Protein domains mediating interactions

W_03 • Protein - Protein interactions domains
Scaffolds and co-incidence
PPI's and networks

W_04 • Protein - Membrane
Lipid-binding domains
Manipulation of location
Activation

"the right molecules at the right place at the right time"

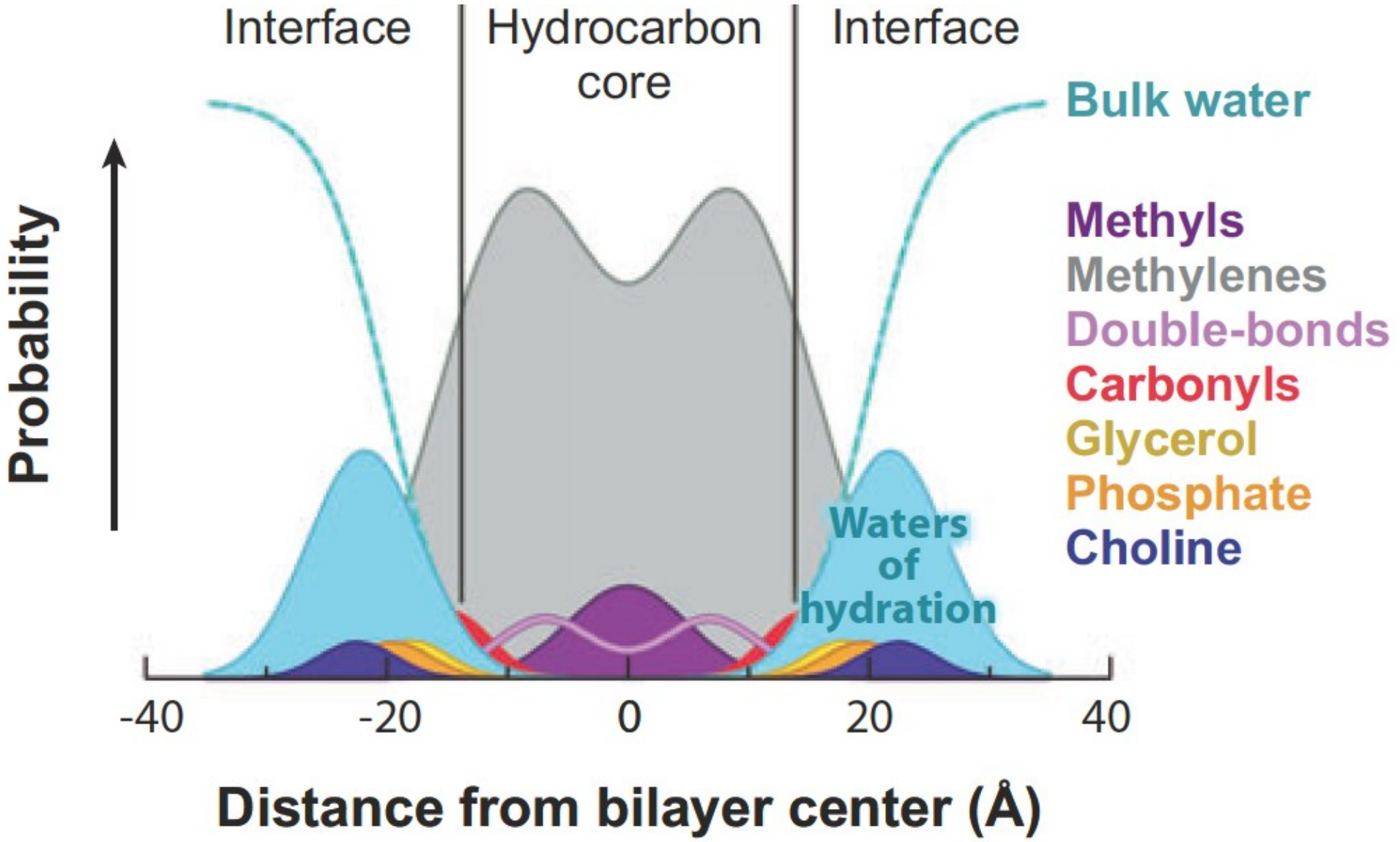
Part 1

Membrane binding domains

- PIP_x interacting domains
- PIP_x imaging
- Polybasic sequences

Membrane architecture

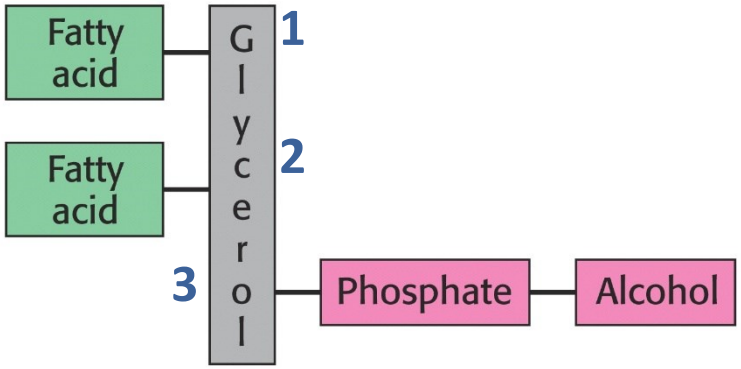
Depth probability profile of lipid functional groups

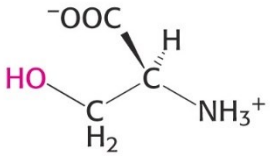


White & Heijne , Ann Rev Biophys 2008

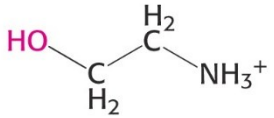
Membrane lipids

Phospholipids

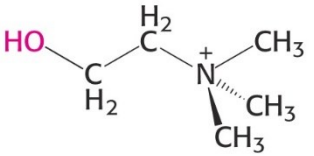




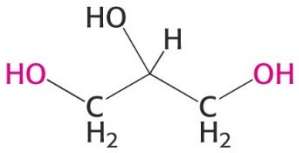
Serine



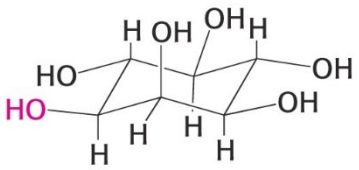
Ethanolamine



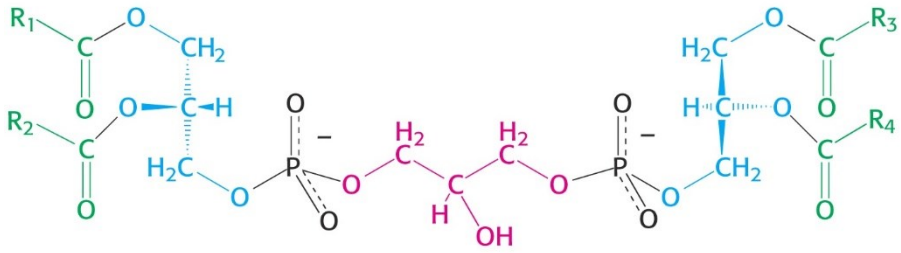
Choline



Glycerol

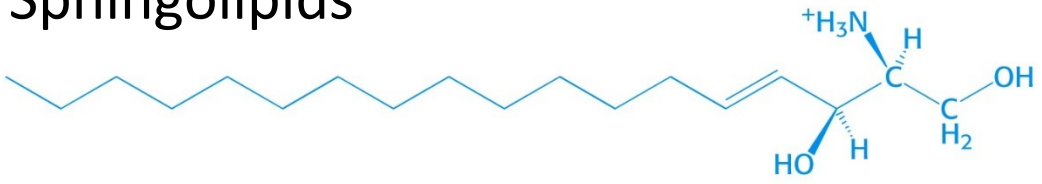


Inositol

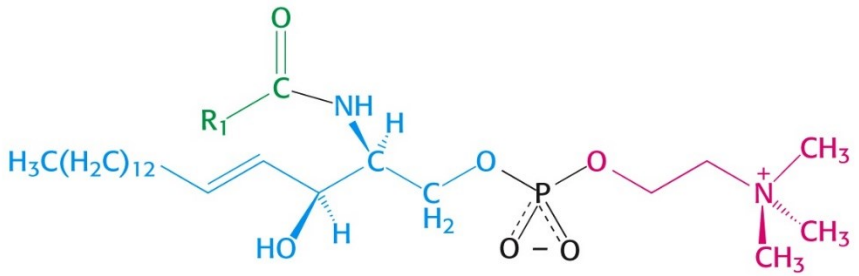


Diphosphatidyl glycerol (cardiolipin)

Sphingolipids

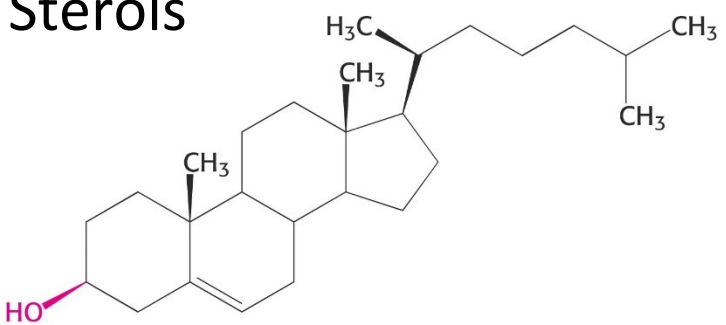


Sphingosine



Sphingomyelin

Sterols



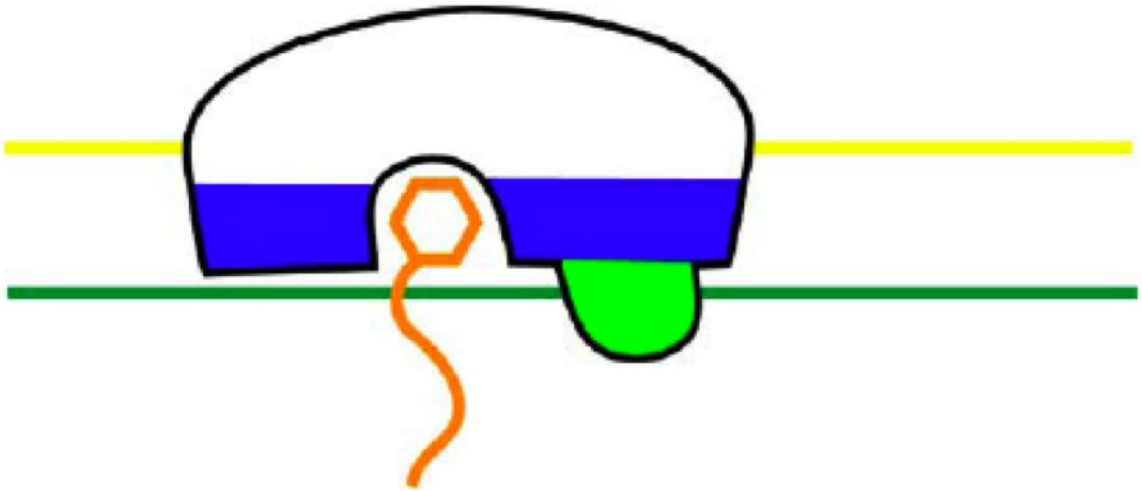
Cholesterol

Membrane binding domains

Schematic representation:

Membrane-water interface

Start hydrophobic core



Three interactions are generally observed:

- Polar stereo-selective binding site for a lipid head group
- Hydrophobic loop inserting in membrane core (green)
- Electrostatic interaction with polar head groups (blue)

Hurley, BBA 2007

Membrane binding domains

In human genome many such domains have been identified:

Domain*	Humans
PH	303 (258)
PKC C2‡	200 (125)
C1	79 (58)
PX	35 (35)
FYVE	27 (26)
Discoidin C2‡§	24 (18)
GRAM	18 (15)
F-BAR‖	14 (14)
Annexin	56 (13)
Gla§	13 (13)
N-BAR	9 (9)
ENTH/ANTH	9 (9)

=> PIPx

=> Ca^{2+}-dependent to phospholipids

=> diacylglycerol

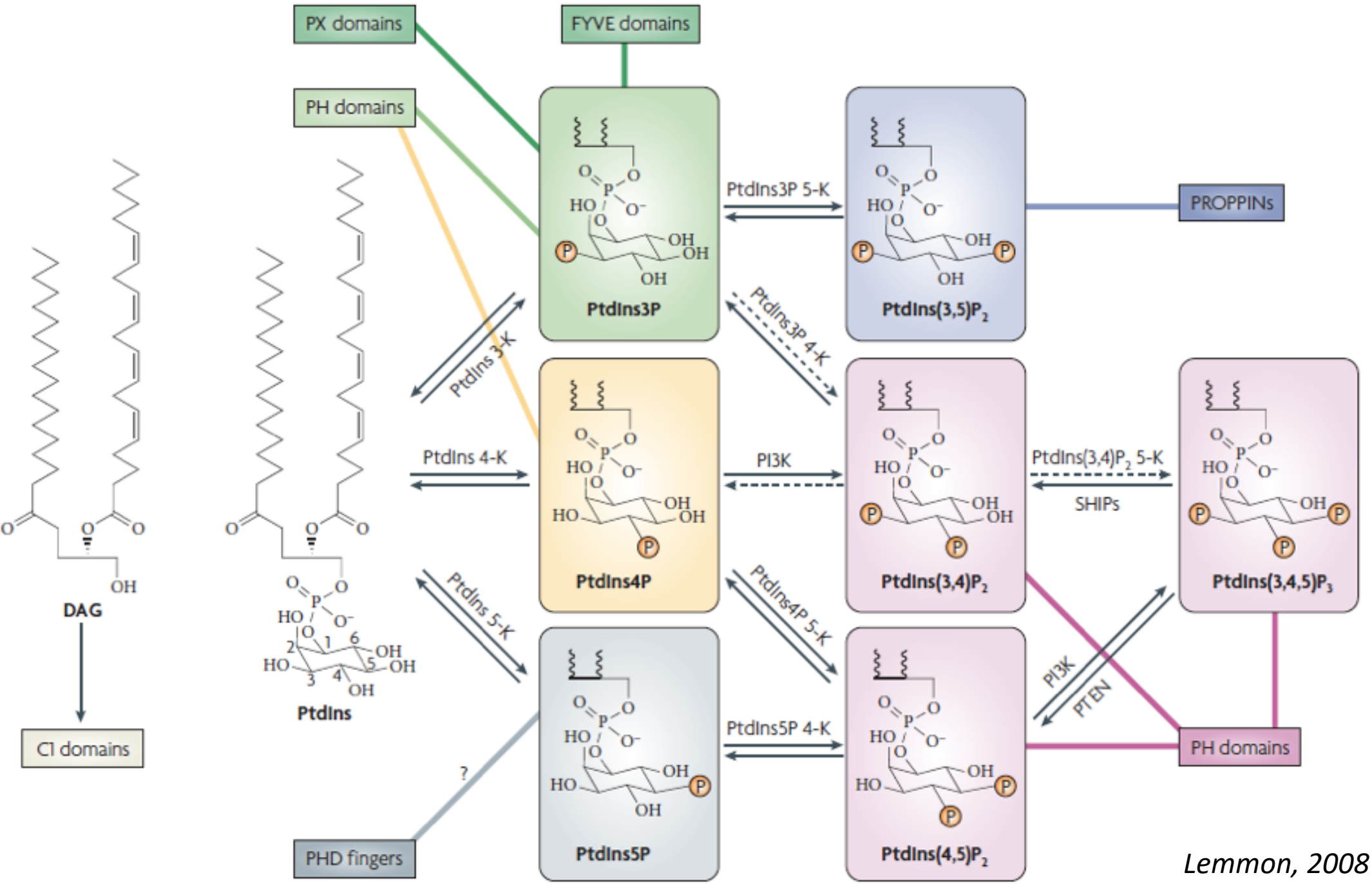
=> PI(3)P

=> PI(3)P

(#) : number of proteins

Lemmon, 2008

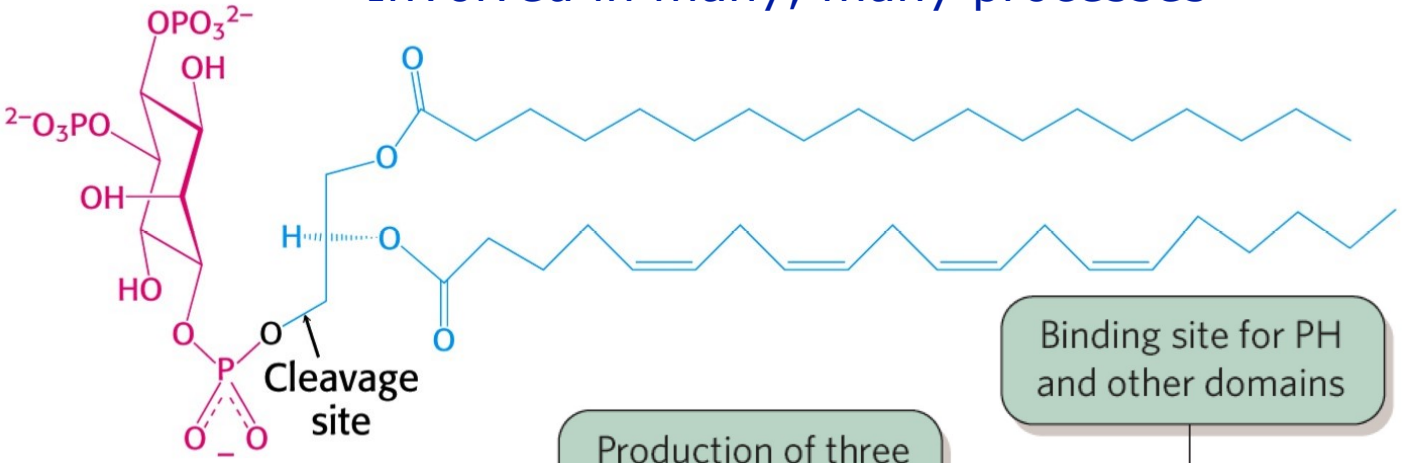
Domains recognising different PIP_x species

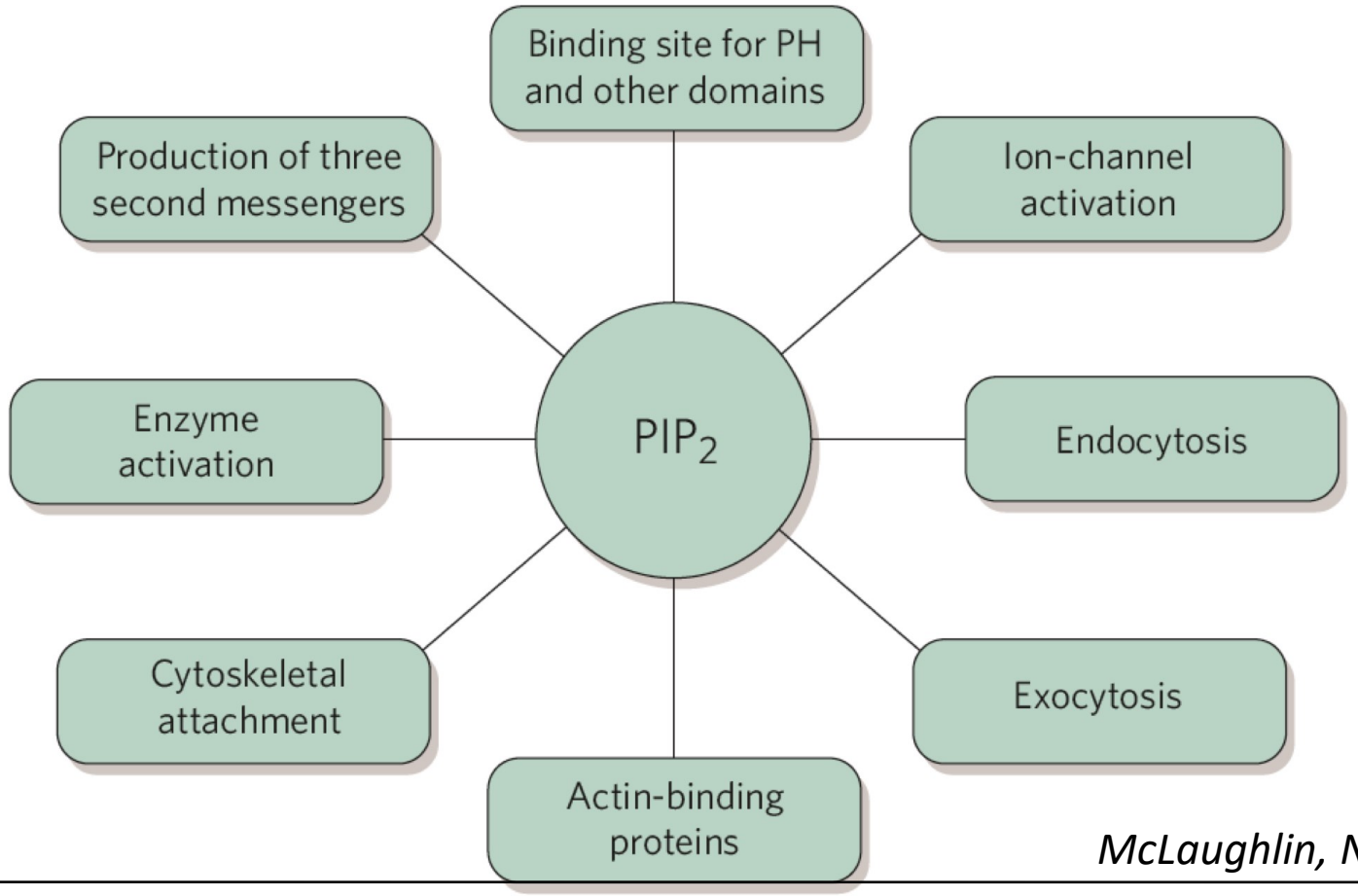


Lemmon, 2008

Phosphatidylinositol-(4,5)-bisphosphate (PIP_2)

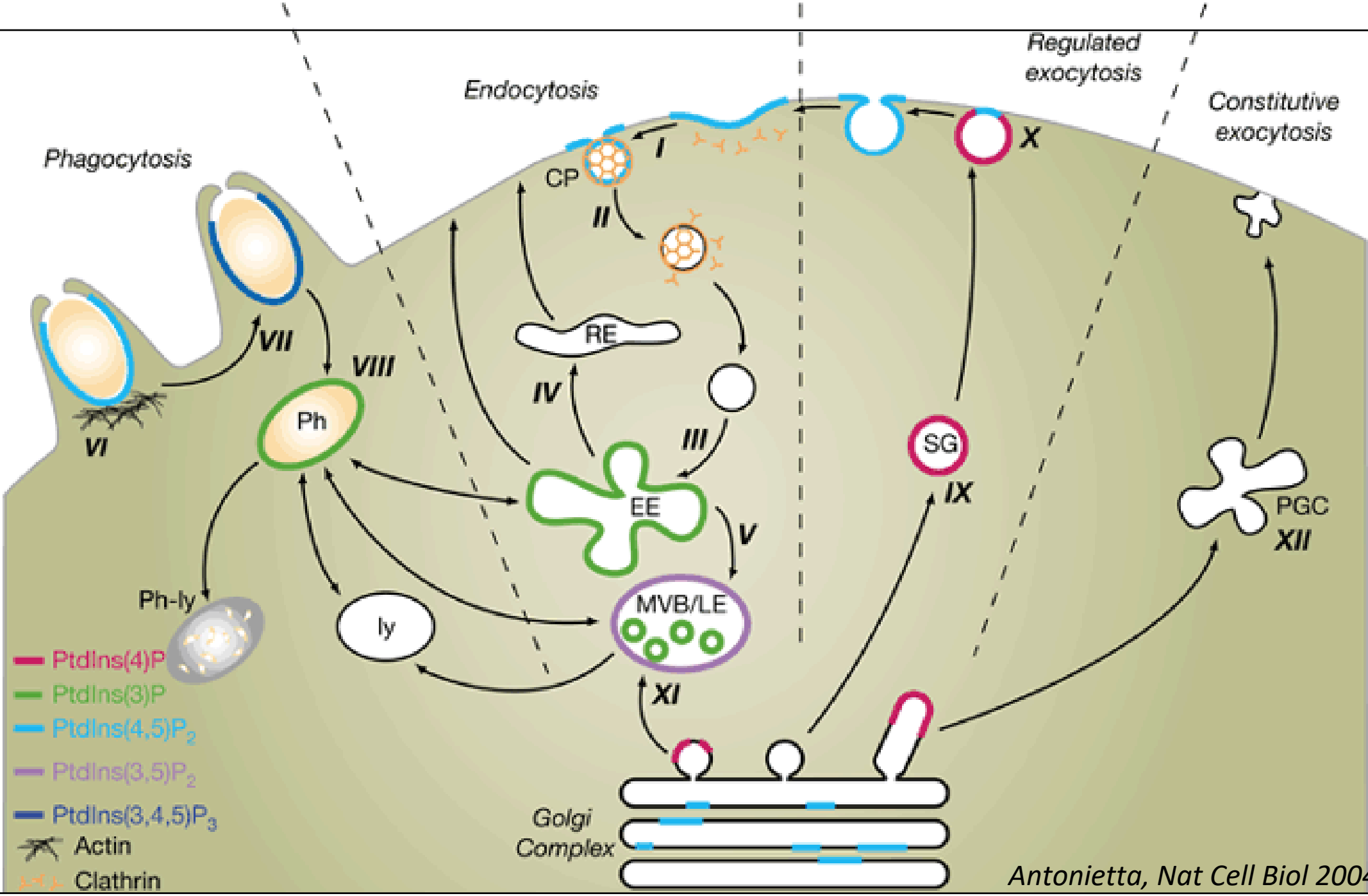
- About 1% of PM lipids (about 1-10 μM); the most abundant of the PIP_x
- Involved in many, many processes





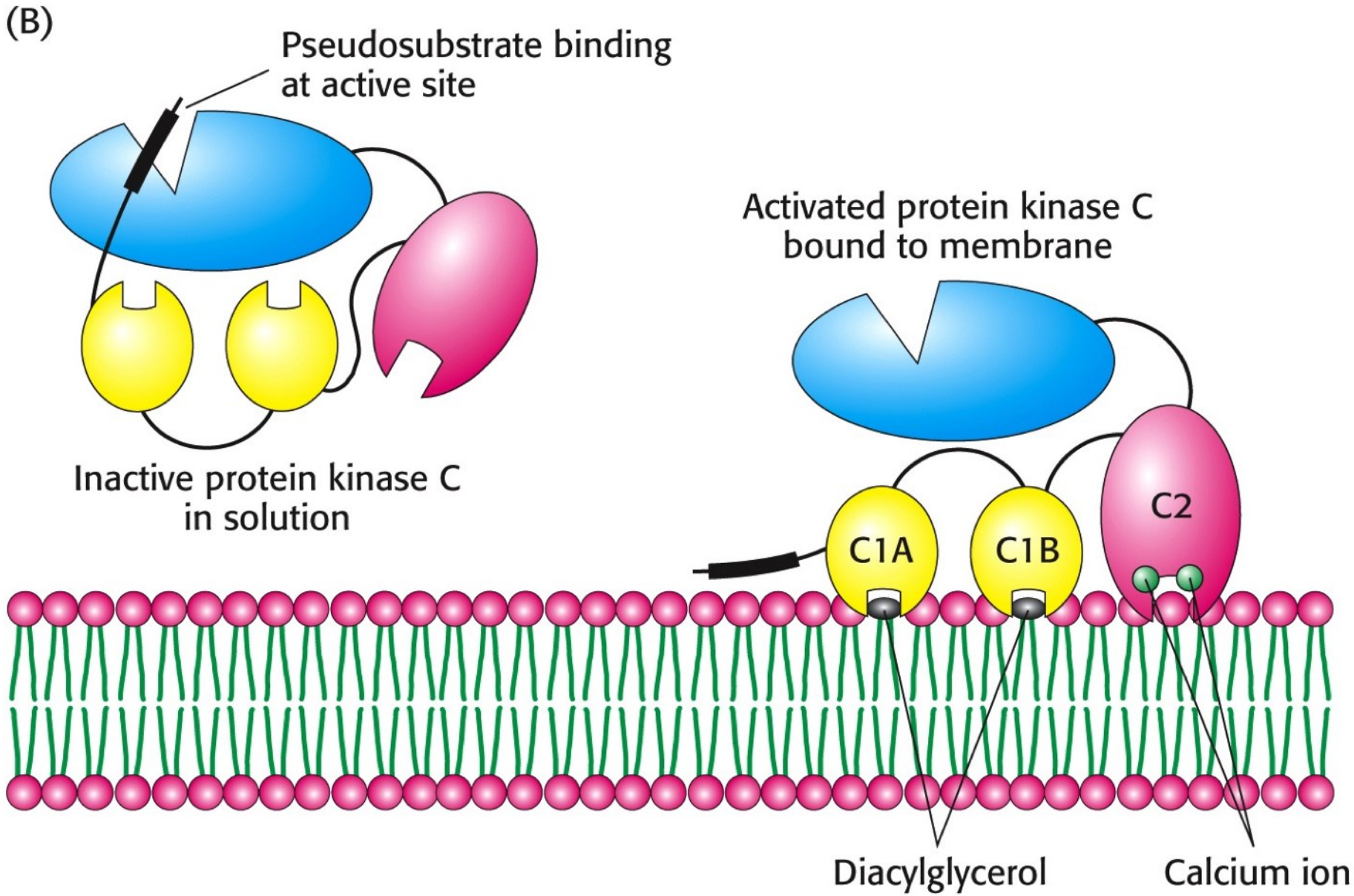
McLaughlin, Nature 2005

PIP_x: Specific locations



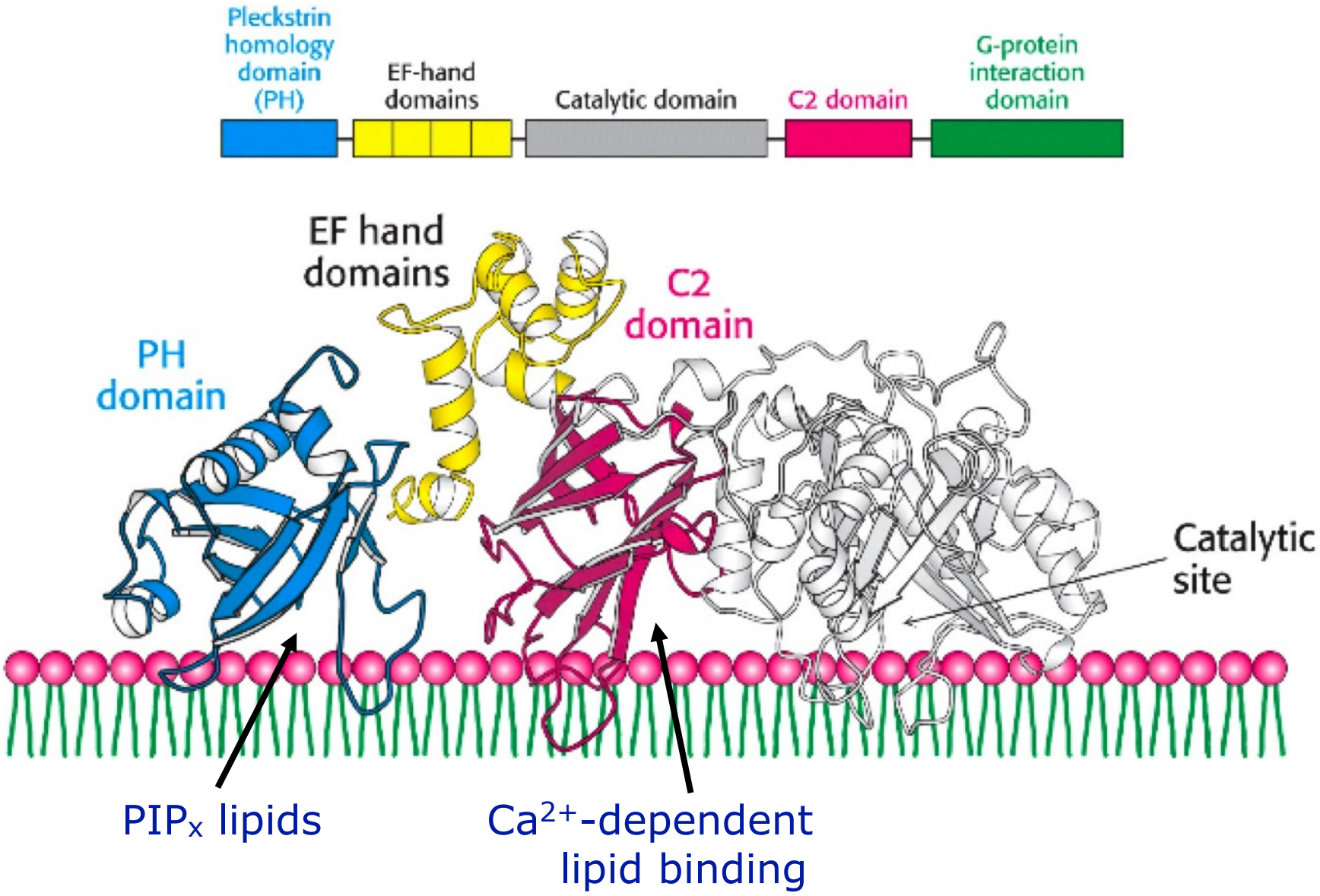
Reversible membrane location and activation

Protein kinase C combines the 2 mechanisms, both for localisation & activation:



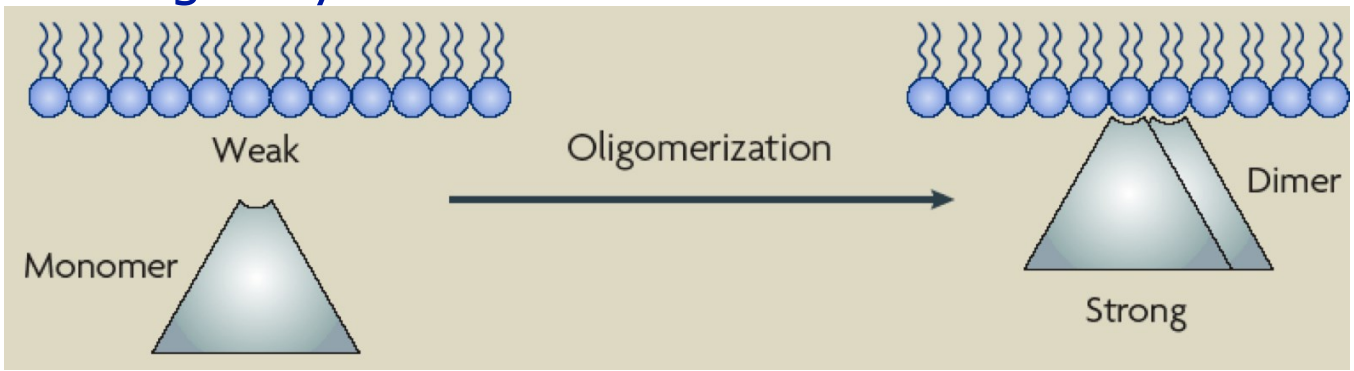
Reversible membrane location and activation

Phospholipase Cβ combines the 2 mechanisms, both for localisation & activation:
but also for it's **in-activation**!!

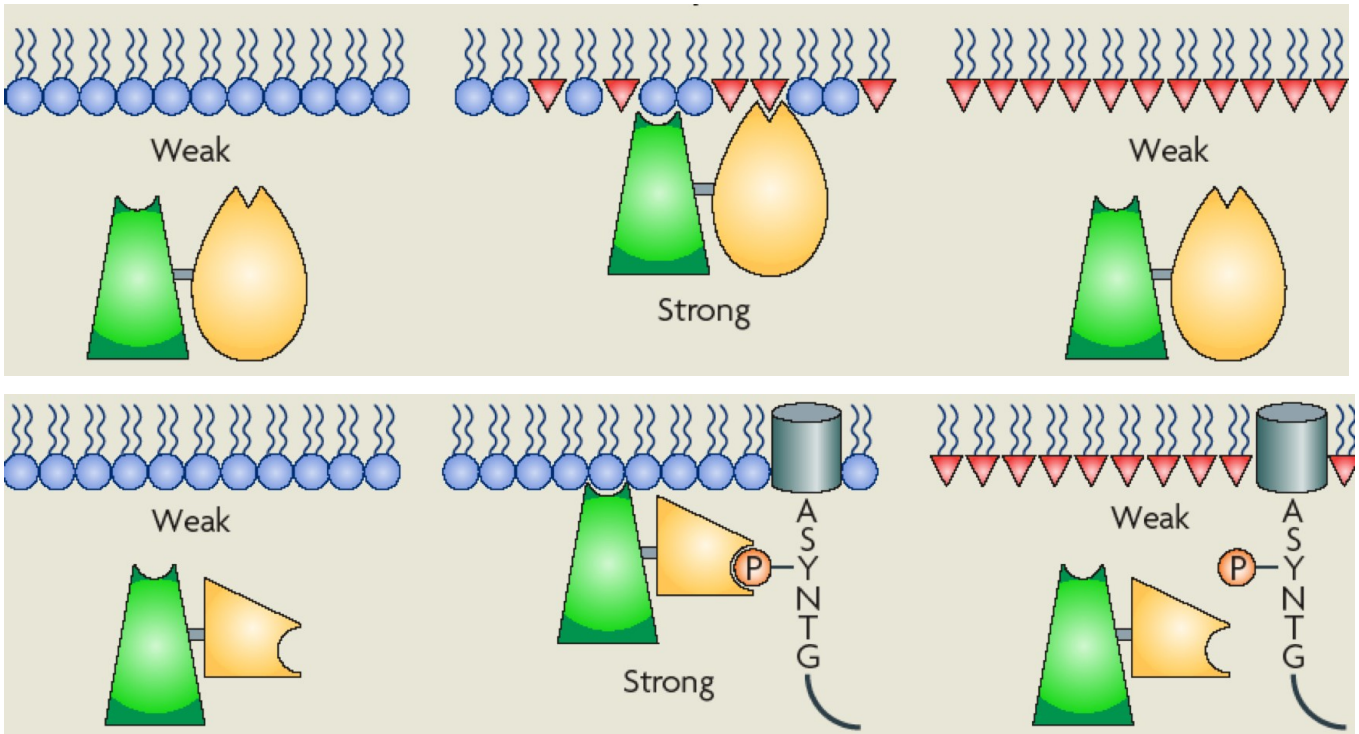


Membrane binding : Combination of domains

=> Stronger by more of the same



=> => Stronger & more selective/specific through **co-incidence**



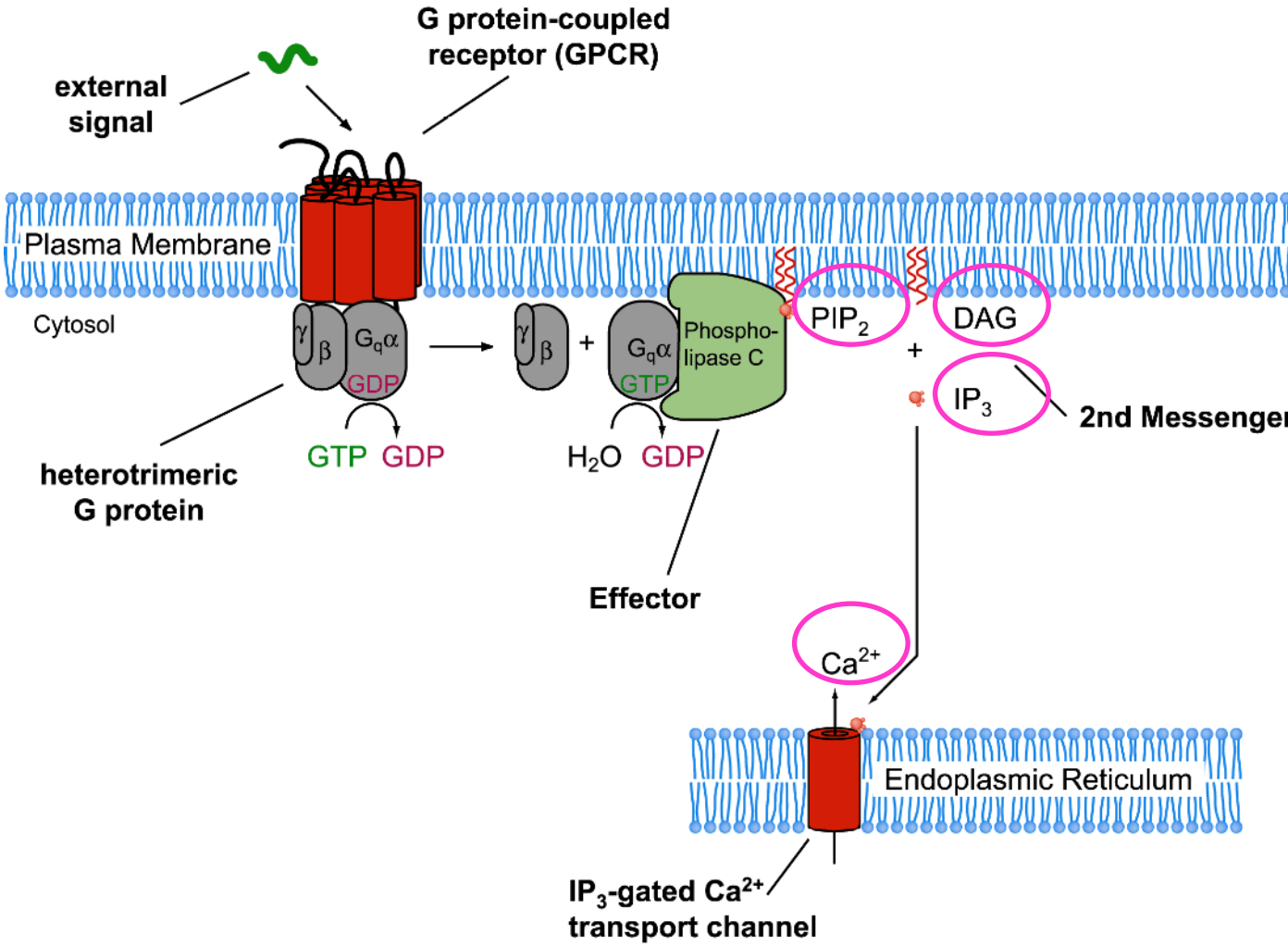
Lemmon, 2008

PIP_x undergo signal-dependent turnover; e.g. $PI(4,5)P_2$

GPCR signalling via $G_{\alpha q}$:

=> PLC activation

$PI(4,5)P_2$ => DAG + IP_3

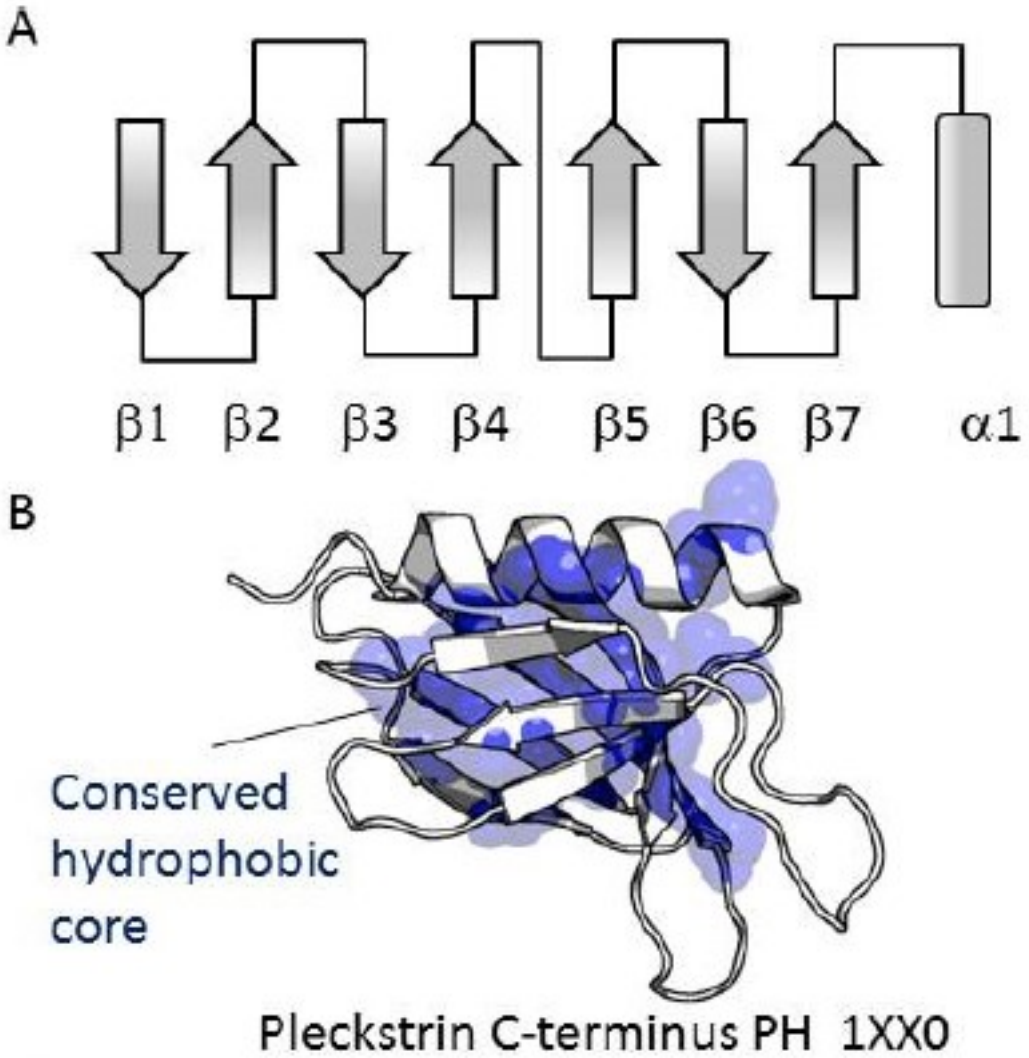


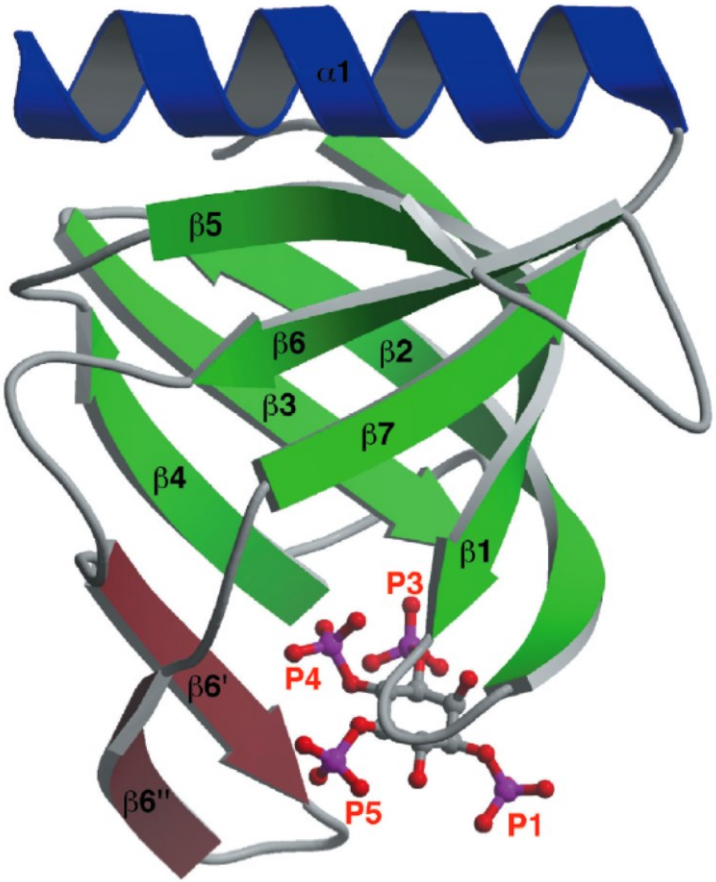
IP_3 = Inositol(1,4,5) triphosphate

DAG = Diacylglycerol

PLC = Phospholipase C

Pleckstrin Homology (PH) domains: Common Structure





Lenoir et al, Membranes 2015

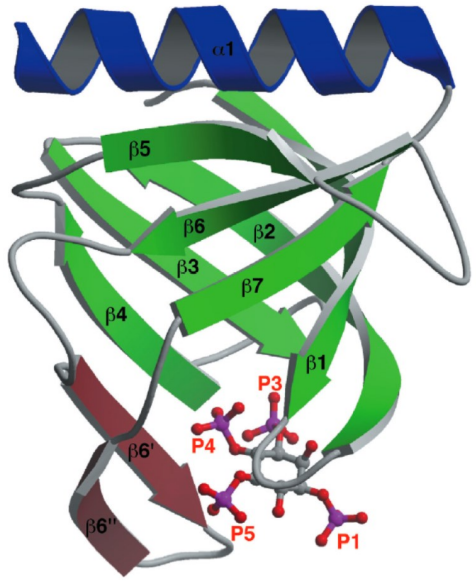
PH domains recognising PI(3,4,5)P_3

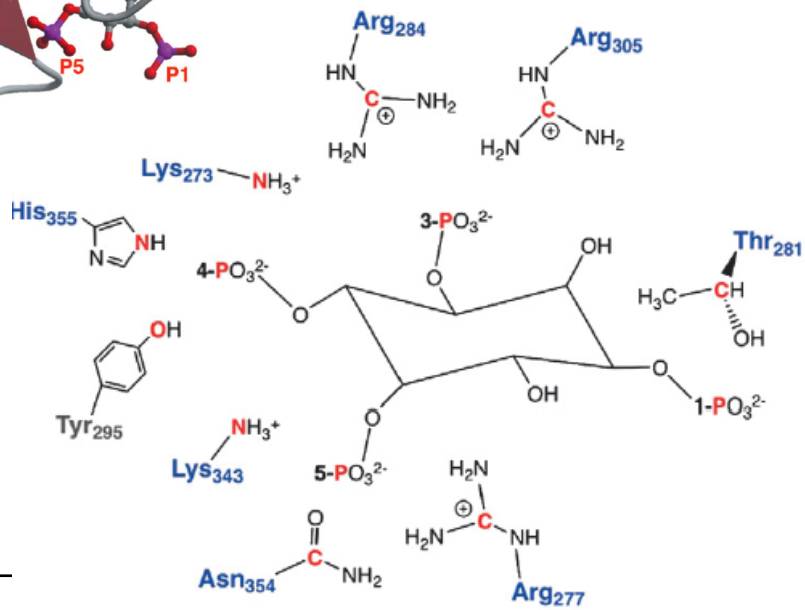
- PI(3,4,5)P_3 has a very low abundance in resting cells
  - upon activation 40-fold increase due to phosphorylation of PI(4,5)P_2 by PI-3-kinase

=> see : 1fgy.pdb

Important regions for interactions:

- β 1-β2 loop is a basic region
- 17-19 H-bonds
- Large hydrophobic loop absent
- K_d = 27 nM => strong enough for firm membrane attachment
- I(1,3,4,5)P_4 binds with same affinity



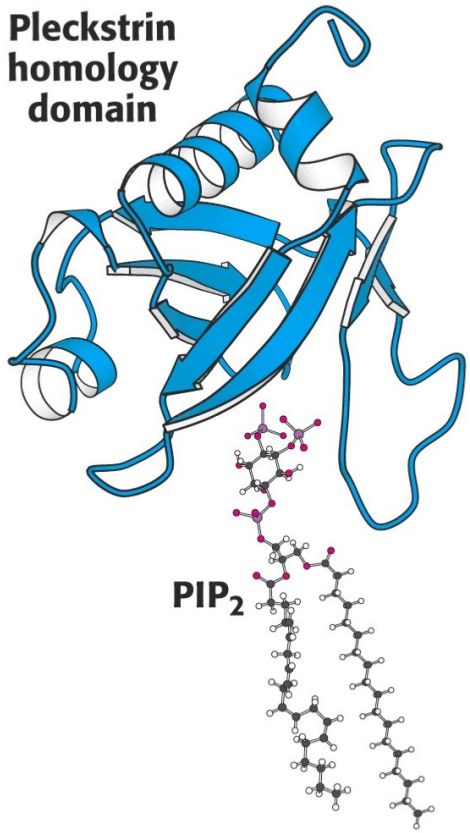


Domains recognising PI(4,5)P_2

- PI(4,5)P_2
  - most abundant PIP_x
    about 1% of plasma membrane PL's
    5 to 100-fold more abundant than other PIP_x's

  - most domains bind with low affinity

  => see : 1mai.pdb



An exception:

- PH domain of phospholipase-Cδ1 binds
  with high affinity, however:
    affinity for I(1,4,5)P_3 is 10-fold higher

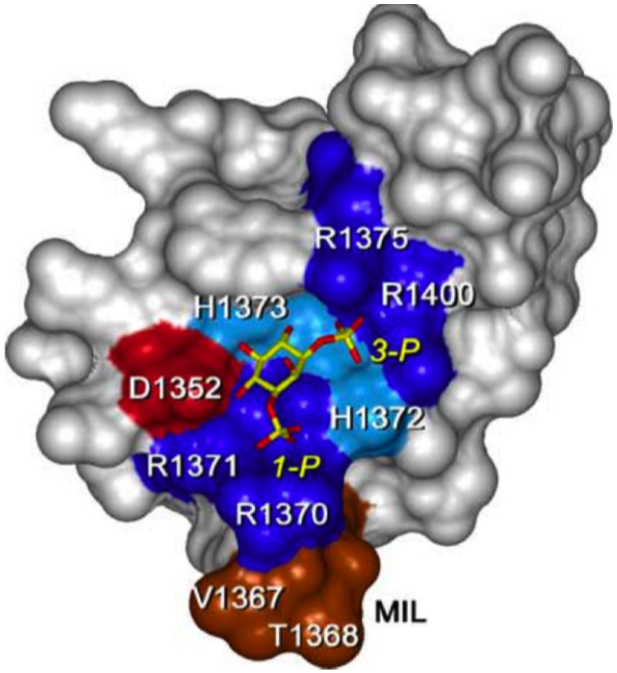
=> The enzyme is removed from the membrane by it's own product.

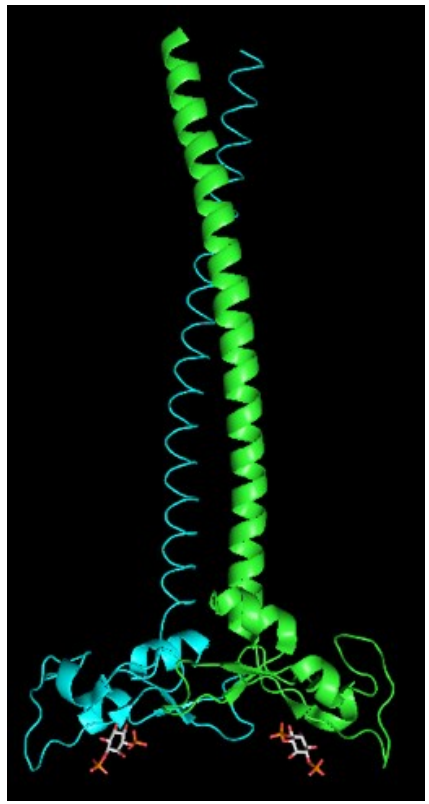
FYVE domains recognising PI(3)P_1

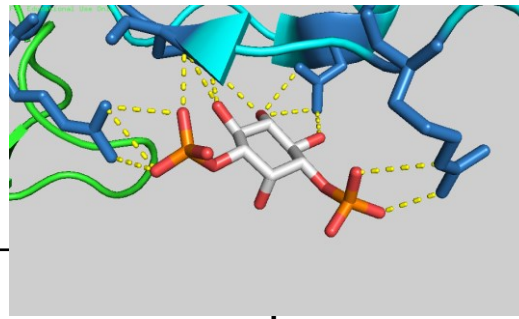
- PI(3)P_1 present in endosomes, 0.25 % of cellular lipid, or 200 µM
  - important in regulating trafficking in endosomal pathway and Golgi-lysosome sorting

◇ FYVE domains : Important for interaction with PI(3)P:

- basic (R/K)(R/K)HHCR in 1st β-strand } => selectivity
- 12 H-bonds with P_i and OH groups
- Monomer too weak for membrane attachment
  => dimers do !!



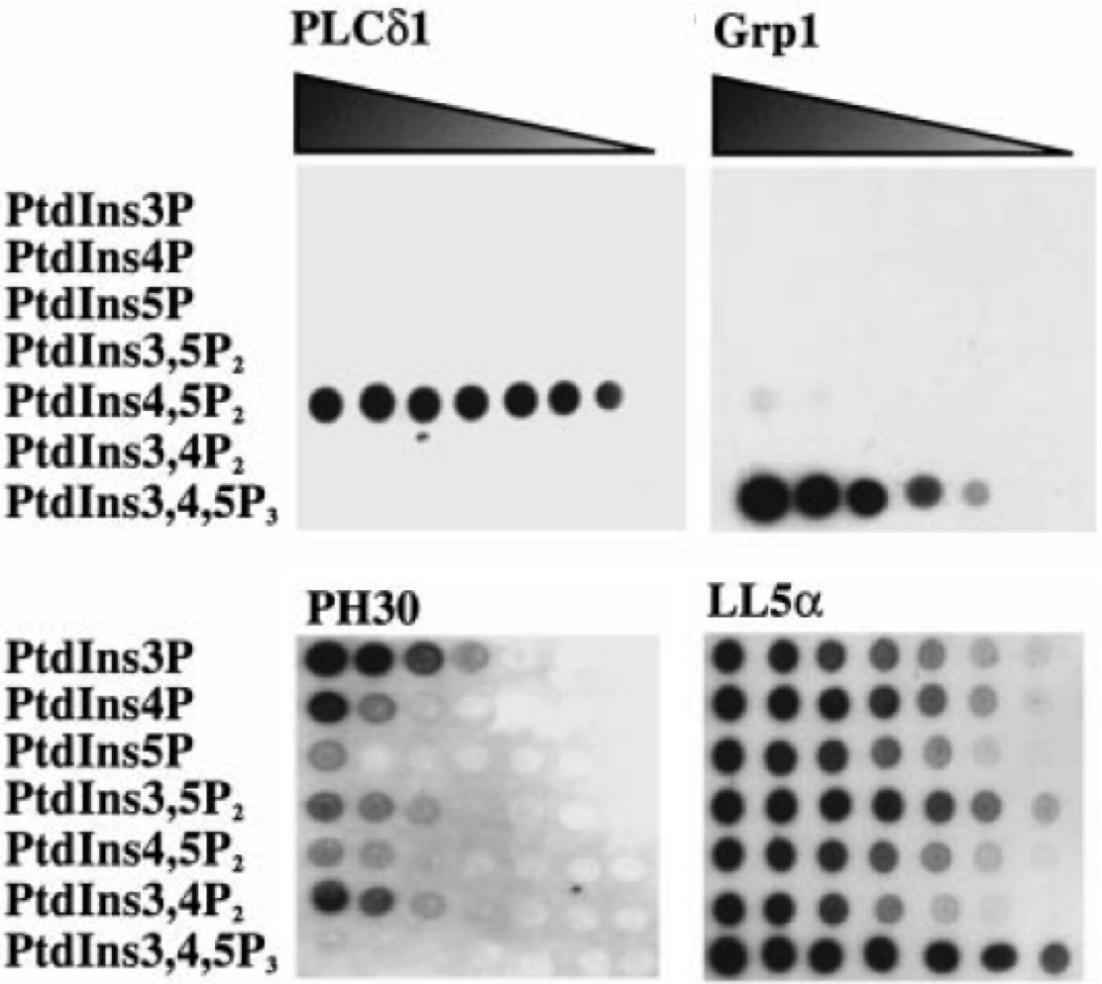




Testing specificity and affinity of PIP_x domains

"Dot blot assay"

- Spot the different lipids on nitrocellulose
- Overlay with PH-domain-GST fusion proteins as dilution series
- Detect bound fusion with an anti-GST antibody



Dowler, Biochem J 2000

GST = Glutathion-S-Transferase
an affinity tag
for purification

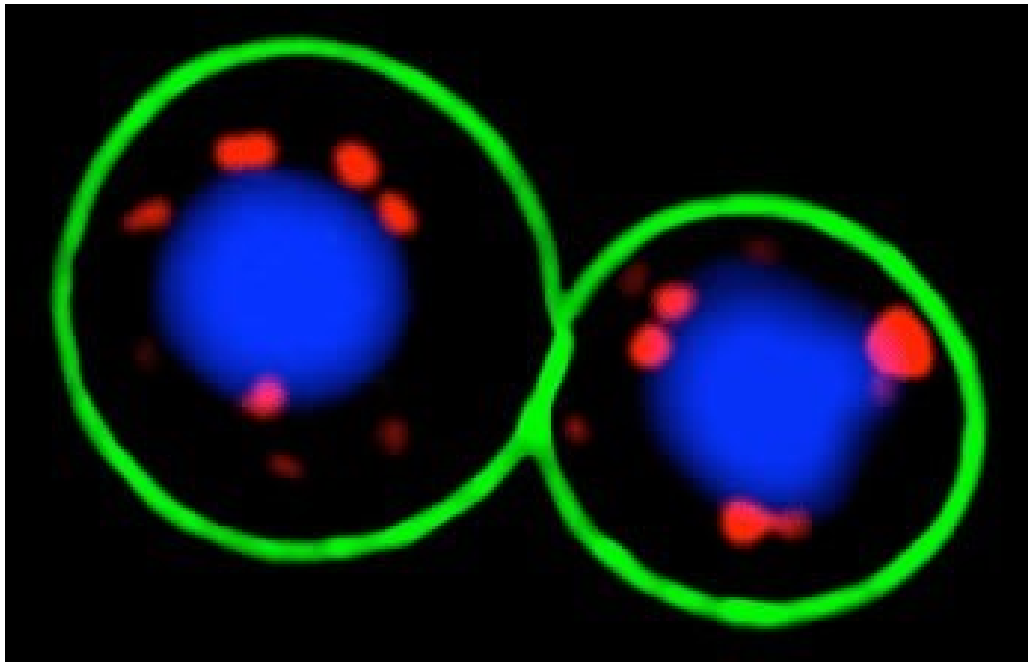
Imaging PIP_x *in vivo* using domain-GFP chimeras

In yeast cells:

Green: PH-domain - GFP fusion => $PI(4,5)P_2$ in plasma membrane

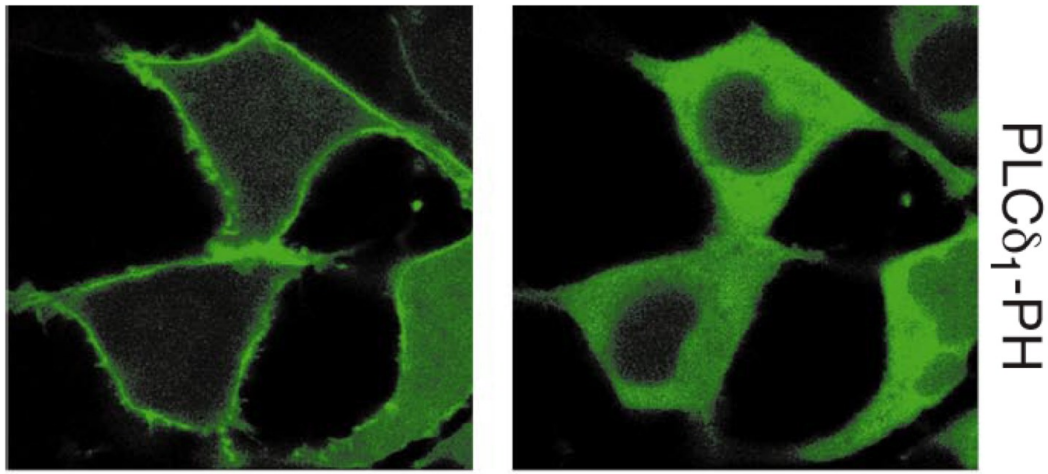
Red: FYVE-domain - DsRed fusion => PI(3)P in endosomes

Blue: Vacuoles



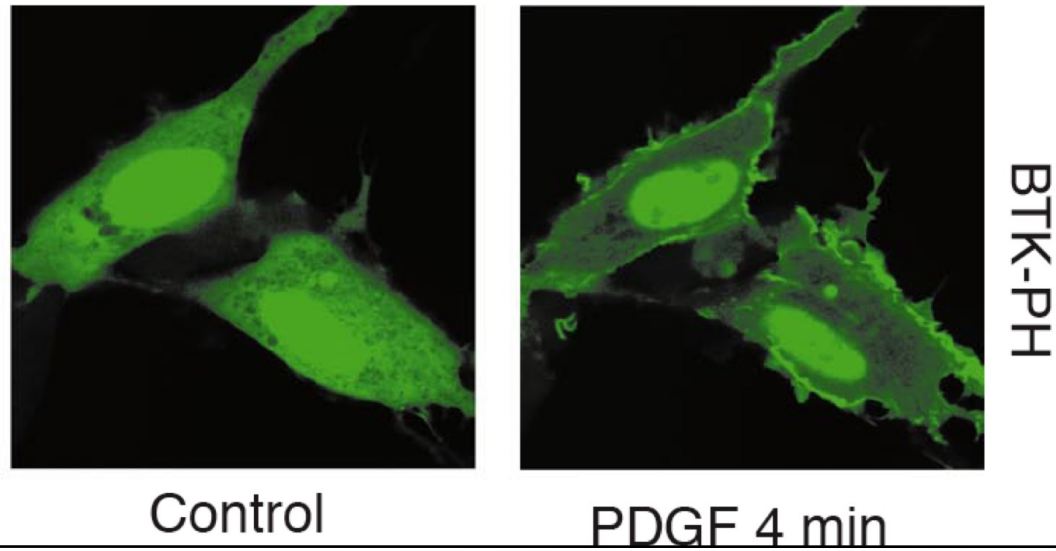
Imaging PIP_x metabolism *in vivo* using PH-GFP chimeras

In mammalian cells:
- If target lipid is present => PH-GFP probe on membrane
- If not: => probe in cytosol



A: PI(4,5)P_2-specific probe
Control: PI(4,5)P_2 in plasma membrane

+ Angiotensin II (AngII) promotes breakdown via PLase-C



B: PI(3,4,5)P_3-specific probe
Control: little PI(3,4,5)P_3 in plasma membrane

+ PDGF stimulate synthesis by activation of PI-3-Kinase

Balla, TiPS 2000

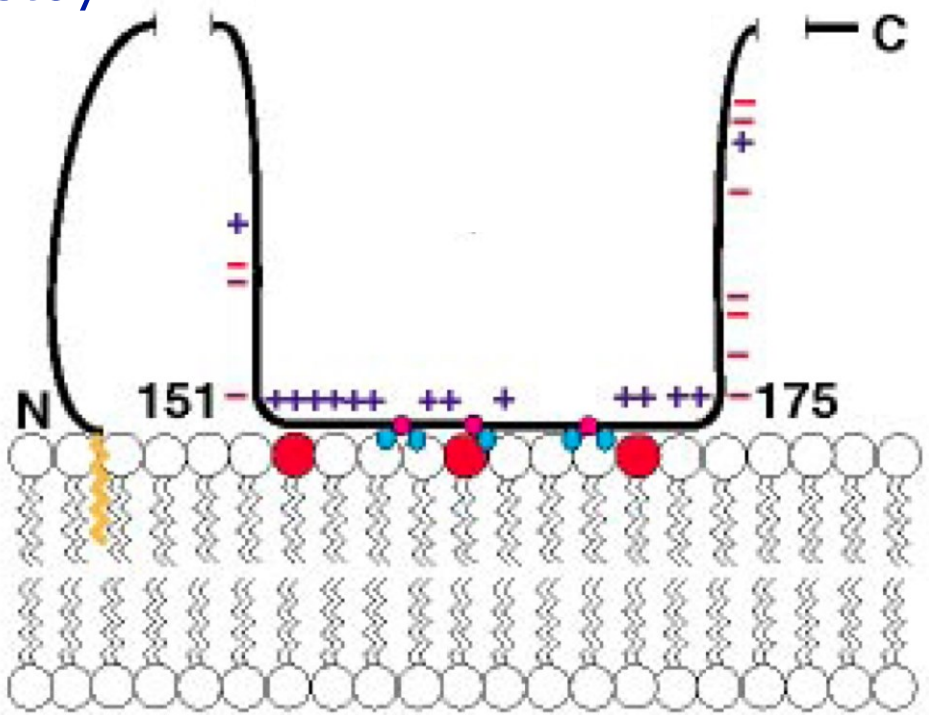
Poly-basic sequence: Membranes, PIP_2 and Ca^{2+2}

Poly-basic sequence: A short stretch of amino acids with many K and/or R

- Bind strongly to negatively charged membranes
- Sequester PIP_2

E.g. MARCKS

- " **M**yristoylated **A**lanine-**R**ich **C**-**K**inase **S**ubstrate"
- Present in the cell at μM concentrations
- Polybasic domain …**KKKKKR**FSF**KK**SF**K**LSGFSF**KK**N**KK**
- N-terminal myristoyl



red dot = PIP_2

McLaughlin, Nature 2005

Poly-basic sequence: Release from membrane

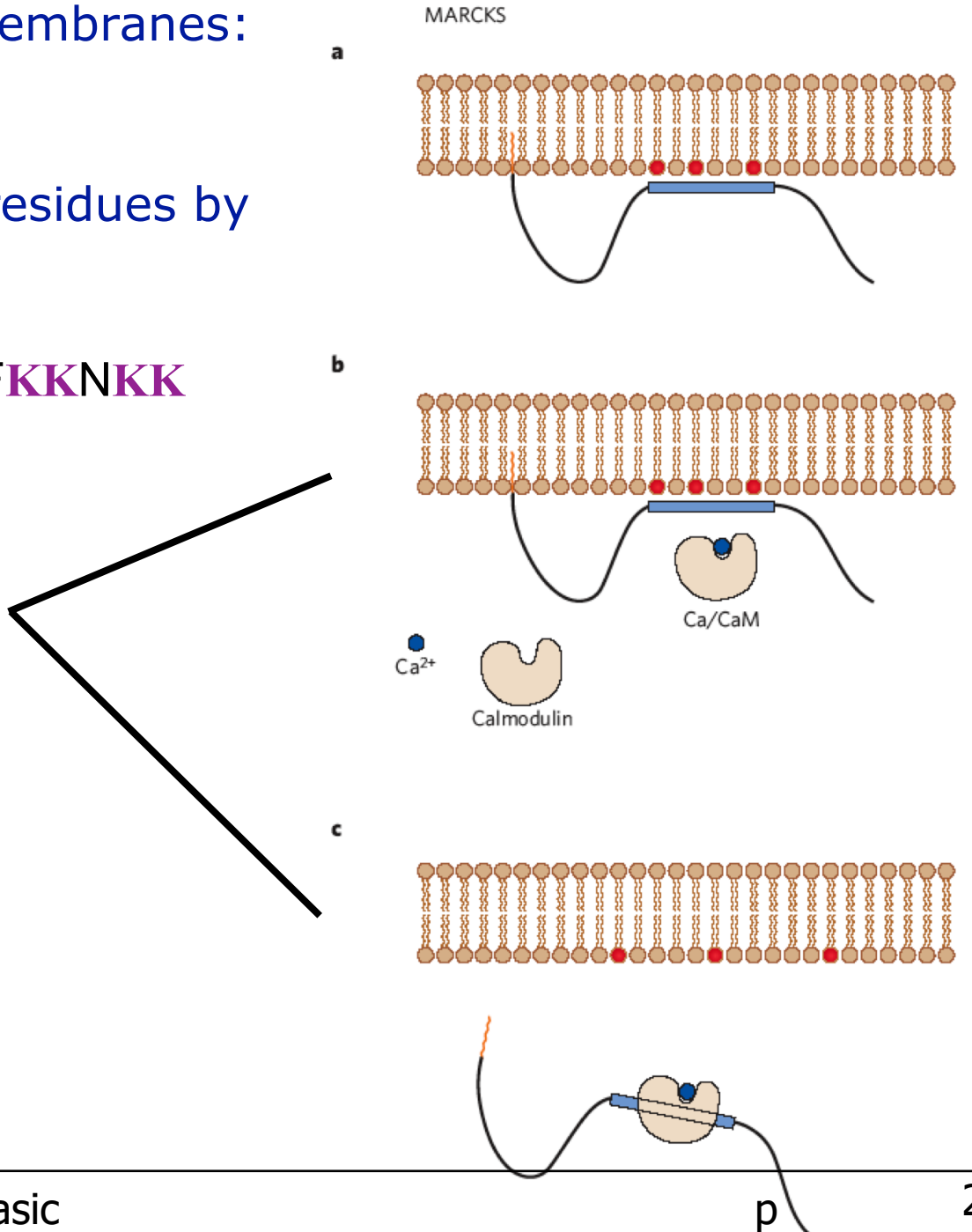
Reduction of MARCKS binding to membranes:

i) Hydrolysis of PIP_2

ii) Phosphorylation of serine **S** residues by protein kinase C (PK-C)

KKKKKRF**S**F**KK**S**K**L**S**GF**S**F**KK**NKK**

iii) Binding of calmodulin-Ca^{2+}



McLaughlin, Nature 2005.

Calcium, a ubiquitous messenger

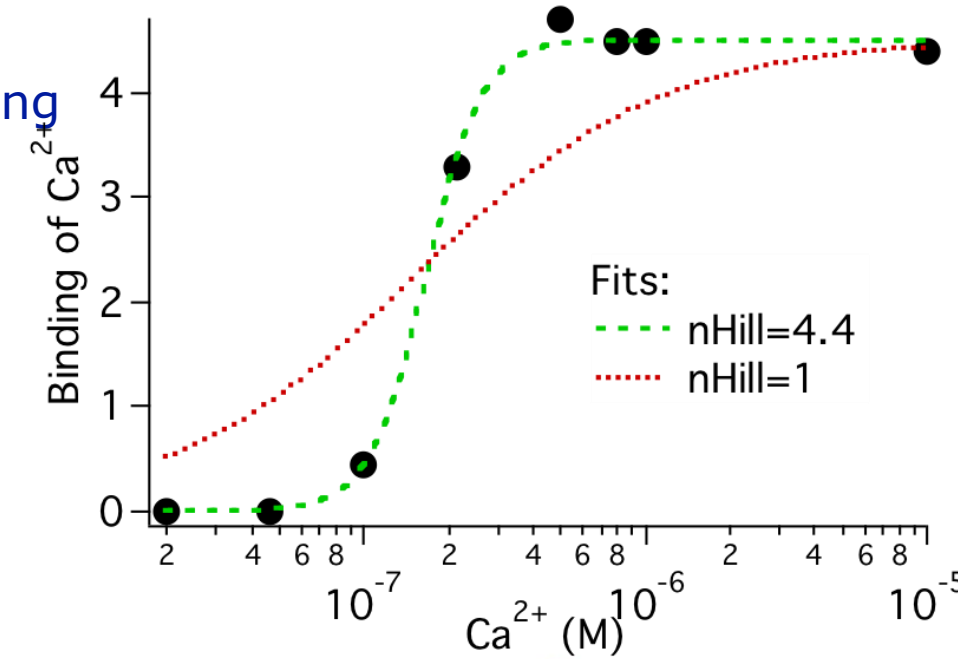
iii) Through a mediator, e.g. calmodulin:

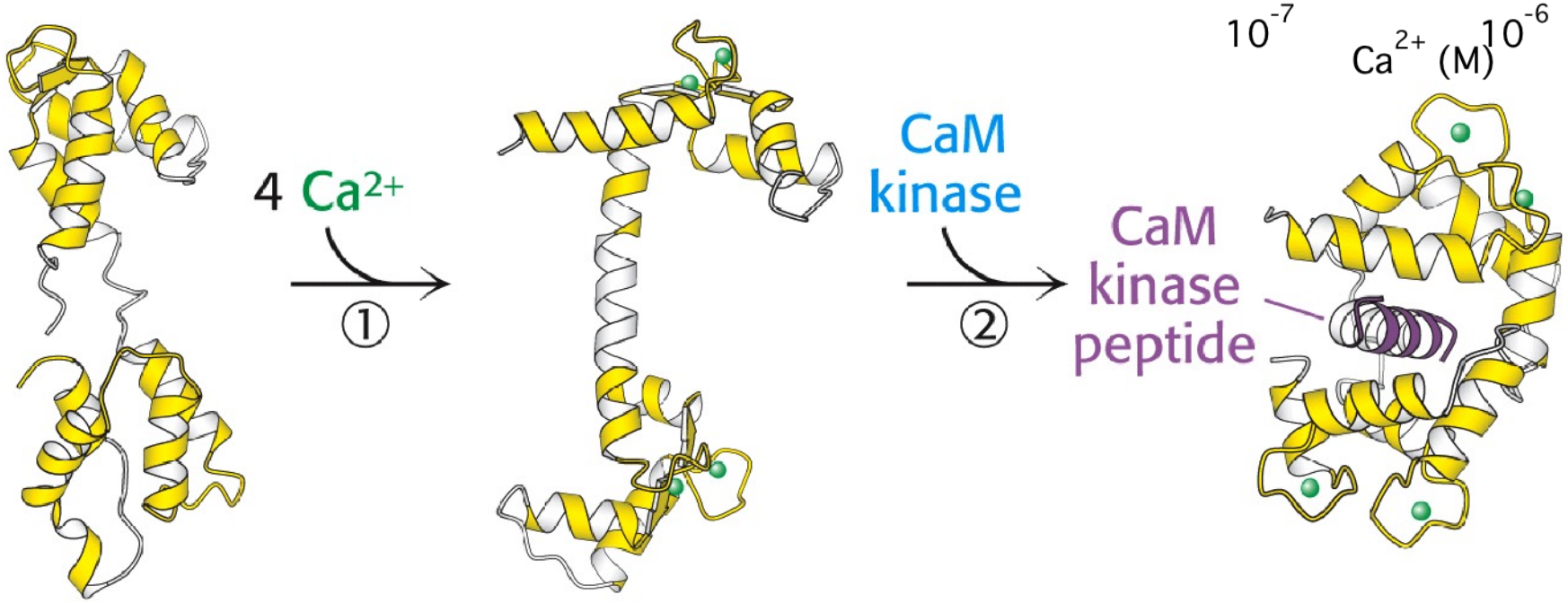
[Ca^{2+}] > 500 nM => steep cooperative binding

=> dramatic structural change

==> binding to calmodulin targets

===> modulation of target's activity





Calmodulin (apo)

Poly-basic sequence: Release from membrane

Regulation of MARCKS binding to membranes:

- Hydrolysis of PIP_2
- Phosphorylation of Ser
- Binding of calmodulin-Ca^{2+}

Crystal structure of complex o

calmodulin-MARCKS

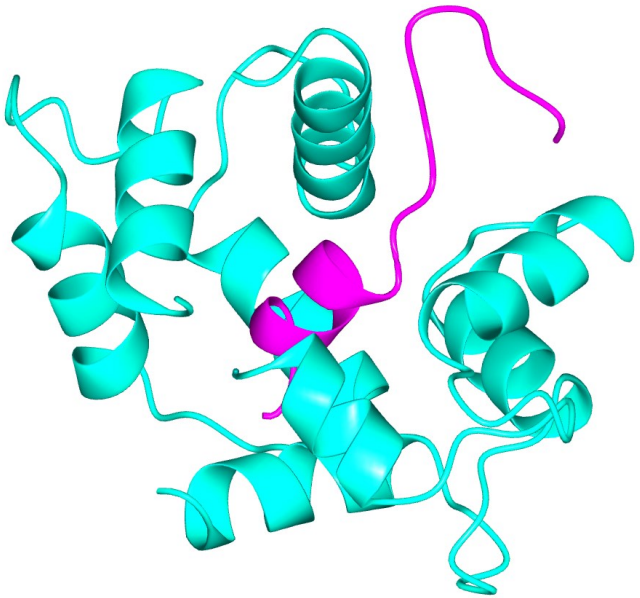
cartoon:

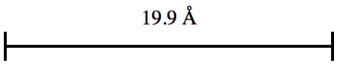
- cyan: calmodulin
- purple: MARCKS

Iso-potential surface:

- red -25 mV
- blue + 25 mV

=> see 1iwq.pdb



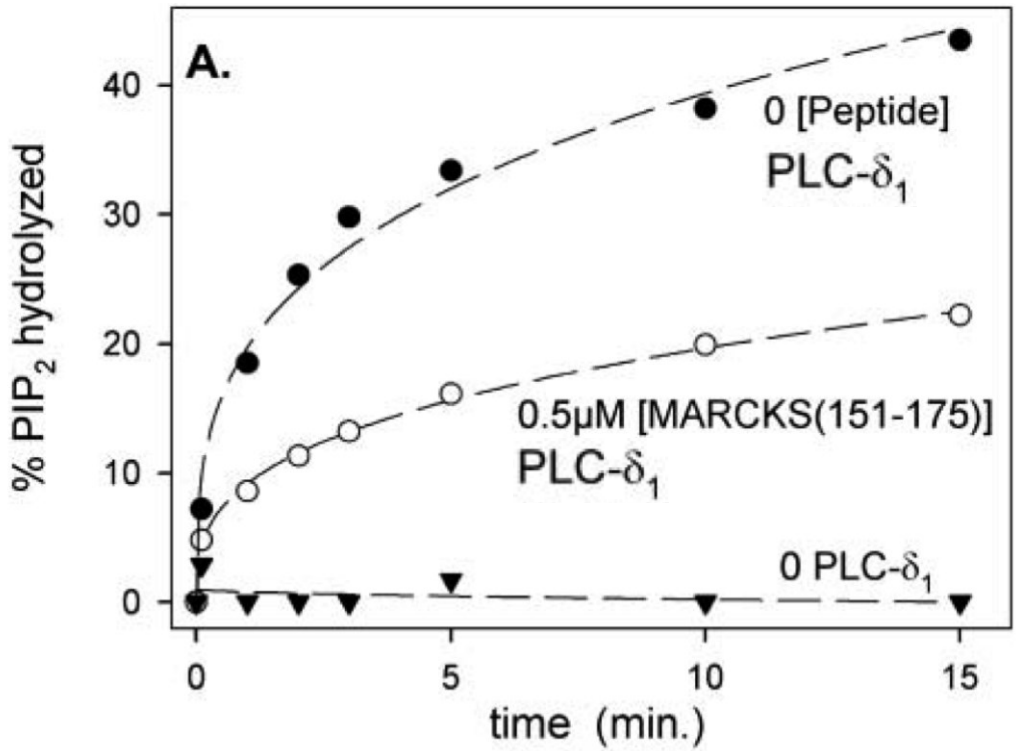


McLaughlin, Nature 2005.

Poly-basic sequence - Effect on other PIP_2 binding proteins

- Inhibition of PIP_2-hydrolysis by MARCKS

Lipid vesicles containing 0.15 % [^{3}H]-PIP_2
Hydrolysis by phospholipase $C_{\delta 1}$

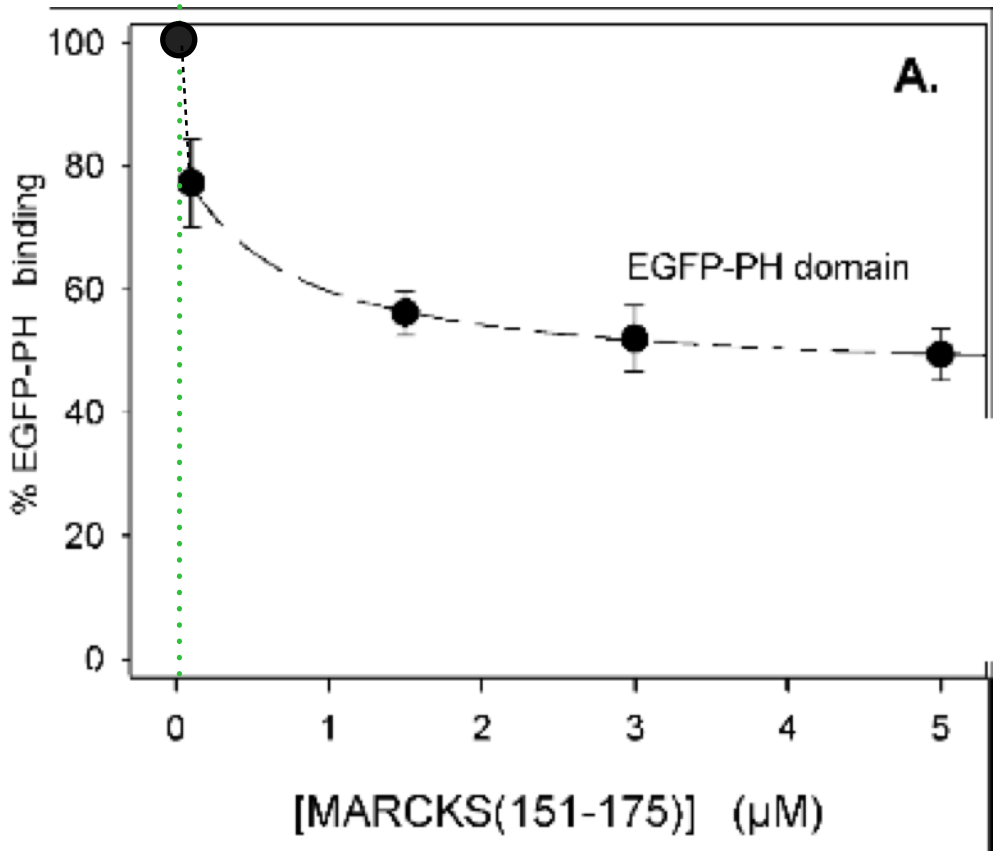


Gambhir, Bp J 2004

Poly-basic sequence - Effect on other PIP_2 binding proteins

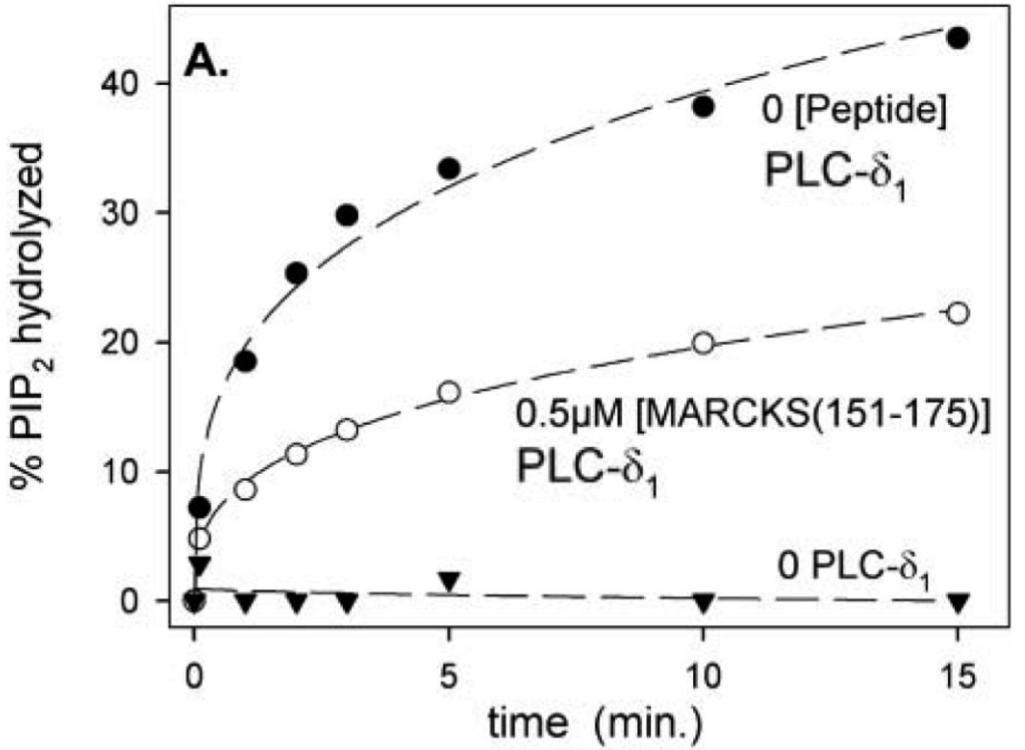
- Inhibition of PH-domain binding to PIP_2 by MARCKS

Lipid vesicles containing 1% PIP_2
10 nM PIP_2 binders



- Inhibition of PIP_2-hydrolysis by MARCKS

Lipid vesicles containing 0.15 % [^{3}H]-PIP_2
Hydrolysis by phospholipase $C_{\delta 1}$



Gambhir, B

=> MARCKS decreasing PLC binding and activity by reducing PIP_2-availability

Localising proteins to and within membranes

Cytosolic proteins can bind in a spatio-temporally regulated manner to the membrane surface through

- lipid binding domains
- poly-basic sequences

Reversible membrane attachment depends

- on **type** and **number** of interactions
- **cofactors** like Ca^{2+}
- **composition** and **concentration** of lipids recognized
- **co-incidence** of different conditions and
- **co-operation** between domains

÷ This is regulated by signalling processes

Further reading:

Balla: "How accurately can we image inositol lipids in living cells?"
TiPS 2000

McLaughlin:"Plasma membrane phosphoinositide organization by protein electrostatics" Nature 2005

Lemmon: "Membrane recognition by phospholipid-binding domains"
Nature Reviews Molecular Cell Biology, 2008

Next week:

Part 2: Lipidation & transmembrane domains