1

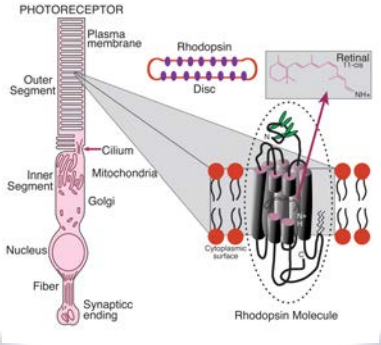
"Structural dynamics of the rhodopsin-transducin complex and the supramolecular organization of the disc membrane"

Thomas P. Sakmar
The Rockefeller University
New York, NY
SakmarLab.org

Cover art by Karina Åberg Sakmar

2

Rod Photoreceptor Cell Schematic



3

Rhodopsin as a "prototypical" heptahelical G protein-coupled receptor

4

The Importance of Heptahelical Receptors in "Drugable Space"

"the most fruitful basis for the discovery of a new drug is to start with an old drug" – Sir James W. Black

A numbers game:

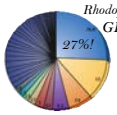
21,000 drug products
1,357 unique drugs
1,204 "small molecules"
803 oral
421 parenteral
275 topical
166 biologicals

Rates of Innovation (past 20 years):

First-against-target – 5.3/year
New target families – 1.9/year

For approved drugs:

324 molecular targets
266 human targets
207 human targets for "small molecules"



Only about 6% of NMEs (new molecular entities) target a previously undrugged target

Genome perspective:

1,620 out of 22,218 human genes have explicit disease associations – 105 are drug targets

726 heptahelical receptors encoded by the human genome. ~200 are "orphan" receptors, most expressed in CNS

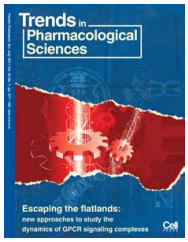
Overington, J. P. et al., Nat Revs Drug Dis 5:993 (2006); Insight Lecture at Rockefeller University, P. N. Goodfellow, Oct. 22, 2007

5

Why Is the Study of Heptahelical Receptors So Challenging?

No modular structure

We can't study individual domains one at a time



Membranes are required

No orthologues in lower organisms:

Only eukaryotic organisms have heptahelicals

Bacteriorhodopsins don't count

Can't express in *E. coli*

Formalisms used to describe GPCR function are 50 years out of date *(i.e., classical pharmacology)*

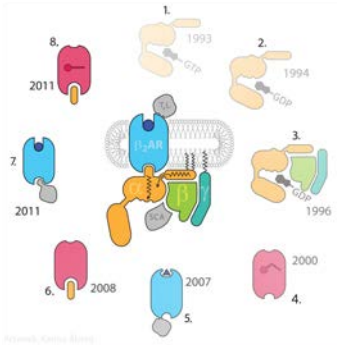
Low abundance in Nature:

It can be argued that only 3 GPCRs have been purified in functional form from tissue

See Huber & Sakmar, *TIPS*, July 2011

6

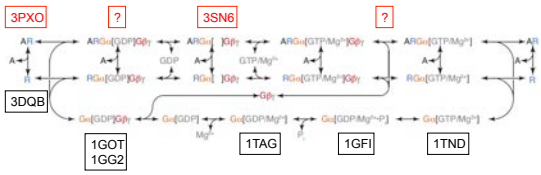
Structural Biology of the GPCR Signaling Complex: A Series of Crystallographic Snapshots



β2AR-Gs complex – Rasmussen *et al.*, *Nature* (2011)

Schwartz & Sakmar *Nature* (2011)

But wait . . . the dynamics of the GPCR-G protein cycle is extremely complicated



- Crystallography has its limitations -- static portraits, often in contrived poses
- Our aim is to complement high-resolution structures with biochemical and biophysical data obtained by other approaches

How will we do that? New technologies are needed . . .

Huber & Sakmar *TIPS* (2011)

New Technologies to Study Receptor Signaling

PART 1

Genetic Encoding of Unnatural Amino Acids in Expressed GPCRs Using Amber Codon Suppression Technology

"Site-directed Unnatural Amino (UAA) Acid Mutagenesis"

(with Shixin Ye, Thomas Huber, Saranga Naganathan, Amy Grunbeck, Sarmistha Ray-Saha, Kelly Daggett)

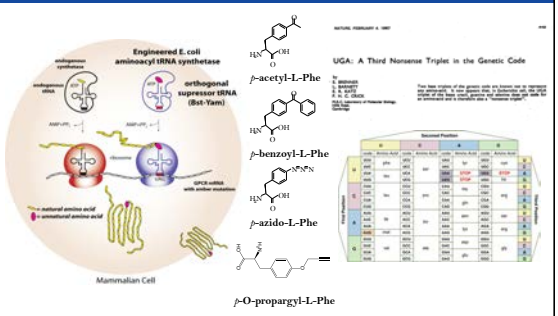
PART 2

Applications for UAA Mutagenesis and Converging Novel Technologies To Study Structural Dynamics of Receptor "Signalosomes"

FTIR spectroscopy of azido groups, site-directed photocrosslinking, bioorthogonal labeling, oriented-tethered bilayers & SMD-TIRF

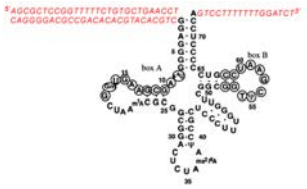
(Adam Knepp, Thomas Huber, Saranga Naganathan, Amy Grunbeck, Kelly Daggett, Sarmistha Ray-Saha, Louise Valentin-Hansen)

Expanding the Genetic Code in Mammalian Cells Using Amber Codon Suppression



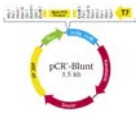
mention Alan Garen

Suppressor tRNA in Various Plasmids

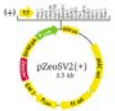


Suppressor tRNA derived from *Bacillus stearothermophilus tyr*-tRNA

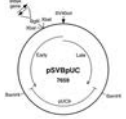
with Caroline Koehler & Tom RajBhandary, M.I.T.



Nucleic Acids Research, 2002, Vol. 30, 4692-4699



PNAS, 2004, Vol. 101, 8883-8887

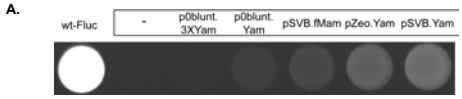


Molecular Cell Biology,1996, Vol. 16, 907-913

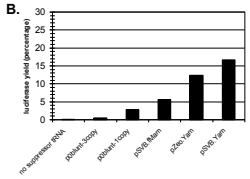
Using a luciferase reporter system to validate and optimize the mutagenesis scheme

Evaluate tRNA constructs

A.



B.

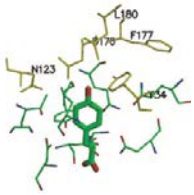


C.

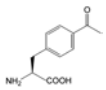
tRNA	F/ug	F%
p0blunt.3xYam	$2.87X10^3$	0.01%
pZeo.Yam	$4.84X10^6$	14.03%
pSVB.Yam	$10.11X10^6$	29.34%

3.5ug Fluc/3.5ug tRNA/0.35ug RS
10cm plate co-transfection

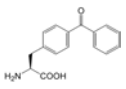
Orthogonal Synthetases



Amino acyl tyr-tRNA Synthetase (*E. coli*)



Acetyl-L-Phe



Benzoyl-L-Phe

Residue #	37	165	182	183	186
E. Coli tyrRS	Tyr	Asp	Asp	Phe	Leu
ap-RS	Ile	Gly	Gly	Met	Ala
bp-RS (v1)	Gly		Gly		Ala
(v2)	Gly		Gly	Tyr	Met

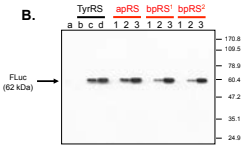
Science, 2003, Vol. 301, 964-967

Evaluate orthogonality of synthetases/unnatural amino acids

A.

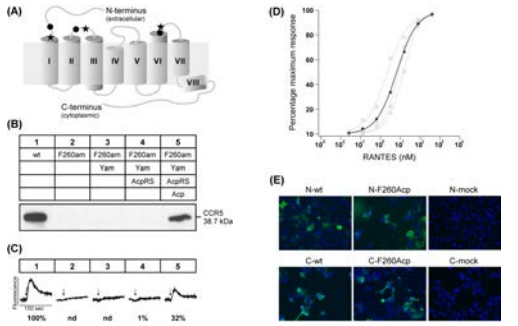
RS	F/ug	F%
ap-RS/ap	$14.69X10^6$	21.3%
ap-RS	$0.95X10^6$	1.26%
bp-RS_1/bp	$3.81X10^6$	5.15%
bp-RS_1	$0.11X10^6$	0.13%
bp-RS_2/bp	$4.31X10^6$	7.02%
bp-RS_2	$0.11X10^3$	0.16%

B.



a. mock
b. FLucY70am
c. FLucY70am/Yam/YRS
d. FLucwt/Yam/YRS
1 FLucY70am/Yam/YRS
2 FLucY70am/Yam/YRS ap/bp
3 FLucwt/Yam/YRS ap/bp

Expression of a functional CCR5 mutant with Ac-Phe at position 260



Ye *et al., J. Biol. Chem. (2008)* with Tom RajBhandary

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- *Unnatural amino acids as targeted photocrosslinkers*
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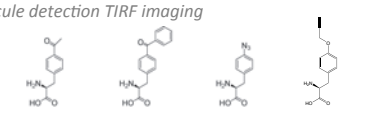
  - *Antibody Epitope Tagging in the native bilayer environment*
  - *Stoichiometric fluorescence labeling for single-molecule detection*
- *Single-molecule detection TIRF imaging*

Tyrosine analogues amenable to genetic encoding

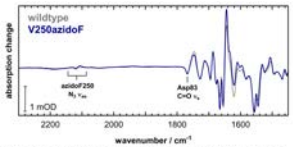


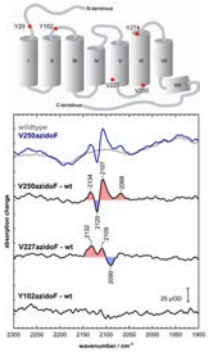
p-acetyl-L-phenylalanine (Acp)	*p*-benzoyl-L-phenylalanine (Bzp)	*p*-azido-L-phenylalanine (Azp)	*p*-O-propargyl-L-phenylalanine (Pgp)
• chemical handle for labeling	• chemical handle for labeling • photocrosslinker	• chemical handle for labeling • photocrosslinker • Infrared probe	• chemical handle for labeling

**Including Rhodopsin & CXCR4/CCR5 as model systems*

Azido-Phe as a Protein Conformational Probe: Proof of Concept Using FT-IR (*Fourier-Transform Infrared Difference Spectroscopy*) of Labeled Rhodopsin

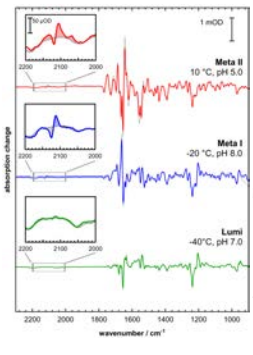
- Environmentally sensitive
- Strong absorption at 2100cm^{-1}
- Stark effect probe

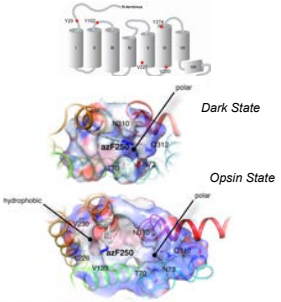




Ye *et al., Nature Chemical Biology (2009)* with Reiner Vogel

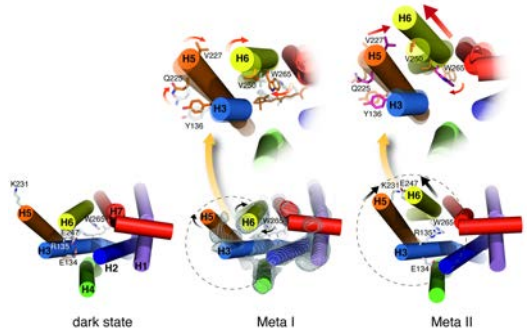
Tracking the *Azido* Probe (*azF250*) Environment Through the Rhodopsin Photocycle





Significant changes in the electrostatic environment of the *azido* probe at codon 250 already appear in Meta I

Significant Helix 5 & 6 Rotation Occurs Even BEFORE the Formation of Meta II



Ye *et al.*, *Nature* (*2010*) with Reiner Vogel, Xavier Deupi, Gebhard Schertler

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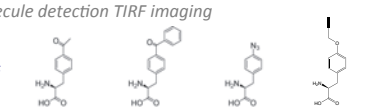
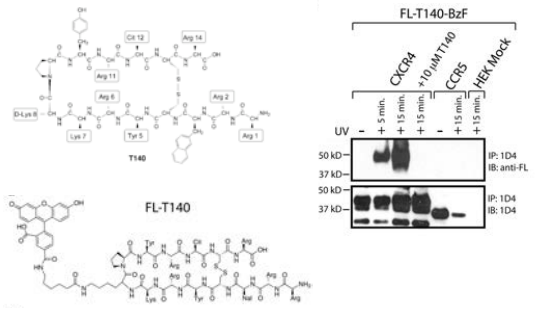


- Acp: chemical handle for labeling
- Bzp: chemical handle for labeling; photocrosslinker
- Azp: chemical handle for labeling; photocrosslinker; infrared probe
- Pgp: chemical handle for labeling

**Including Rhodopsin & CXCR4/CCR5 as model systems*

Unnatural amino acids as targeted photocrosslinkers: CXCR4-T140 receptor-ligand interaction as a test case



M. Teintze, Montana State

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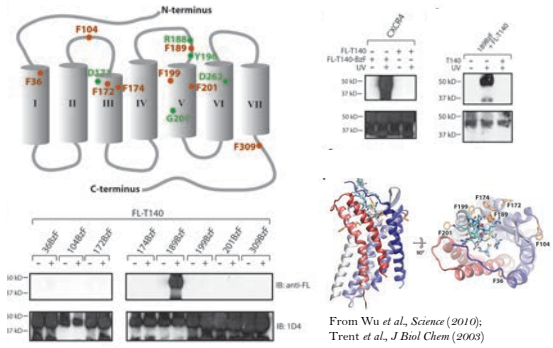
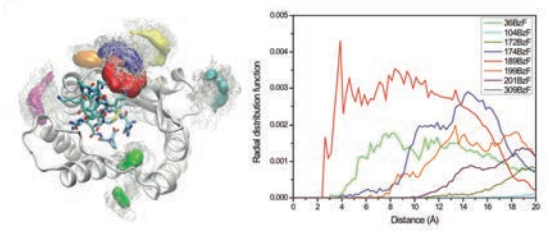


From Wu *et al.*, *Science* (*2010*); Trent *et al.*, *J Biol Chem* (*2003*)

Unnatural amino acids as targeted photocrosslinkers: CXCR4-T140 receptor-ligand interaction as a test case

STRUCTURAL RATIONALE FOR SPECIFIC CROSSLINKING AT POSITION 189

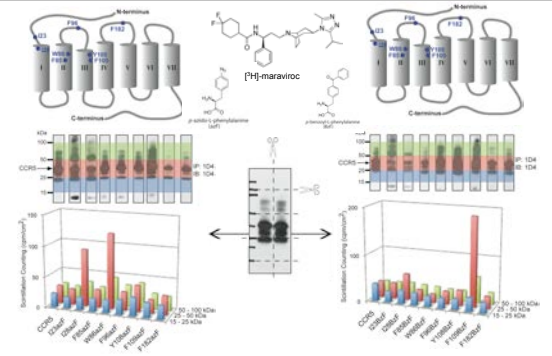


Homology Modeling using CVX15-CXCR4 structure to create T140-bound CXCR4-BzF models – *all possible BzF orientations*

Radial distribution function *versus* distance – *BzF carbonyl atom to closest atom of bound T140 for each possible BzF orientation*

Grunbeck *et al.*, (2011) *Biochemistry*

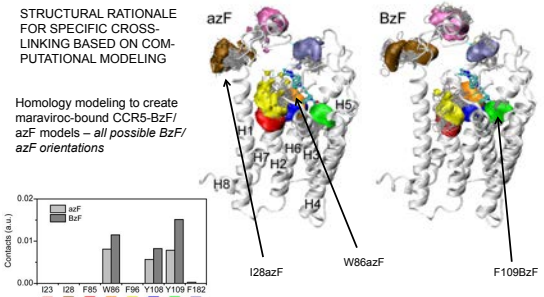
Unnatural amino acids as targeted photocrosslinkers: Identification of the maraviroc binding pocket on CCR5



Unnatural amino acids as targeted photocrosslinkers: Identification of the maraviroc binding pocket on CCR5

STRUCTURAL RATIONALE FOR SPECIFIC CROSS-LINKING BASED ON COM-PUTATIONAL MODELING

Homology modeling to create maraviroc-bound CCR5-BzF/azF models – *all possible BzF/azF orientations*



With Bill Goddard & Ravi Abrol, Cal Tech.

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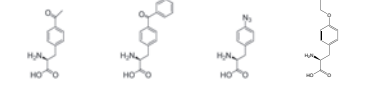
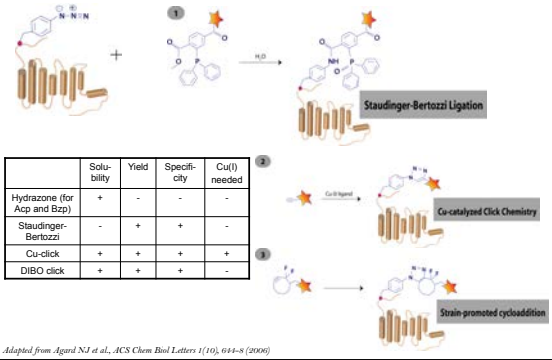


p-acetyl-L-phenylalanine (Acp)	*p*-benzoyl-L-phenylalanine (Bzp)	*p*-azido-L-phenylalanine (Azp)	*p*-O-propargyl-L-phenylalanine (Pgp)
• chemical handle for labeling	• chemical handle for labeling • photocrosslinker	• chemical handle for labeling • photocrosslinker • Infrared probe	• chemical handle for labeling

**Including Rhodopsin & CXCR4/CCR5 as model systems*

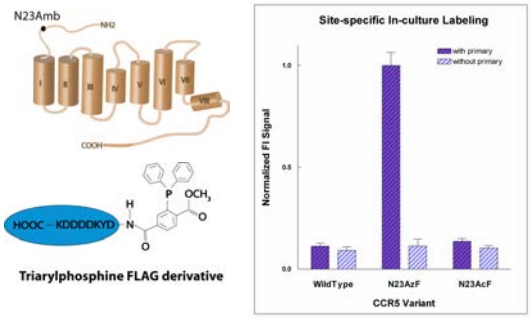
Site-specific incorporation of AzidoPhe into GPCRs: Bio-orthogonal Chemistries



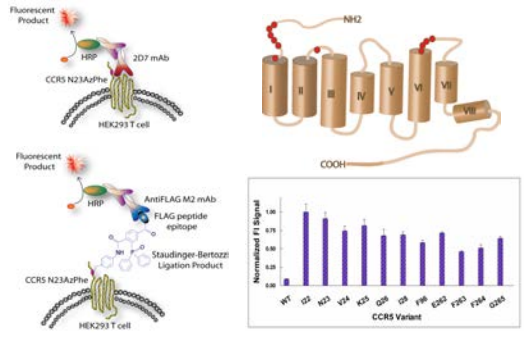
	Solu-bility	Yield	Specifi-city	Cu(I) needed
Hydrazone (for Acp and Bzp)	+	-	-	-
Staudinger-Bertozzi	-	+	+	-
Cu-click	+	+	+	+
DIBO click	+	+	+	-

Adapted from Agard NJ et al., ACS Chem Biol Letters 1(10), 644-8 (2006)

Specific in-culture labeling of CCR5-AzidoF via Staudinger-Bertozzi ligation

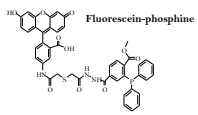


Labeling CCR5 in cell culture: a high-throughput cell-based ELISA

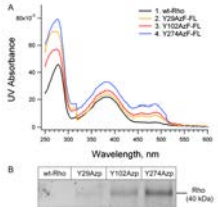


Application of Staudinger-Bertozzi Ligation

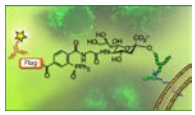
Site-specific incorporation of fluorescent probes into GPCRs



Ye S et al., (2010) in preparation



Fluorescent labeling of sugars in live cell



Bertozzi Group @ UC Berkeley
Prescher JA et al., Nature 430, 873-877 (2004)

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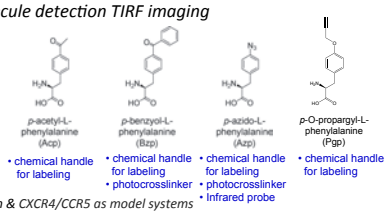
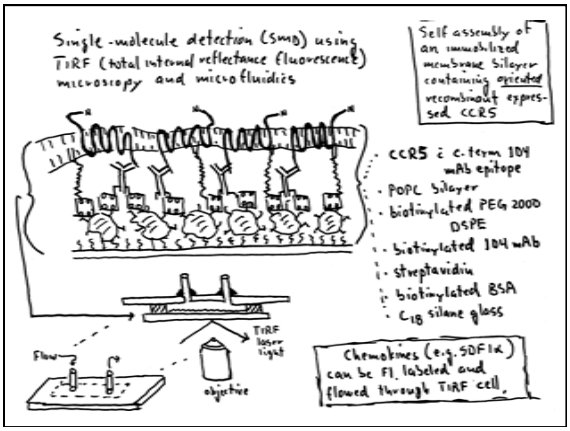
Tyrosine analogues amenable to genetic encoding



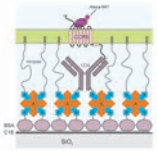
p-acetyl-L-phenylalanine (Acp)	*p*-benzoyl-L-phenylalanine (Bzp)	*p*-azido-L-phenylalanine (Azp)	*p*-O-propargyl-L-phenylalanine (Pgp)
• chemical handle for labeling	• chemical handle for labeling • photocrosslinker	• chemical handle for labeling • photocrosslinker • Infrared probe	• chemical handle for labeling

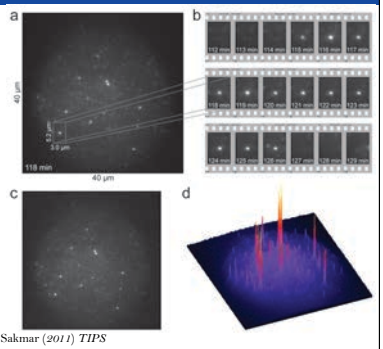
**Including Rhodopsin & CXCR4/CCR5 as model systems*



Toward SMD Fluorescence Microscopy of GPCRs in Live Cell Membranes

A stable oriented "tethered" bilayer can be assembled in a flow-cell "chip" for SMD-TIRF microscopy





Huber & Sakmar (*2011*) *TIPS*

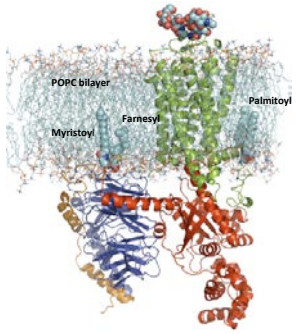
Back to the Vertebrate Visual System . . .

Some thoughts on the supramolecular structure of the rod outer segment disc membrane

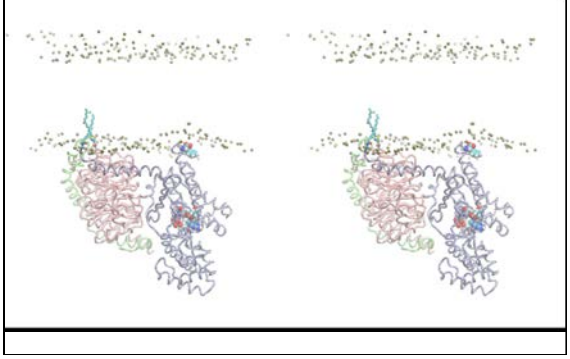
"How does the G protein (transducin) find an active receptor (R)? And then what?*

(Adam Knepp, Thomas Huber –
With Xavier Periole and Siewert-Jan Marrink, Groningen, The Netherlands)

Model of a heptahelical receptor-G protein complex ("Signalosome") in a membrane bilayer

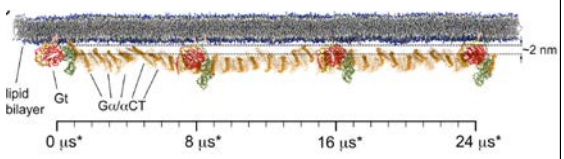


But How Does the G Protein Find an Active Receptor in the Bilayer?

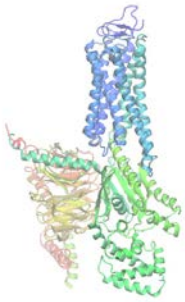


But How Does the G Protein Find an Active Receptor in the Bilayer?

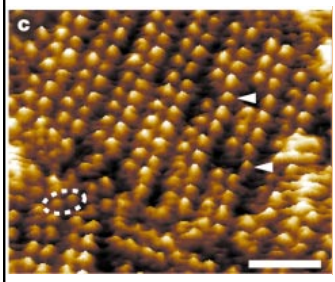
"Coarse-grain" simulations extend the effective interrogation period by 2-orders of magnitude!

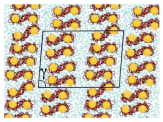


And then what happens?



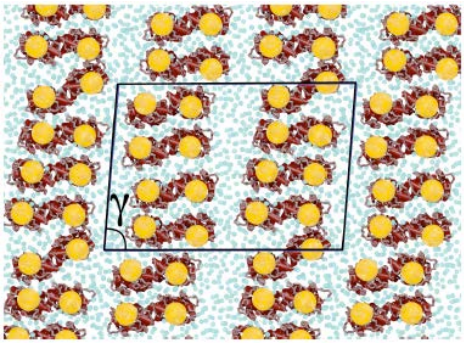
Insights from earlier Atomic Force Microscopy of the ROS disc membrane



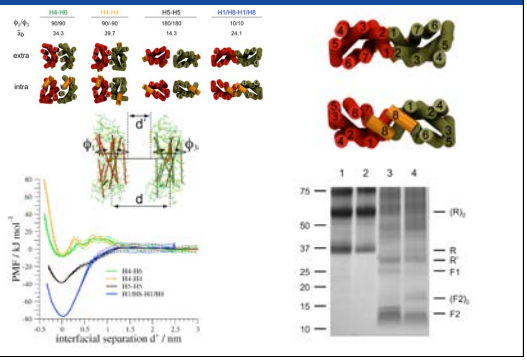


Fotiadis, D. *et al. Nature* **421**:127–128 (2003)

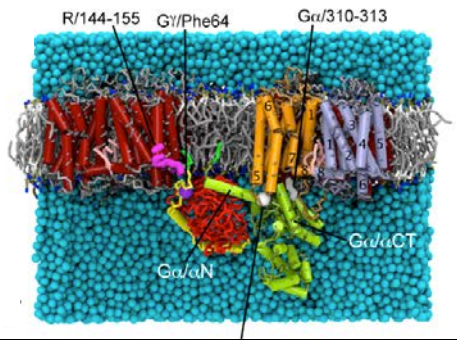
Extension of CG DM simulations to build a disc membrane with 16 rhodopsins (8 dimer pairs)

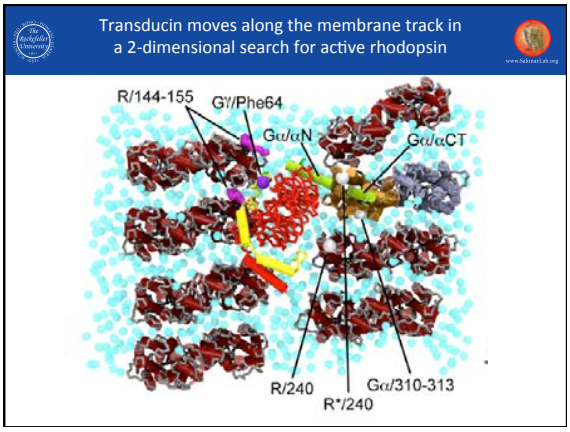


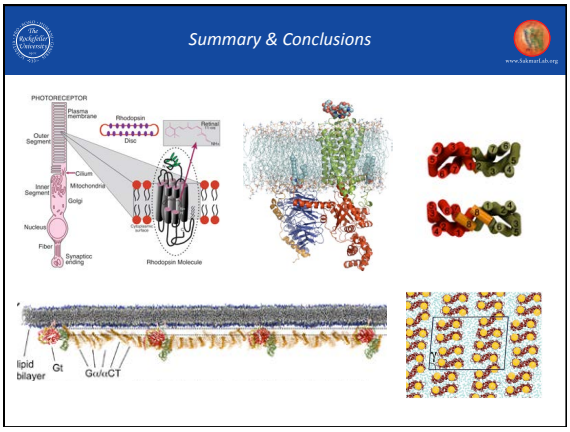
Validation of the correct rhodopsin dimer-dimer interface



Myrsitoylation and prenylation anchor transducin to the disc membrane between rows of rhodopsin dimers




Transducin moves along the membrane track in a 2-dimensional search for active rhodopsin
R/144-155
Gγ/Phe64
Gαα N
Gαα CT
R/240
R*/240
Gα/310-313


Summary & Conclusions
PHOTORECEPTOR
Plasma membrane
Rhodopsin
Disc
Outer Segment
Cilium
Mitochondria
Golgi
Inner Segment
Nucleus
Fiber
Synaptic ending
Rhodopsin Molecule
lipid bilayer
Gt
GαCT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LABORATORY OF MOLECULAR BIOLOGY AND BIOCHEMISTRY
Transmembrane Signal Transduction by Heptahelical Receptors


46


47


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