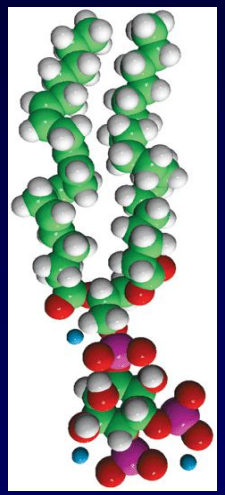
Les phosphoinositides: des lipides intégrateurs de la dynamique cellulaire

27ème Séminaire Pédagogique CNBBMM: Lipides, signalisation et physiopathologie Anglet 19-21 Mai 2015

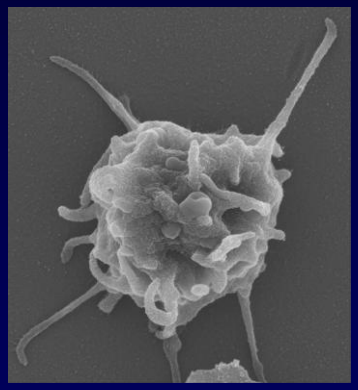
B. Payrastre *et collègues*

INSERM U1048, I2MC,

Toulouse, France



PI(4,5)P2



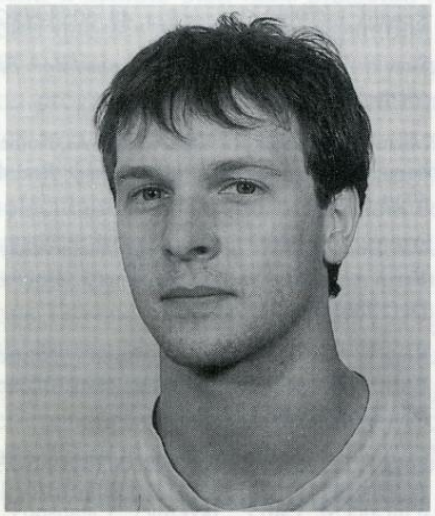
Activated mouse platelet

Phosphoinositides et transduction du signal

Bernard Payrastre, Monique Plantavid et Hugues Chap.
INSERM Unité 326, Hôpital Purpan
31059 Toulouse Cedex.

Regard sur la Biochimie 1992

Les auteurs



Bernard Payrastre

Docteur Bernard Payrastre, chercheur post-doctoral à l'Unité INSERM 326.

Monique Plantavid

Docteur Monique Plantavid, maître de conférence, praticien hospitalier

Hugues Chap

Professeur Hugues Chap, directeur de l'Unité INSERM 326

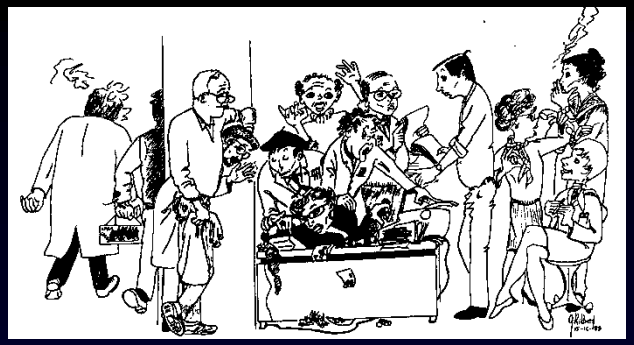
Merci Hugues pour la puissance et l'élégance de la démarche de recherche, toujours lumineuse, que vous su nous révéler et que nous avons suivi tels de petits poissons qui suivent le maître dans sa remontée vers les sources des mécanismes du vivant, depuis la physiopathologie, la biochimie et jusqu'à la chimie des molécules.

LA DEPECHE DU MIDI

FOOTBALL

AURIAC SUR VENDINELLE

H. Chap emmène son équipe vers les sommets



INSTITUT EPICURIEN DU LIPIDE

(Dir Pr H. Chap)

Toulouse, France

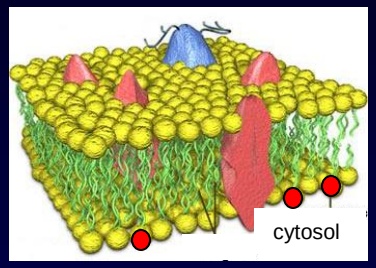
Instituts thématiques Inserm

Institut national de la santé et de la recherche médicale

Le Professeur Hugues CHAP devant ses étudiants qu'il emmène vers les sommets. Un humaniste au parcours impressionnant, au service de la faculté et de son excellence.

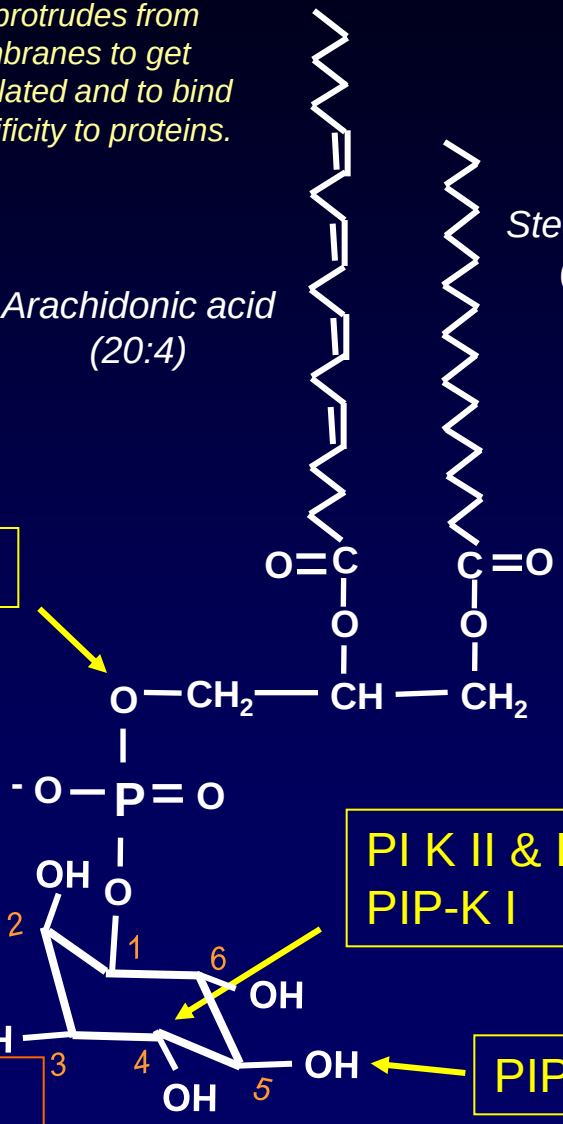
Phosphoinositides are minor constituents of cell membranes with a highly dynamic metabolism

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.

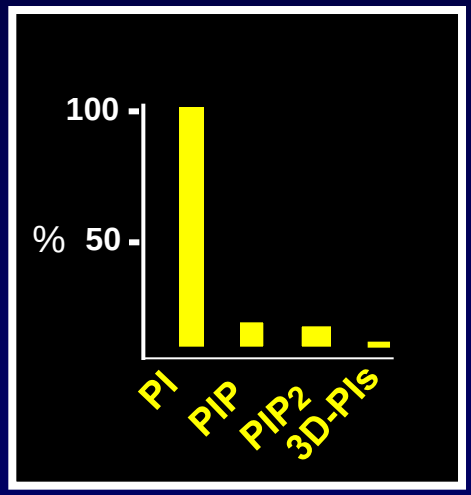


Arachidonic acid (20:4)

Stearic acid (18:0)



Relative amounts of phosphoinositides



The PI cycle in the seventies/eighties

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

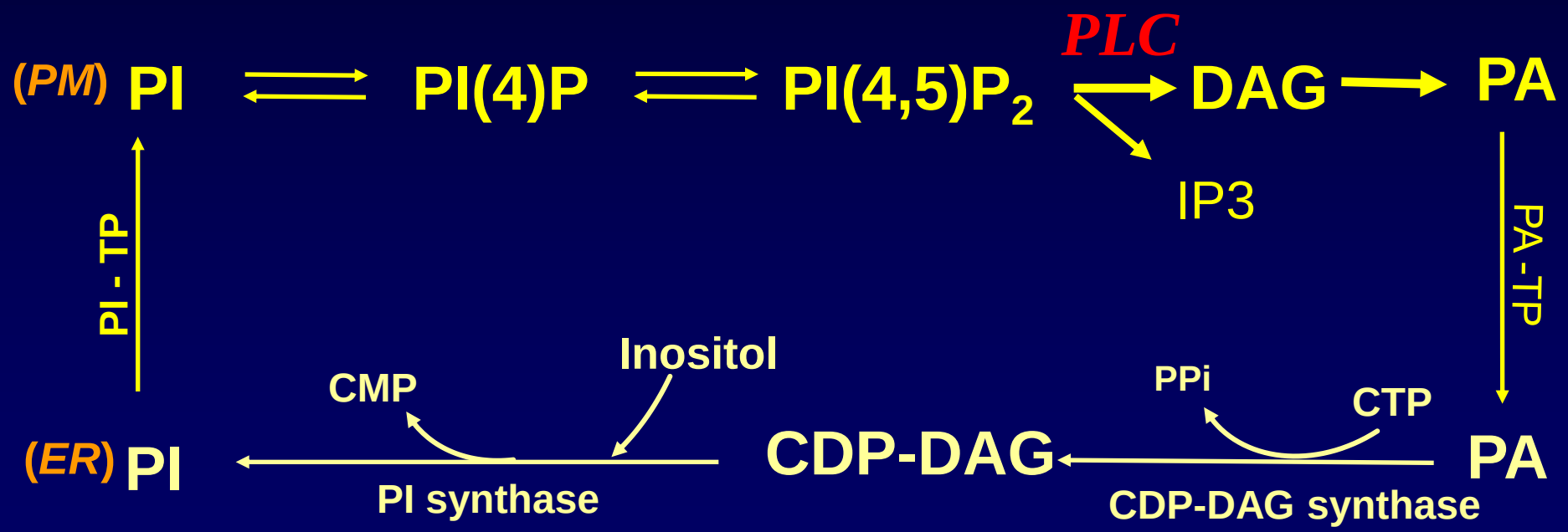
USA 1969

M. Berridge
R.H. Michell
R. Irvine

UK 1979

Y. Nishizuka
K. Mikoshiba

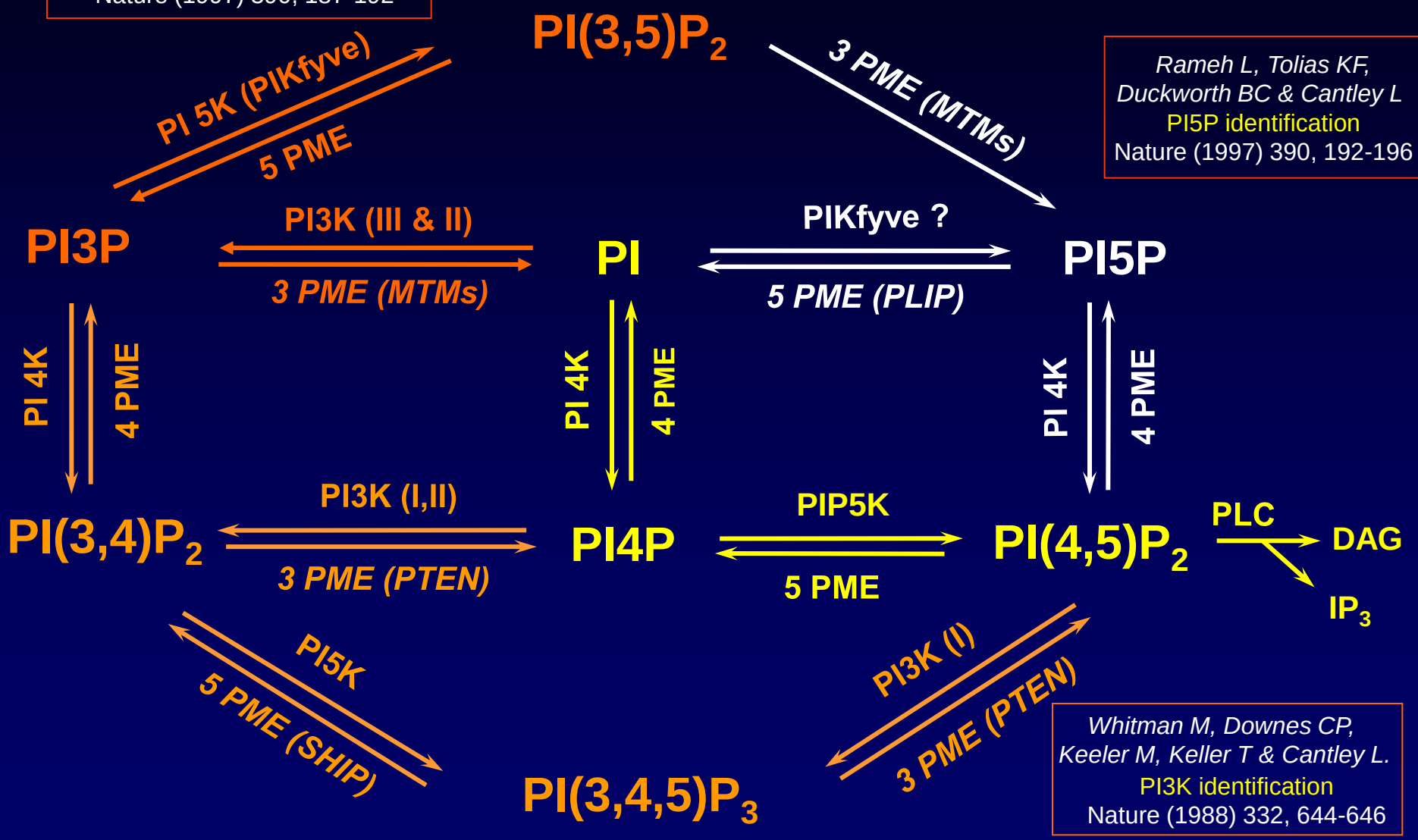
Japan 80's



Michell R.H. Biochimica Biophysica Acta 1975

Various pathways of phosphoinositide synthesis and interconversions

Dove et al. $PI(3,5)P_2$ pathway identification
Nature (1997) 390, 187-192

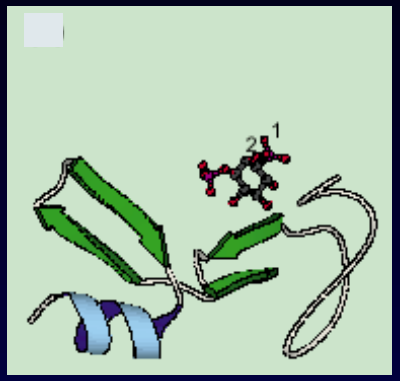


Rameh L, Tolias KF, Duckworth BC & Cantley L
PI5P identification
Nature (1997) 390, 192-196

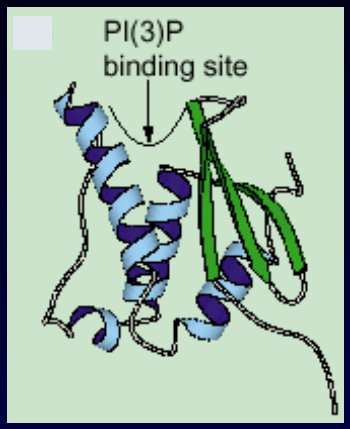
Whitman M, Downes CP, Keeler M, Keller T & Cantley L.
PI3K identification
Nature (1988) 332, 644-646

Phosphoinositide binding domains

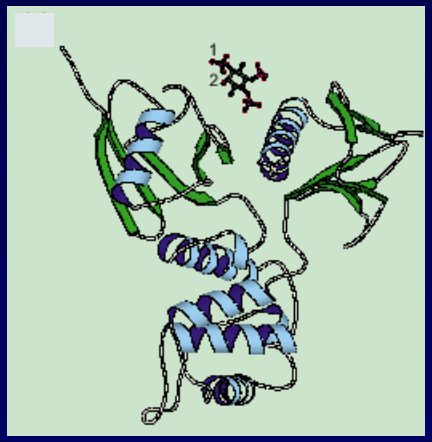
PIP3-binding **PH** domain from Grp1



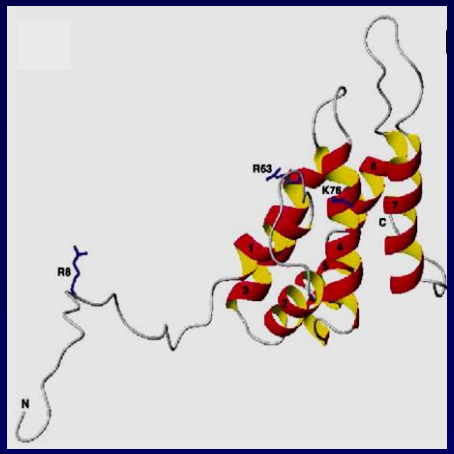
PI(3)P-binding **FYVE** domain from EEA1



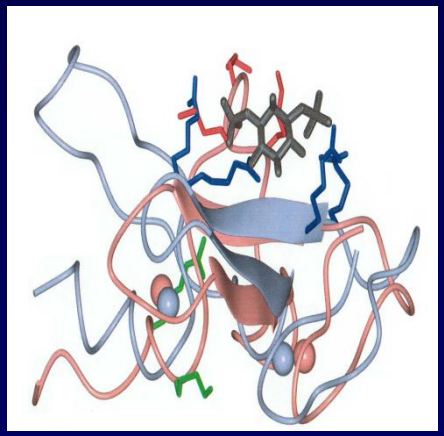
PI(3)P-binding **PX** domain from p47phox



PIP2-binding **FERM** domain from radixin



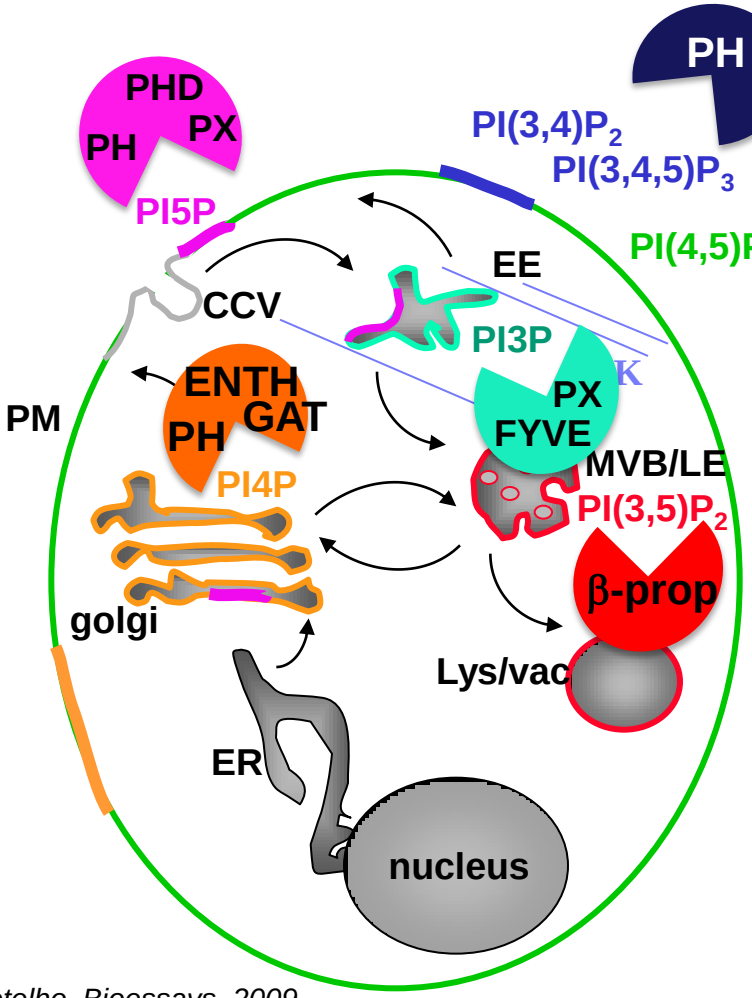
PIP2-binding **ENTH** domain from epsin



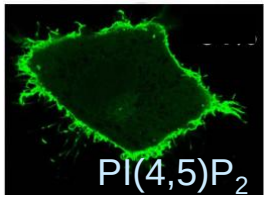
PI5P-binding domain **PHD** from ING2

Itoh T & Takenawa T, Cell Signal 2002

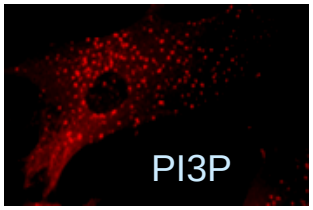
Localization of phosphoinositides with specific probes : do phosphoinositides define organelle identity ?



PLCδ (PH)



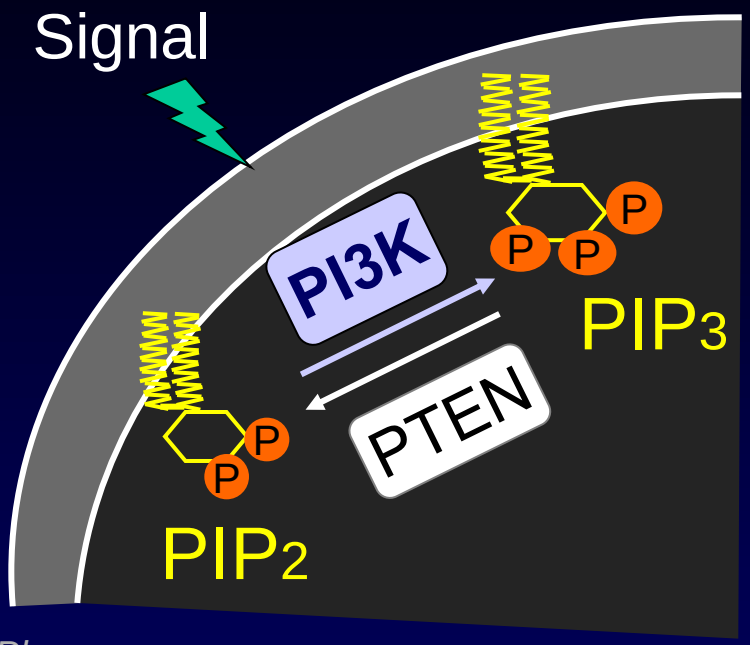
EEA1(FYVE)



PM : plasma membrane
EE: Early endosomes
LE : Late Endosomes
MVB : Multivesicualr bodies
CCV : Clathrin Coated vesicles
Lys: lysosomes
ER: Endoplasmic reticulum

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

A critical role for Class I PI3Ks and PI(3,4,5)P_3 in cell regulation



PH

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., Annals Med 2014

Phosphoinositide 3-kinase (PI3K) : from discovery to targeting in cancer therapy

1985

- Unknown phosphoinositide kinase associated with the polyoma middle T protein

- PI3-kinase is activated and produces $PI(3,4,5)P_3$ in response to growth factors and oncogenes

- Cloning and characterization of the different Class I PI3-kinases and, later on, of Class II and III PI 3-kinases

- Multiple roles of the different PI3-kinases in cell biology

- Identification of oncogenic mutations in PI3-kinase α in various cancers

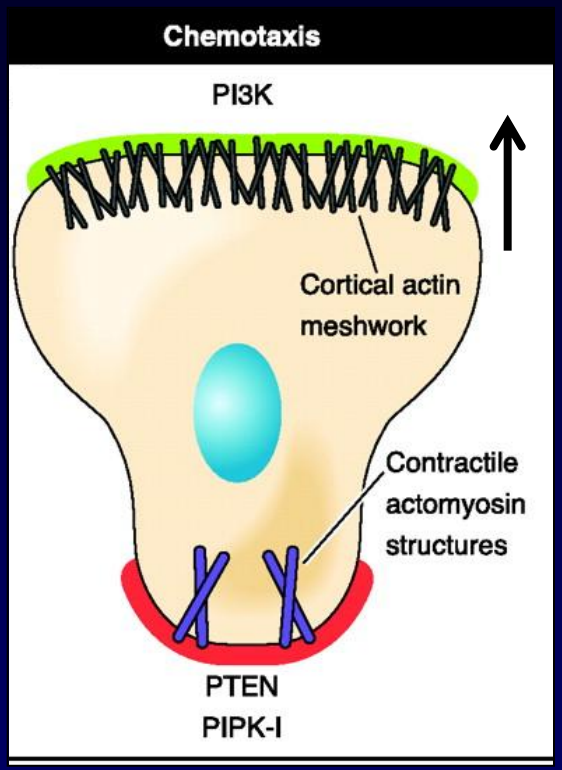
2014

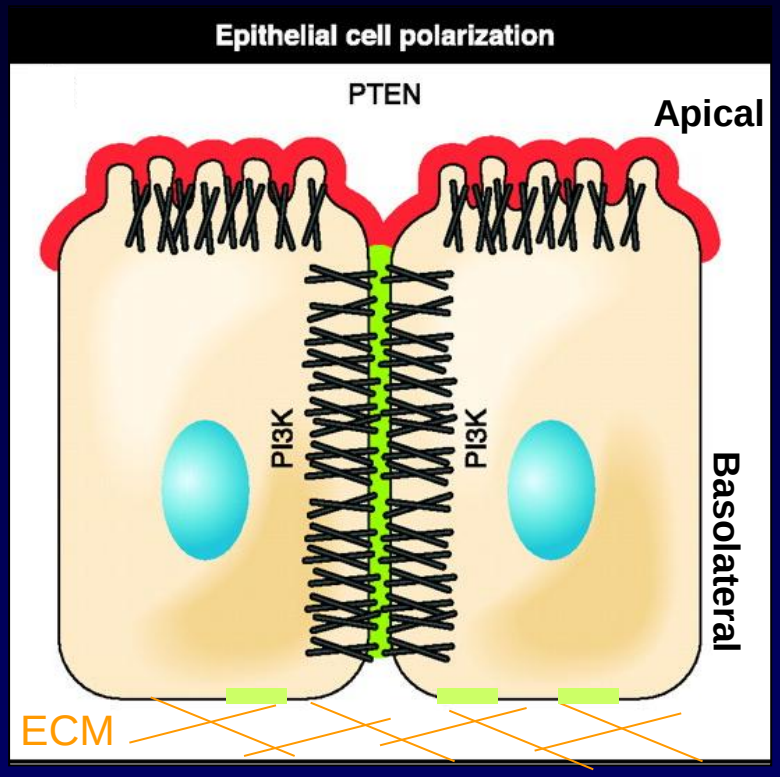
- First PI 3-kinase (δ) selective inhibitor in clinic (Idelalisib - approval by the FDA on July 2014 for CLL and B cell lymphomas)

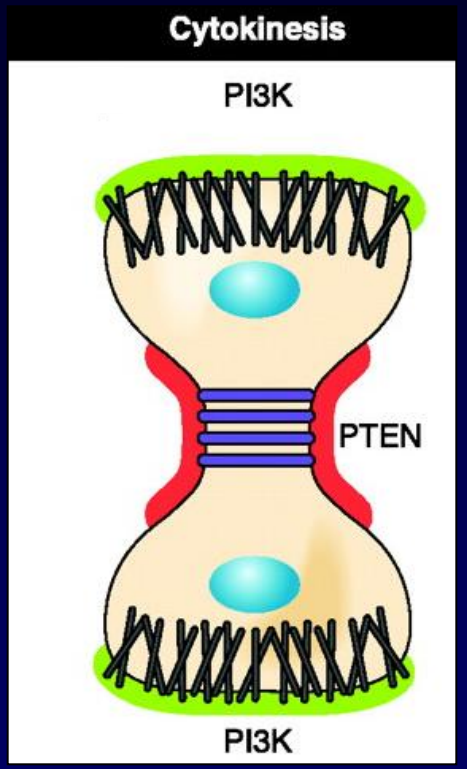
L. Cantley / M. Waterfield / P. Vogt/ B. Vanhaesebroeck / E.Hirsch

Phosphoinositides in the dynamics of cell organization and polarity

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

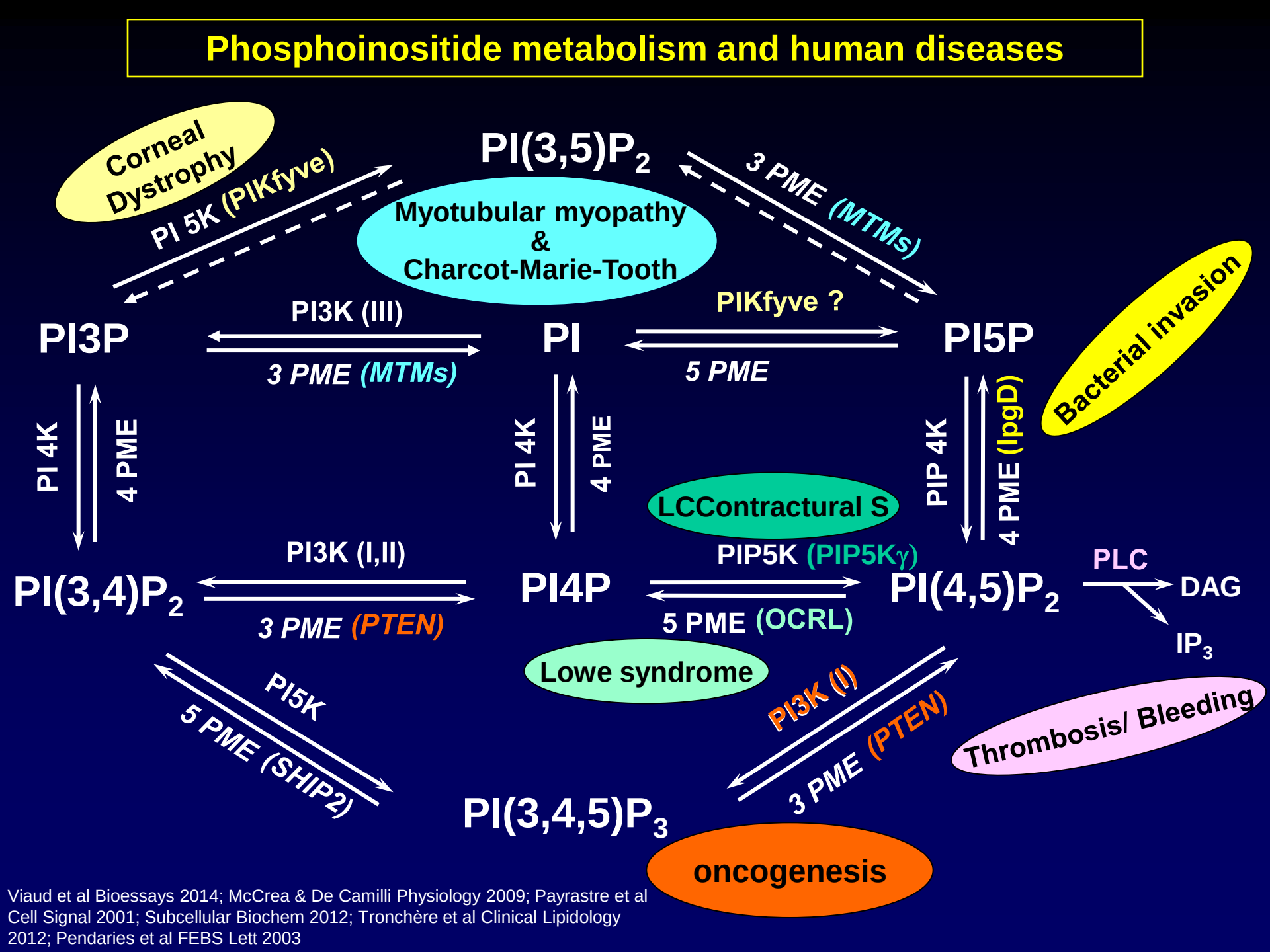






Di Paolo & Camilli, Nature Rev 2006
Sasaki et al., Progress Lipid Res 2009
Gassama & Payrastre, Int Rev Cell Mol Biol 2009
Saarikangas et al., Physiol Reviews 2010

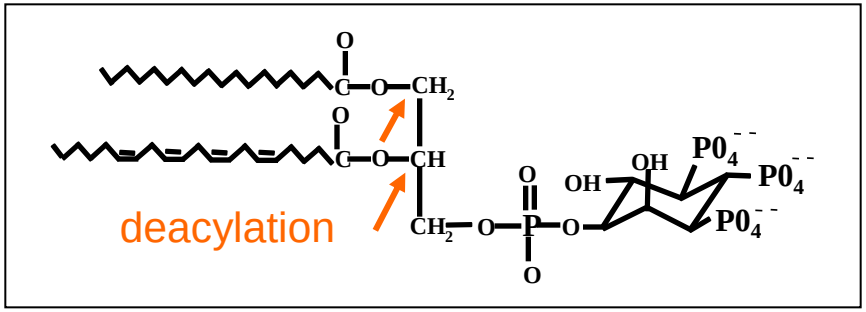
Phosphoinositide metabolism and human diseases



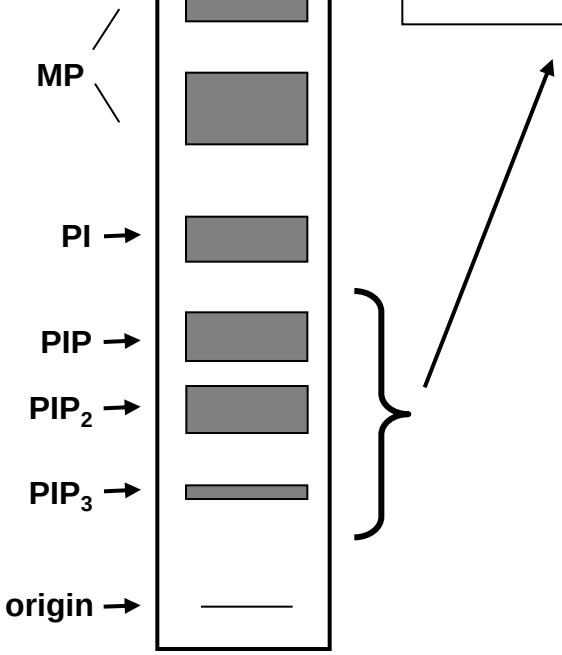
Viaud et al Bioessays 2014; McCrea & De Camilli Physiology 2009; Payrastre et al Cell Signal 2001; Subcellular Biochem 2012; Tronchère et al Clinical Lipidology 2012; Pendaries et al FEBS Lett 2003

Analysis of phosphoinositides: a specialized lipid biochemistry

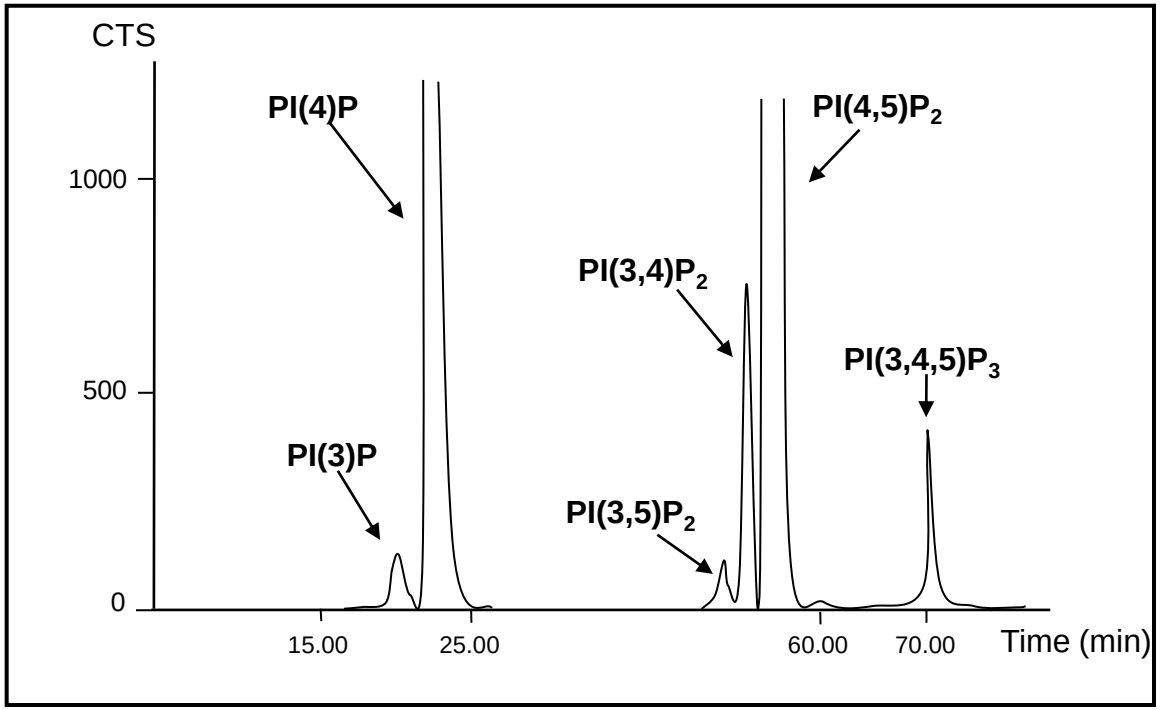
➡ *Isotopic labelling (^{3}H-inositol or ^{32}Pi) followed by TLC and HPLC techniques :*



TLC



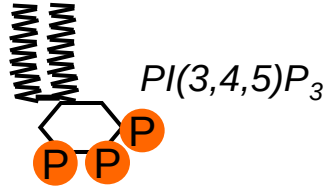
HPLC



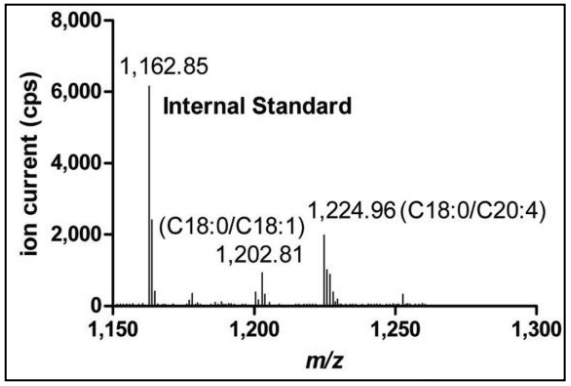
B. Payrastre, Methods Mol Biol 2004

Development of new methods to analyze phosphoinositides

⇨ **Mass spectrometry method to mesure PI, PIP, PIP_2 and $PI(3,4,5)P_3$** (Collaboration L. Stephens/P. Hawkins, Cambridge and the I2MC lipidomic platform)



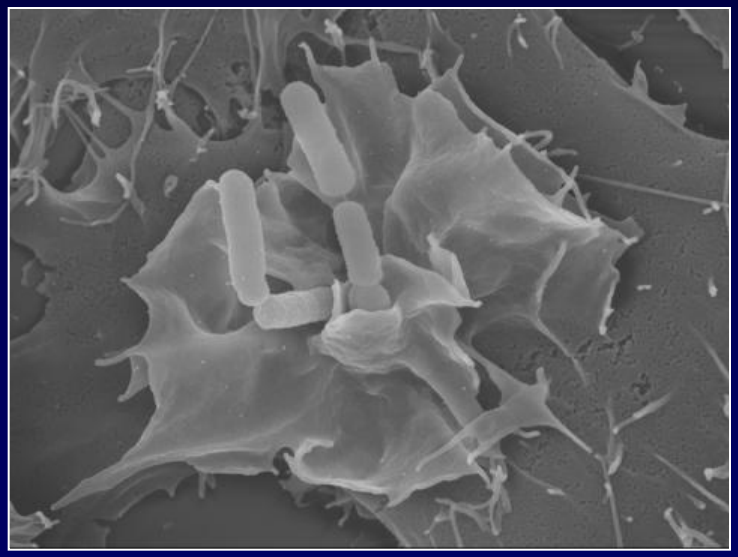
$PI(3,4,5)P_3$



⇨ **Specific mass assay to quantify PI3P** (Chicanne et al. Biochem J 2012 and patent Inserm-Transfert WO2014023436A1)

⇨ **PLIF: a new rapid and efficient method to study lipid-protein interactions and screen for inhibitors** (Viaud et al. article and patent in prep.)

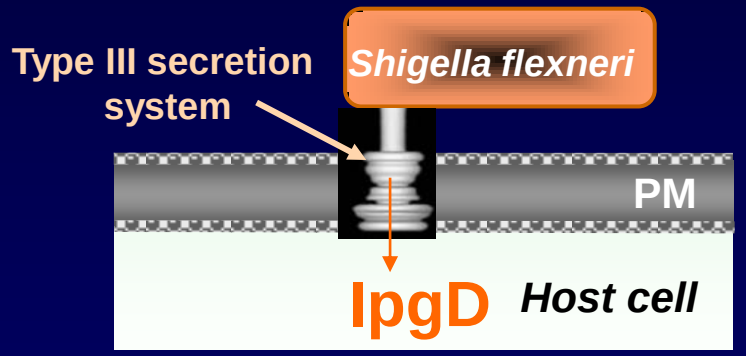
The bacterial pathogen Shigella flexneri uses PI5P to hijack the signaling machinery of host cell and establish its virulence



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

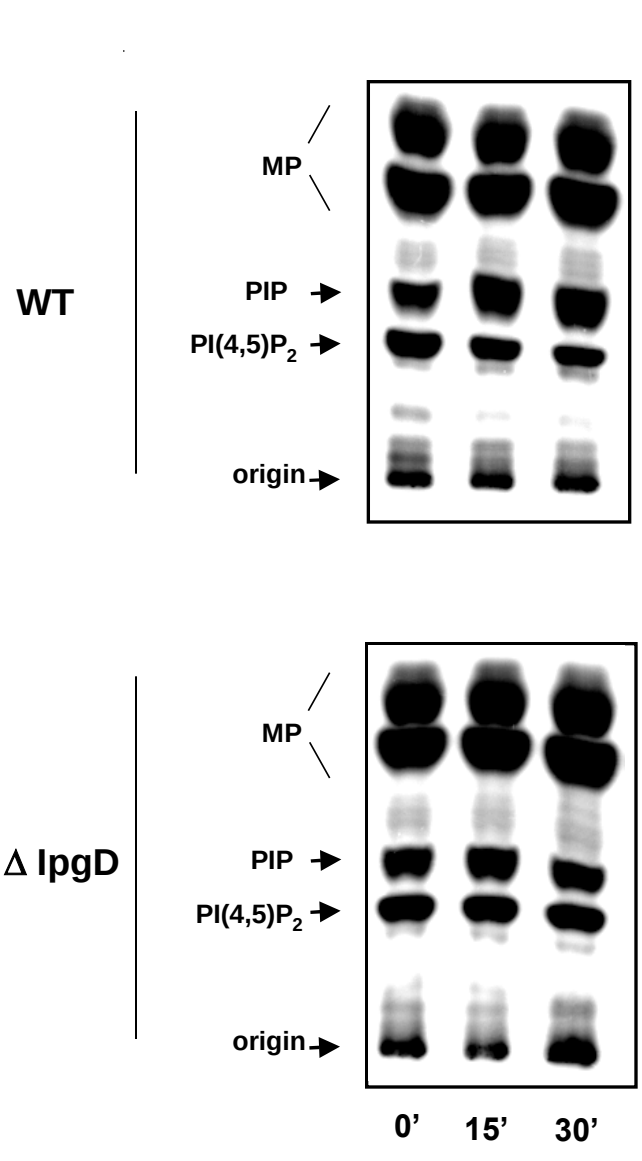
The *Shigella flexneri* effector IpgD has a motif related to the active site of mammalian phosphoinositide phosphatases

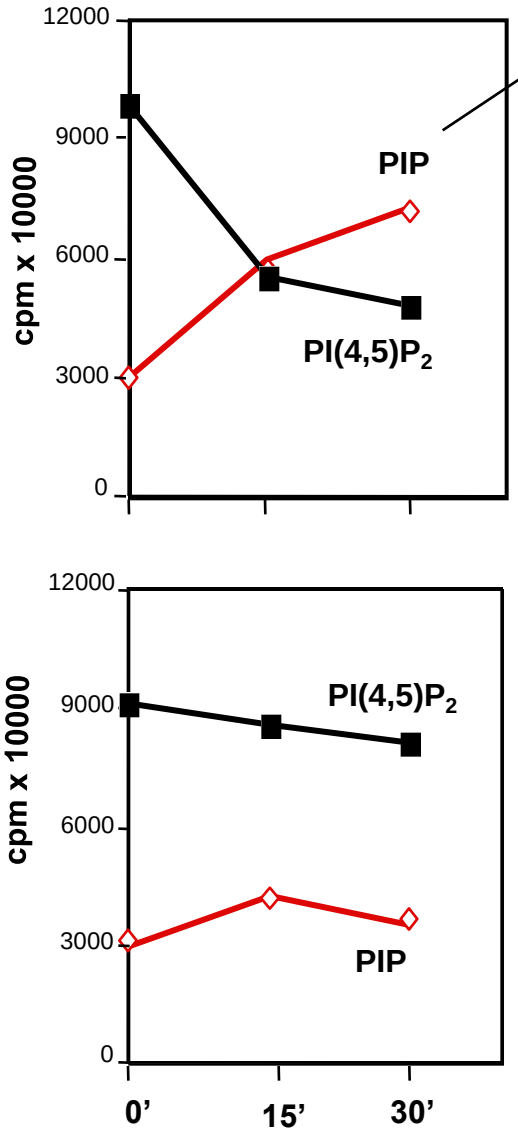
IpgD	PCWNCKSGKDRTGMQDAEIKREIIRK
PTEN	AAIHCKAGKGRTGVMICAYLLHRGKF
MTM1	VLVHCSDGWDRTAQLTSLAML-M-LD
MTMR3	VLVHCSDGWDRTPQIVALAKL-L-LD

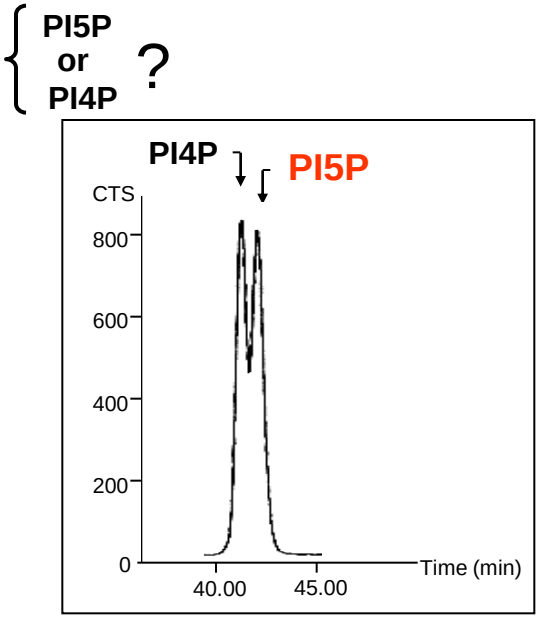


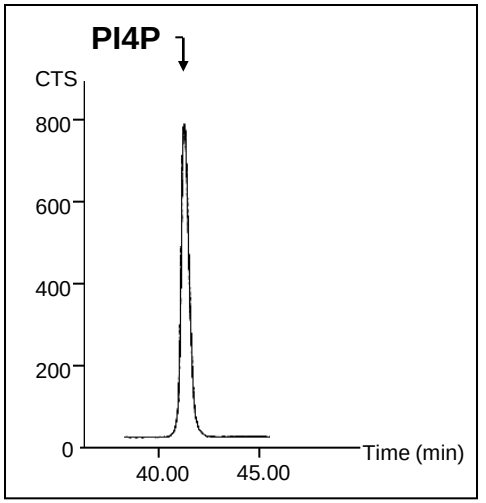
➡ ***The IpgDC438S is an inactive phosphatase***

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



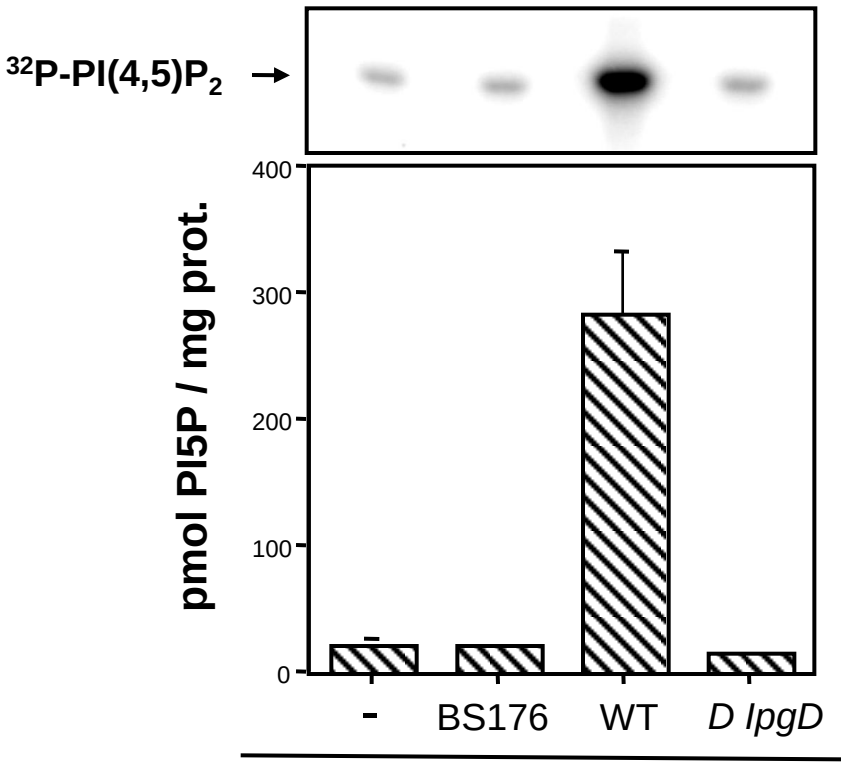




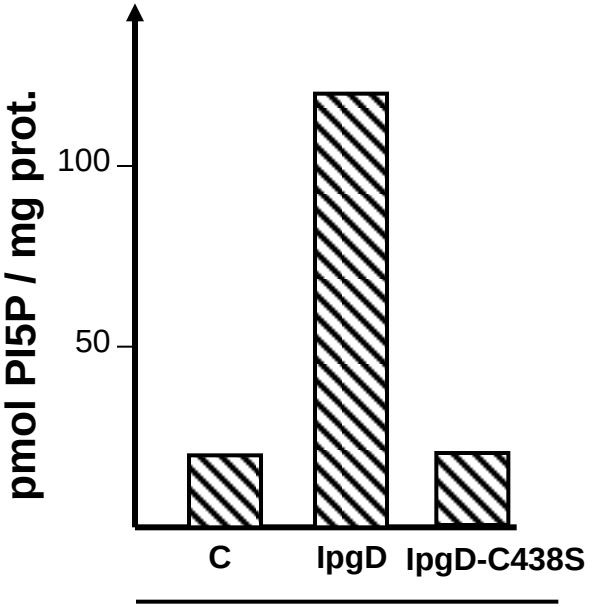


The *Shigella* effector IpgD is sufficient to increase PI5P levels in host cells

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

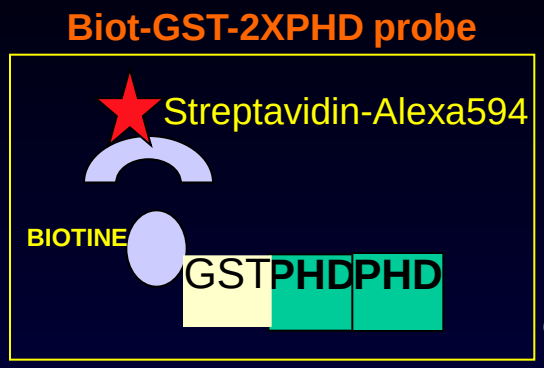


Hela cells infected with wild type *shigella* and mutants

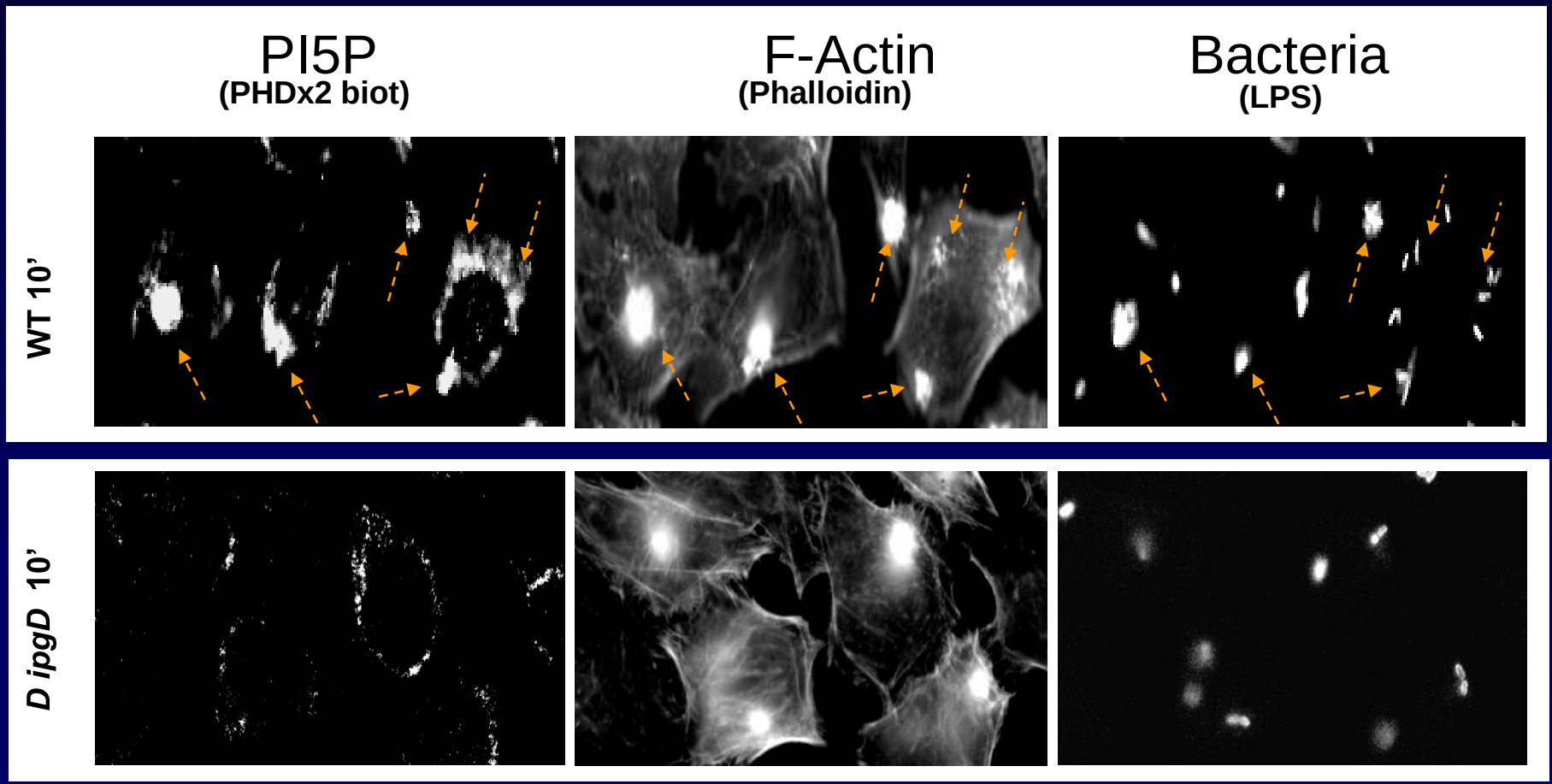


Expression of GFP-IpgD in Hela cells

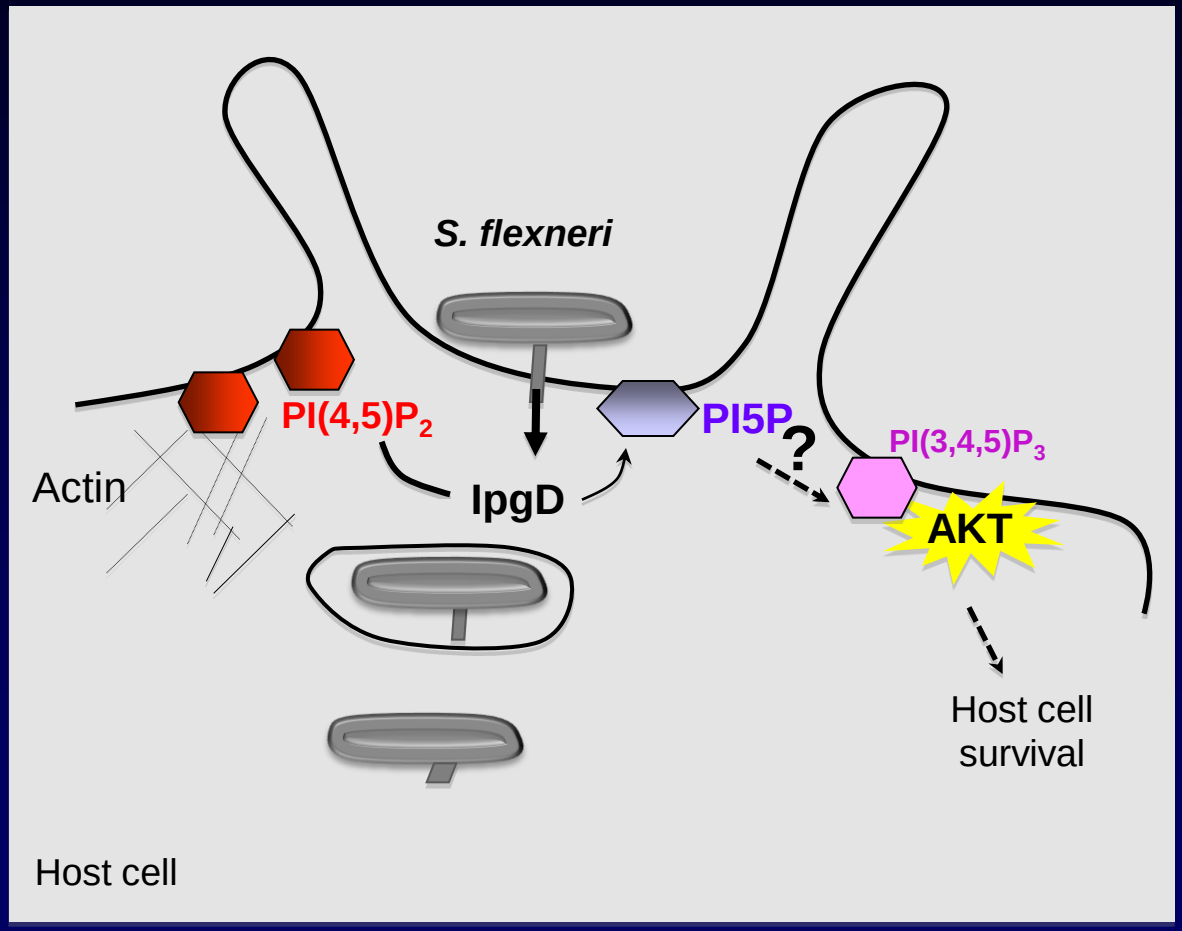
PI5P is produced in host cells at the entry site of *S. flexneri*



O. Gozani et al Cell 2003

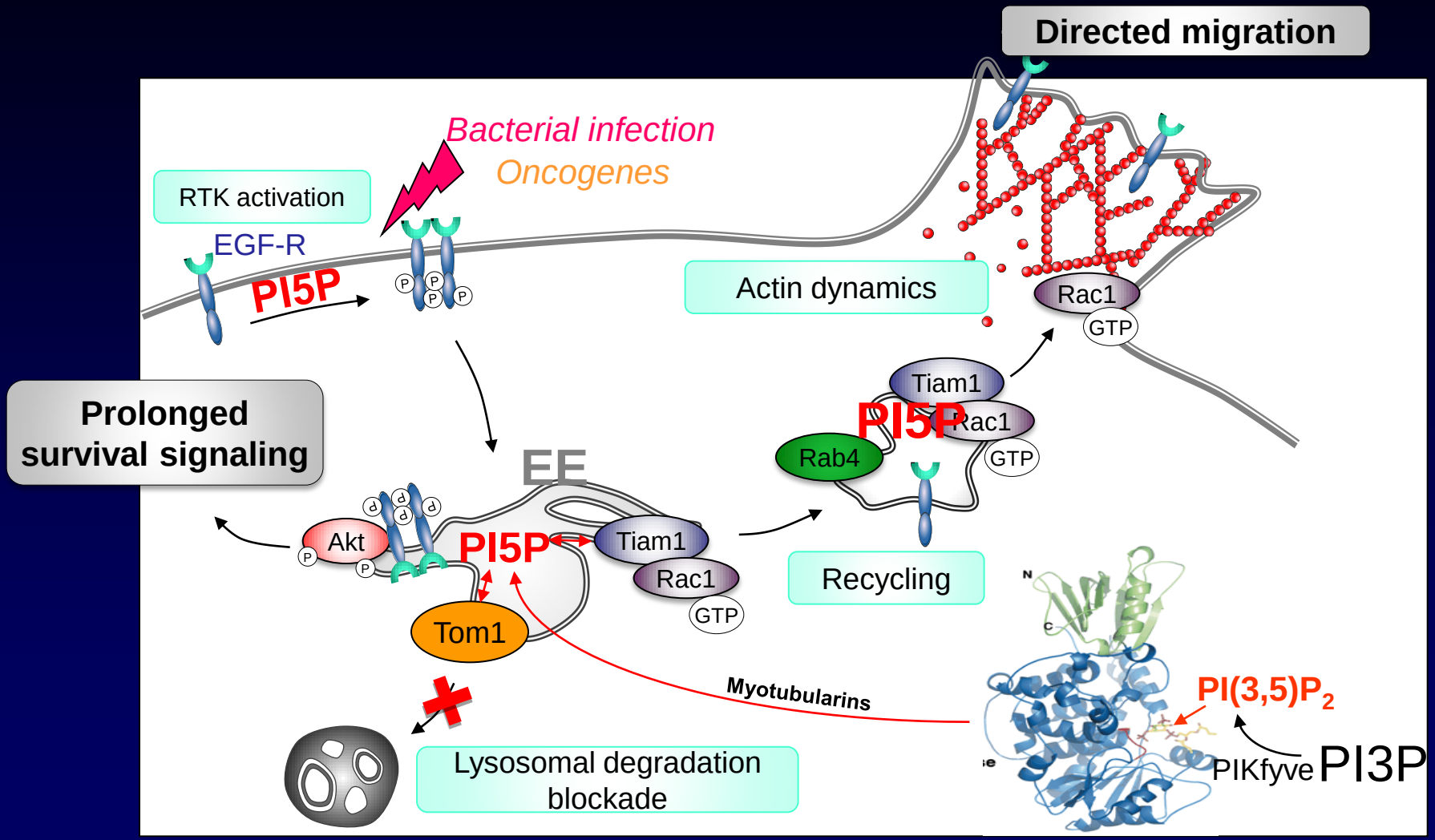


Shigella flexneri injects the PI-phosphatase IpgD in the host cell through its Type III secretion system : a model to study the role of PI5P



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

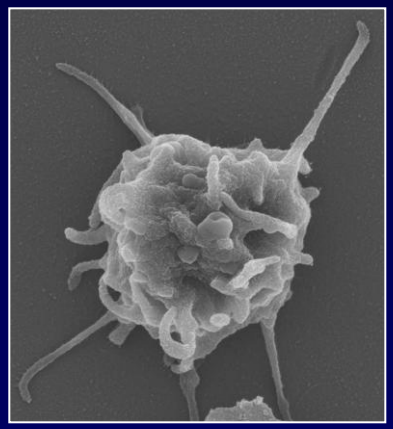
PI5P integrates membrane and cytoskeleton dynamics



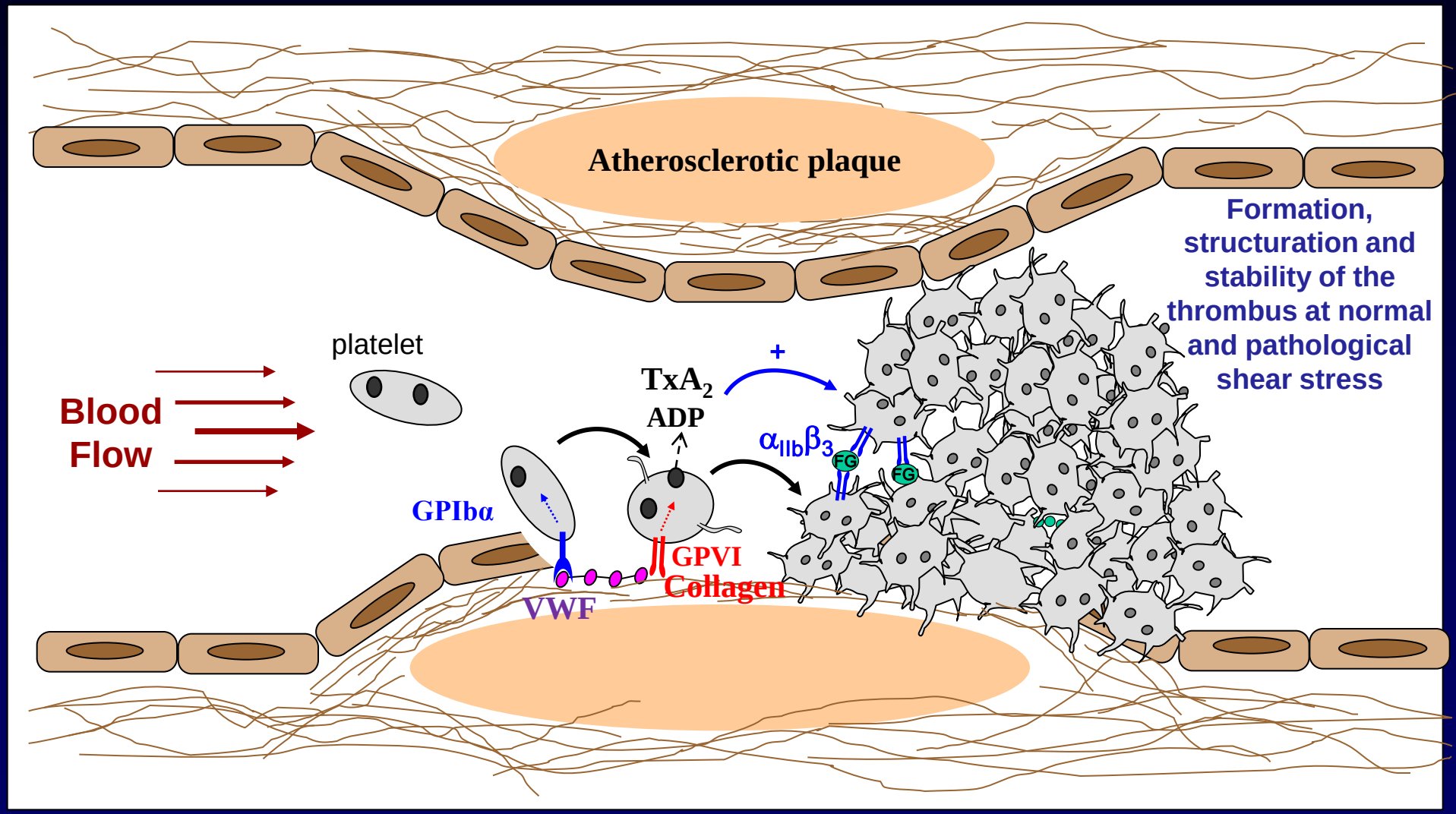
Viaud et al. Nature Commun 2014
Boal et al. J Cell Sci 2015
Ramel et al. Science Signaling 2011

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

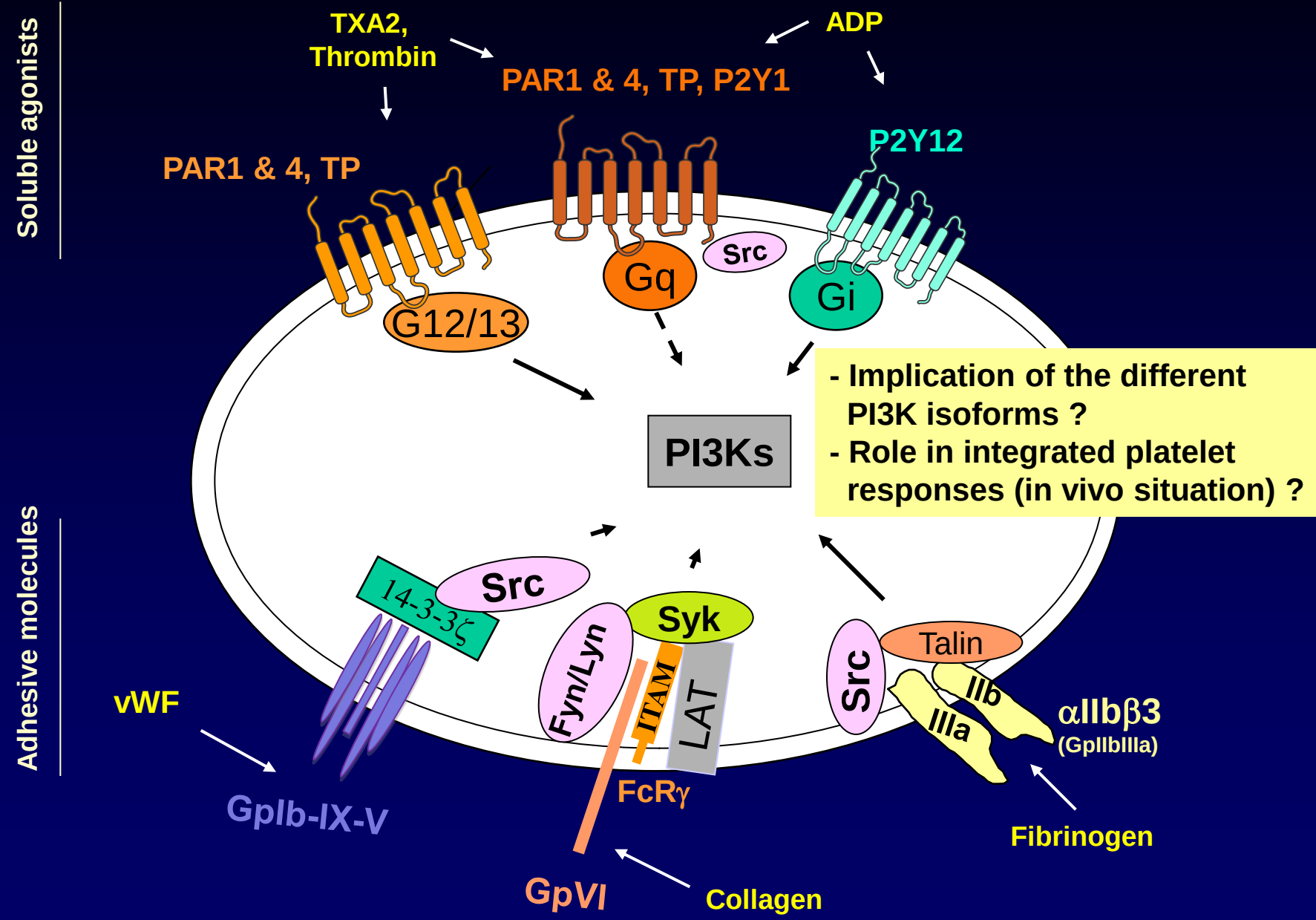
A role for PI3Kβ in platelet functions and thrombosis



Regulation of platelet activation and thrombus formation



PI3Ks are activated downstream of most activatory human and mouse platelet receptors



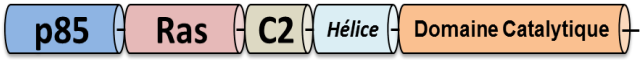
Blood platelets contain 7 different PI3Ks

PI(3,4,5)P_3
(plasma membrane)

PI3P
(endosomal membranes)

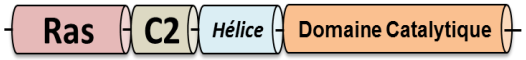
Class IA

p110 α, β, δ



Class IB

p110 γ



Class II

PI3KC2 α,β,~~γ~~

Class III

Vps34p

*PF4*Cre-p110β$^{lox/lox}$

*PF4*Cre-p110α$^{lox/lox}$

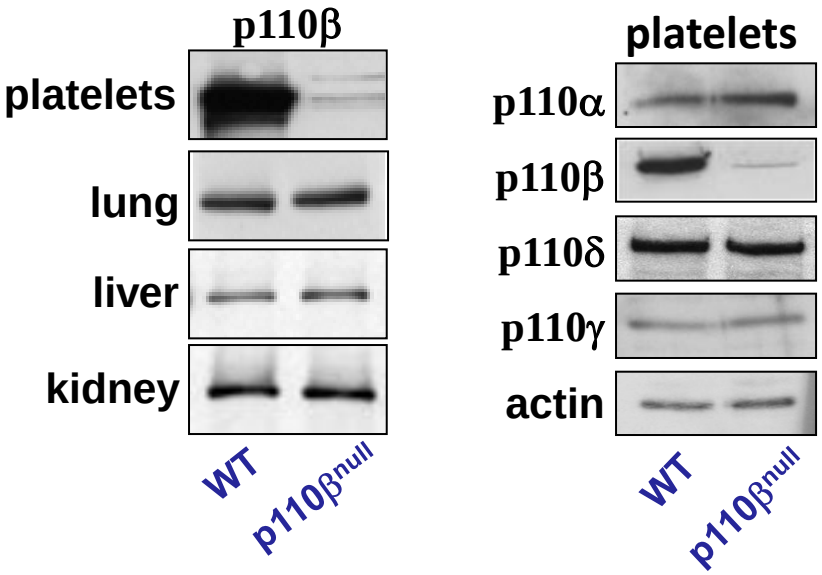
Vanhaesebroeck et al., Nat Rev Mol Cell Biol 2012
Laurent et al., Advance Biol Reg 2013

Mouse model of PI3Kα and β invalidation specifically in the megakaryocyte/platelet lineage

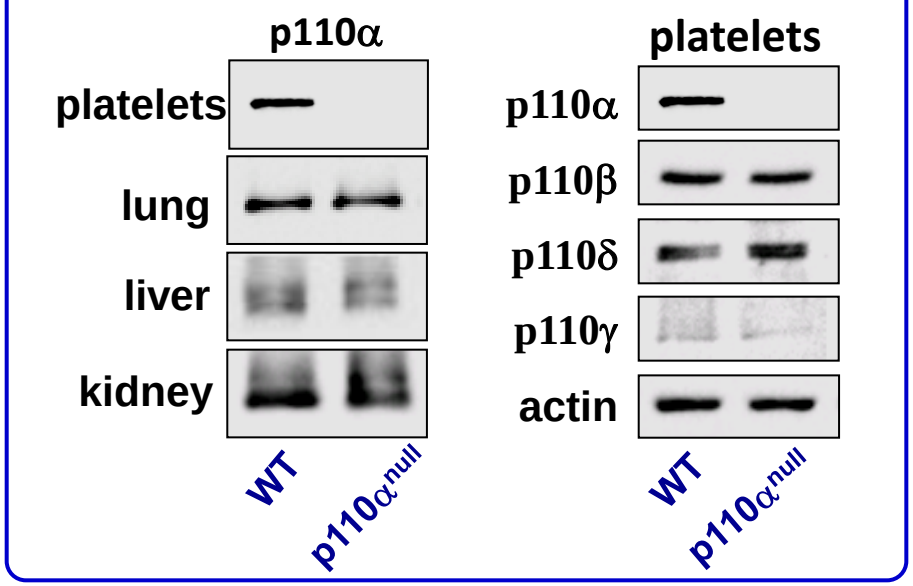
PI3Kβ

PI3Kα

PF4Cre-p110β lox/lox

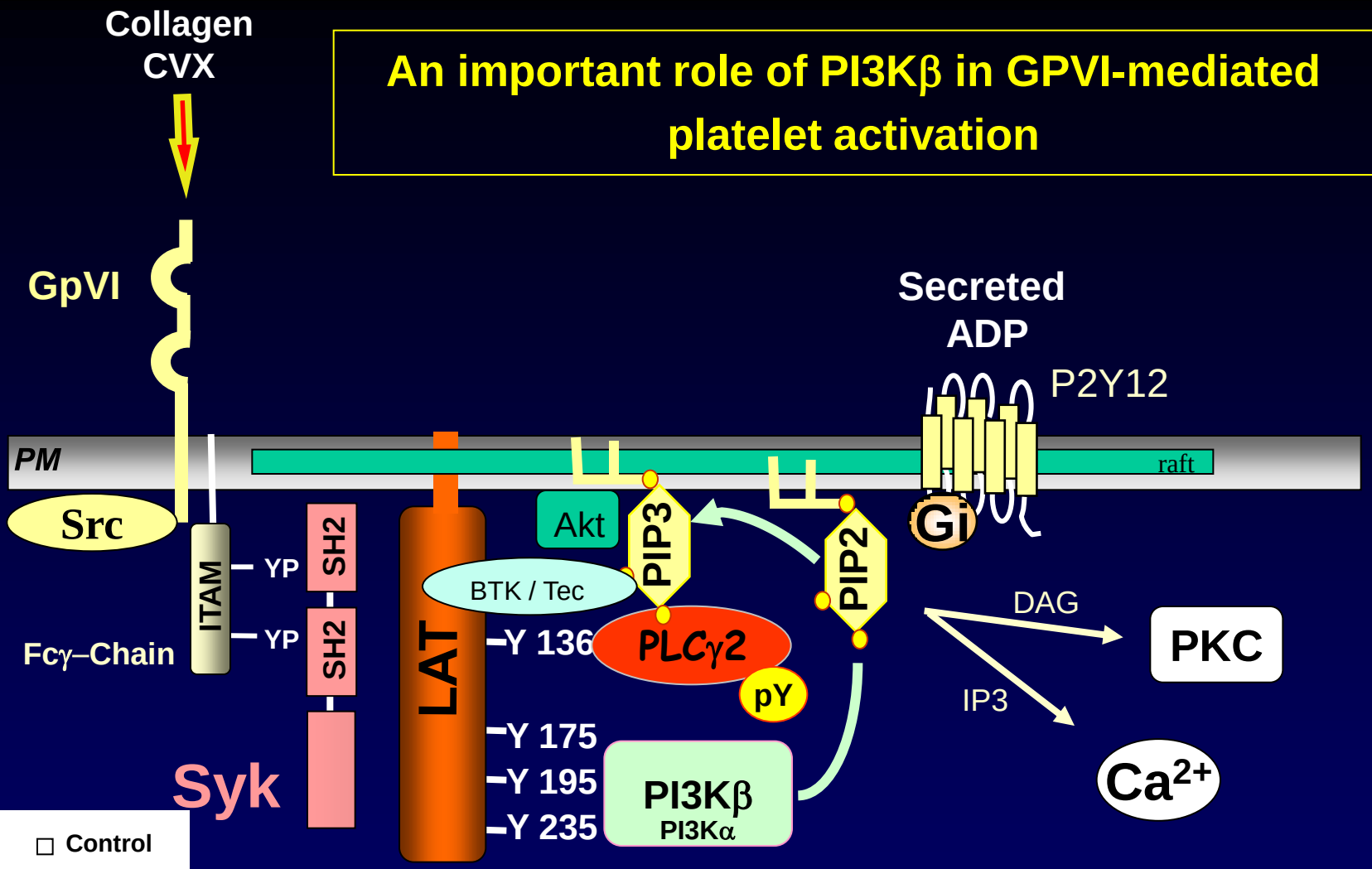


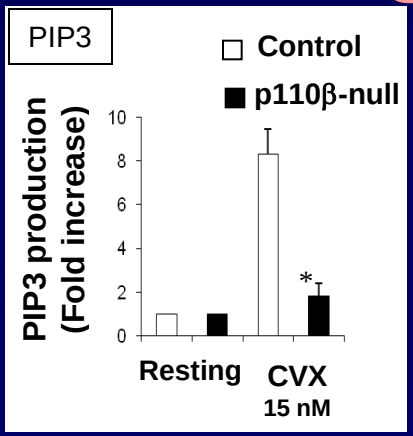
PF4Cre-p110α lox/lox

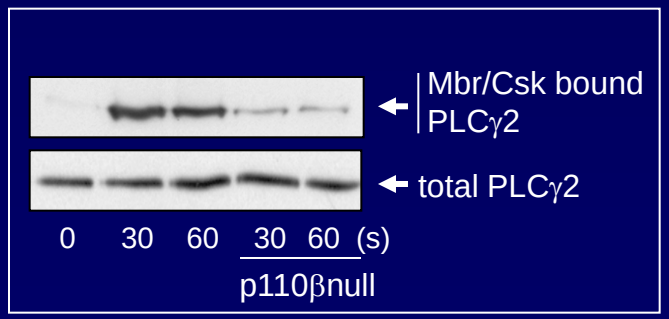


Platelet count and morphology : normal

An important role of PI3Kβ in GPVI-mediated platelet activation





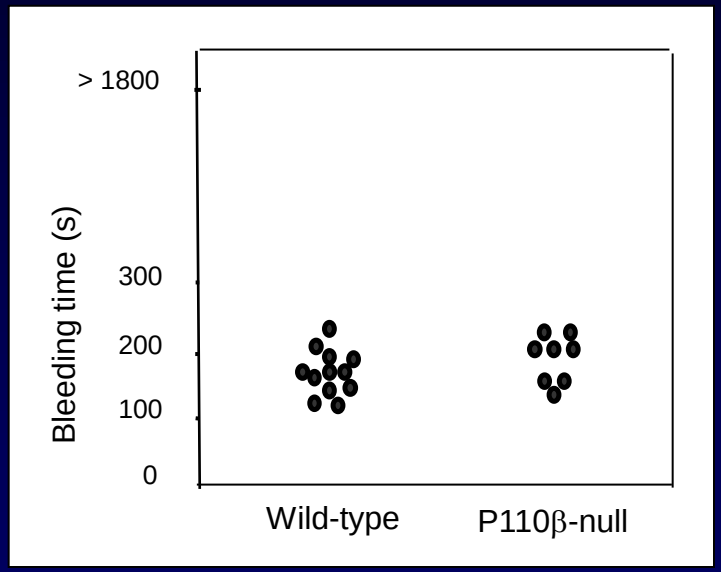


Secretion / Aggregation

Ragab et al. Blood 2007
Gratacap et al. Blood 2009
Martin et al. Blood 2010

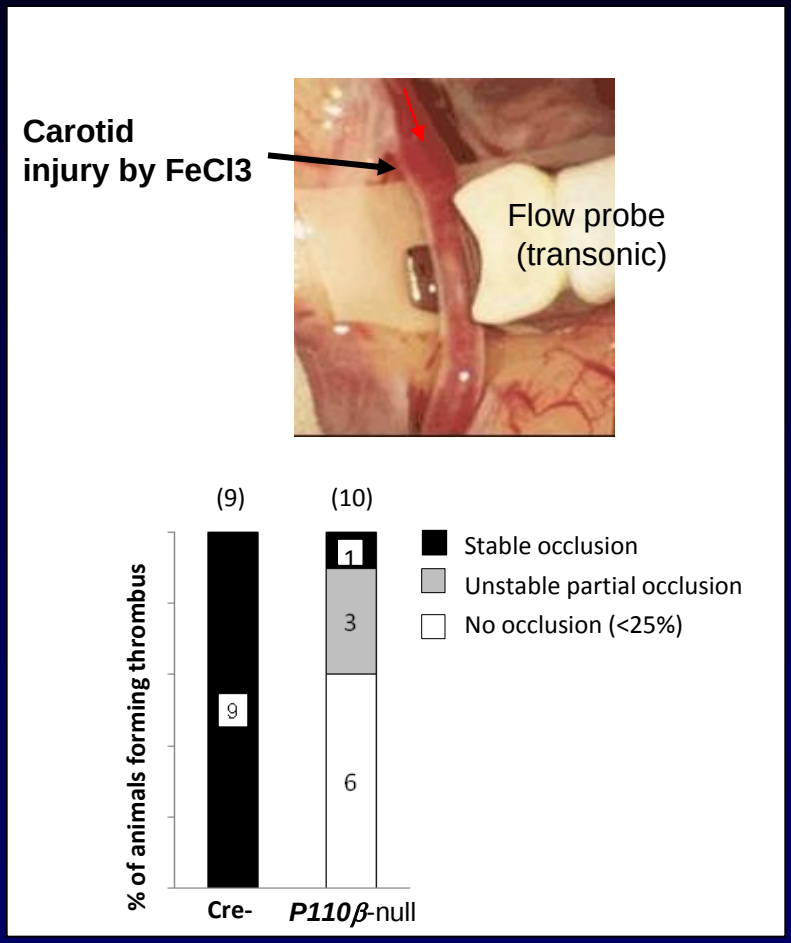
A role for PI3Kβ in arterial thrombus formation

Tail bleeding time:



Thrombus formation in vivo:

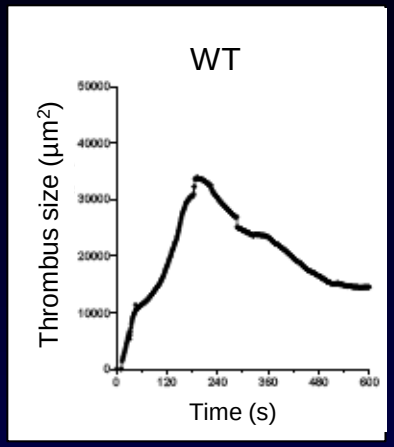
(FeCl3 carotid injury and monitoring of blood flow)

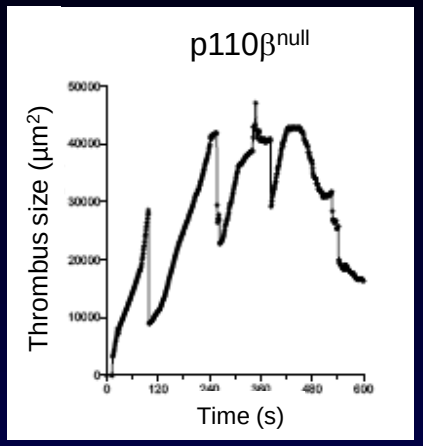


➡ PI3Kβ-null mice have a normal tail bleeding time but are protected against the formation of occlusive thrombus

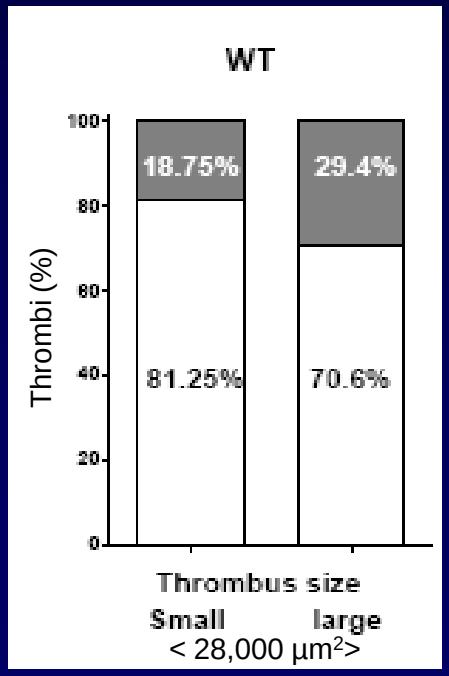
PI3Kβ regulates arterial thrombus stability in vivo

Laser injury of mesenteric arteriole and intravital microscopy :

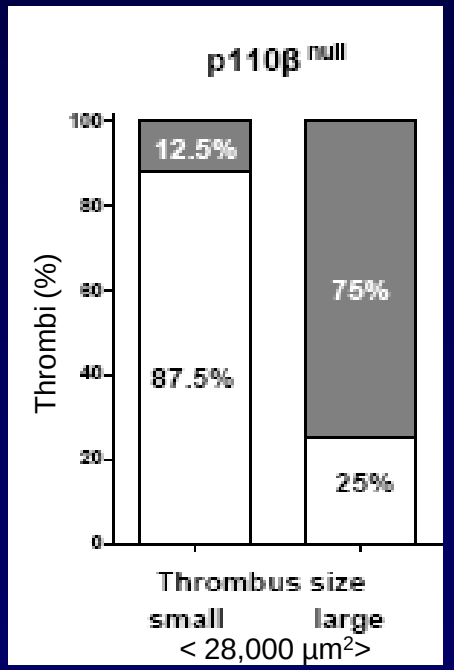




Quantification :

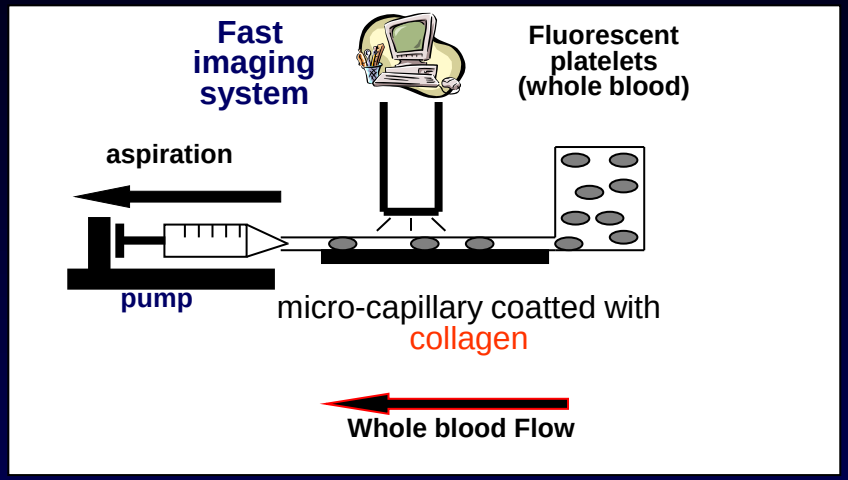


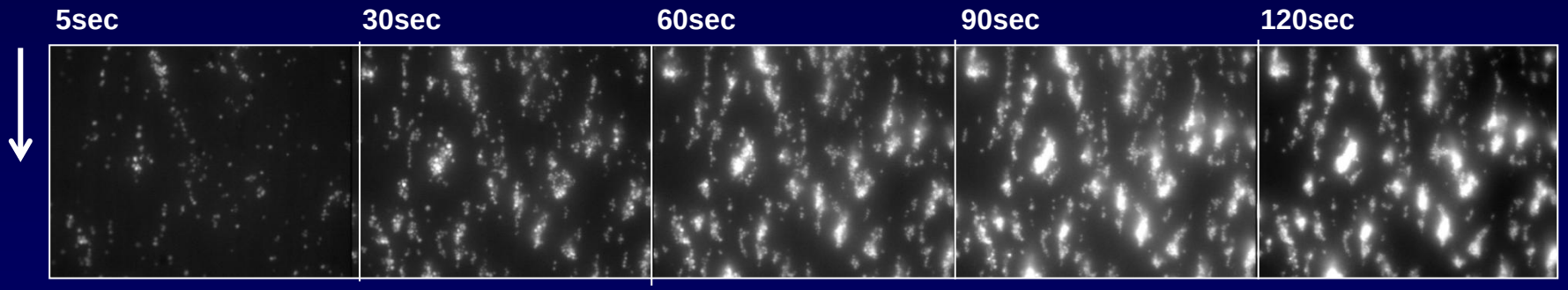
Without emboli
With emboli



Role of the platelet PI3Kβ in thrombus stability

Microfluidic



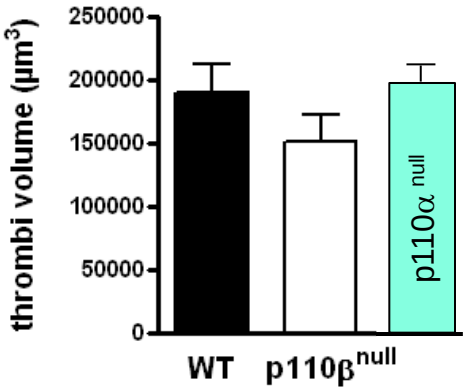


$500s^{-1}$ (120s) → Thrombus formed → $4000s^{-1}$ (60s) → Imaging/quantification

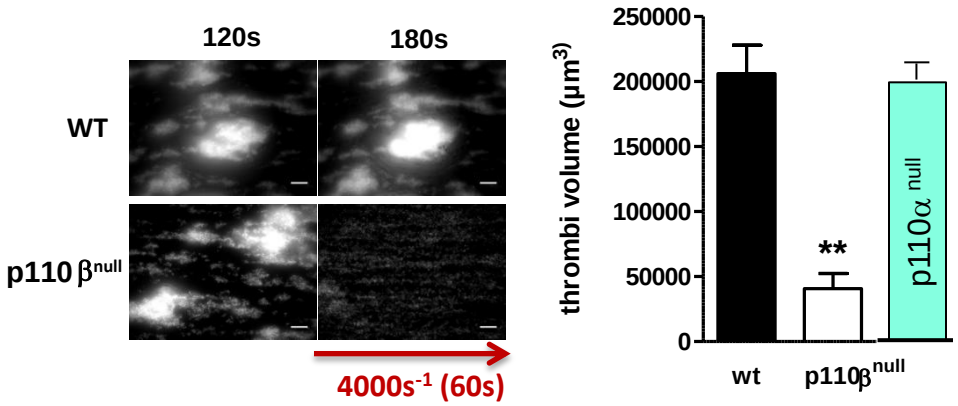
PI3Kβ regulates thrombus stability under high shear rate

500s^{-1} (120sec) → Thrombus formed → 4000s^{-1} (60s) → Imaging/quantification

Physiological shear (500s^{-1})

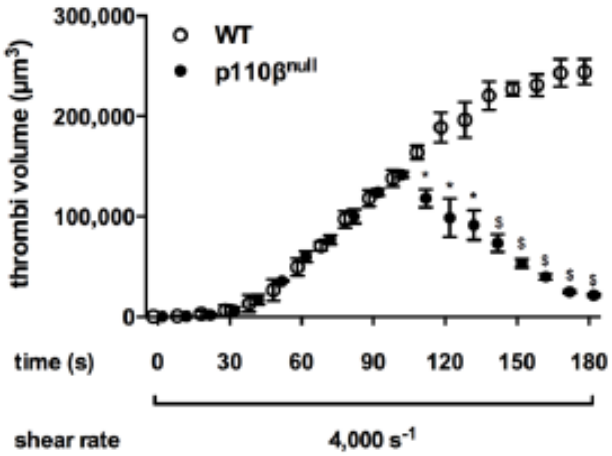


High shear (4000s^{-1})



Measurement of thrombus volume in real time

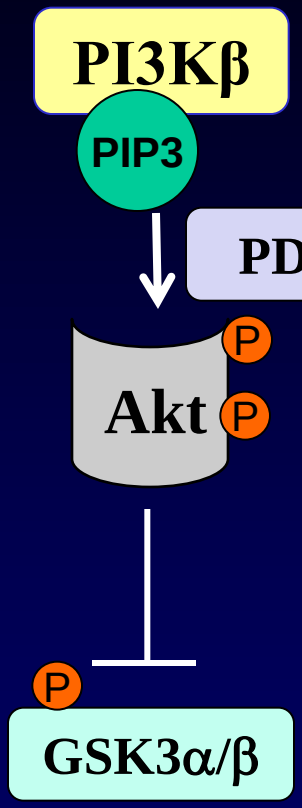
High shear (4000s^{-1})

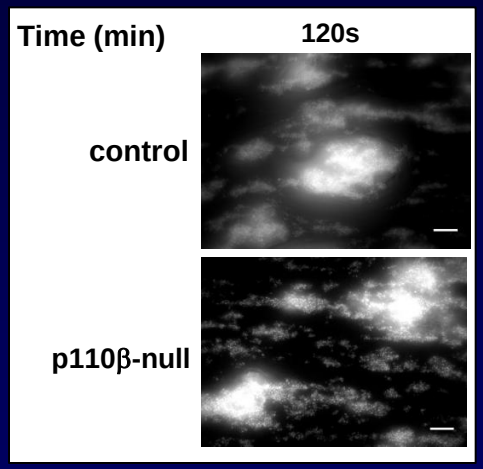


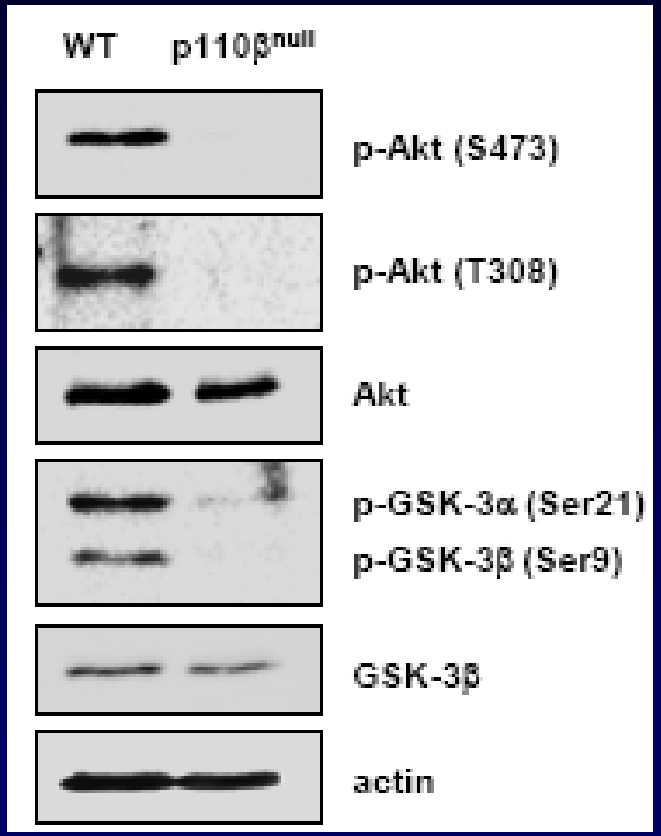
Similar results obtained with human blood treated or not with AZD6482

Laurent et al. Blood 2014, in press

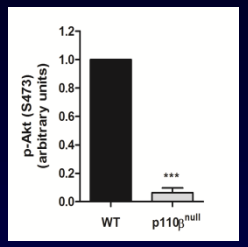
PI3Kβ is required for GSK3α/β inhibitory phosphorylation within the thrombus

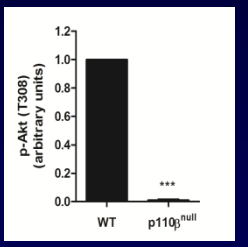


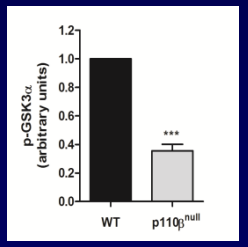


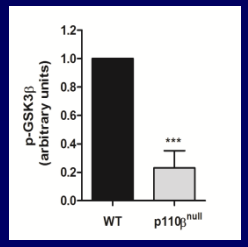


Quantification



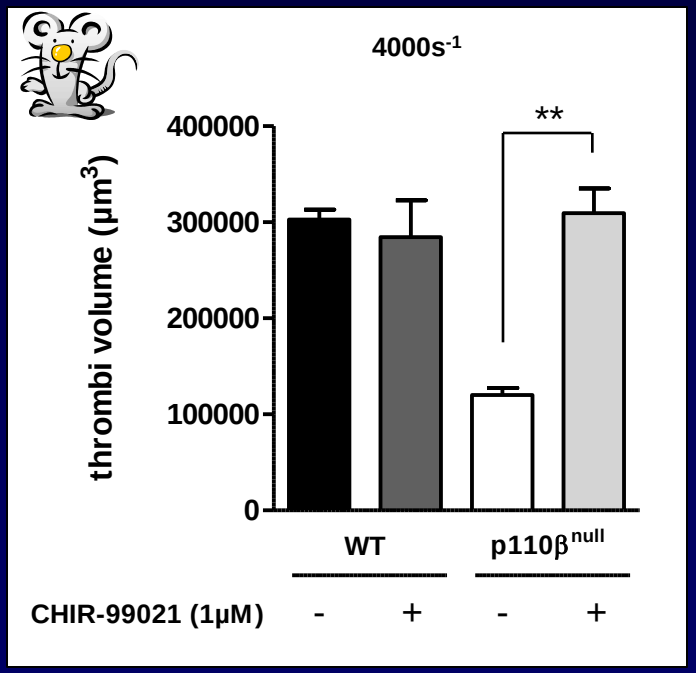


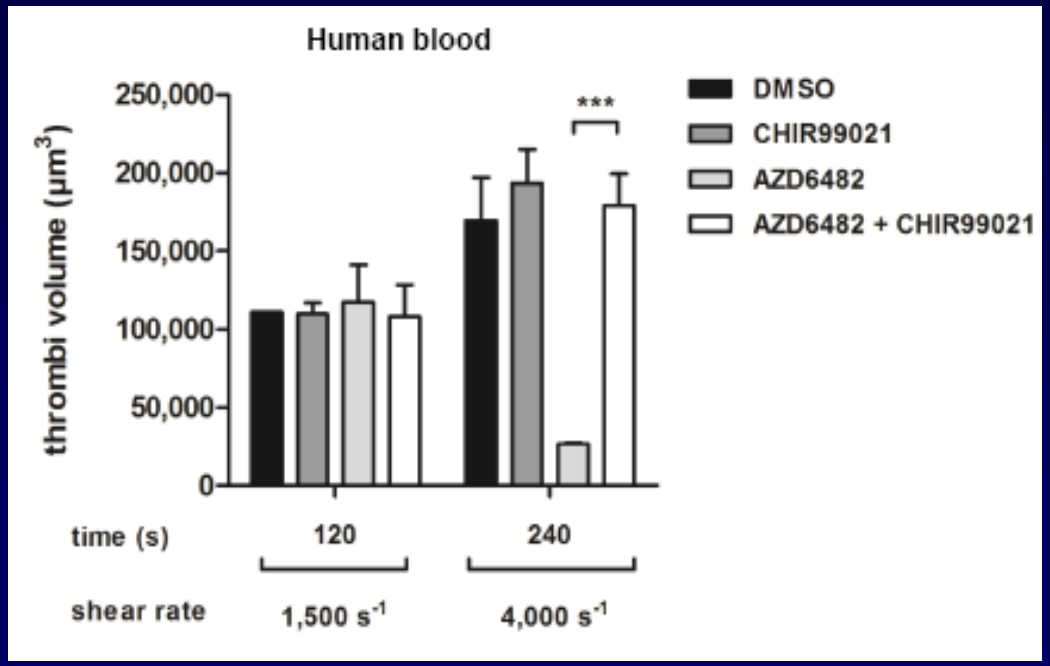




Inhibition of GSK3 restores thrombus stability of PI3Kβ null platelet mice under high shear stress

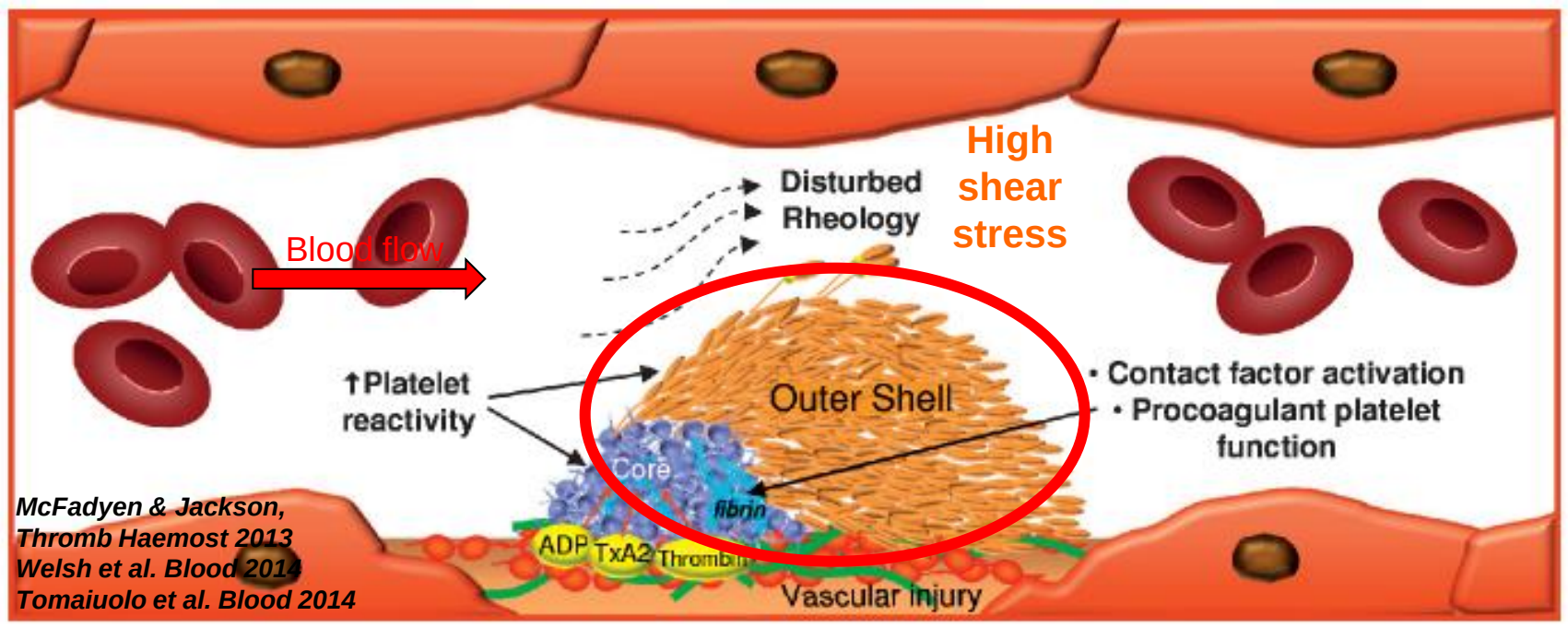
Thrombus formed at 500s^{-1} or 1500s^{-1} (2 min) → 4000s^{-1} (1 min) → Imaging/quantification





Laurent et al. Blood 2015

Thrombus growth and stability is a highly structured and dynamic process: role of PI3Kβ

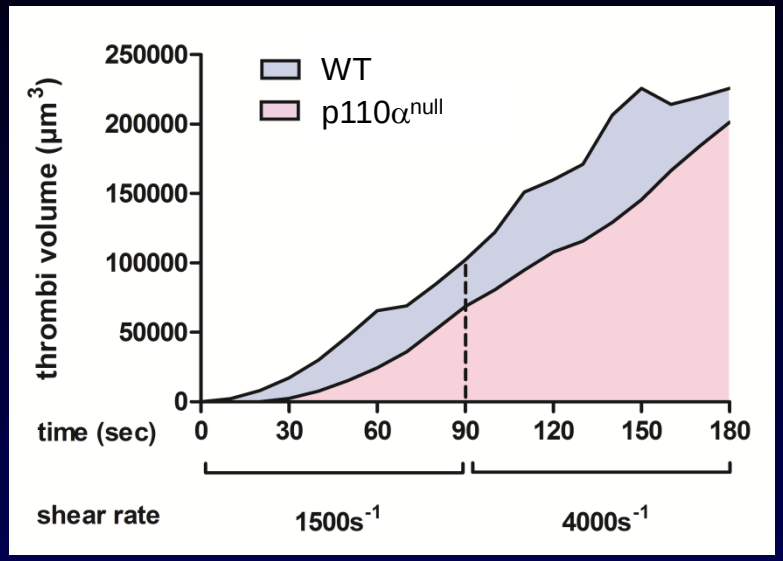


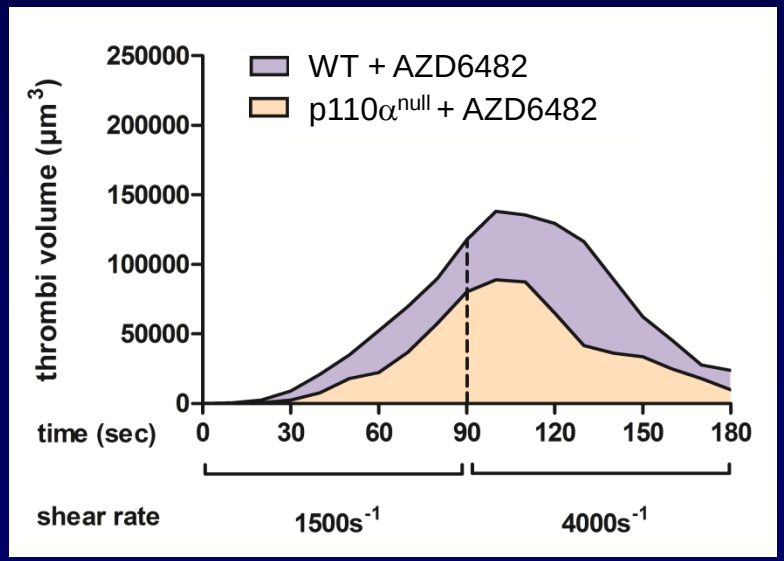
➡ PI3Kβ regulates thrombus stability at high (pathological) shear rate.

- PI3Kβ is involved in mechano-transduction /sensing and thrombus porosity

- Potential antithrombotic target : decrease the thrombotic risk without increasing the bleeding risk **but** possible distal embolization.

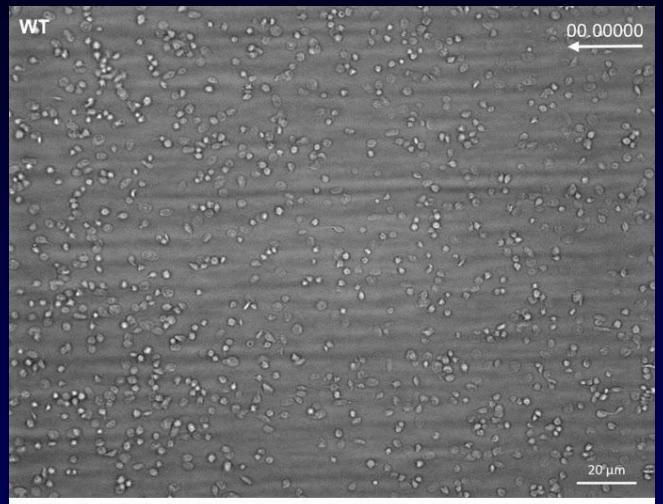
Are PI3Kα and PI3Kβ redundant in the regulation of platelet functions ?





Role of PI3Kα (but not PI3Kβ) in platelet rolling and adhesion on vWF *ex vivo*

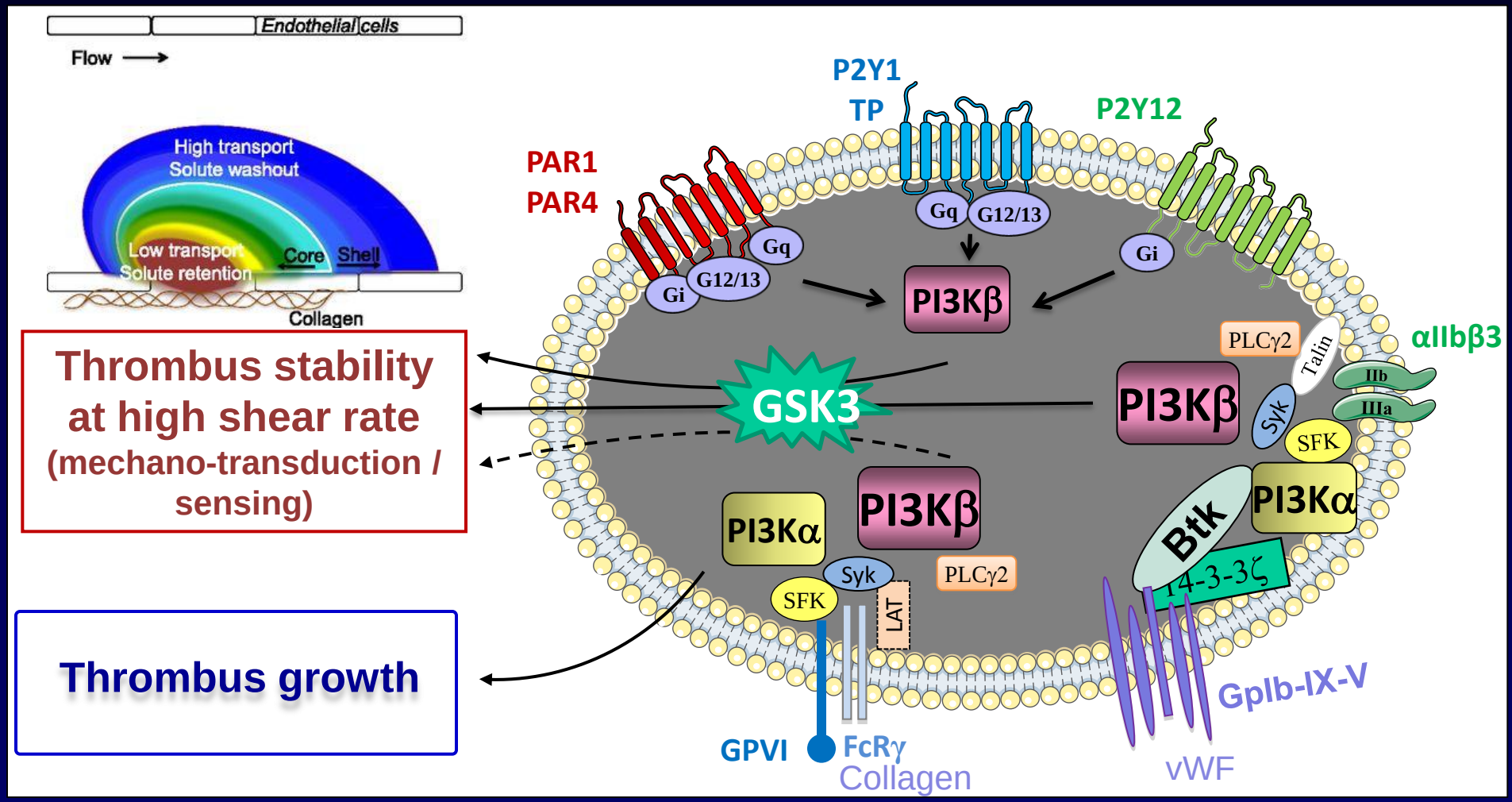
WT



p110α^null

Laurent et al. In preparation

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



Inserm U1048, I2MC, Toulouse, France

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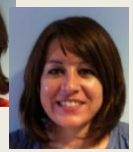
Laurie Bonnafe





Thanks

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Plateformes d'imagerie (TRI)

Plateforme de lipidomique (I2MC)

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- **Queen Mary University of London, UK**
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