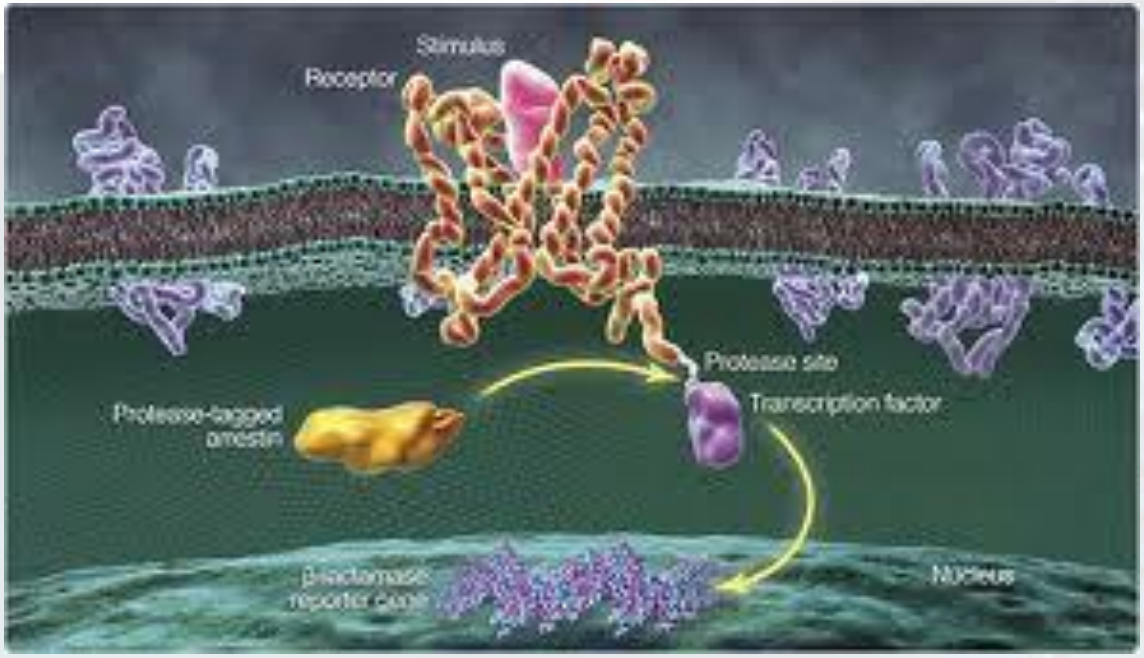


G-Protein Coupled Receptors: Rhodopsin and (engineered) β2AR

Thursday, Jan 31st

MCDB 4810

Presented by: Matt

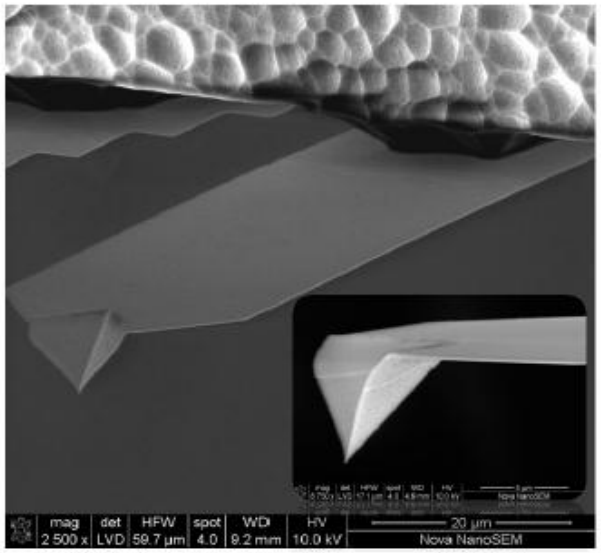
Objectives

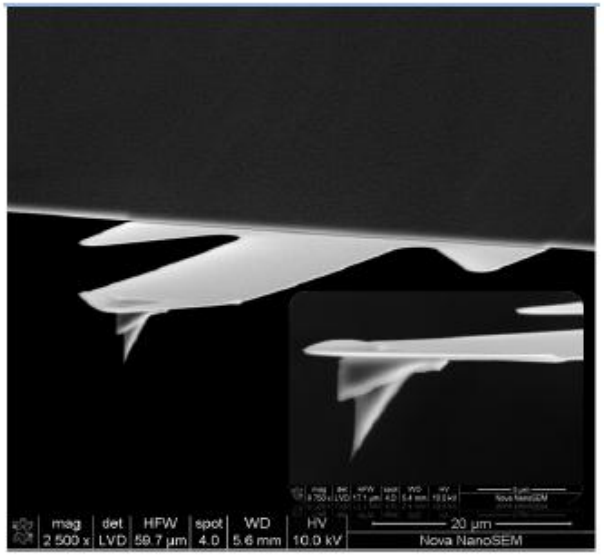
- Further our understanding of some of the details of x-ray crystallography of membrane proteins
- Facilitate quick assessment of crystallography data quality
- Speak to the structure of Rhodopsin and β2AR as informed by crystallographic methods

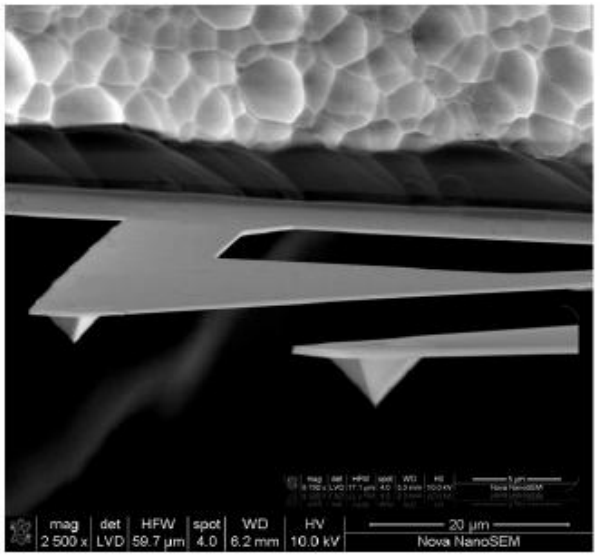
Outline

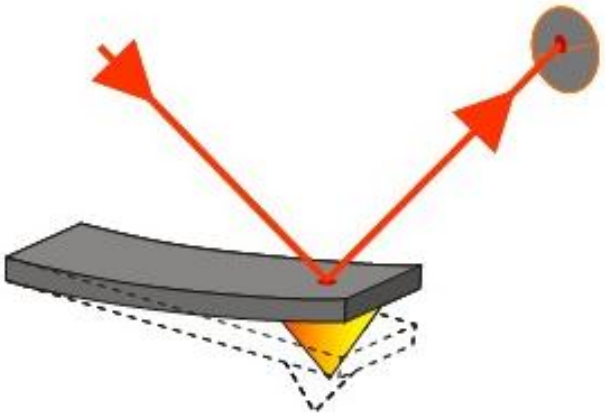
- Revisit from Tuesday: AFM & Electron Density Representations in crystallography
- The seminal identification of Rhodopsin's structure
- The creative endeavor after the structure of β2AR

Fundamentals of Atomic Force Microscopy

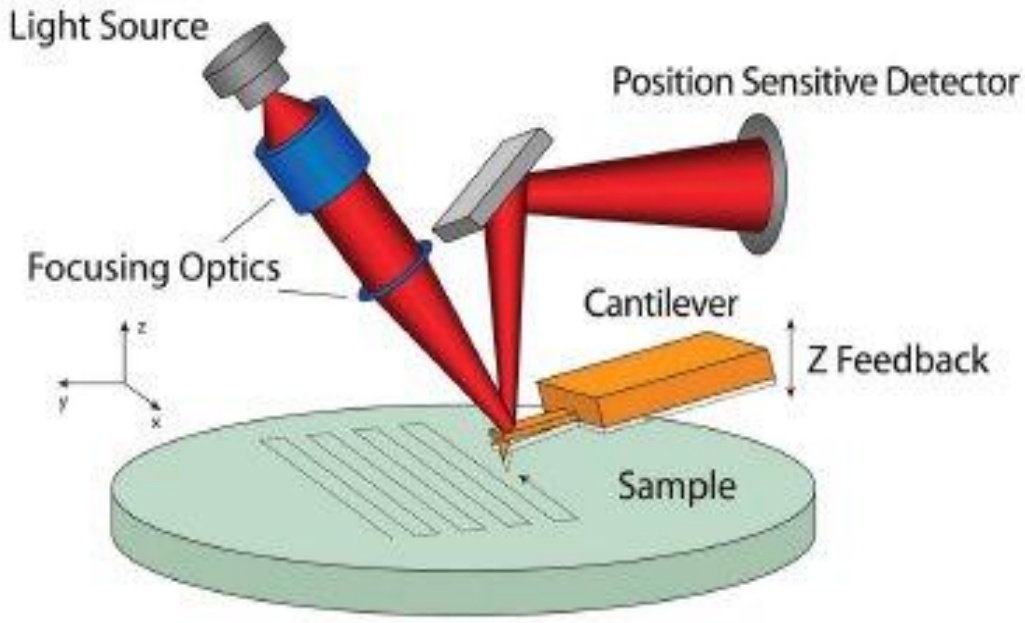




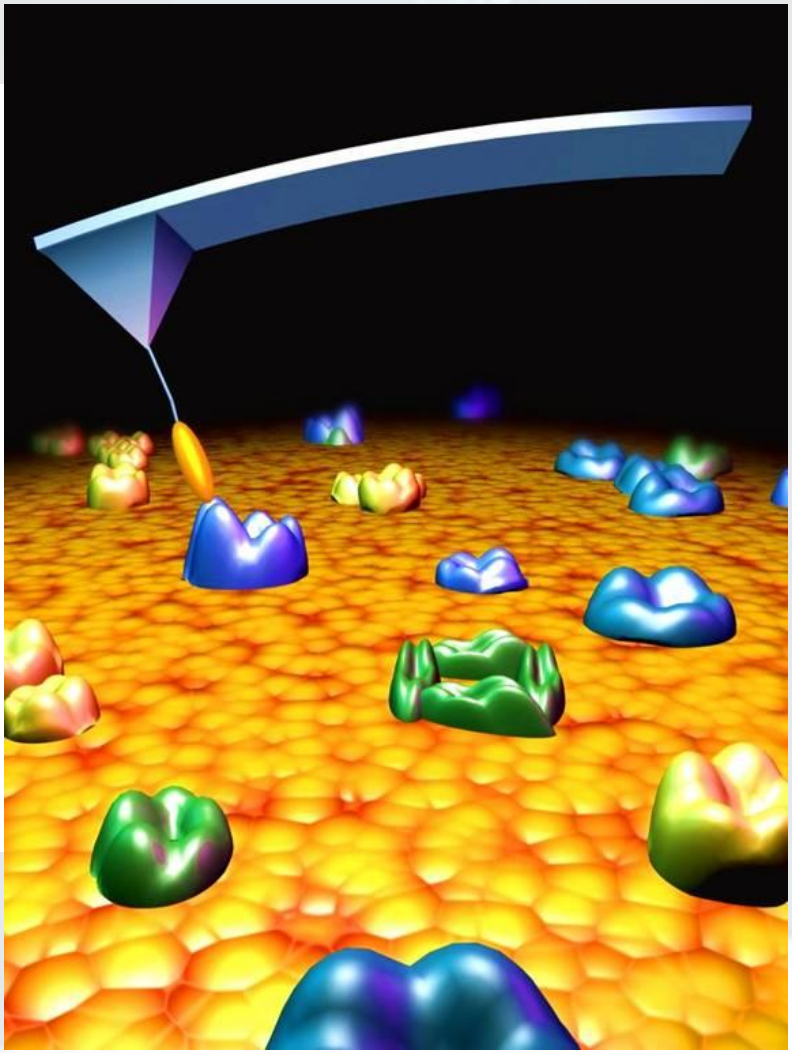




Two modalities: Imaging and Force Spectroscopy



Unrestricted Optical Access from Below the Sample Plane

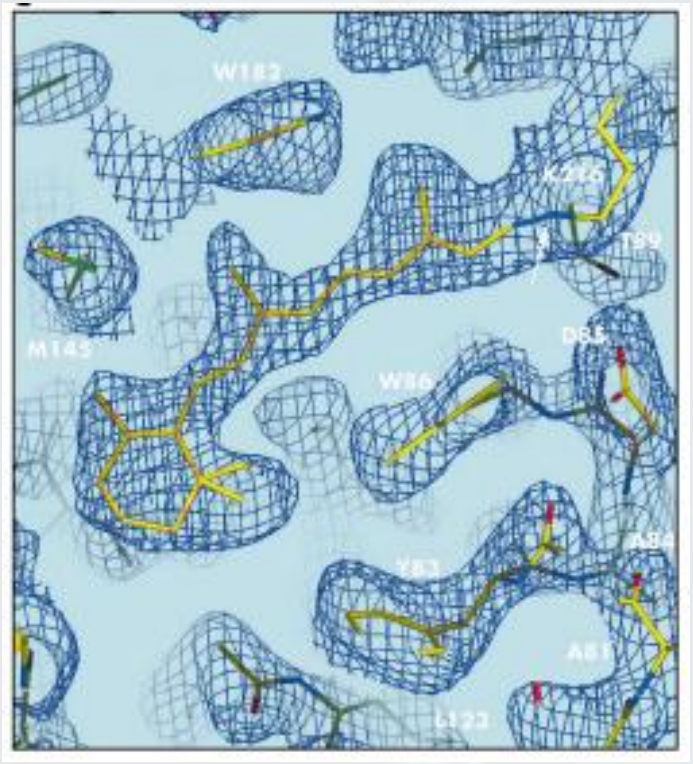


Discussion

- When are circumstances you might want to use AFM in place of electron beam imaging or x-ray diffraction?
- What are the limits of AFM? Can it be used to inform structural biology on a residue by residue basis?

Be careful: there are three types of electron density maps

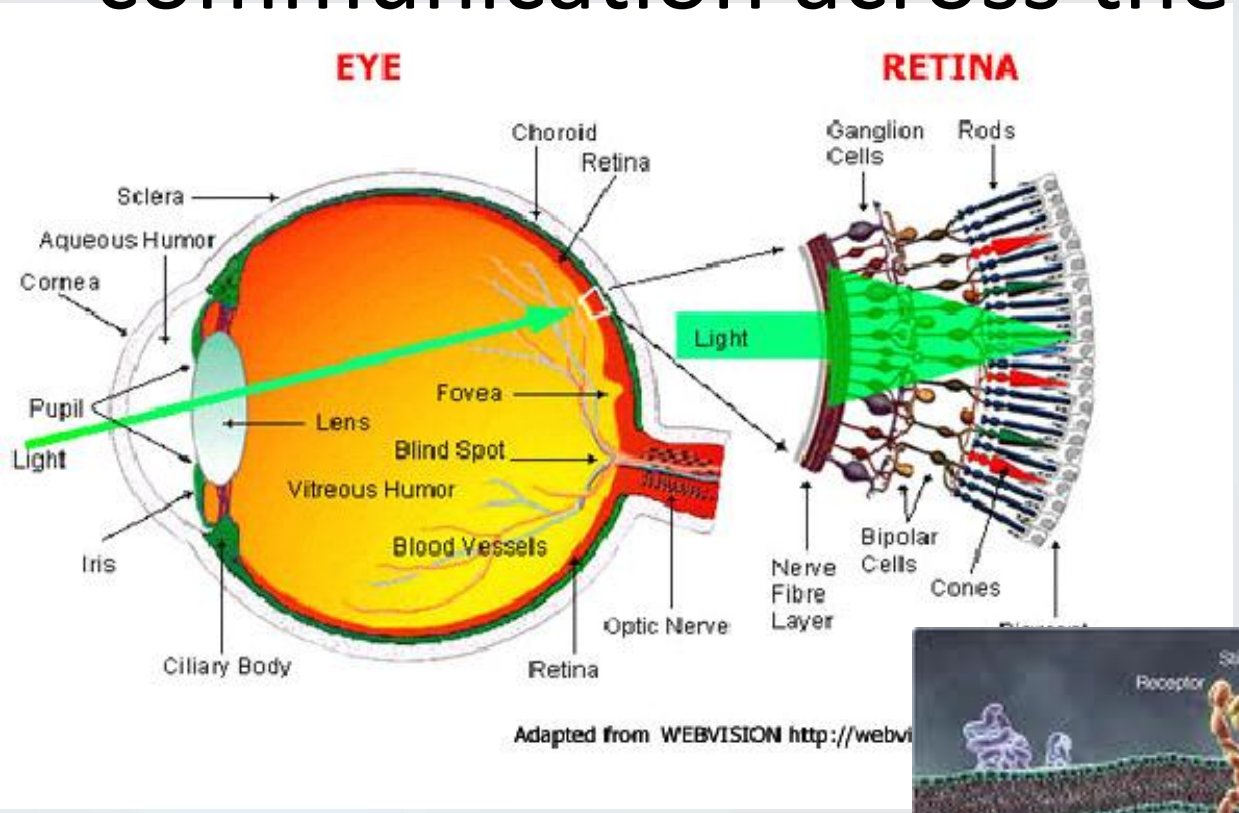
- F_obs, φ_calc
  - Contours plotted to σ
- (2F_obs – F_calc), φ_calc
  - Contours Plotted to σ
- (F_obs – F_calc), φ_calc
  - Contours Plotted to 3σ

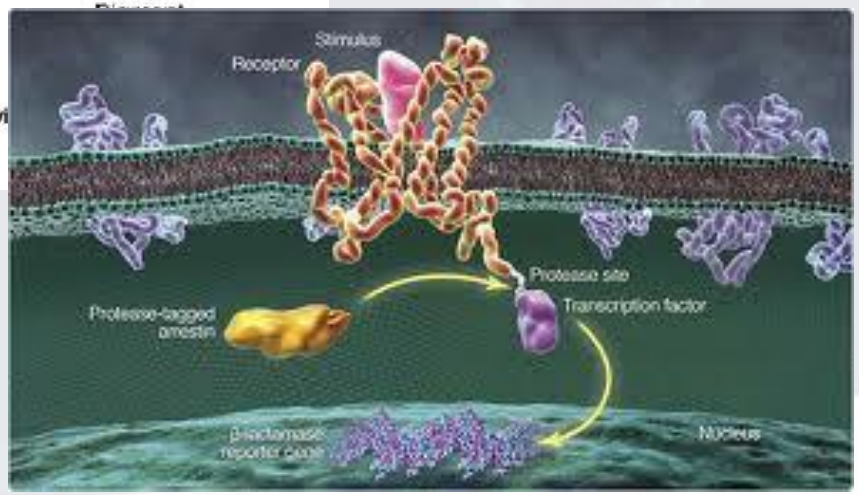


Discussion

- Why might you consider plotting the difference of your observed and calculated reflections?

GPCRs permit cells high fidelity communication across the membrane





Science
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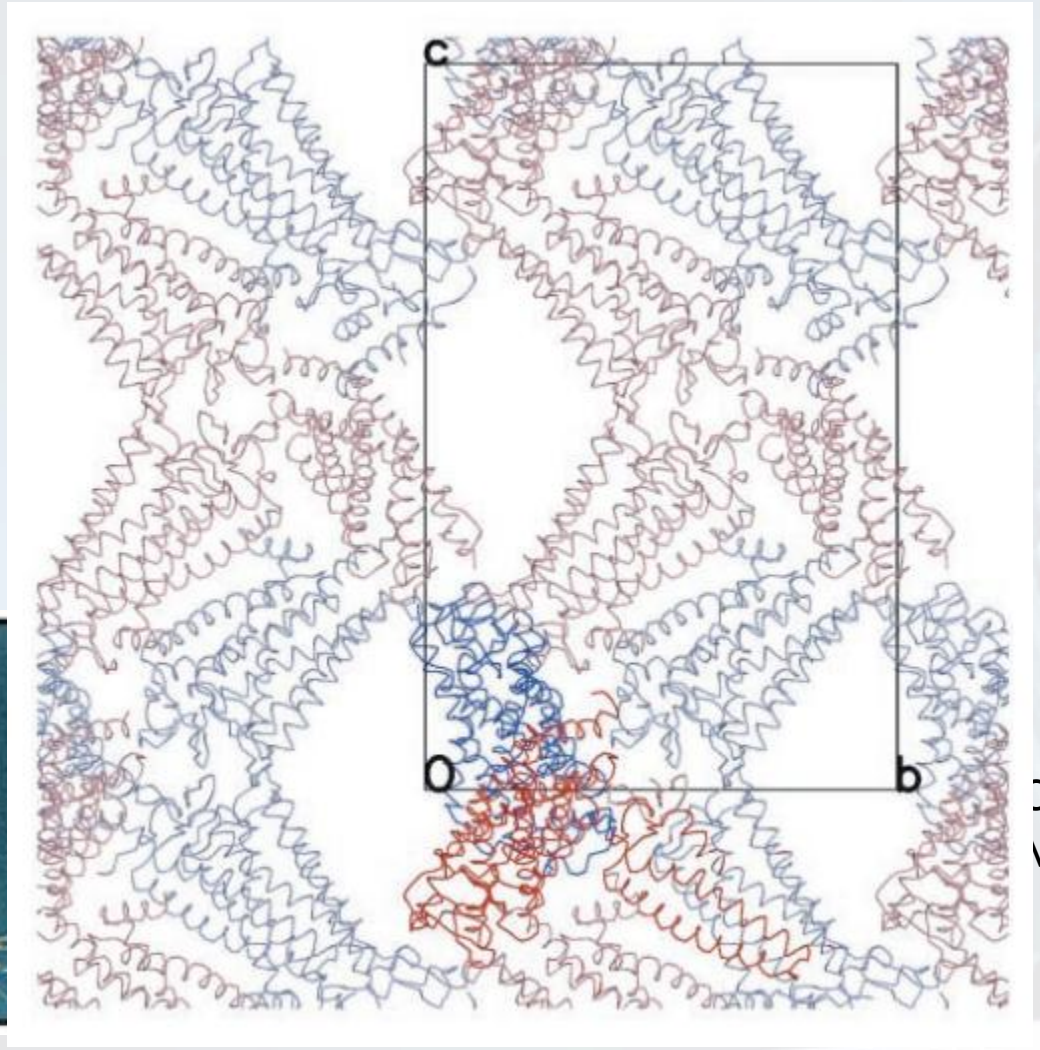
Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor
Krzysztof Palczewski, *et al.*
Science **289**, 739 (2000);
DOI: 10.1126/science.289.5480.739

Crystal Structure of Rhodopsin: A G Protein–Coupled Receptor

Krzysztof Palczewski,[1,2,3*] Takashi Kumasaka,[7] Tetsuya Hori,[7,8] Craig A. Behnke,[4,6] Hiroyuki Motoshima,[7] Brian A. Fox,[4,6] Isolde Le Trong,[5,6] David C. Teller,[4,6] Tetsuji Okada,[1] Ronald E. Stenkamp,[5,6*] Masaki Yamamoto,[7] Masashi Miyano[7*]

www.sciencemag.org SCIENCE VOL 289 4 AUGUST 2000

Mixed micelles enable crystallization of uniform crystals of bovine Rhodopsin

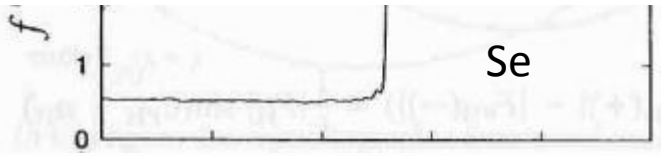


Cross Polarization
Microscopy

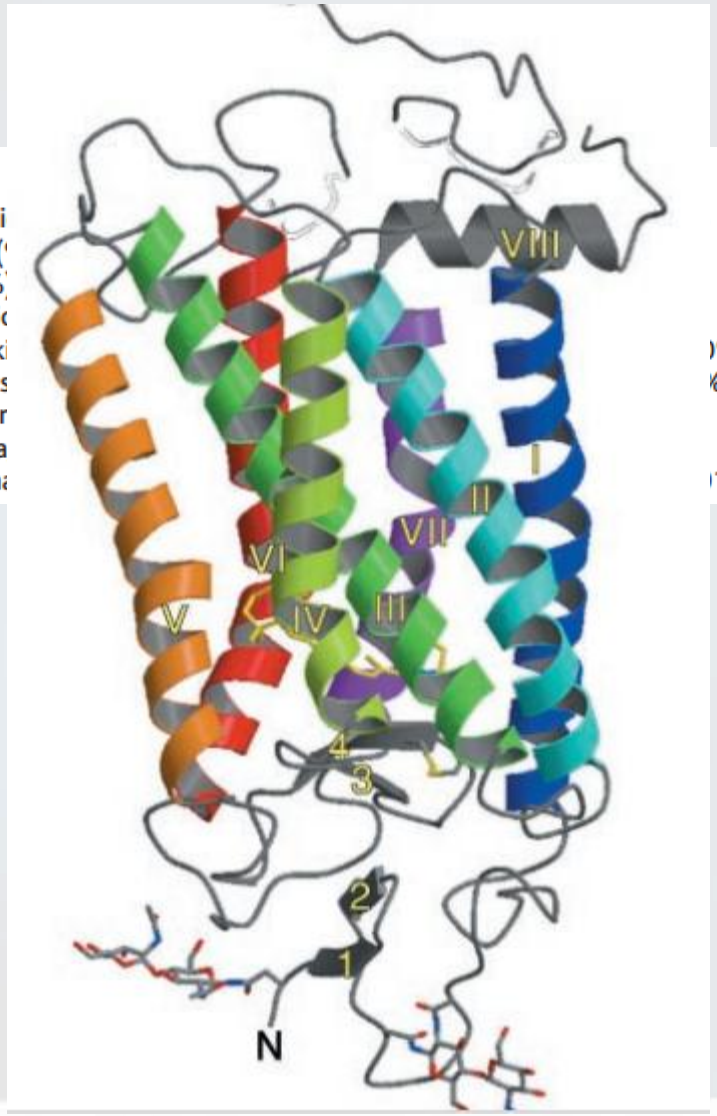
Experimental phasing information was gathered using Multi-wavelength Anomalous Diffraction

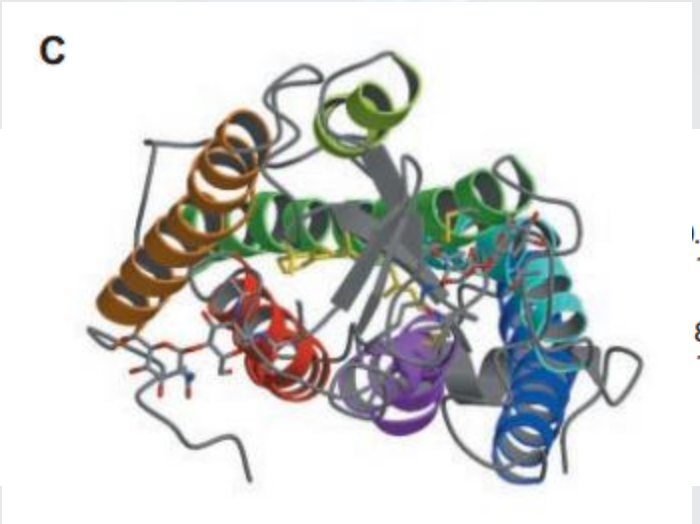
Data collection and phasing

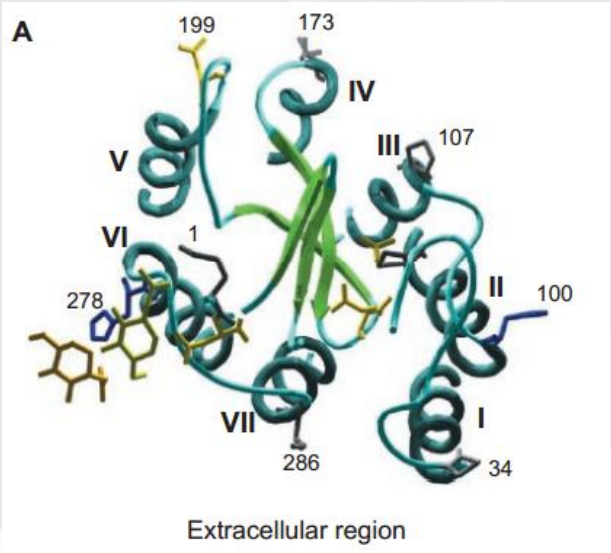
Space group	$P4_1$						
Beamline	SPring-8 BL45XU						APS 19-ID
Unit cell	a = 96.73, c = 149.63 Å						a = 97.25, c = 149.54 Å
Resolution (Å)	3.3 (MAD)						2.8 (Refine)
Data set	Remote 1	Edge 1	Peak 1	Remote 2	Edge 2	Peak 2	High resolution
Wavelength (Å)	0.96000	1.00876	1.00800	1.04000	1.00866	1.00700	1.000
Observed reflections	66,421	66,589	66,651	75,521	74,715	73,063	111,245
Unique reflections	20,499	20,529	20,541	20,636	20,613	20,624	33,221
Completeness*	99.0 (99.5)	99.2 (99.7)	99.1 (99.4)	99.4 (99.8)	99.3 (99.7)	99.3 (99.8)	97.1 (80.7)
I/σ	12.6 (3.6)	11.9 (3.3)	10.6 (2.6)	11.1 (2.4)	11.7 (3.6)	11.6 (3.2)	7.8 (1.2)
R_{merge}*†	8.5 (39.3)	9.2 (43.6)	10.0 (55.3)	10.6 (54.6)	10.3 (51.3)	10.4 (53.5)	12.1 (69.3)
Phasing power‡	0.0/1.1/–	0.6/1.0/0.4	0.9/1.0/0.7	1.6/1.0/1.1	1.5/1.1/1.1	1.4/1.2/1.0	
Figure of merit	0.37/0.31						
Twin fraction							0.288



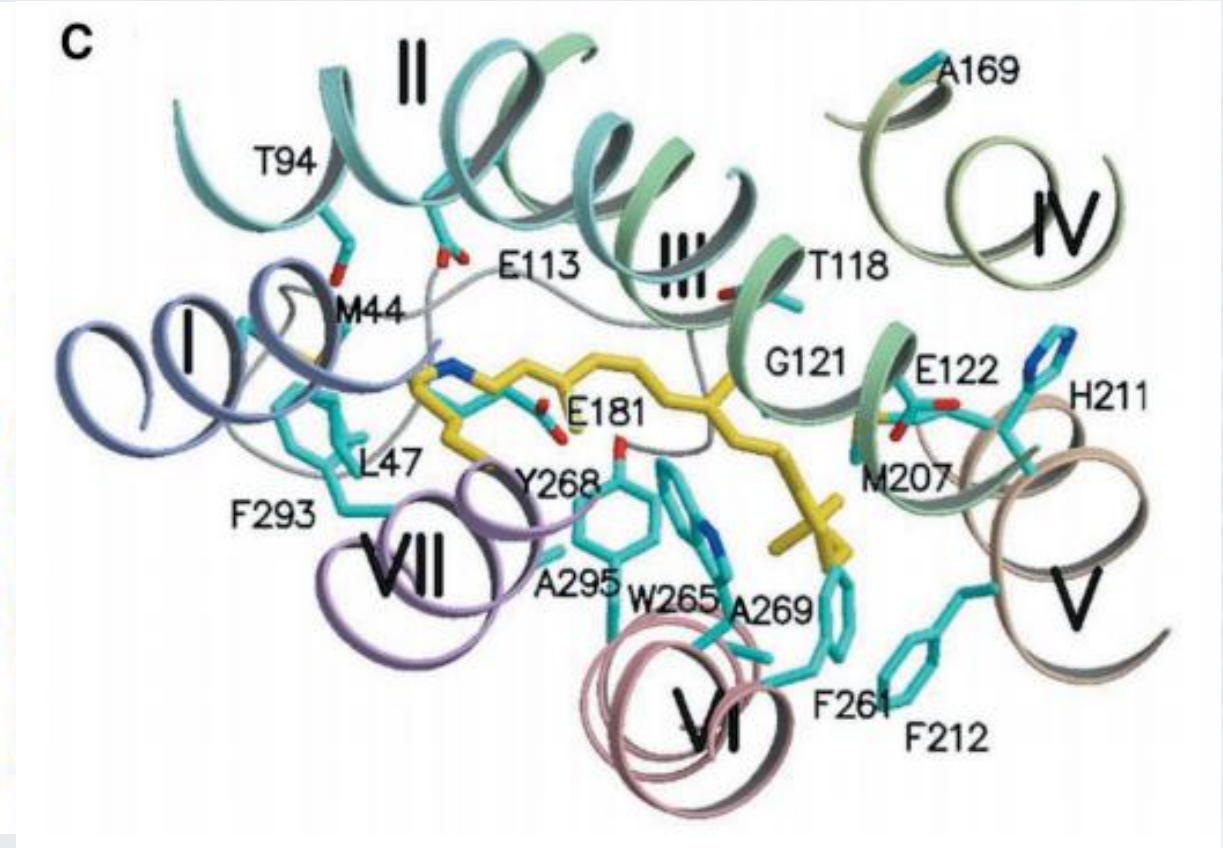
The first high resolution structure of a GPCR: Bovine Rhodopsin







The active element: the role of the 11-cis-Retinal



Discussion

- Does experimental phasing deserve more weight than molecular replacement?
- How would experimental error of light exposure alter show up in the data?

Science
AAAS

High-Resolution Crystal Structure of an Engineered Human ² 2-Adrenergic G Protein Coupled Receptor
Vadim Cherezov, *et al.*
Science **318**, 1258 (2007);
DOI: 10.1126/science.1150577

High-Resolution Crystal Structure of an Engineered Human β_2-Adrenergic G Protein–Coupled Receptor

Vadim Cherezov,[1*] Daniel M. Rosenbaum,[2*] Michael A. Hanson,[1]
Søren G. F. Rasmussen,[2] Foon Sun Thian,[2] Tong Sun Kobilka,[2] Hee-Jung Choi,[2,3]
Peter Kuhn,[4] William I. Weis,[2,3] Brian K. Kobilka,[2†] Raymond C. Stevens[1†]

23 NOVEMBER 2007 VOL 318 **SCIENCE** www.sciencemag.org

GPCR Engineering Yields High-Resolution Structural Insights into β_2-Adrenergic Receptor Function

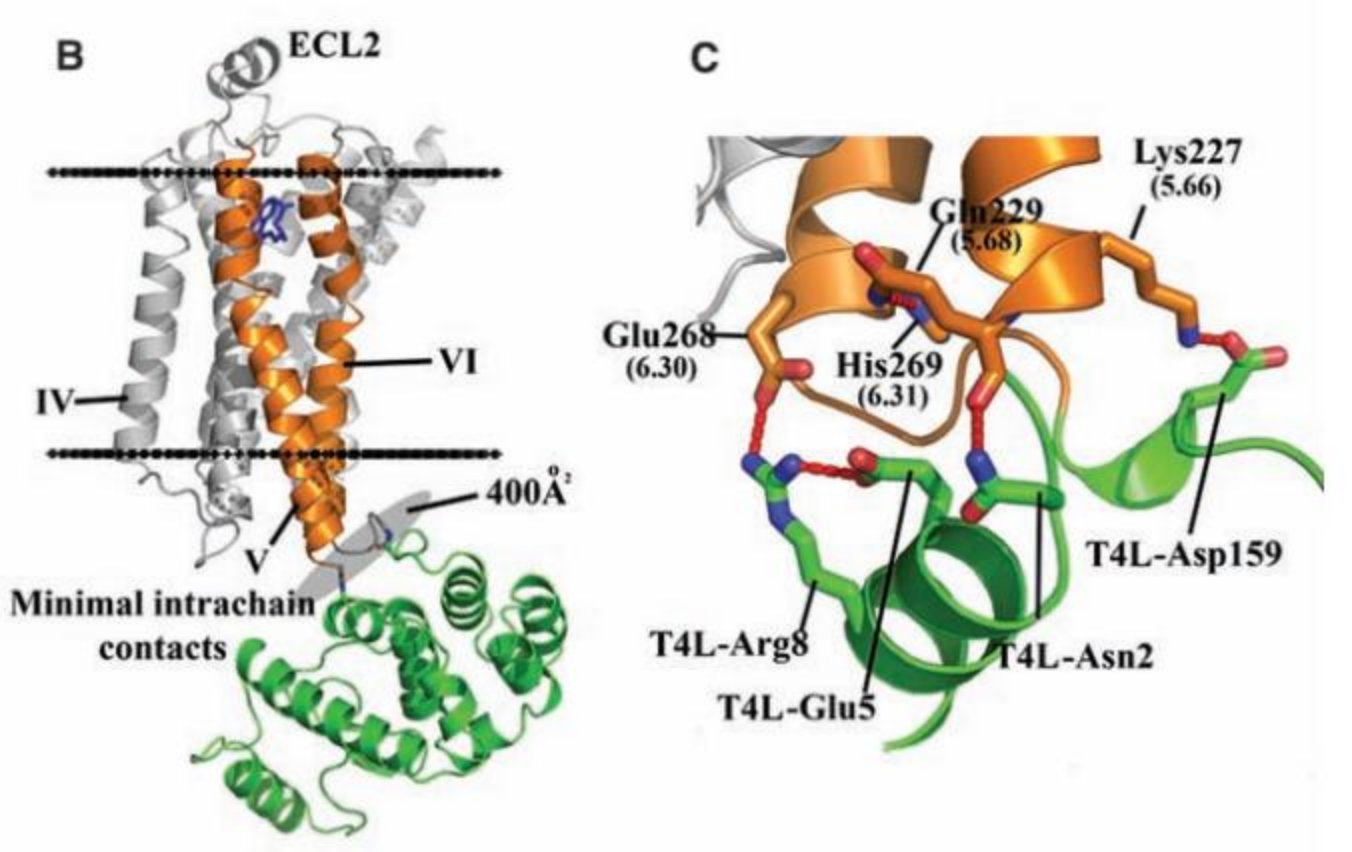
Daniel M. Rosenbaum[1,*], Vadim Cherezov[2,*], Michael A. Hanson[2], Søren G. F. Rasmussen[1], Foon Sun Thian[1],
Tong Sun Kobilka[1], Hee-Jung Choi[1,3], Xiao-Jie Yao[1], William I. Weis[1,3], Raymond C. Stevens[2,†], Brian K. Kobilka[1,†]

More practice surveying modern data standards

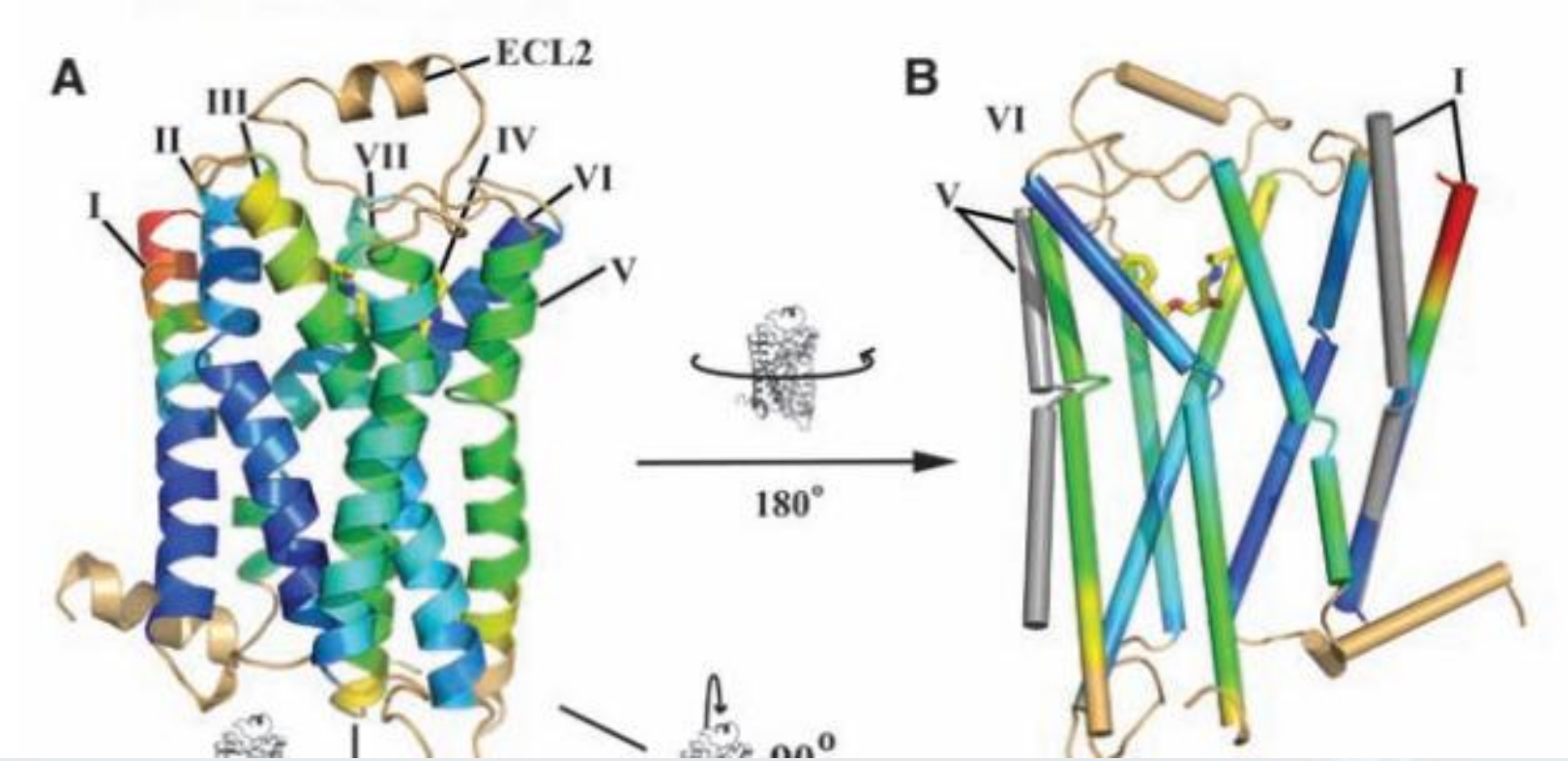
	β_2AR-T4L
*Data collection (APS GM/CA CAT 23ID-B, 10-μm beam)**	
Space group	*C*2
Cell dimensions	
a, *b*, *c* (Å)	106.3, 169.2, 40.2
β (°)	105.62
Number of reflections processed	245,571
Number of unique reflections	26,574
Resolution (Å)	50 to 2.4 (2.5 to 2.4)
R_{sym}†	12.7 (67.8)
Mean $I/\sigma(I)$	9.6 (2.2)
Completeness (%)	99.5 (99.1)
Redundancy	9.4 (4.8)
*Refinement**	
Resolution (Å)	20 to 2.4 (2.46 to 2.4)
Number of reflections (test set)	25,247 (1310)
R_{work} / R_{free}	19.8 (27.0) / 23.2 (30.1)
Number of atoms	3805
Protein	3544
Ions, lipids, ligand, and other	213
Water	48
Overall *B* values (Å^2)	82
β_2AR	77
T4 lysozyme	75
Carazolol	55
Lipid	100
RMSD	
Bond lengths (Å)	0.013
Bond angles (°)	1.5
Ramachandran plot statistics (%) (excluding Gly, Pro):	
Most favored regions	94.8
Additionally allowed regions	5.0
Generously allowed regions	0.2
Disallowed regions	0

*Highest-resolution shell is shown in parentheses. †$R_{sym} = \Sigma_{hkl}\, I(hkl) - \langle I(hkl)\rangle | I/\Sigma_{hkl}(hkl)$, where $\langle I(hkl)\rangle$ is the mean of the symmetry-equivalent reflections of $I(hkl)$.

The β2AR-T4L Structure by Lipidic Cubic phase crystallography



The remarkable plasticity of GPCRs enables their diverse roles in communication



Discussion Questions

- Why does the lysozyme help crystallization?
- Why is carazolol important for β2AR-T4L structure?
- What implications does GPCR diversity have for drug design?

Thank you for your time

I hope you feel this class has been worthwhile.