G protein-coupled receptors

- Introduction
- Classical signaling scheme
- Shut down
- Diversity & complexity
- Signaling pathways testing
- Localisation
- Monomer vs dimer

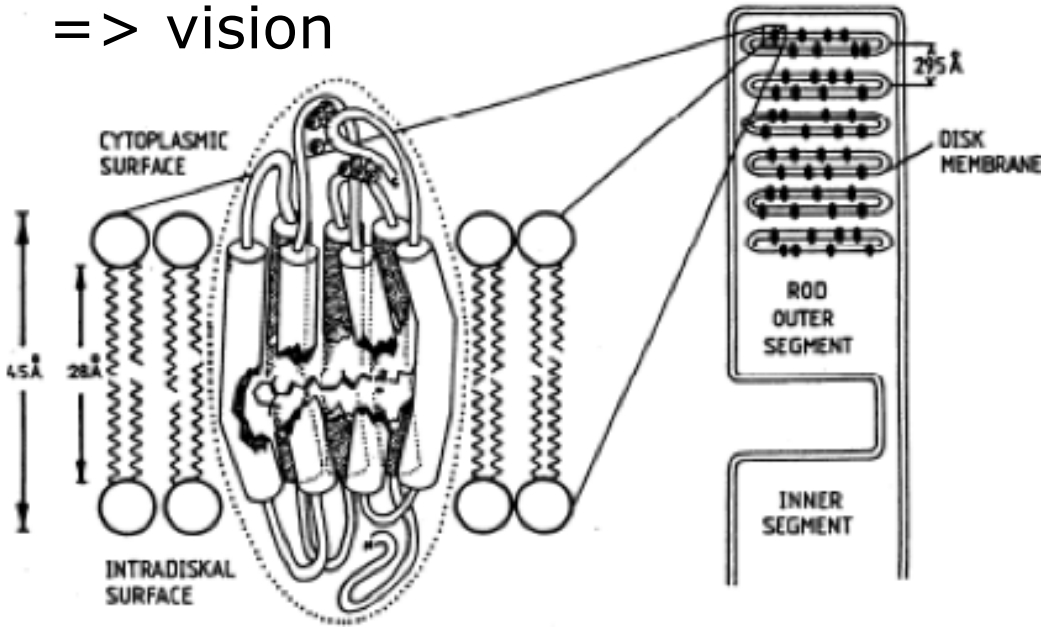
The magnificent seven

GPCR's or 7TM-receptors

A very short history of G protein-coupled receptors

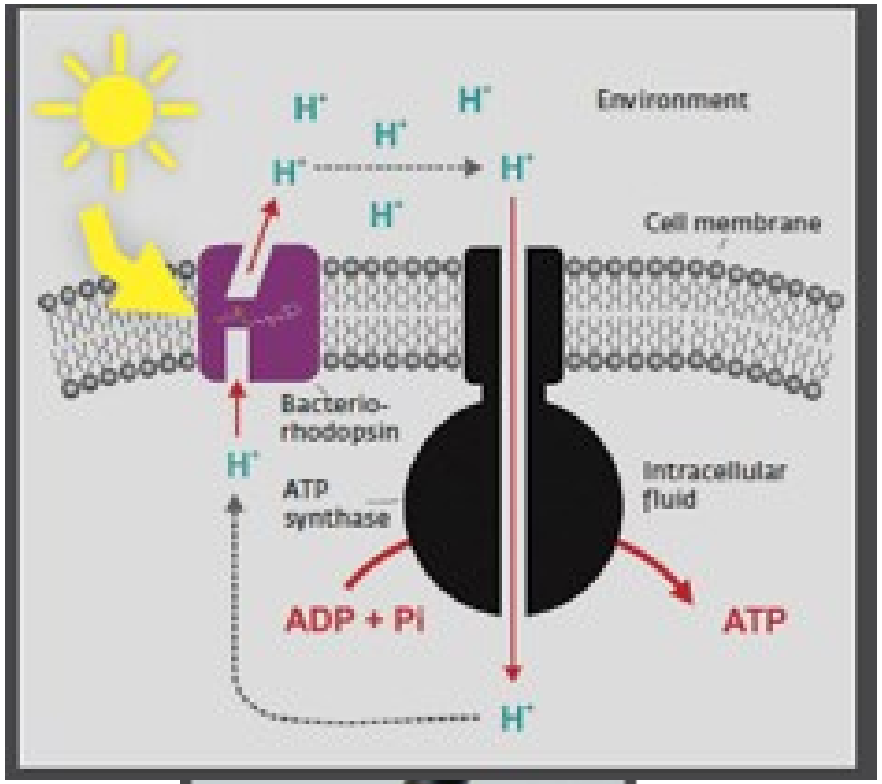
Rhodopsins : Light-activated proton pumps

mammalian retina => vision

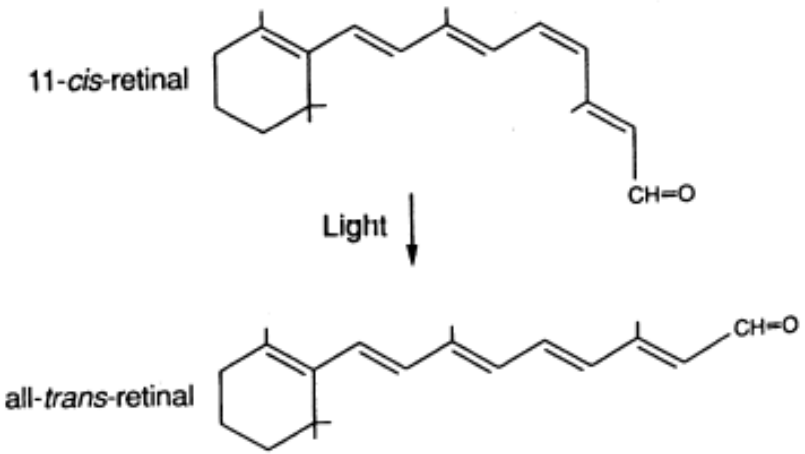


vs

bacterial purple membrane => energy production



The chromophore : Retinal



A very short history of G protein-coupled receptors

The first structures :

Bacterio-Rhodopsin

1986 : electron microscopy 3.5Å

1990 X-ray crystallography 3.5Å

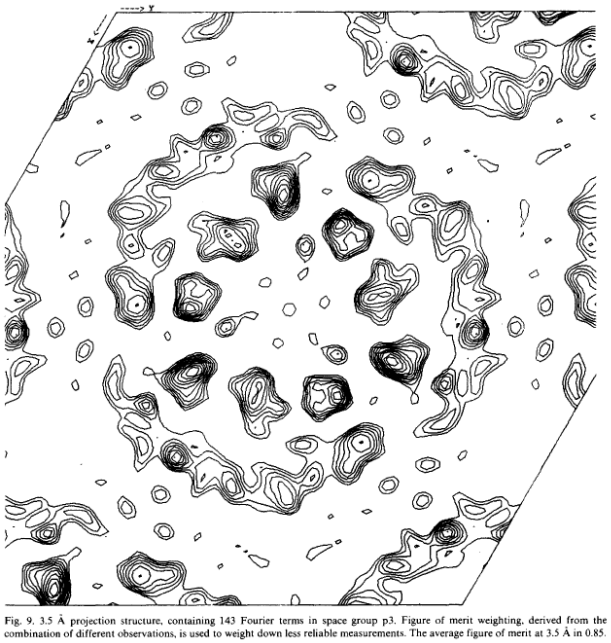
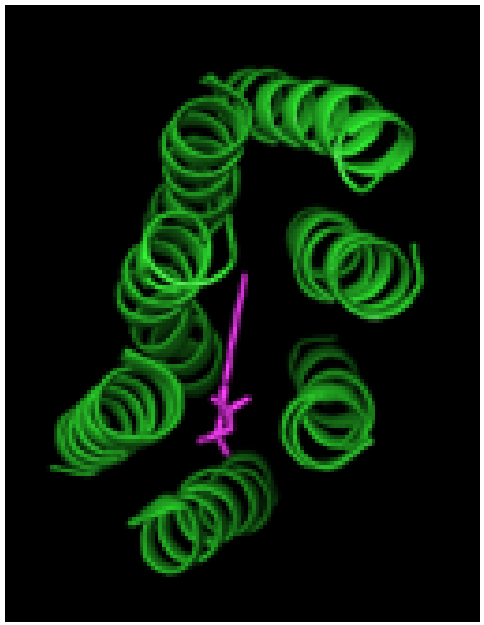


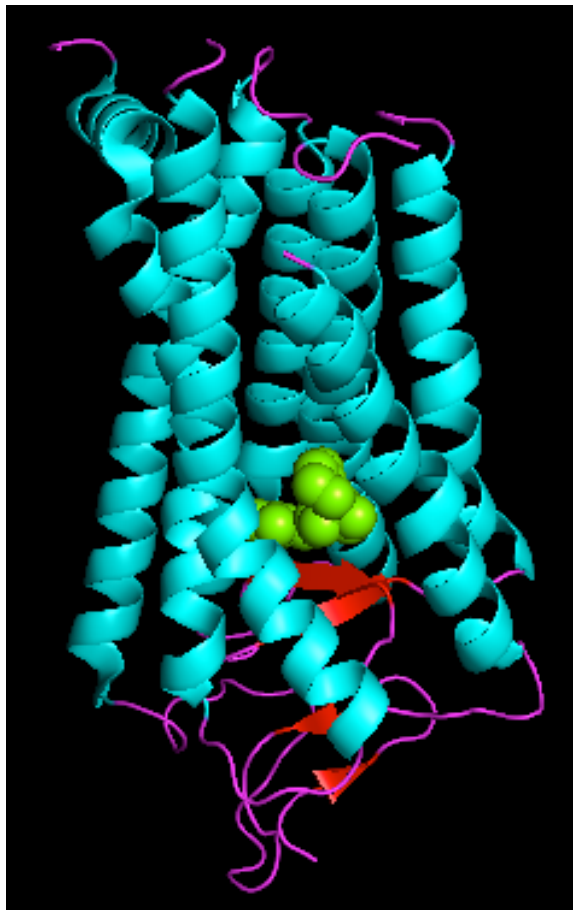
Fig. 9. 3.5 Å projection structure, containing 143 Fourier terms in space group p3. Figure of merit weighting, derived from the combination of different observations, is used to weight down less reliable measurements. The average figure of merit at 3.5 Å in 0.85.



R. Henderson et al.

2000 : Rhodopsin

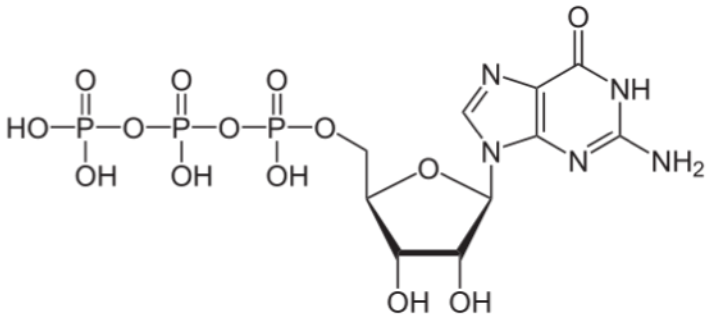
X-ray crystallography 2.8 Å



K. Palczewski et al.

A very short history of G protein-coupled receptors

GTP-binding proteins :

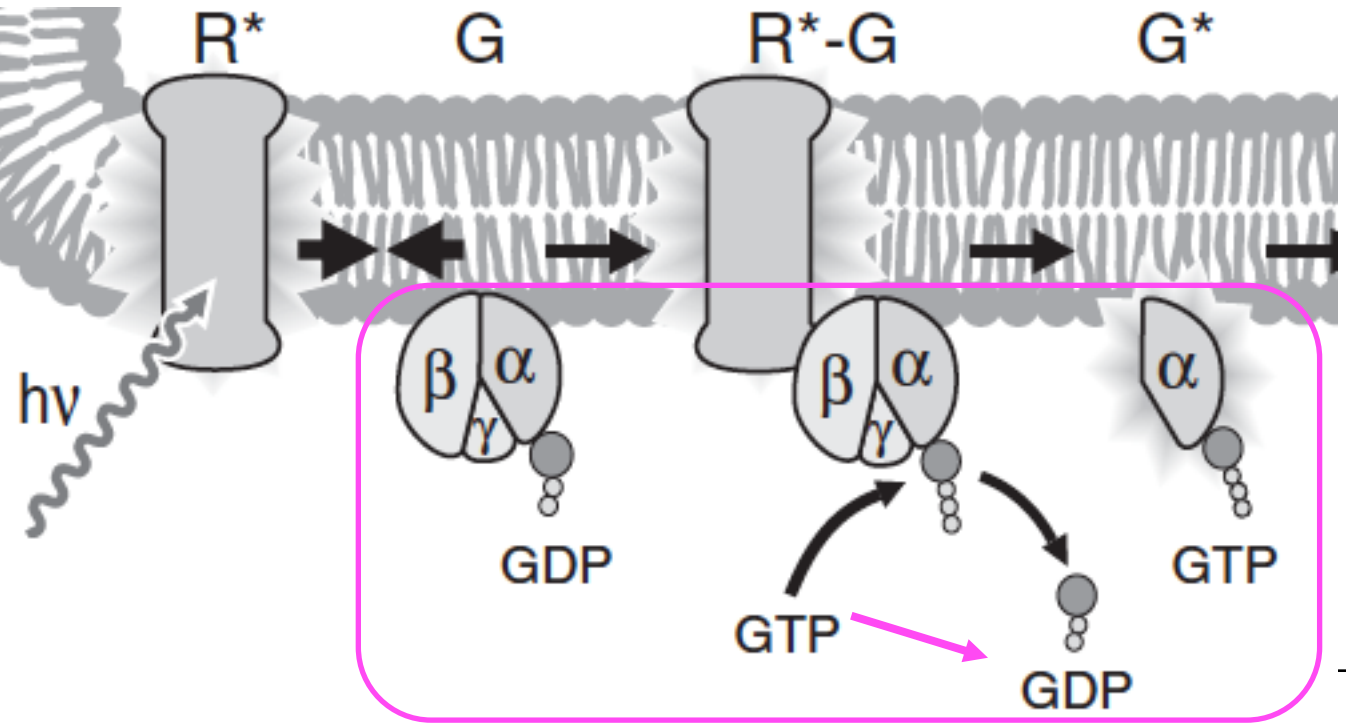


Proc. Natl. Acad. Sci. USA
Vol. 77, No. 5, pp. 2500–2504, May 1980
Biochemistry

Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments

(visual transduction/bovine retinas/amplification/light-activated GTPase)

BERNARD KWOK-KEUNG FUNG AND LUBERT STRYER



÷ G-Proteins with GTP are active !

÷ G-Proteins with GDP are in-active !

÷ G-proteins are αβγ–hetero-trimers

A very short history of G protein-coupled receptors

Recent years :

÷ Enormous advance in biochemistry and pharmacology

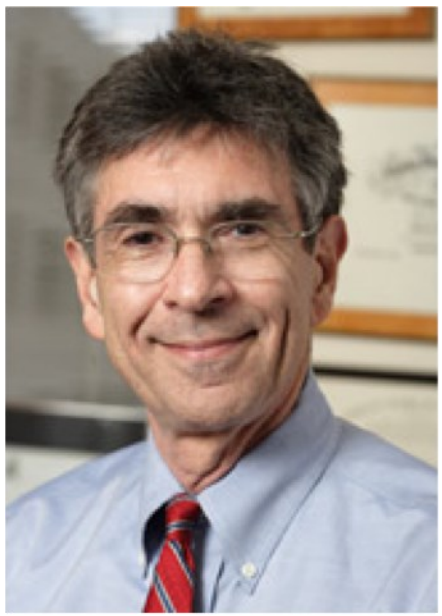
÷ An overwhelming number of structures

=> Nobel price 2012 Chemistry

Ray Stevens

USC

High-throughput

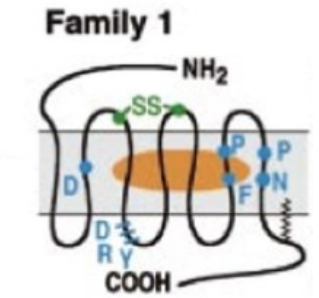


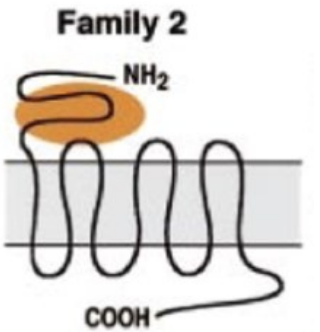
Robert J Lefkowitz

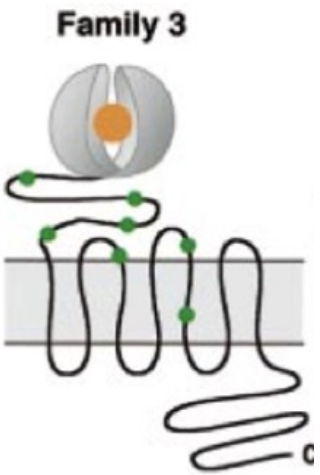
Duke

Brian K. Kobilka

Stanford







G Protein-Coupled Receptors (GPCRs) as Drug Targets

- Transmembrane receptor proteins critical to biological function and the most important family of drug targets in industry
- Source of >40% approved drugs across multiple disease areas

Neuroscience
Seroquel (AZ) - $5.8Bn (2011)
Abilify (BMS/Otsuka) - $5.7Bn (2012)
Imitrex (GSK) - $1.4Bn (2007)

Respiratory
Advair (GSK) - $8.3Bn (2013)
Singulair (Merck) - $5.5Bn (2011)
Spiriva (BI/Pfizer) - $3.2Bn (2015)

Cardiovascular
Diovan (Novartis) - $6Bn (2010)
Plavix (Sanofi) - $6Bn (2008)
Tracleer (Actelion) - $1.7Bn (2010)

Metabolic
Myrbetriq (Astellas) – ~$1.2Bn (2020)
Victoza $2bn (2014)

Cancer
Zoladex - $924m (2014)
Erivedge (Roche) - $140m (2014)

Source: Bloomberg Industries, Globe Data

GPCR structures : Brute force structural biology

gpcr.usc.edu/tracking_status.htm

Success **In Progress** **Collaborator Progress** **Failed** **Work Stopped**

GPCR Net ID	Protein Name	Uniprot	Ligand/Lattice	Cloning	Expression	Purification	Crystallization	Diffraction	Structure	Refinement	PDB
1*	5-hydroxytryptamin (serotonin) receptor 1A	P08908									
2	5-hydroxytryptamin (serotonin) receptor 2C	P28335									
4	5-hydroxytryptamine receptor 2B	P41595	Ergotamine								4IB4

Protein Cell 2016, 7(5):325–337
DOI 10.1007/s13238-016-0263-8

CrossMark

Protein & Cell

RESEARCH ARTICLE

In vitro expression and analysis of the 826 human G protein-coupled receptors

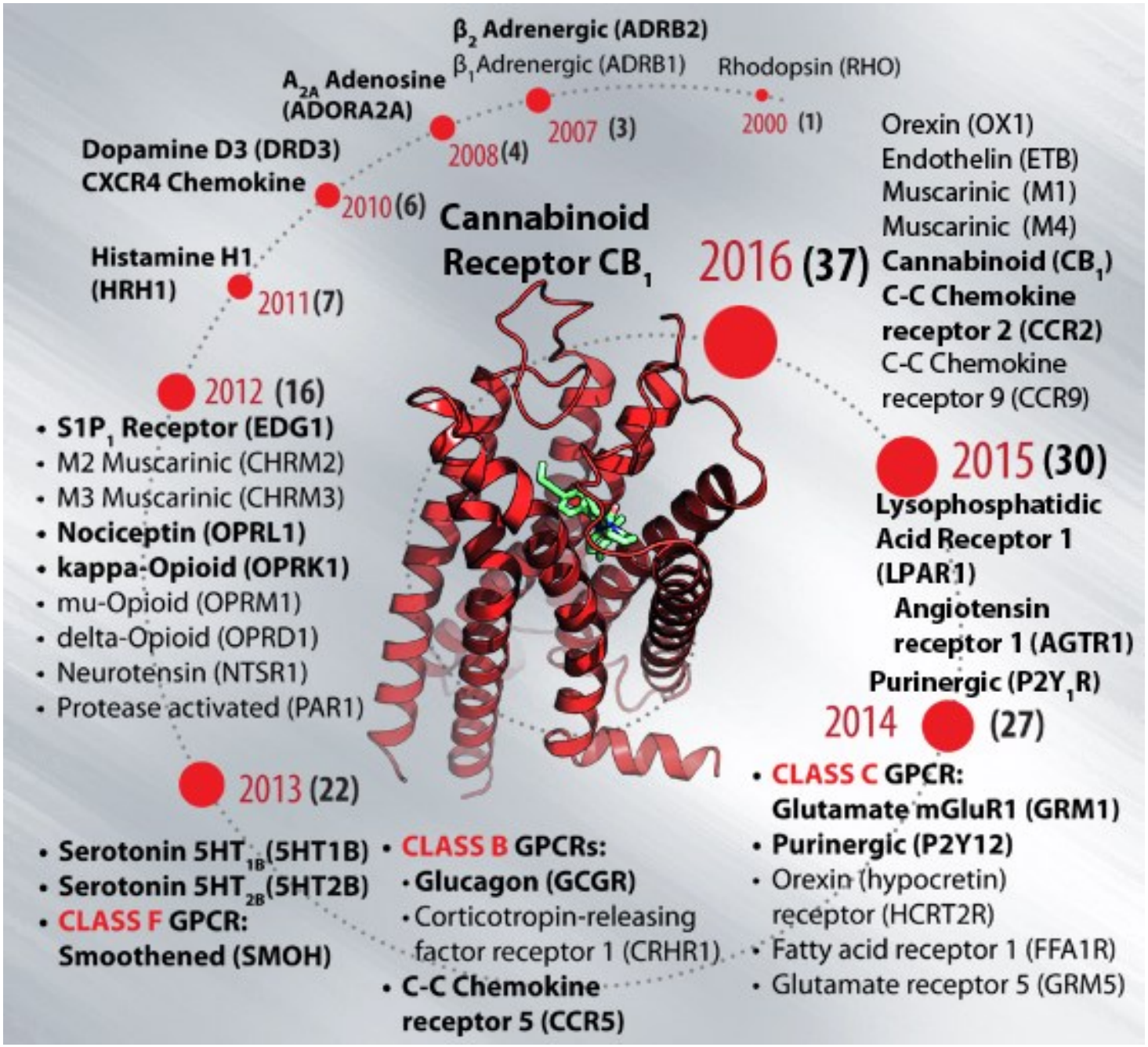
Xuechen Lv[1], Junlin Liu[1], Qiaoyun Shi[1], Qiwen Tan[1], Dong Wu[1], John J. Skinner[1], Angela L. Walker[2], Lixia Zhao[1], Xiangxiang Gu[1], Na Chen[1], Lu Xue[1], Pei Si[1], Lu Zhang[1], Zeshi Wang[1], Vsevolod Katritch[2], Zhi-jie Liu[1], Raymond C. Stevens[1]✉

[1] iHuman Institute, ShanghaiTech University, Shanghai 201210, China
[2] Department of Biological Sciences, Bridge Institute, University of Southern California, Los Angeles, CA 90089, USA
✉ Correspondence: stevens@shanghaitech.edu.cn (R. C. Stevens)

Received February 26, 2016 Accepted March 9, 2016

50	Glucose-dependent insulinotropic receptor	Q8TDV5									
51*	Gonadotropin-releasing hormone receptor	P30968									
53	Histamine receptor H1	P35367	Doxepin								3RZE
56*	kappa opioid receptor	P41145	JDTic								4DJH
65	Melanocortin 4 receptor	P32245									
68	Metabotropic glutamate receptor 1	Q13255									
71*	mu opioid receptor	P35372									
74	Neurokinin 1 receptor (Substance P receptor)	P25103									
79*	nociceptin opioid receptor (ORL-1)	P41146	Compound-24								4EA3

GPCR structures : Brute force structural biology



Today about >50 crystal structures have been published

Many unpublished are suspected

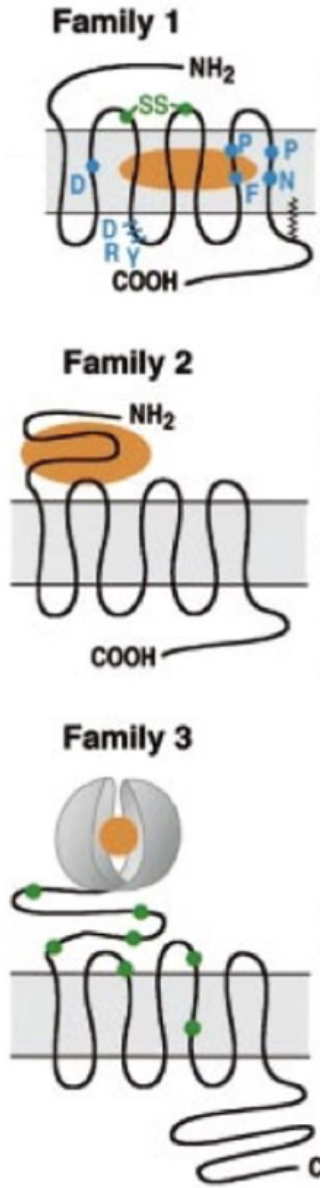
http://stevenslab.usc.edu

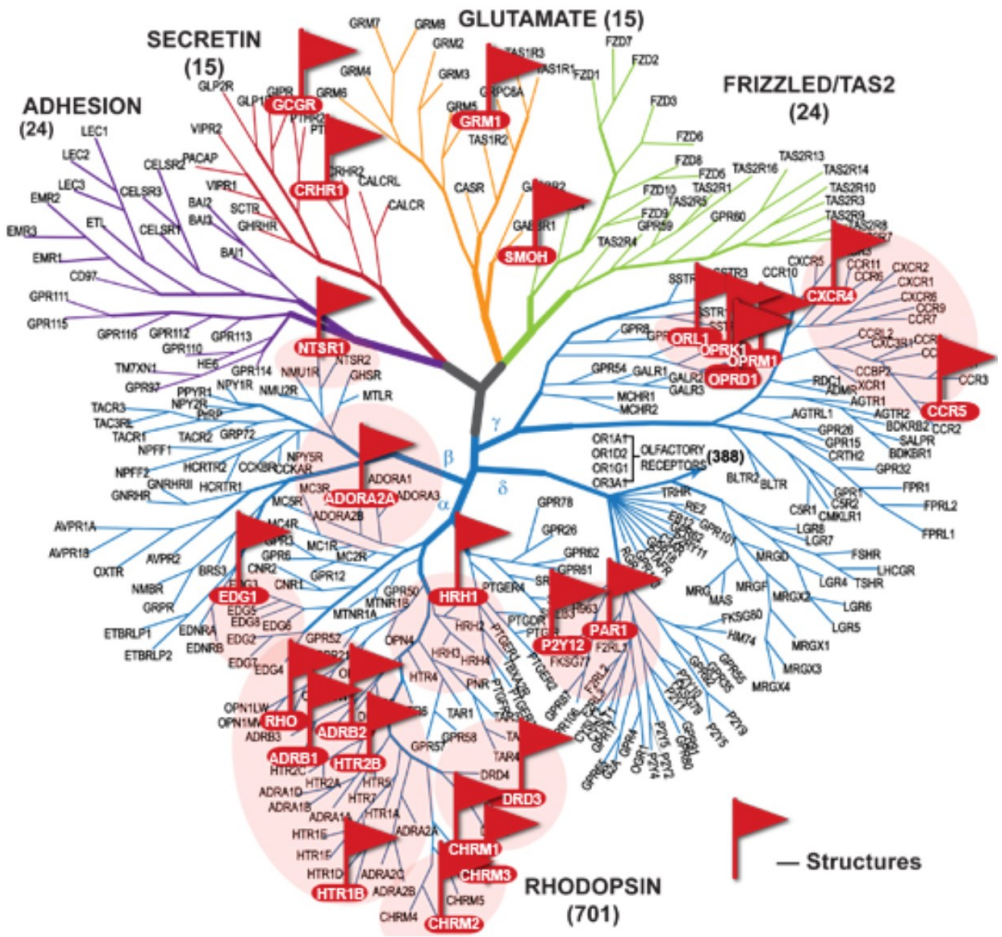
GPCR's and their structures

The human genome encodes ~923 GPCR's; about 1/3 are used to detect odorants

Brian Kobilka
Raymond Stevens

Kurt Wüthrich

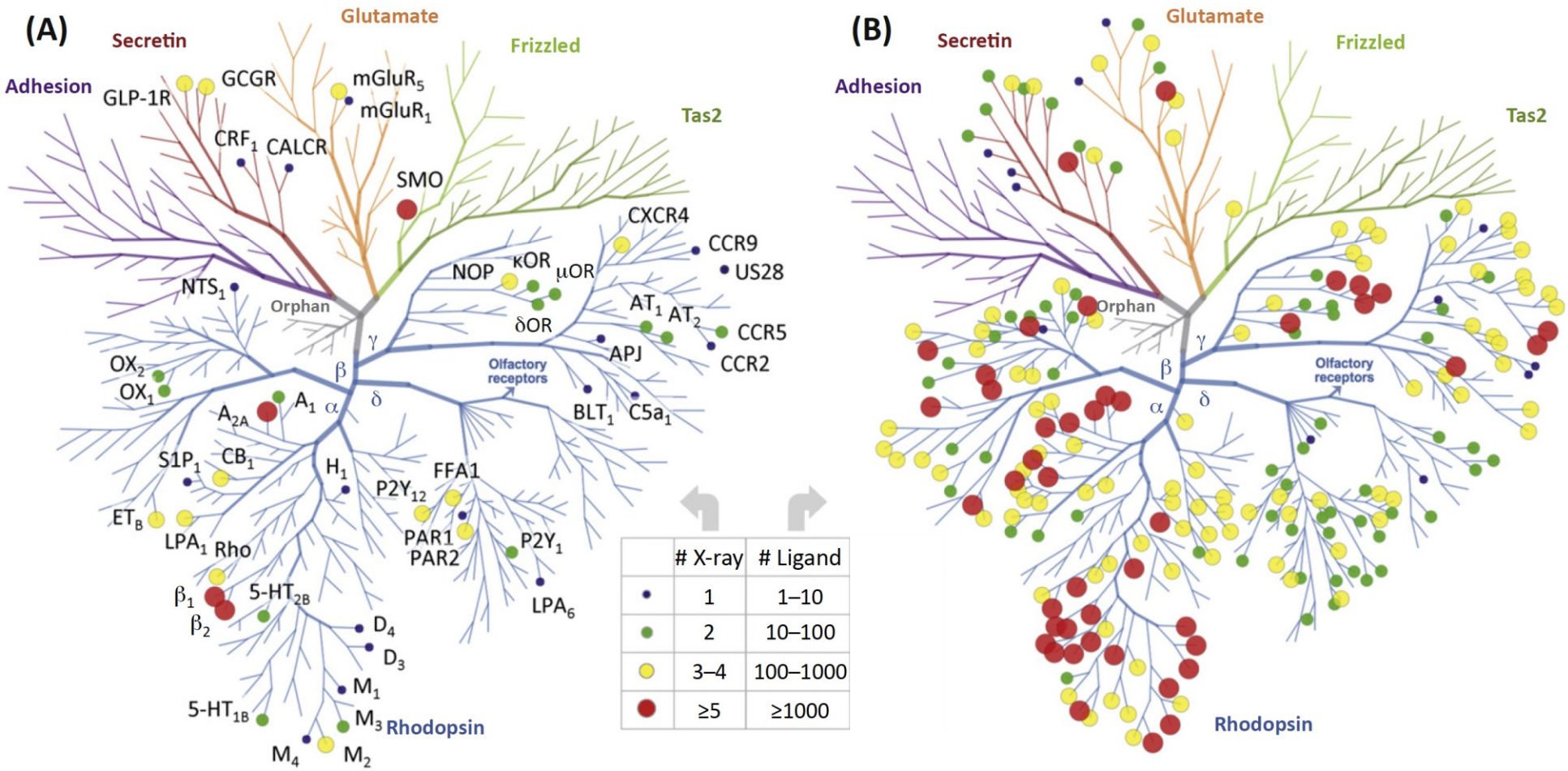




Phylogenetic tree

GPCR structures and small molecule binders

Structural Coverage of Chemical G Protein-Coupled Receptor Ligand Space



Vass et al. TiPS (2018) 39(5) p 494

Not just the big established companies

E.g. HEPTARES therapeutics founded in 2007 (UK)

Founders: Chris Tate & Richard Henderson

Technology & Business model:
÷ expression of thermostable GPCR mutants, crystallisation & structure
÷ ligands: structure-based drug design

Heptares : an enormous success !

Pipeline deals with big pharmaceutical companies:

Allergan : global rights to muscarinic receptor agonists to treat ***neurological diseases***……Heptares received **$125 million** *upfront*…eligible for milestone payments of up to approximately **$3.25 billion**, plus up to **double-digit royalties**.

AstraZeneca : adenosine A_{2A} receptor-inhibitors for ***immuno-therapies***…..Heptares received **$10 million** *upfront*..eligible to receive milestone payments …..eligible to receive more than **$500 million**, as well as up to d**ouble-digit tiered royalties**

Technology deals:

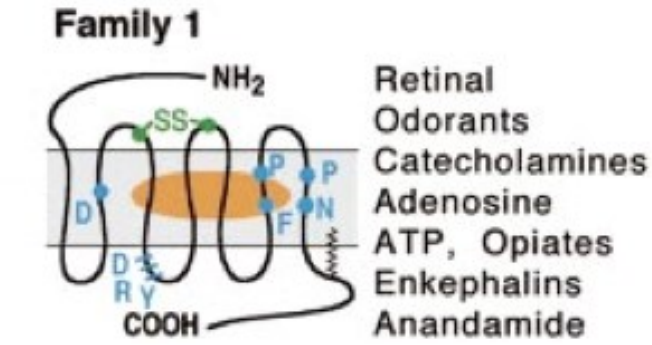
Pfizer : **$189 million** per target up to 10 targets

AstraZeneca : **$6 million**, up to **$180 million**

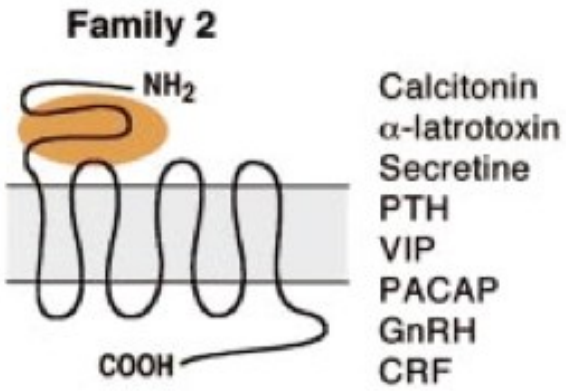
Takeda : up to **$100 million** + **royalties**

Novartis Fund : up to **$200 million + royalties**

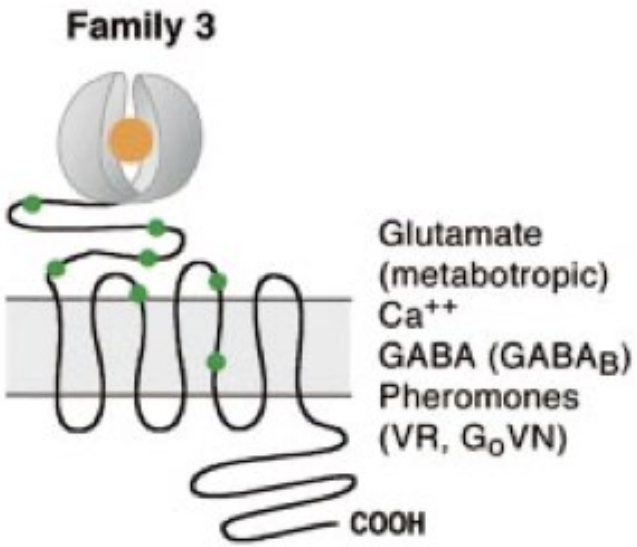
3 families : primary sequence & ligand binding site



Family 1 contains most GPCRs
- Palmitoylation of C-terminus closed to TM7
- Conserved residues (blue, disulfide)
- Small ligands bind between TM's;
  larger ones more towards exterior



Family 2:
- Similar to family 1, but no sequence homology.
- **high molecular weight peptide hormones** such as glucagon,

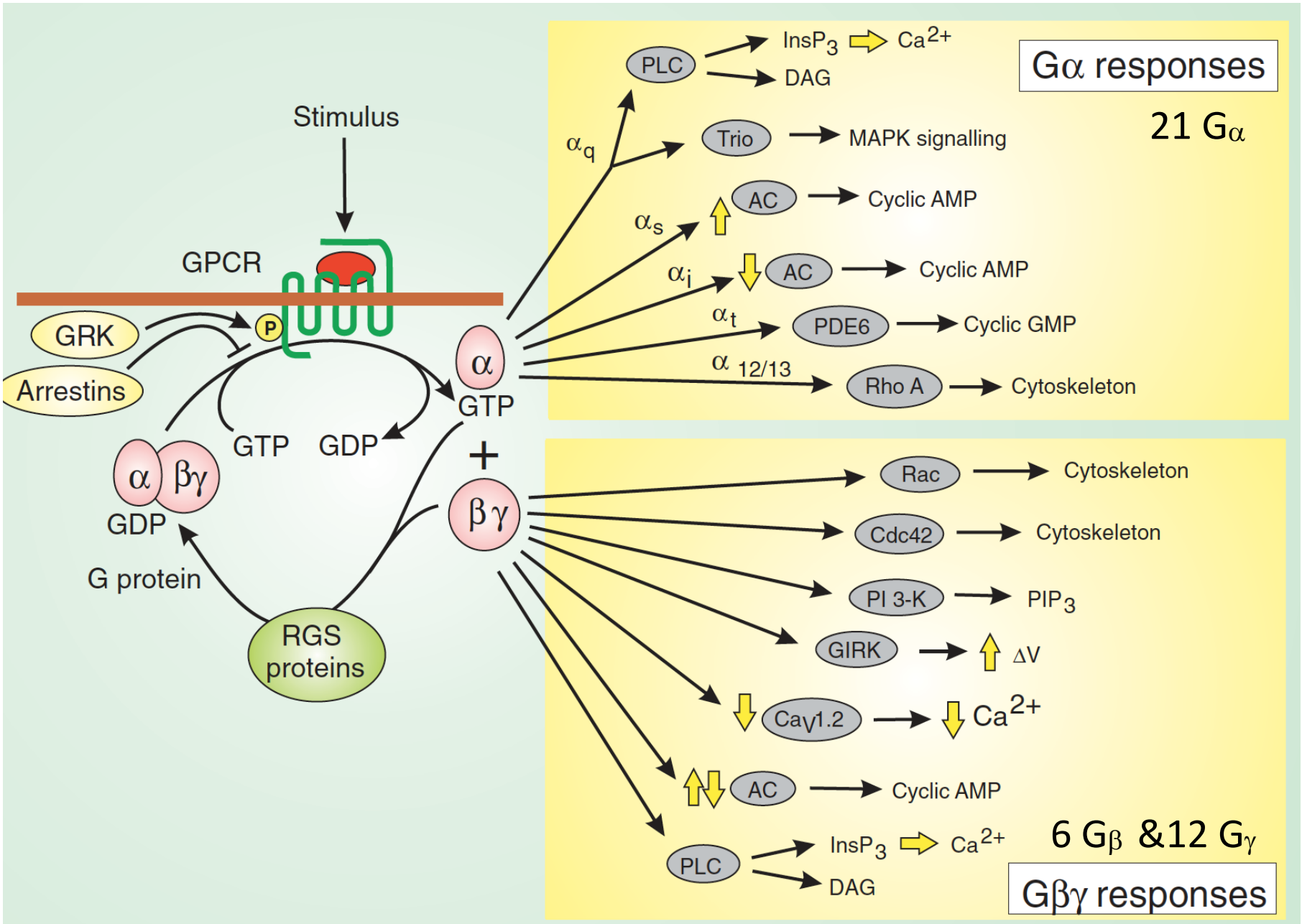


Family 3:
- very large N-terminal ligand binding domains with fly-trap-like structure
- **small ligands**, like glutamate

Orange ellipse: Ligand

The GPCR cycle : Ligand-induced GDP-GTP exchange & GTP hydrolysis

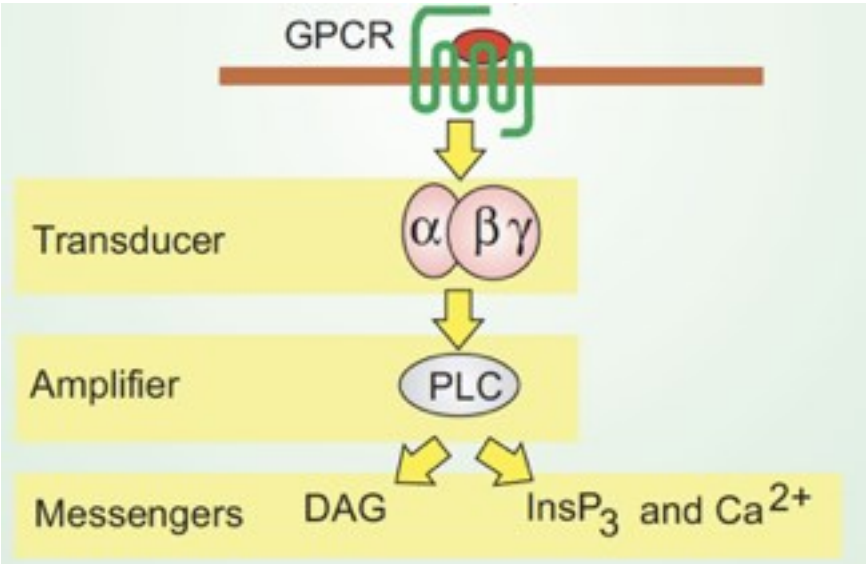
GPCR signalling : A host of Gα and Gβγ



Signalling via Gα_q : Increasing [Ca^{2+}]

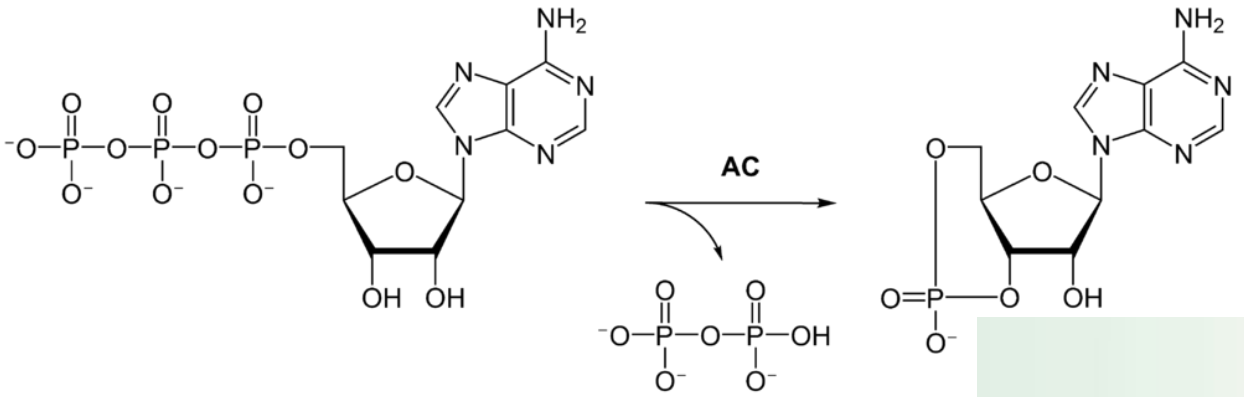
- G_{α_q} stimulates phospholipase C

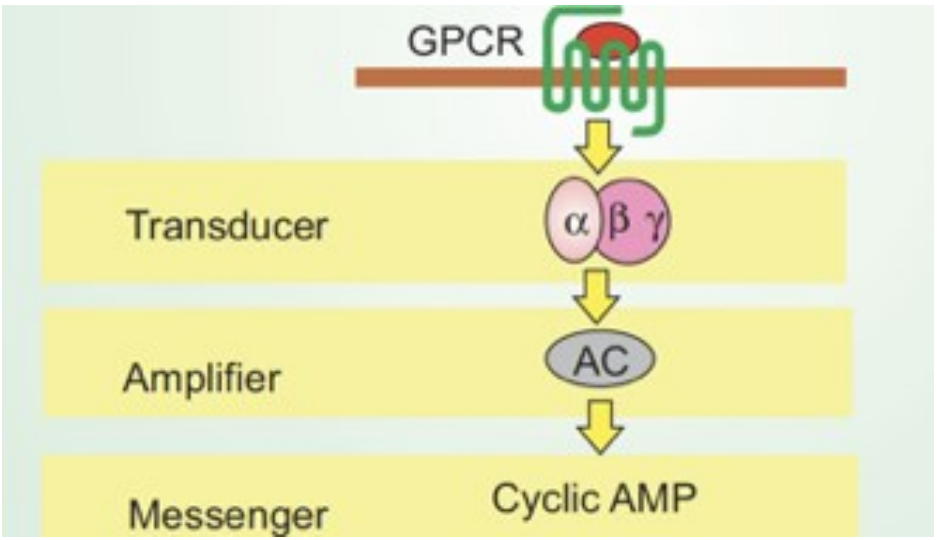
Ca^{2+} is an important 2nd messenger



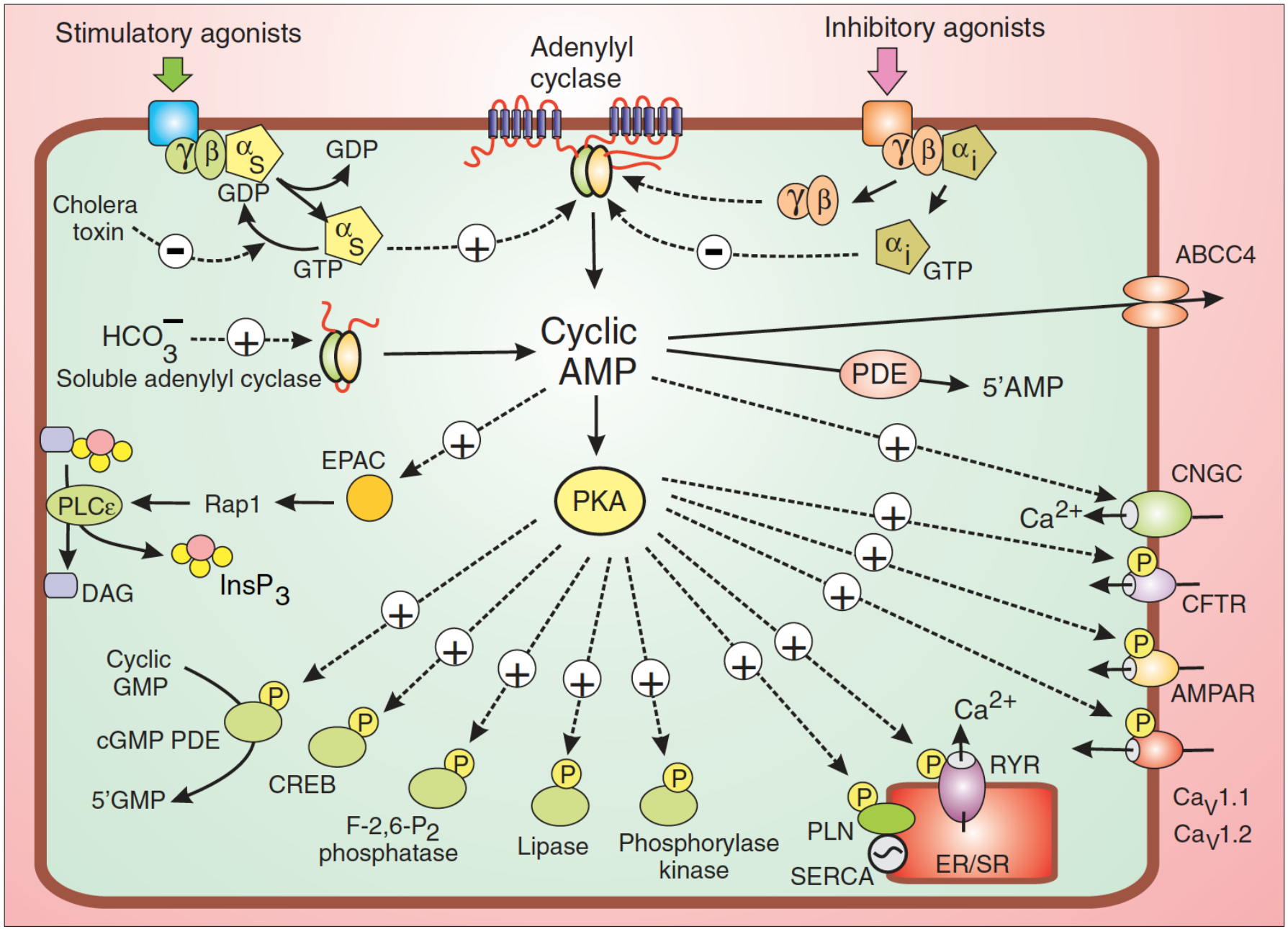
Signalling via Gα_s or Gα_i : Acting on [cAMP]

- cAMP is an important 2nd messenger
- Produced by the enzyme adenylate cyclase AC :

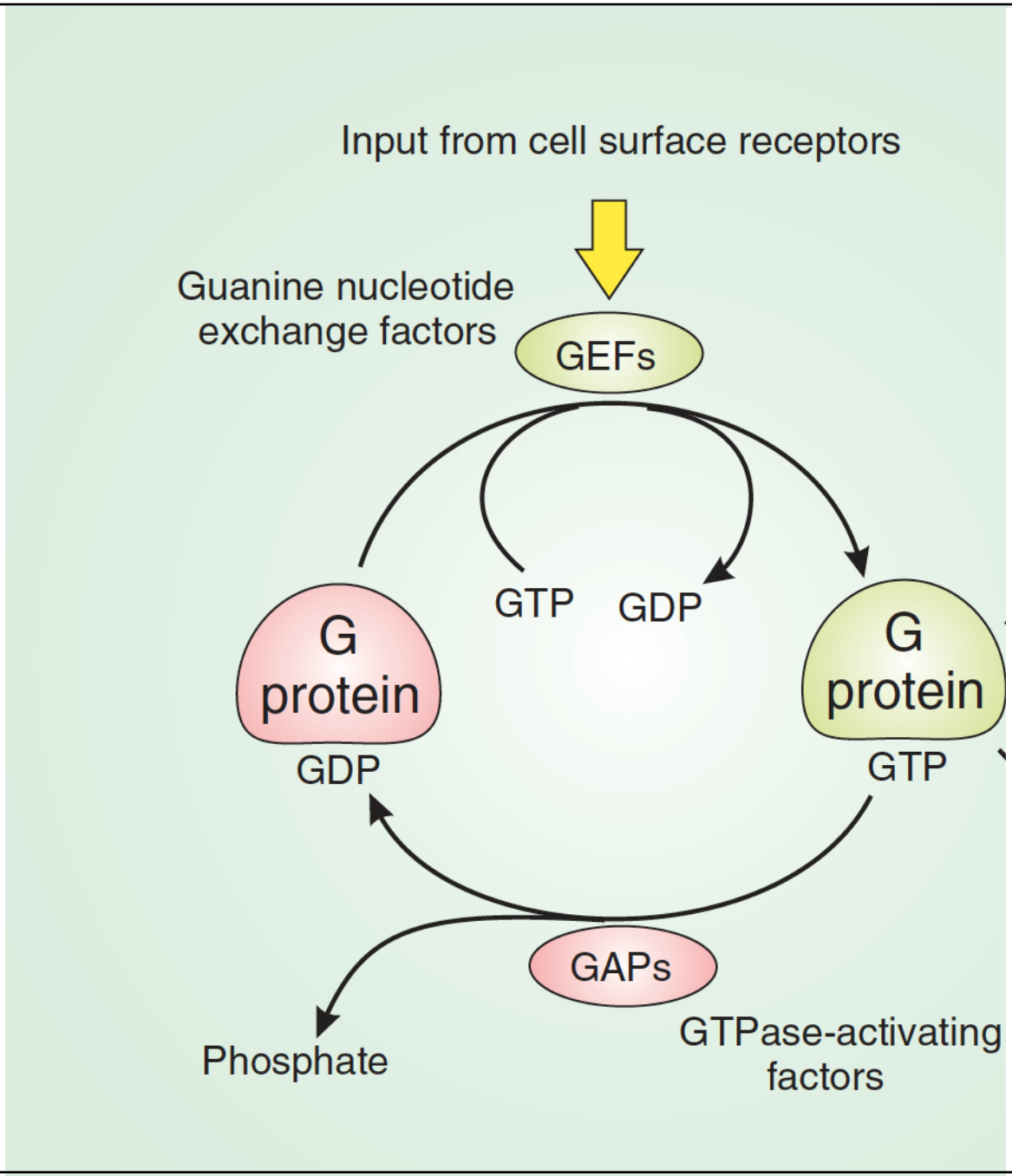




Signalling via cAMP – Proteinkinase-A is major target



Regulation of **G**-protein **S**ignalling by RGS proteins



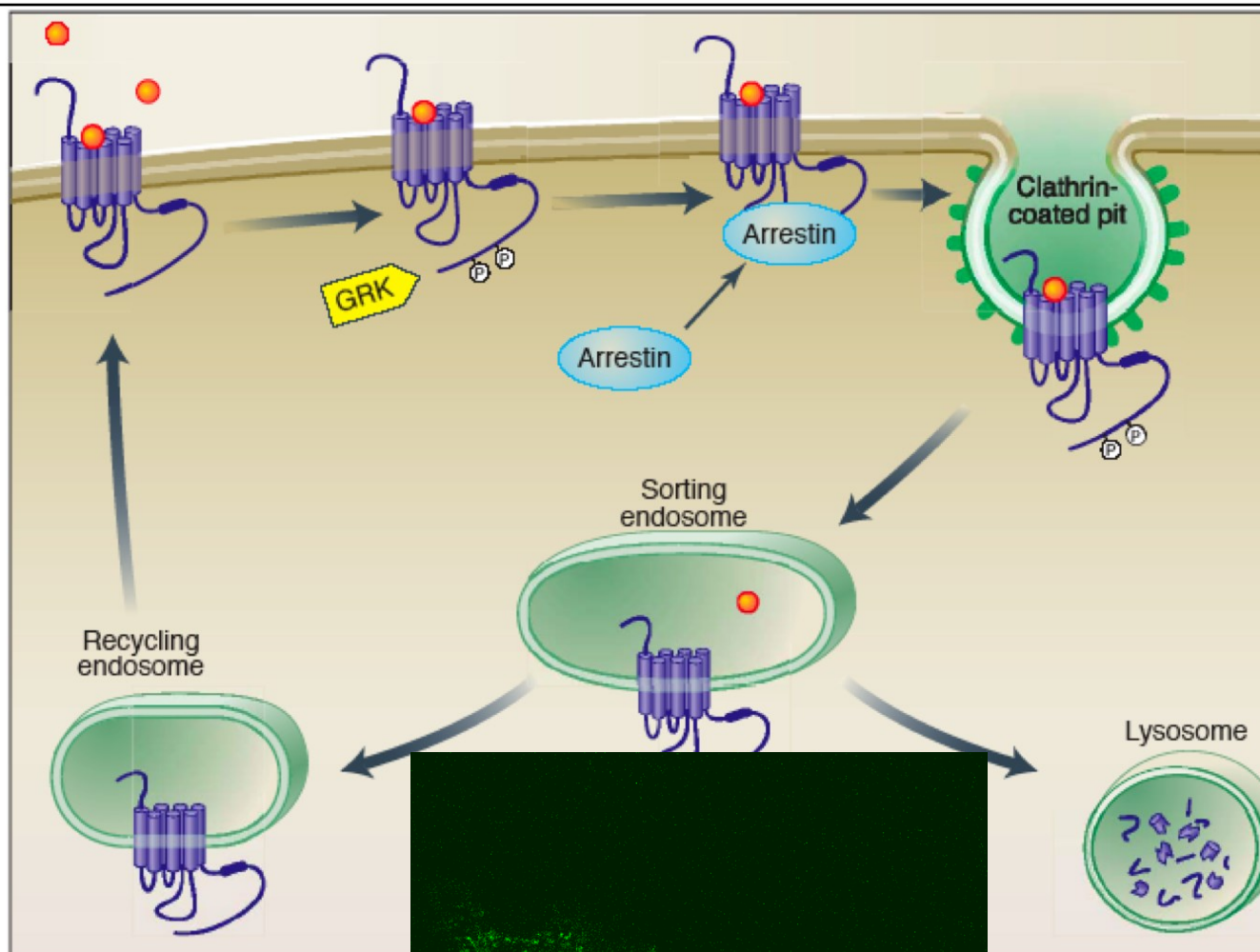
Shut-down of GPCR activity

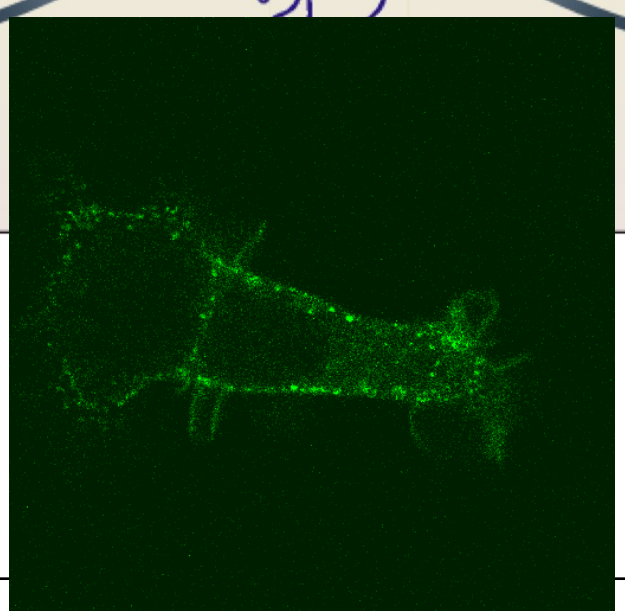
i Activated receptor is phosphorylated by GPCR kinase (**GRK**)

ii **Arrestin** binding
=> **end of G protein activation**

iii Sequestration in coated pits
- Internalisation to endosome followed by either

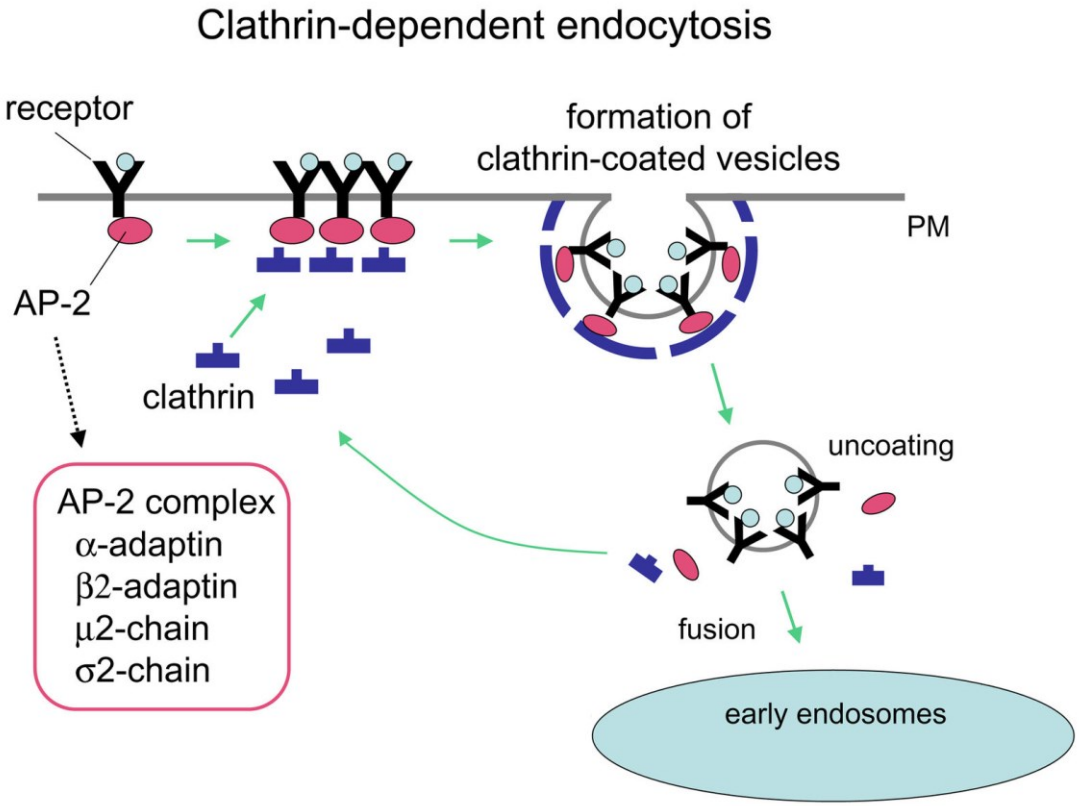
÷ recycling to cell surface
or
÷ degradation in lysosomes

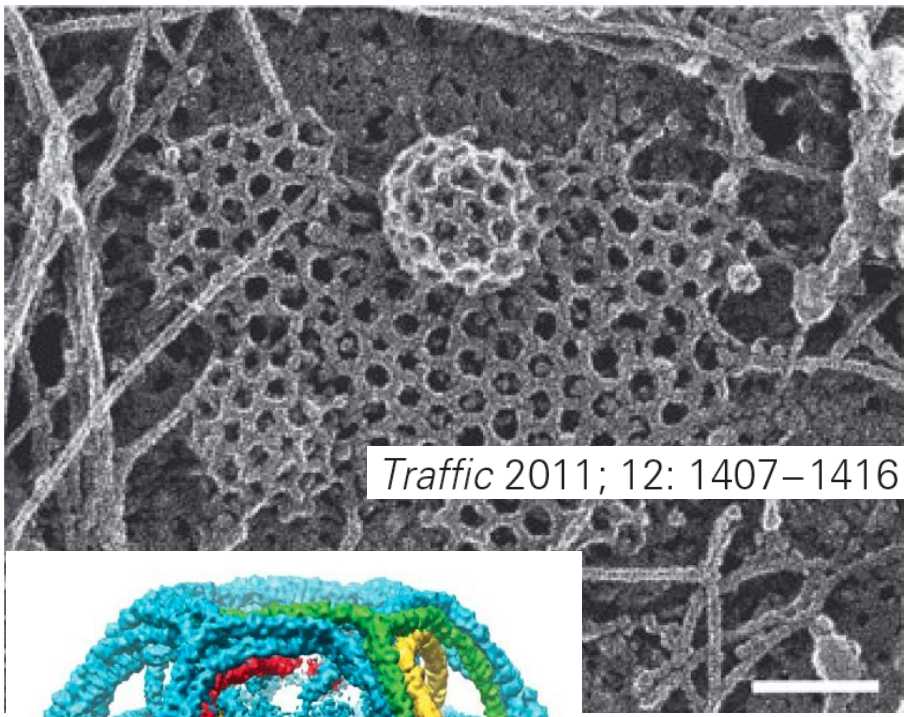


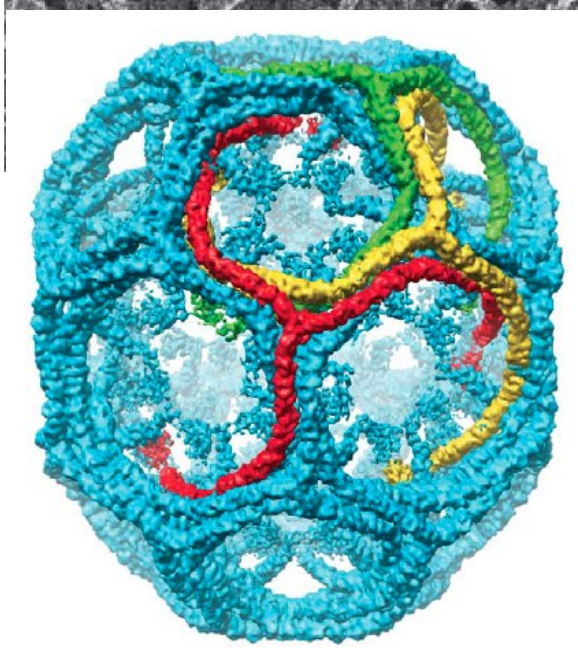


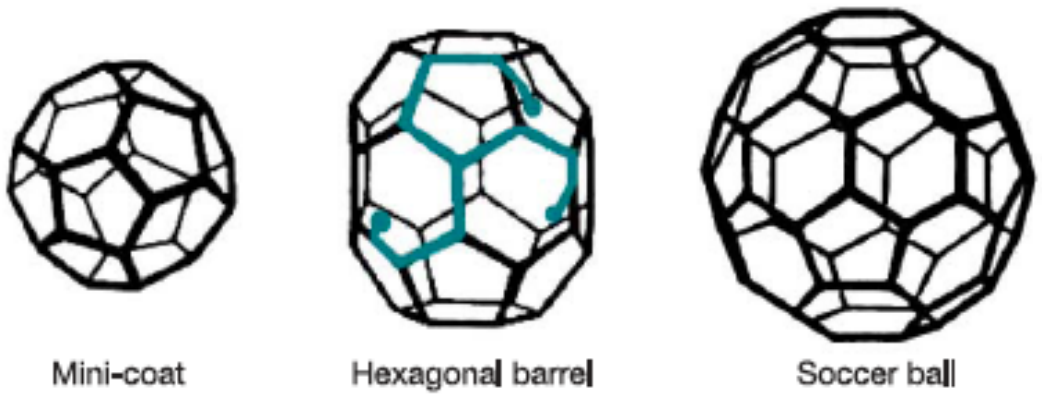
Luigino Grasso
30 x accele rated

Internalisation via clathrin-coated vesicles









Tomas Kirchhausen5 & Thomas Walz2
NATURE |VOL 432 | 2 DECEMBER 2004

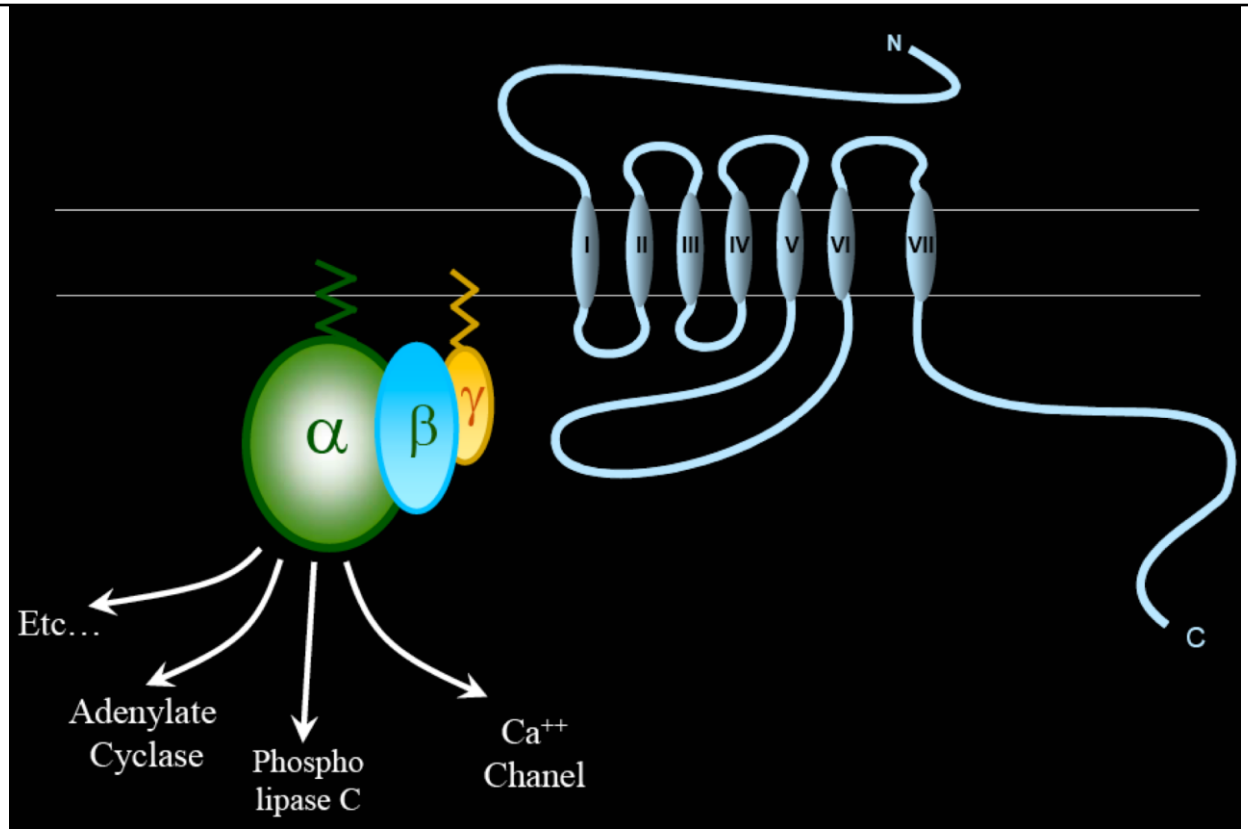
G protein-coupled receptors

- Introduction
- Classical signalling scheme
- Shut down
- Diversity & complexity
- Signalling pathways testing
- Localisation
- Monomer vs dimer

The magnificent seven

GPCR’s or 7TM-receptors

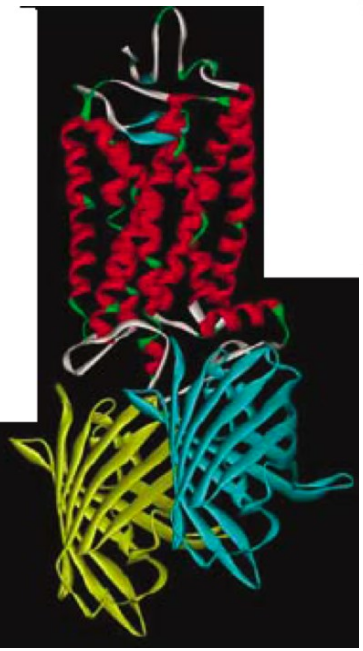
Conventional GPCR signalling : Questions remain..

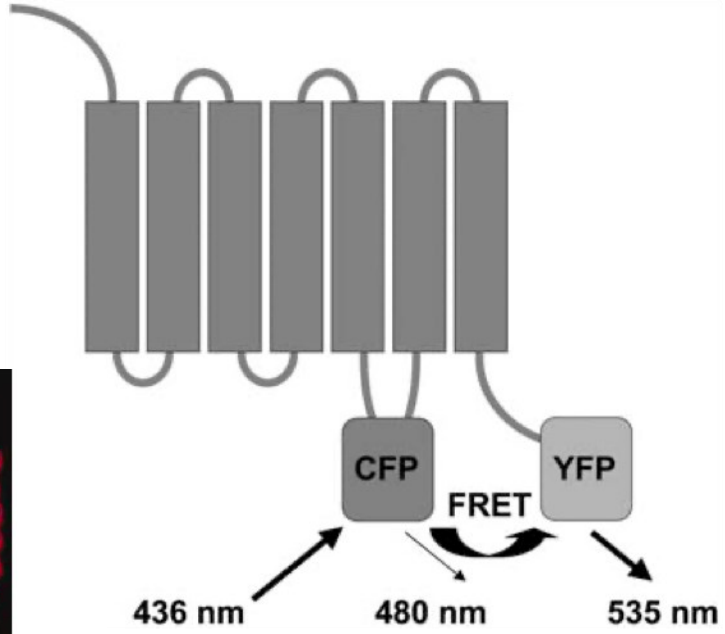


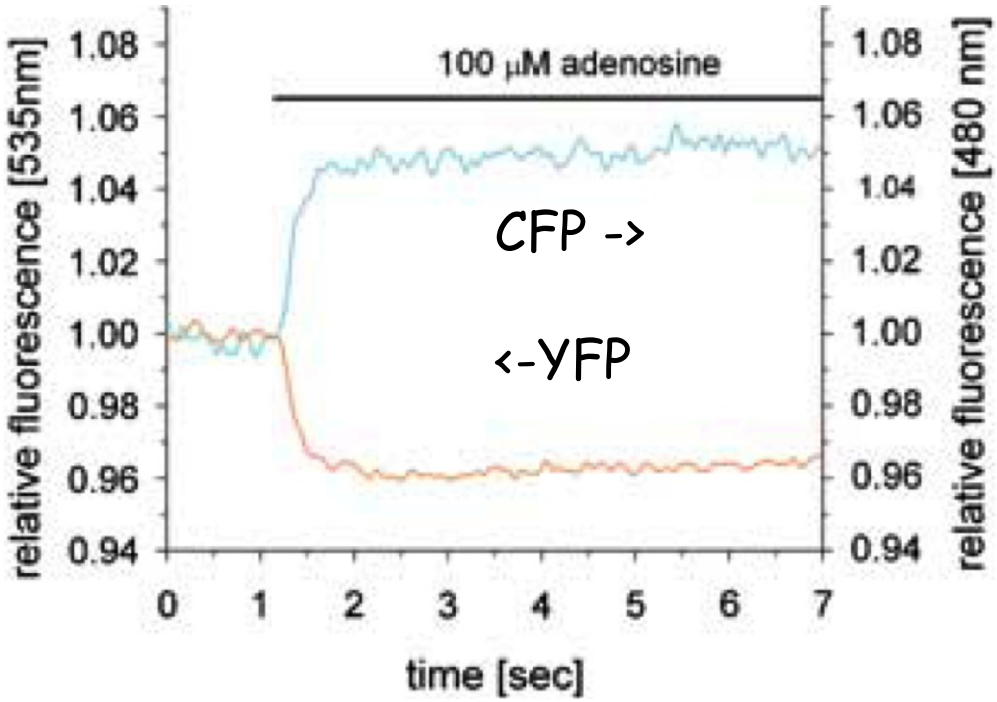
- How fast is GPCR signalling ?
- Does $G_{\alpha(GTP)\beta\gamma}$ really dissociate into $G_{\alpha(GTP)}$ and $G_{\beta\gamma}$?
- Origin of signal diversity ?
- GPCR's act as monomers or dimers ?
- Where are GPCR's active ?

GPCR signalling : How fast does it go?

FRET experiments to measure molecular interactions or conformational changes:
e.g. **conformational change in GPCR** using CFP in 3rd loop & YFP on C-terminus:







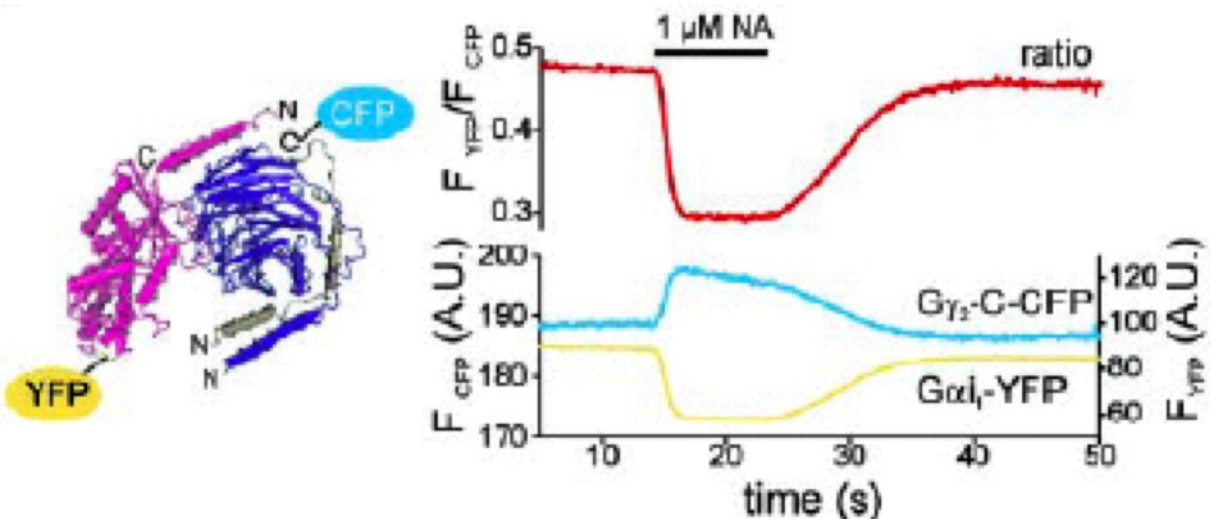
Step	*Half-life $t_{1/2}$ (ms)*
Receptor conformational change	30–50
Receptor–G-protein interaction	30–50
G-protein activation	300–500
cAMP accumulation	20 000–50 000

Lohse & Bünemann

Does the hetero-trimeric G protein dissociate?

Accepted model claims a dissociation into G_{α} and $G_{\beta\gamma}$,
as shown for transducin

Experiments using FRET between labelled G protein subunits:



FRET decreases with agonist addition!!
=> dissociation!!

Bunemann, M., M. Frank, et al. (2003). PNAS 100(26): 16077-82.

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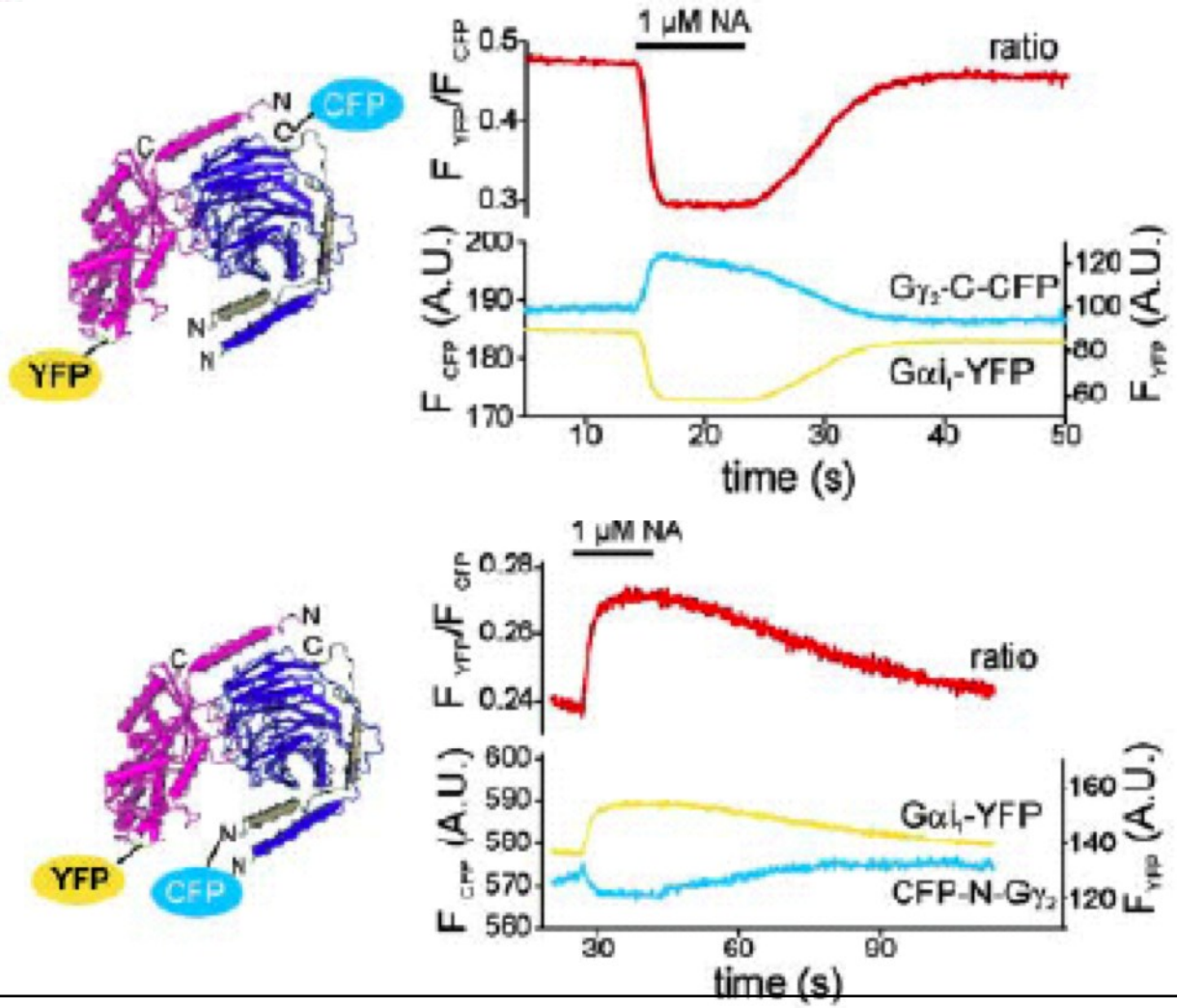


FRET decreases with agonist addition!!
=> dissociation!!

FRET increases!!
=> ???
- no dissociation ?
- re-orientation ?

Bunemann, M., M. Frank, et al. (2003). PNAS 100(26): 16077-82.

G protein-coupled receptors

- GPCR's are a very prominent class of receptors
- GPCR structure is 7TM
- Receptor activation induces activation of G protein through GDP-GTP exchange
- G proteins propagate the signal
- Shut down of signalling
- Ligand-determined effects : Bias

Signalling diversity : Numerous receptors for one ligand

Often multiple receptors are activated by a given ligand.

E.g. there are 16 serotonin receptors :

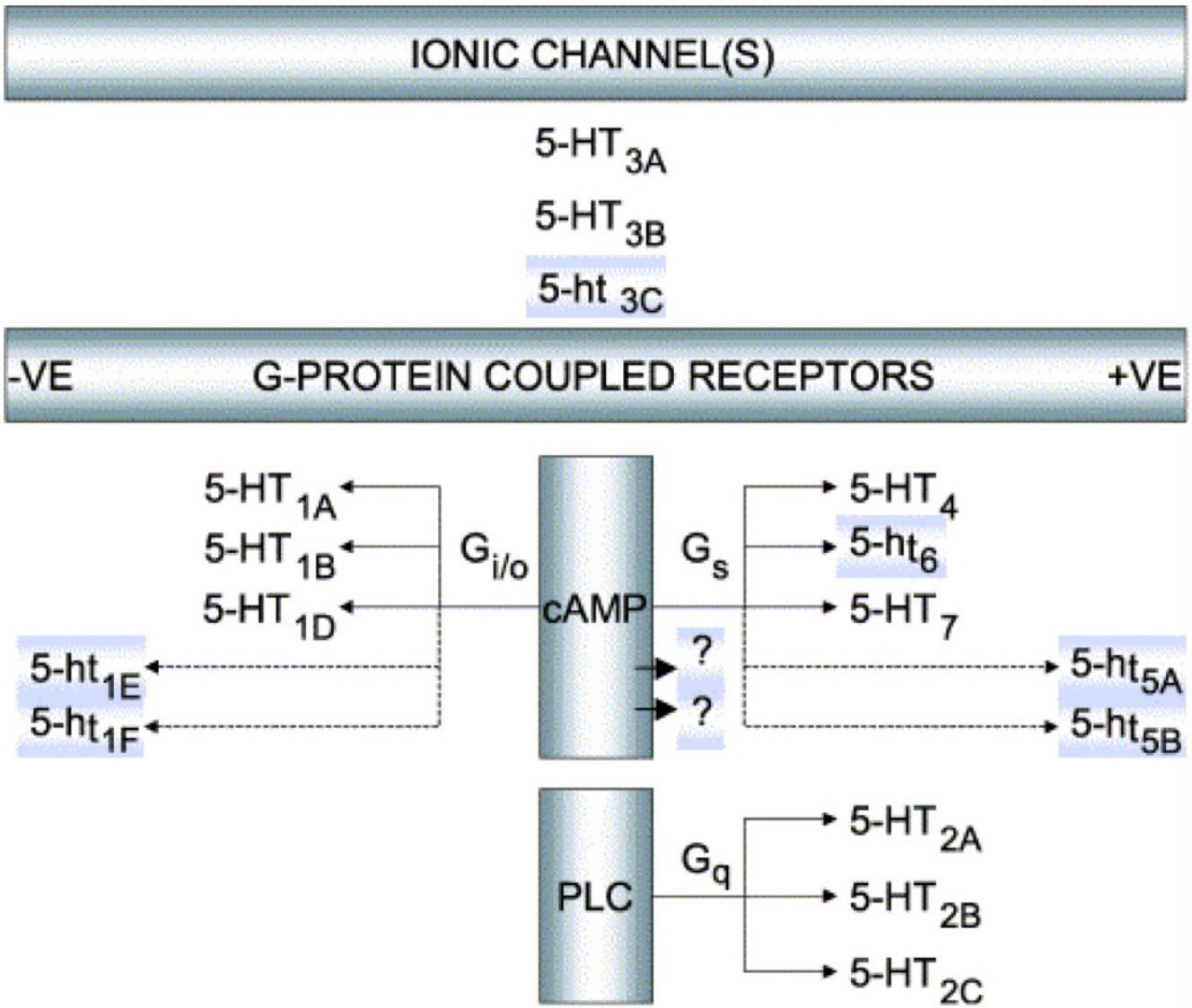
- 3 LGIC's
- 13 GPCR's

↓

couple to different G proteins

↓

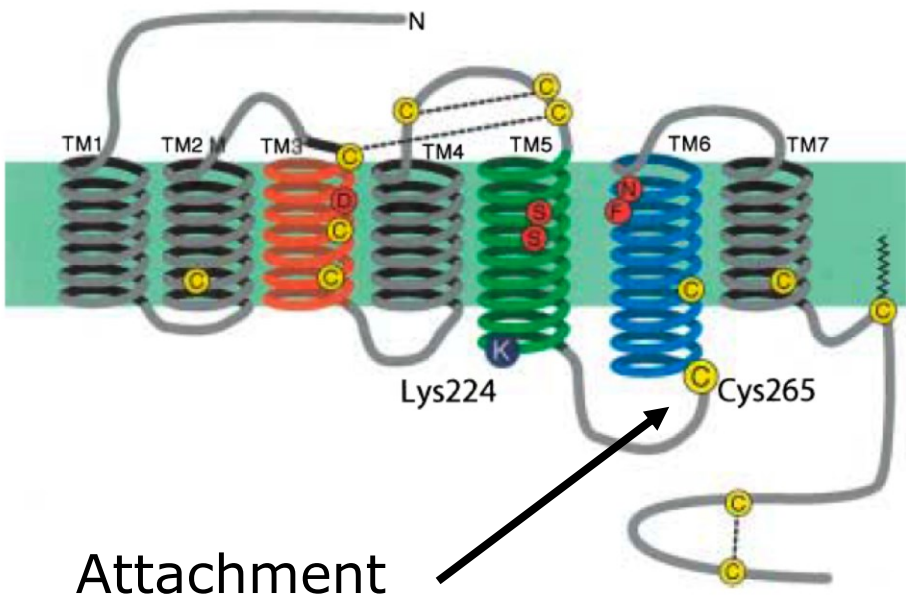
diversity of effects for 1 ligand



Signalling diversity - Who rules, the ligands?

Observation: different ligands for same receptor can yield different responses
=> do the ligands stabilise different receptor conformations?

Experiment: Fluorescently label a **purified adrenergic** receptor, and look to the fluorescence changes upon ligand binding.



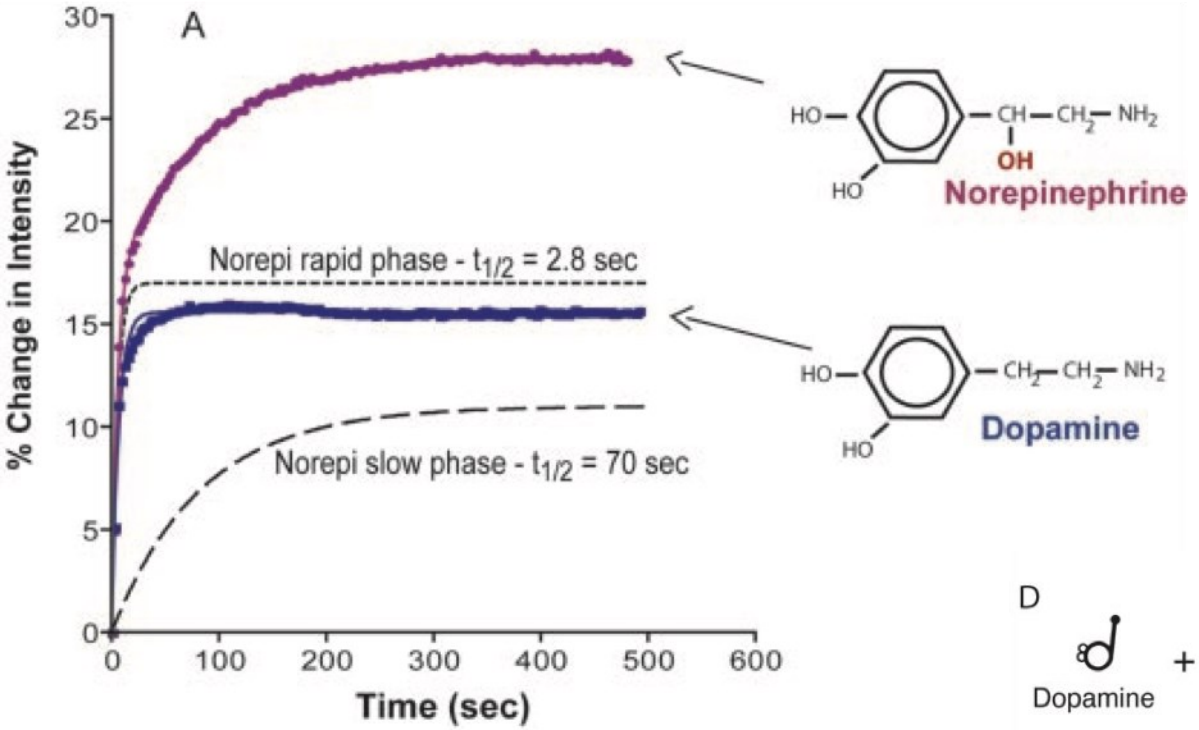
Attachment of fluorophore to Cys265

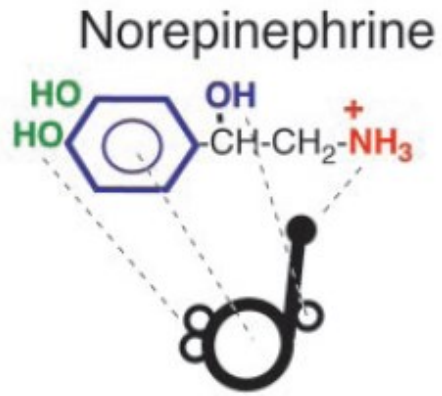
Brian Kobilka

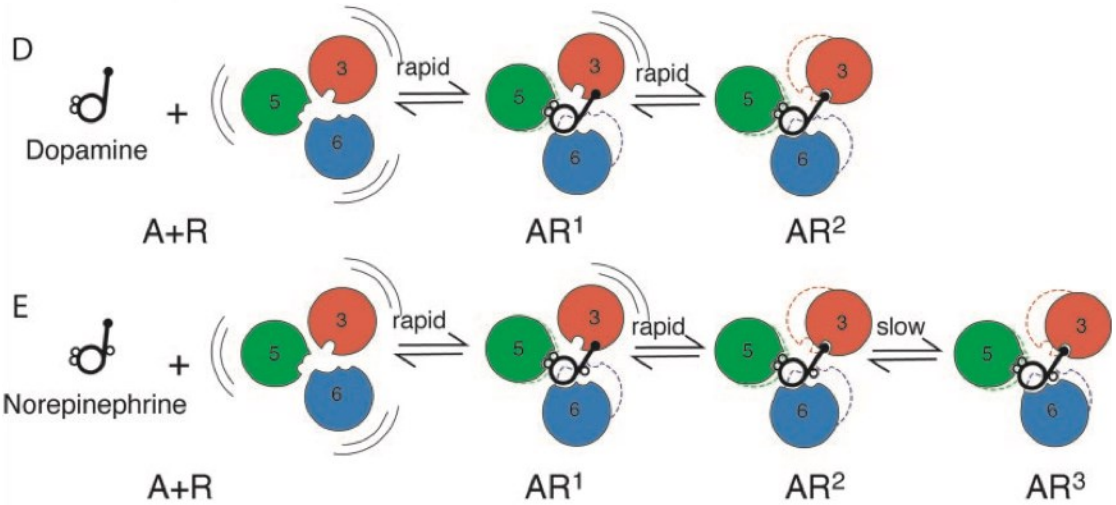
Signalling diversity - Who rules, the ligands?

Observation:
- some ligands feature only a rapid binding
- others have a biphasic (slow & fast) binding

=> Binding to different GRPC conformations





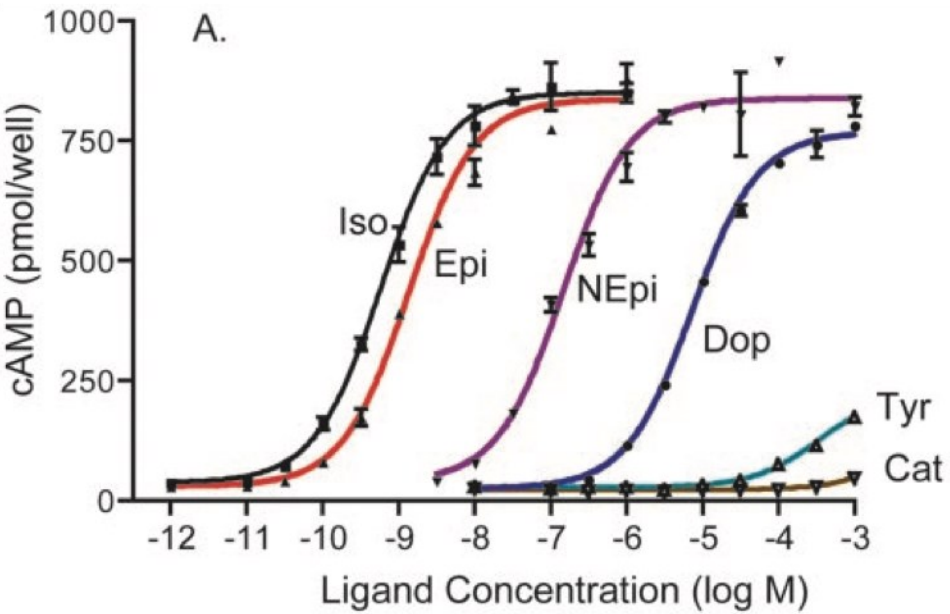


Swaminath, 2004 JBC

Signalling diversity - Who rules? The ligands too!

What happens in a live cell?

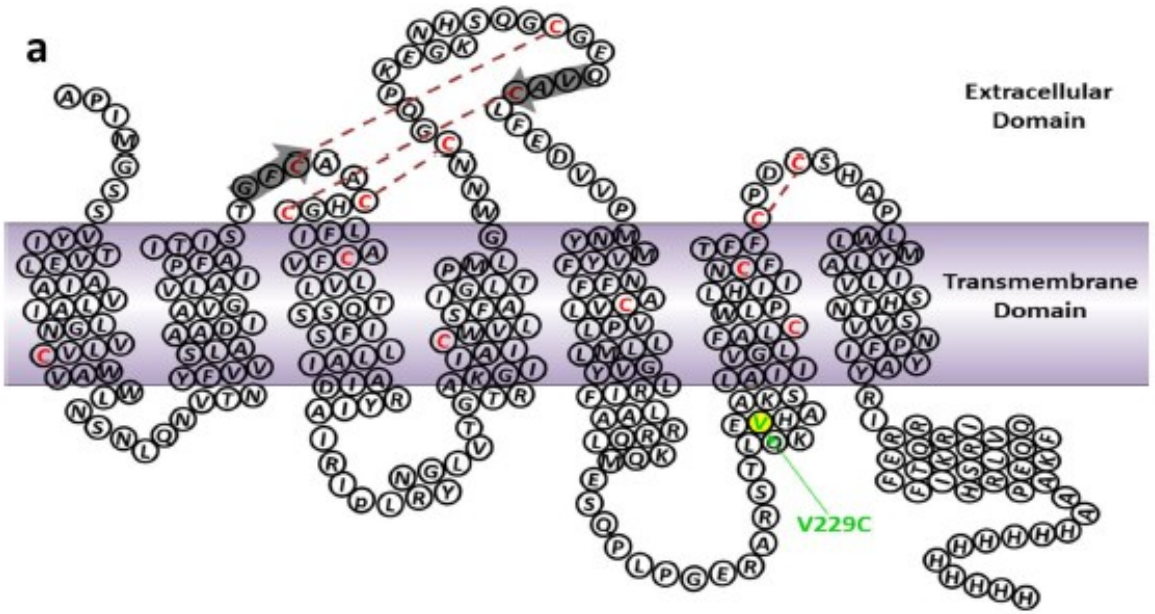
cAMP production:

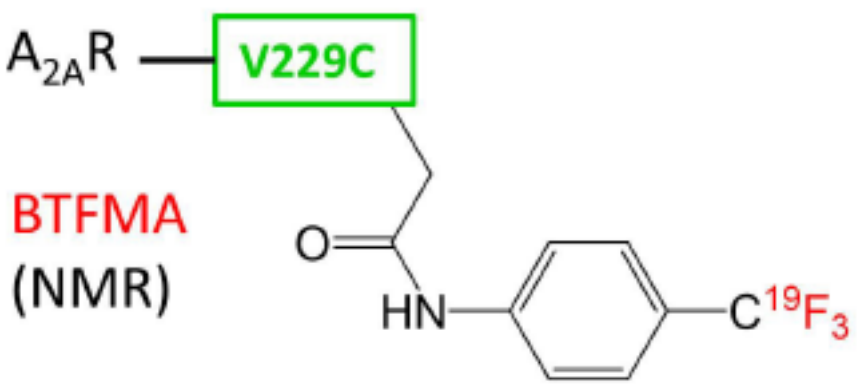


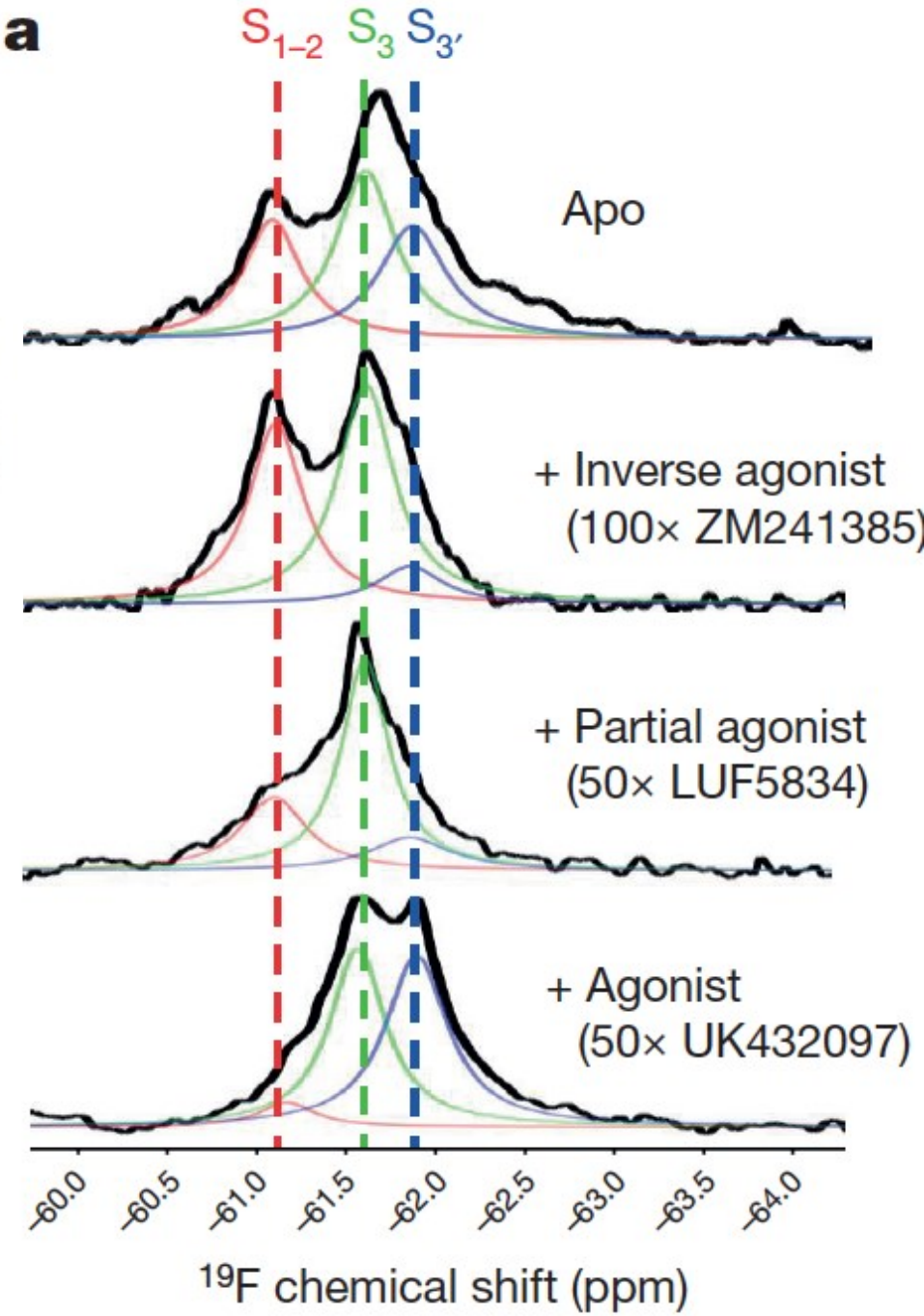
Iso, **Epi**, **NEpi** and **Dop** all stimulate cAMP production to same extent

Different ligands do stabilise different structures

Adenosine receptor A2A



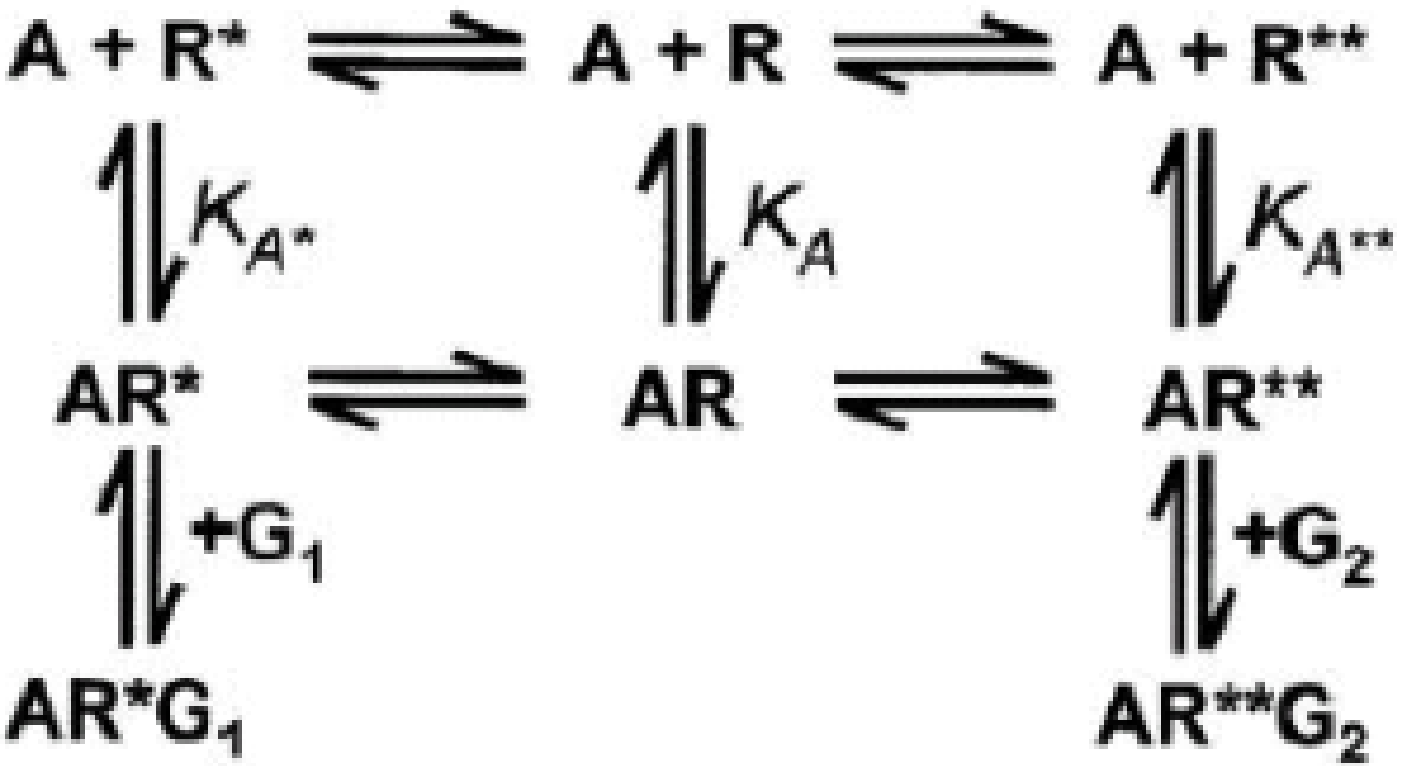




Ye et al; Nature 2016 (533) p265

Signal diversity : Conformational dynamics of receptors

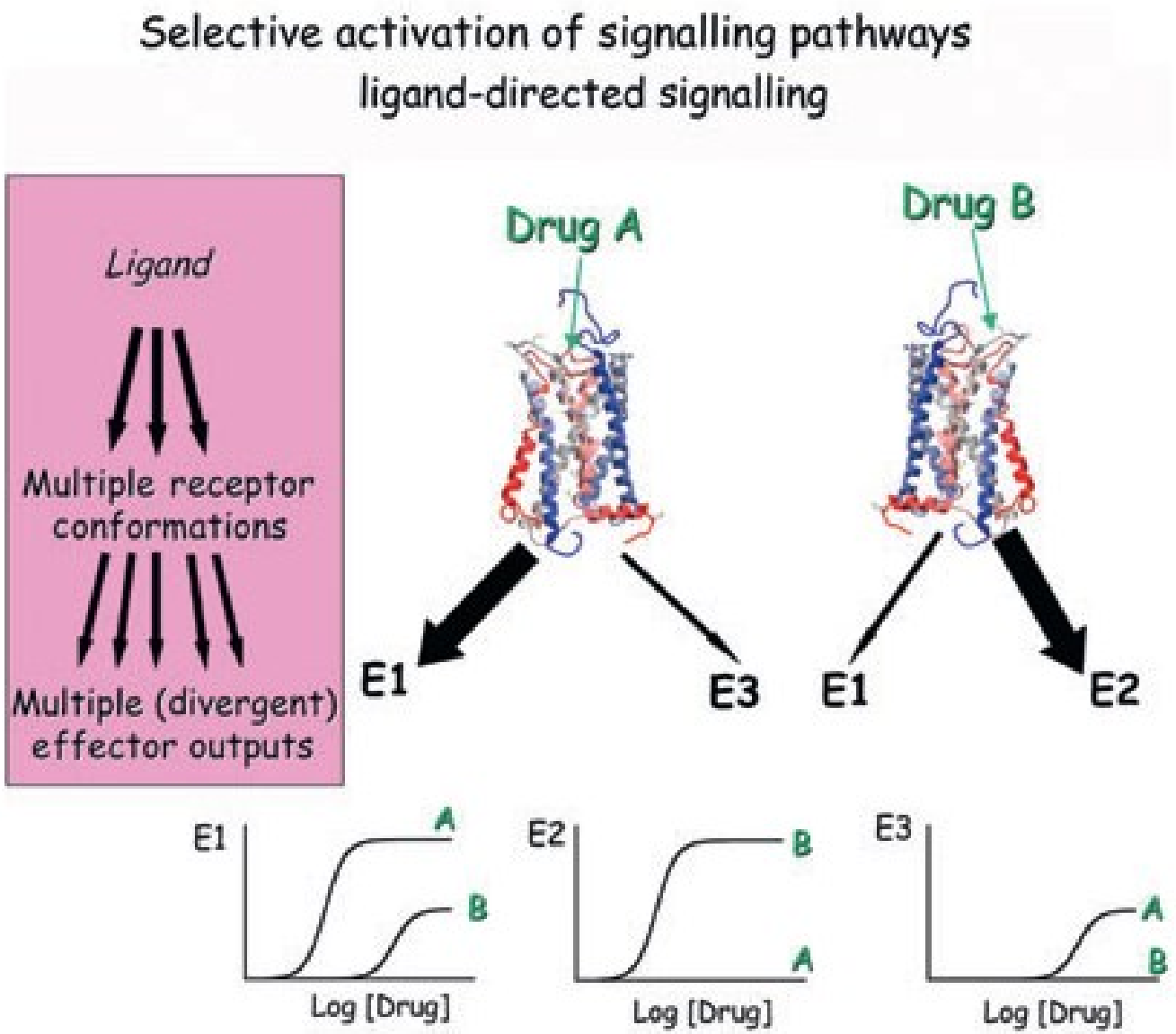
Observation: different ligands for same receptor can yield different responses as different ligands can stabilise different receptor conformations that can interact with diverse G-proteins



Signal diversity - Who rules, the ligands ?

Possible implications of different receptor ***conformations*** stabilised by different ligands changing cellular response patterns !

=> Relative ligand efficacies might change = **<u>Ligand bias</u>**.



G protein-coupled receptors

- GPCR's are a very prominent class of receptors
- GPCR structure is 7TM
- Receptor activation induces activation of G protein through GDP-GTP exchange
- G proteins propagate the signal
- Shut down of signalling
- Ligand-determined effects : Bias

Hilger 2018 GPCR signalling & Structure
Tan 2018 Biased ligands
Weis 2018 GPCR activation

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Hilger 2018 GPCR signalling & Structure
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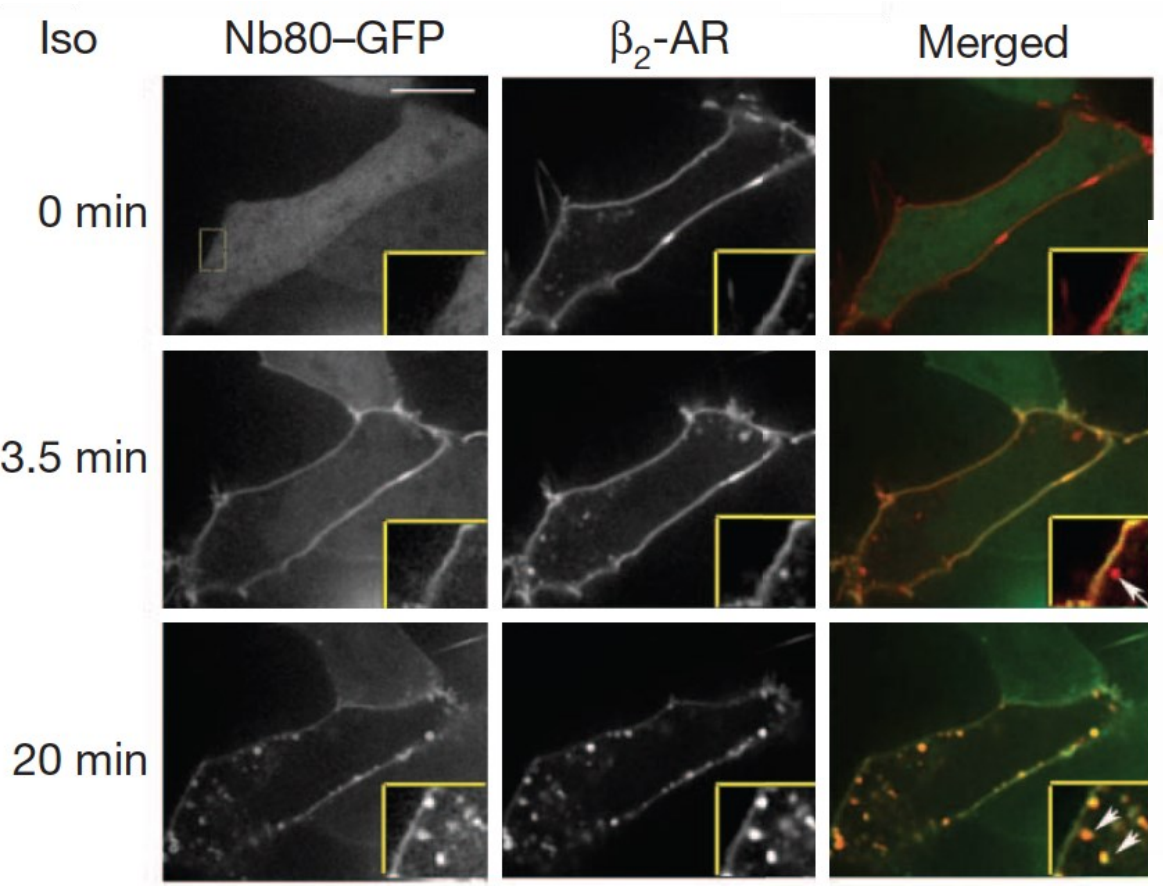
Weis 2018 GPCR activation

GPCR activity upon internalisation

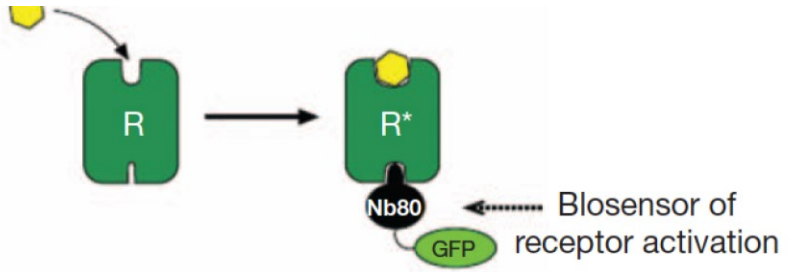
"Conformational biosensors reveal GPCR signalling from endosomes"

Conformationally-sensitive antibody fragments "**Nb**" fused to GFP

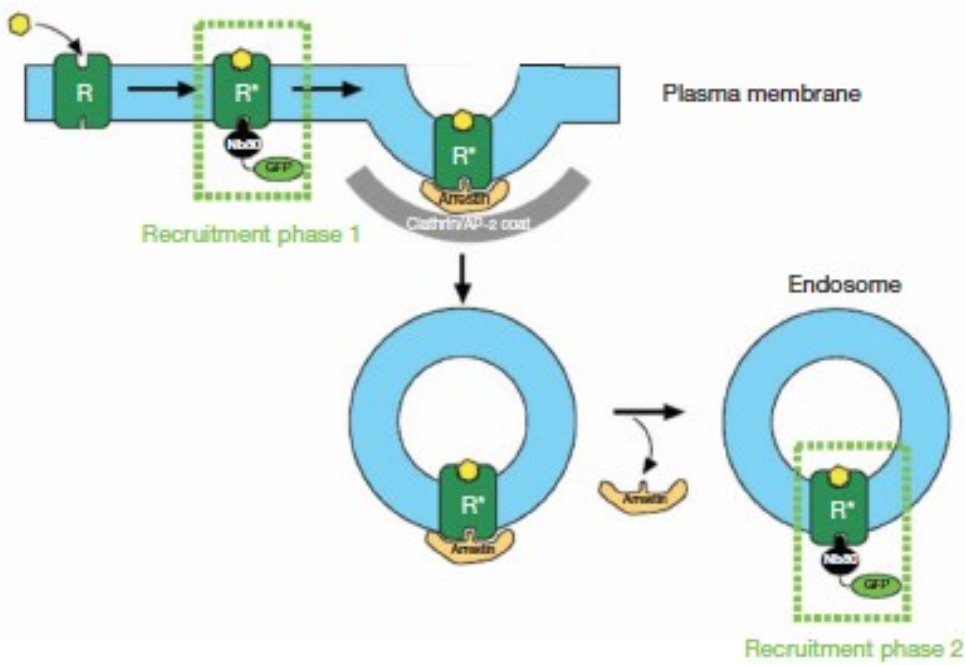
÷1 **Nb80 :: Agonist-activated adrenergic receptor (β_2-AR)**



red : 2β-AR green : Nanobody 80



=> Internalised receptor is ligand-bound !

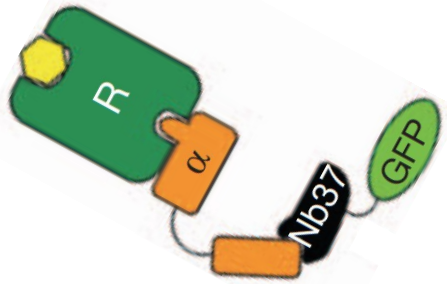


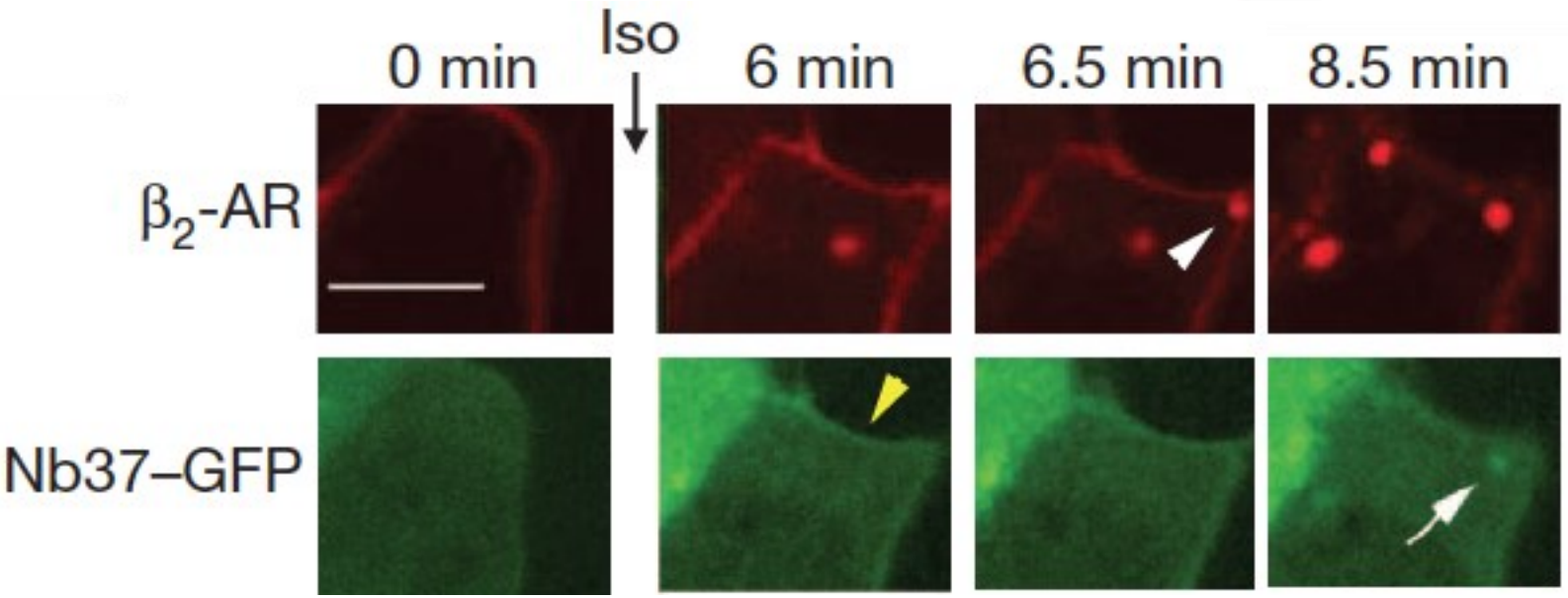
Irannejad et al 2013 Nature

GPCR activity upon internalisation

Conformationally-sensitive antibody fragments fused to GFP

÷2 **Nb37 :: Activated G-protein**





red : 2β-AR

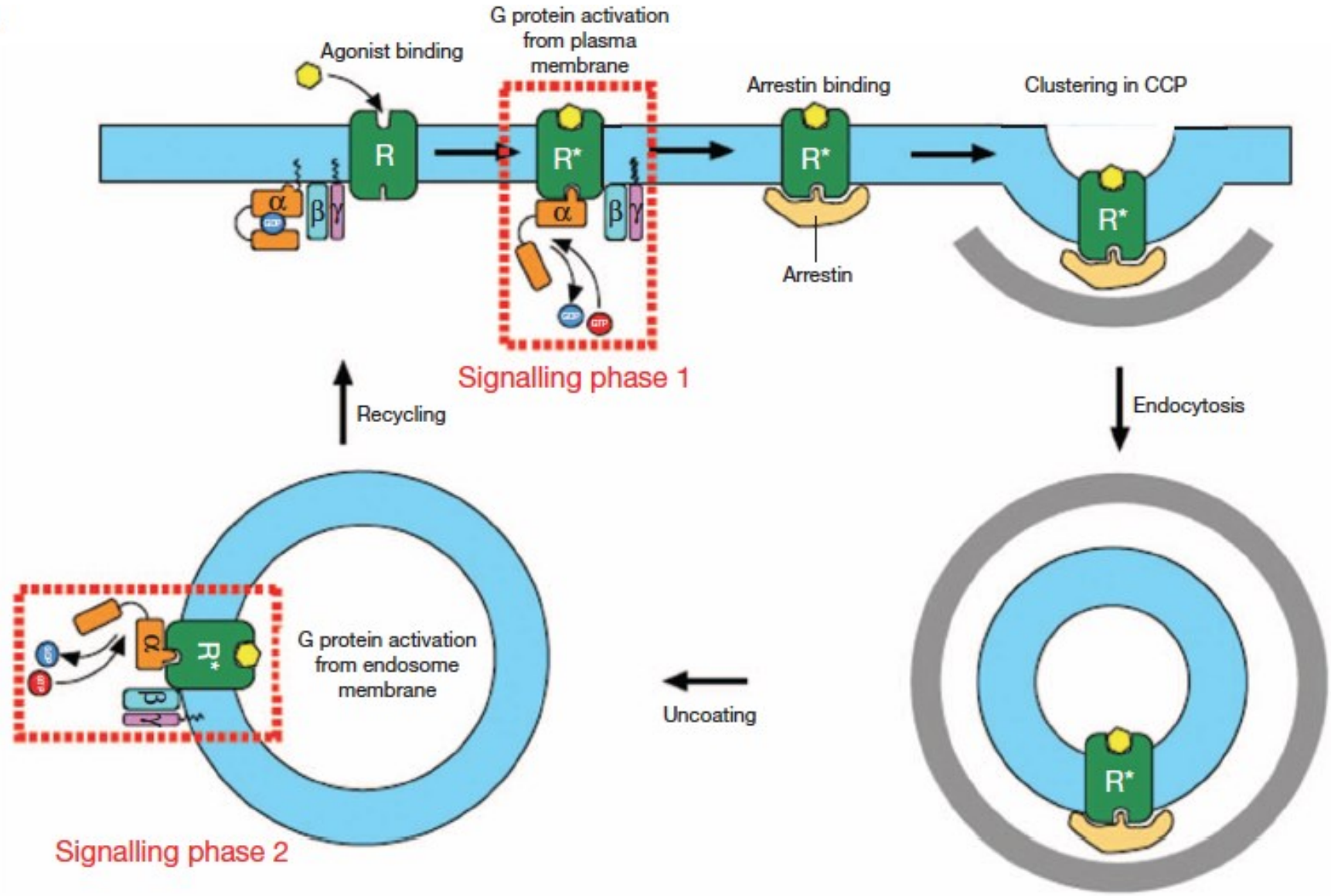
green : Nanobody 37

=> Internalised receptor interacts with activated G-protein

Irannejad et al 2013 Nature

GPCR activity upon internalisation

Internalised receptor has agonist bound & activates G-protein



Irannejad et al 2013 Nature

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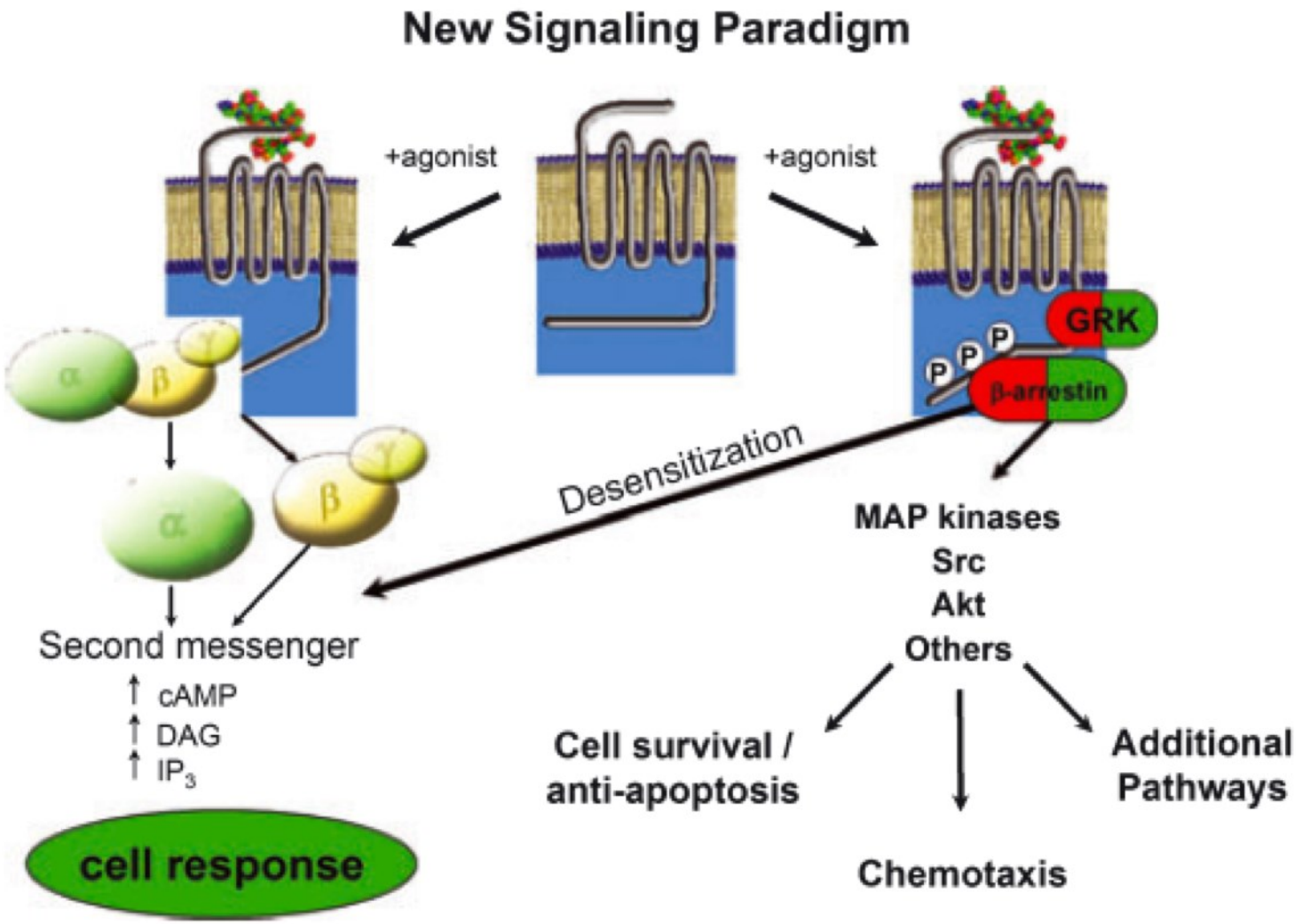
- Signalling upon internalisation
- Arrestin-mediated signals

Signalling specificity - Some ligands prefer arrestin

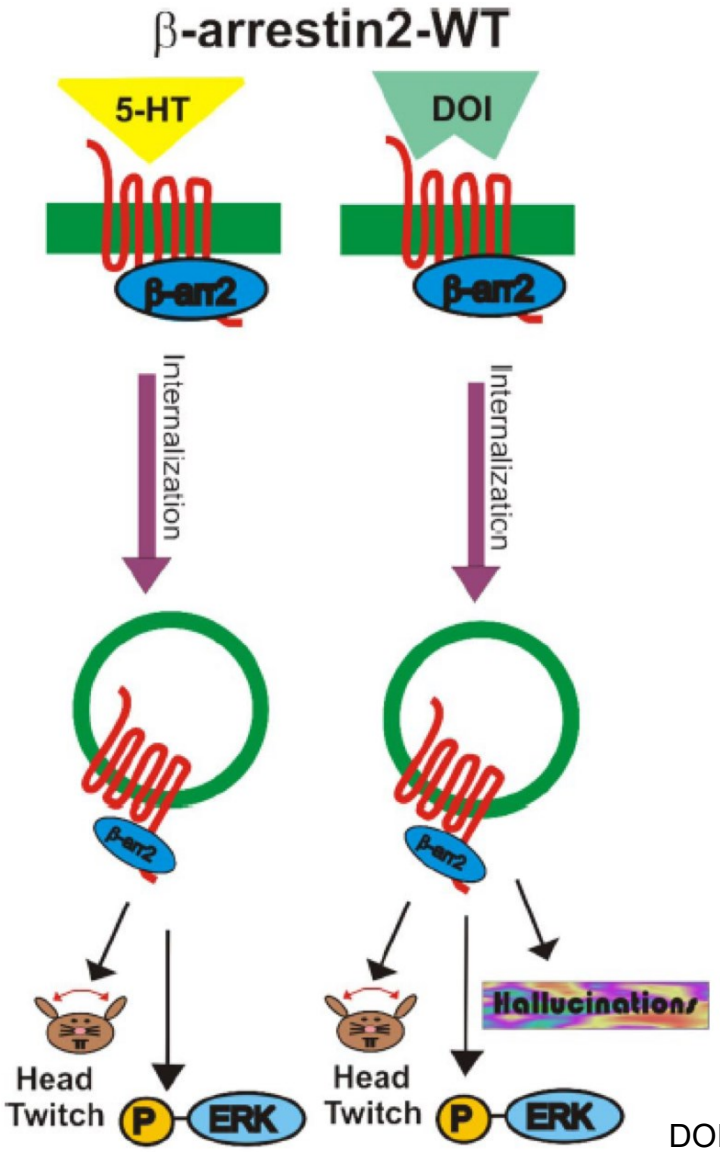
Observation : Some ligands signal in a G protein ***independent*** manner through an ***arrestin bound state***!!

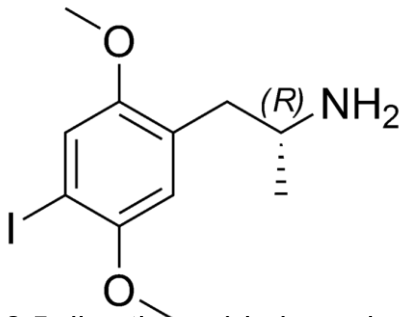


Lefkowitz, 2007

Signalling specificity - Some ligands do without G protein & arrestin

- Serotonin (5HT) and amphetamine DOI signal through the 5HT-2A receptor





DOI = 2,5-dimethoxy-4-iodoamphetamine

- Knock-out of arrestin suppresses effect serotonin (5HT), but not DOI

Roth, 2008

G protein-coupled receptors

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- Arrestin-mediated signals
- Finding ligands

Exploiting ligand-induced bias for tailored medication

Ligand bias: the ligand prefers one pathway more than another
e.g. the Angiotensin receptor AT_1

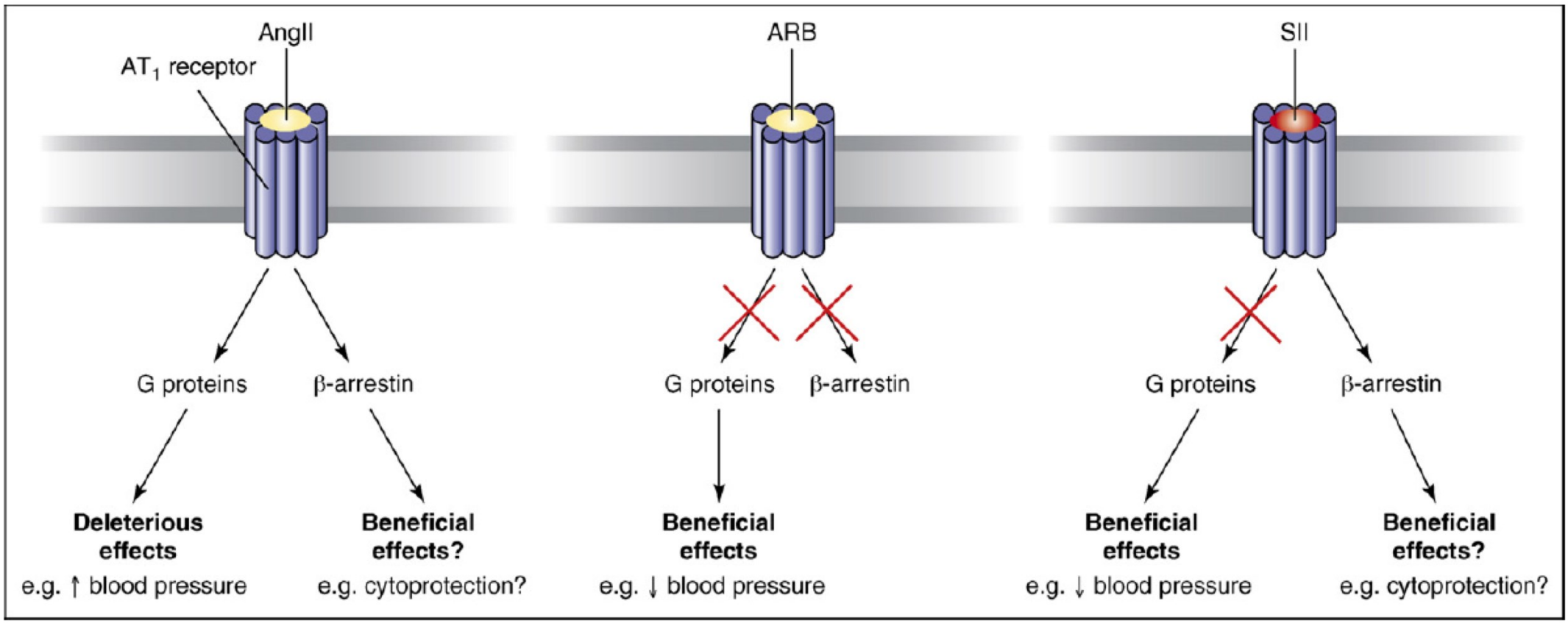
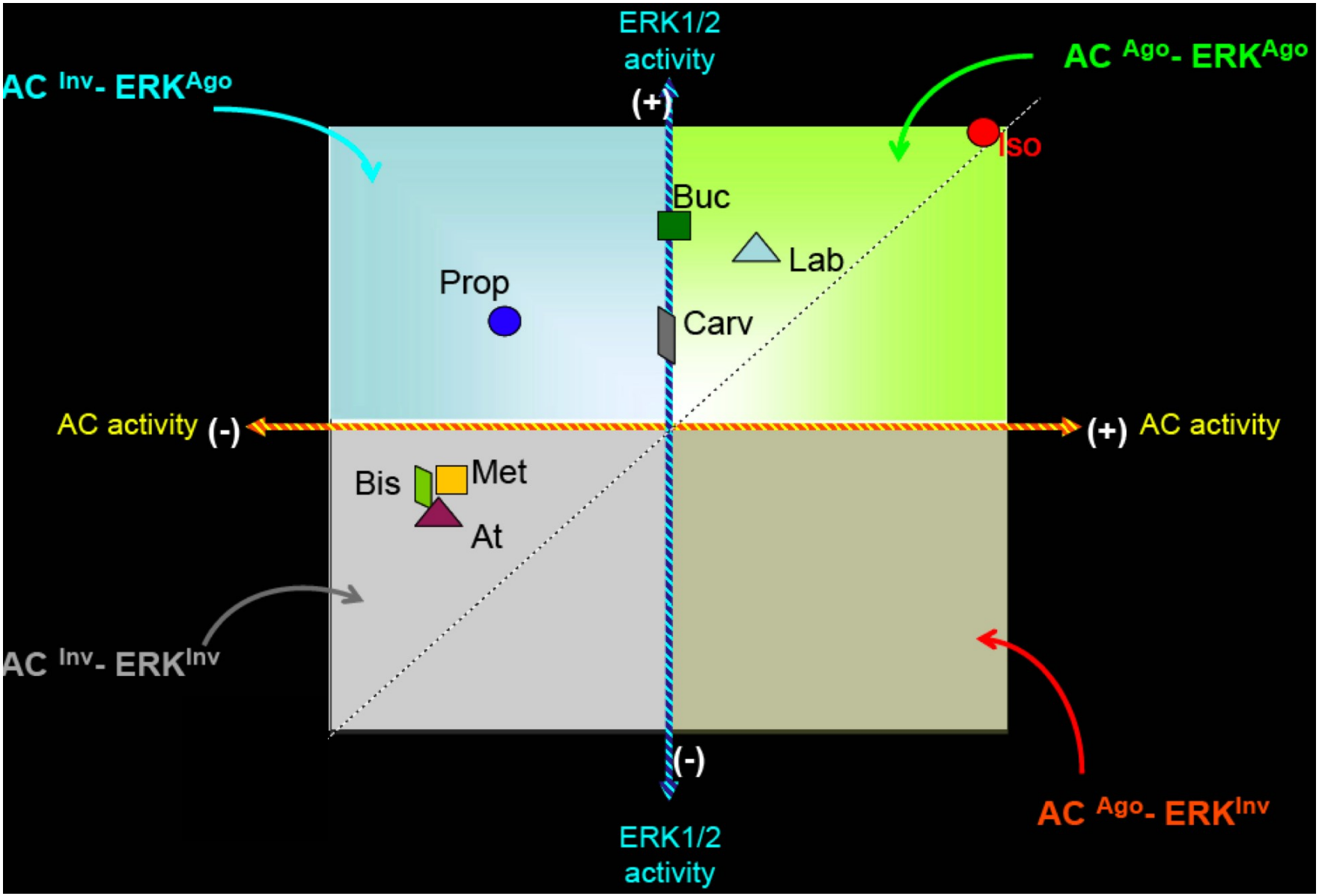


Figure 3. Biased agonism at the angiotensin II type 1 (AT_1) receptor. The endogenous agonist angiotensin II (AngII) stimulates both G-protein and β-arrestin signals from the AT_1 receptor and thus increases blood pressure and stimulates β-arrestin signals, such as ERK, Akt and PI3K, that might be beneficially cytoprotective.

AngII: Angiotensin II , natural agonist **ARB**: AT_1 receptor blockers **SII**: AngII derivative

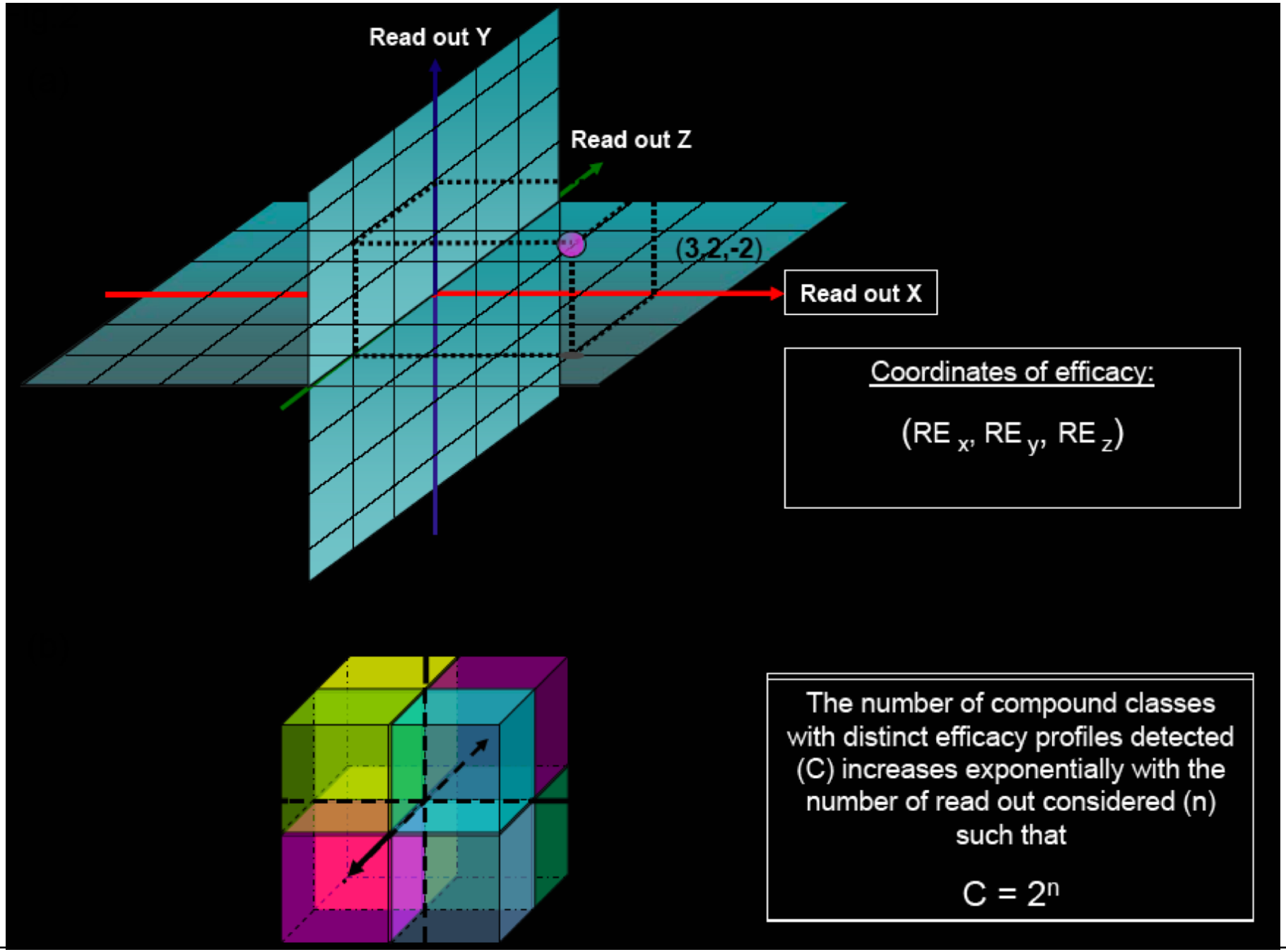
Receptor signalling - Multi-dimensional efficacy patterns

Adrenergic receptor signalling: Ligand bias for AC or ERK pathways



Bouvier, 2009

Multidimensional efficacy patterns - Headache or bonus?

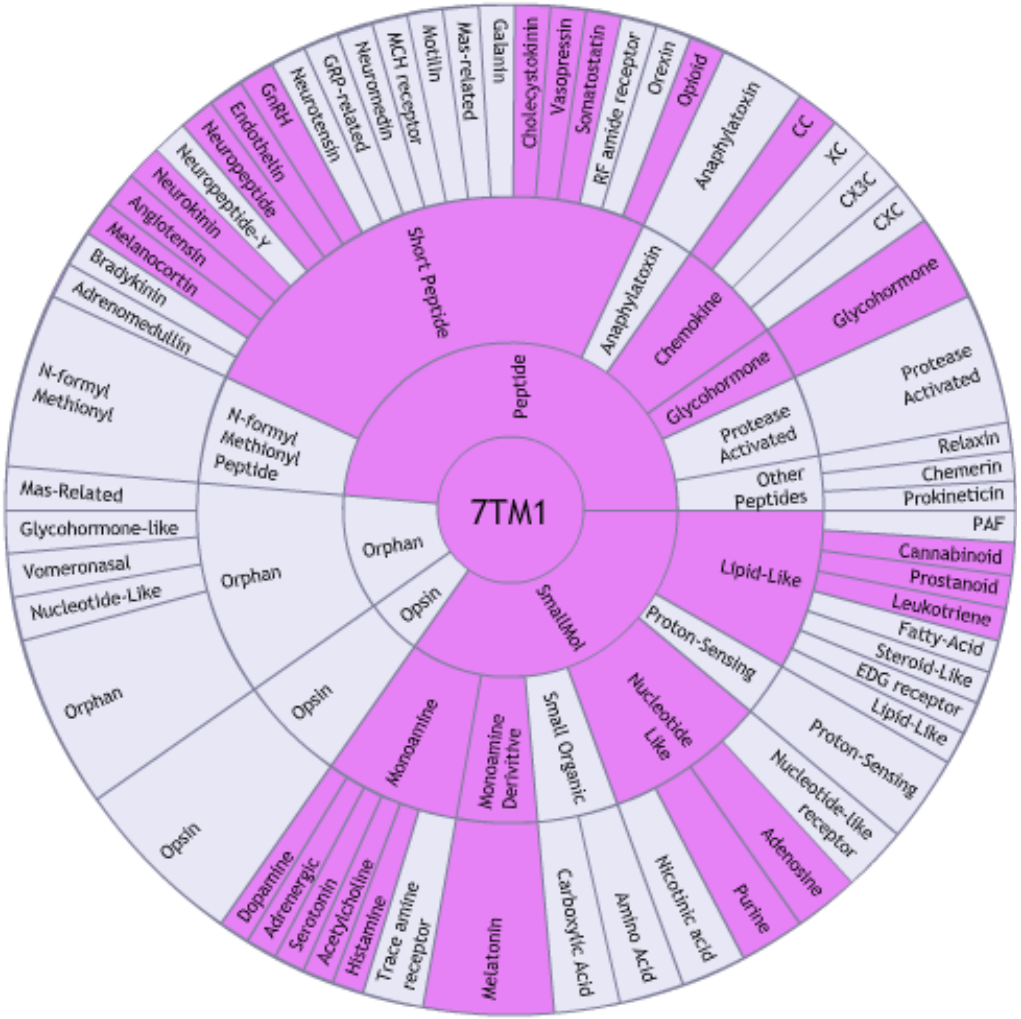


Bouvier, 2009

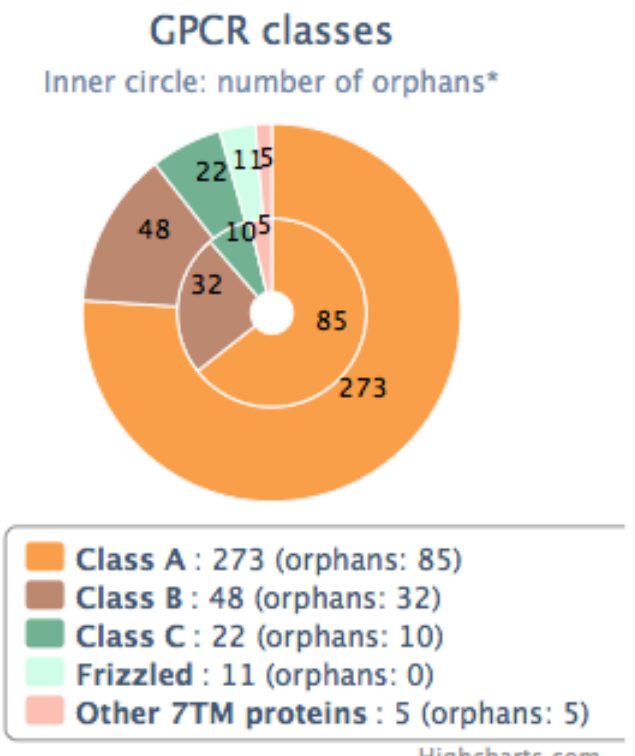
Screening : Looking for a needle in a haystack

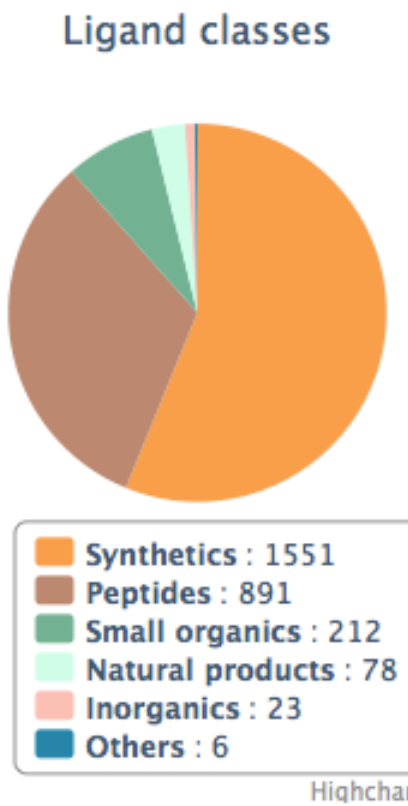
923 genes for GPCR's

Drugs for only 359 ….



ChEMBL | Clinical Candidate | Approved Drug | Reset





Approved drugs

www.ebi.ac.uk/chembl/sarfari/gpcrsarfari/family

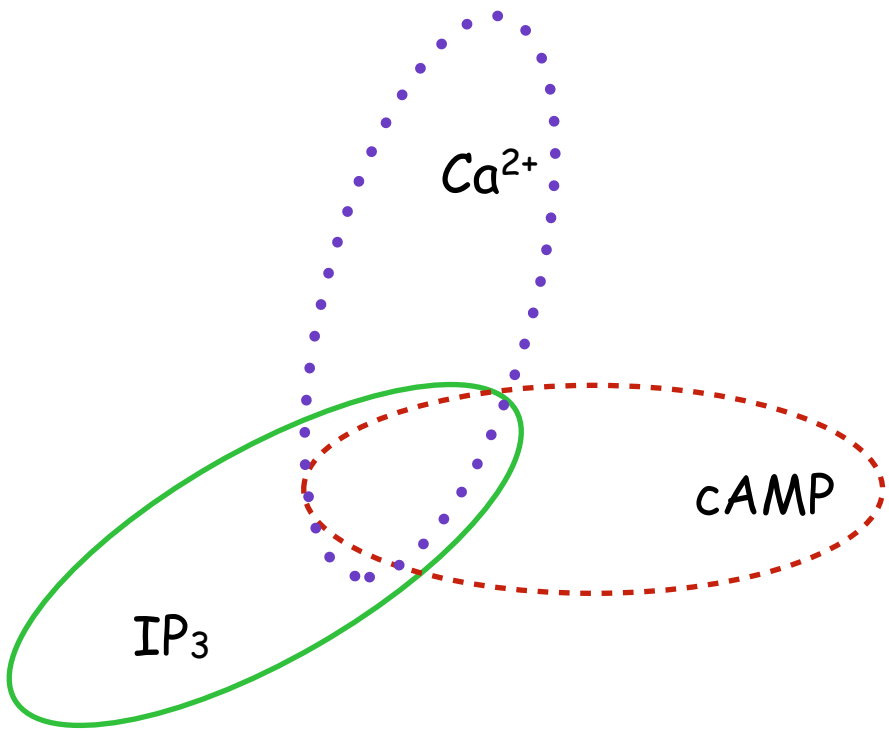
Source: ChEMBL

Screening,
for what ?
how to do ?

Screening : Looking for a needle in a haystack

High throughput screening (HTS) or High content screening (HCS)

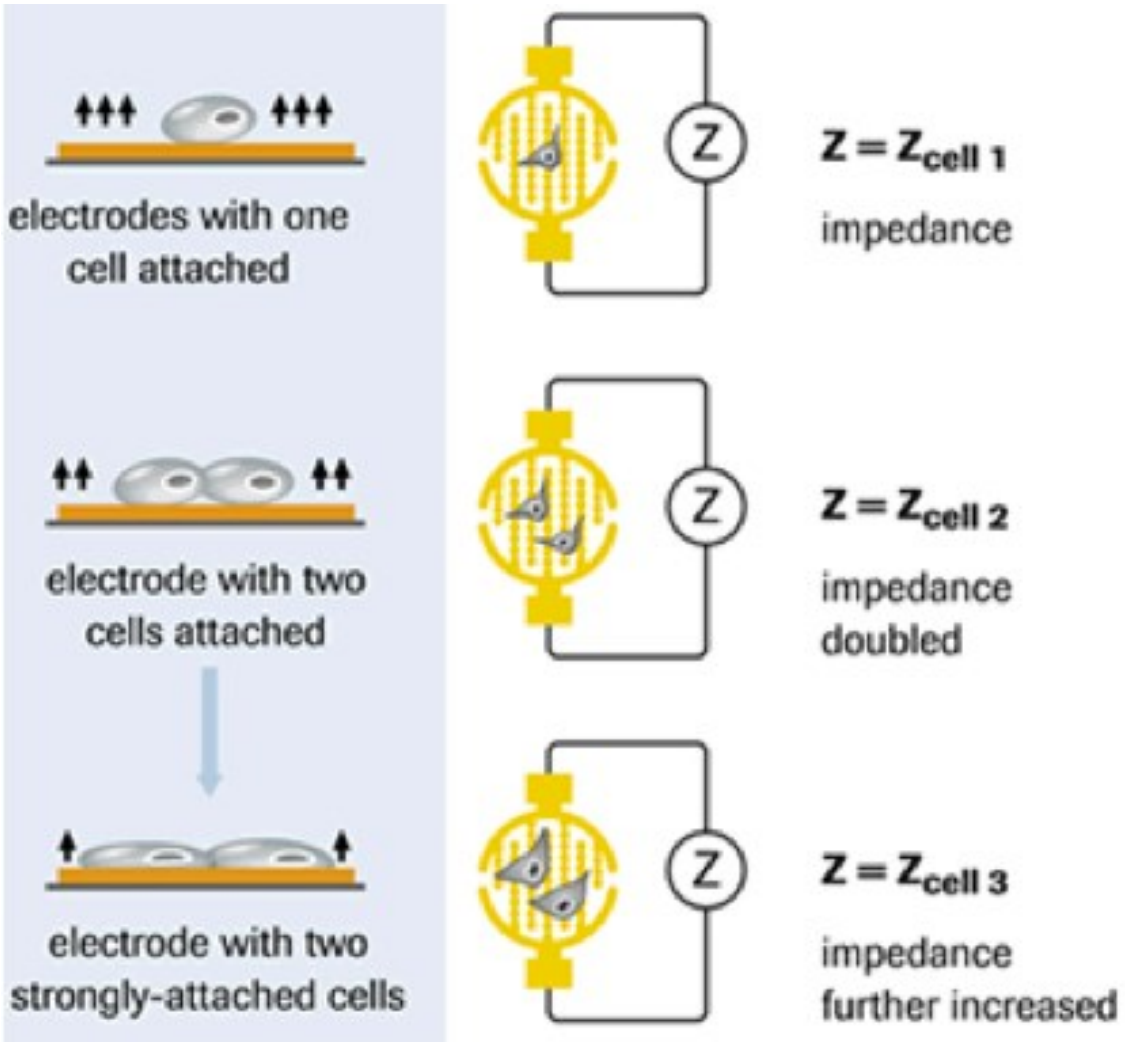
- quantification of a **specific** parameter, e.g.
  - Concentration of second messenger
    - ÷ Ca^{2+}
    - ÷ cAMP
    - ÷ IP_3
  - Displacement
    - ÷ arrestin recruitment
    - ÷ receptor internalisation



Screening : Looking for a needle in a haystack with holistic assays

Cell growth

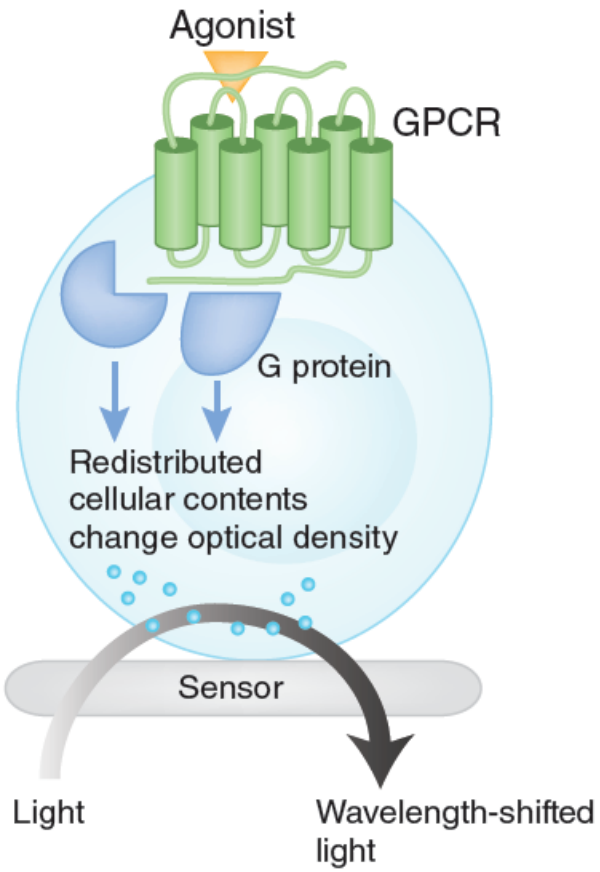
- Physionometer (Harden McConnel, 1991) => to early...
- Impedance Spectroscopy (~2005)

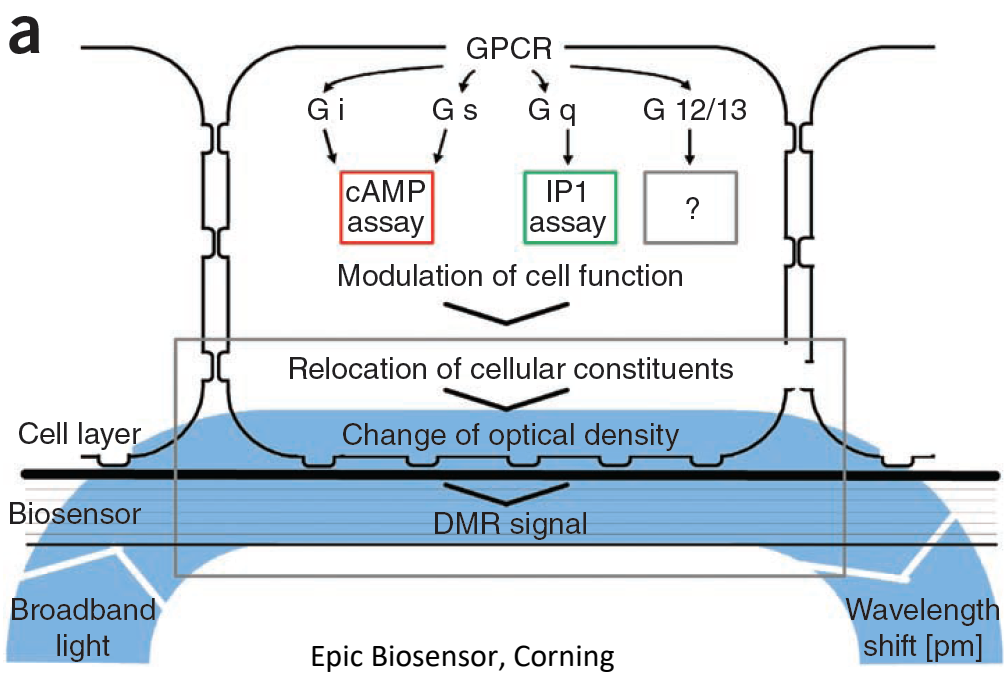


Roche : xCELLigence System

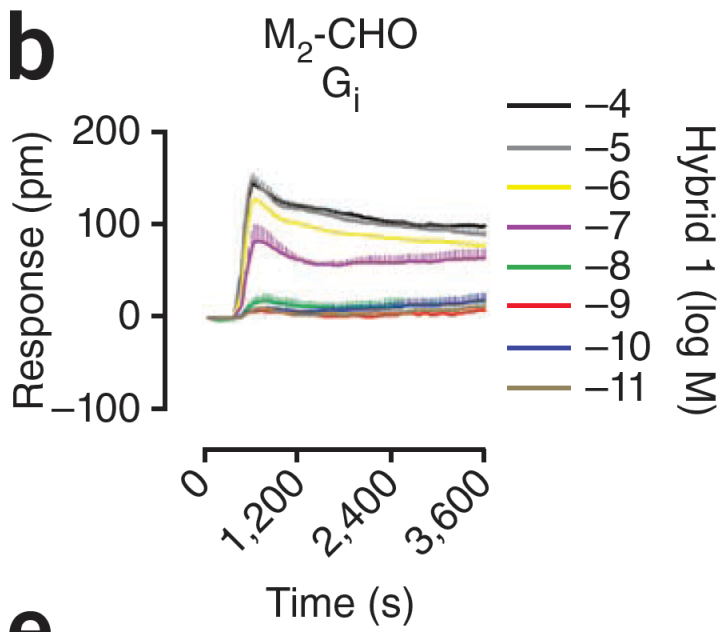
Screening : Looking for a needle in a haystack with holistic assays

- Dynamic Mass Redistribution (2010)





Epic Biosensor, Corning



Kenakin & Schröder (2010) Nat Biotech

Finding small molecule binders

- The cellular effect depends on a complex interplay between
  - ligand structure
  - receptor
  - intracellular signalling components

- The assays can be separated in two groups:

  - detailed vs global assay methods

  or

  deterministic vs holistic

G protein-coupled receptors

- GPCR's are a very prominent class of receptors
- GPCR structure is 7TM
- Receptor activation induces activation of G protein through GDP-GTP exchange
- G proteins propagate the signal
- Shut down of signalling
- Ligand-determined effects : Bias
- Signalling upon internalisation
- Arrestin-mediated signals
- Finding ligands
- Location

GPCR's : Active only in the plasma membrane?

Generally accepted view :

"GPCRs are cell surface receptors and are generally assumed to signal to second messengers such as cAMP exclusively from the plasma membrane"

Calebiro 2010 TiPS

Adopted view:
"….and might also signal once internalized" *Irannejad 2013 Nature*

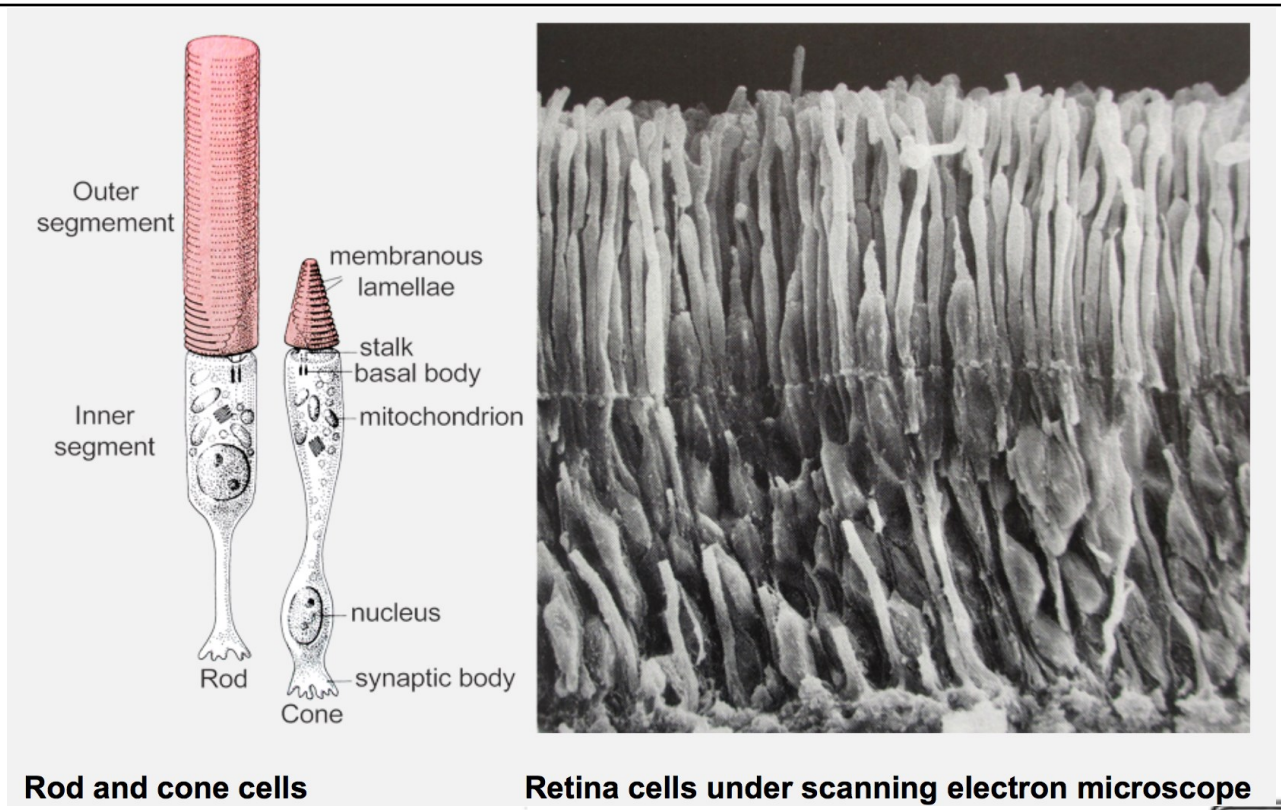
However,
Several observations extend on this:

I • Rhodopsin

II • Odorant receptors

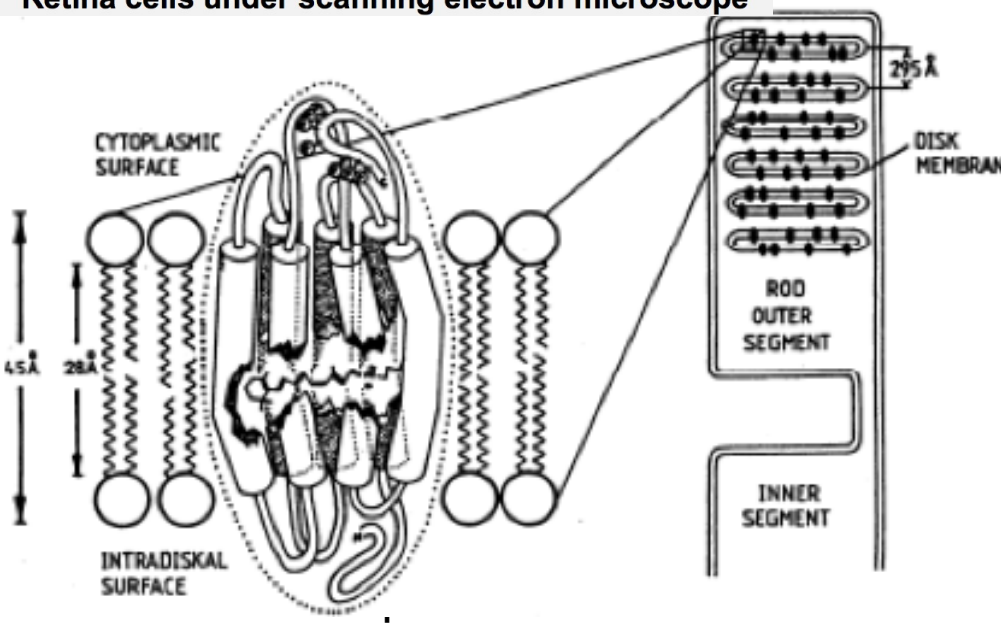
III • Nuclear, ER or Golgi resident GPCR's

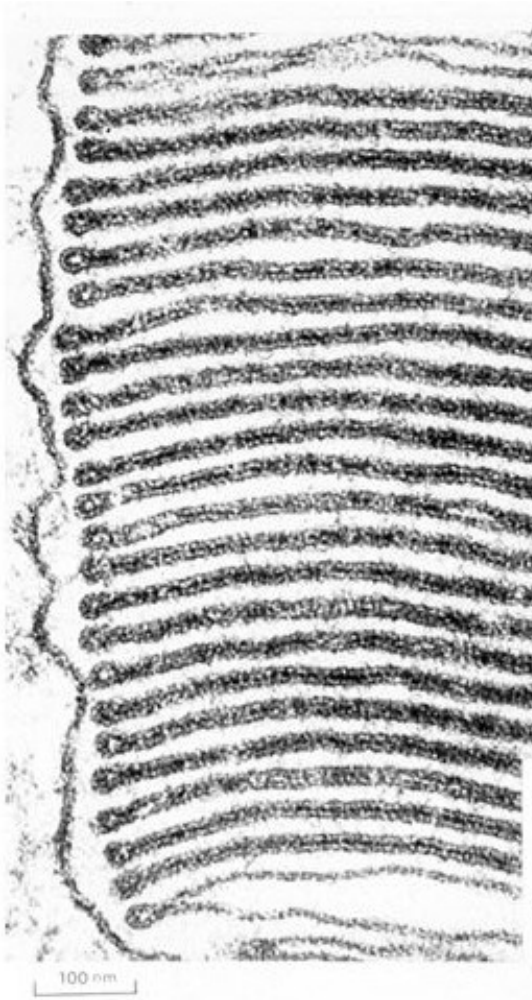
I - Rhodopsin



Rod and cone cells

Retina cells under scanning electron microscope



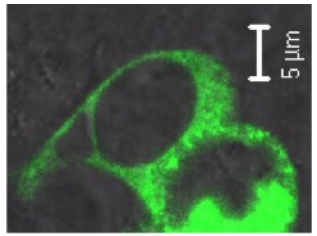


Rod-outer segment
transmission EM

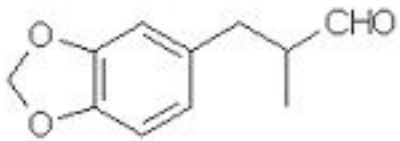
II - Odorant receptors

Odorant receptors:

- Predominant intracellular localisation upon heterologous expression



e.g. OR17-40, activated by e.g. helional

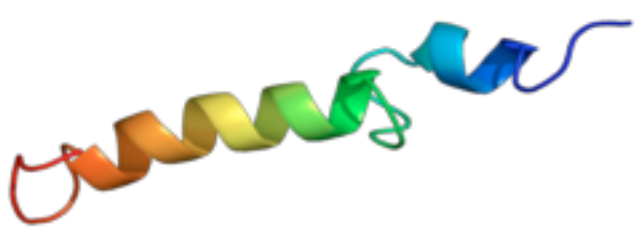


III -Some GPCR's are resident within the cell

"Intracellular" GPCR's of all 3 families have been found

	GPCR subfamilies	Cell/Tissue	Function
1989-93	Family 1 mAChR	Cornea, corneal epithelial/endothelial cells	↑DNA and RNA Pol II activity,
2000-06	Family 2 PTH_1R	Kidney, liver, gut, uterus, ovary,	DNA synthesis, mitosis?
2003	Family 3 mGluR5	Neuronal cells, transfected HEK293 cells	↑nuclear (Ca^{2+})
2012	Cannabinoid receptor in mitochondria		Affects energy metabolism

PTH : parathyroid hormone



Boivin 2008 J Receptor Signal Transduction

GPCR's resident within the cell

≥ 30 GPCR's have been shown to never go to the PM, and to be fully active.

Even within the inner nuclear membrane.

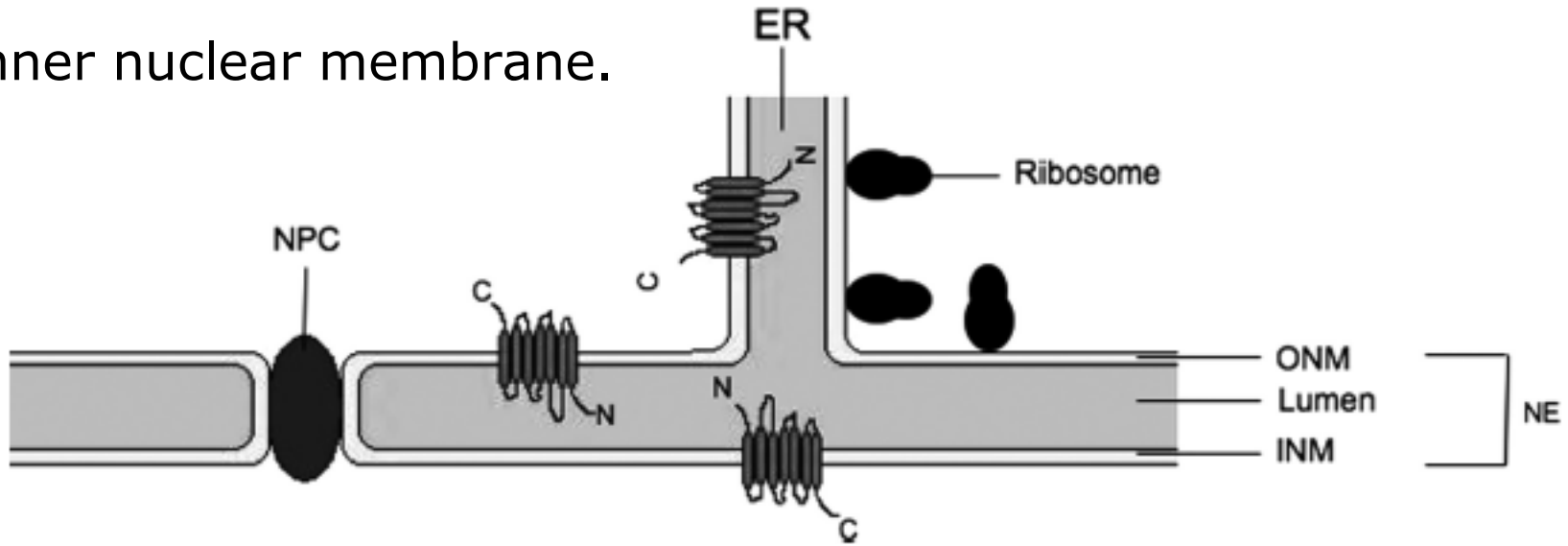


Figure 1: Schematic representation of the endoplasmic reticulum (ER) and nuclear envelope (NE). The outer nuclear membrane (ONM) is continuous with both the ER and the inner nuclear membrane (INM), with which it is joined at the level of nuclear pore complex (NPC) insertion. Also depicted is the predicted topology of G protein-coupled receptors (GPCRs) located either on the ONM or INM as well as in the ER.

Questions:

- How do ligands get to these GPCR's ?
- Which signalling pathways ?
- Are GPCR interacting proteins present ?

Boivin 2008 J Receptor Signal Transduct

Cellular localisation of GPCRs

More and more GPCR's have been shown to be active within the cell

- some are still active after internalisation
  often with a different signalling pathway

- some are only active within the cell

G protein-coupled receptors

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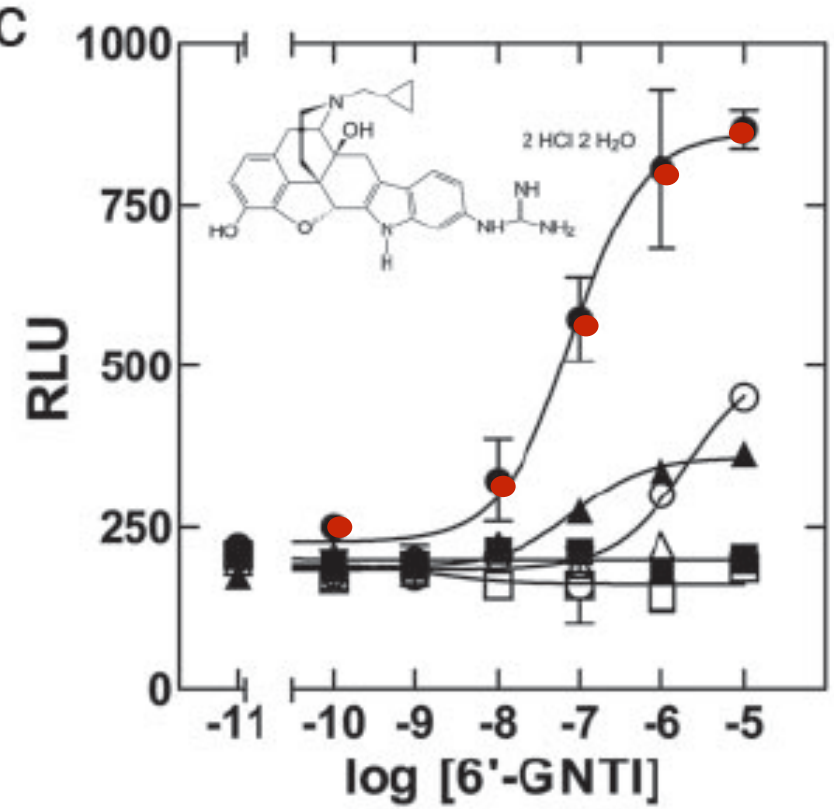
- Shut down of signalling
- Ligand-determined effects : Bias
- Signalling upon internalisation
- Arrestin-mediated signals
- Finding ligands
- Location
- Loners or collaborators ?

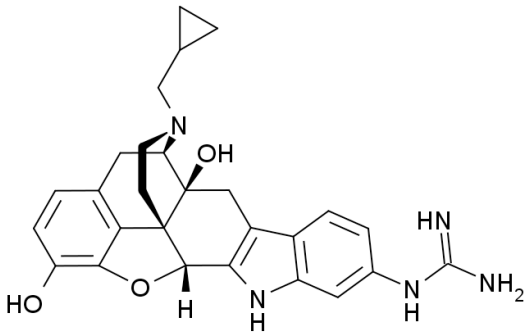
1 Opioid receptors : Functional oligomers

- Pharmacology can be affected by co-expression of GPCRs, e.g.

δ, **κ** or **μ** opioid receptors form homo- and heterodimers upon (co-)expression

-> Quantify Ca^{2+}-signalling ("RLU") induced by the OP-R agonist 6'GNTI

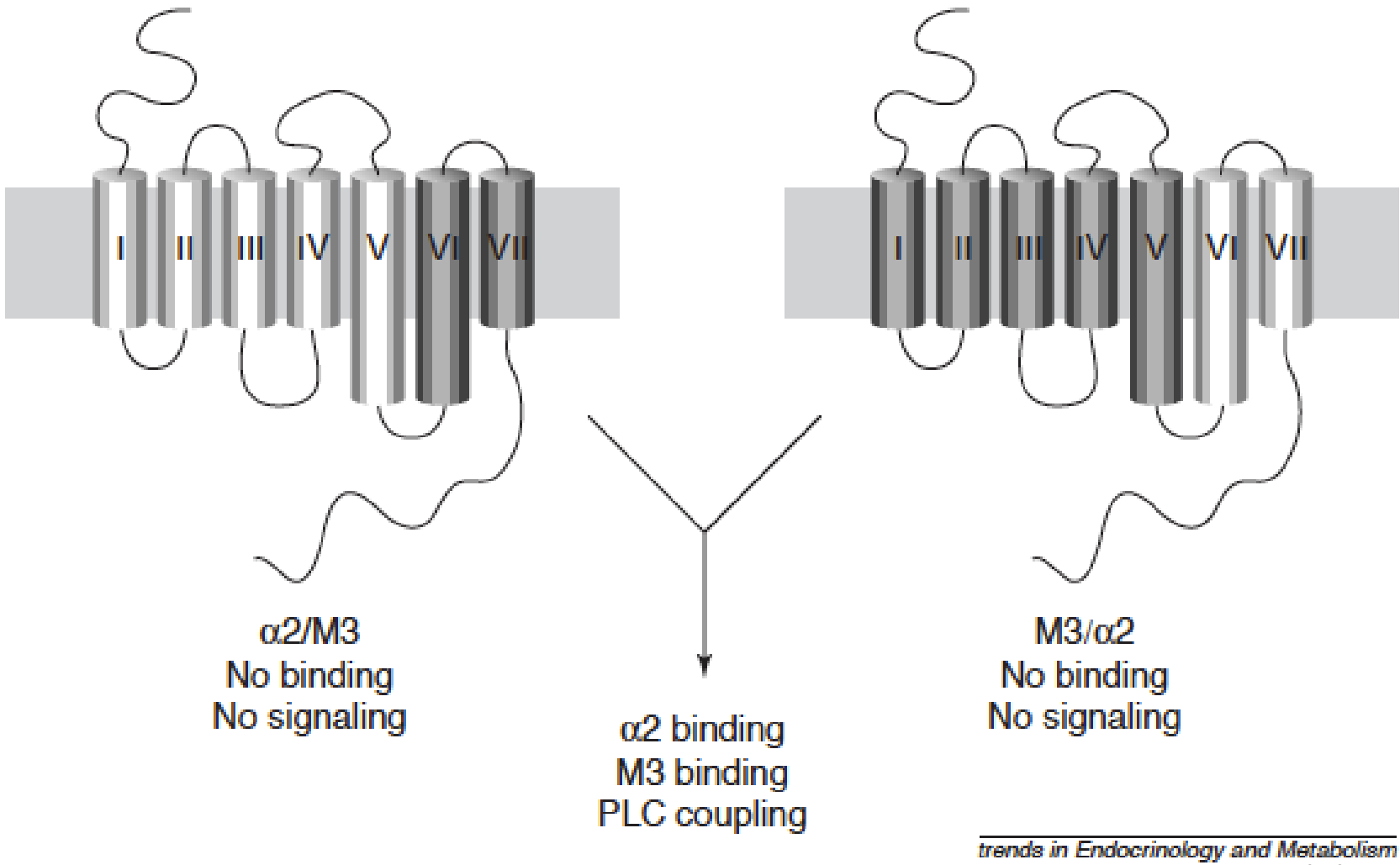




Waldhoer 2005 PNAS

2 - Structural oligomers of chimera's

- Pharmacology can be affected by co-expression, e.g. a model experiment:
  Trans-complementation upon the expression of complementary chimera's of α2-adrenergic and M3-muscarinic acetylcholine receptors:

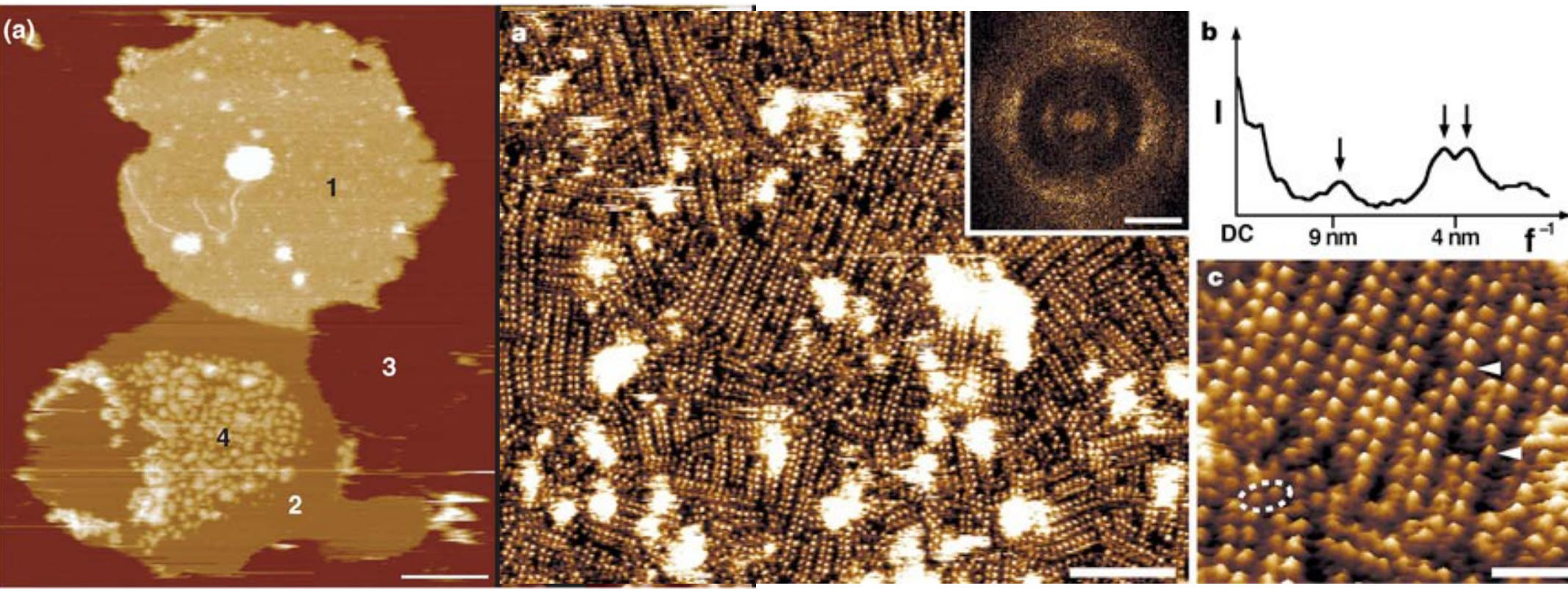


trends in Endocrinology and Metabolism

Salahpour TEM *2000*

3 - Rhodopsin : Structural dimer

- Microscopic imaging, e.g
  atomic force microscopy on disk membranes from cone cells



50 nm

15 nm

Fotiadis, 2006, Curr Op Struc Biol

Fotiadis, 2003, Natur

III - Rhodopsin : Rather monomers than dimers !?!

However, recent experiments with purified Rho in detergent indicate that monomers are perfectly active..

Transducin Activation by Nanoscale Lipid Bilayers Containing One and Two Rhodopsins*[S]

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Monomeric G protein-coupled receptor rhodopsin in solution activates its G protein transducin at the diffusion limit

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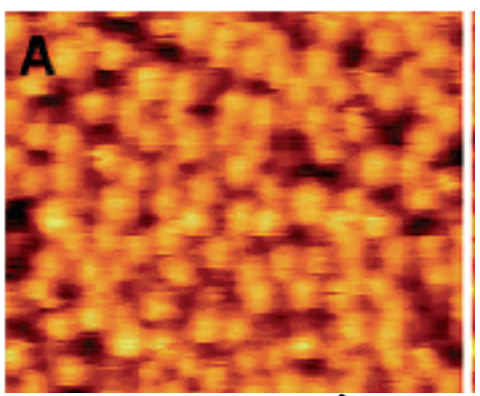
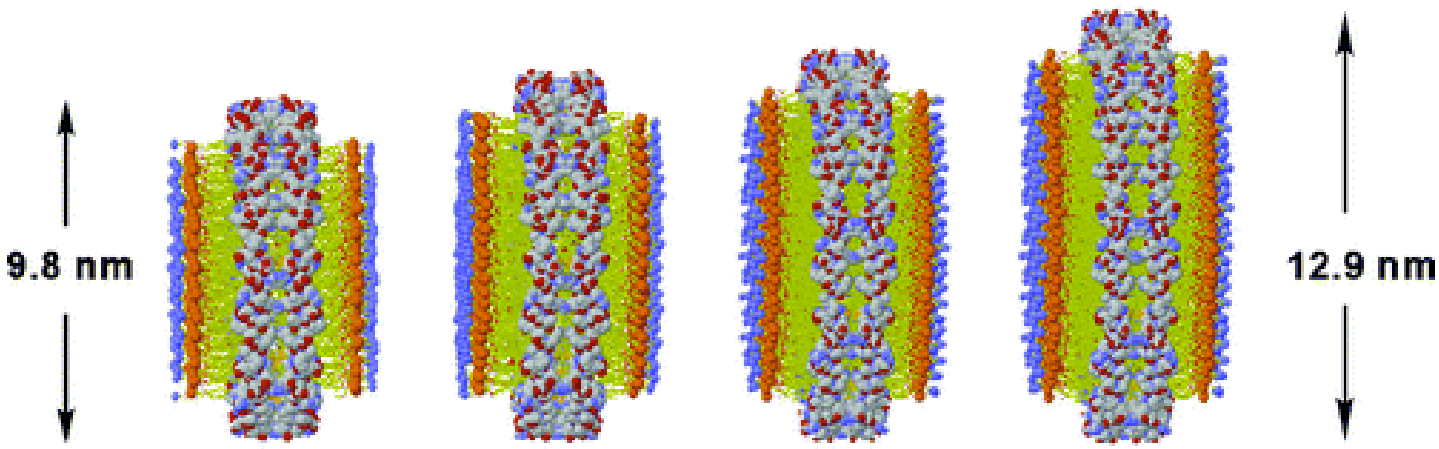
Nanodiscs : Small model membranes

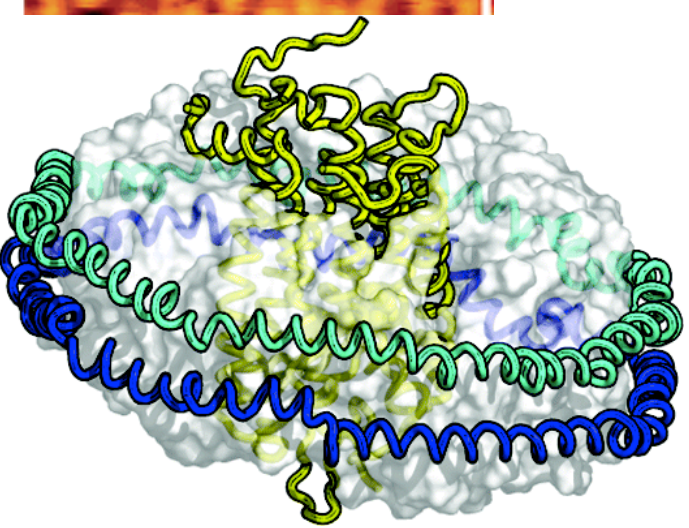
Mix a specific amphipathic peptide with lipids => nanodisc

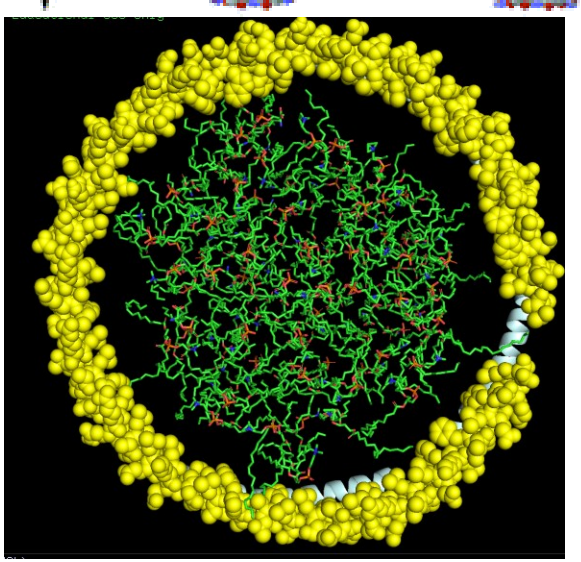
AFM image:

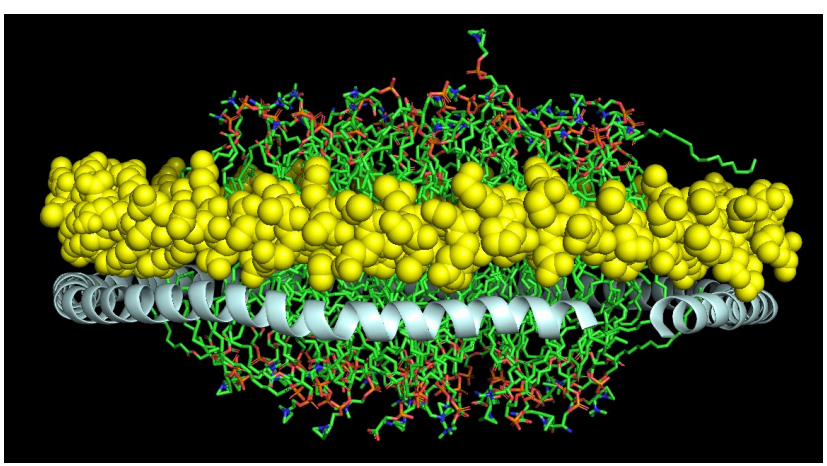


Models:





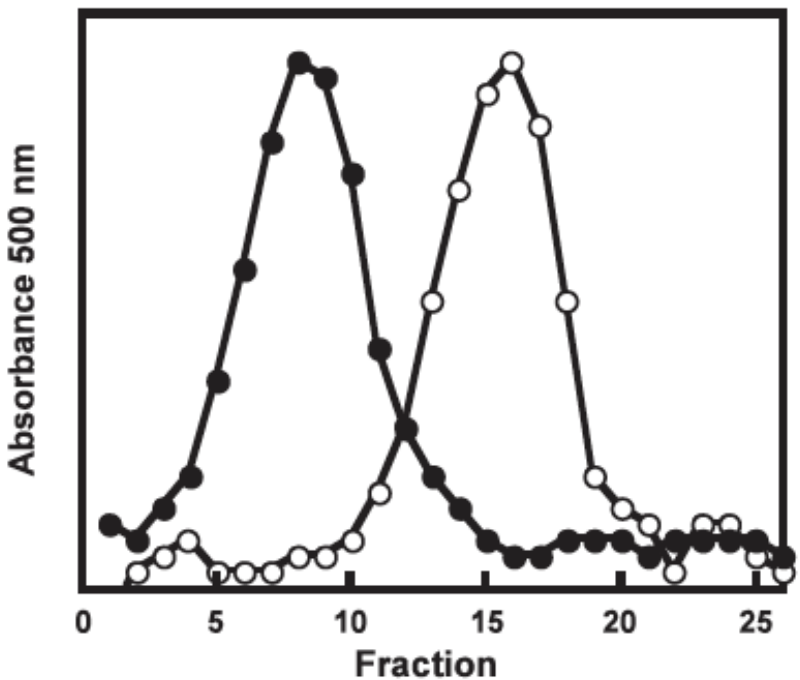




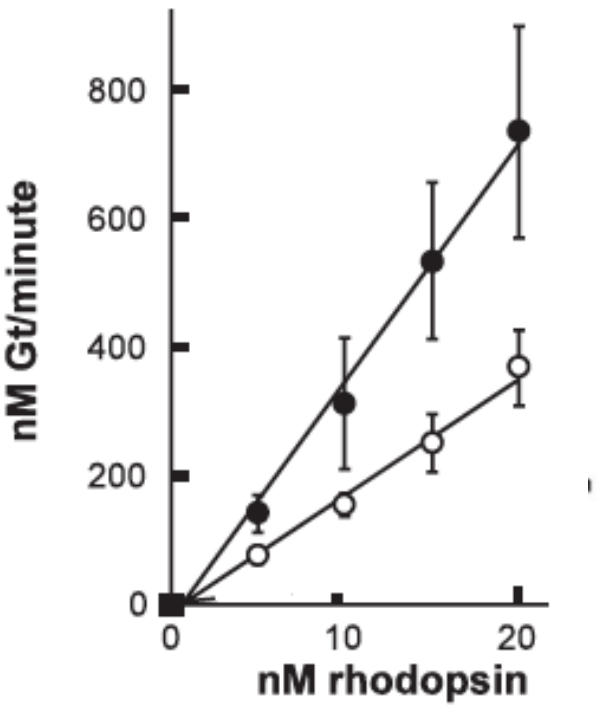
Rhodopsin : Rather monomers than dimers !?!

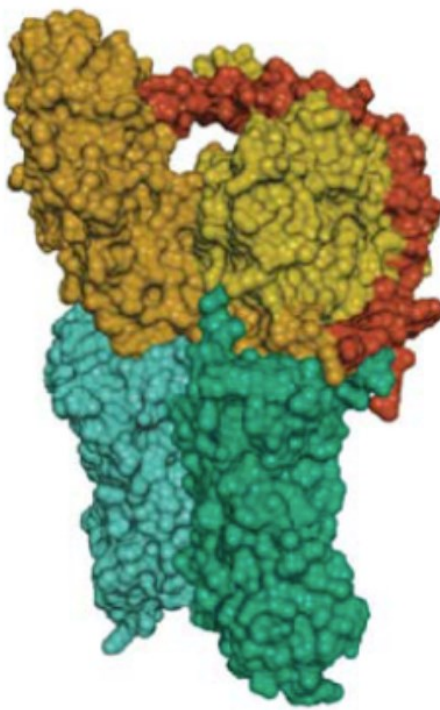
However, recent experiments with purified Rhodopsin in nanodisc model membranes indicate that monomers are perfectly active, but as mono- or di-mer ?

Density gradient separation of nanodiscs with 1 (●) or 2 (☼) Rho's



GTPγS exchange by nanodiscs with 1 (●) or 2 (☼) Rho's





Fotiadis, 2006, Curr Op Struc Biol

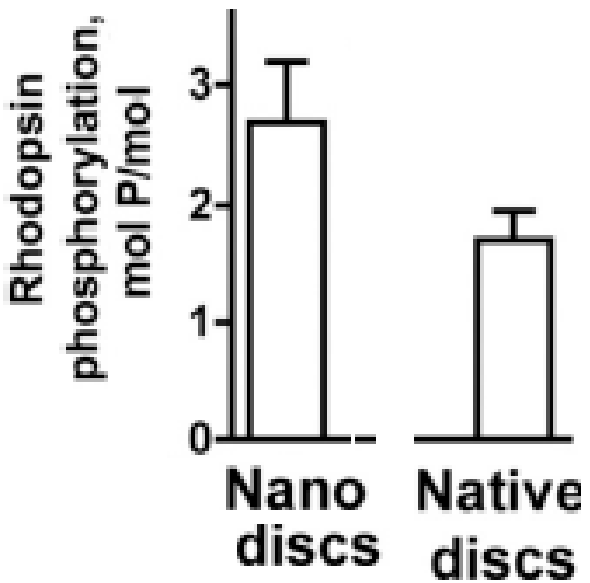
=> 1 Rho / disk => fully active

=> 2Rho's/disk is not more active as only one can couple to G protein

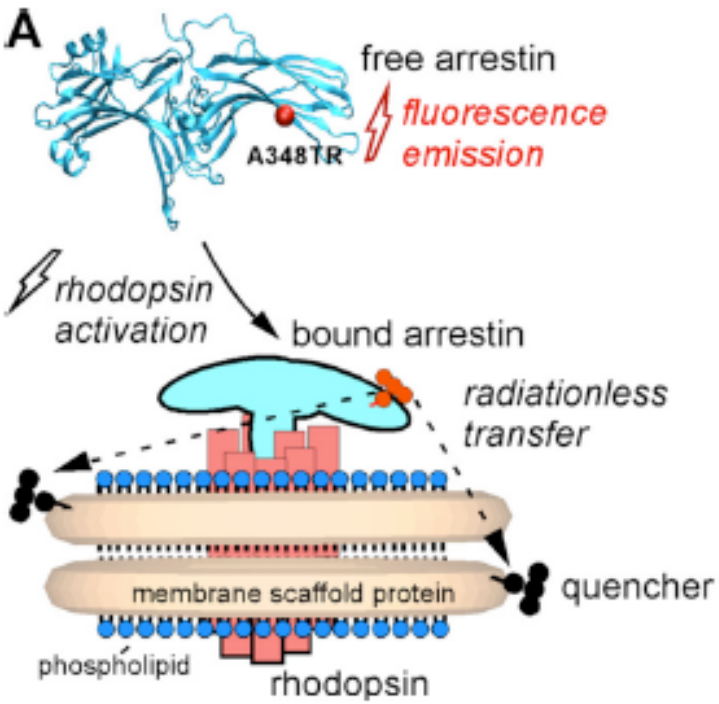
Rhodopsin : Rather monomers than dimers !?!

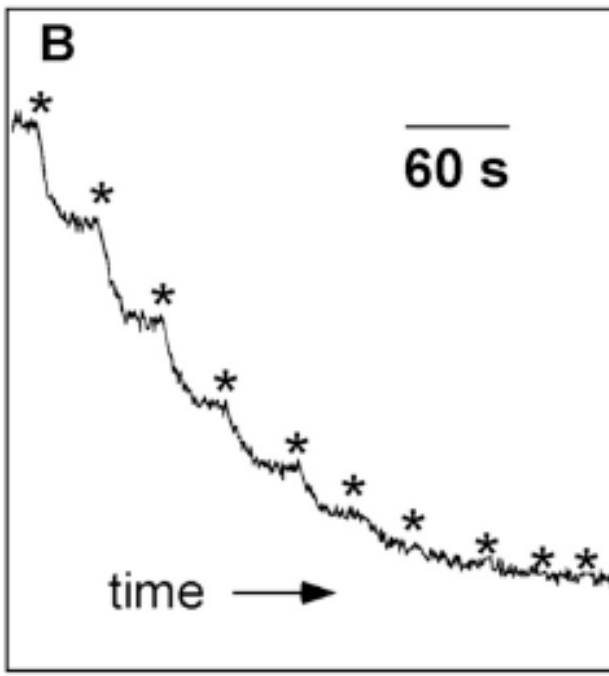
Nanodisc with a rhodopsin monomer are perfectly active..

- Efficiently phosphorylated by GPCR Kinase GRK1



- Binding of arrestin to rhodopsin upon activation by light





=> 1 Rho monomer => couples to G protein, GRK and arrestin

Bayburt 2011 JBC

G protein-coupled receptors

- GPCR's are a very prominent class of receptors
- GPCR structure is 7TM
- Receptor activation induces activation of G protein through GDP-GTP exchange
- G proteins propagate the signal
- Shut down of signalling
- Ligand-determined effects : Bias
- Signalling upon internalisation
- Arrestin-mediated signals
- Finding ligands
- Location
- Loners or collaborators ?

In short

The classical model of GPCR signalling is a good basis,
but there are many variations on the theme,and
has to be adapted to account for :

- ligand bias
- receptor dimer or monomer
- coupling to different G-proteins
- G protein or arrestin (in-)dependent pathways
- modulating proteins
- cellular location

....

Further reading

Hilger 2018 GPCR signalling & Structure
Tan 2018 Biased ligands
Weis 2018 GPCR activation