

Deorphanizing G Protein Coupled Receptors

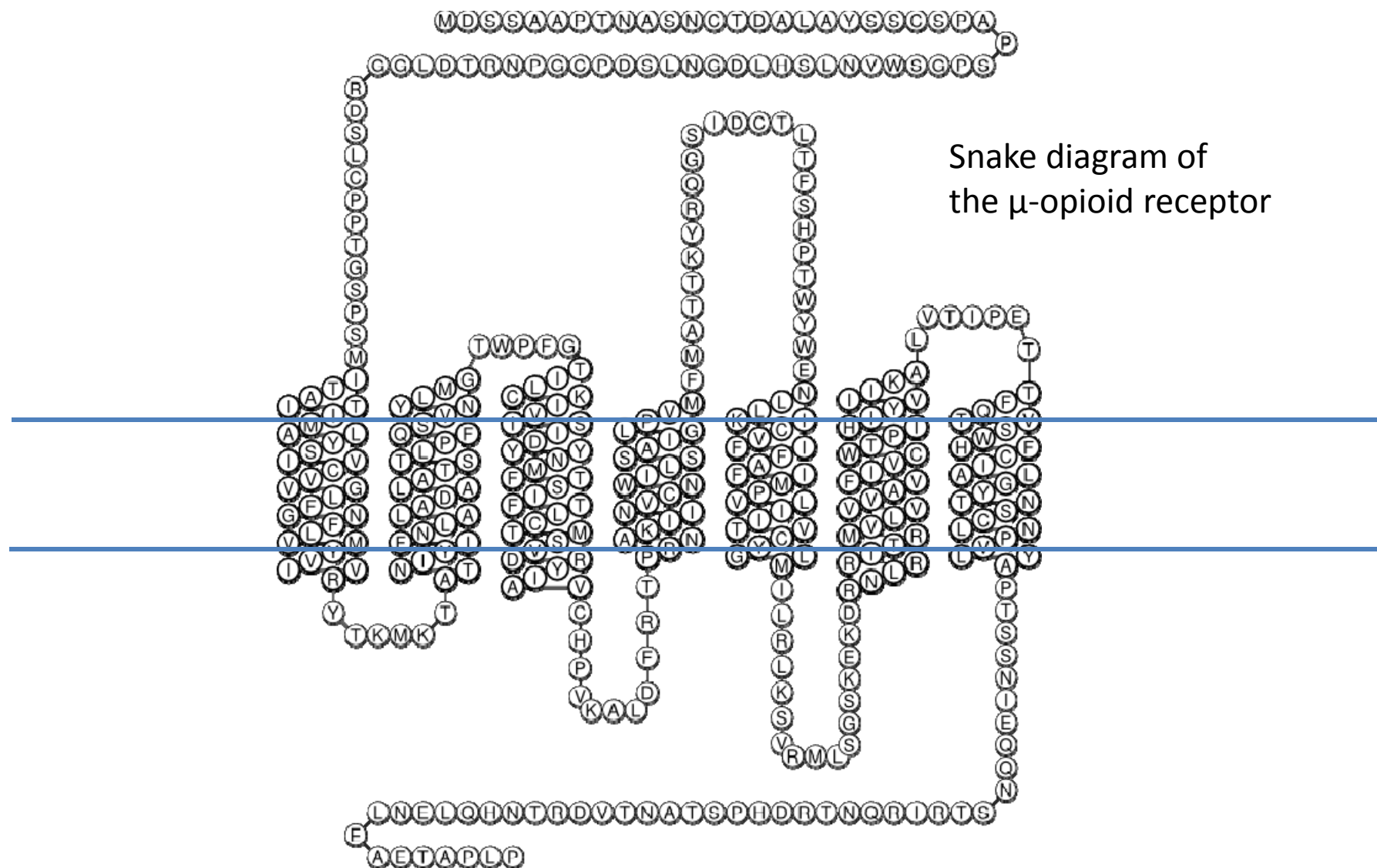
Mark Connor

Australian School of Advanced Medicine

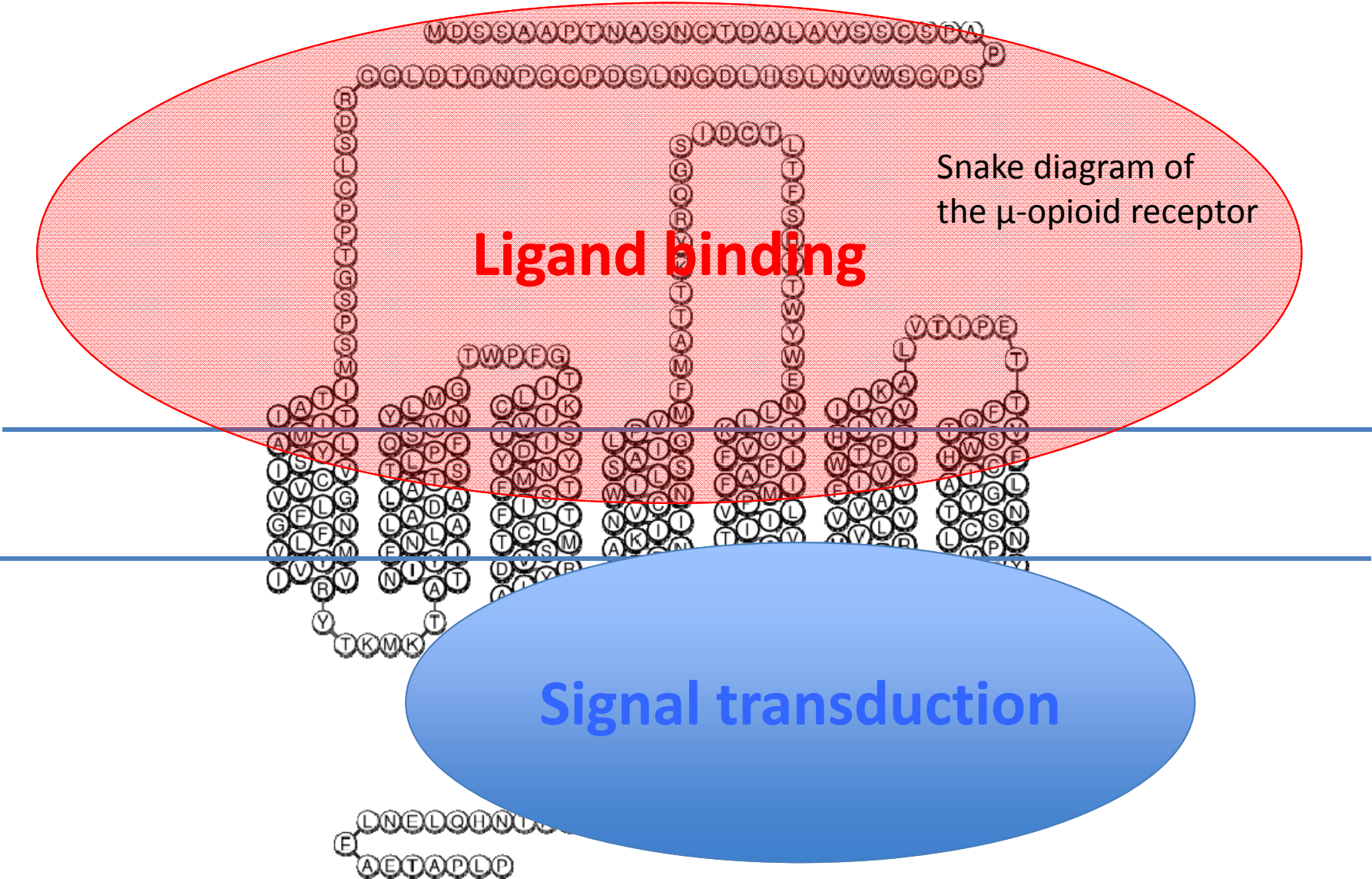
Macquarie University

mark.connor@mq.edu.au

What is a G protein coupled receptor ?



What is a G protein coupled receptor ?



G protein coupled receptors

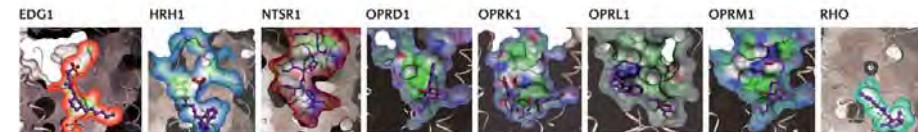
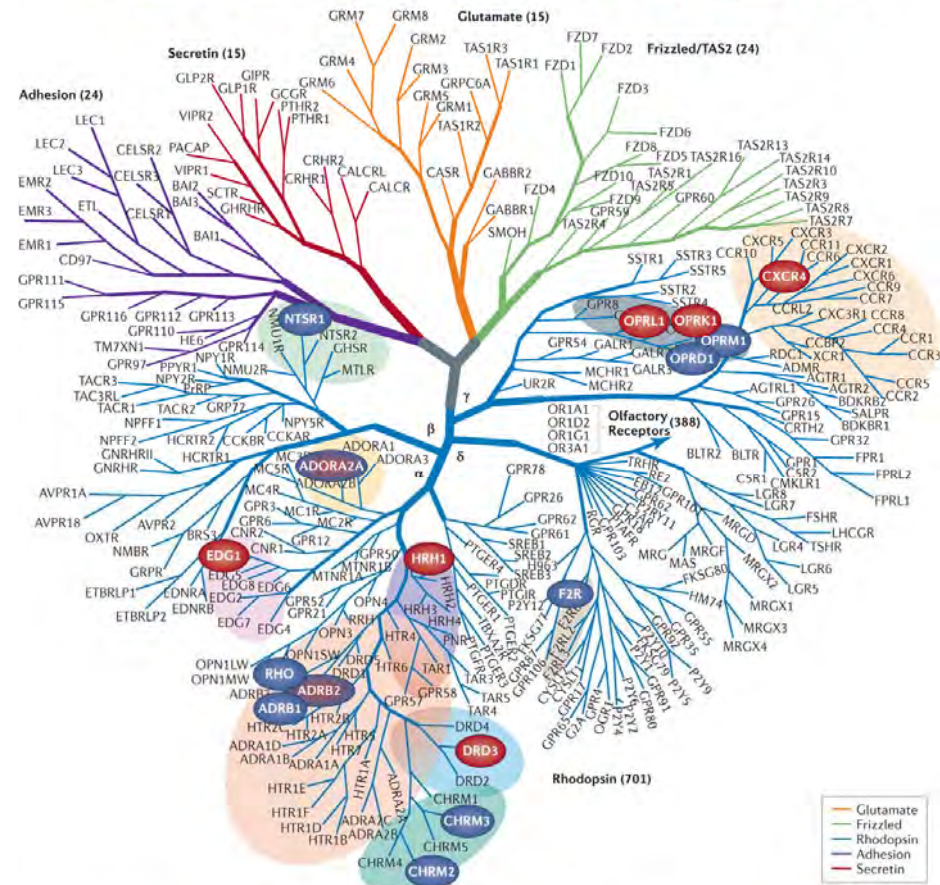
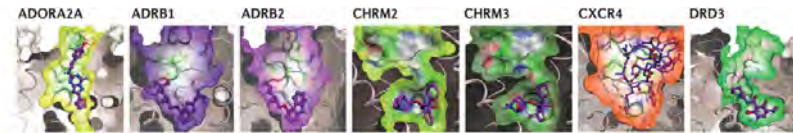
....

1265 in human genome
CBRC Sevens Database

325 to > 6000 a.a.

photons, protons to 30 kD peptides

- Lipids
- amino acids
- Ca
- Nucleotides
- Catechols
- Steroids
- themselves.....(proteases)



Drugs Targeting GPCR

Directly

opioid agonists

angiotensin receptor
antagonists

β -adrenoceptor ligands

CaSR ligands

Antipsychotics

Antihistamines

Caffeine

Cannabis

Indirectly

COX inhibitors

ACE inhibitors

Antidepressants

cocaine

methamphetamine

Drugs Targeting GPCR

Directly

opioid agonists

angiotensin receptor
antagonists

β -adrenoceptor ligands

CaSR ligands

Antipsychotics

Antihistamines

Caffeine

Cannabis

Indirectly

COX inhibitors

ACE inhibitors

Antidepressants

cocaine

methamphetamine

5 of the top 10 prescription drugs act through mechanisms dependent on GPCR....

Missing GPCR

~ 550 in all

~ 430 are “olfactory” GPCR

~ 15 are taste-related GPCR

*(courtesy of the Missing Protein Master List,
Lydie Lane (SIB), via Mark Baker)*

Not all missing GPCR are orphans...

β 3 adrenoceptor (adrenaline/noradrenaline, *Mirabegron*)

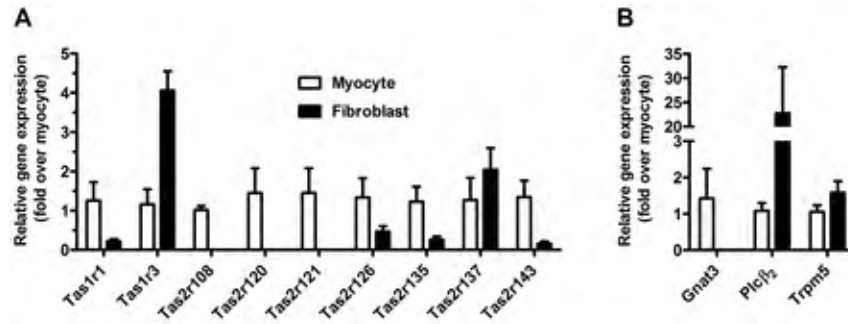
Orexin receptor 1 & 2 (orexin, *Suvorexant*)

5-HT_{1E/F} receptors (serotonin, *Lasmiditan*)

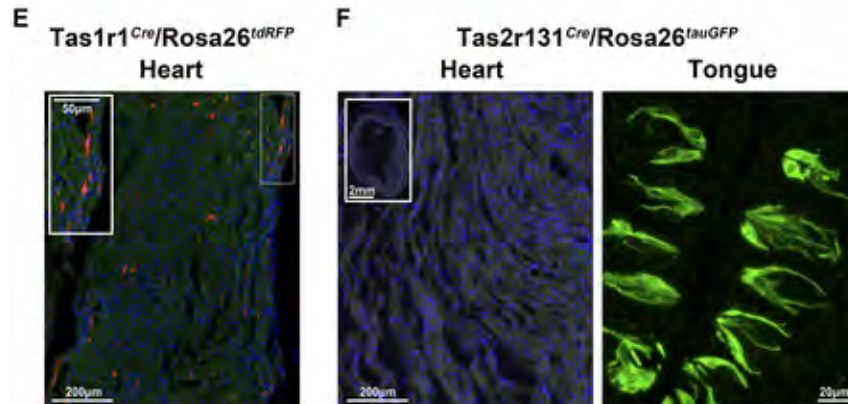
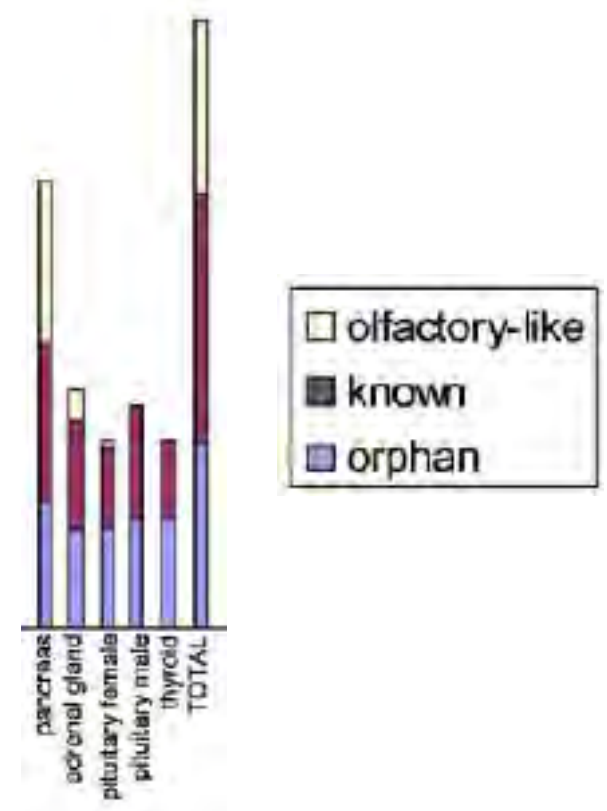
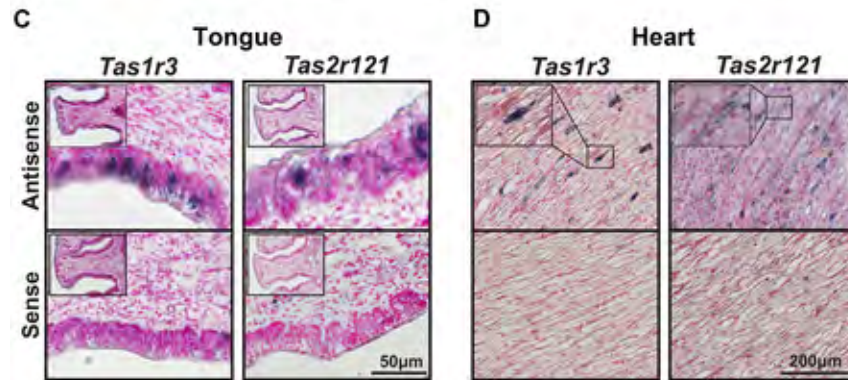
Prostaglandin D₂ receptor 1 (prostaglandin D₂, *Laropiprant*)

Also adenosine, glutamate, NPFF, somatostatin....

Not all olfactory/taste receptors are up our nose....



Hakak , FEBS Lett 550 11-17, 2003



Foster et al, PLOS One 2013

De-orphanizing - what do you need to know ?

- Is your receptor expressed ?
- How does your receptor signal ?
- The ligand.....

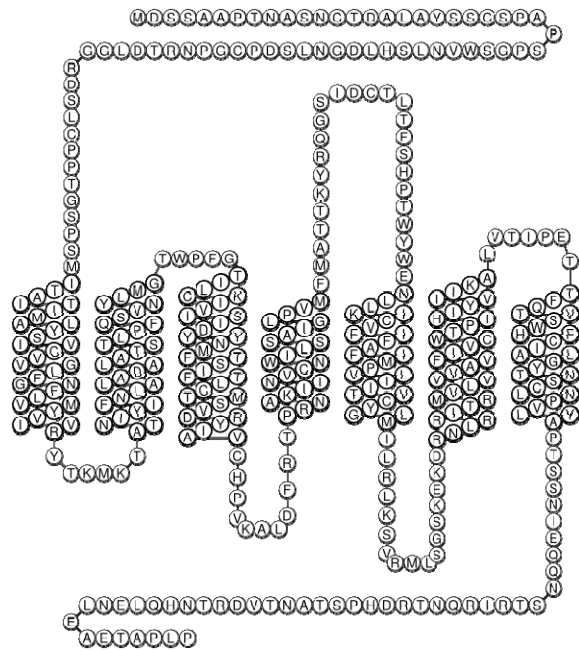
Is your receptor expressed ?

- Epitope tag the clone (usually N-terminus) and then check for surface expression with immunohistochemistry

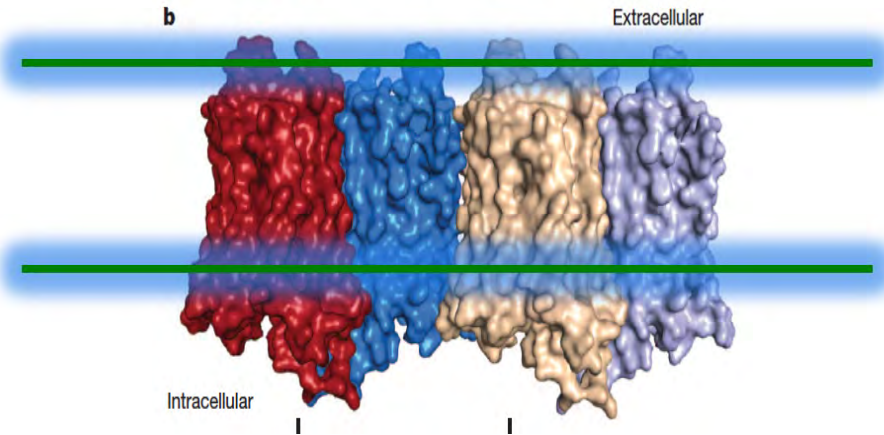
But what if it isn't there ?

- Some GPCR are obligate heterodimers

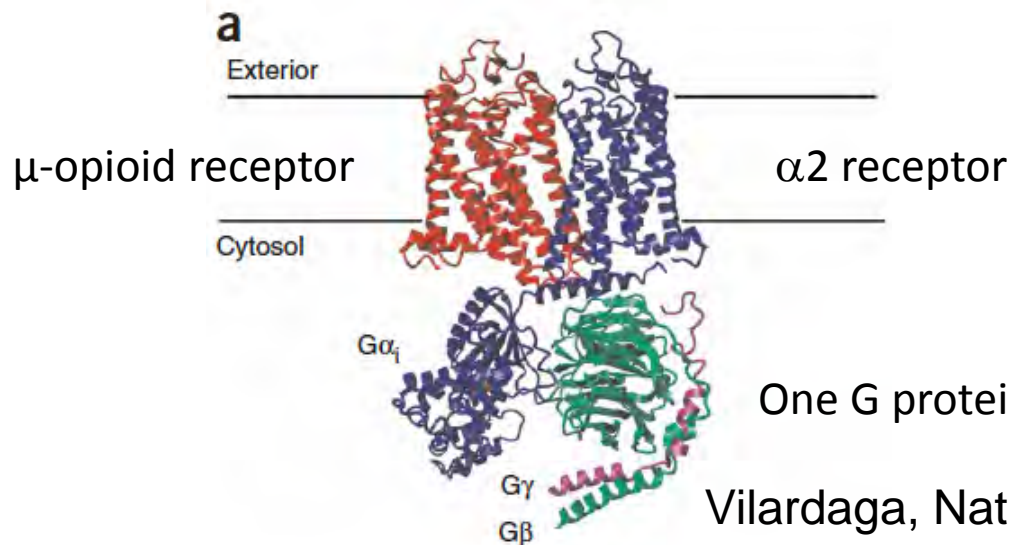
My Snake



The μ -opioid receptor *complex*



Manglik *et al.*, Nature 485, 321 (2012)



One G protein heterotrimer....

Villardaga, Nat Chem Biol 4, 126 (2008)

Is your receptor expressed ?

- Epitope tag the clone (usually N-terminus) and then check for surface expression with immunohistochemistry

But what if it isn't there ?

- Some GPCR are obligate heterodimers
- Some GPCR require accessory proteins for functional expression

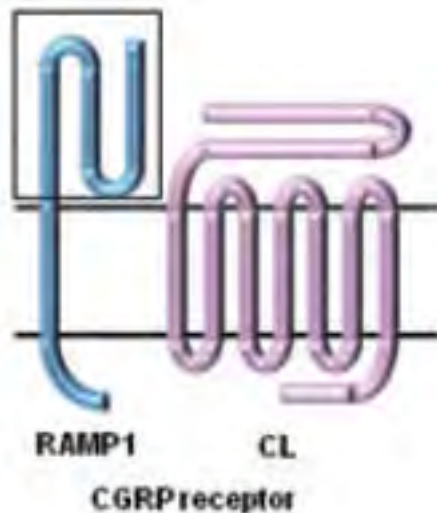
Calcitonin receptors/RAMP proteins

Calcitonin receptor (CTR) **alone** binds calcitonin

CTR + RAMP1 binds: rat amylin > CGRP > calcitonin (AMY₁ R)

CTR + RAMP2 binds: not really known... (AMY₂ R)

CTR + RAMP3 binds: rat amylin > CGRP > human amylin (AMY₃ R)

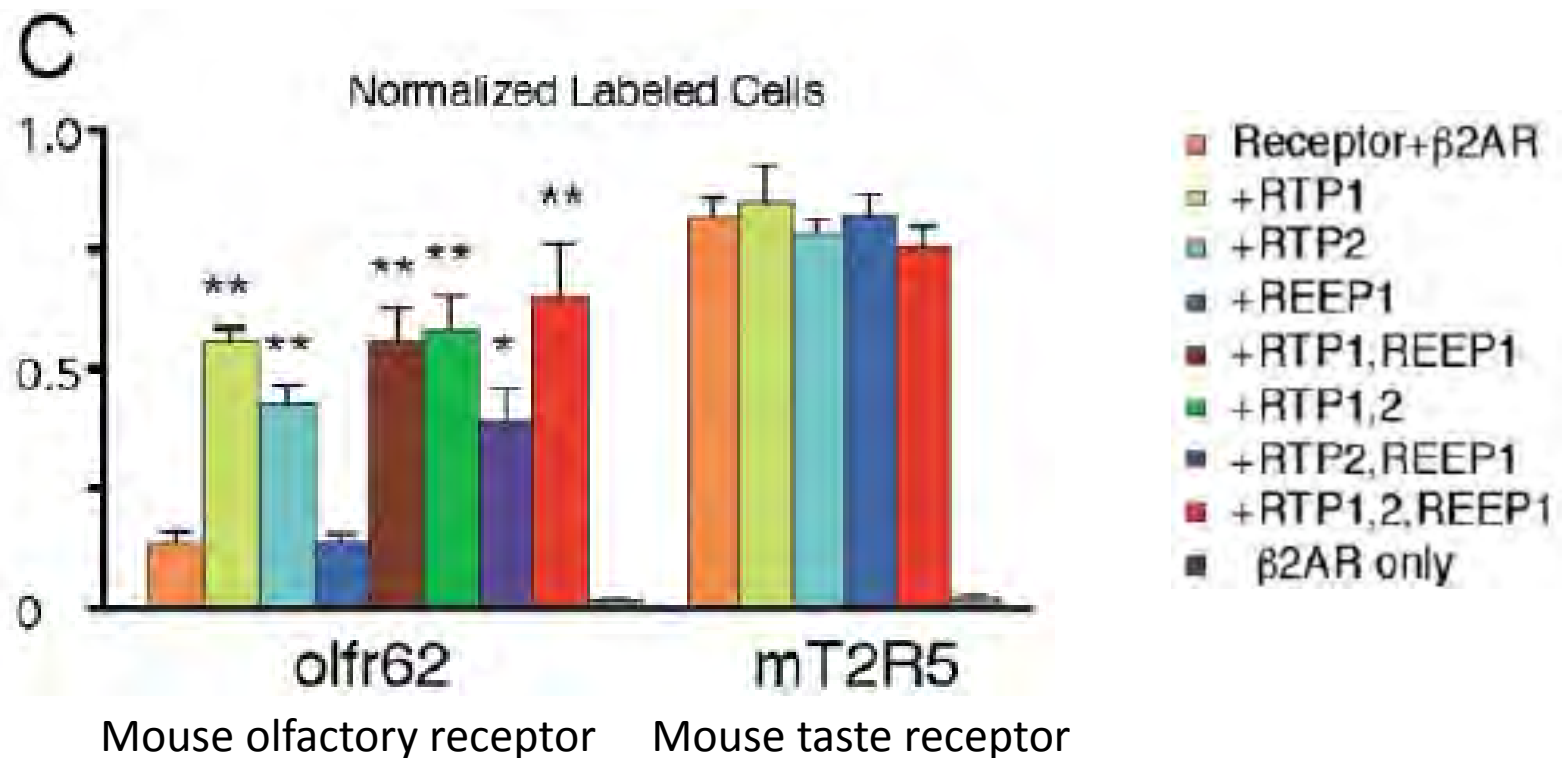


Also form complexes with:
calcitonin-like receptor (CL receptor)
VIP/PACAP receptors
PTH1 and PTH2 receptors...etc

Regulate pharmacology, trafficking to and from surface

Olfactory receptors

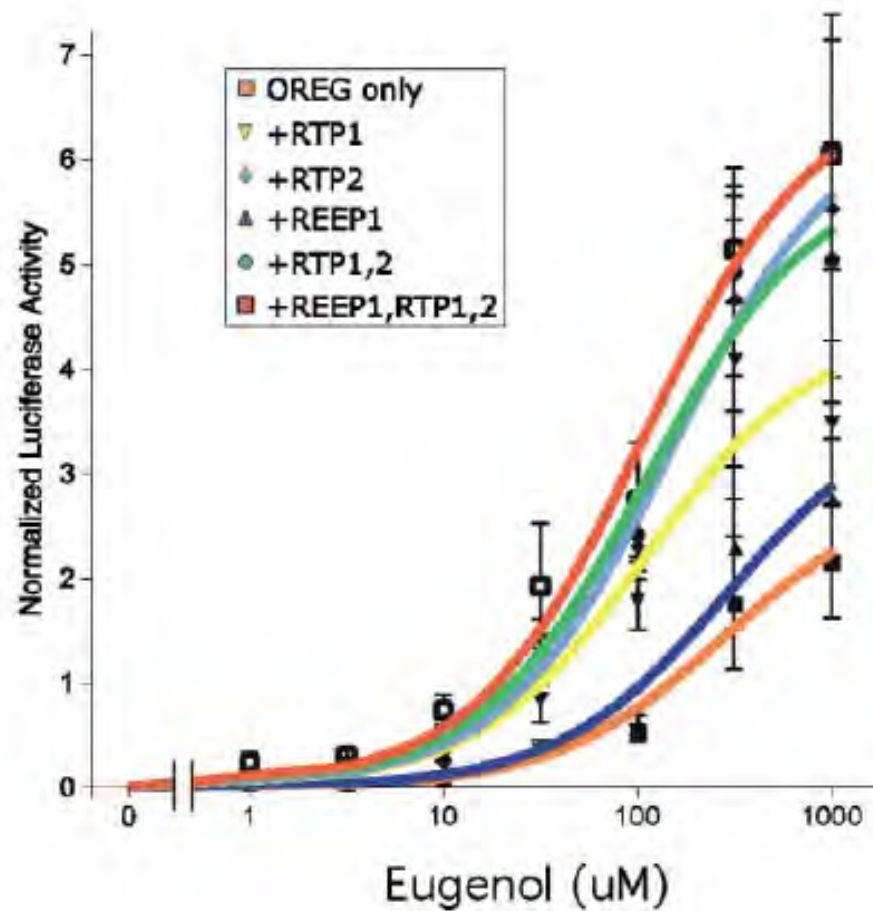
Can require accessory proteins for expression:



Olfactory receptors

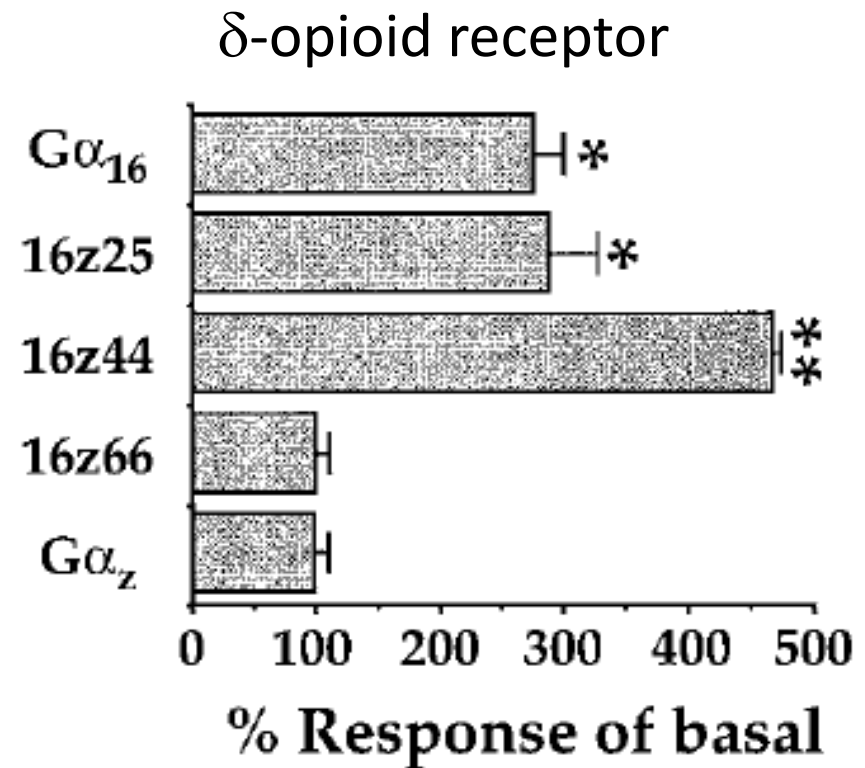
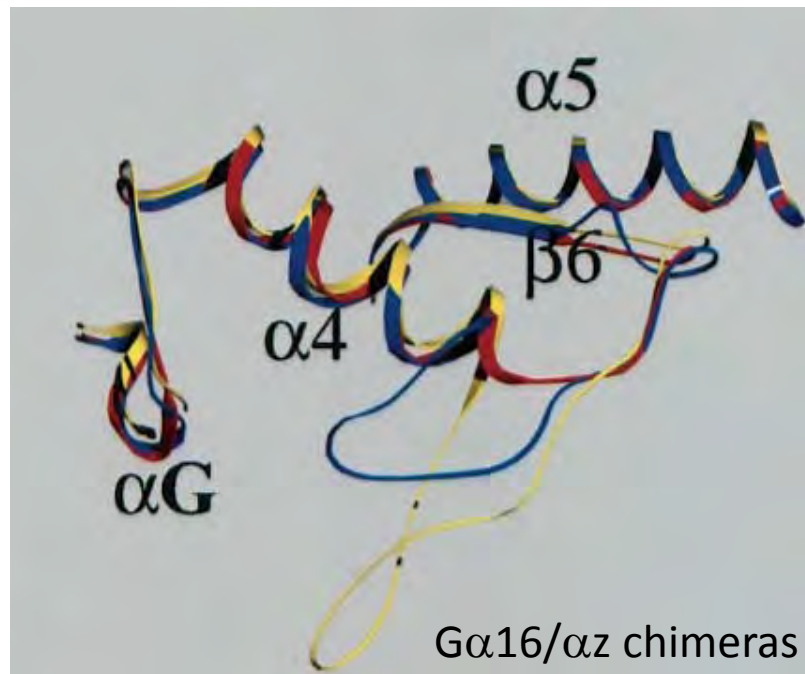
Can require accessory proteins for expression *and function*:

B



What do you look for ?

- There are many G proteins
- Not all give rise to rapid signals in cell lines
- Rises in calcium are easiest



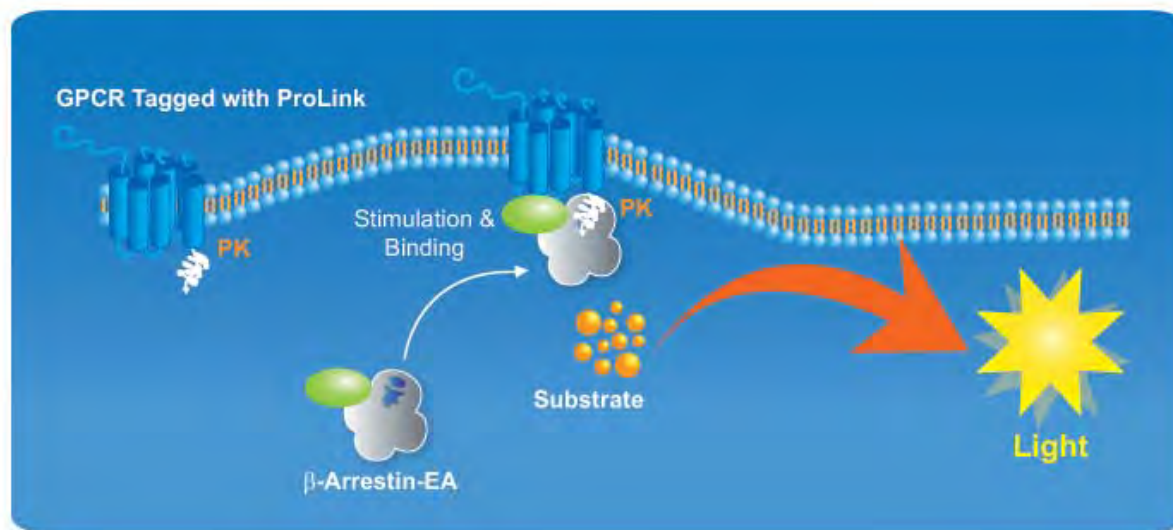
Mody et al., Mol Pharm 57, 13-23, 2000

What do you look for ?

- Do all agonists recognize the $G_{\alpha 1644z}$ conformation ?
(ligand biased signalling –false positives OR negatives)
- Are the receptors always in the compartment as $G_{\alpha 1644z}$?

Or, Non G protein-mediated responses

e.g. arrestin recruitment

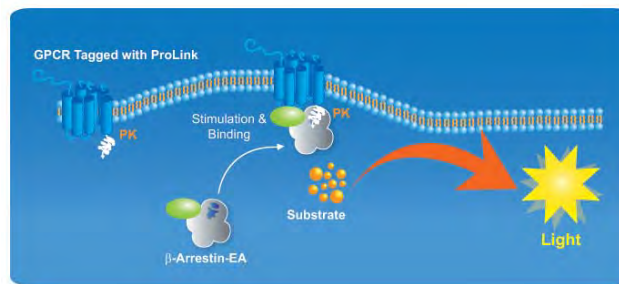


<http://www.discoverx.com>

Screening β -Arrestin Recruitment for the Identification of Natural Ligands for Orphan G-Protein-Coupled Receptors

Or, Non G protein-mediated responses

e.g. arrestin recruitment



Screening β -Arrestin Recruitment for the Identification of Natural Ligands for Orphan G-Protein-Coupled Receptors

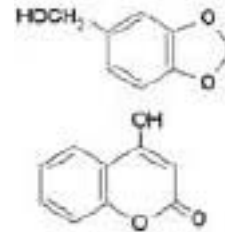
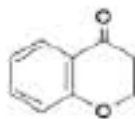
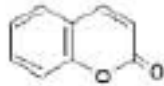
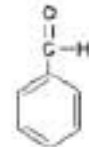
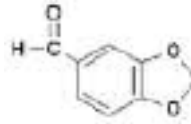
Southern et al *Journal of Biomolecular Screening*
18(5) 599–609

82 Orphans, 10500 ligands....one potential deorphanization....

Er, what about the ligands ?

For olfactory receptors...olfr62

2-Coumaranone	100uM	
	30uM	
	10uM	
	3uM	
	1uM	
Piperonal	100uM	
	30uM	
	10uM	
Benzaldehyde	100uM	
	30uM	
	10uM	
Coumarin	100uM	
	30uM	
	10uM	
4-Chromanone	100uM	
	30uM	
	10uM	
Piperonyl alcohol	100uM	
4-Hydroxycoumarin	100uM	



misses

Ligands

- Is there only one ?
- What is the “true” ligand
- What if it is in serum ?
- What if it is allosteric, not orthosteric....
- Many false positives....

Summary

Many orphans

Many Ligands

Many reasons why it is not simple to match them up

Thought not *just* force is required...