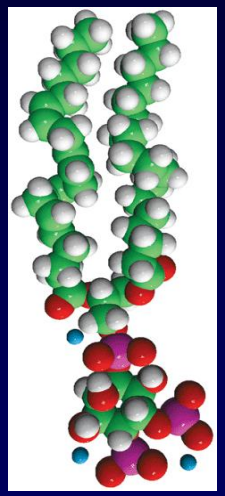
Rôles des phosphoinositides dans les pathologies cardiovasculaires

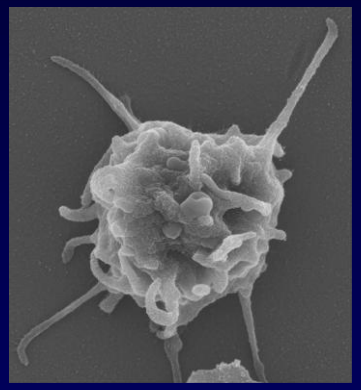


PI(4,5)P2

B. Payrastre *et al.*

INSERM U1048, I2MC,

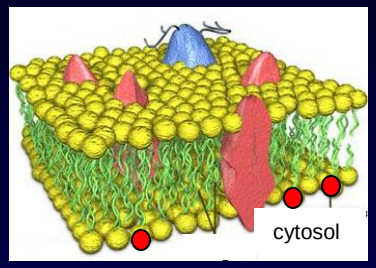
Toulouse, France

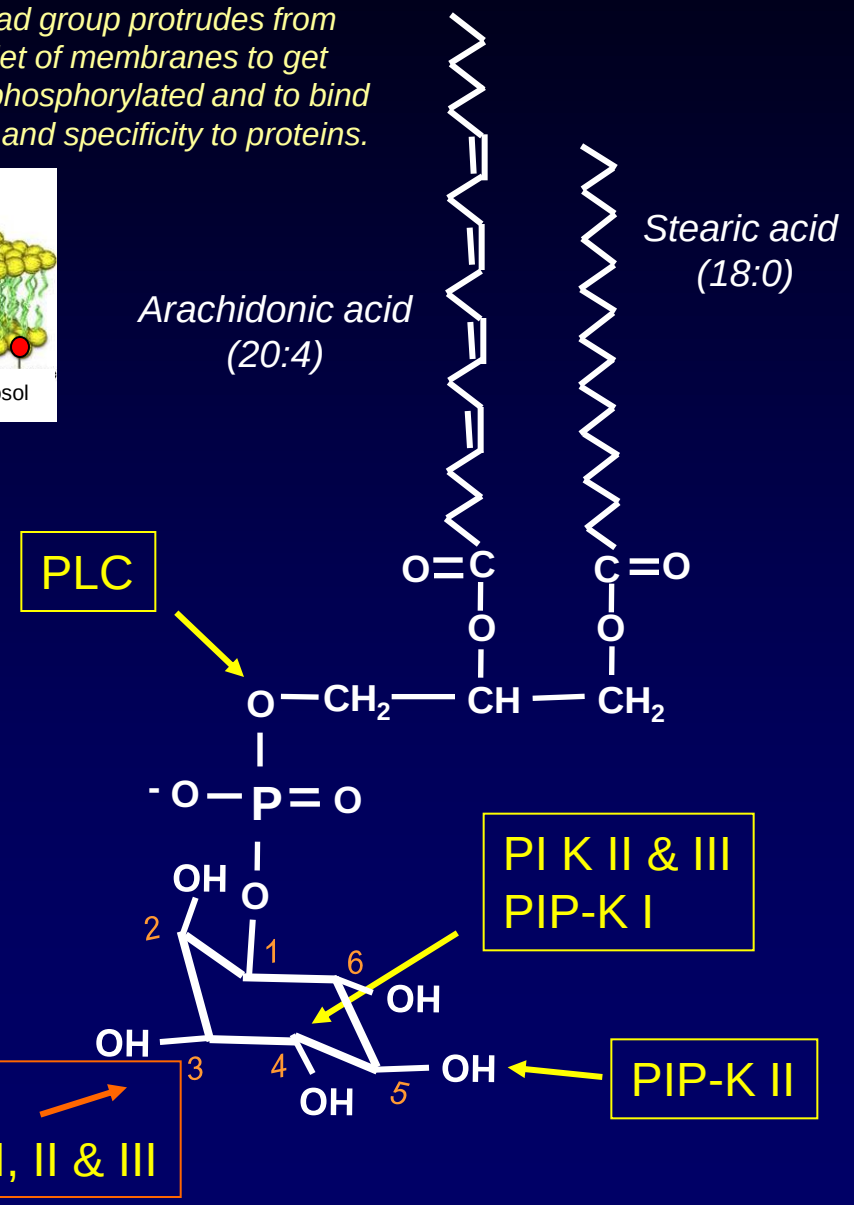


Activated mouse platelet

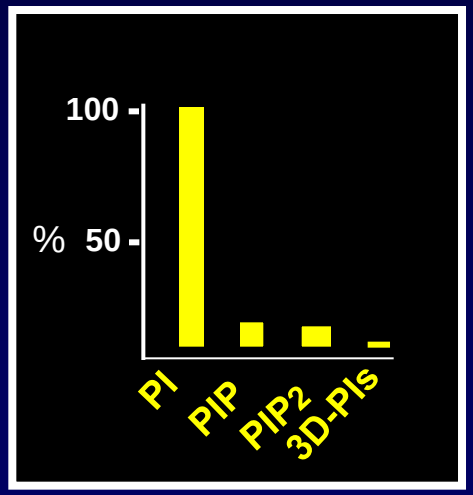
Phosphoinositides are minor constituents of cell membranes with a highly dynamic metabolism

In general their head group protrudes from the cytosolic leaflet of membranes to get phosphorylated/dephosphorylated and to bind with various affinity and specificity to proteins.





Relative amounts of phosphoinositides



The PI cycle in the seventies/eighties

USA 1969

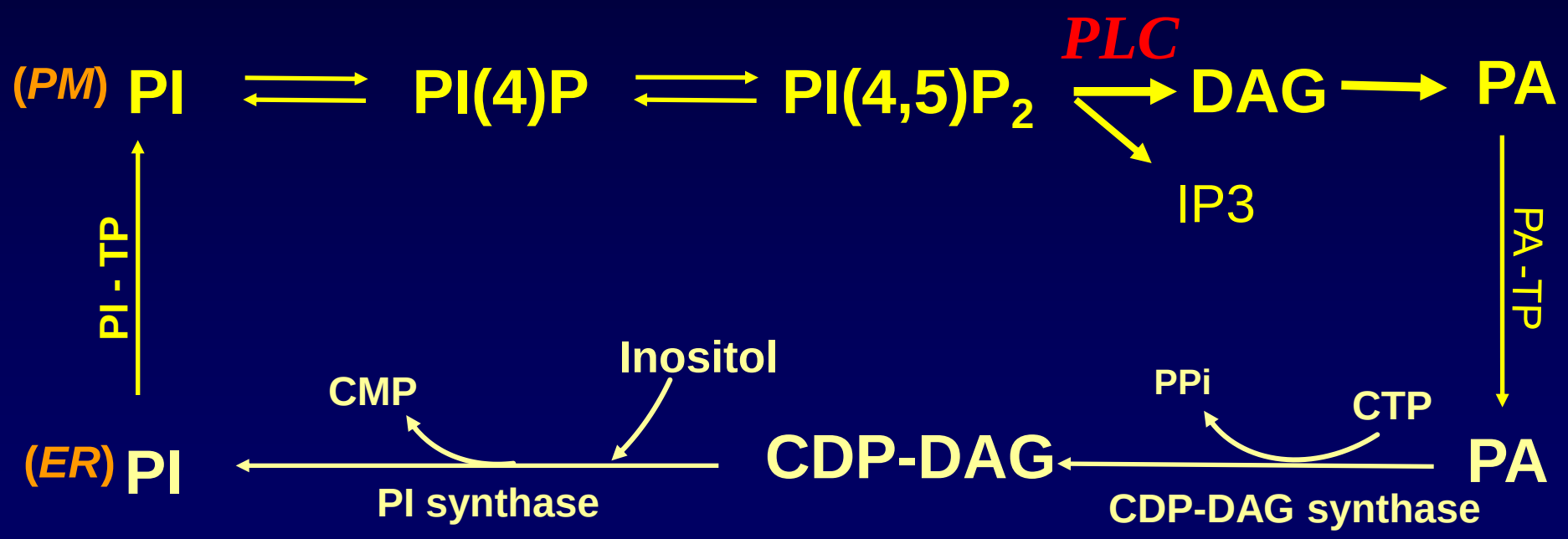
M. & L. Hokin
R. Dawson
J. Folch

UK 1979

M. Berridge
R.H. Michell
R. Irvine

Japan 80's

Y. Nishizuka
K. Mikoshiba

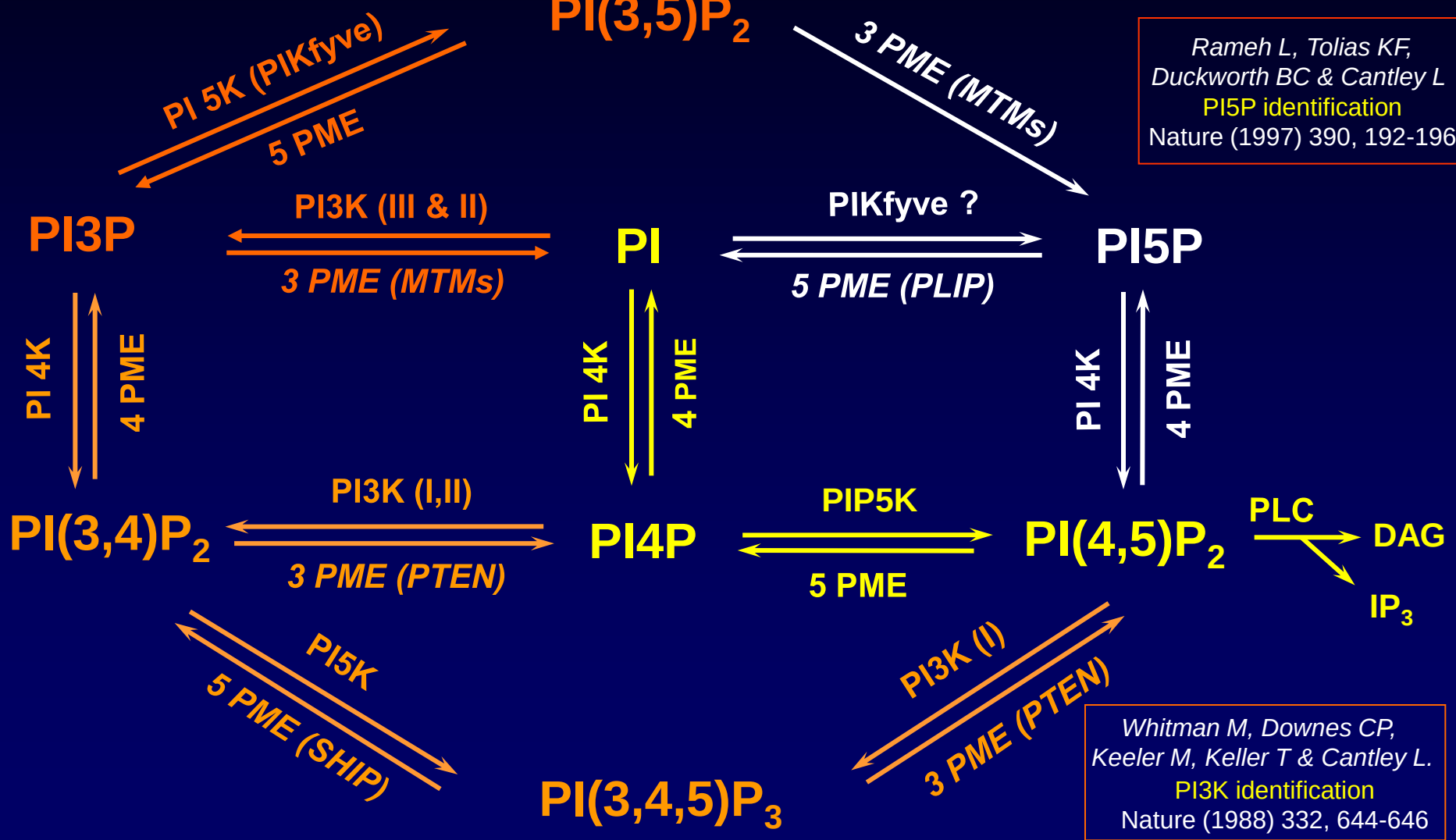


Michell R.H. Biochimica Biophysica Acta 1975

Various pathways of phosphoinositide synthesis and interconversions

Dove et al. PI(3,5)P2 pathway identification
Nature (1997) 390, 187-192

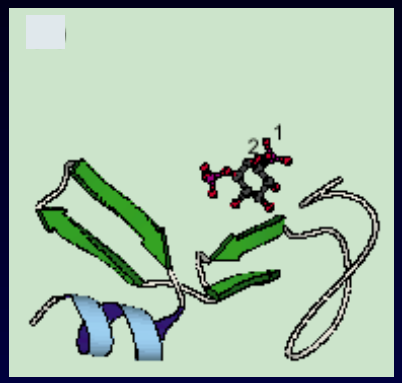
Rameh L, Tolias KF, Duckworth BC & Cantley L
PI5P identification
Nature (1997) 390, 192-196



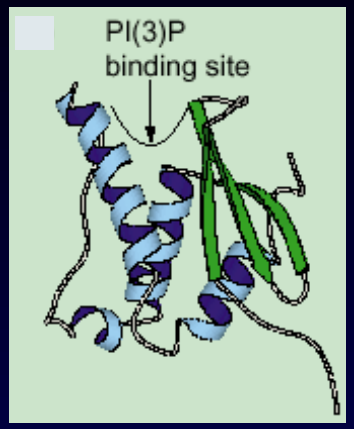
Whitman M, Downes CP, Keeler M, Keller T & Cantley L.
PI3K identification
Nature (1988) 332, 644-646

Phosphoinositide binding domains

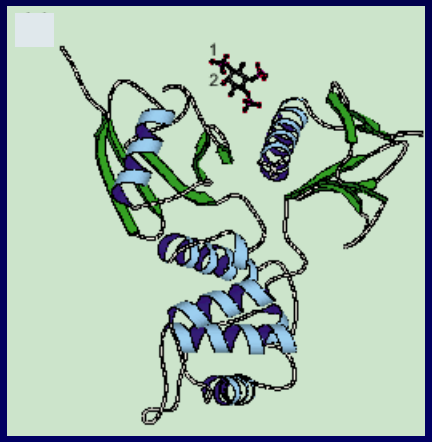
PIP3-binding **PH** domain from Grp1



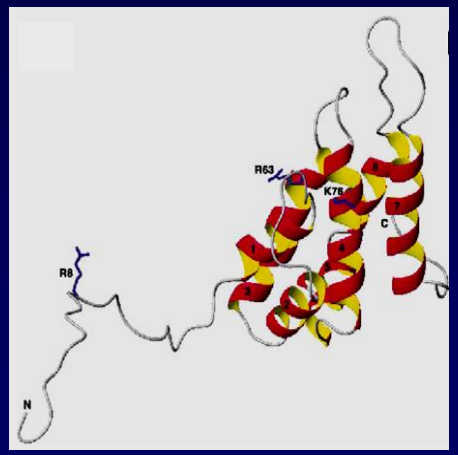
PI(3)P-binding **FYVE** domain from EEA1



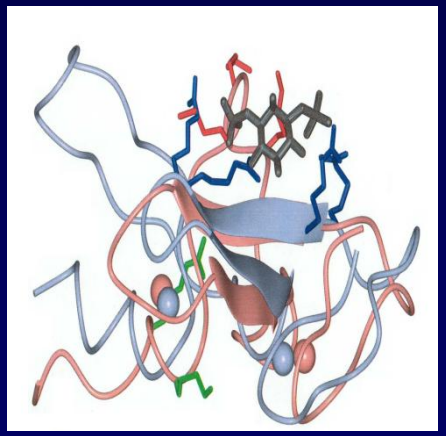
PI(3)P-binding **PX** domain from p47phox



PIP2-binding **FERM** domain from radixin



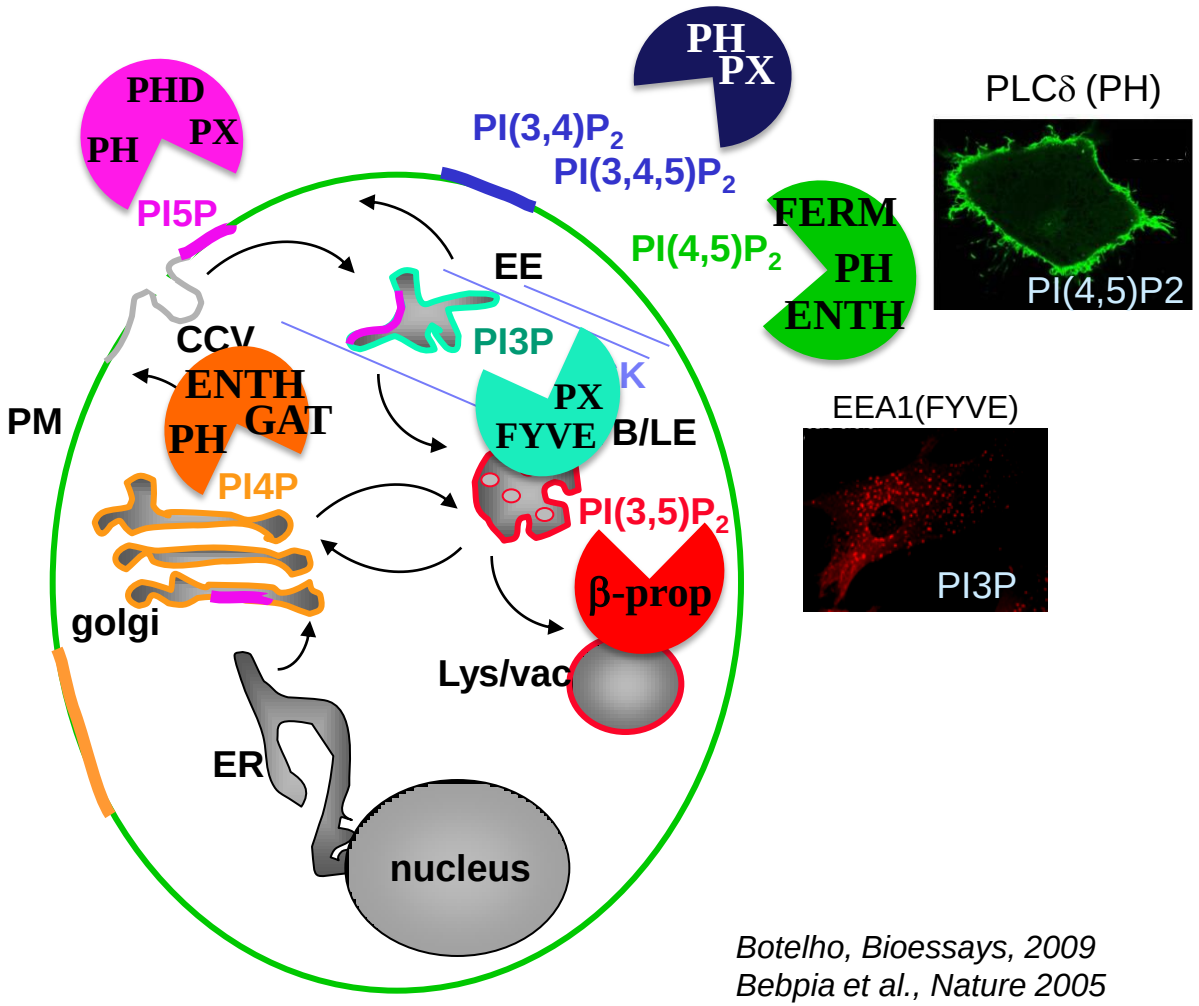
PIP2-binding **ENTH** domain from epsin



PI5P-binding domain **PHD** from ING2

Itoh T & Takenawa T, Cell Signal 2002

Phosphoinositides define organelle identity ?



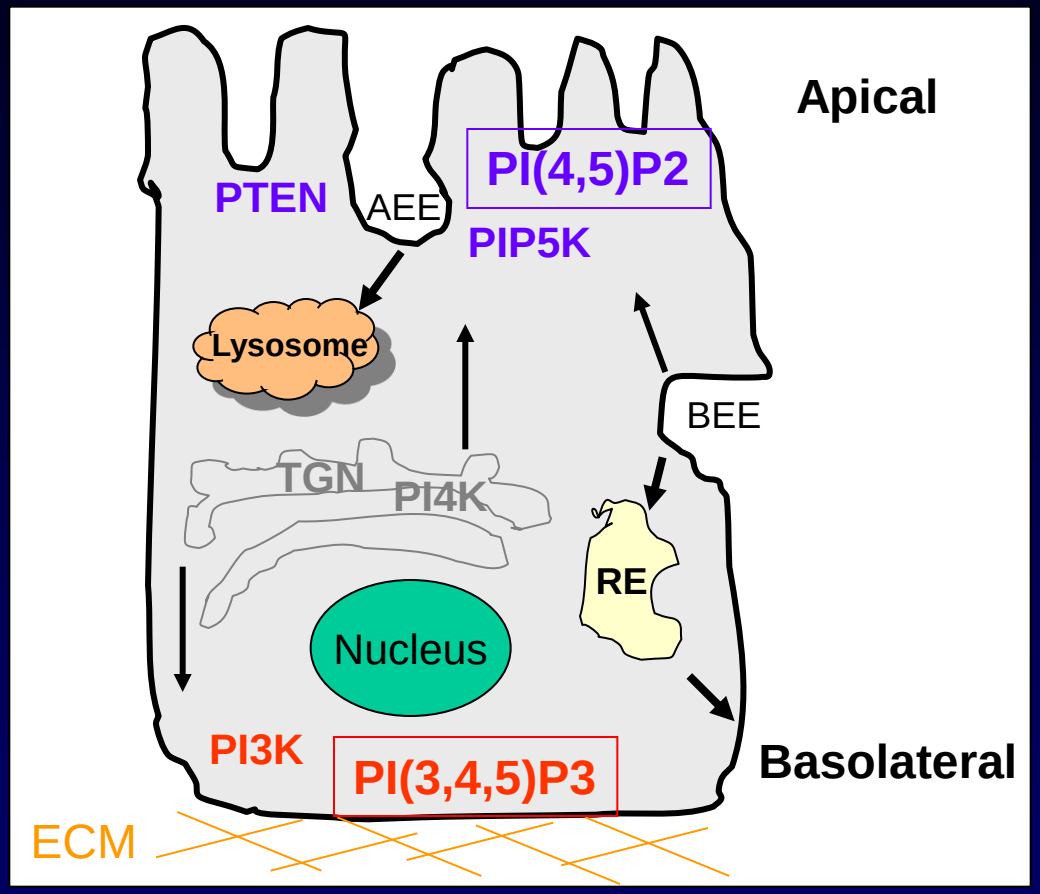
PLCδ (PH)

EEA1(FYVE)

Botelho, Bioessays, 2009
Bebpia et al., Nature 2005
Carlton & Cullen, Trends Cell Biol 2005
Balla & Varnai, Curr Protoc cell Biol 2009

Phosphoinositides in the dynamics of cell organization and polarity

Epithelial cell polarity



Migrating cells :

- $PI(3,4,5)P_3$ at the leading edge

Dividing cells :

- $PI(3,4,5)P_3$ at the midsection of the cortex to properly orientate the spindle

- PI3P at the midbody during cytokinesis to recruit cytokinesis regulatory machinery

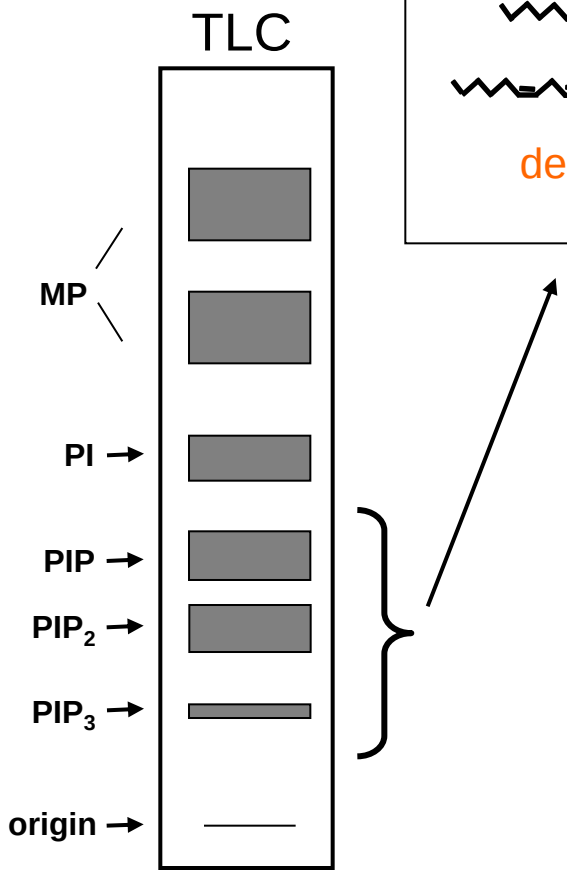
- $PI(4,5)P_2$ at the mitotic cortex as a spatial regulator of its stability

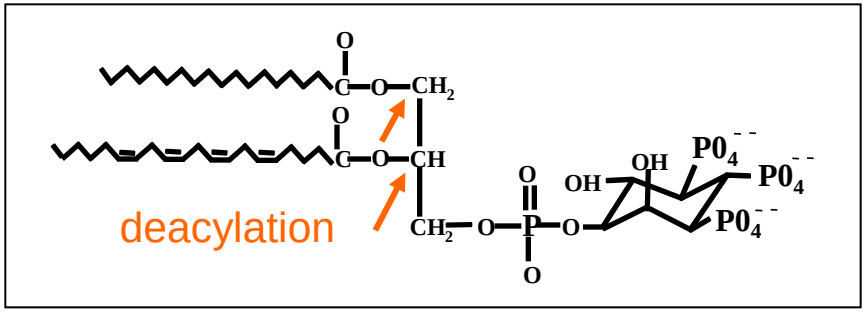
The regulation and localization of PI-kinases and -phosphatases is critical to ensure adequate cell response to environmental cues.

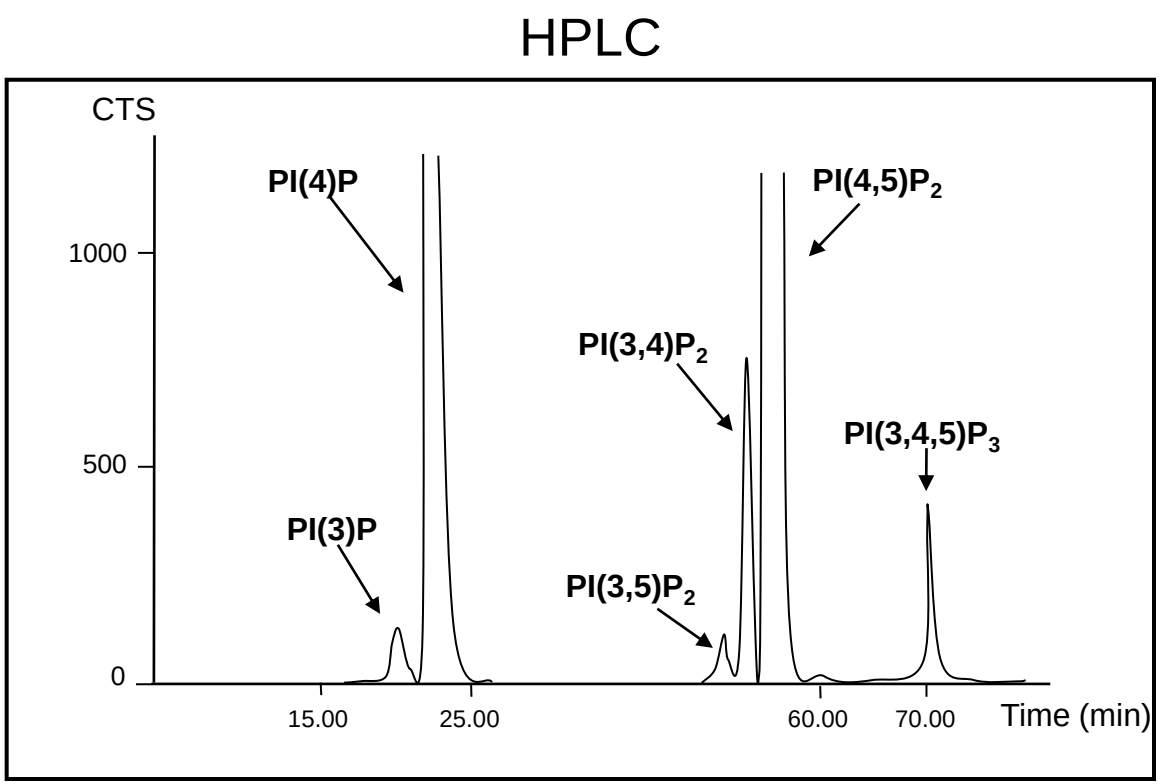
Di Paolo & Camilli, Nature Rev 2006
Sasaki et al., Progress Lipid Res 2009
Gassama & Payrastre, Int Rev Cell Mol Biol 2009

Analysis of phosphoinositides: a specialized lipid biochemistry

➡ *Isotopic labelling (^{3}H-inositol or ^{32}Pi) followed by TLC and HPLC techniques :*



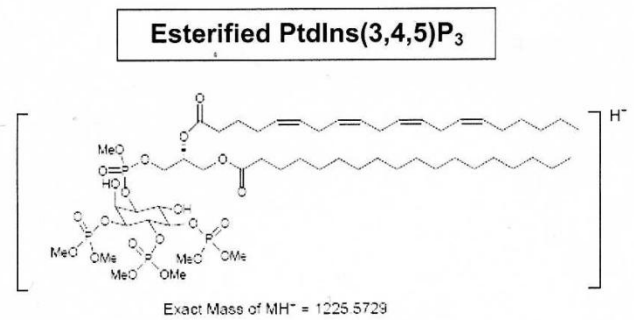




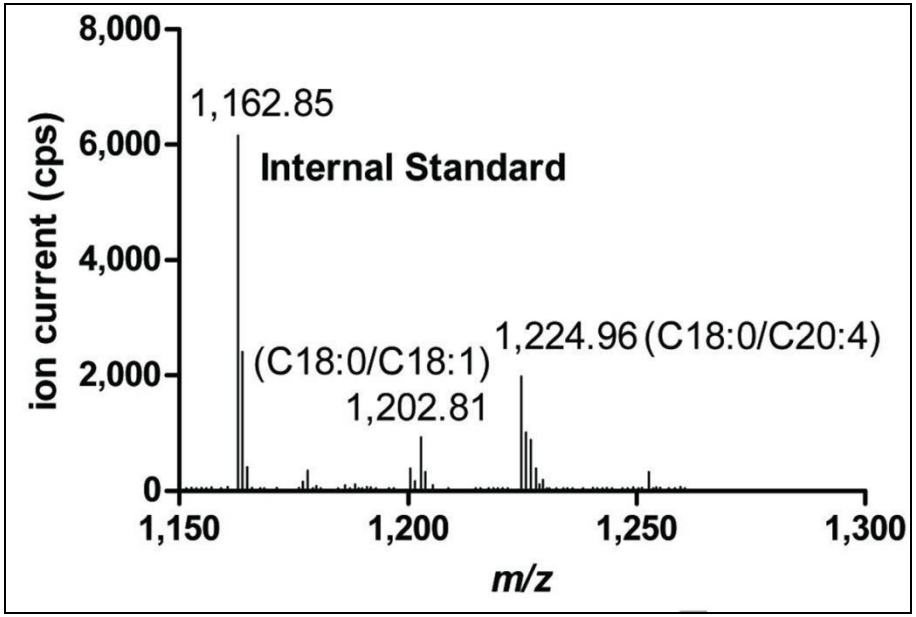
B. Payrastre, Methods Mol Biol 2004

Analysis of phosphoinositides : towards mass spectrometry methods

➡ *Chemical derivation (phosphate methylation with TMS-diazomethane) coupled to HPLC-MS techniques :*

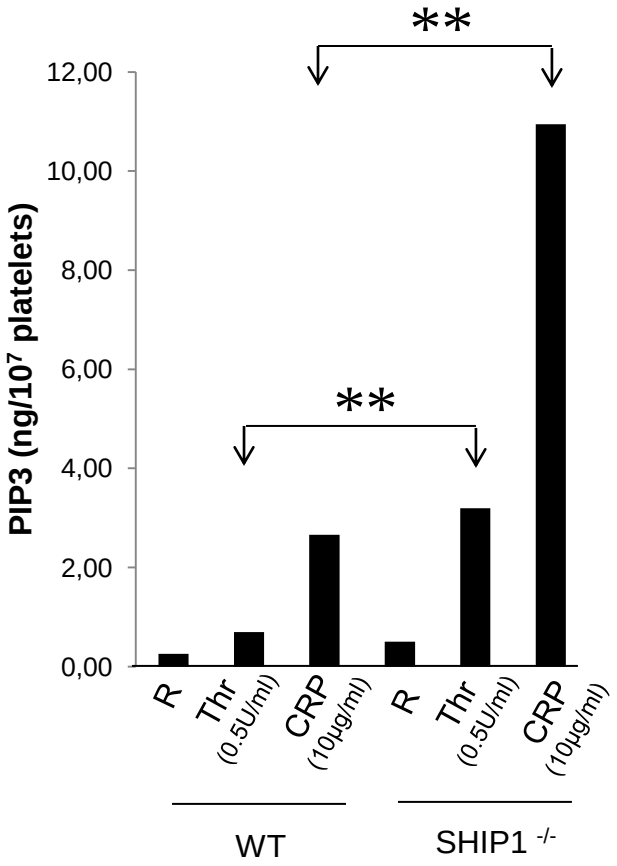


PIP3 molecular species



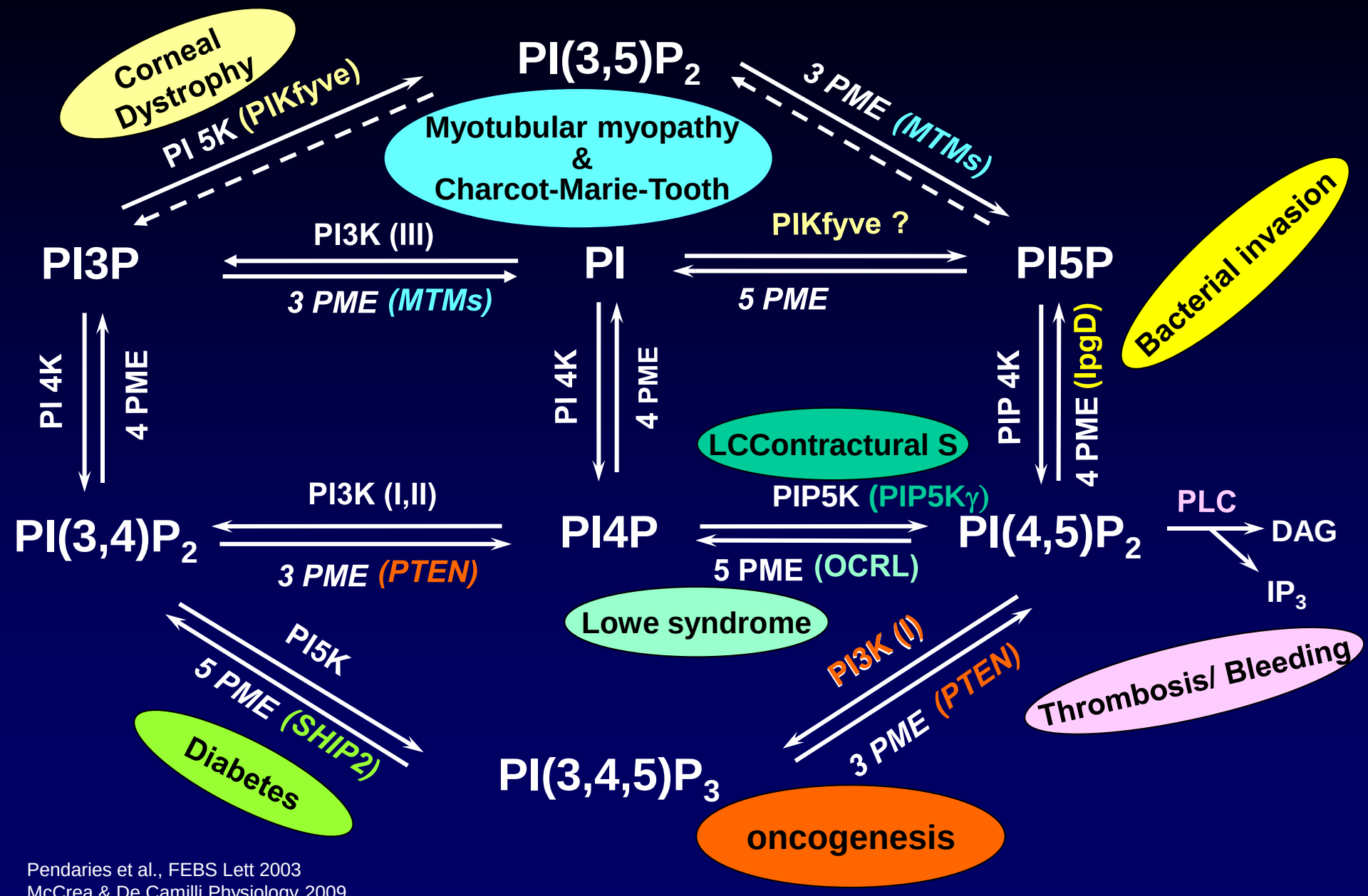
J. Clark et al., Nature Methods 2011

PIP3 level in WT versus SHIP1 -/- platelets:



Plateau de lipidomique MétaToul
I2MC, Toulouse (http://www.i2mc.inserm.fr)

Phosphoinositide metabolism and human diseases

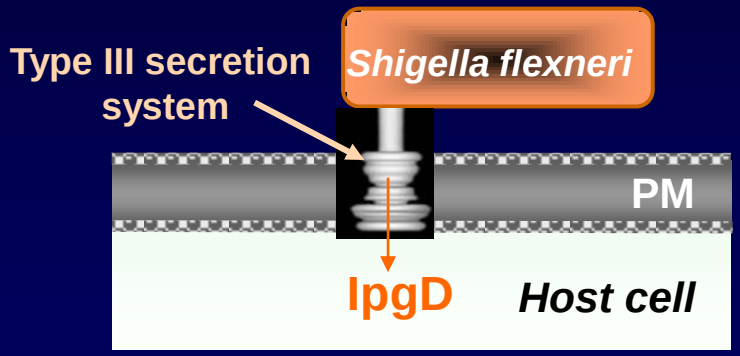


Pendaries et al., FEBS Lett 2003
McCrea & De Camilli Physiology 2009

The *Shigella flexneri* effector IpgD is a PI-phosphatase

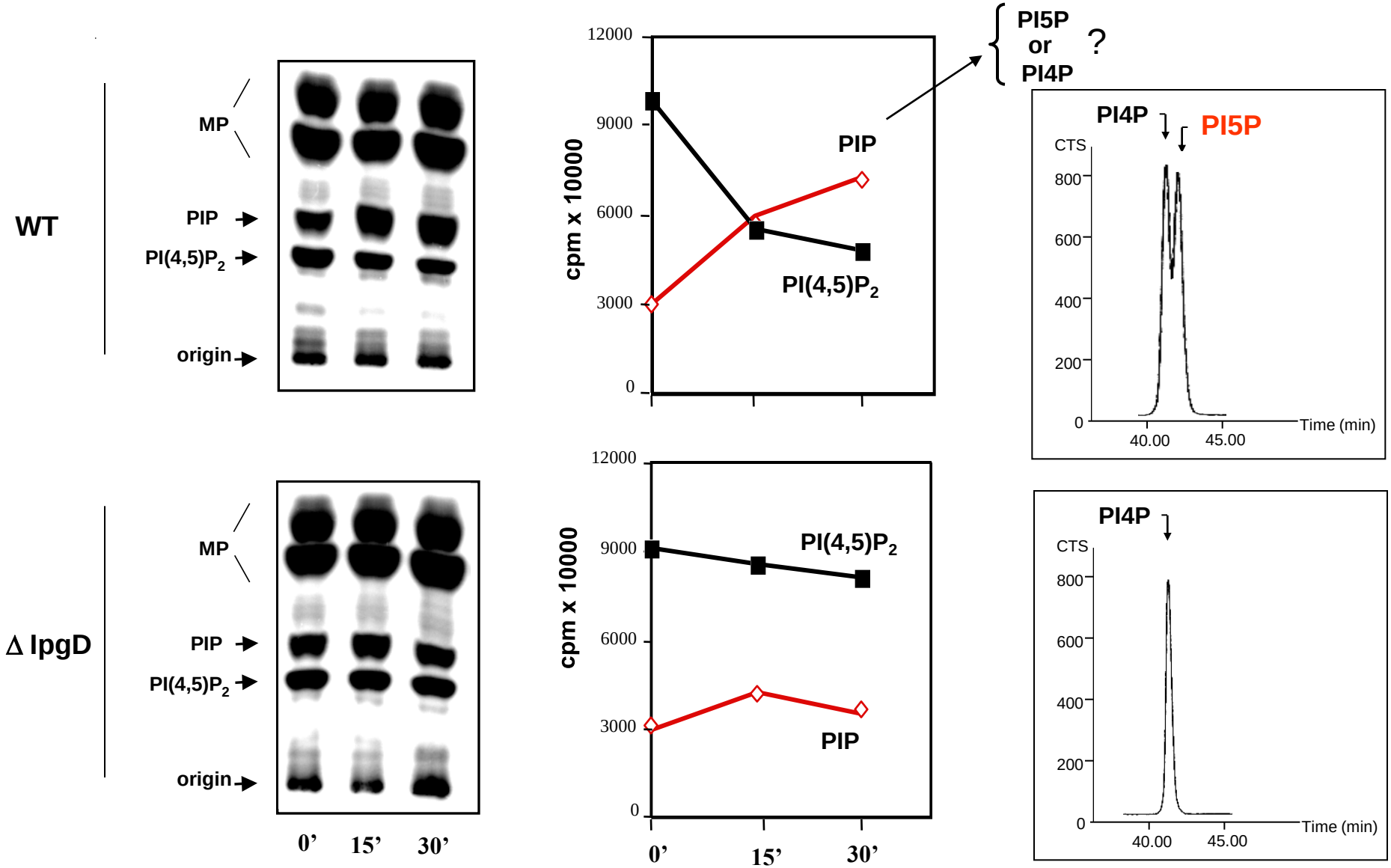
The *Shigella flexneri* effector IpgD has a motif related to the active site of mammalian phosphoinositide phosphatases

IpgD	PCWNCKSGKDRTGMQDAEIKREIIRK
PTEN	AAIHCKAGKGRTGVMICAYLLHRGKF
MTM1	VLVHCSDGWDRTAQLTSLAML-M-LD
MTMR3	VLVHCSDGWDRTPQIVALAKL-L-LD



➡ ***The IpgDC438S is an inactive phosphatase***

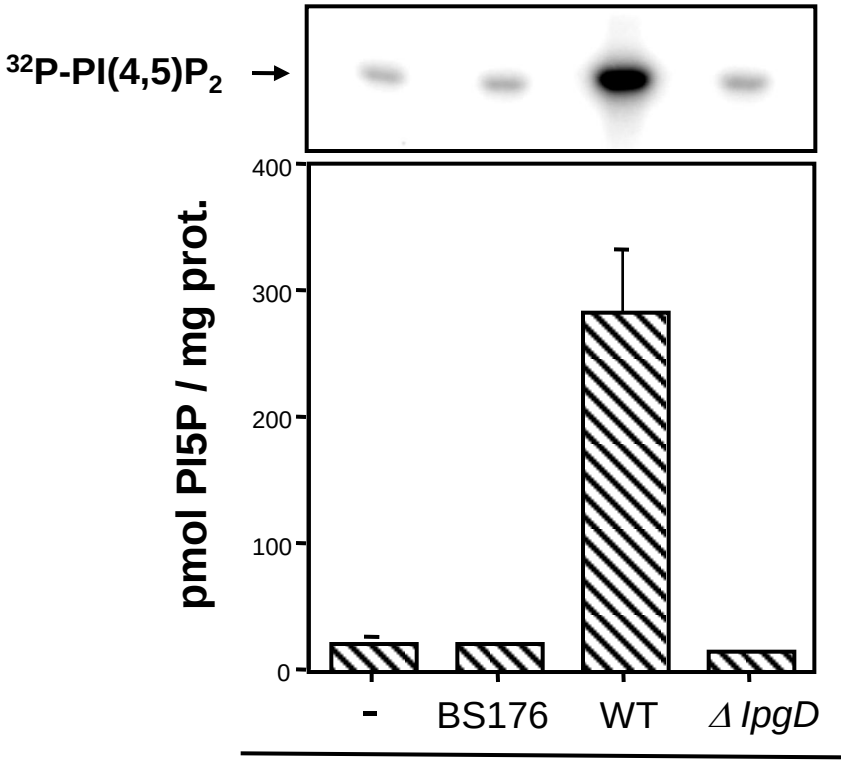
IpgD-dependent PI(4,5)P_2 hydrolysis in Hela cells infected with *Shigella flexneri*



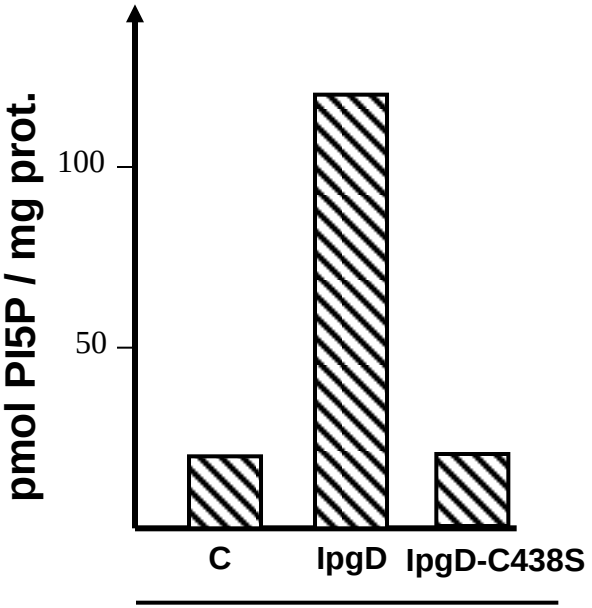
The *Shigella* effector IpgD is sufficient to increase PI5P levels in host cells

PI5P mass assay

PI3P
PI4P
PI5P + PIP-kinase IIα $\xrightarrow{\gamma^{32}\text{P-ATP}}$ **^{32}P-PI(4,5)P_2**



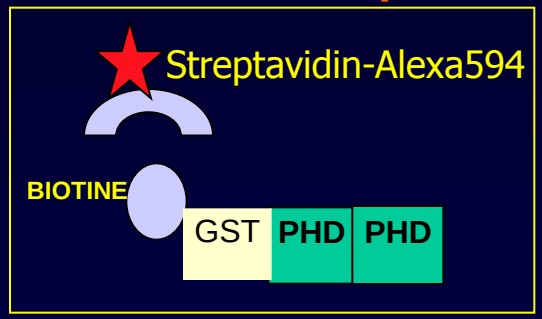
Hela cells infected with wild type *shigella* and mutants



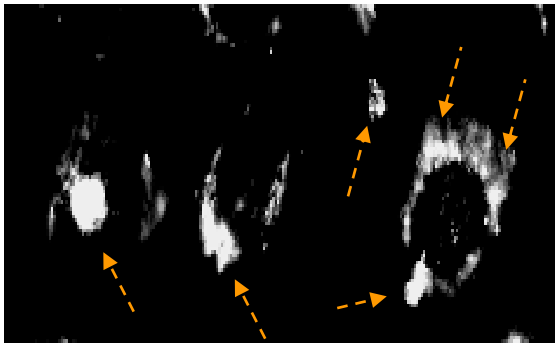
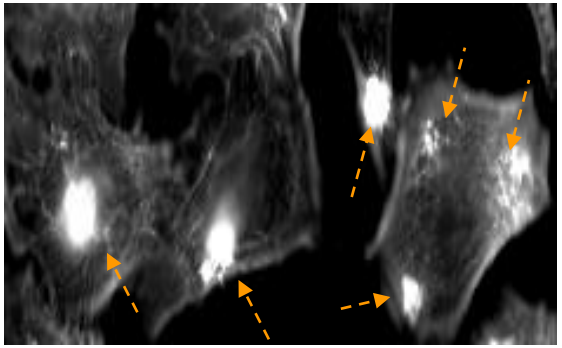
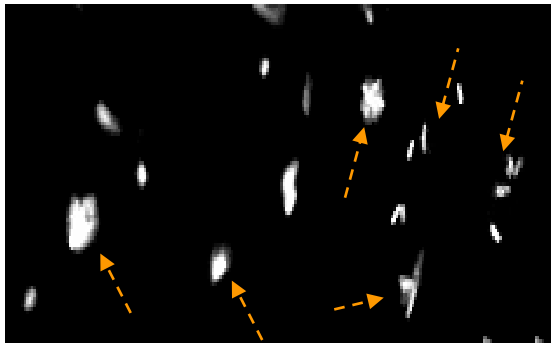
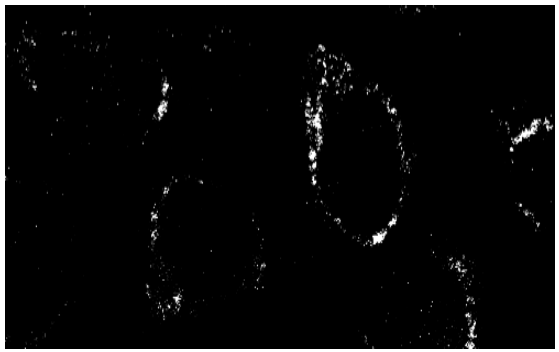
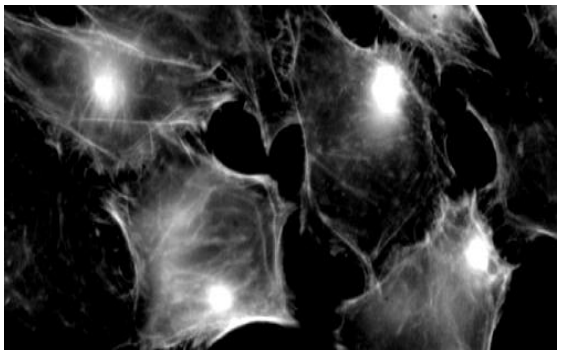
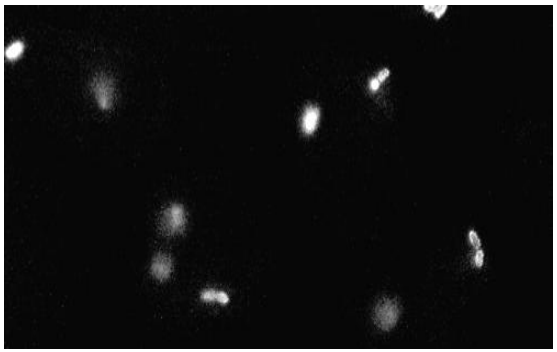
Expression of GFP-IpgD in Hela cells

PI5P is produced in host cells at the entry site of *S. flexneri*

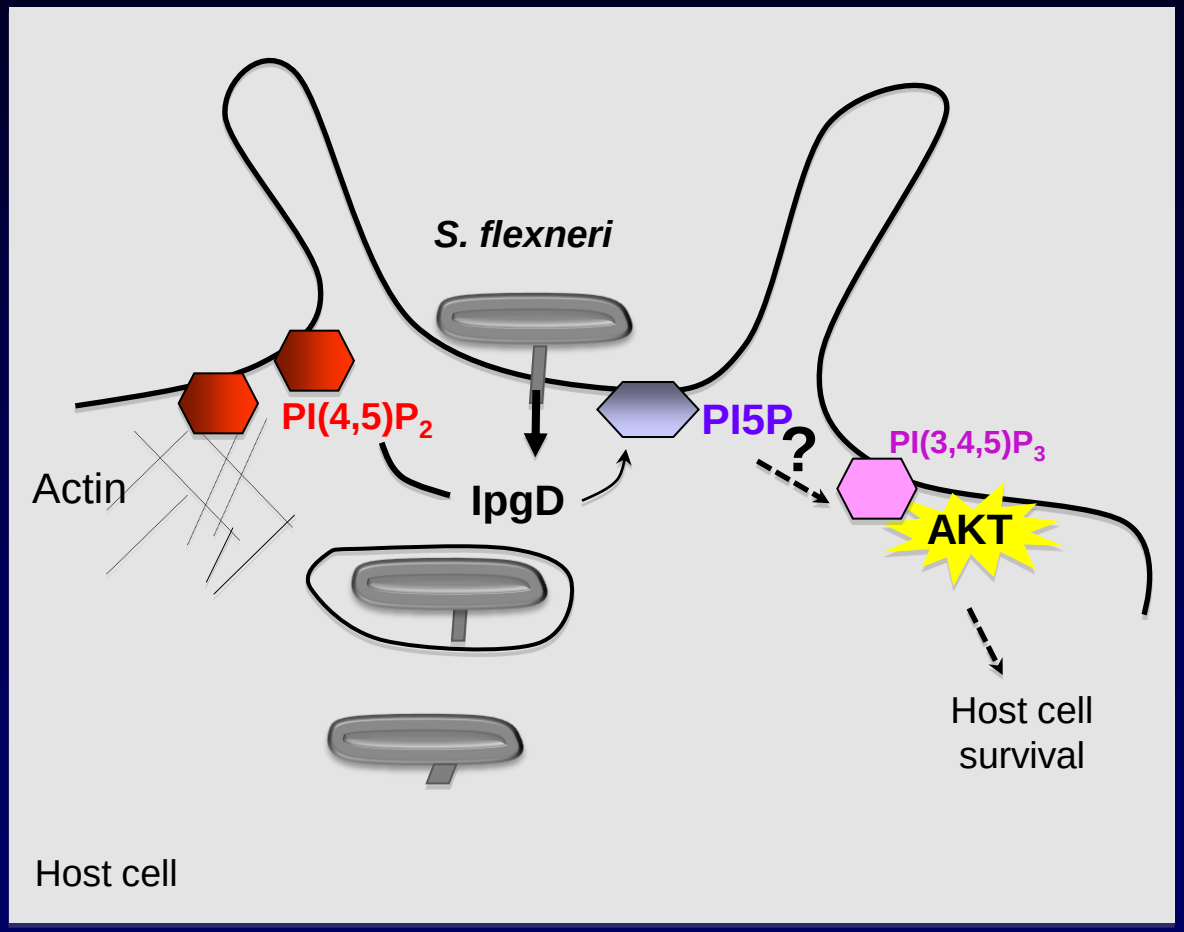
Biot-GST-2XPHD probe



O. Gozani et al Cell 2003

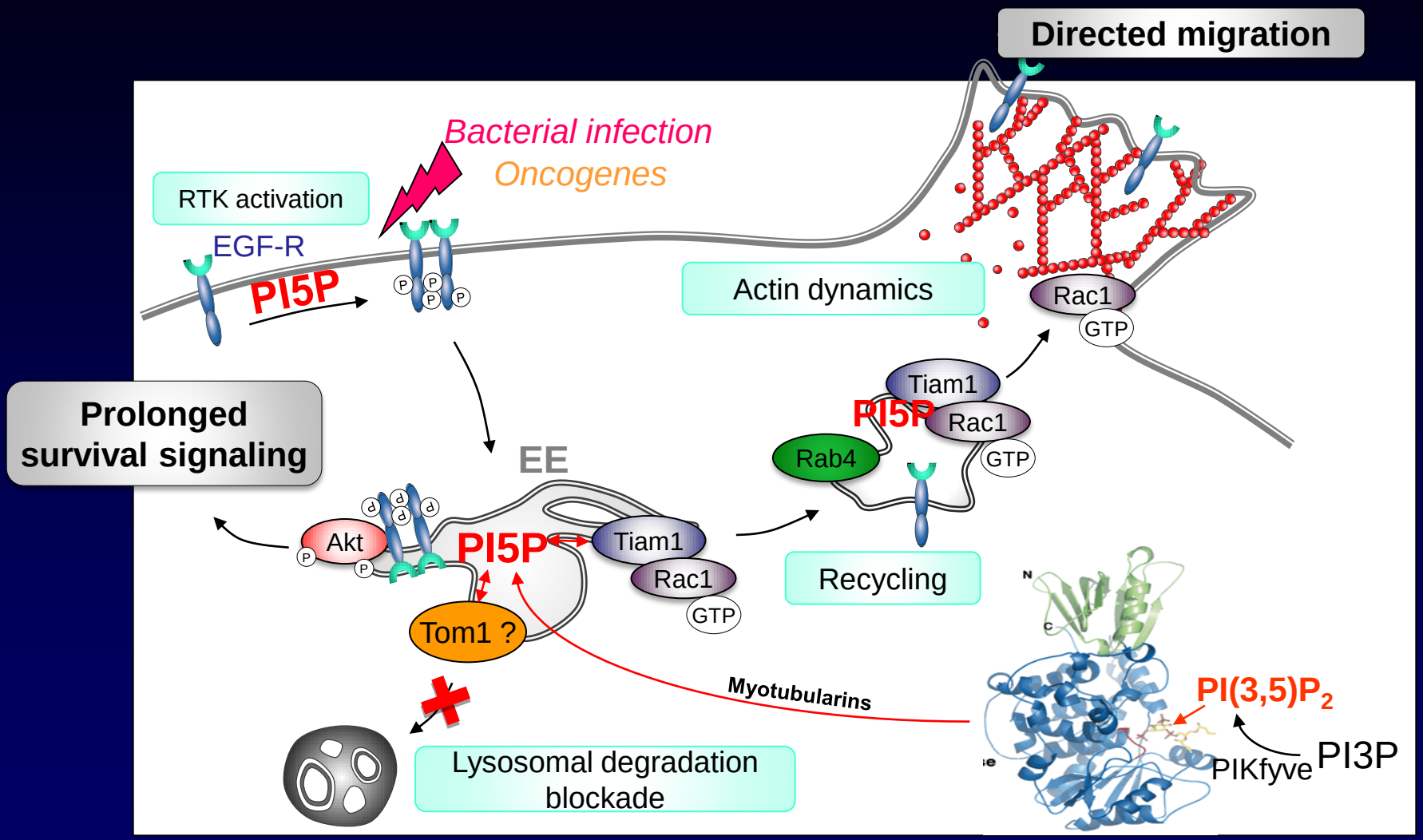
	PI5P (PHDx2 biot)	F-Actin (Phalloidin)	Bacteria (LPS)
WT 10'			
Δ *ipgD* 10'			

Shigella flexneri injects the PI-phosphatase IpgD in the host cell through its Type III secretion system : a model to study the role of PI5P



Niebuhr et al EMBO J. 2002
Pendaries et al. EMBO J. 2006
Coronas et al. Biochem Soc Symp 2007
Ramel et al. BBRC 2009

PI5P integrates membrane and cytoskeleton dynamics



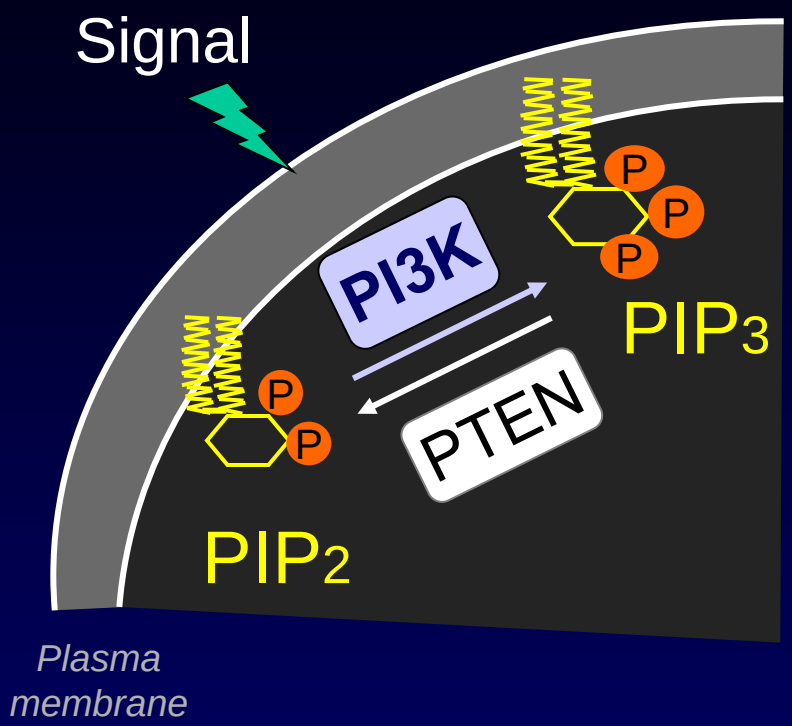
Ramel et al, 2011

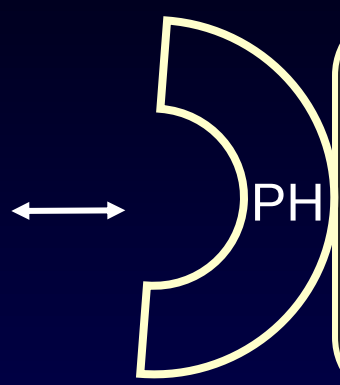
Science Signaling
The Leading Journal for Cell Signaling

Chicanne et al Biochem J 2012
Dupuis-Coronas et al. J Biol Chem 2011
Bertazzi et al. PLoS Genetics 2012

PI3Kβ in platelet activation and thrombus growth

A critical role for PI3K and PIP3 in cell regulation





Kinases (Akt,PDK)
Phospholipase (PLCγ)
Adaptors (Gab)
GEFs (Vav, ARNO)

Increase protein concentration to generate a steric environment favorable for protein interactions

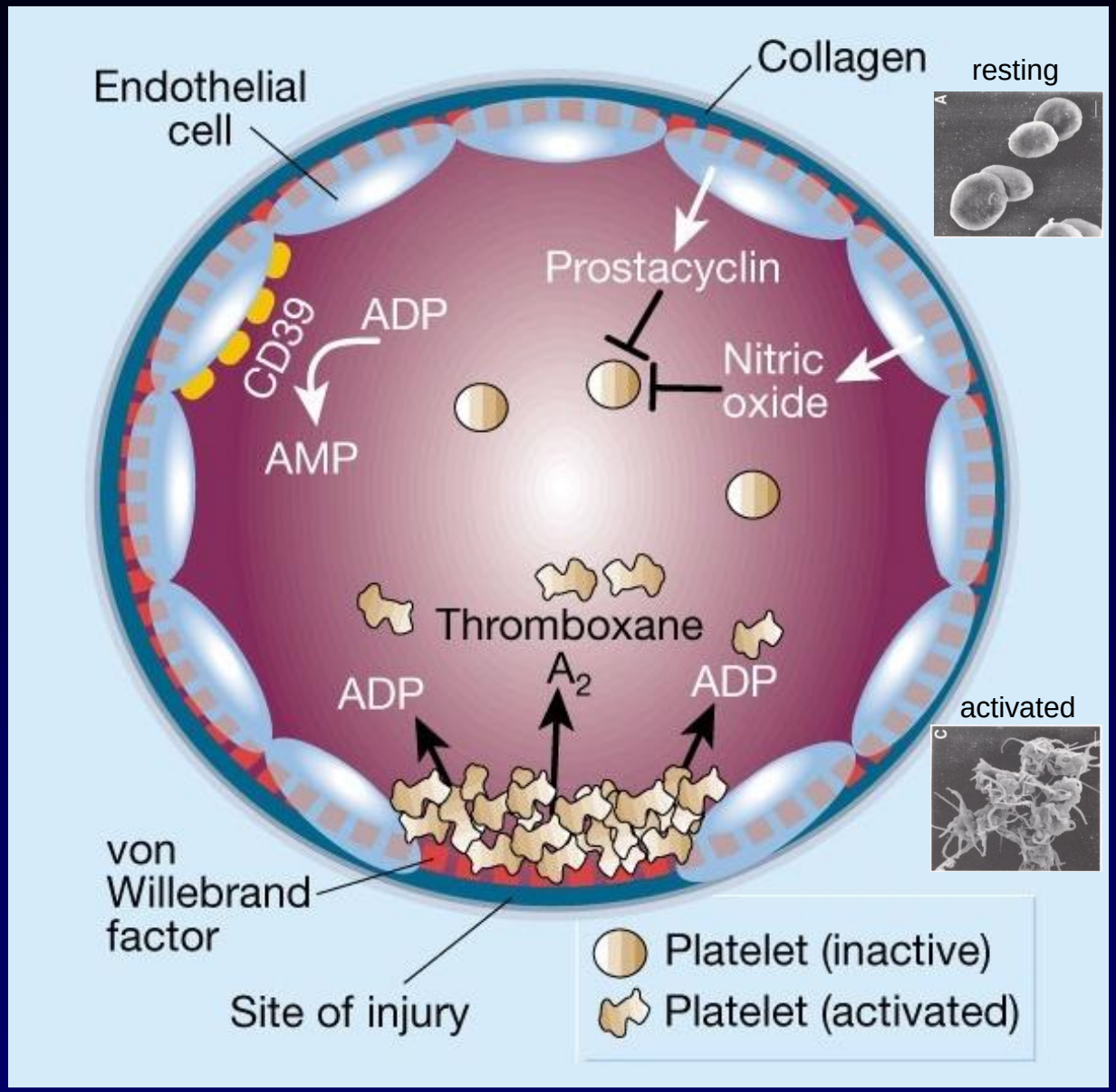
↓

Cancer
Immunity
Diabetes
Haemostasis

Growth
Proliferation & Survival
Metabolism
Differentiation
Polarisation & Motility

Vanhaesebroeck B. et al., Nature Review 2010
Sasaki et al., Progress Lipid Res 2009
Hirsch E. et al., Trends Biochem Sci 2009

The key role of platelets in haemostasis and thrombosis



THROMBOSIS

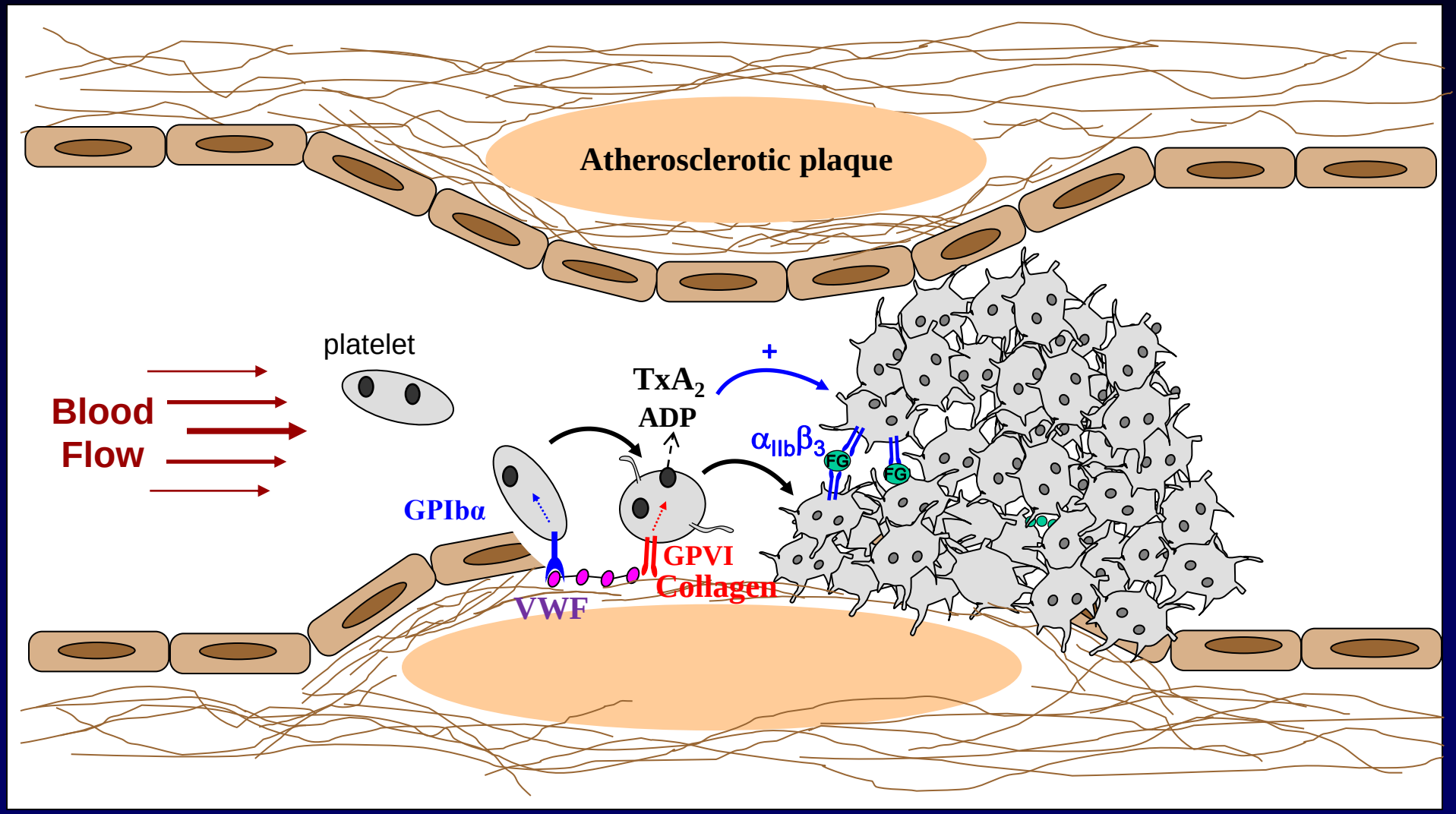
BLEEDING

Interactions with blood cells and endothelial cells, secretion of cytokines, growth factors and matrix proteins

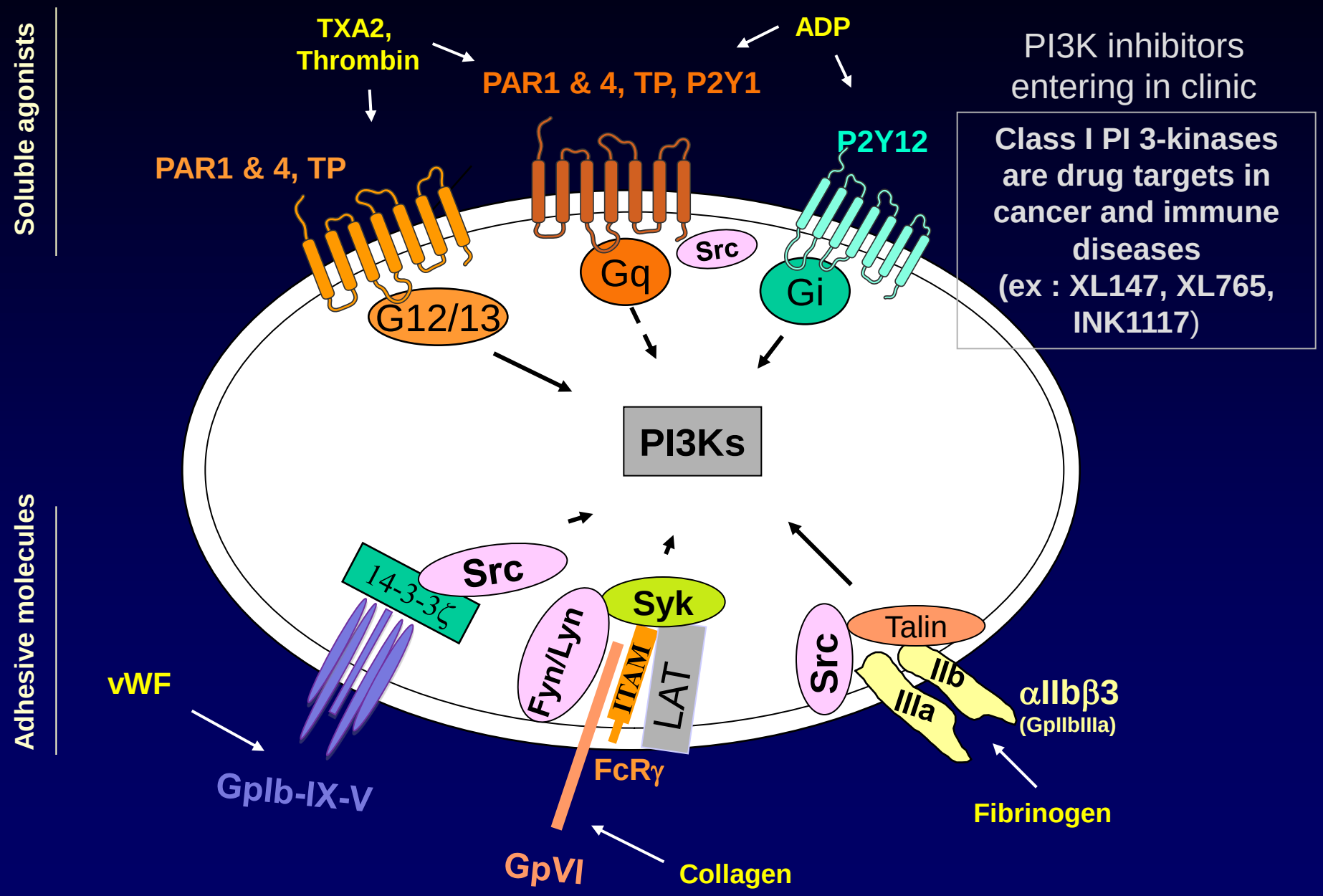
- Inflammation
- Atherosclerosis
- Angiogenesis
- Metastasis

Adapted from Brass, Nature 2001

Platelet activation and ischemic vascular events during atherothrombosis



PI3Ks are activated downstream of most activatory human platelet receptors



PI3K inhibitors entering in clinic

Class I PI 3-kinases are drug targets in cancer and immune diseases (ex : XL147, XL765, INK1117)

Blood platelets contain all the different PI3K

Class Ia — p110 α, β, δ

p85 | Ras | C2 | *Hélice* | Domaine Catalytique

Class Ib — p110 γ

Ras | C2 | *Hélice* | Domaine Catalytique

Class II — PI3KC2 α,β,γ

Ras | C2 | *Hélice* | Domaine Catalytique | PX | C2

Class III — Vps34p

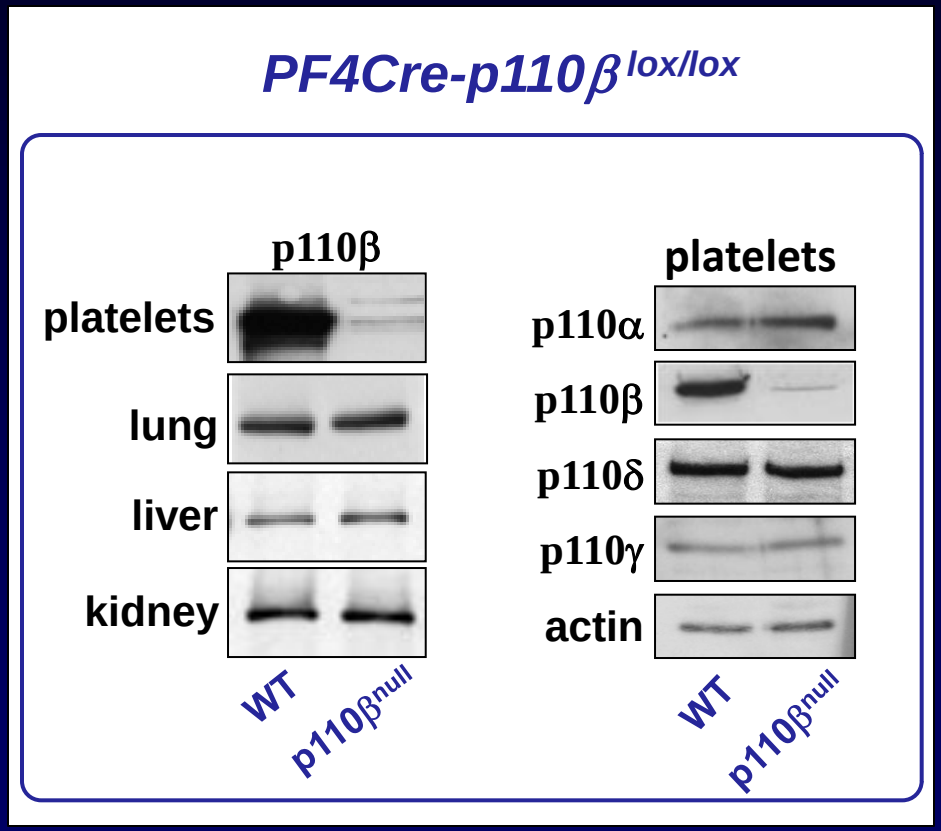
C2 | *Hélice* | Domaine Catalytique

***PF4*Cre-p110β$^{lox/lox}$**

***PF4*Cre-p110α$^{lox/lox}$**

Vanhaesebroeck et al., Nat Rev Mol Cell Biol 2012
Laurent P.A. et al., Advance Biol Reg 2013

Mouse model of PI3Kβ invalidation specifically in the megakaryocyte/platelet lineage



PI3Kβ in platelet activation in vitro

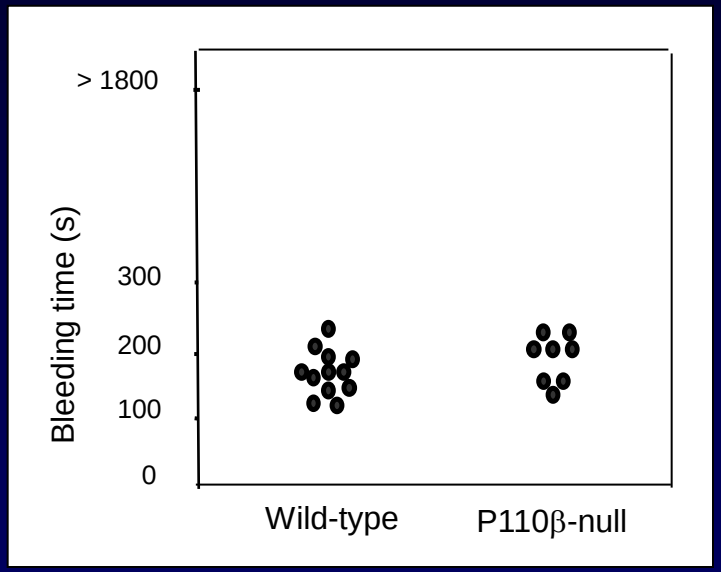
- **critical role in GPVI-mediated platelet activation (PLC activation, Ca2+ mobilization, PKC activation, PLA2 activation & TXA2, 12-HETE, 8-HETE synthesis)**
- **not mandatory to induce platelet aggregation in response to thrombin or U46619 stimulation but required for the formation of large platelet aggregates**
- **required for αIIbβ3 -mediated platelet adhesion on fibrinogen under flow conditions and spreading in static conditions, involved in fibrin clot retraction**

Jackson et al Nature Med 2005
Ragab et al., Blood 2007
Gratacap et al. Blood 2009
Martin et al., Blood 2010
Canobbio et al Blood 2009

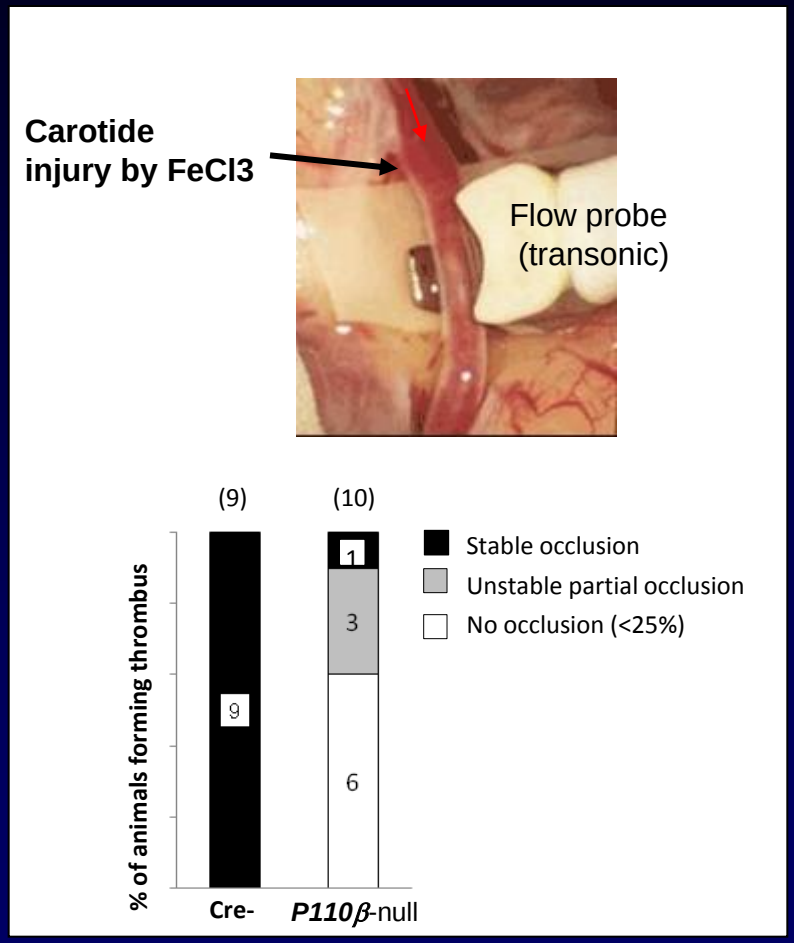
Role in vivo ?

A role for PI3Kβ in arterial thrombus formation

Tail bleeding time :



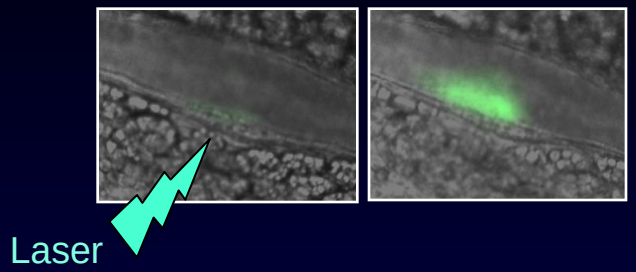
Thrombus formation in vivo:
(carotide injury and monitoring of blood flow)

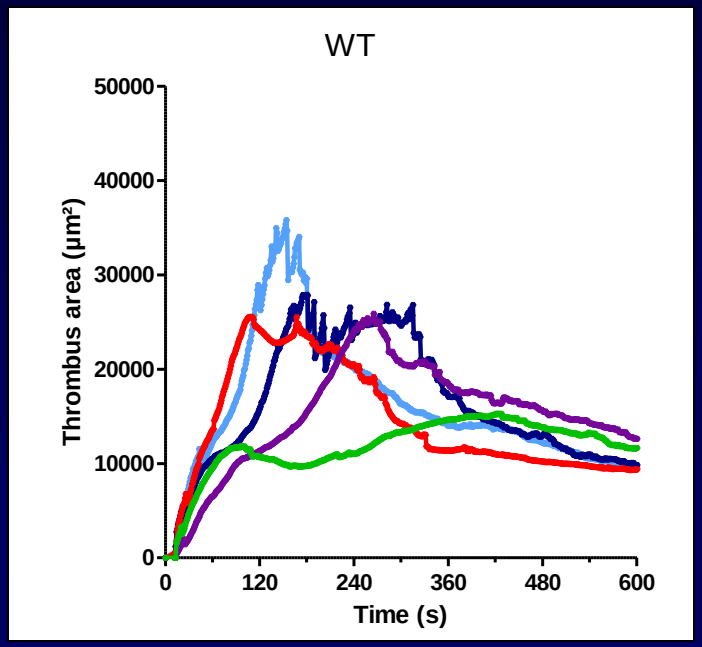


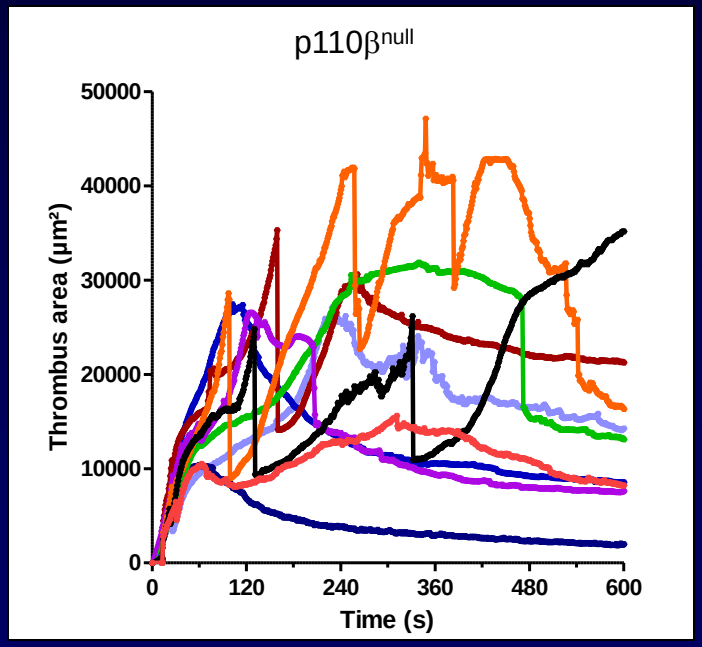
➡ PI3Kβ-null mice have a normal tail bleeding time but are protected against the formation of occlusive thrombus.

PI3Kβ regulates thrombus growth and stability under high shear rate

Laser injury of mesenteric arteriole and intravital microscopy :



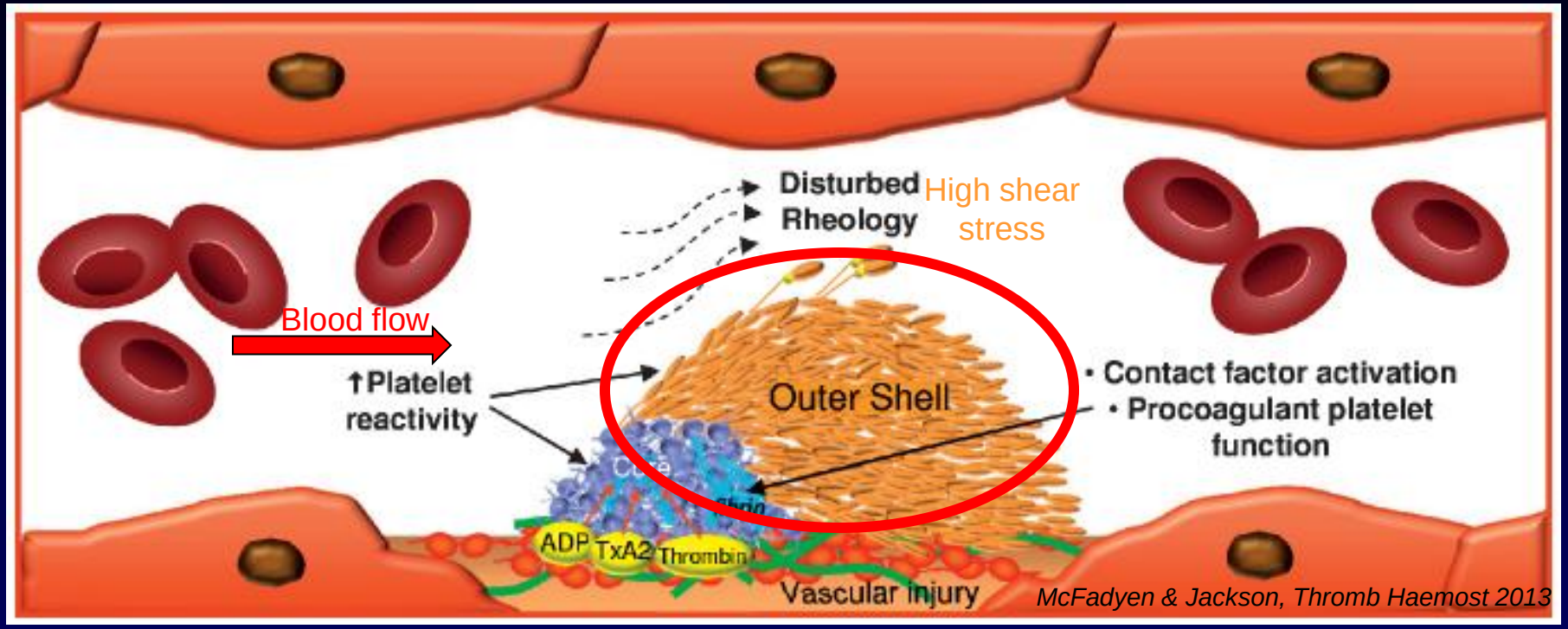




PI3Kβ plays a key role in thrombus stability

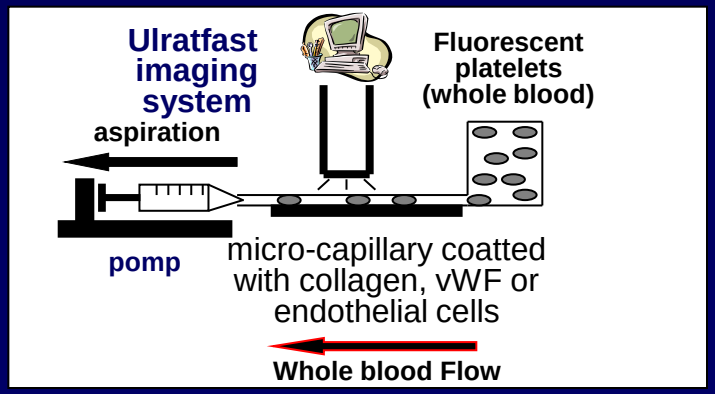
Laurent et al., submitted

Thrombus growth and stability is a highly dynamic process

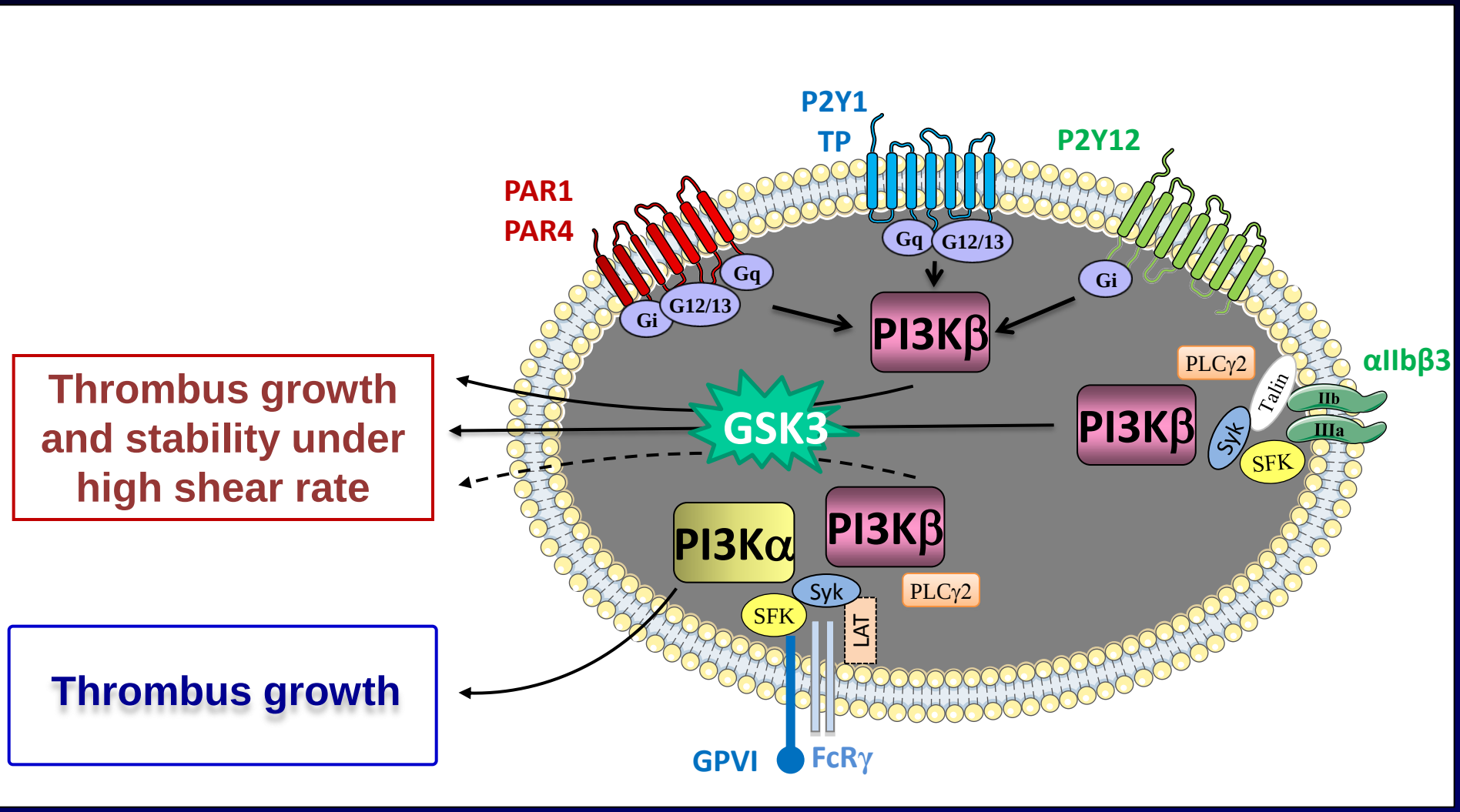


McFadyen & Jackson, Thromb Haemost 2013

Study of platelet adhesive interactions and thrombus growth in whole blood under flow conditions



PI3Kα and PI3Kβ have distinct and important roles in platelet activation and thrombus stability



“Platelet production and function, signaling and phosphoinositides ”

Inserm U563, Toulouse, France

B. Payrastre
F. Gaits-Iacovoni
H. Tronchère
M.P. Gratacap
A.D. Terrisse
G. Chicanne
A. Ragab
J.M. Xuereb

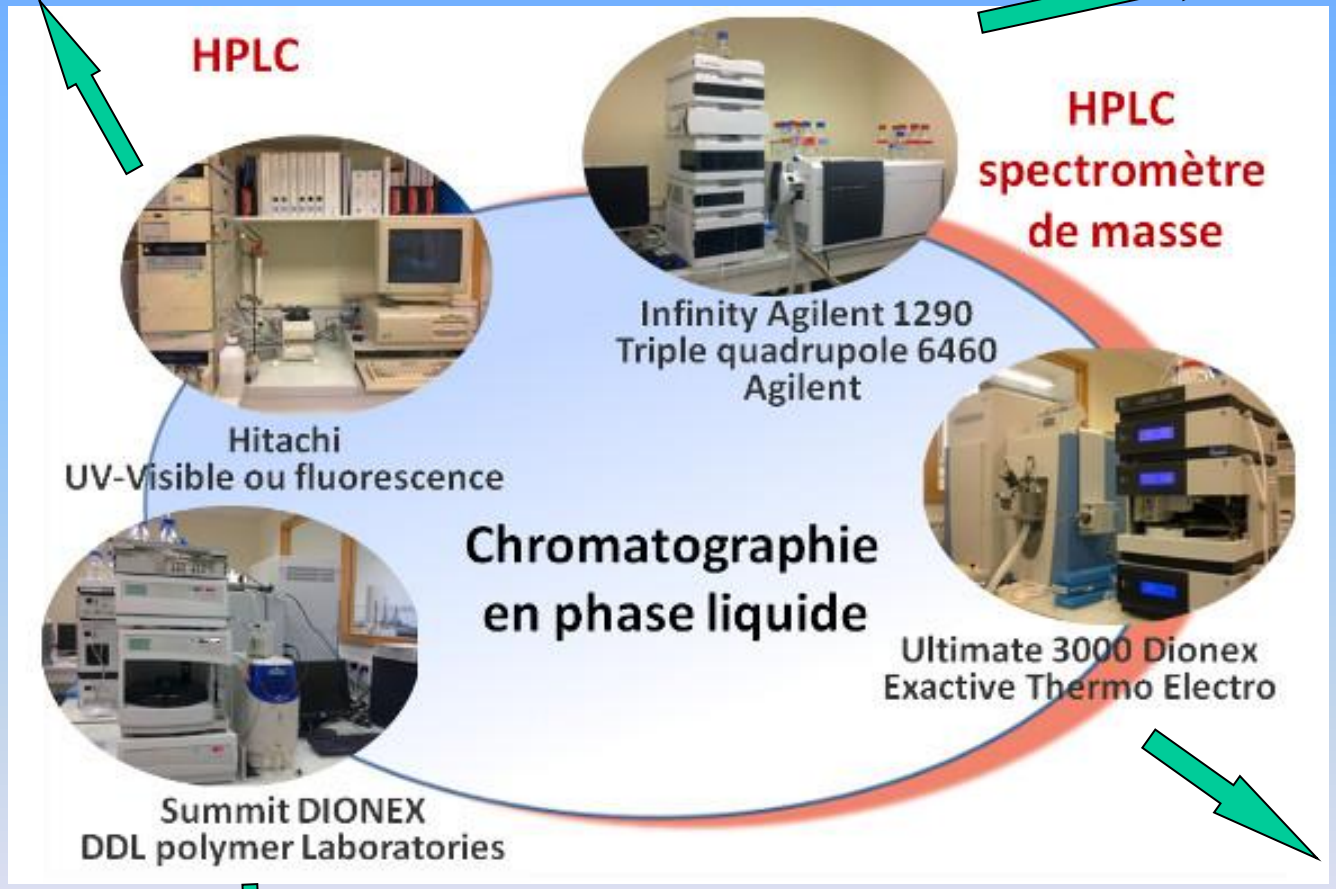
S. Severin
J. Viaud
F. Boal
P.A. Laurent
A. Antkowiak
(D. Ramel & F. Lagarigue)

Collaborations *:*
B. Van Haesebroeck
C. Gachet
P. Sansonetti

Animal , lipidomic and Cell Imaging Facilities

Instruments analytiques et molécules analysées

Lipoprotéines



- **Eicosanoides**
- **Phospholipides**
- **Céramides, Sphingomyéline**
- **Stérol C4**
- **PIP**
- *Sphingosine-Phosphate*
- *Isoprostanes*
- *LPA,PA,LPC*

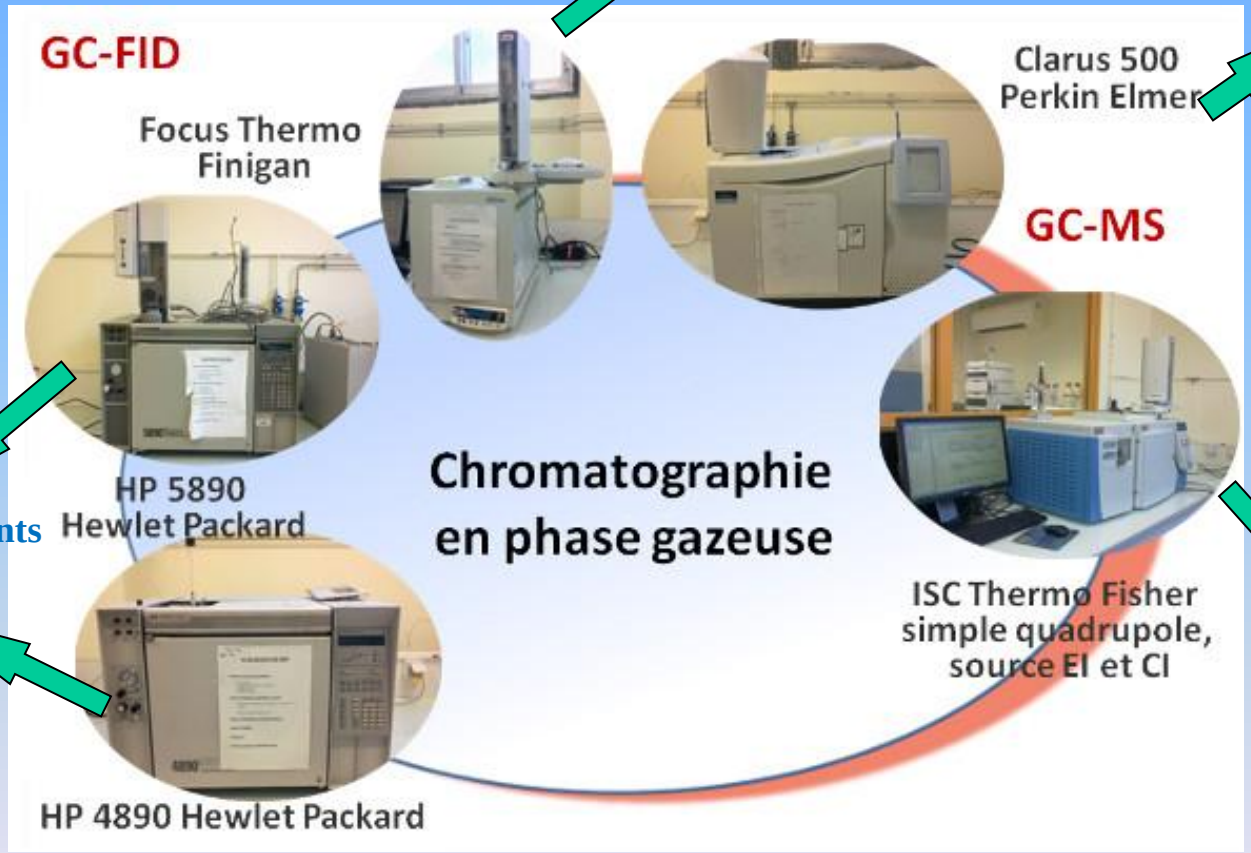
- *Sphinganine, sphingosine*
- **Céramides**
- **Sphingomyéline**
- *Ganglioside, GluCer, GalCer*

Acides biliaires (bile)

Instruments analytiques et molécules analysées

Acides Gras libres et Totaux

Lipides neutres
- TG
- DG
- Cholestérol
- Chol esters



Développements Essais

- *Oestradiol*
- **Stérols**
- *Oxystérols*
- **Acides gras hydroxylés (non conventionnels)**

MetaToul-Lipidomique

✓ Le **personnel** du plateau

Justine BERTRAND MICHEL
IR, responsable plateau

Véronique ROQUES
Technicienne UPS

Pauline
LE FAOUDER
CDD IR

Aude
DUPUY
CDD IE

Fabien
RIOLS
CDD IE

✓ Les membres du **conseil scientifique**

Bernard Payrastre (Président), Xavier Collet, Thierry Levade, Marc Poirot, Hervé Guillou, Nicolas Cénac, Joost Schanstra, Nathalie Auge, François Tercé, Jean Sébastien Saulnier-Blache

Plateau de lipidomique MétaToul
I2MC, Toulouse (http://www.i2mc.inserm.fr)