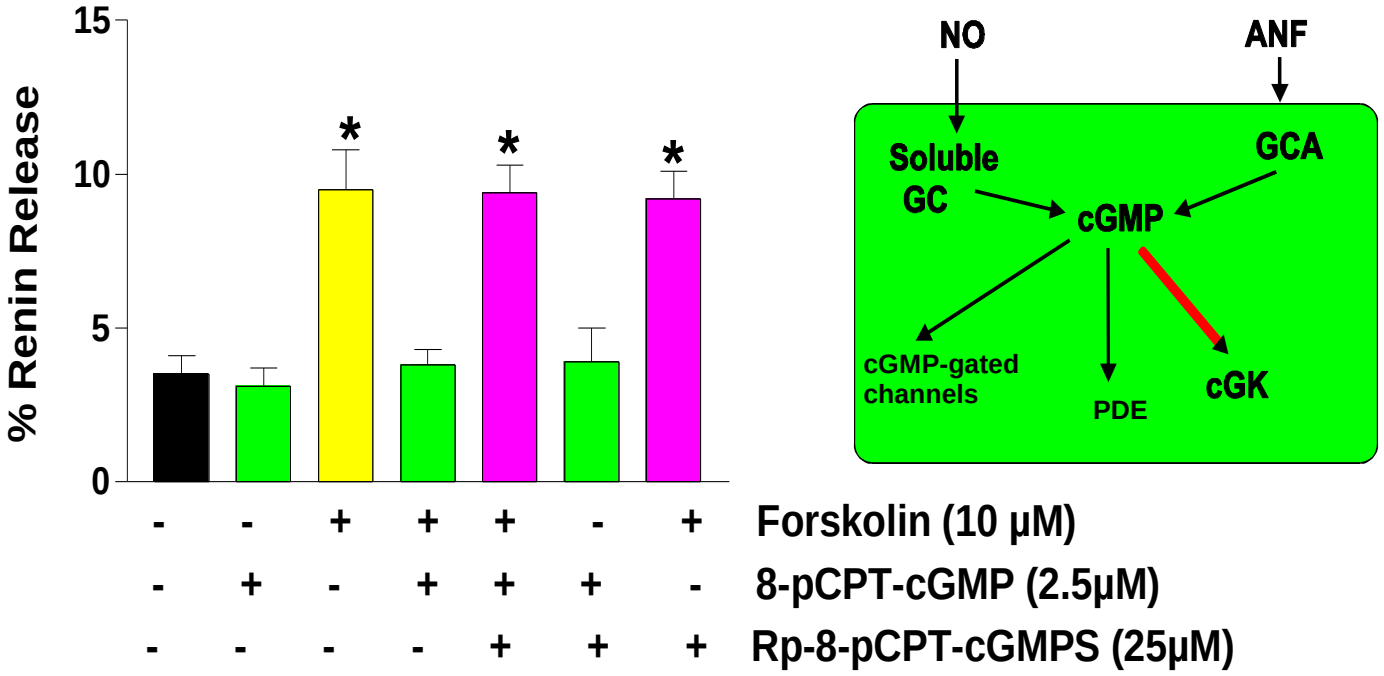
Gambaryan S et al. PNAS 1998

Inhibition of isoproterenol-stimulated renin release by 8-pCPT-cGMP in the isolated perfused rat kidney.



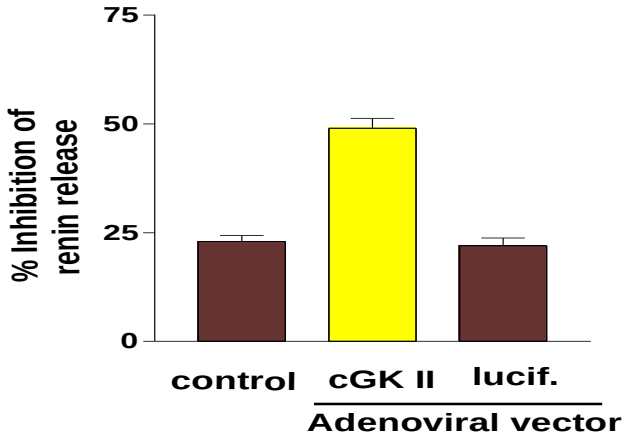
Gambaryan S et al. PNAS 1998

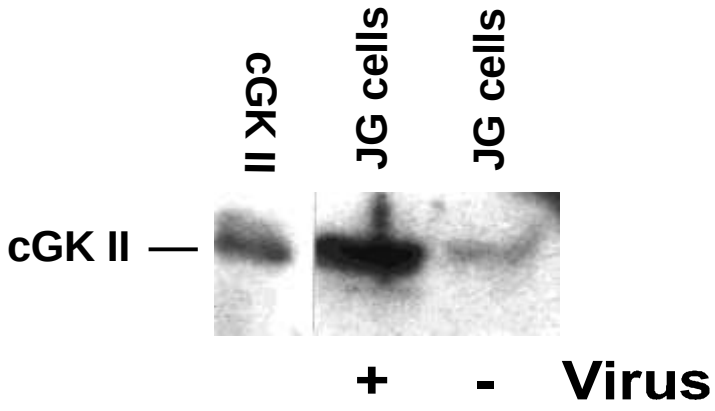
cGK activation inhibits cAMP stimulated renin release



Gambaryan S et al. PNAS 1998

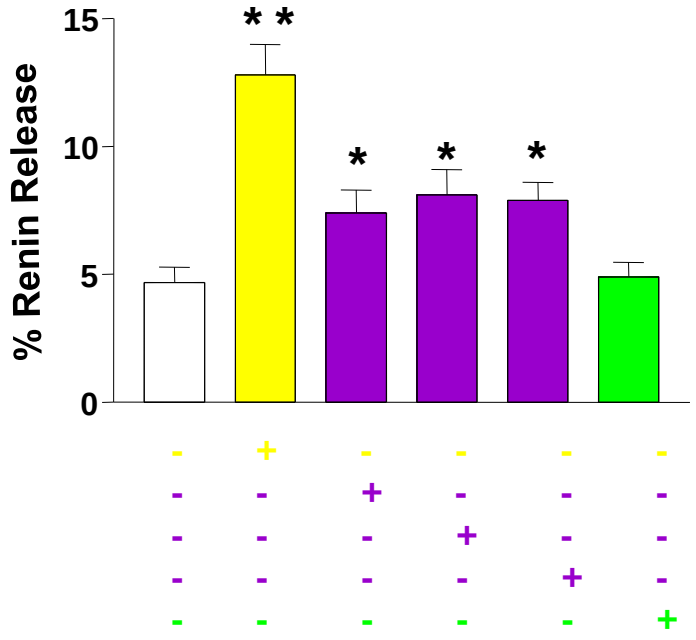
cGK II inhibits cAMP stimulated renin release

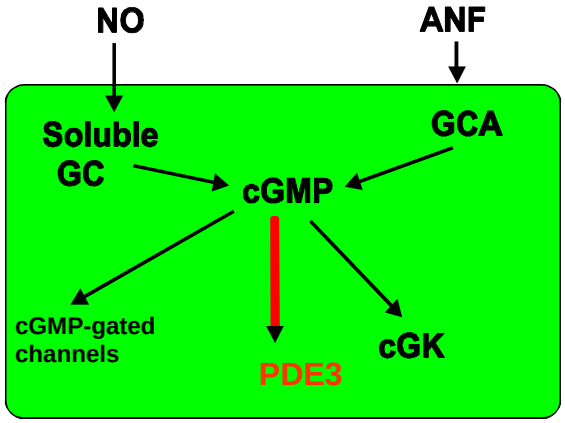




Gambaryan S et al. PNAS 1998

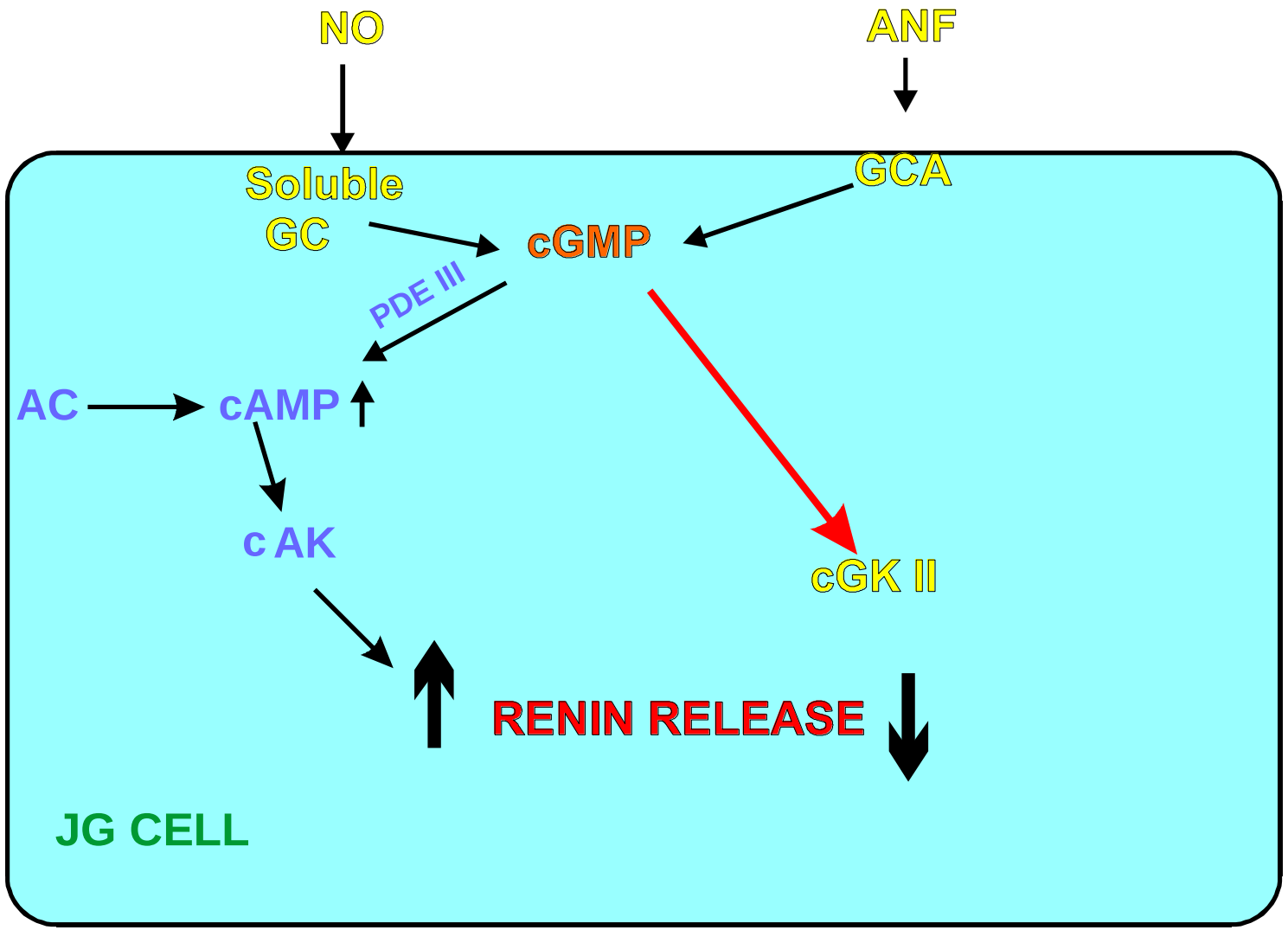
NO donors and ANP but not 8-pCPT-cGMP stimulating renin release





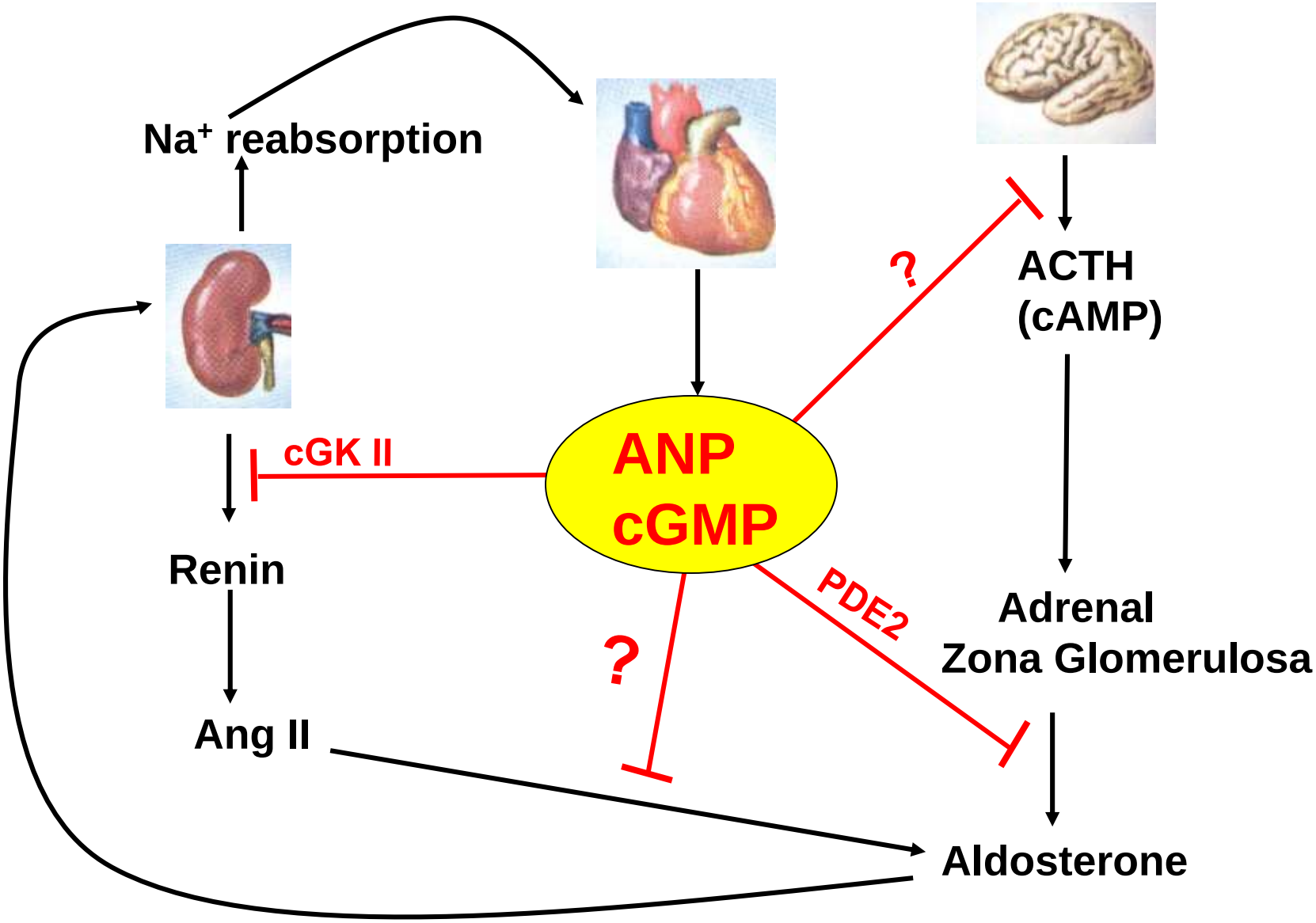
Gambaryan S et al. PNAS 1998

Dual role of cGMP in Regulation of Renin Release from JG Cells

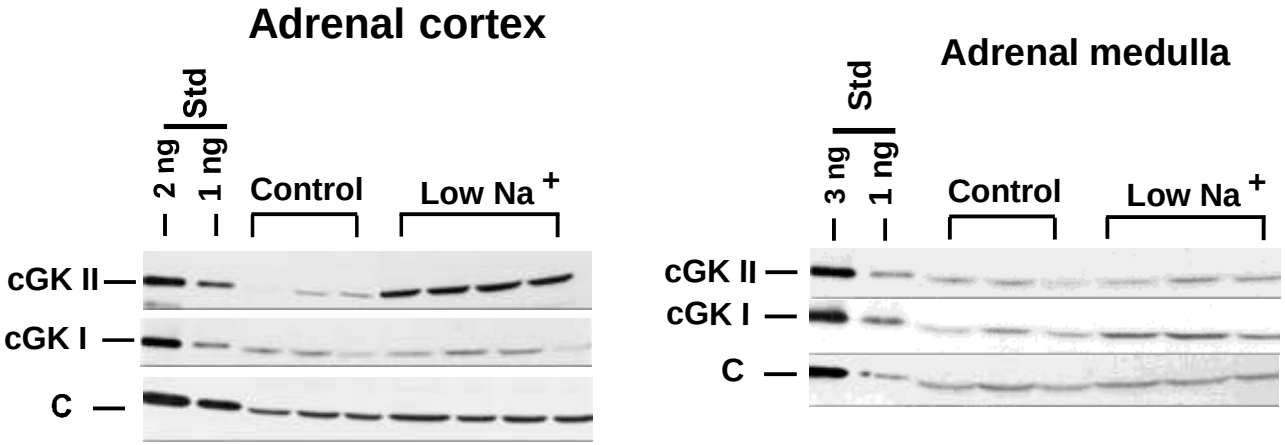


Part 3 Aldosterone

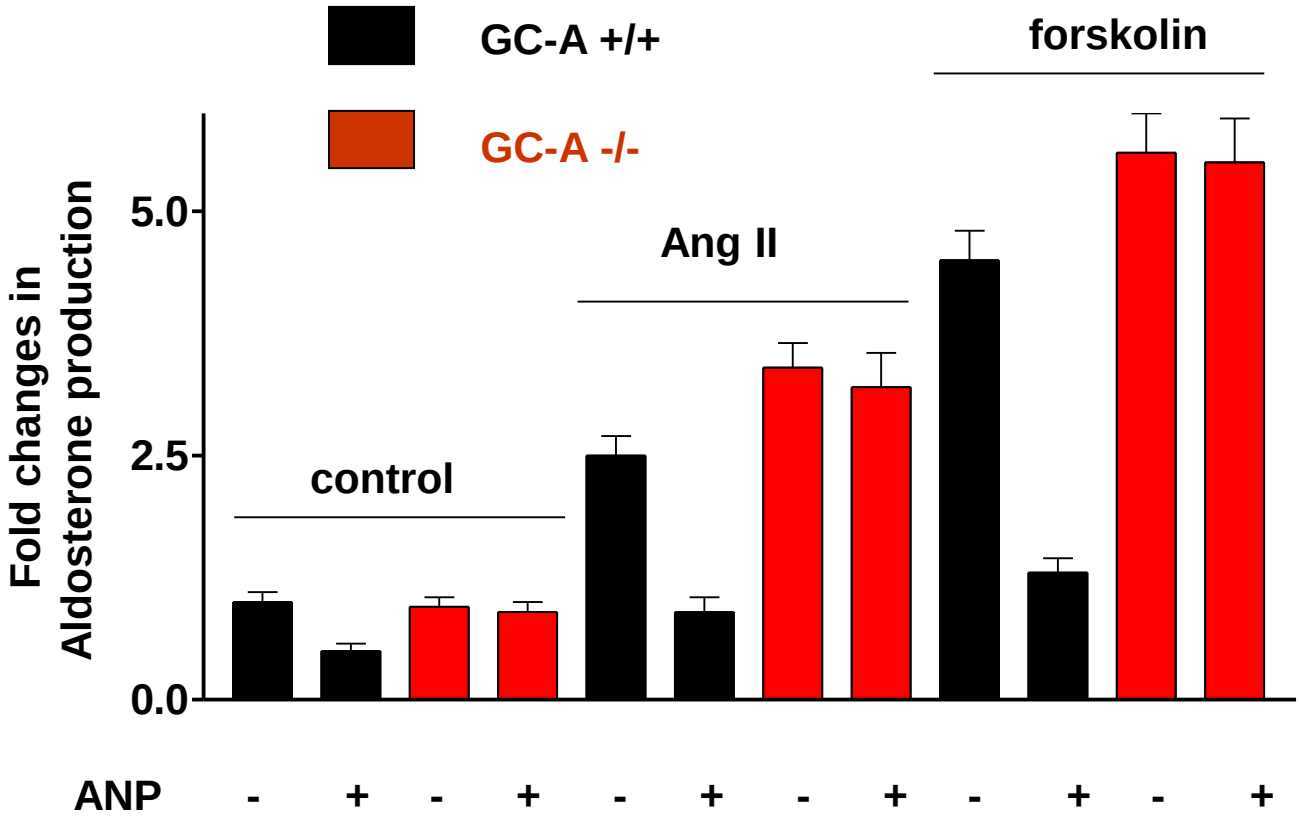
Inhibition of RAAS activity by ANP (cGMP)



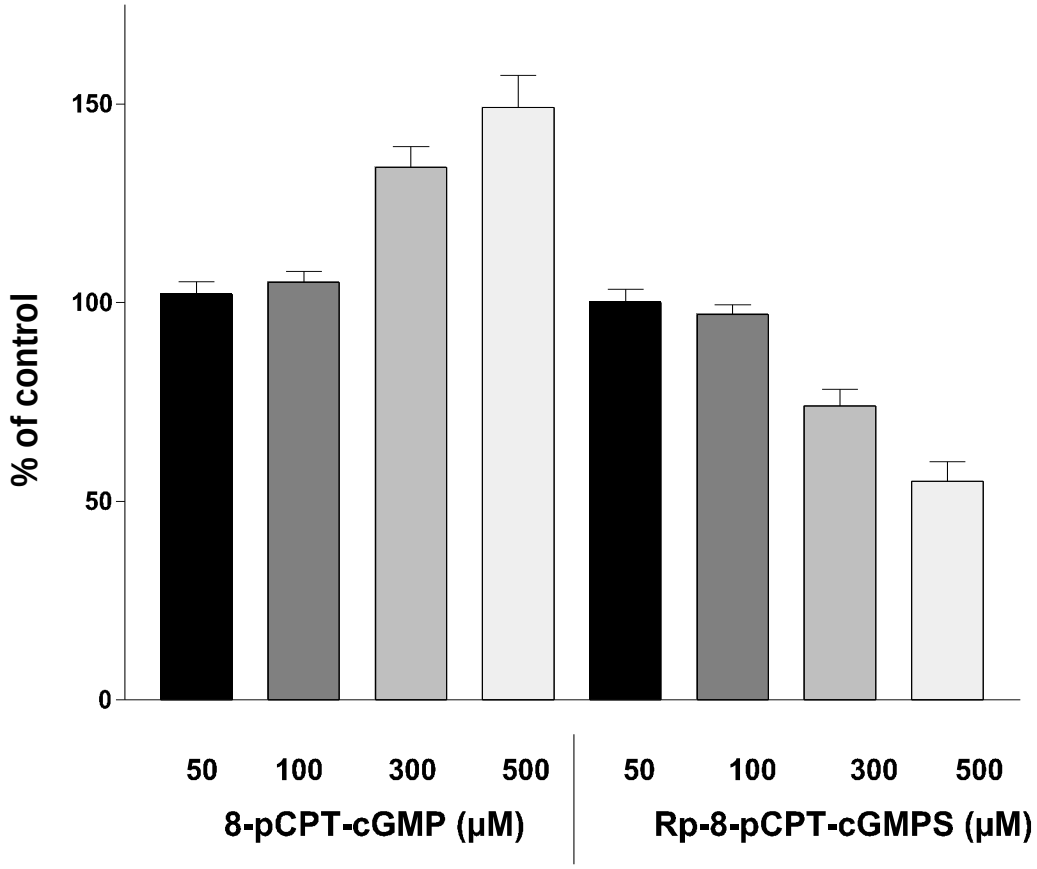
Low Na^+ diet activates expression of cGK II, in rat adrenal cortex



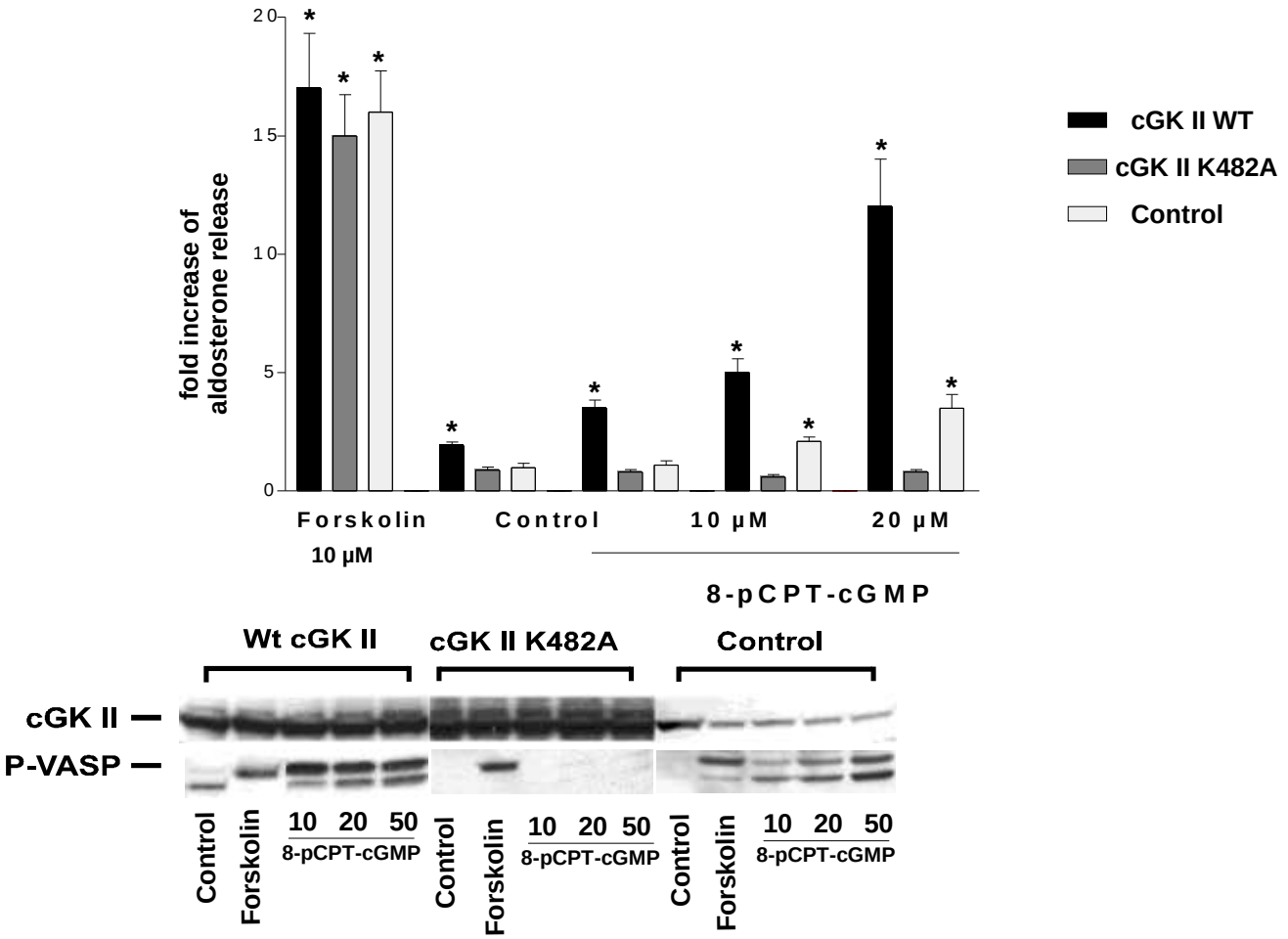
Inhibition of aldosterone production by ANP is mediated by GC-A receptor



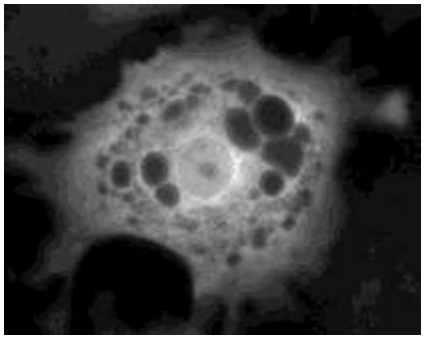
activation of PKG II stimulate basal aldosterone production from rat zona glomerulosa cells

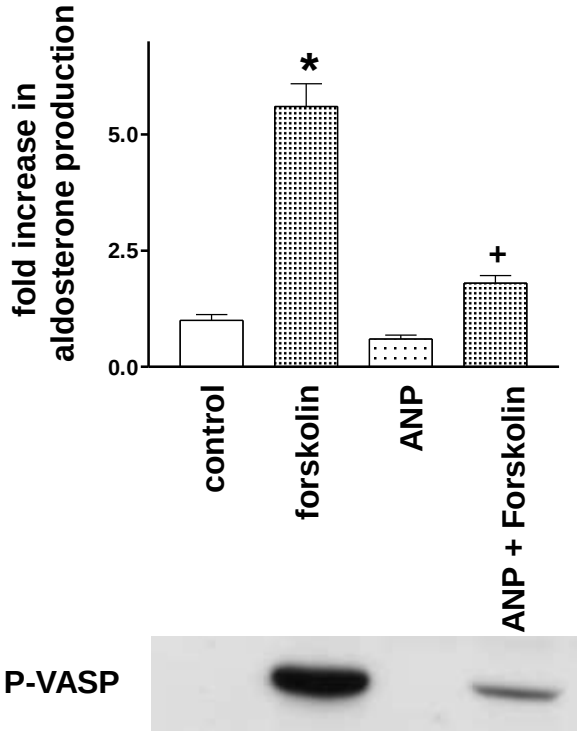


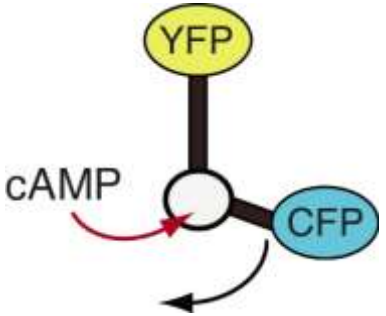
Adenoviral gene transfer of cGK II enhances cGMP effect on aldosterone release from rat zona glomerulosa cells

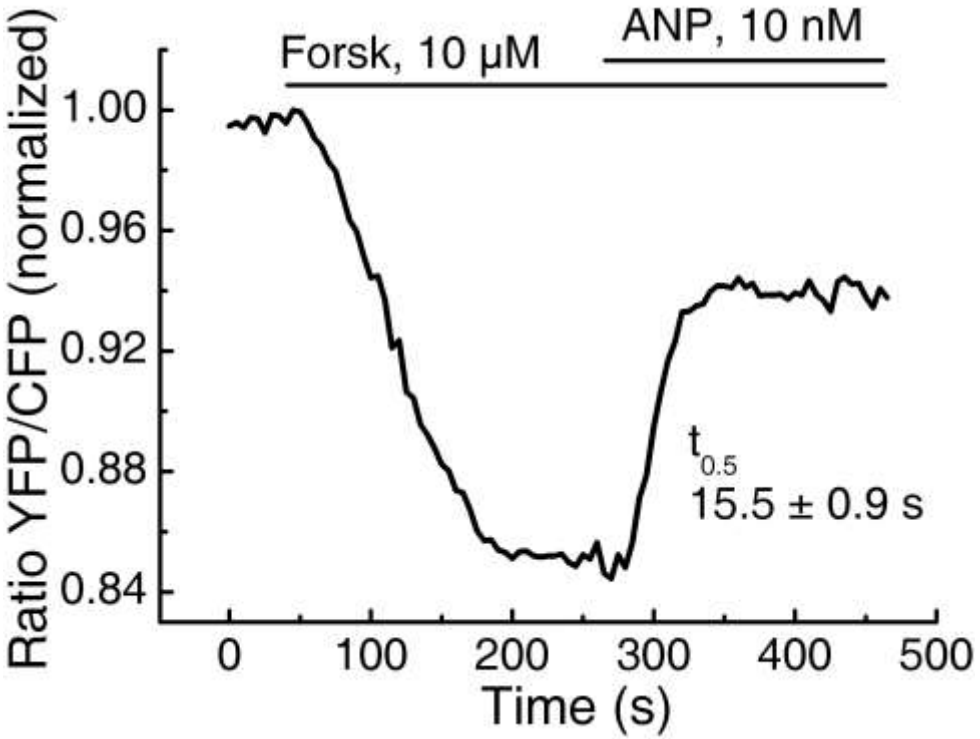


ANP inhibits cAMP stimulated aldosterone production by activation of PDE2

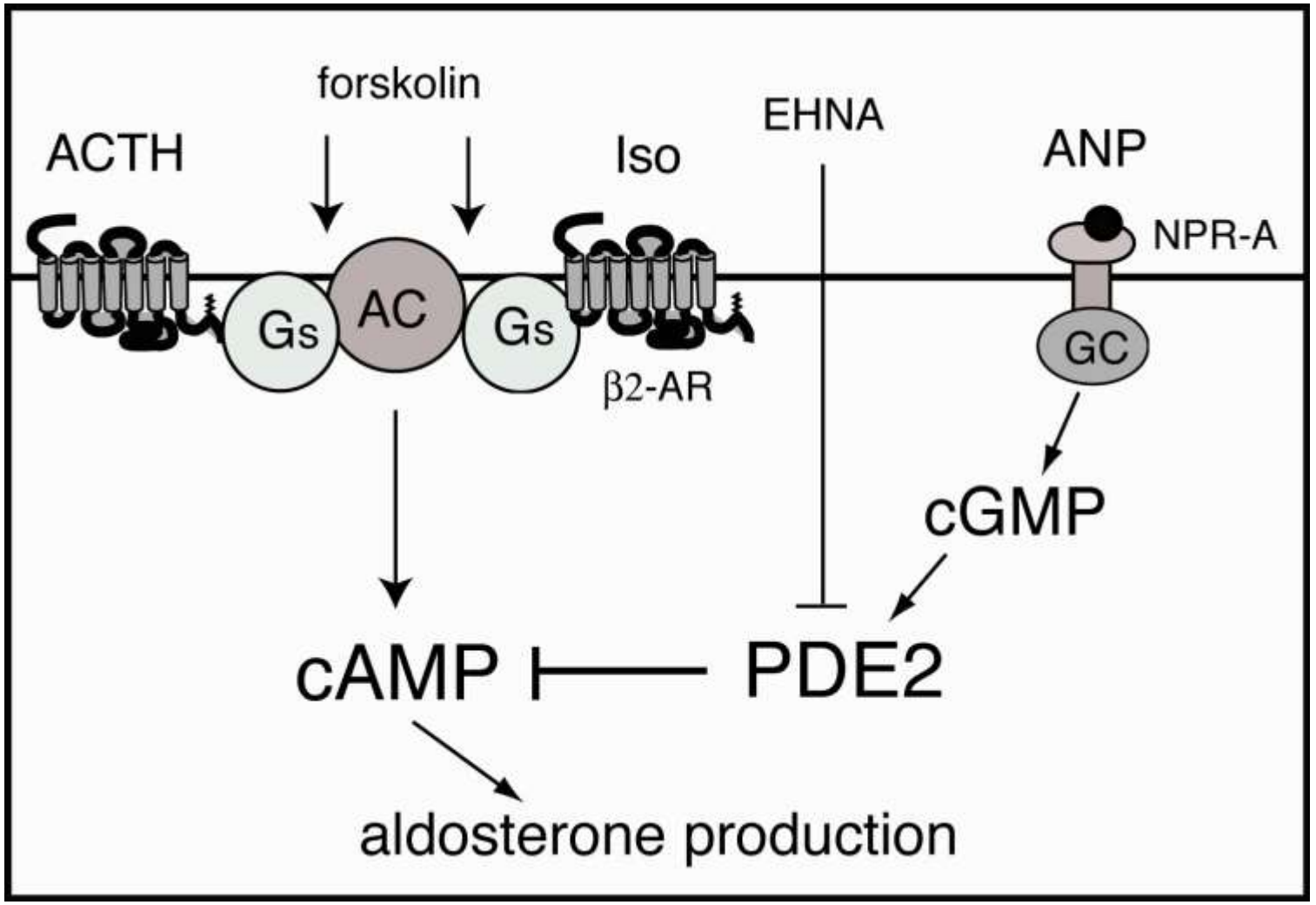




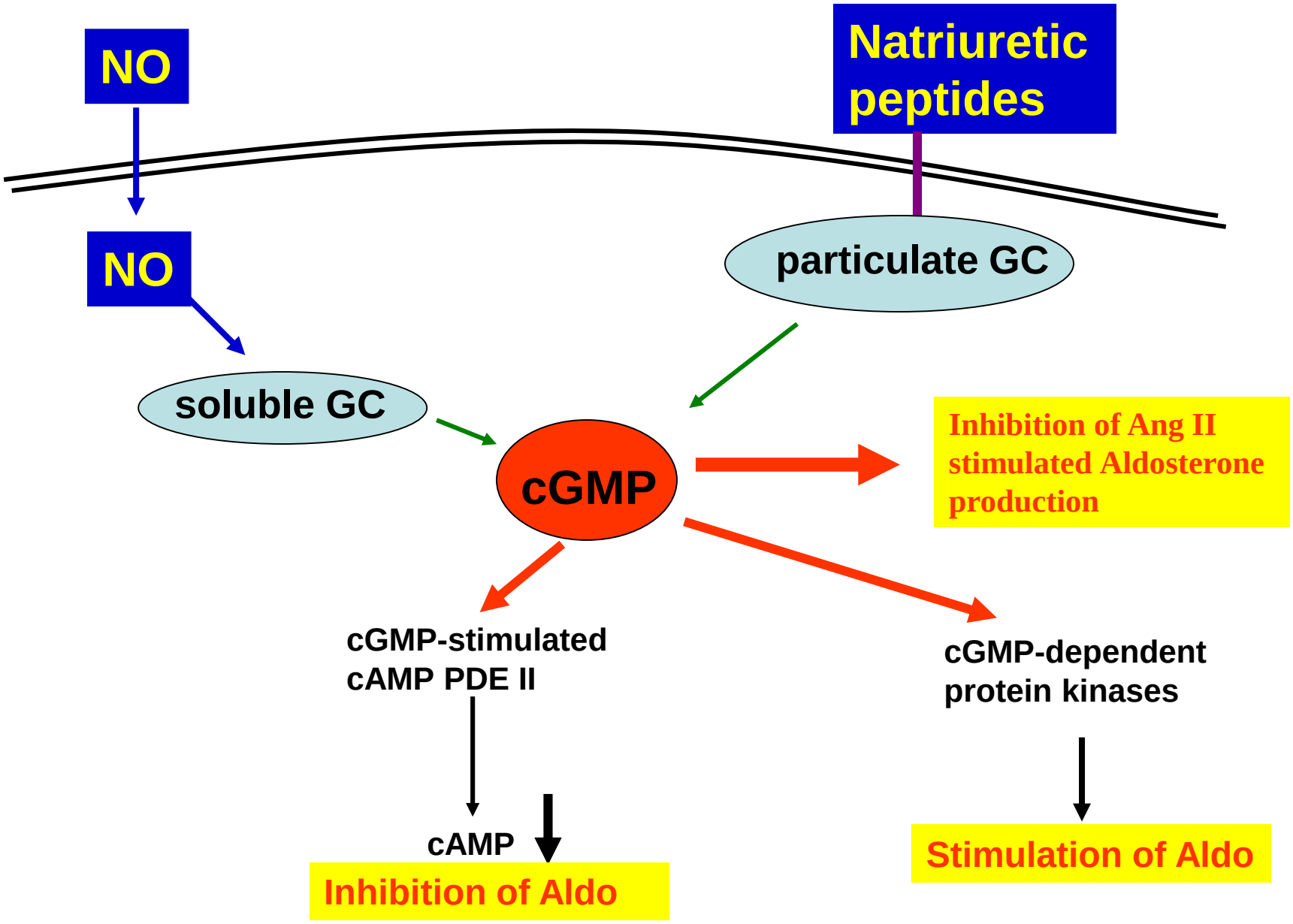




Nikolaev, Gambaryan et al. 2005, JBC

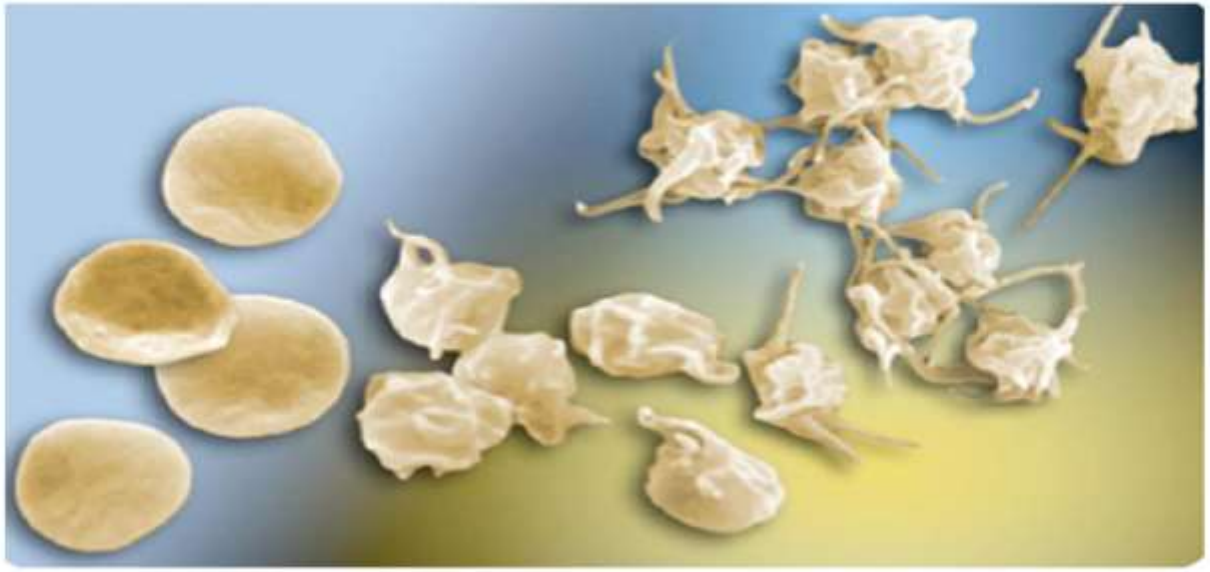

forskolin
ACTH
Iso
EHNA
ANP
NPR-A
Gs
AC
Gs
β2-AR
GC
cGMP
cAMP
PDE2
aldosterone production

Dual mechanisms of cGMP signaling in regulation of aldosterone release



Part 4 Platelet

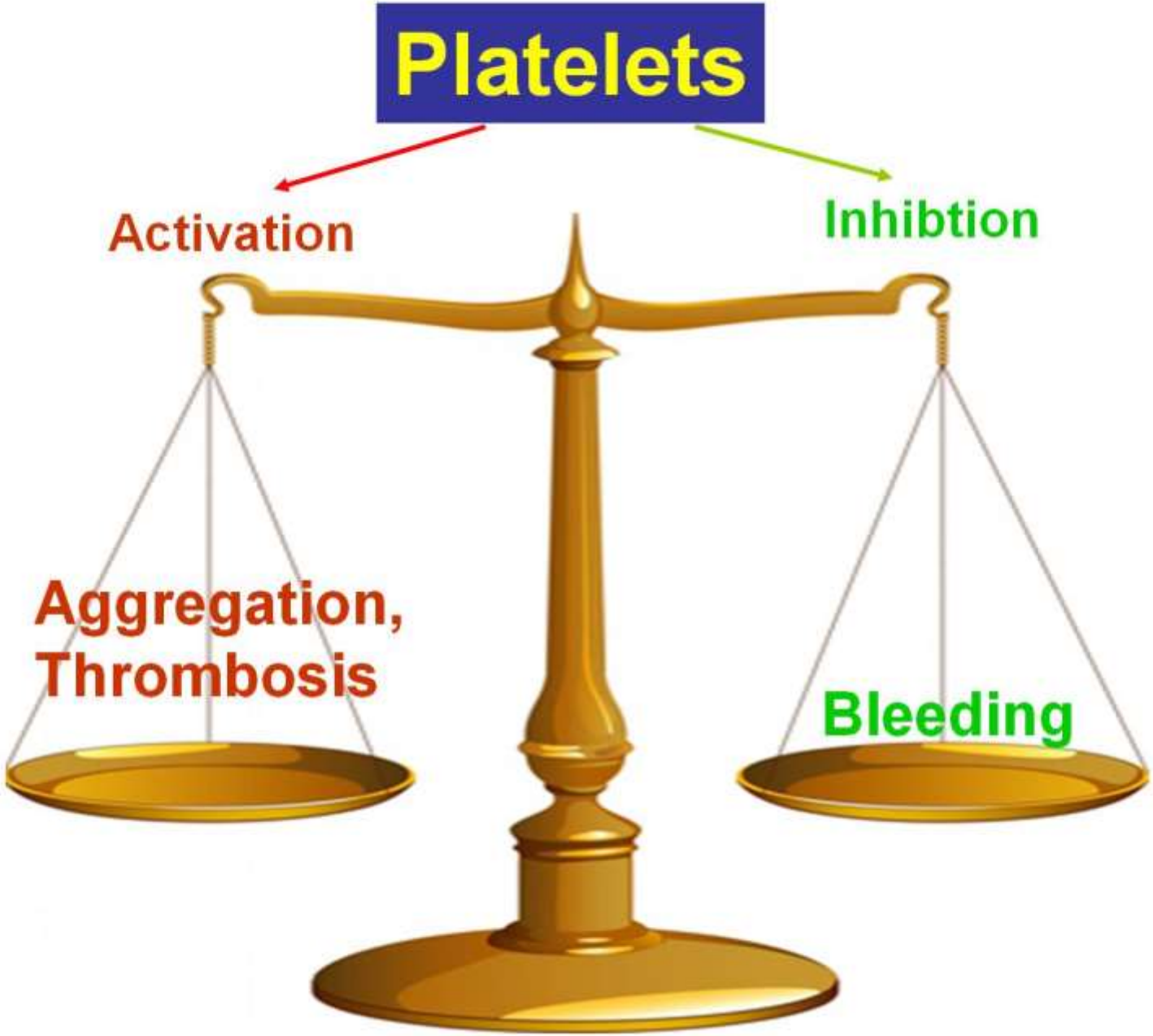
Part 4. Platelets

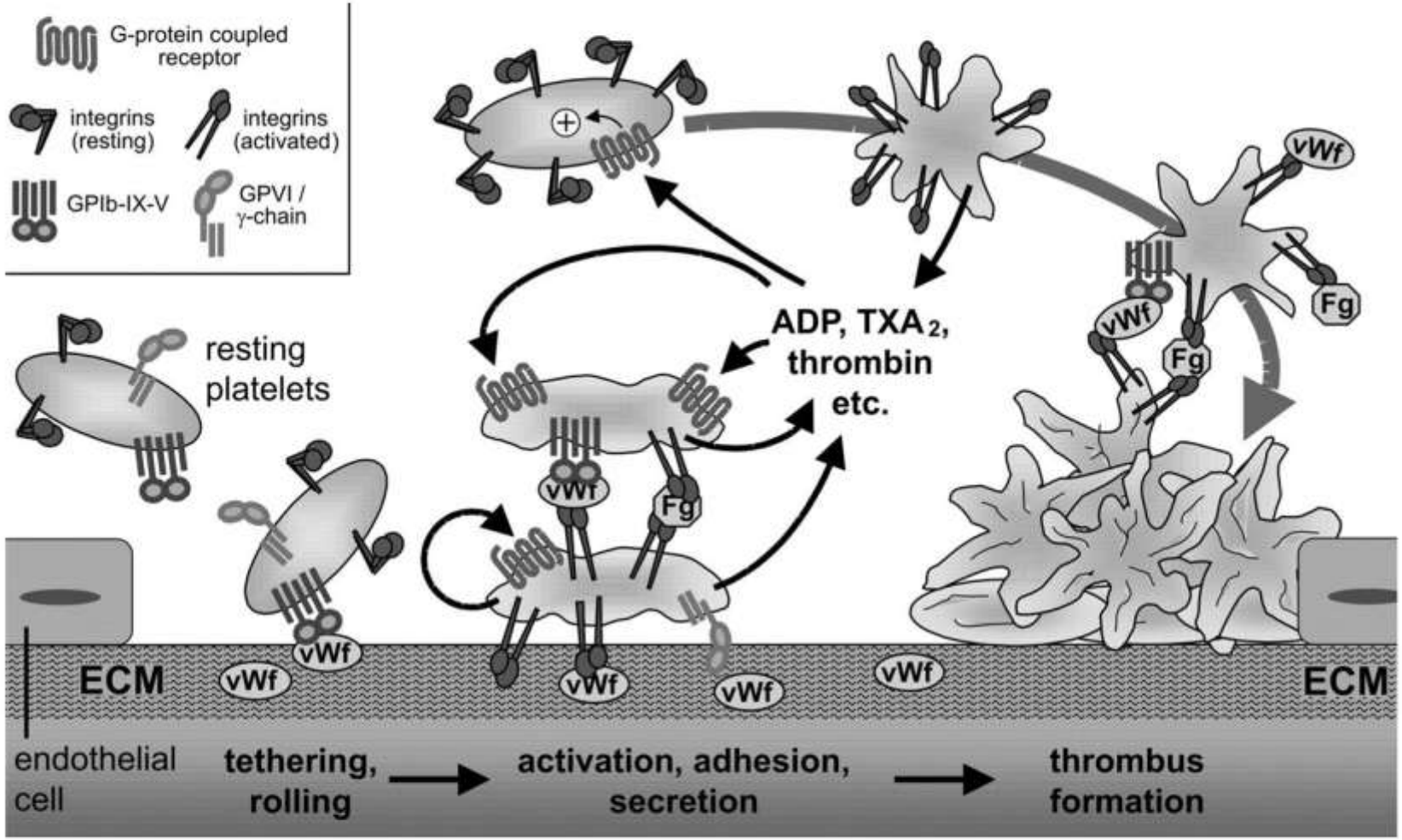


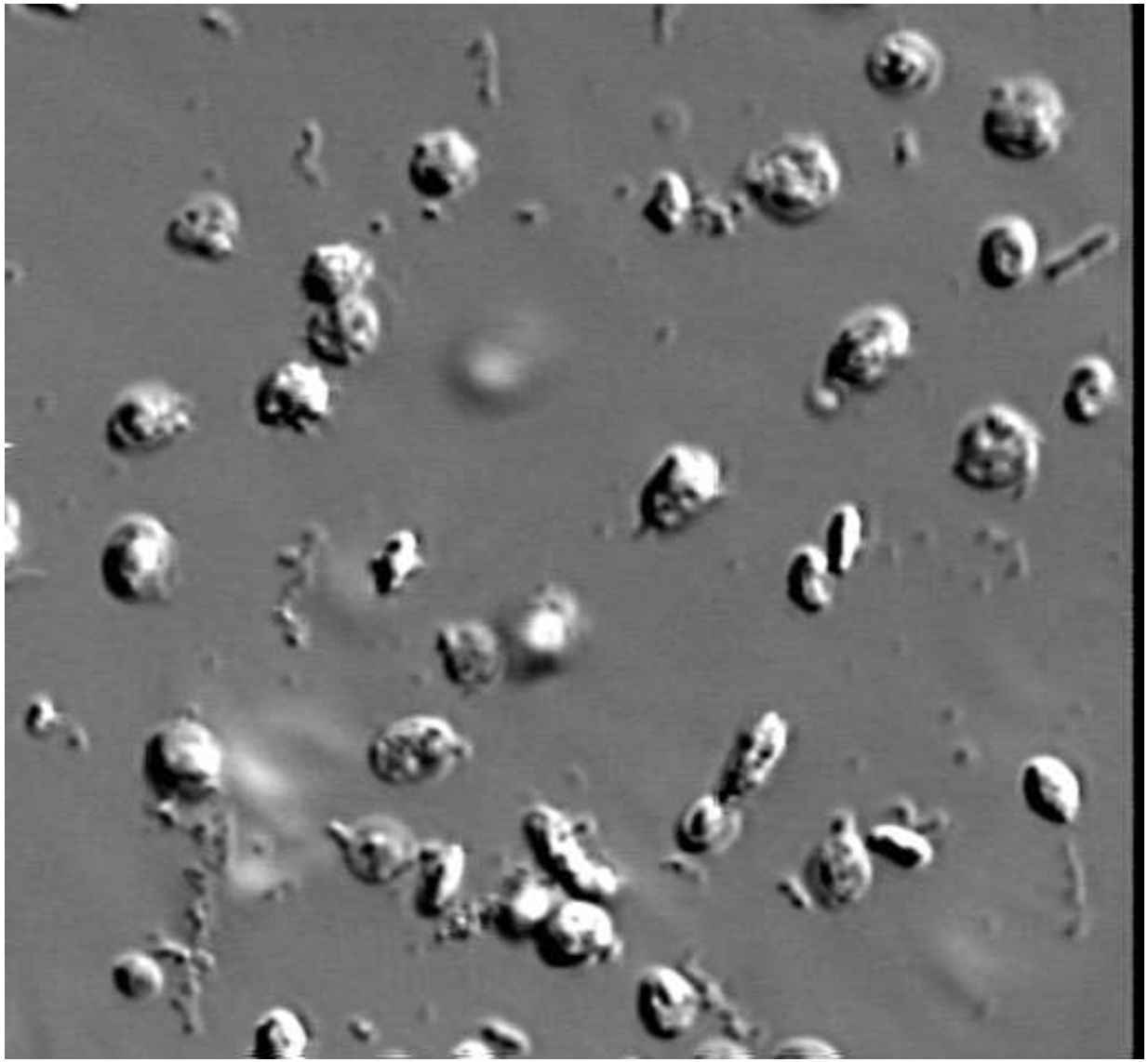
Clinical significance

- 1 x 10^{12} platelets in circulation
- 8 – 10 days life span of platelets
- 10 x 10^{10} new platelets per day
- 150 – 400 x 10^{8}/ml normal range in blood

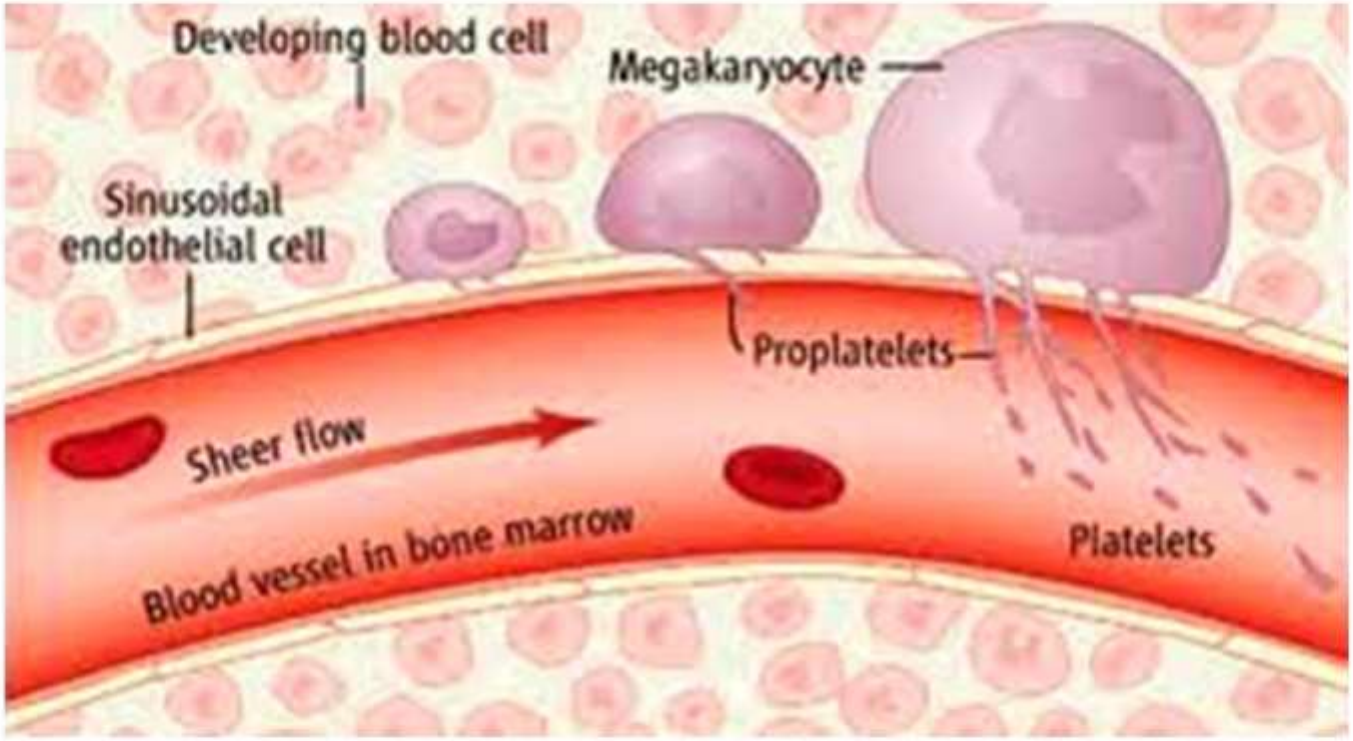
- Thrombocytosis – high number of platelets (thrombosis)
- Thrombocytopenia – low number of platelets (bleeding)

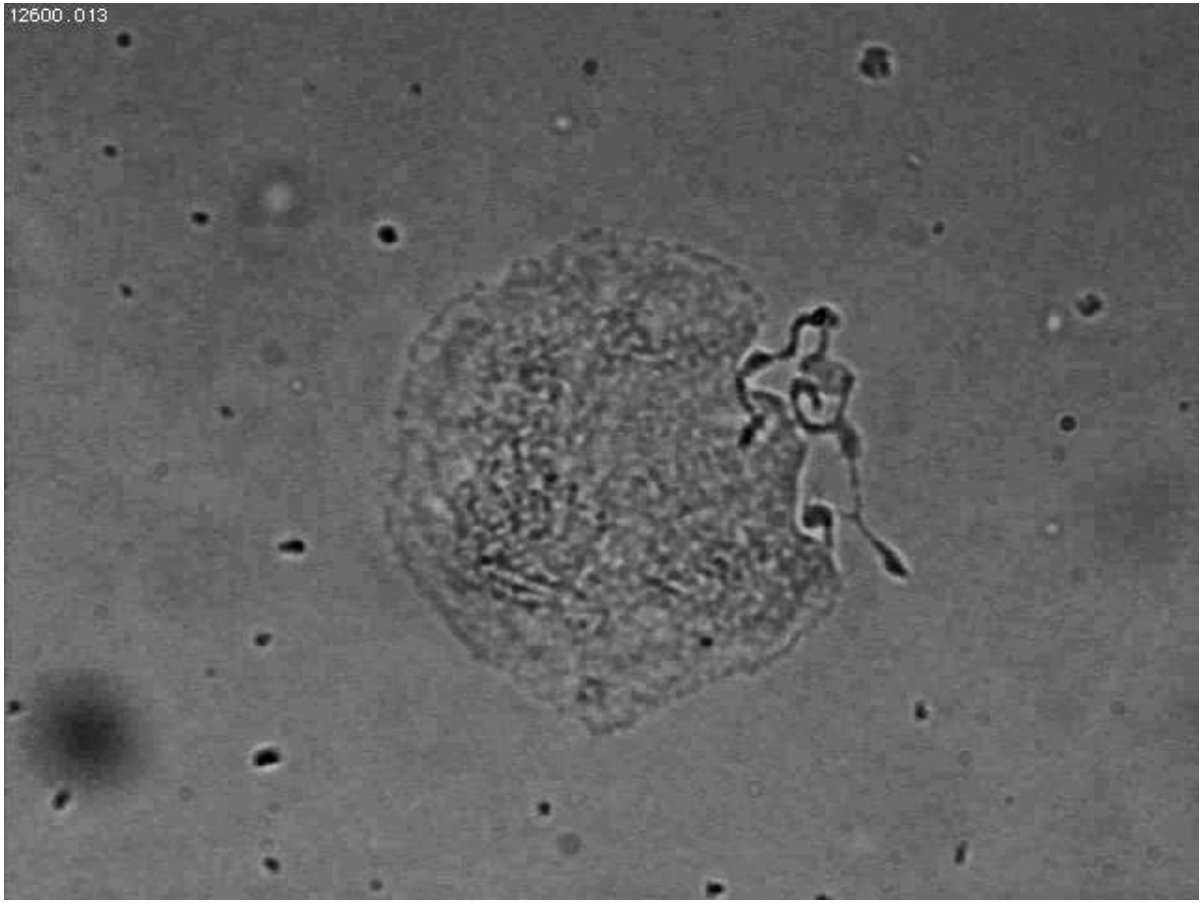

Platelets
Activation
Inhibtion
Aggregation,
Thrombosis
Bleeding


G-protein coupled receptor
integrins (resting)
integrins (activated)
GPIb-IX-V
GPVI / γ-chain
resting platelets
ADP, TXA2, thrombin etc.
vWf
Fg
ECM
endothelial cell
tethering, rolling
activation, adhesion, secretion
thrombus formation

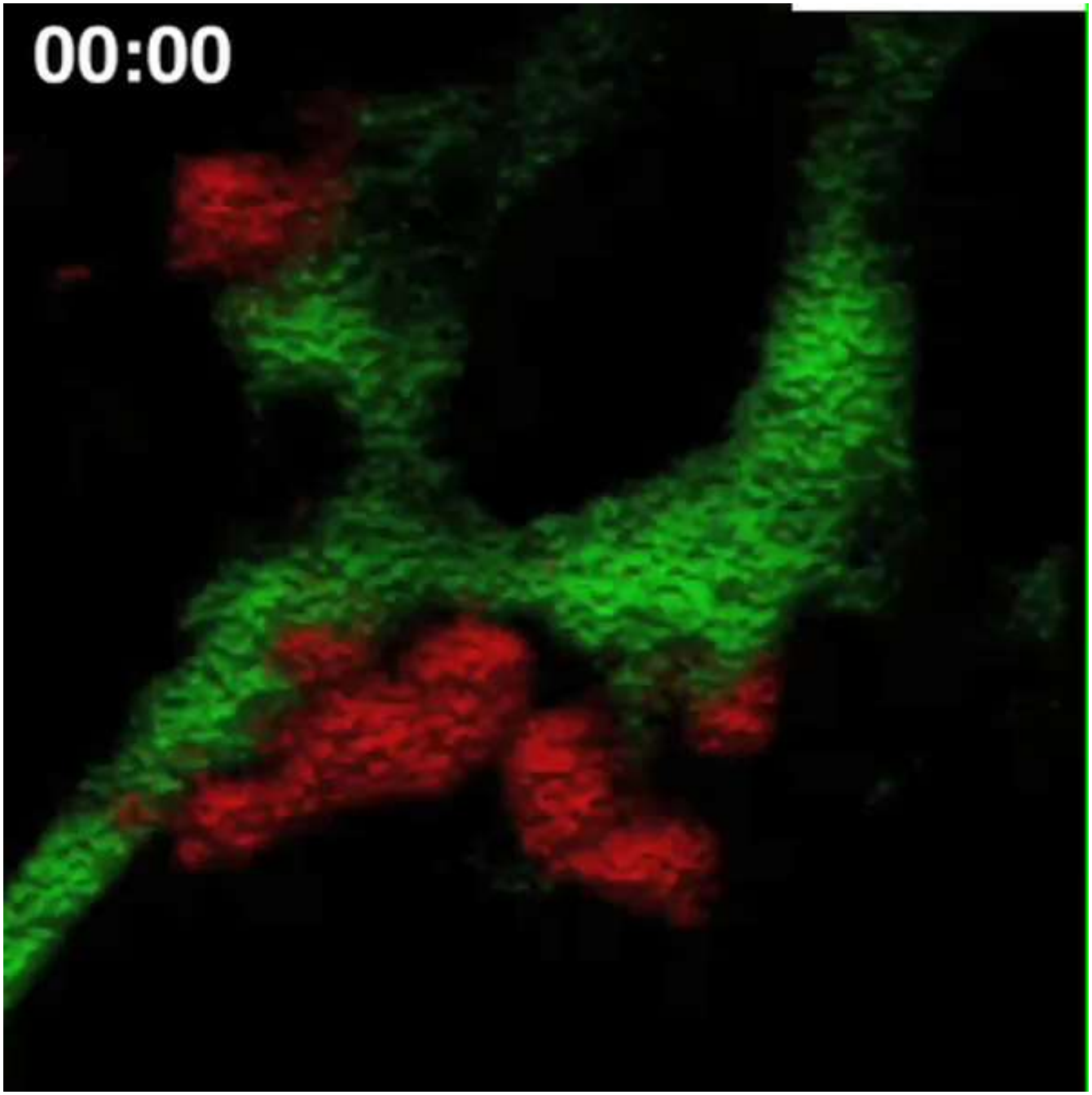


Platelet production from megacaryocytes





Italiano, 1999 JCB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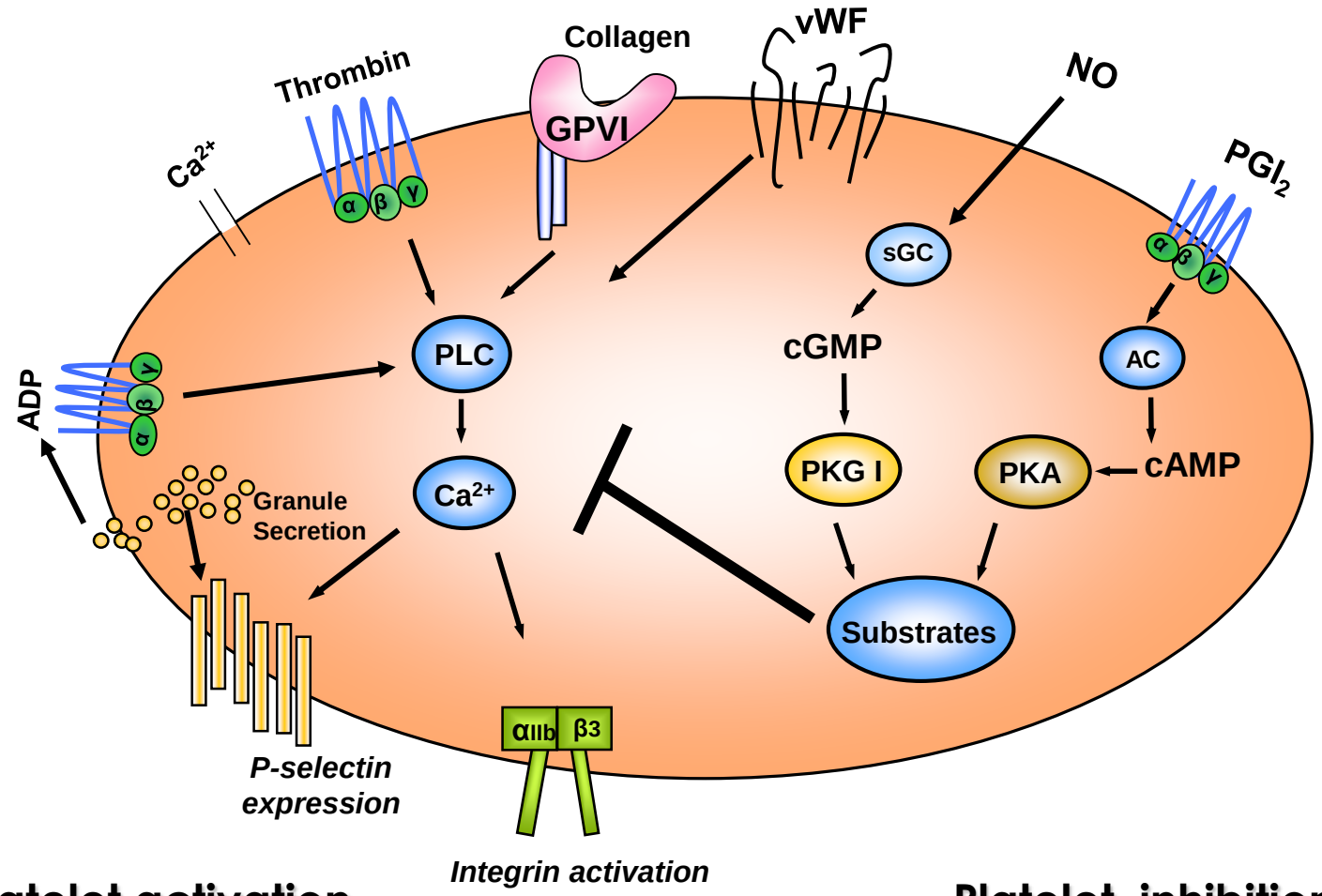
Stimulatory and inhibitory pathways in platelets

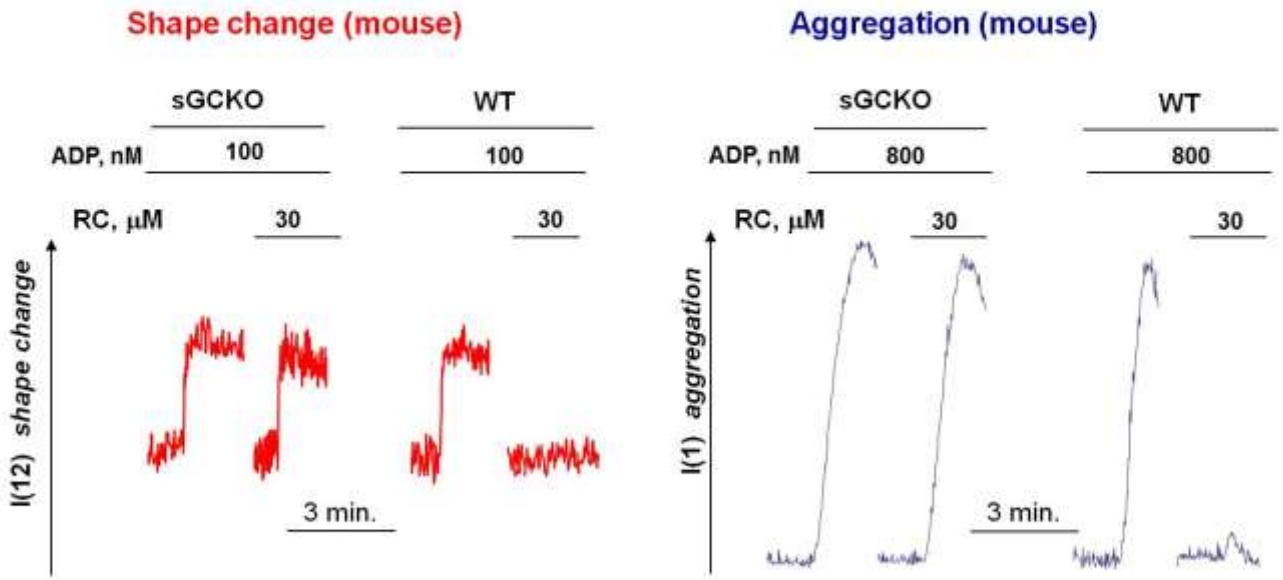


Platelet activation

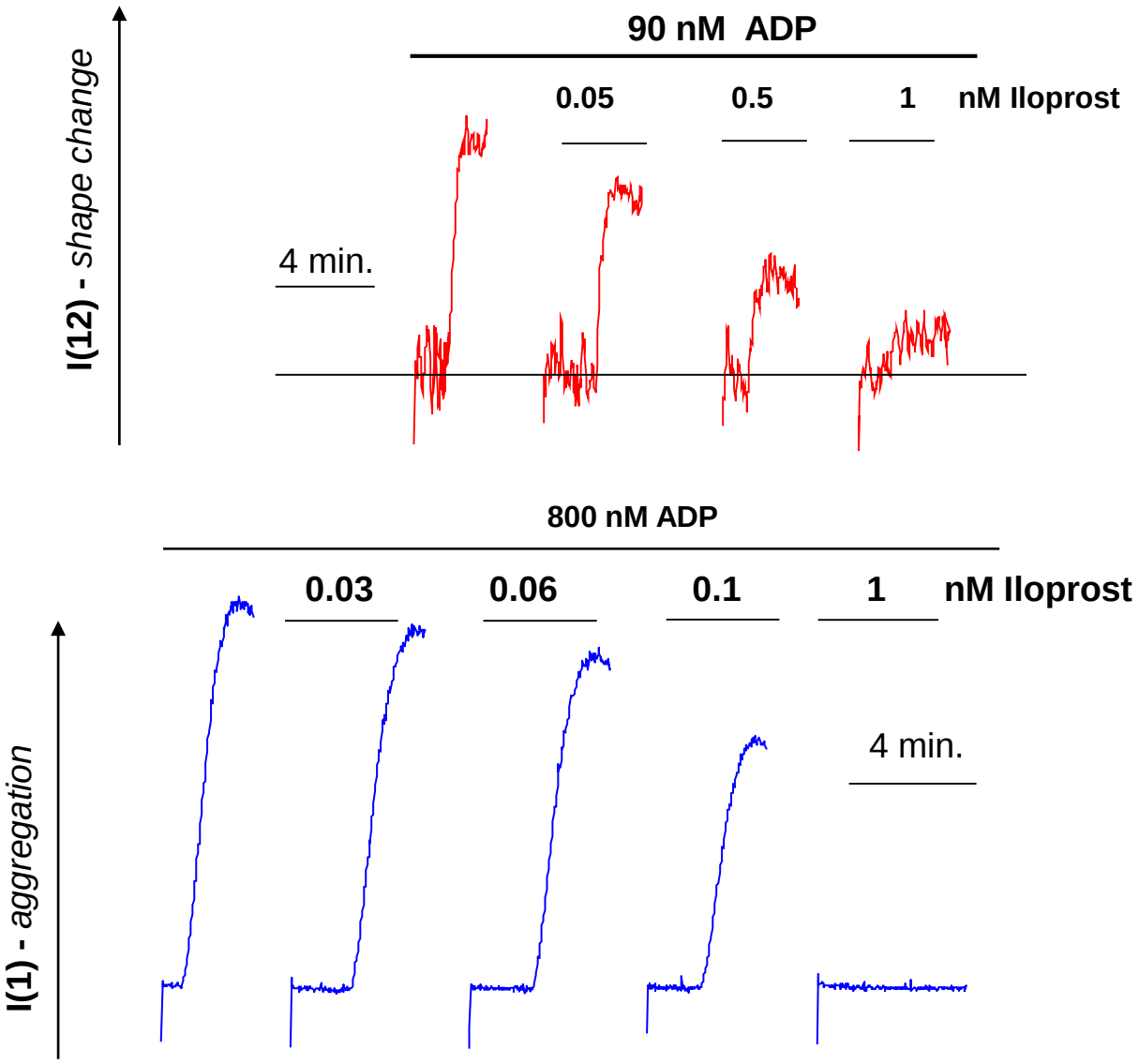
Platelet inhibition

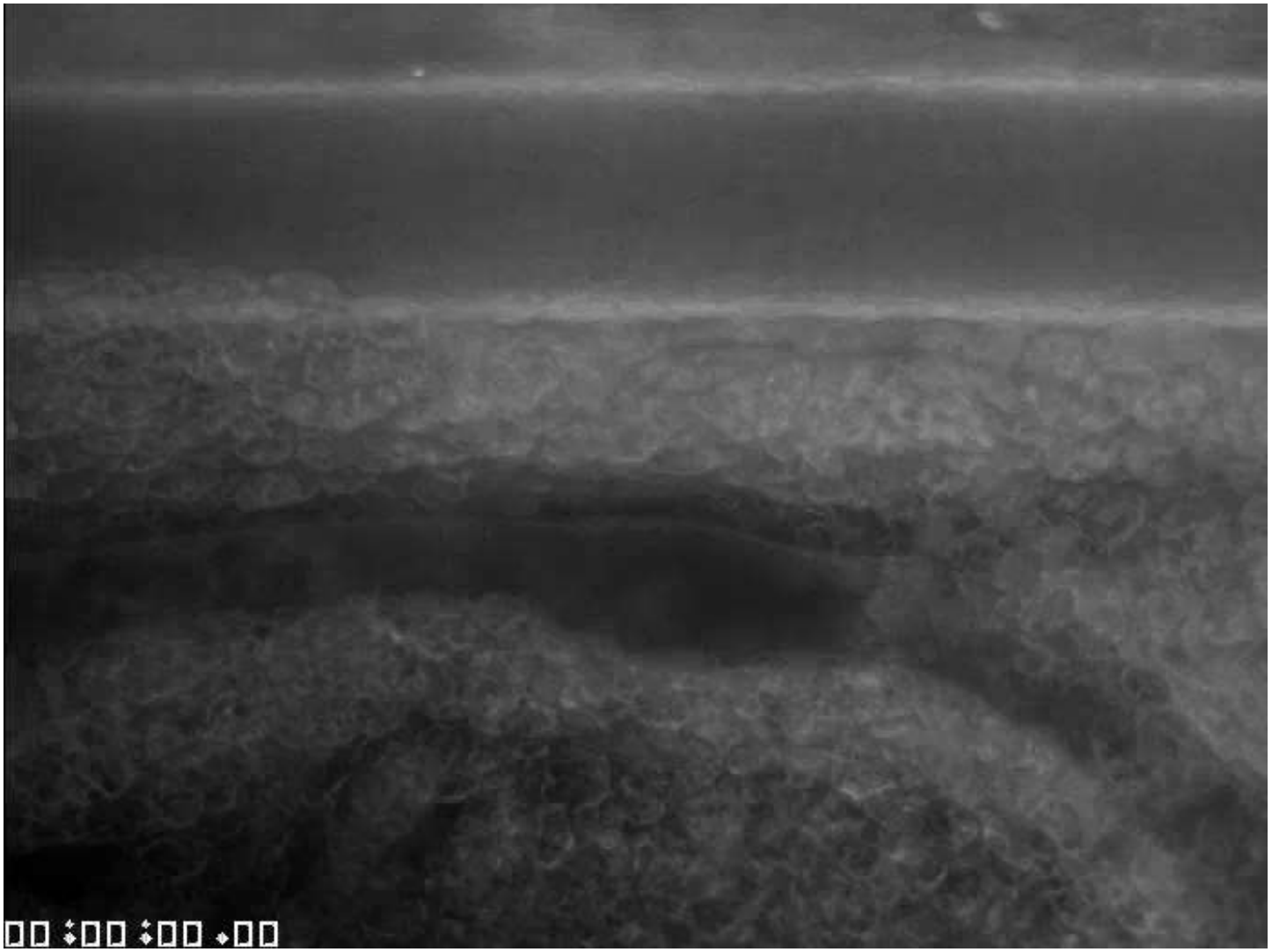
Platelet inhibition by cGMP pathway

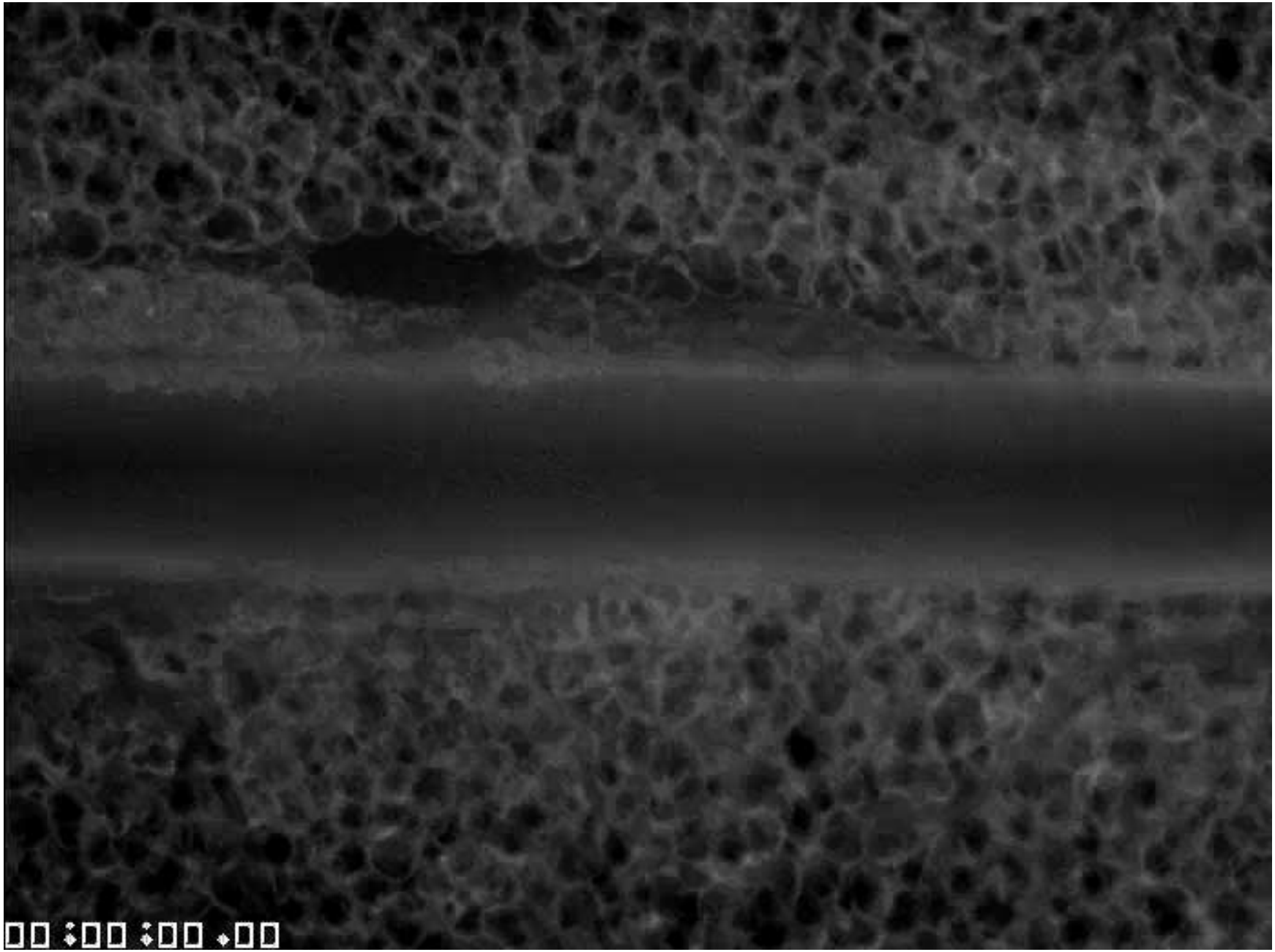
Activation of sGC by riociguat (RC) inhibits shape change and aggregation only in WT, but not sGCKO mouse platelets



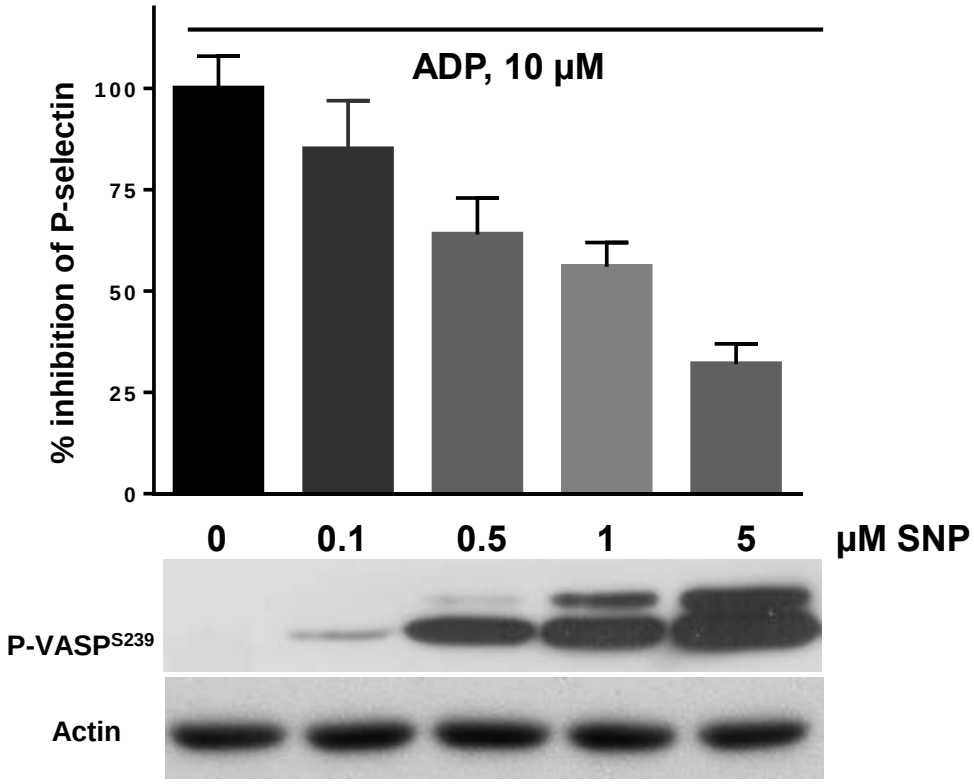
Platelet inhibition by cAMP pathway



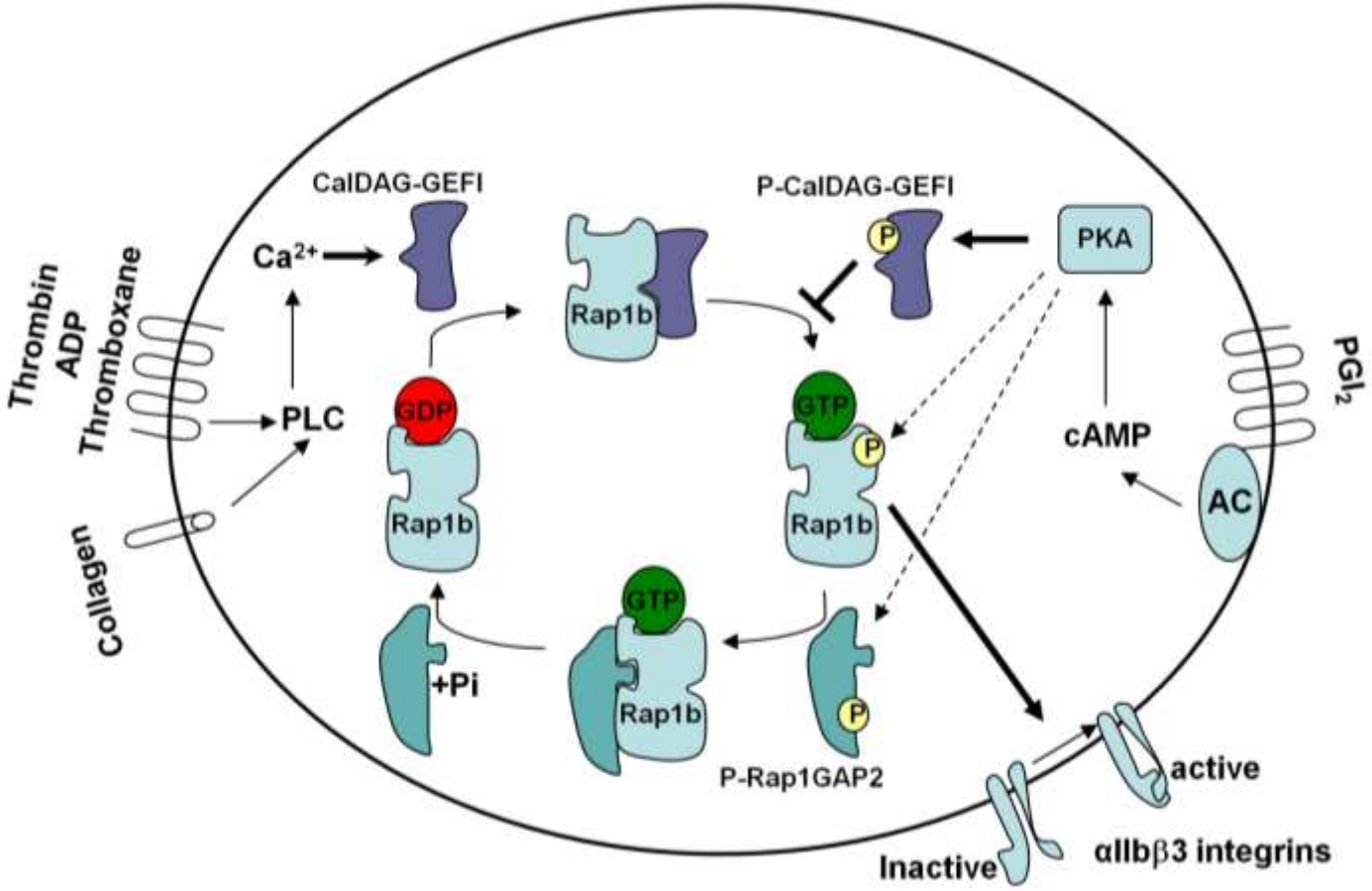




Inhibition of ADP-stimulated platelet activation by NO/sGC/cGMP/PKG pathway

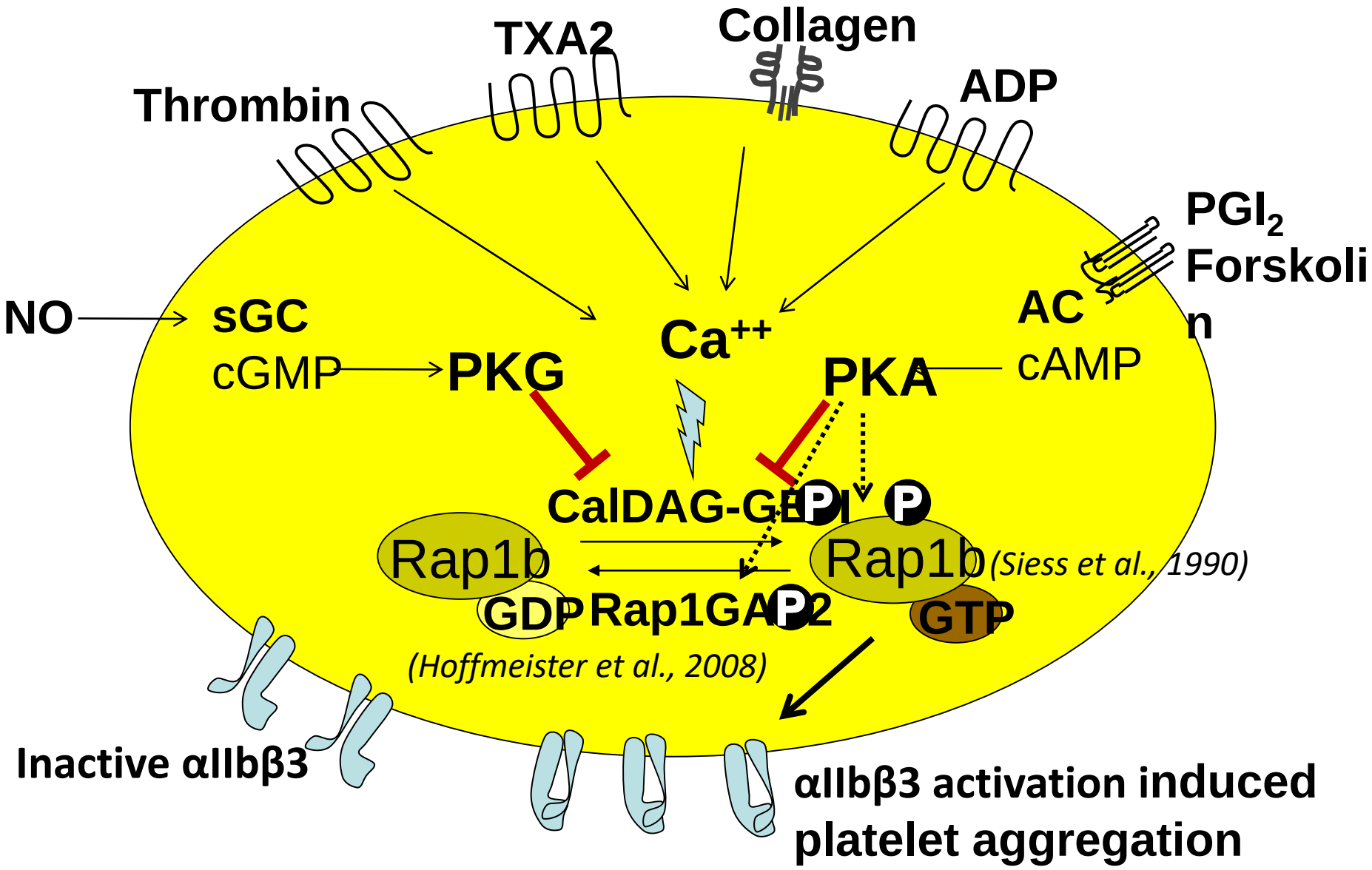


Phosphorylation of CalDAG-GEFI is a major PKA-mediated inhibitory pathway

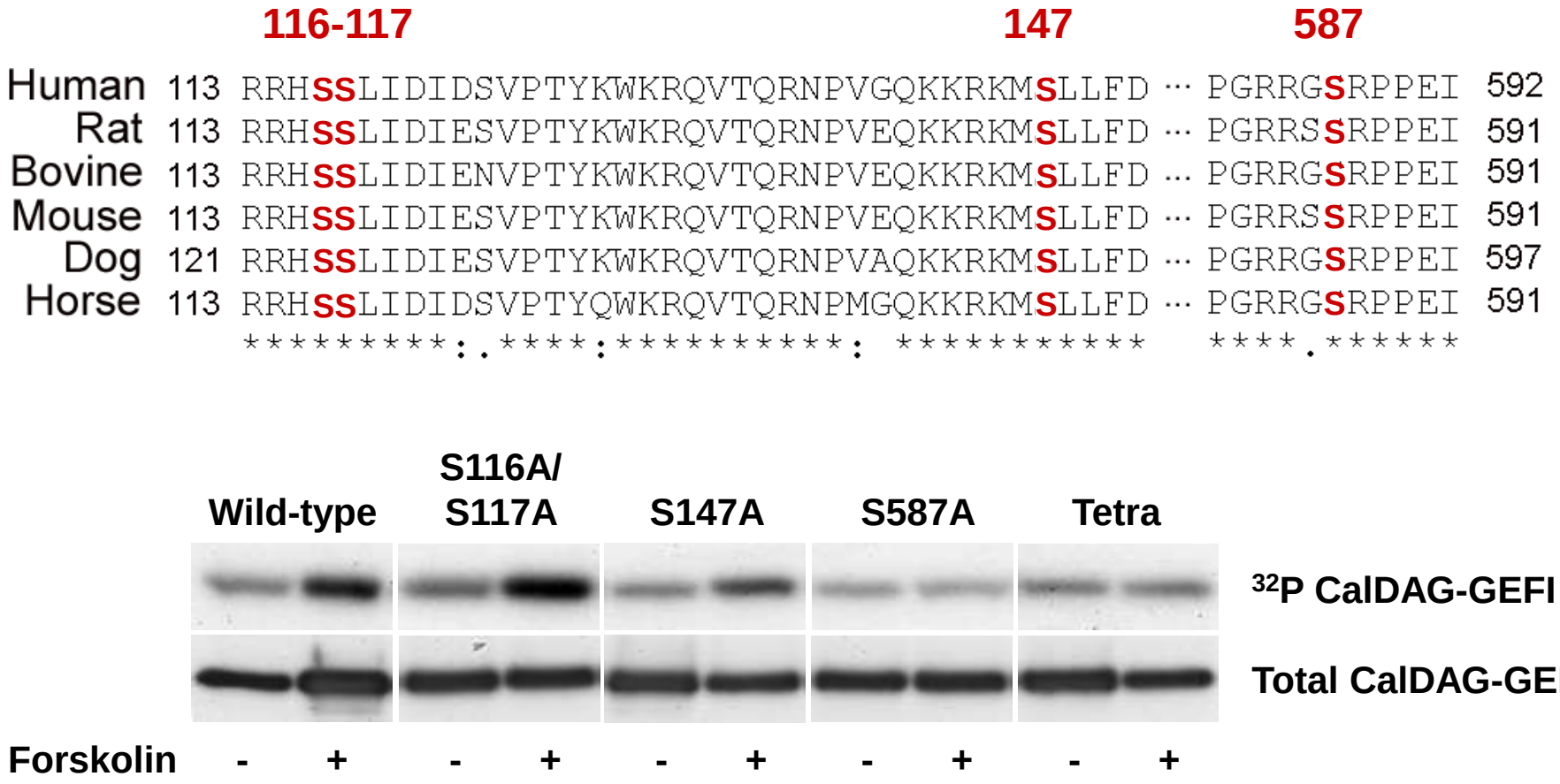


Cover figure, JTH, August 2013

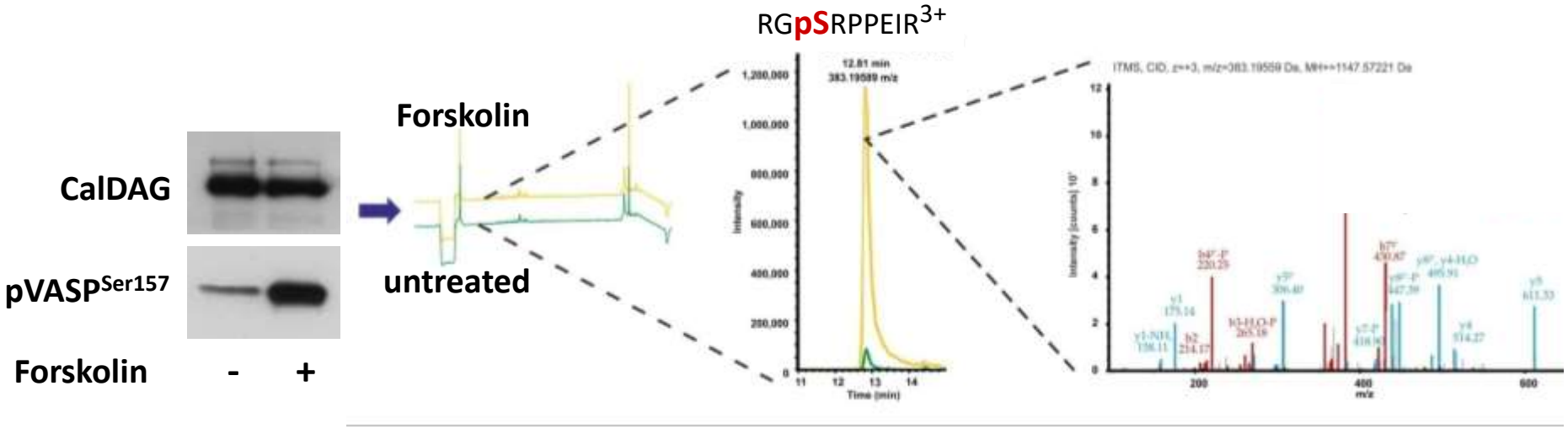
Regulation of Rap1b activation in platelets



S587 is the major PKA phosphorylation site in CalDAG-GEFI

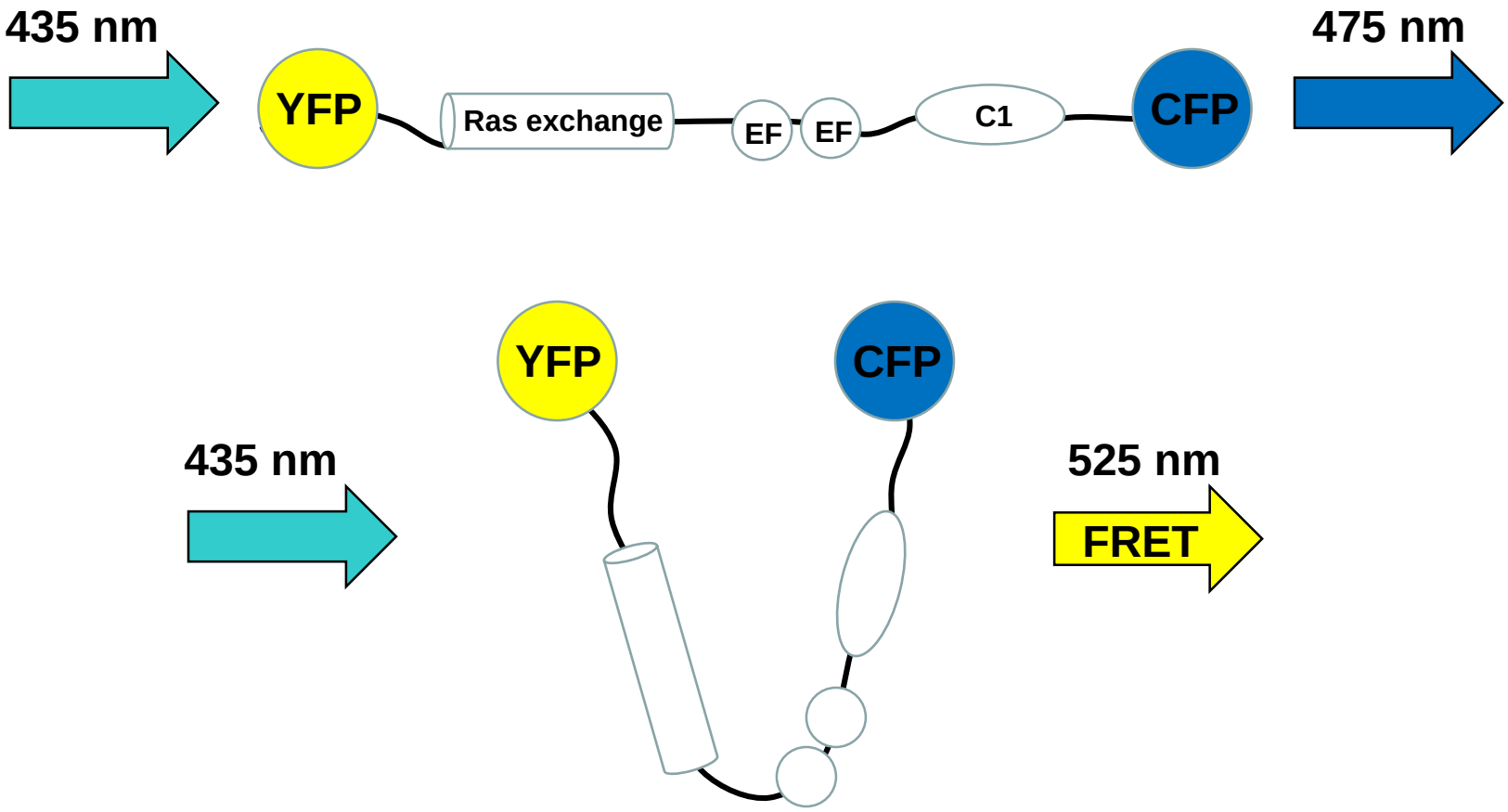


CalDAG-GEFI^{S587} is strongly phosphorylated in PKA activated human platelets



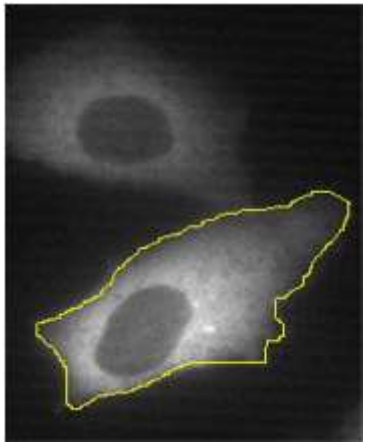
- S587 phosphorylation is 35-fold higher in PKA activated platelets.
- S116 is weakly phosphorylated.

FRET based CalDAG-GEFI sensor

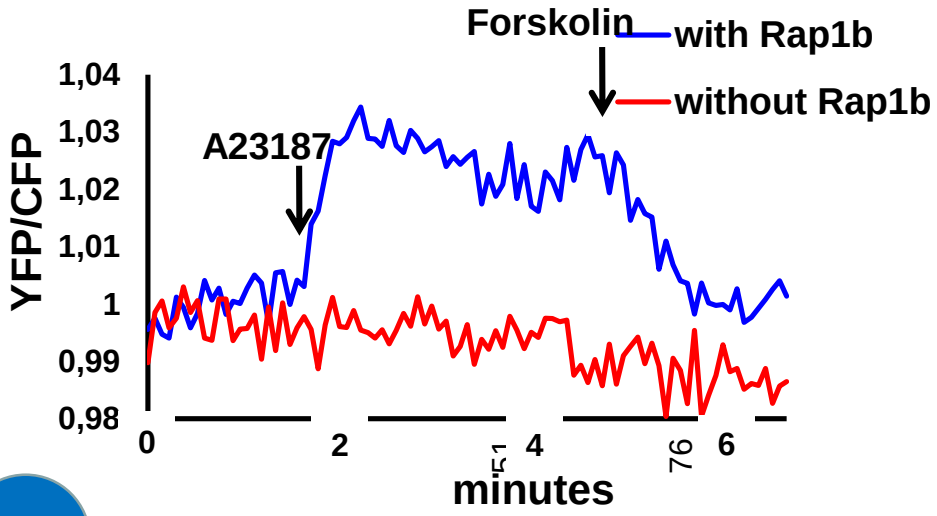


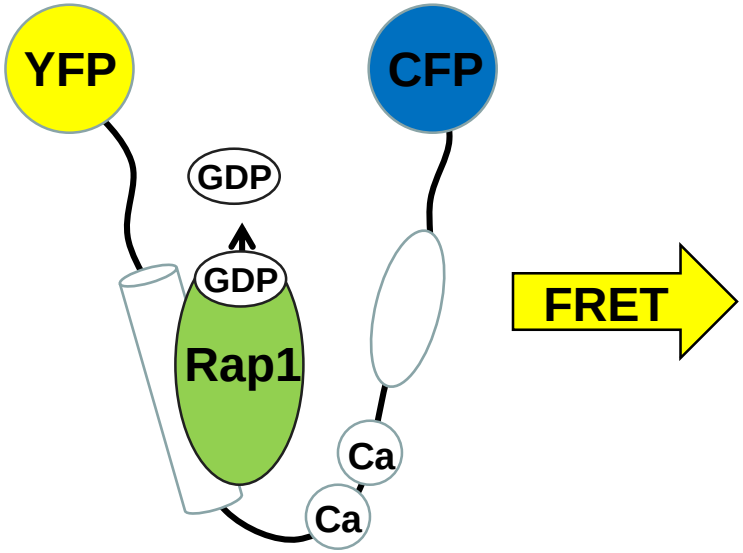
FRET analysis and CalDAG-GEFI conformation

Sensor and Rap1b in HEK293 cells

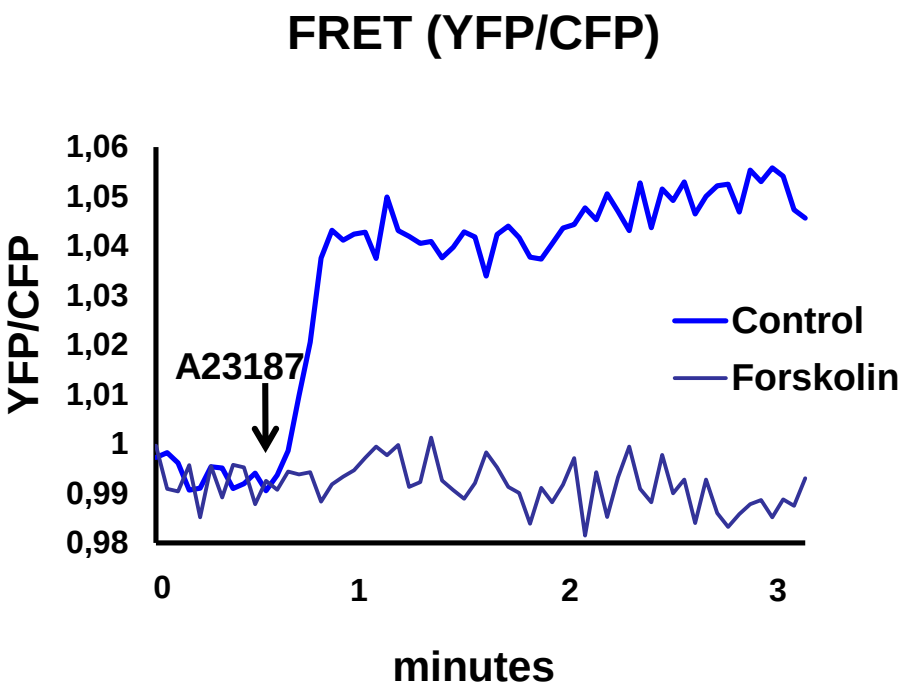


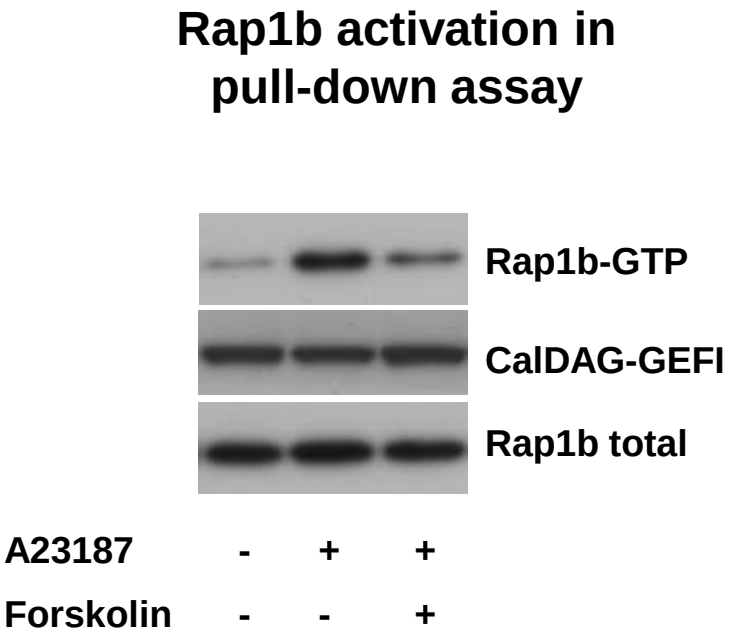
FRET (YFP/CFP)



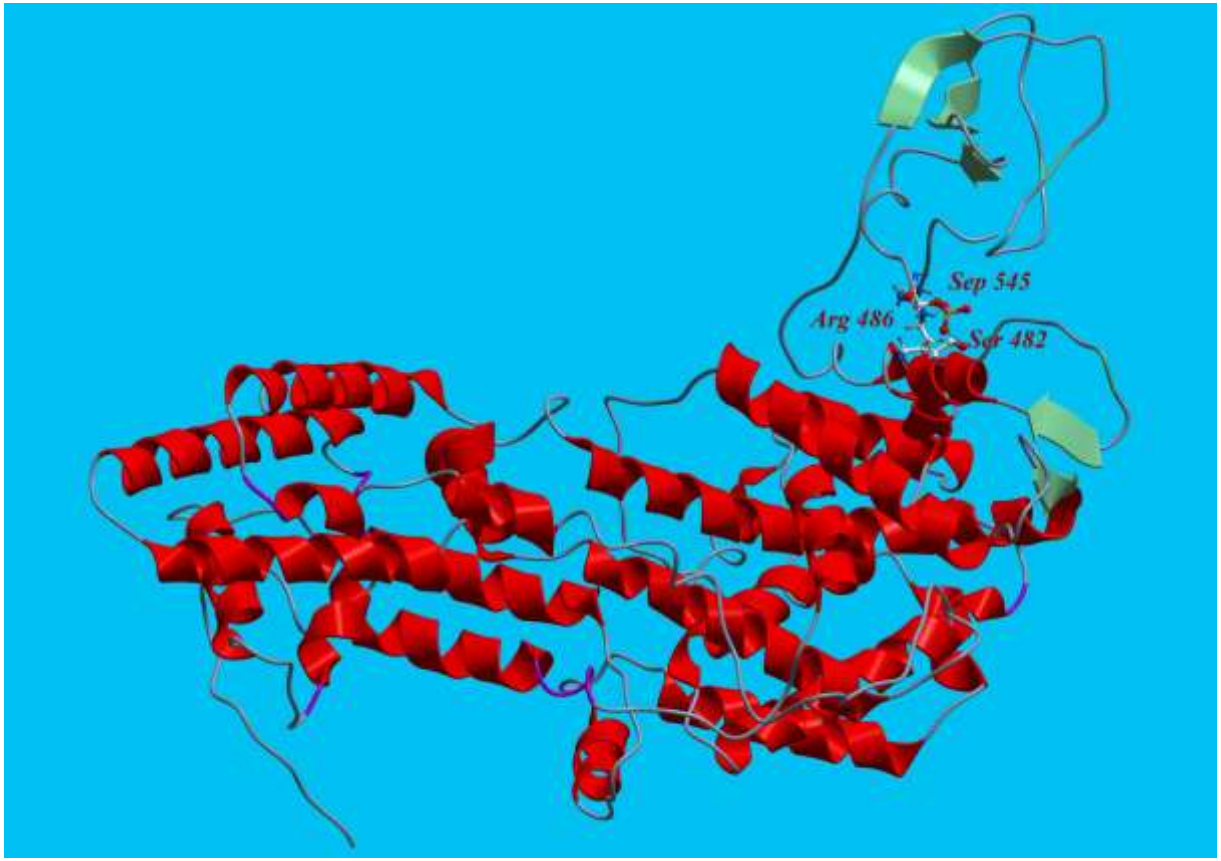


PKA activation prevents conformation change in CalDAG-GEFI and inhibits Rap1b activation





Molecular structure of CalDAG-GEF I



Collaboration with M. Petuchov, Yu. Orlov (SPbSPU)

SUMMARY

- **In JG cells cGMP by activation of PKG inhibits renin release, and by stimulation of PDE3 potentiates cAMP stimulatory effect.**

- **In ZG cells cGMP by activation of PKG stimulates aldosterone production; by activation of PDE2 inhibits cAMP-mediated aldosterone production.**

- **In platelets, both PKG and PKA are major inhibitory mechanisms.**

Thanks to all who were involved in these projects and thanks for your attention