

Marine Biological Laboratories  
Workshop in Molecular Evolution

# Adaptive protein evolution: Introduction

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How do protein sequences evolve?

Can we identify evolutionary patterns of selection associated with adaptive shifts in protein function?

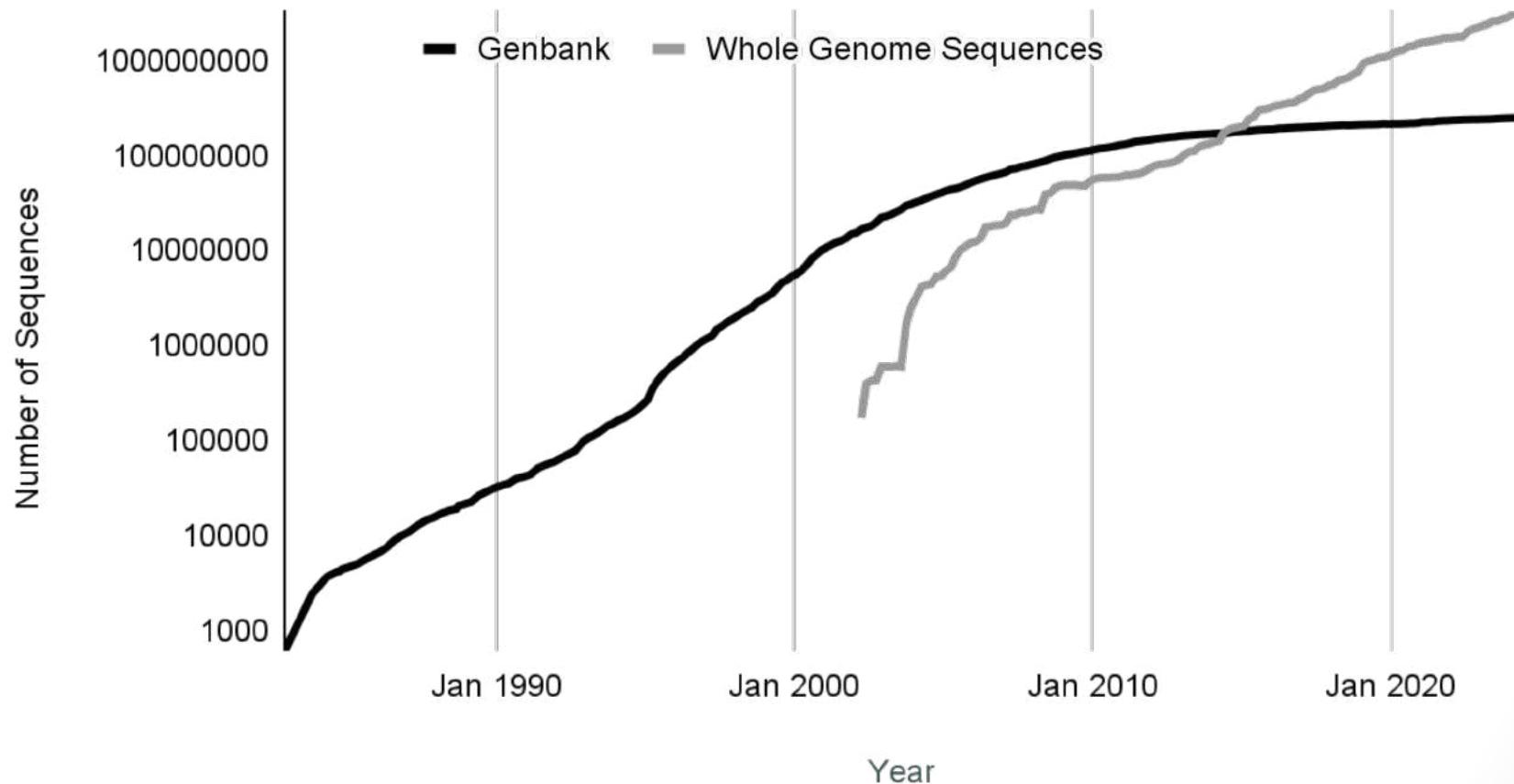
Can we identify the underlying mechanisms associated with adaptive shifts?

# Evolution of protein function

$$Q_{ij} = \begin{cases} 0 \\ \pi_j \\ \kappa\pi_j \\ \omega\pi_j \\ \omega\kappa\pi_j \end{cases}$$

(3)

# Rapid accumulation of sequence data



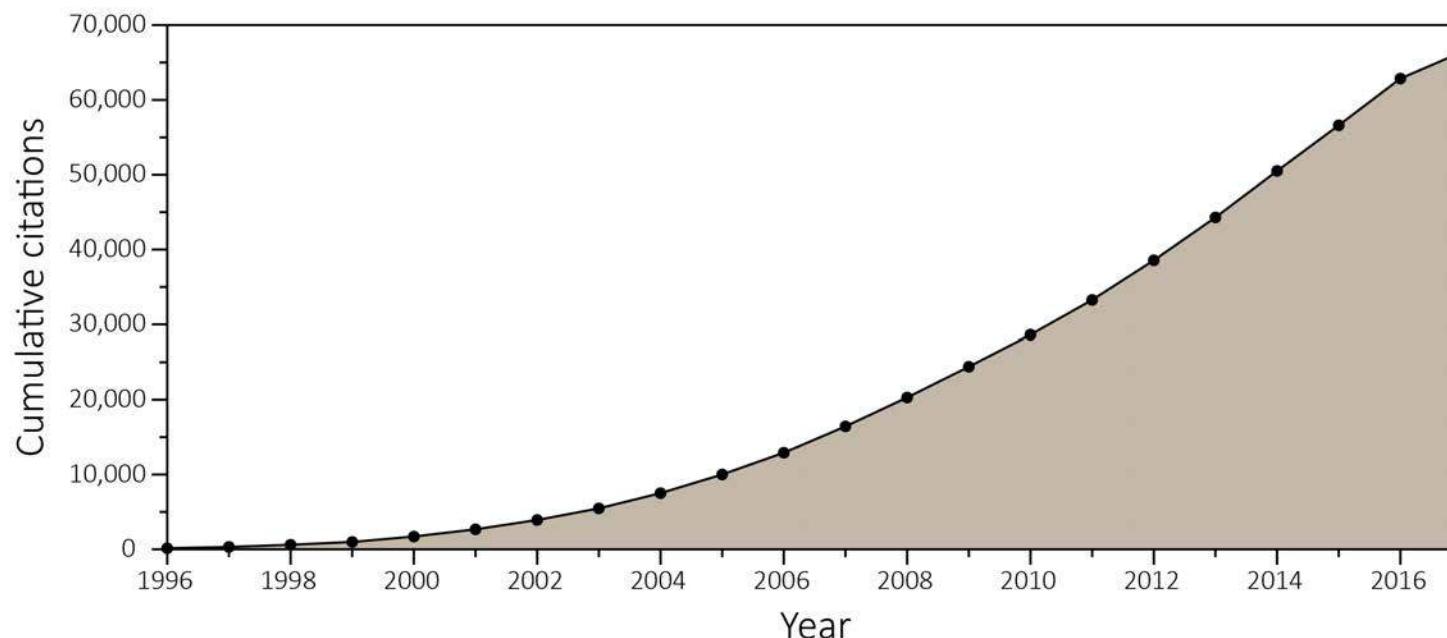
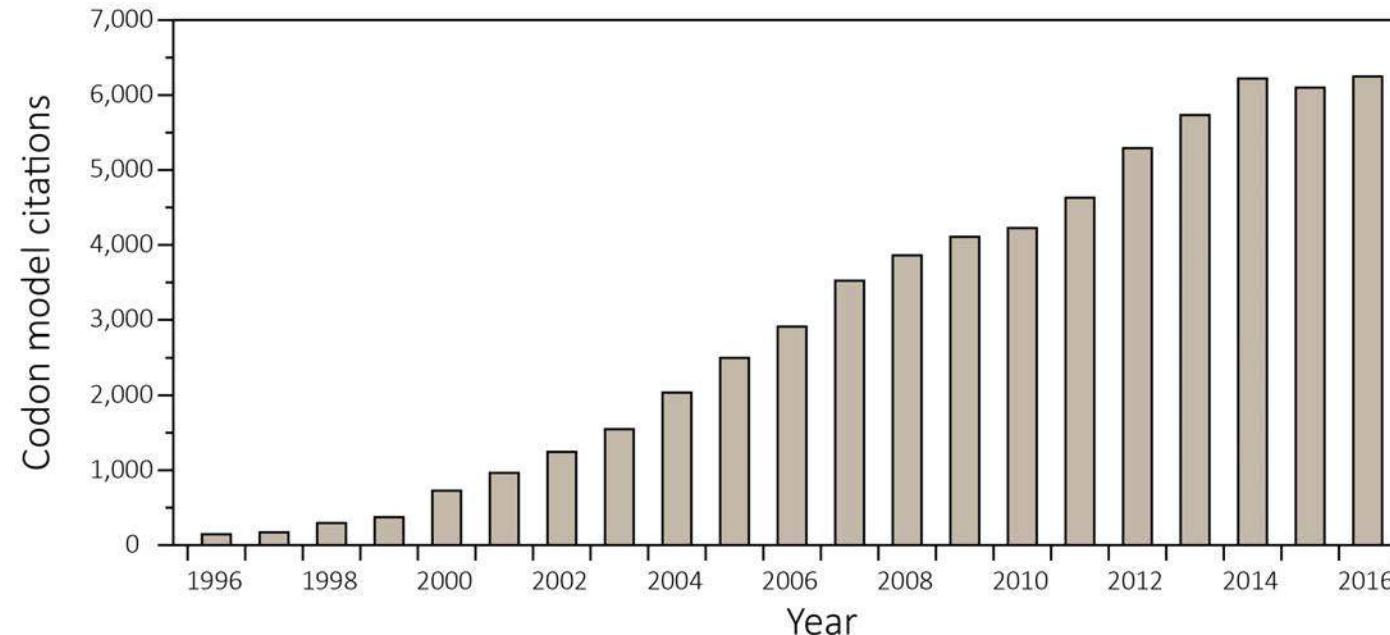
Comparative sequencing can be used to address questions at many different levels

- Evolution of organisms, systematics
- Evolution of genomes
- Evolution of gene regulation
- Evolution of proteins

# Evolution of protein-coding genes: Comparative sequence analysis

- Phylogenetically based methods
- Models of evolution (nucleotide, amino acid, codon)
- Hypothesis testing of theories of selection
- $dN/dS$  as a measure of the strength of selection

# Increased use of codon models



# Codon-based testing for positive selection: Why so popular? Hypothesis testing!

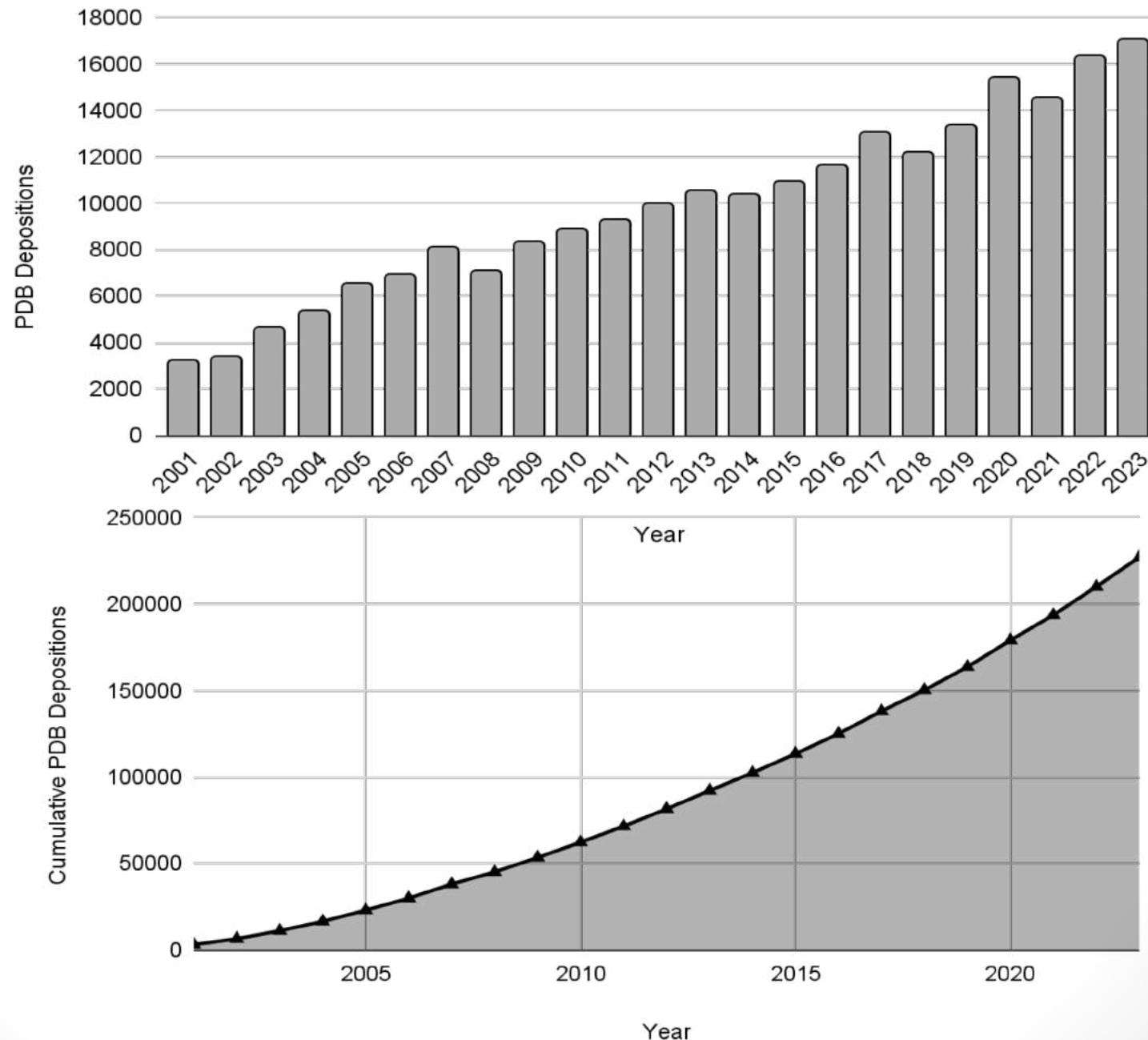
- WHEN selection occurred in evolution
  - Episodic, pervasive, lineage-specific selection
- WHICH proteins were targets of selection
  - Physiology: sensory, metabolic, developmental
- WHICH regions of the protein
  - Mechanisms underlying evolution of function
- Hints as to WHY selection occurred

# Evolution of protein function

$$Q_{ij} = \begin{cases} 0 \\ \pi_j \\ \kappa\pi_j \\ \omega\pi_j \\ \omega\kappa\pi_j \end{cases}$$

[ 9 ]

# Rapid accumulation of protein structures



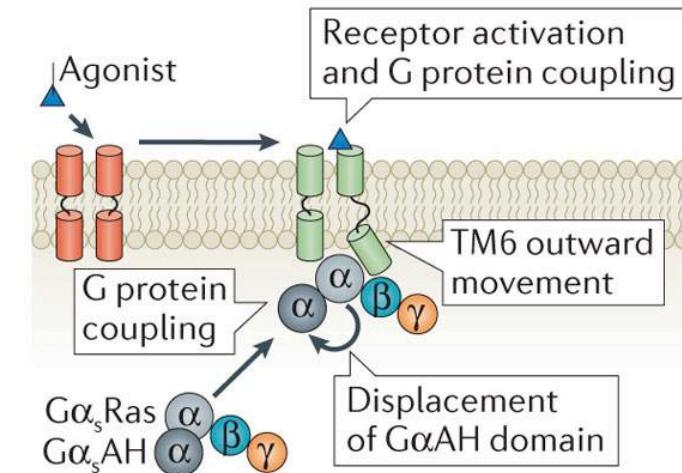
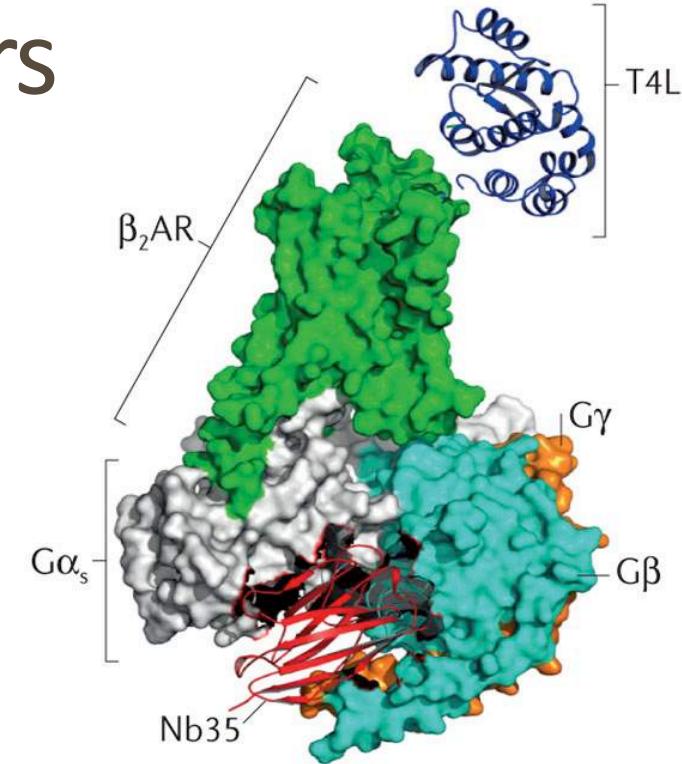
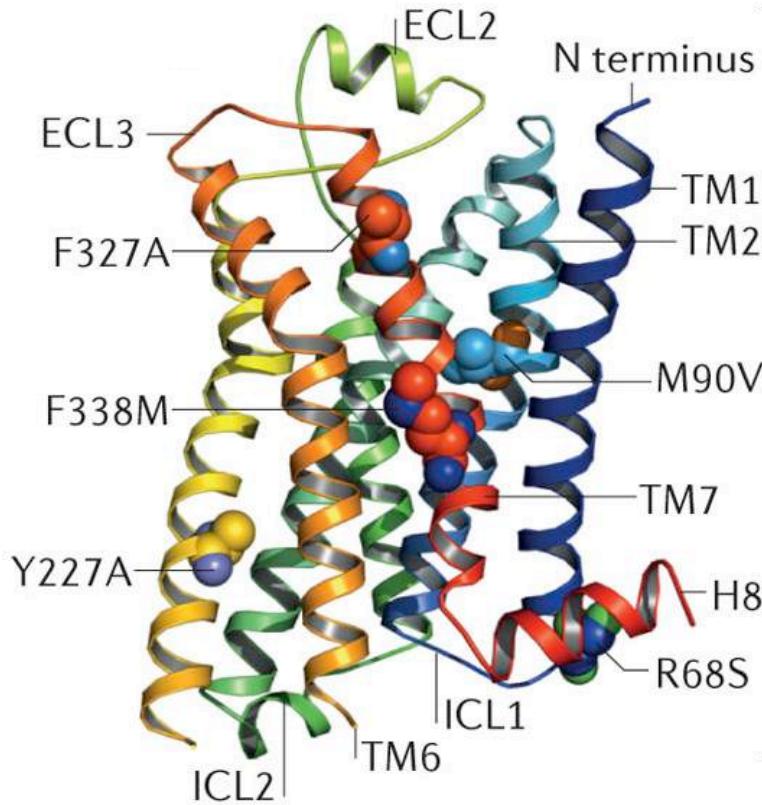
Adaptive protein evolution

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# Rapid accumulation of protein structures

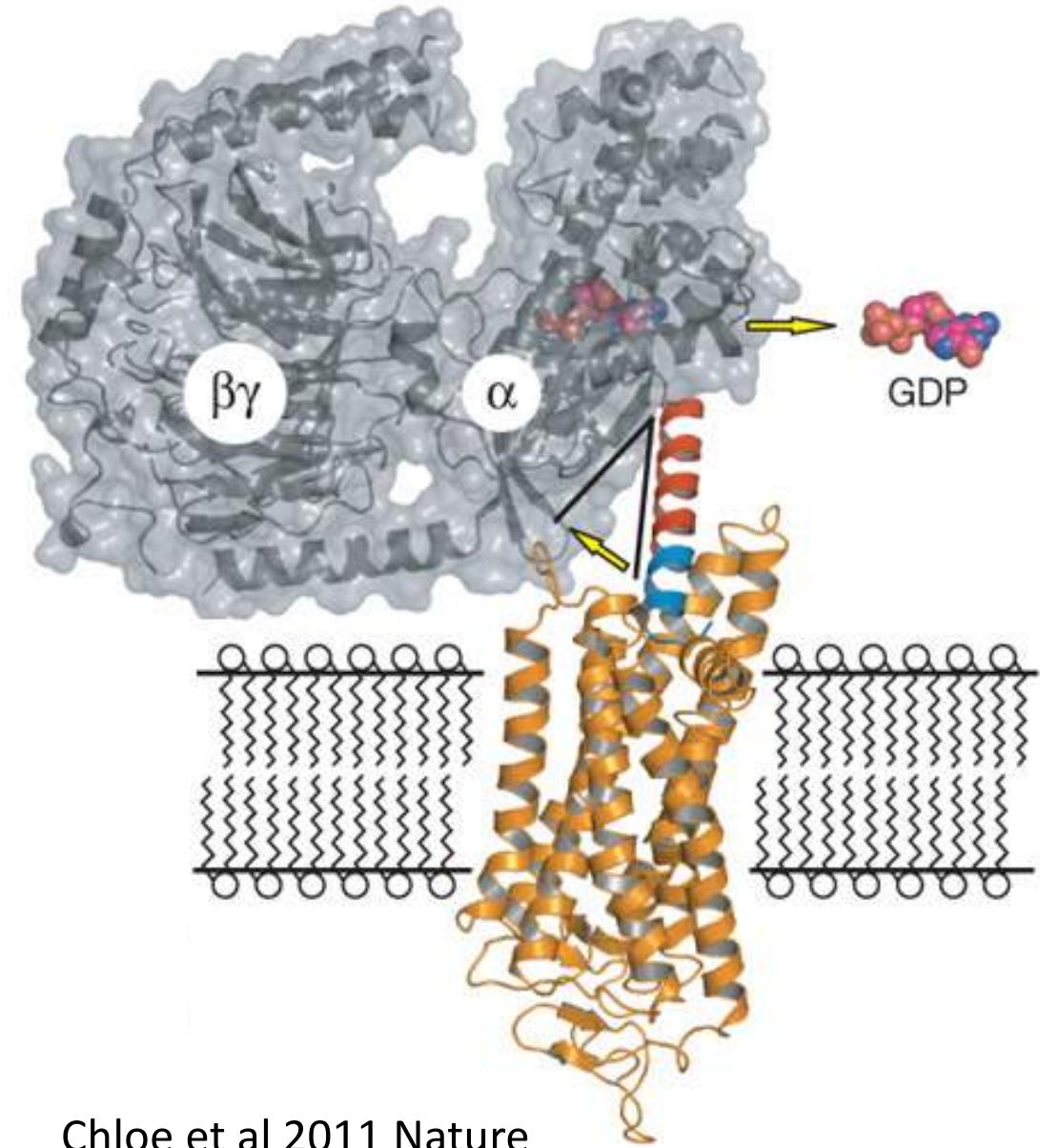
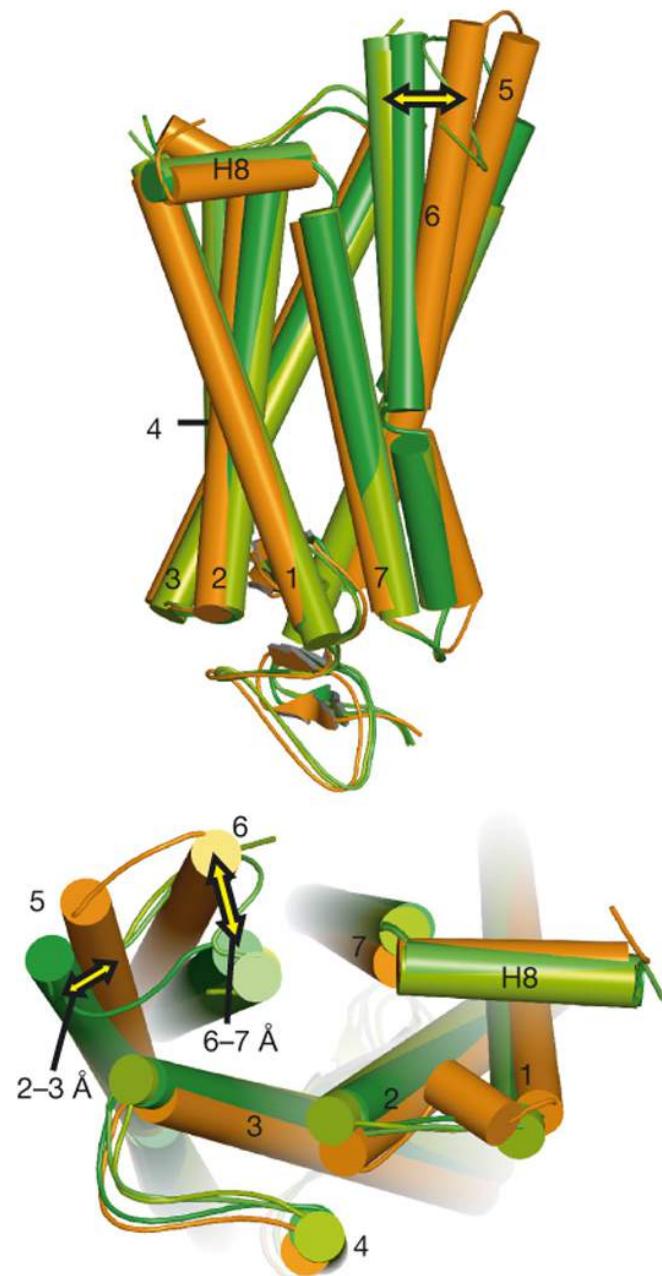
- Driven by interest in high-throughput crystallography
- Advances in protein structure determination methods
- Programs such as the Protein Structure Initiative
- Targeted difficult to crystallize proteins such as membrane proteins and large macromolecular assemblies

# G protein-coupled receptors



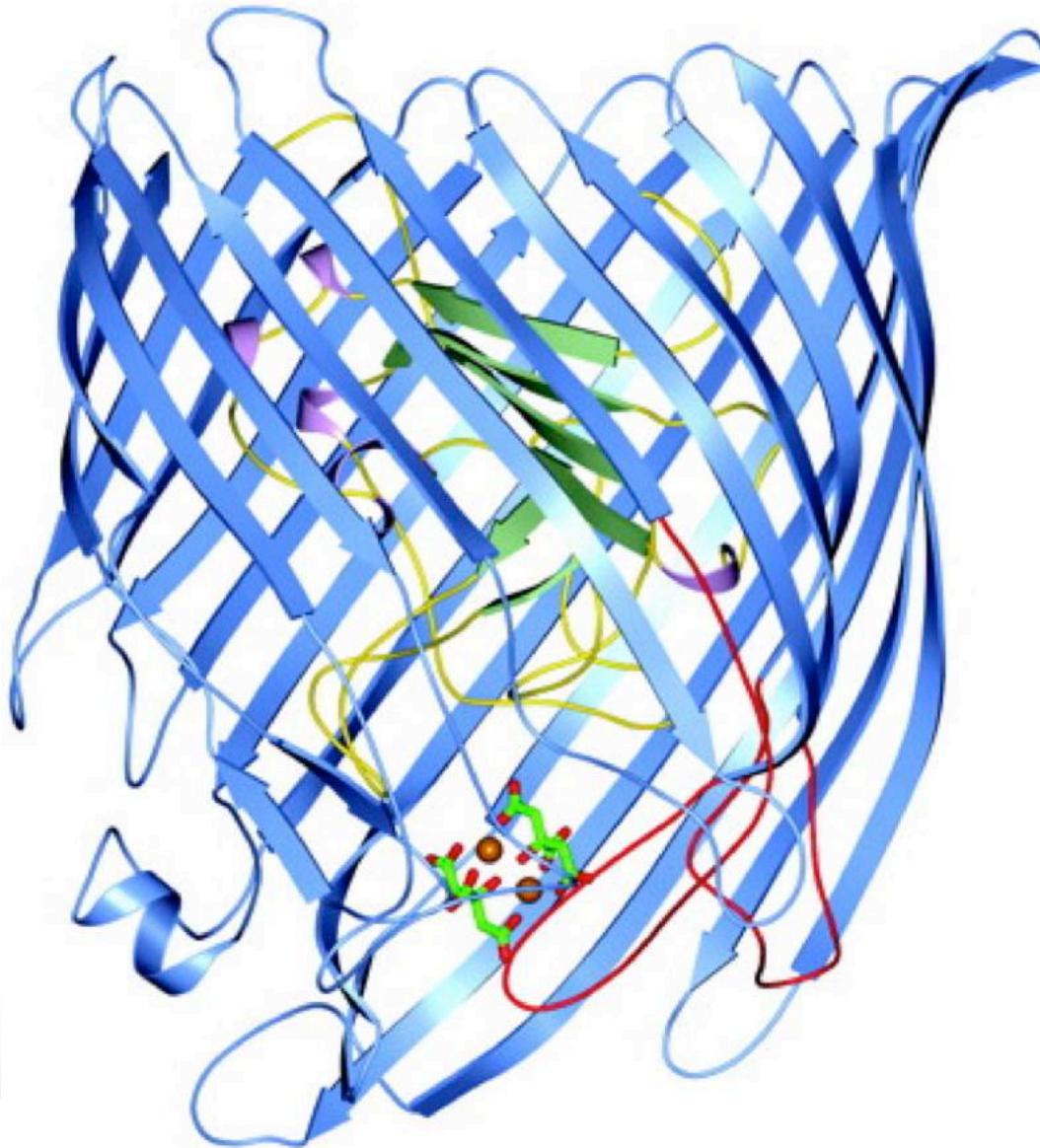
- Largest family of TM signaling proteins
- Extremely difficult to crystallize

# Conformational changes upon activation

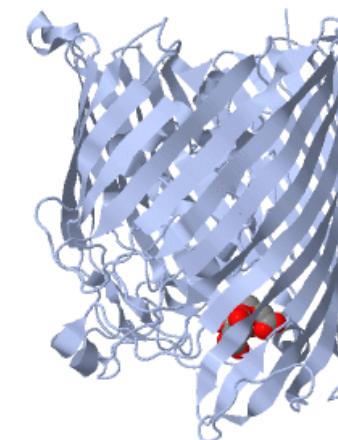


Chloe et al 2011 Nature

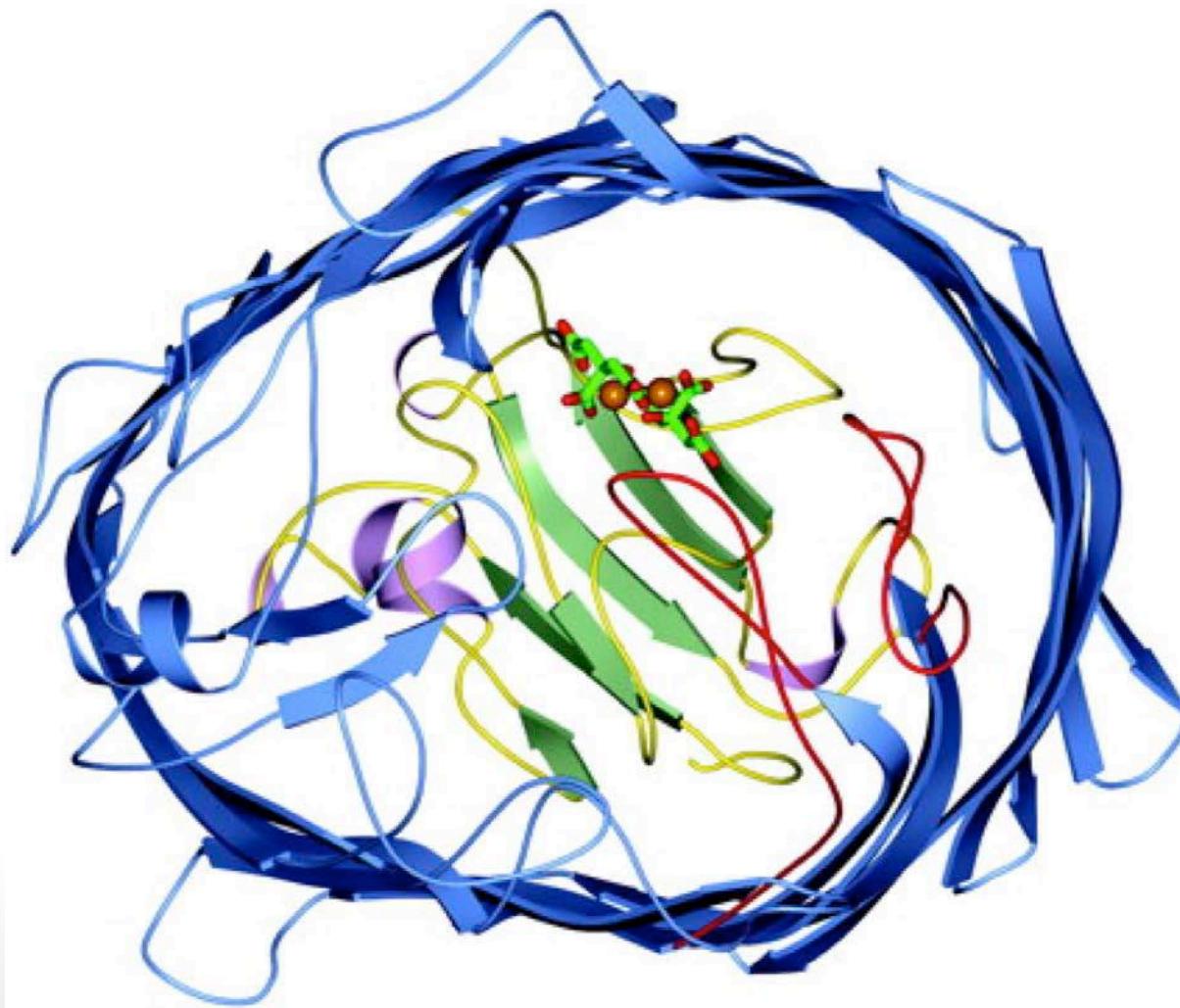
# Largest monomeric TM protein: FecA



- Bacterial ion transporter
- Large transmembrane protein
- 22 beta strands

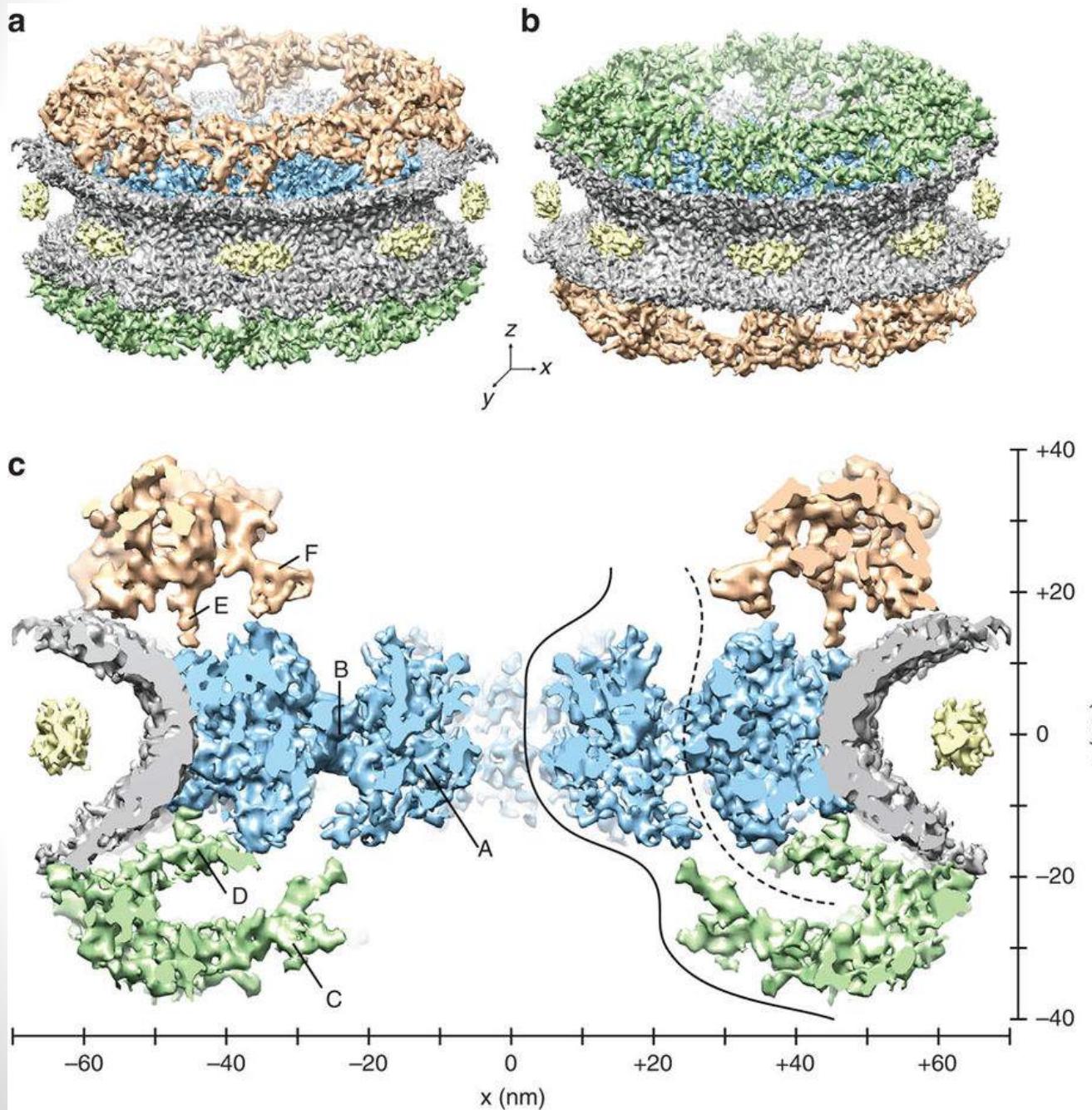


# Largest monomeric TM protein: FecA



Bacterial ion  
transporter  
Large transmembrane  
protein  
22 beta strands

# Nuclear pore complex

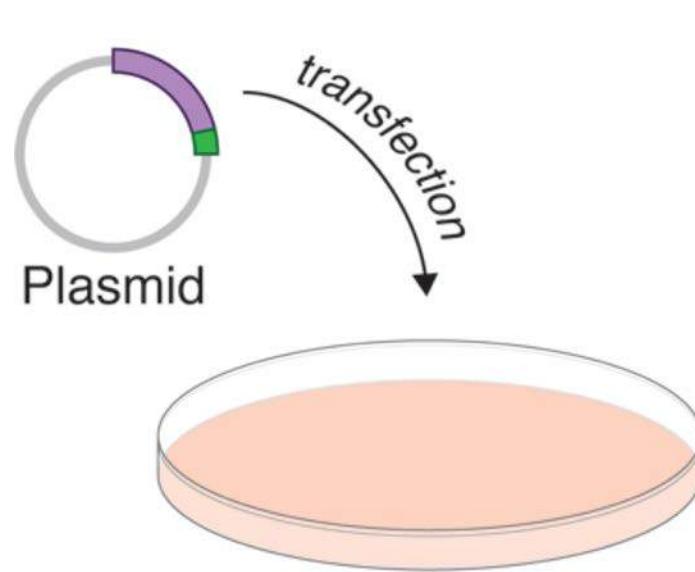


- Largest membrane bound structure
- About 30 different proteins
- Diameter of 98 nm, 50 MDa

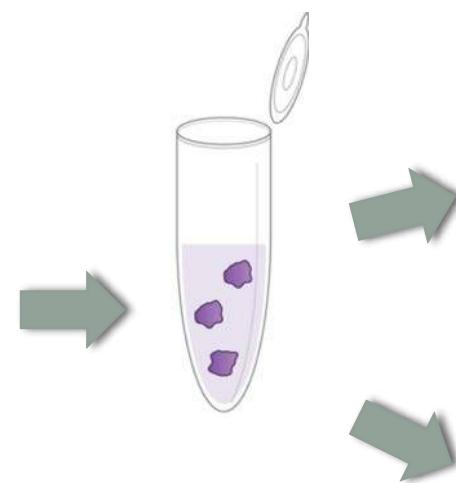
# Recent advances in protein structure studies

- Difficulties of working with proteins
- Required the development of expression methods to obtain large amounts of properly folded protein
- Mostly X-ray crystallography, but also NMR, and more recently cryo-electron microscopy
- Homology modeling, molecular dynamics
- Structure predictions

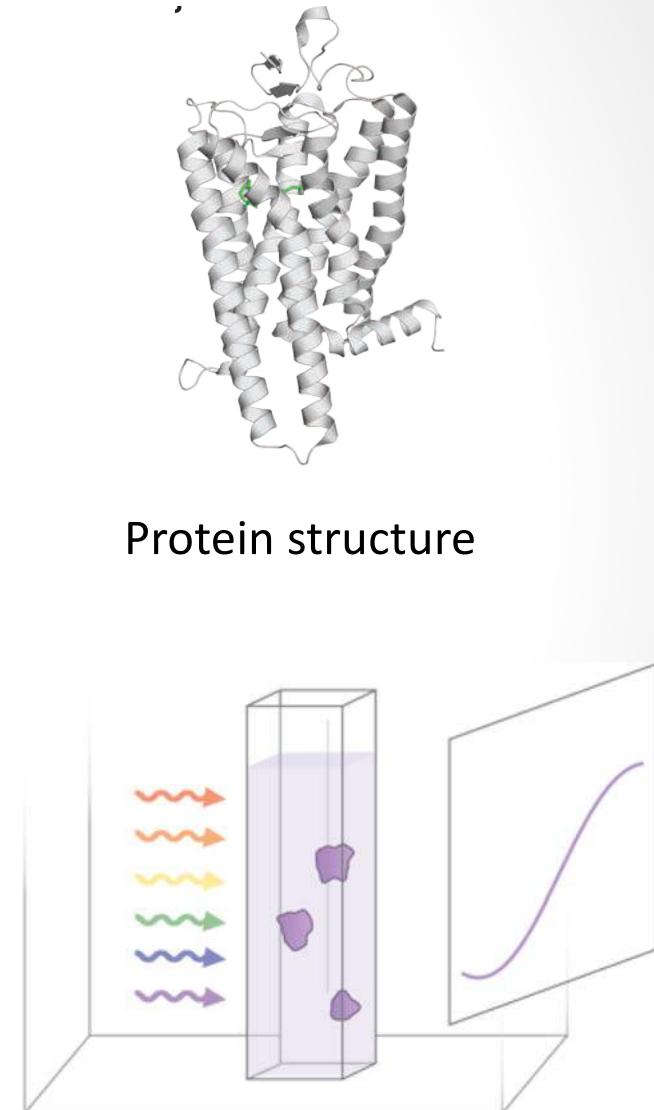
# In vitro protein expression methodologies



Grow cells containing  
gene of interest



Purify  
protein



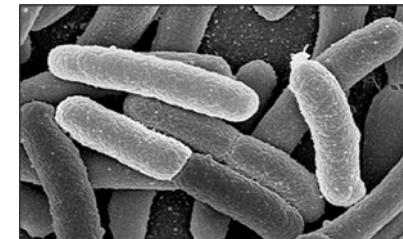
Functional assays

# In vitro expression vs. purification from tissue

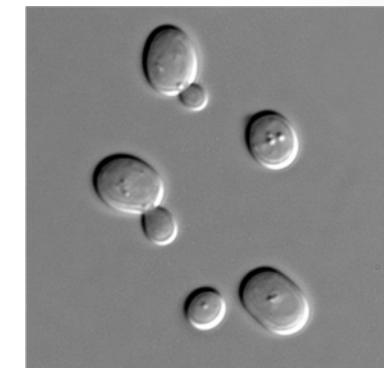
- Many proteins only present in small amounts in tissue
- Purity of sample may be an issue with complex tissues
- Purification from tissue samples does not allow for site-directed mutagenesis studies
- In vitro expression allows for testing of evolutionary hypotheses of protein structure and function

# In vitro protein expression methodologies

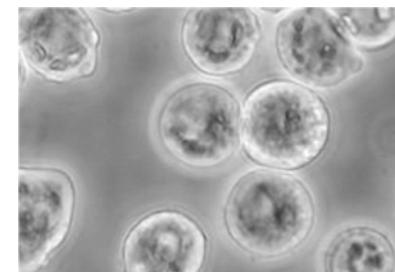
- Bacteria: *E. coli*



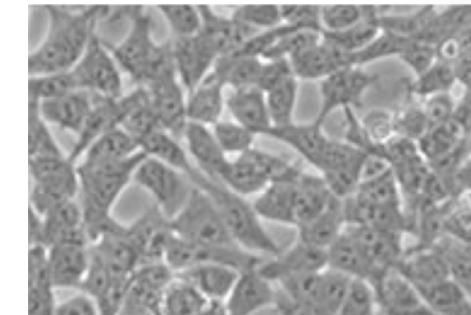
- Yeast cells: *S. cerevisiae*



- Insect cells: SF9

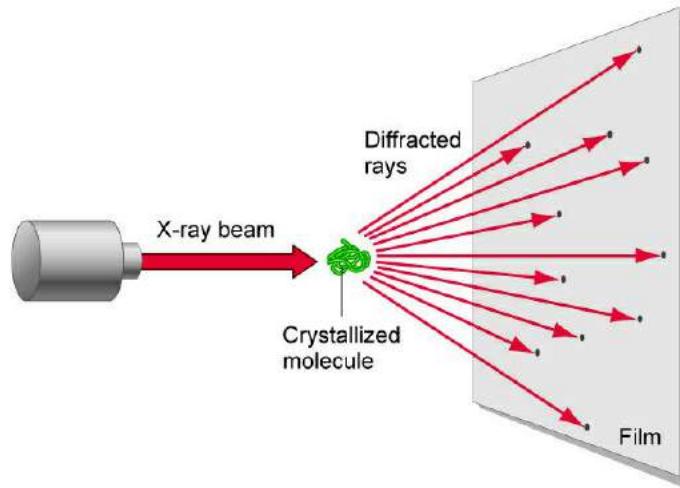


- Mammalian cell culture: HEK293

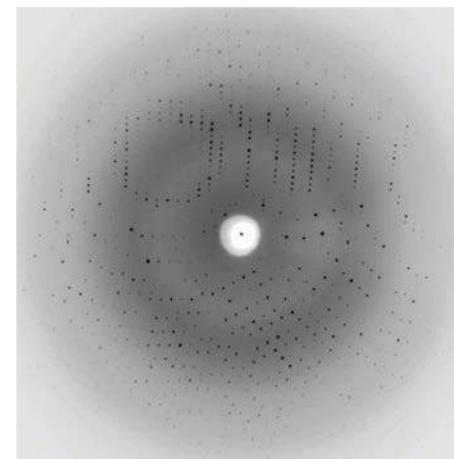


# Protein structure methodologies

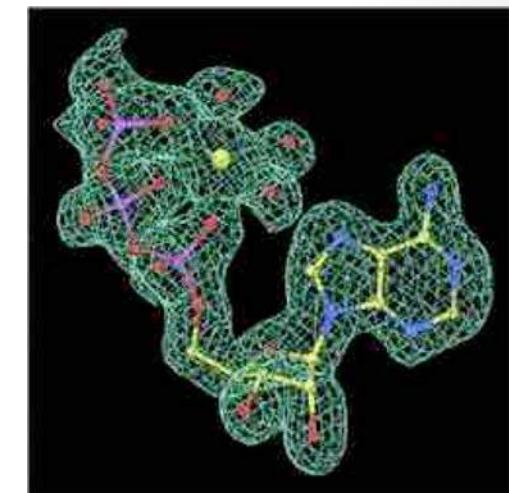
## X-ray crystallography



Synchotron



Diffraction pattern

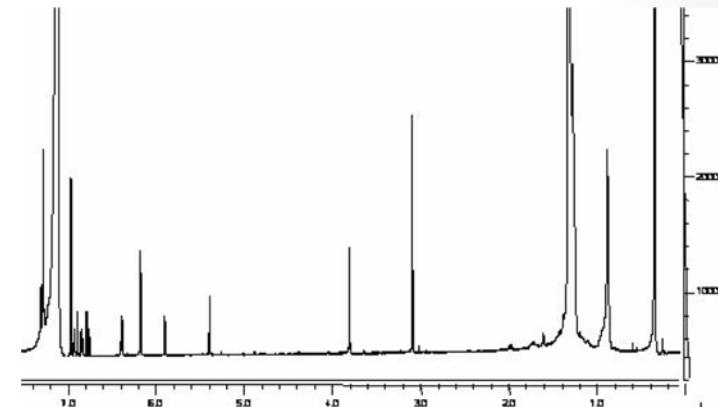
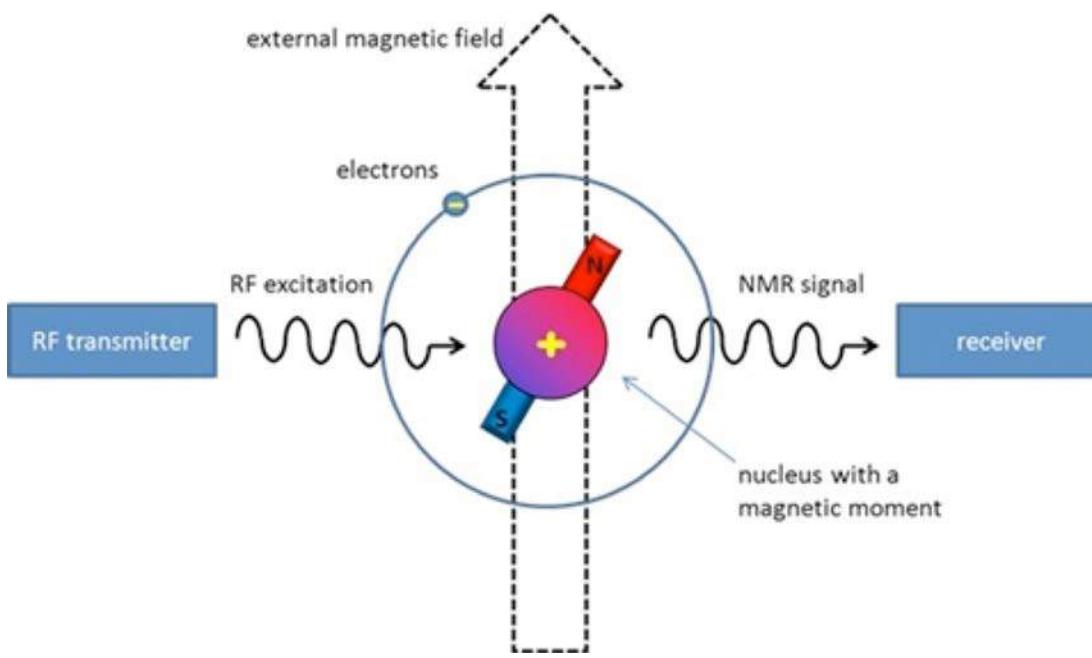


Electron density map

- Multiple conformations, flexible regions often unresolved
- Crystallization conditions not found in nature
- Serial femtosecond crystallography

# Protein structure methodologies

## NMR spectroscopy

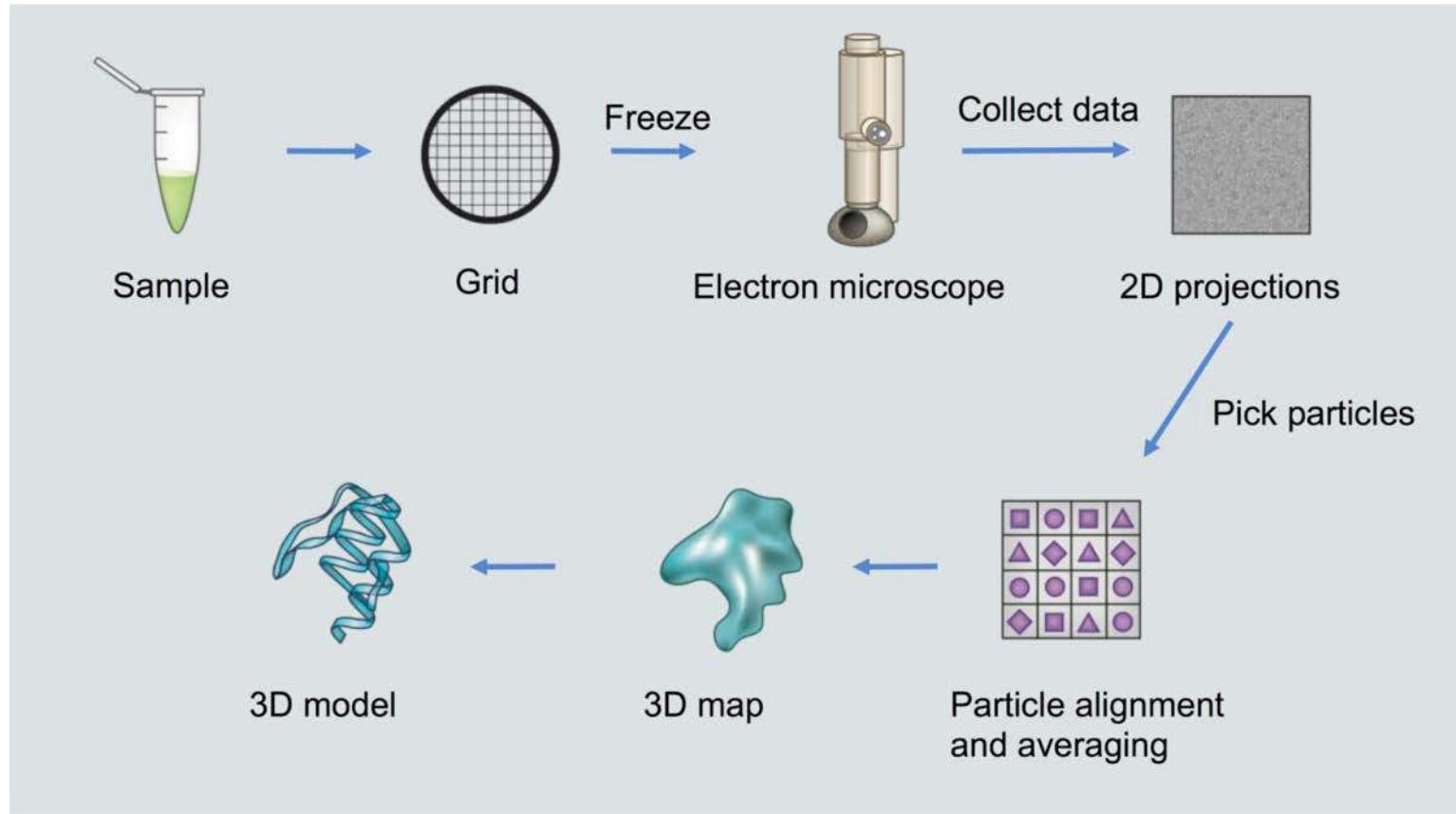


NMR spectra

- Advantage of measuring proteins in solution
- Great for studying flexible proteins
- Limited to small proteins

# Protein structure methodologies

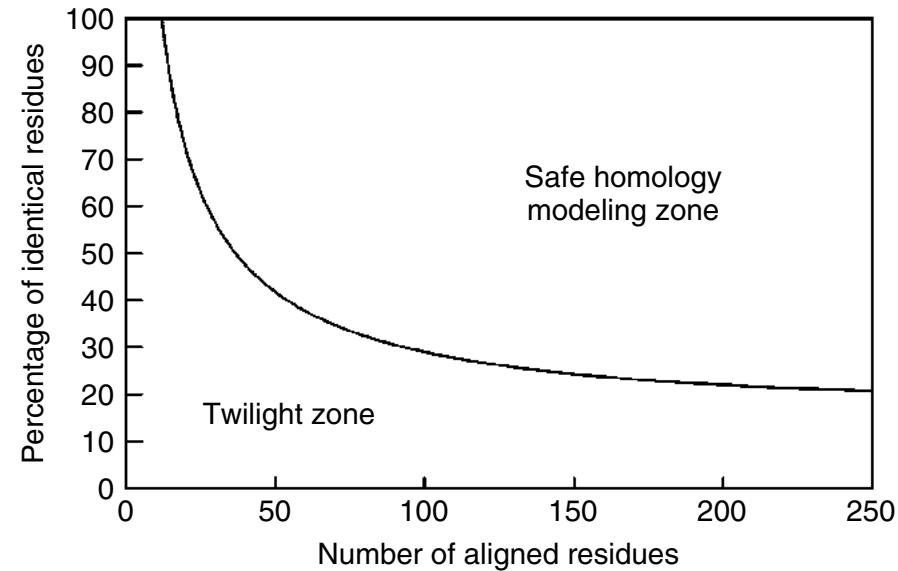
## Cryo-electron microscopy



- Advances in direct detection, sample prep, and instrumentation have achieved high resolution for larger protein complexes
- This technique offers high resolution of larger proteins in a native state
- Requires highly specialized instrumentation

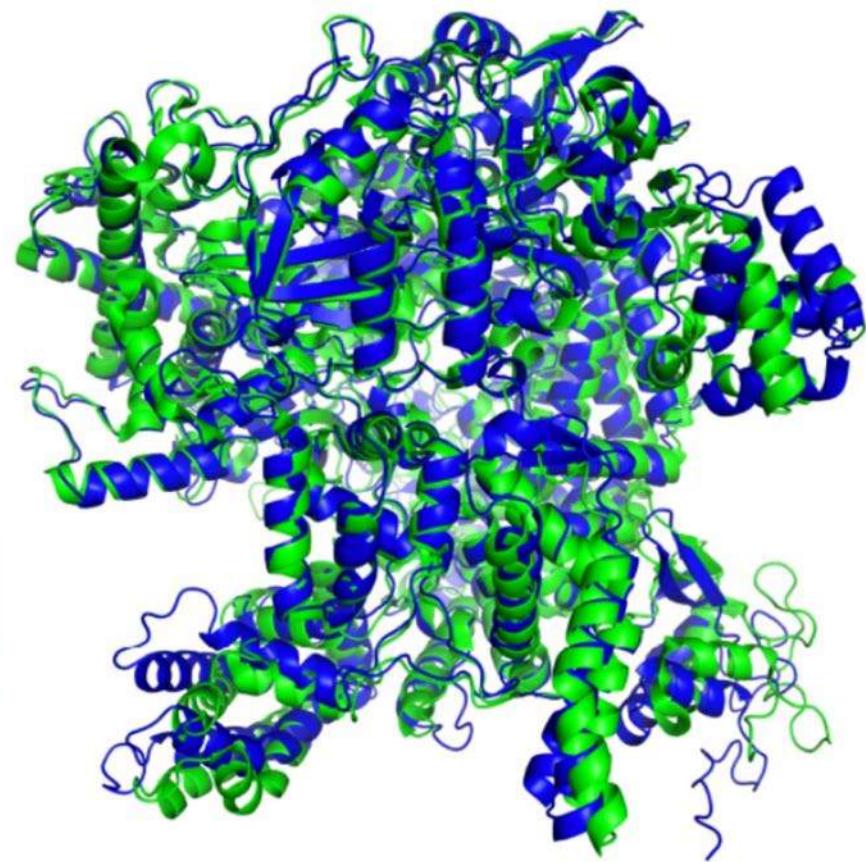
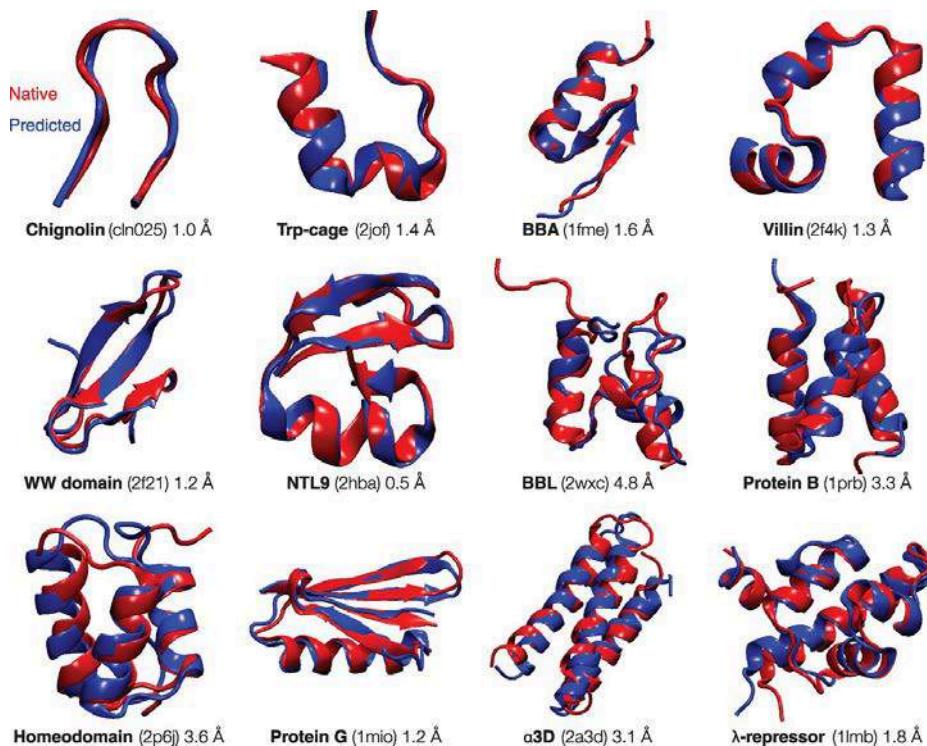
# What about protein structure prediction?

Where the twilight zone  
is may depend on your  
of view



Krieger et al. 2003

# The twilight zone for ab initio protein folding predictions



Dill & MacCallum, Science 2012

Jumper et al, Nature 2021

# Protein structure prediction

- Homology modeling
  - MODELLER (<https://salilab.org/modeller/>)
  - Rosetta suite (<http://robetta.bakerlab.org/>)
  - SWISS-MODEL (<https://swissmodel.expasy.org/>)
- Molecular dynamics simulations
- Machine learning (AlphaFold)

# Molecular evolution: Evolution of protein function

## DATA

- Genomic sequencing
- Protein structures

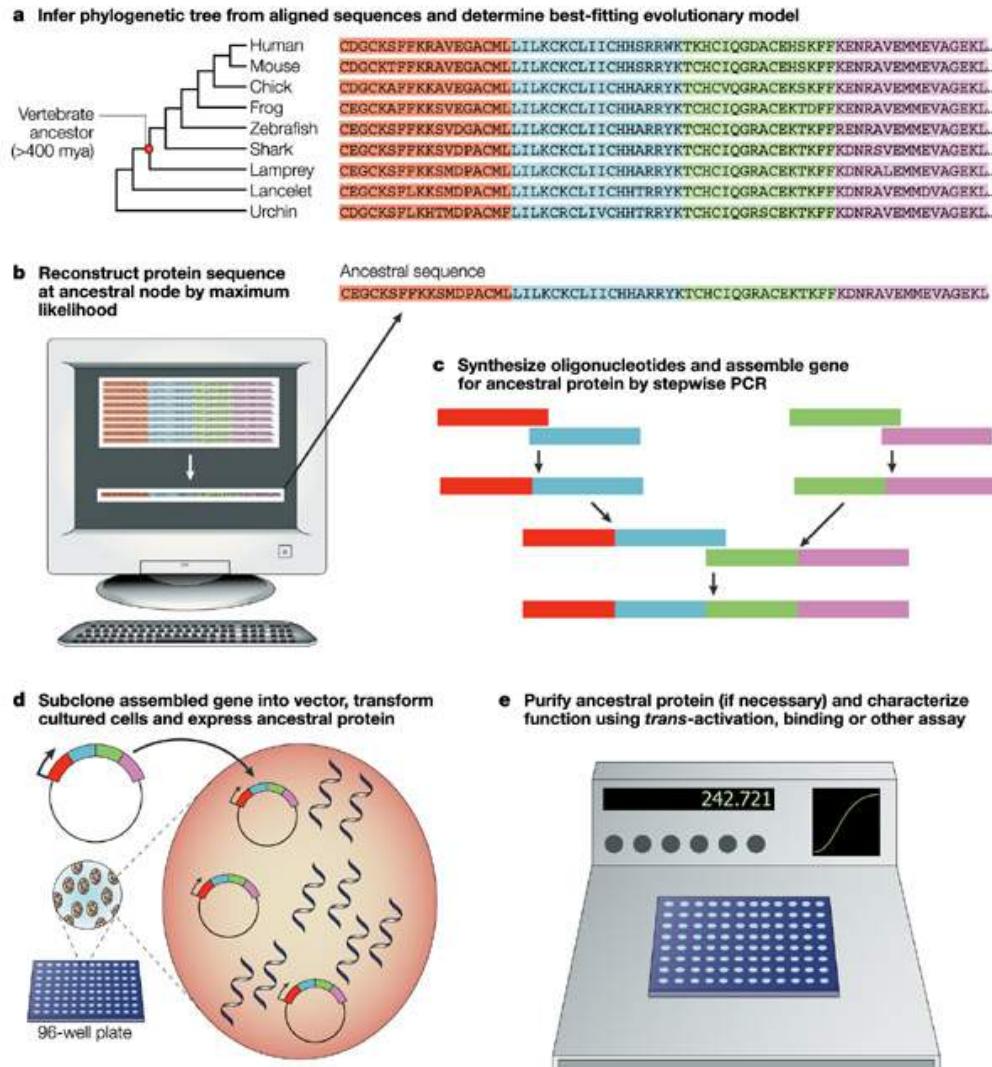
## TOOLS

- Phylogenetic models of coding sequence evolution
- Experimental studies, mutagenesis and ancestral resurrection

# Phylogenetic approaches to the study of protein structure and function

- Ancestral protein reconstruction
  - Computational analyses of selection ( $dN/dS$ )
- > Combining computational with experimental approaches allows us to test hypotheses of selection in protein evolution

# Resurrecting ancestral proteins



Nature Reviews | Genetics

Thornton, 2004  
*Nat. Reviews Genet.* (5):366

# Ancestral reconstruction: considerations

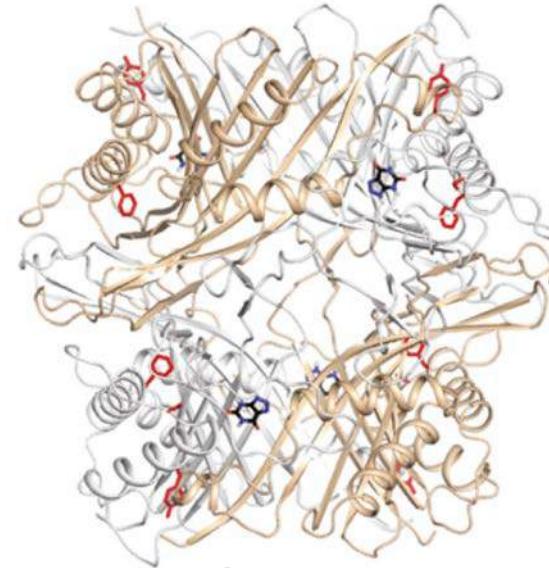
- Most studies use ML/Bayes methods to infer ancestral sequence with highest probability, single point estimate
- Violations of model assumptions, e.g. shifts in equilibrium frequencies
- Uncertainty in tree topology
- Statistical bias towards states with highest equilibrium frequencies
- This may also result in functional bias towards more stable proteins (Goldstein et al. 2013)

-> How to assess robustness of reconstruction in a functional context?

# Assessing robustness of reconstruction in a functional context

- Alternate tree topologies, species tree topology
  - Alternate approaches, models of evolution
  - Sampling alternate ancestors from the posterior distribution (Pollack & Chang 2012)
  - Sampling of near ancestor sequences (Bar-Rogovsky et al. 2015)
  - Uncertainty in genotype does not necessarily reflect uncertainty in phenotype (Gaucher et al. 2008)
- > Need for experimental data to inform effects of uncertainty in reconstruction on function

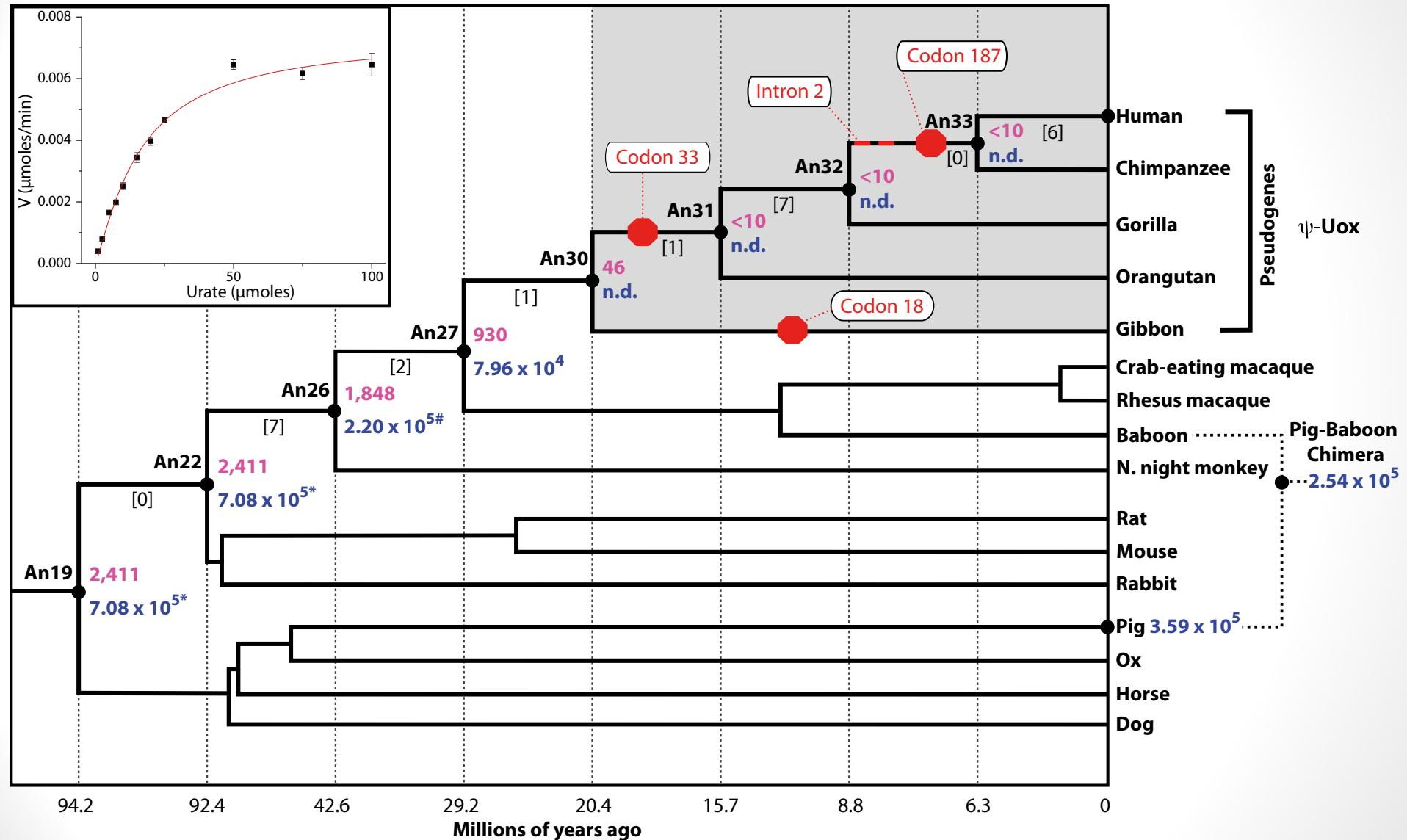
# Uricase evolution in primates



- Key enzyme metabolizing uric acid in vertebrates
- Lost in some primates, including humans
- Prevalence of diseases such as gout, hypertension, obesity, cardiovascular disease
- Uricase knockouts in mice result in mortality in first 4 weeks

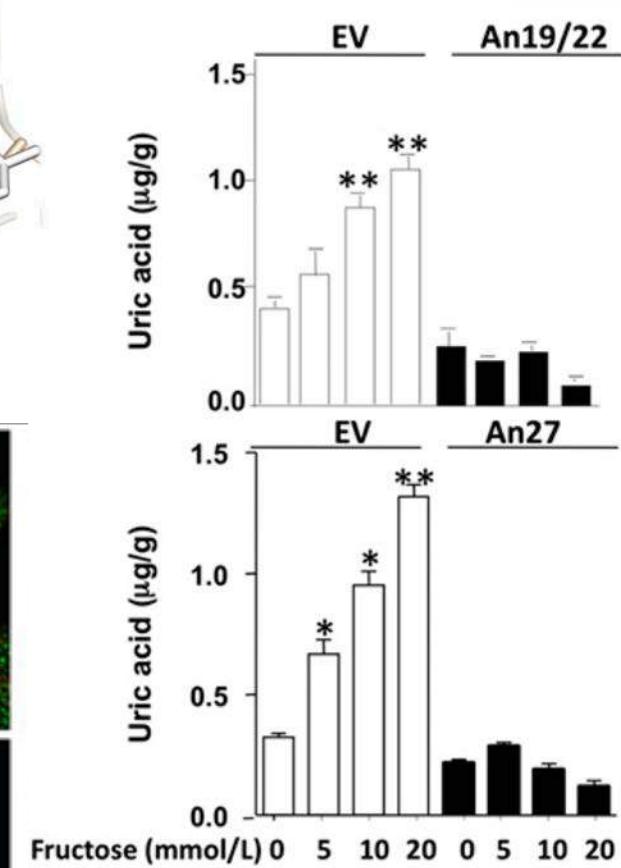
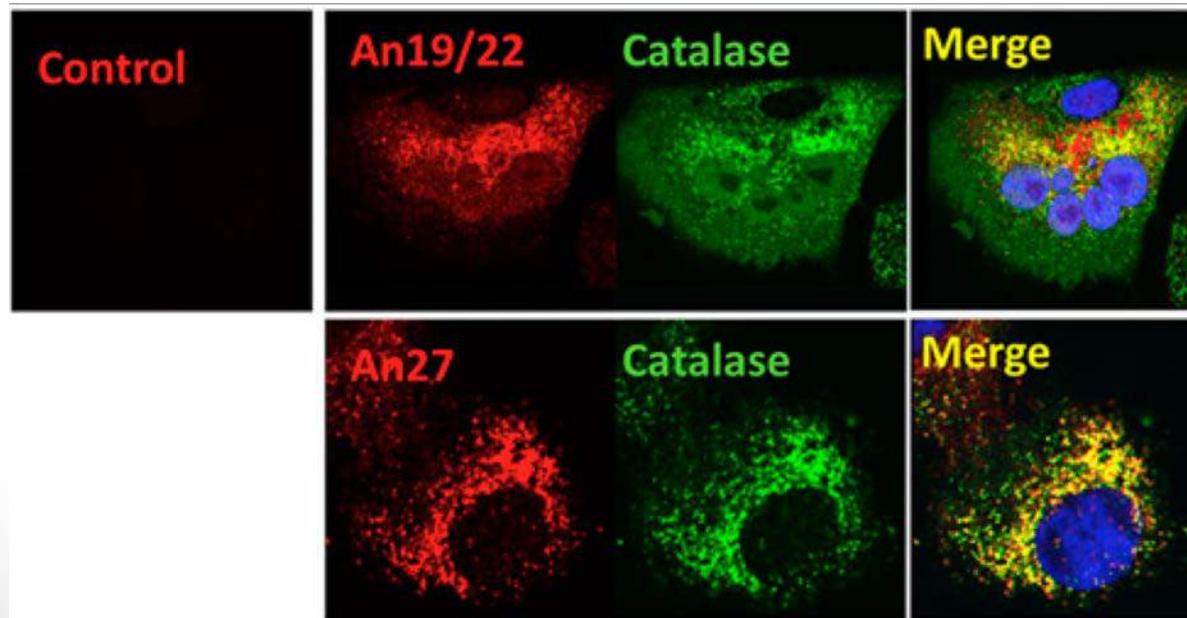
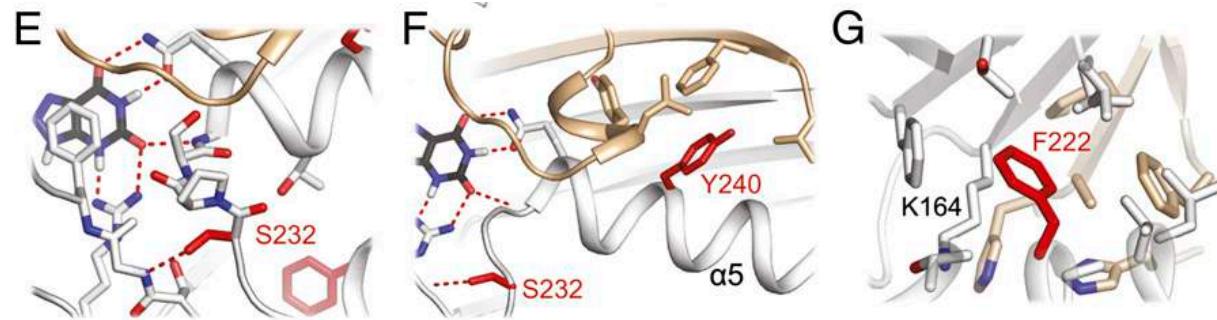
-> Kratzer *et al.* 2014 (PNAS) used experimentally recreated ancient uricases to determine exactly when, and how, uricase function was lost in primates.

# Uricase evolution in primates



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# Uricase evolution in primates



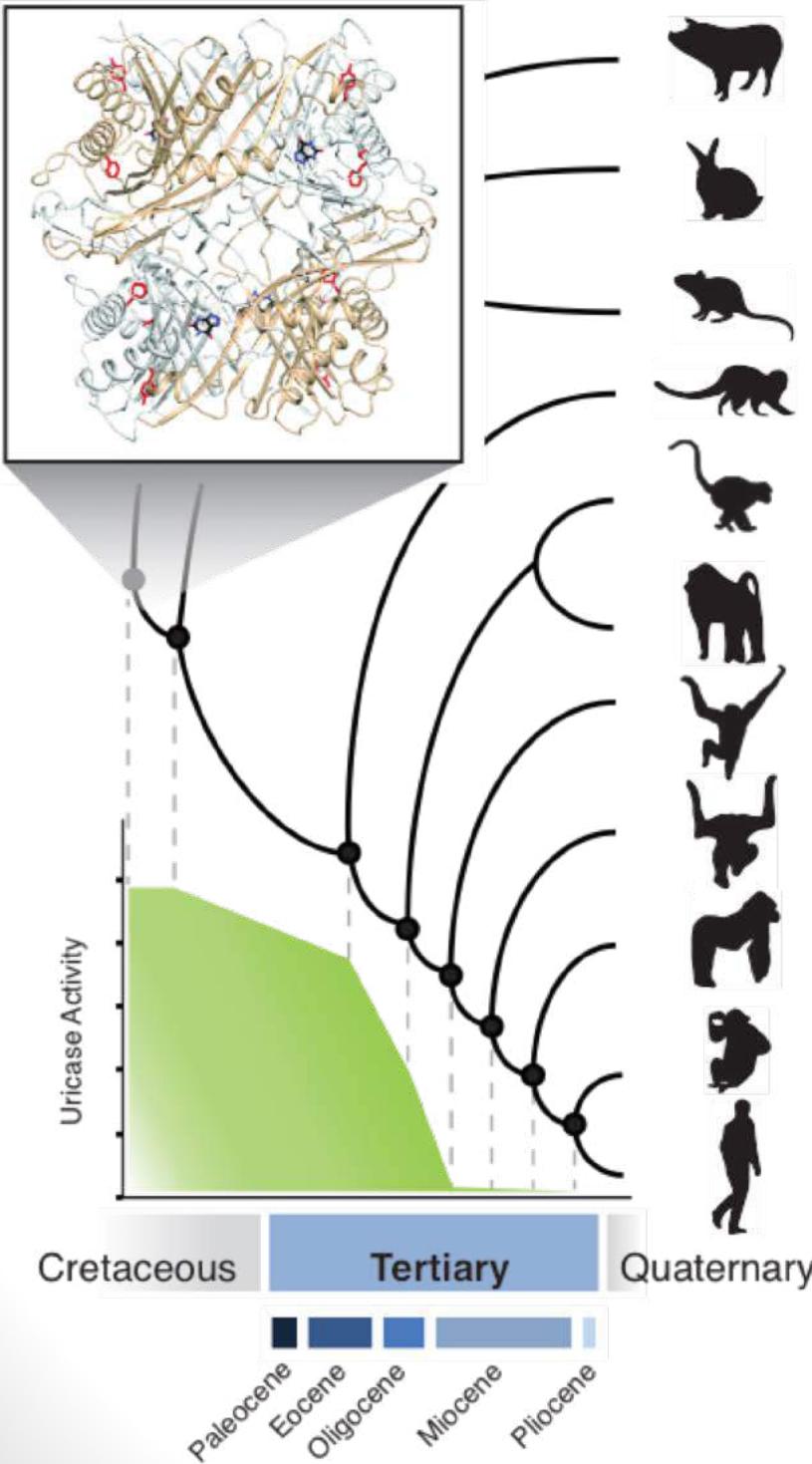
Kratzer et al., 2014, PNAS

# Uricase evolution in primates

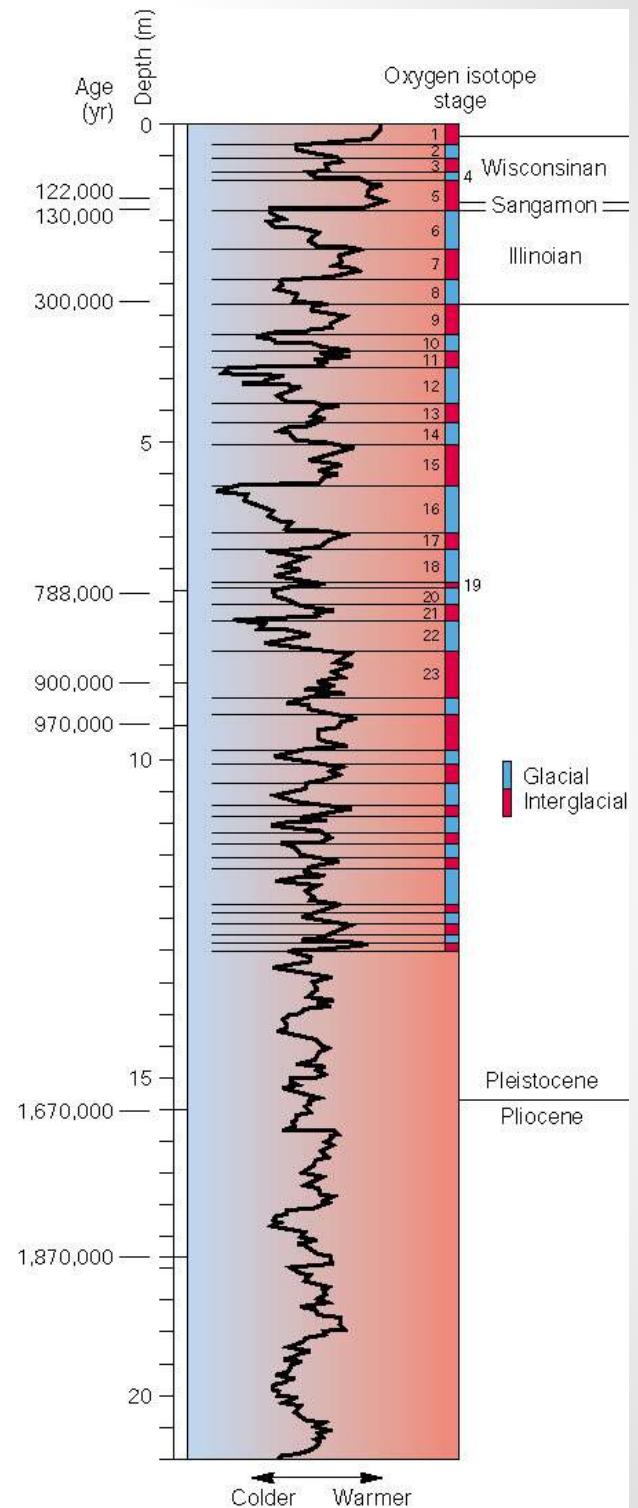
Why was uricase lost in the great apes?

Thrifty genes vs.  
“drifty” genes

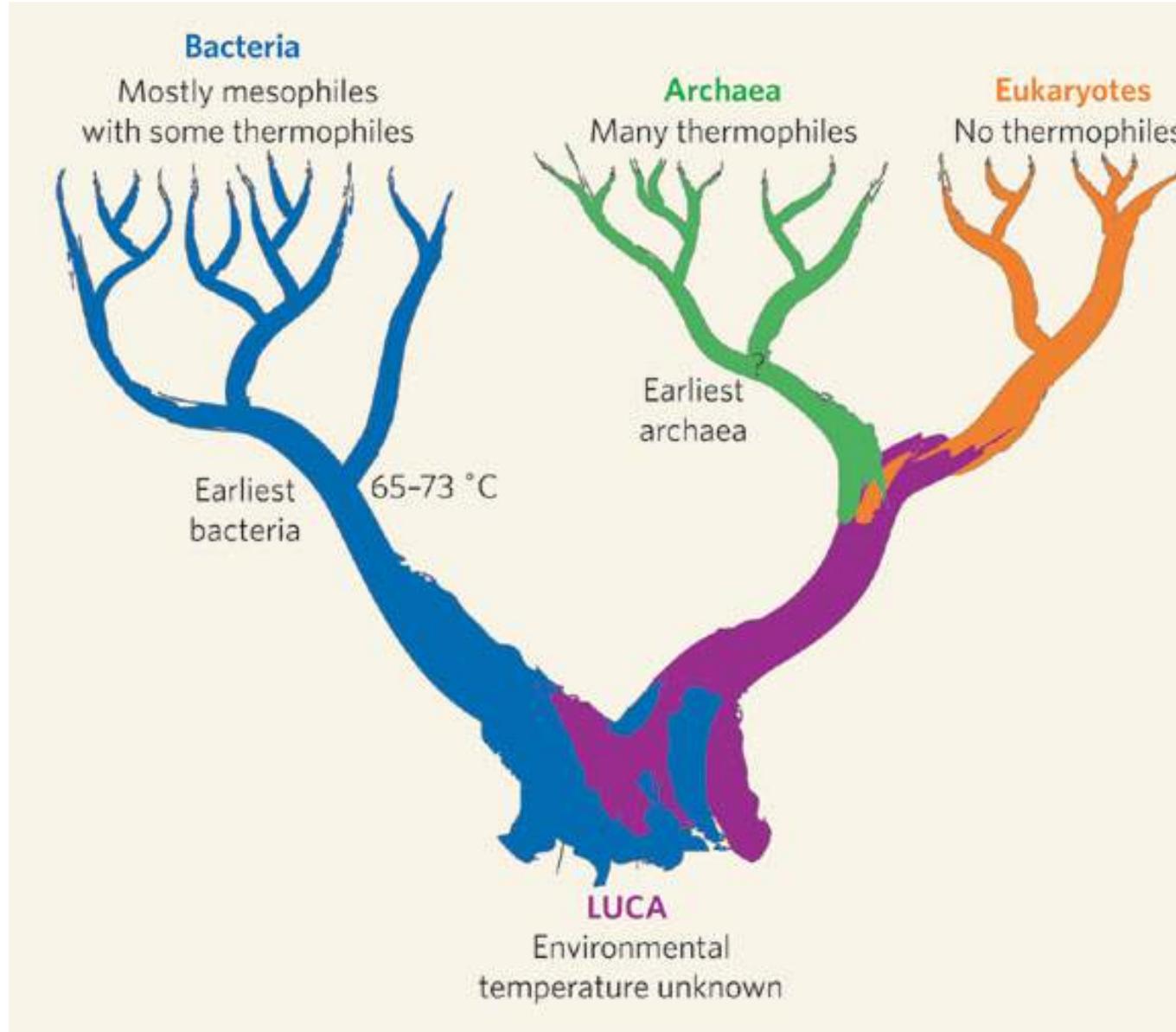
Kratzer et al., 2014, PNAS



# Oceanic paleoenvironments

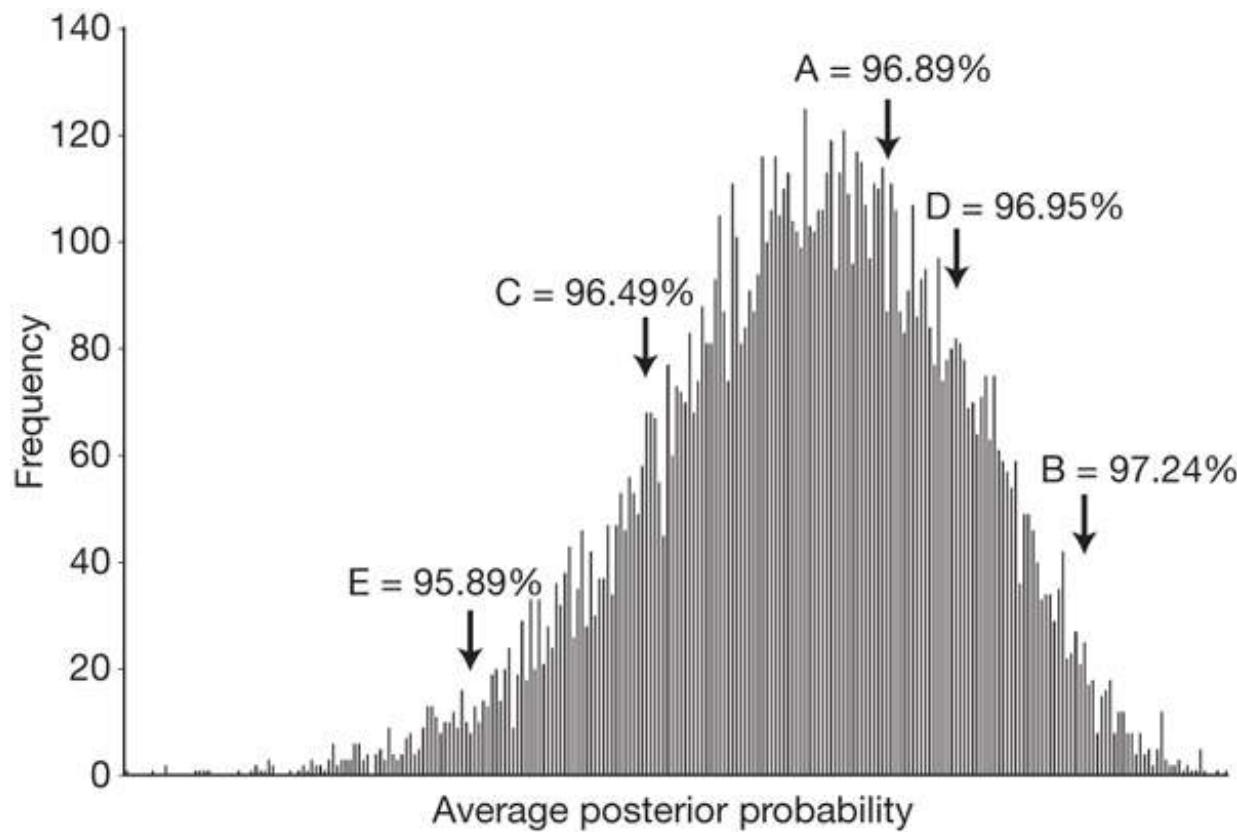


# Paleoenvironments (EF-Tu)



# Paleoenvironments (EF-Tu)

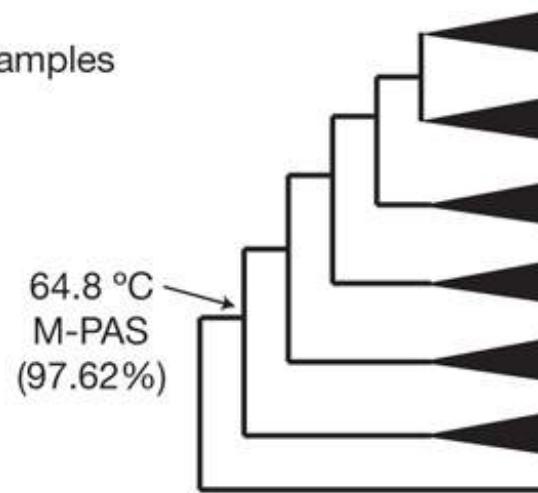
- Resurrected proteins can provide clues about the temps at which ancient organisms lived
- EF-Tu, an elongation factor crucial for protein synthesis in all cells throughout evolutionary history
- Present day organisms have EF-Tu's which are highly correlated to temp at which organisms live
- Express resurrected gene in E coli, measure thermostability ( $T_m$ ) of proteins using CD
- Bacterial ancestors appear to be thermophilic (60-80 deg C)



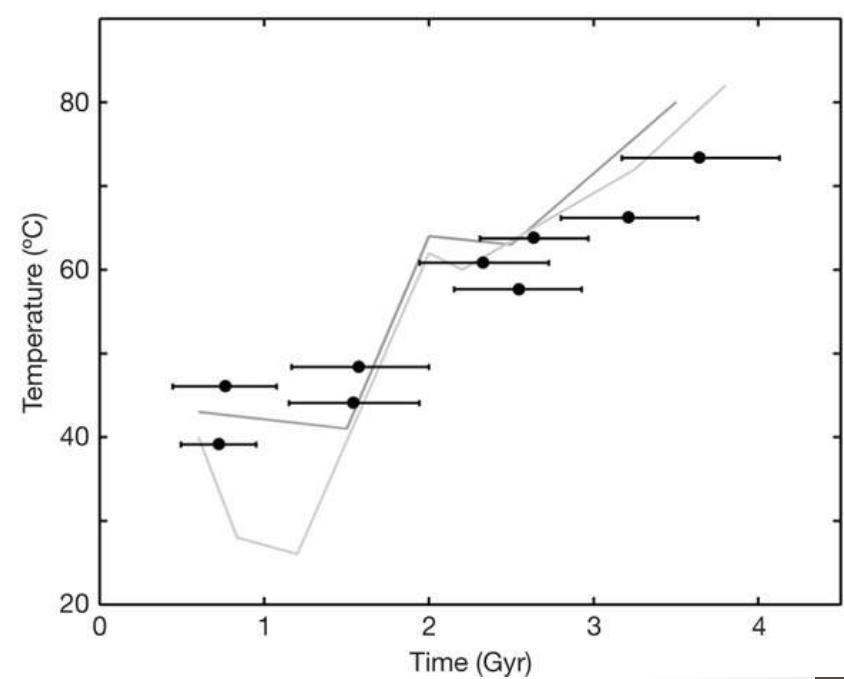
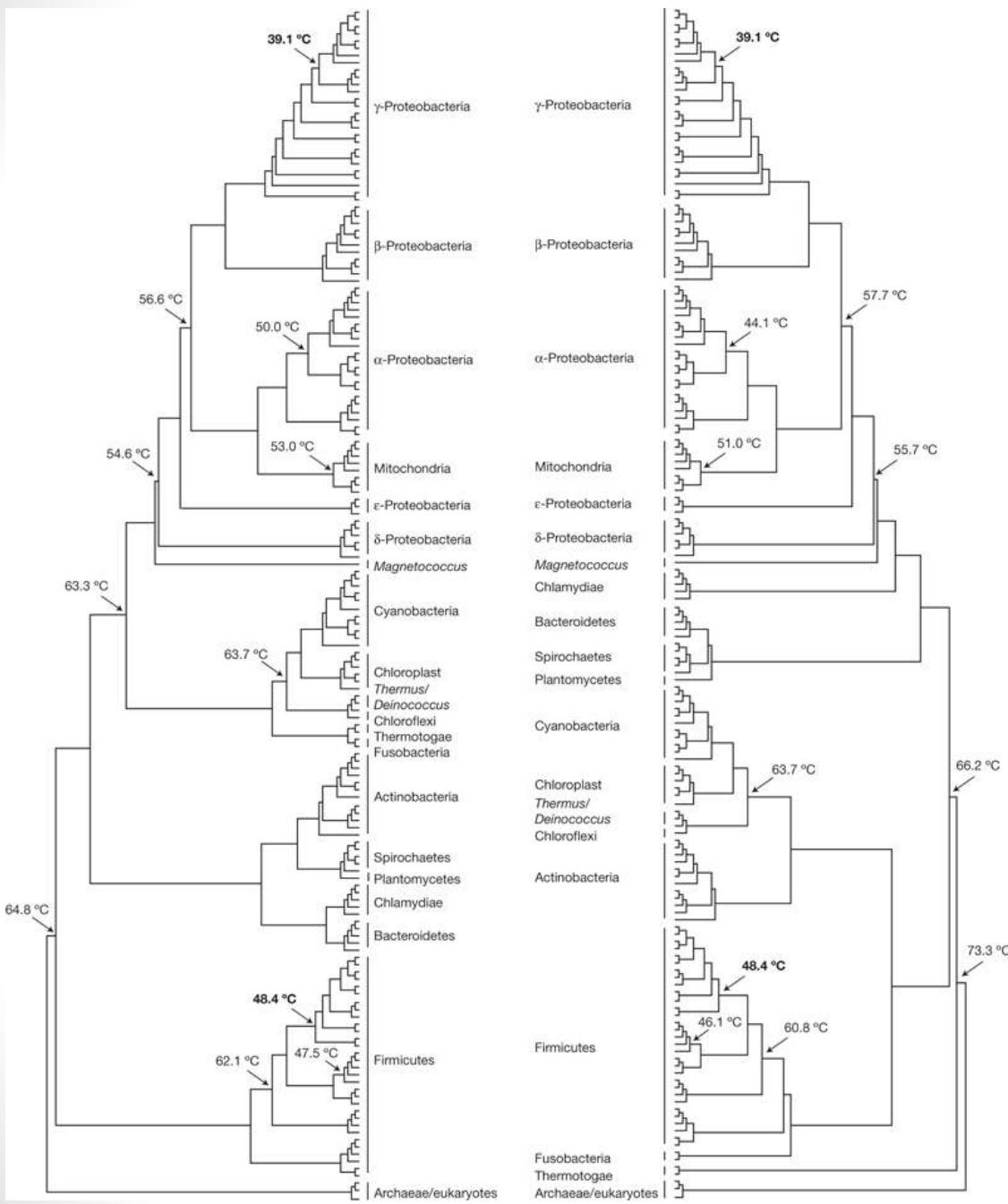
$T_m$  values for weighted random samples  
from the posterior distribution:

- A = 66.3 °C
- B = 62.2 °C
- C = 64.6 °C
- D = 60.5 °C
- E = 60.0 °C

Ancestral  $\pi_{eq} T_m = 61.4$  °C



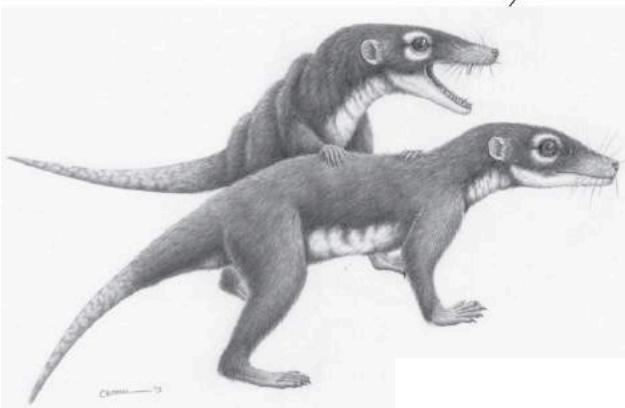
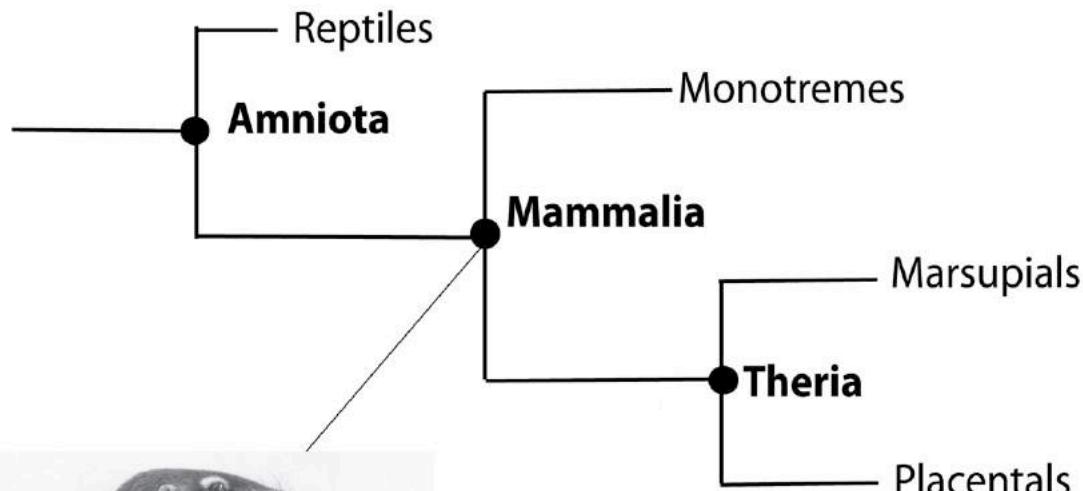
Gaucher et al., 2008  
Nature (451):704



(42)

Gaucher et al. 2008 Nature

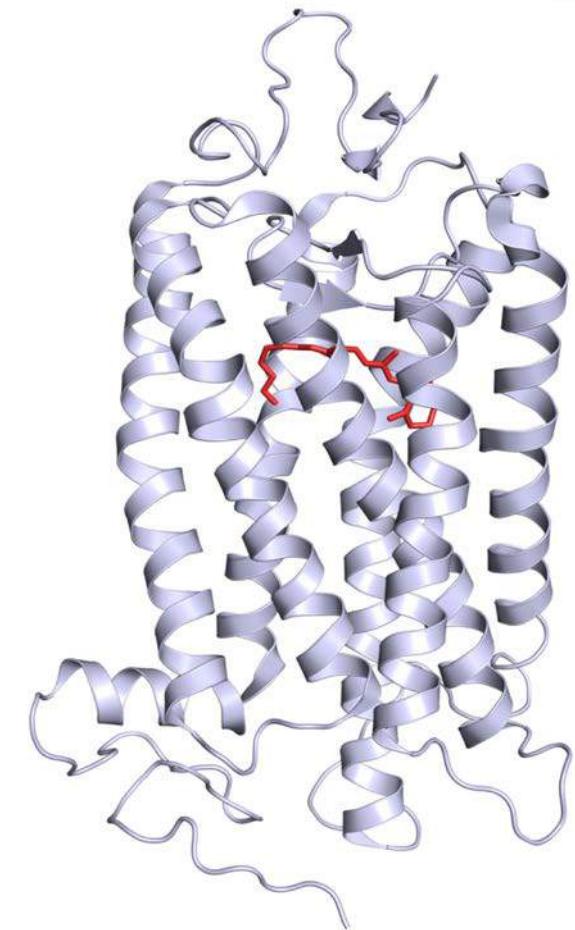
# Rhodopsin evolution: Nocturnality of early mammals?



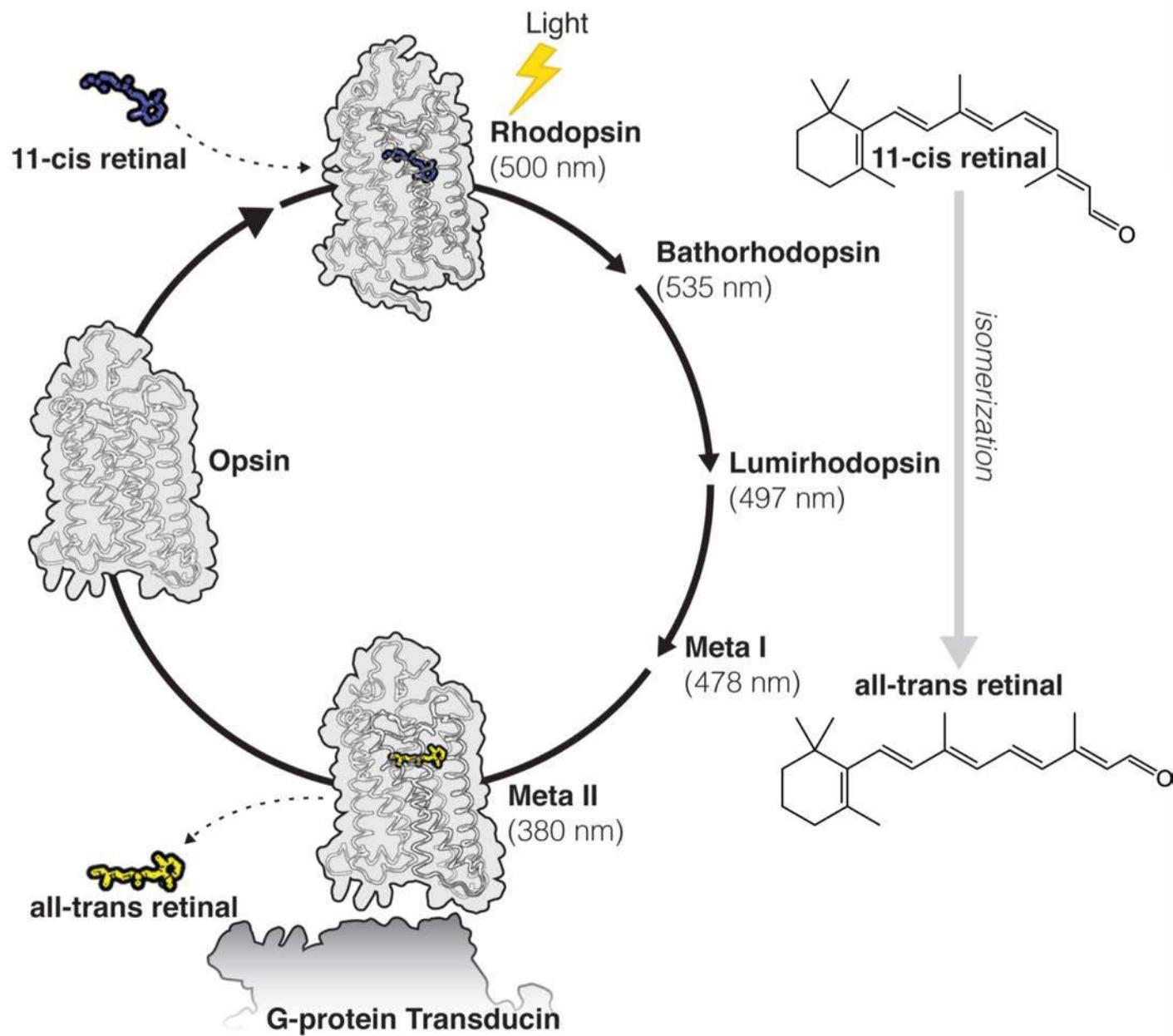
<http://www.seirim.net/Cont/Draw/Vert/Morganucodon.jpg>

## Previous Hypothesis about early mammals:

1. Living in **Nocturnal Niche**  
(Crompton, Taylor and Jagger 1978 *Nature*)
2. **Adaptive Changes In Rod Photoreceptors** to improve dim-light vision  
(Walls 1942 ; Ahnelt and Kolb 2000 *Prog. Retinal and Eye Res.*)

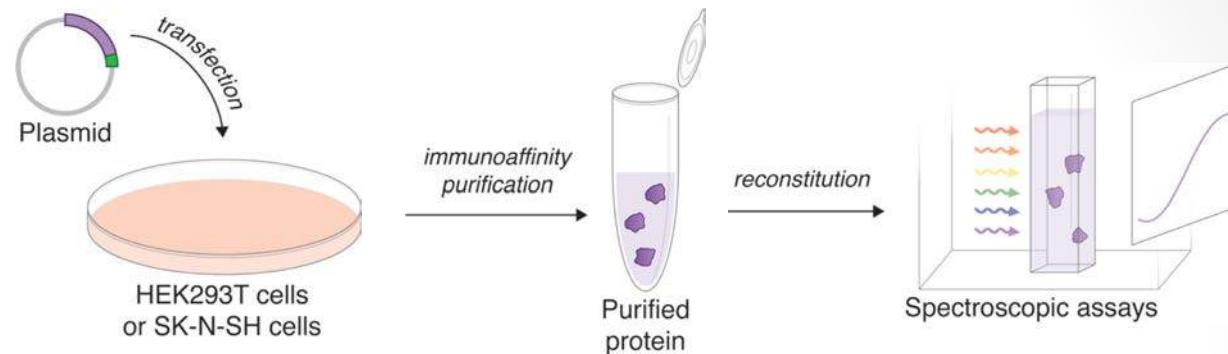


# Visual cycle: conformational changes in rhodopsin

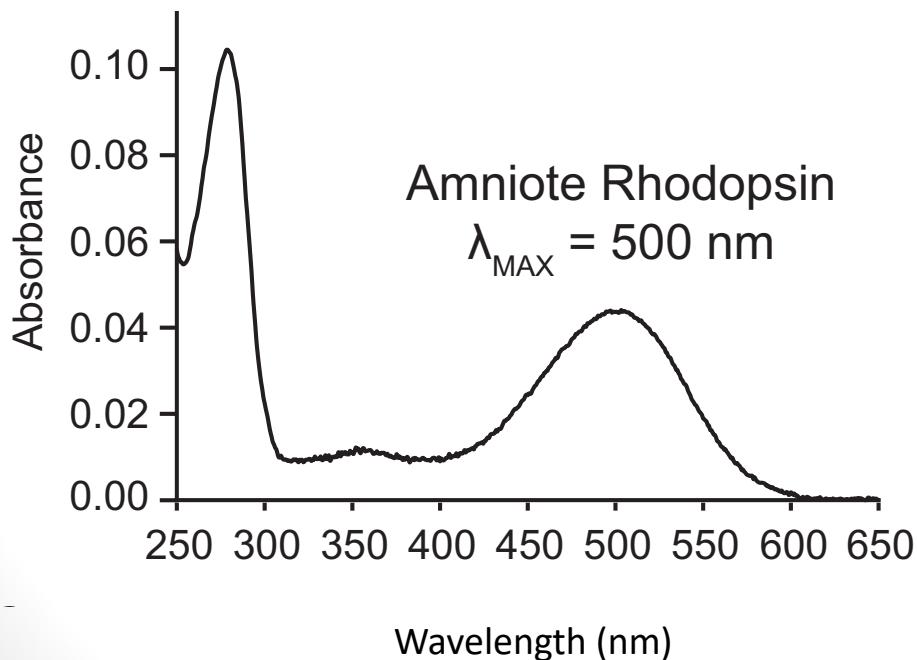


# Spectroscopic assays of rhodopsin function

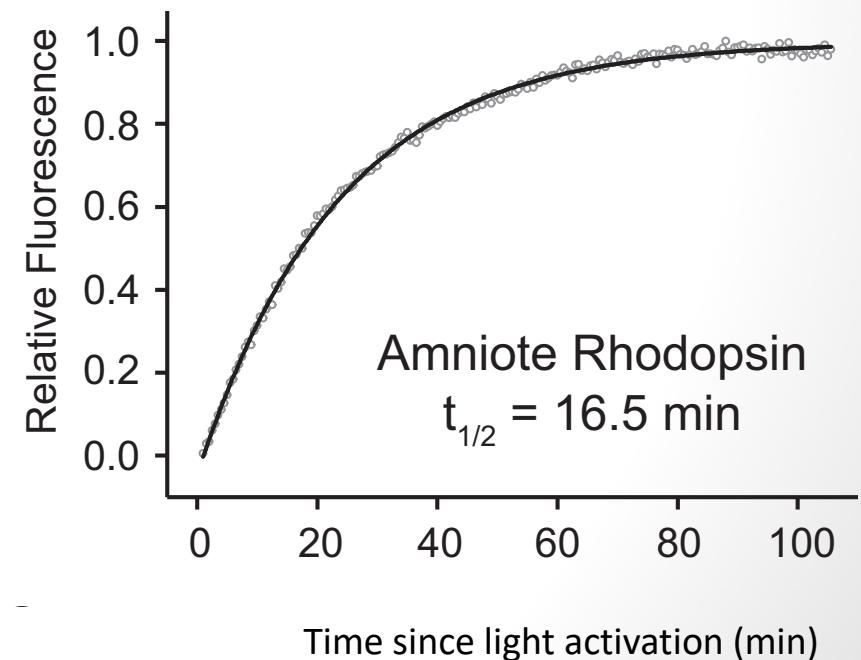
*In vitro* expression  
& purification



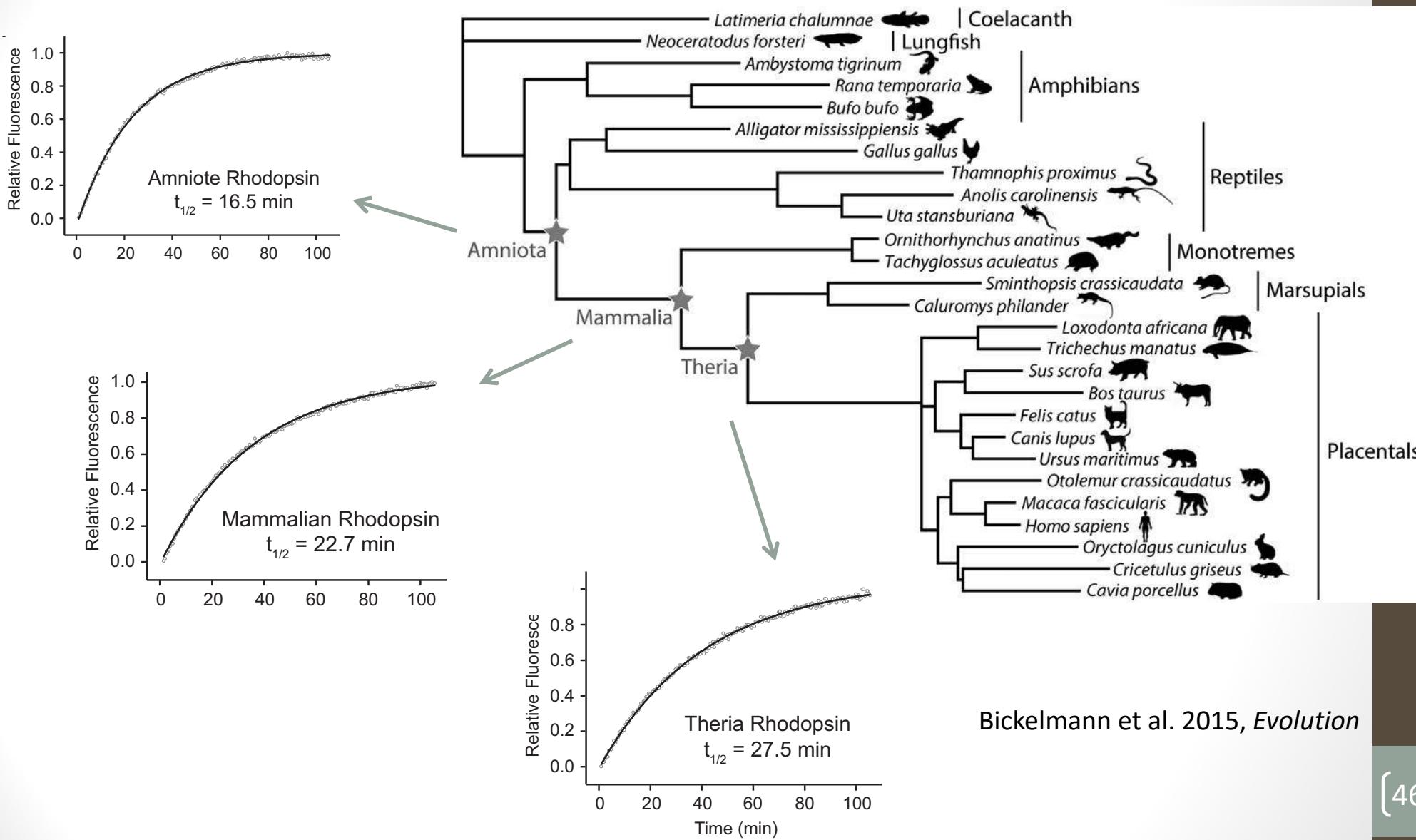
Rhodopsin spectral tuning



Lifetime of activated state



# Kinetic rates of light-activated rhodopsin lifetimes



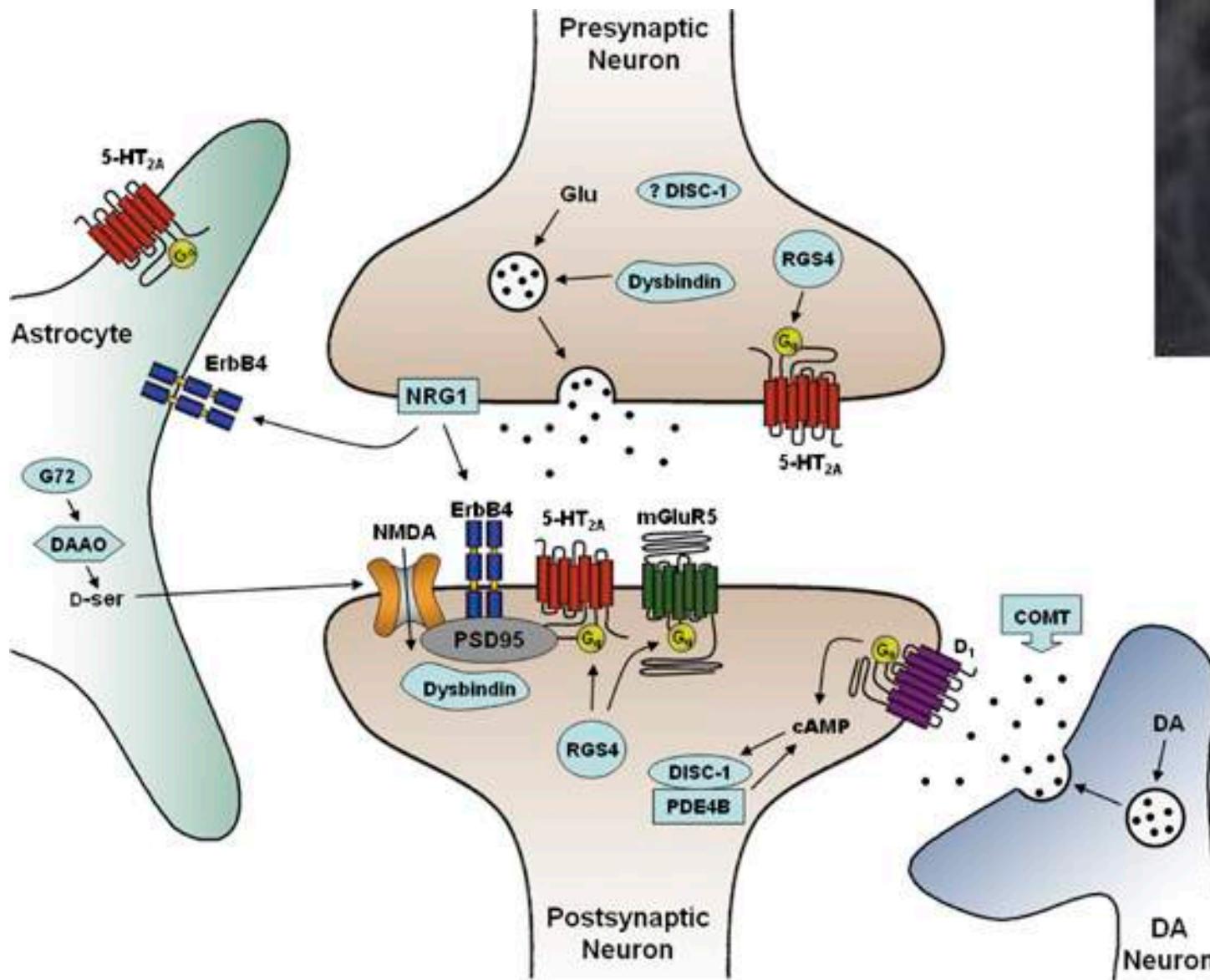
-> Increased lifetime of activated state of rhodopsin in mammalian and therian ancestors

# Synaptic neurotransmission

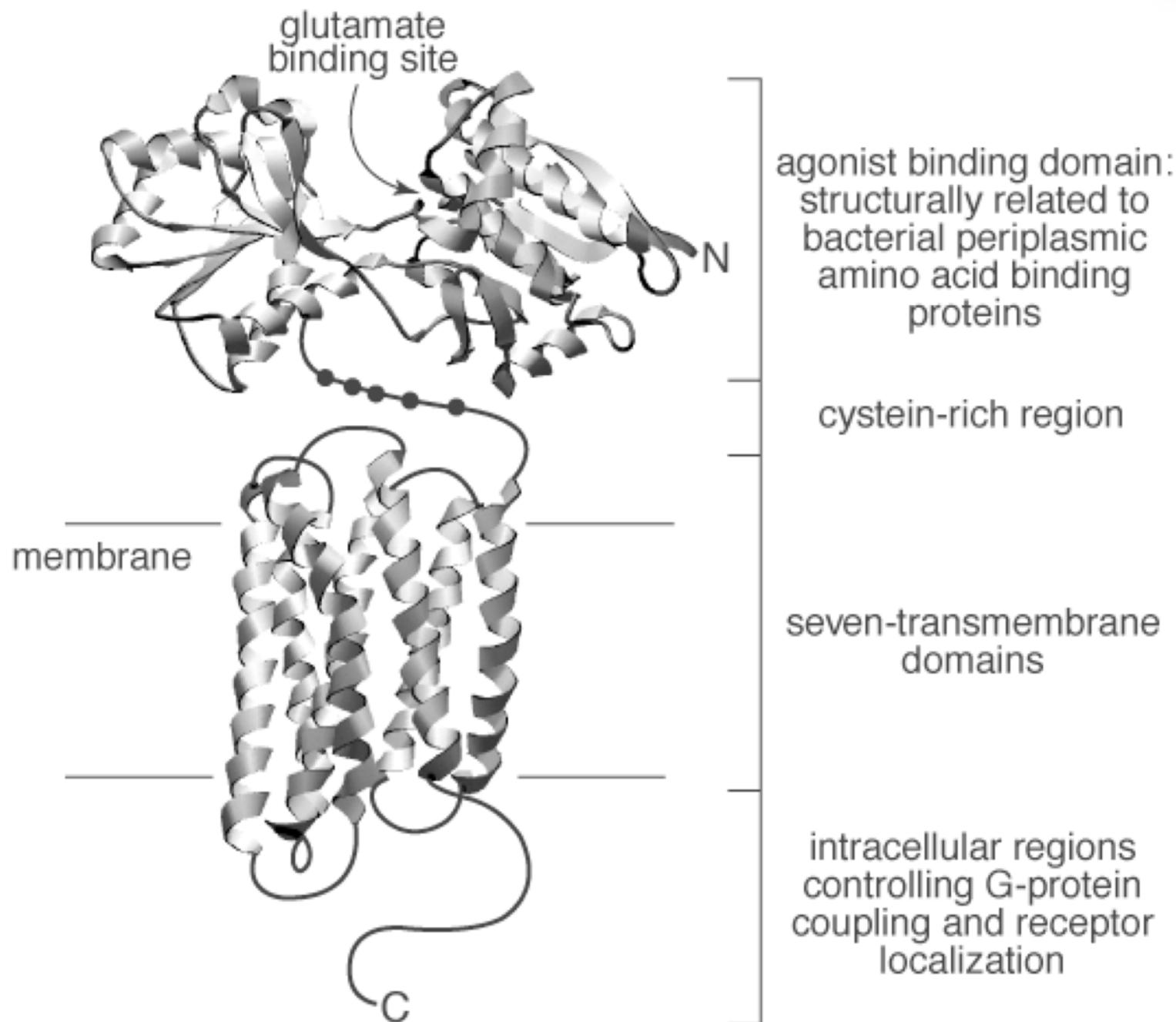


Adaptive protein evolution

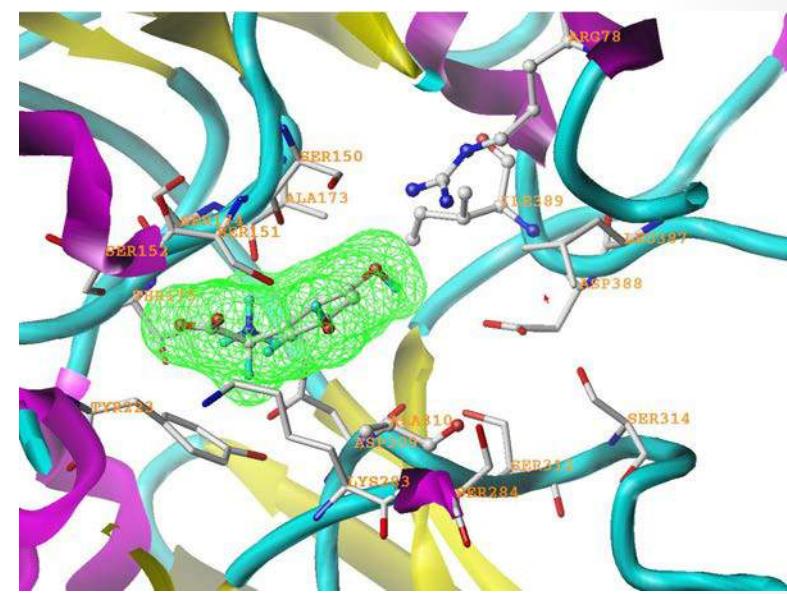
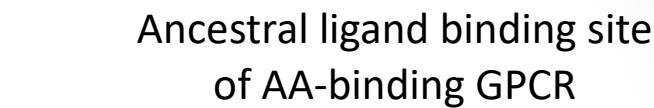
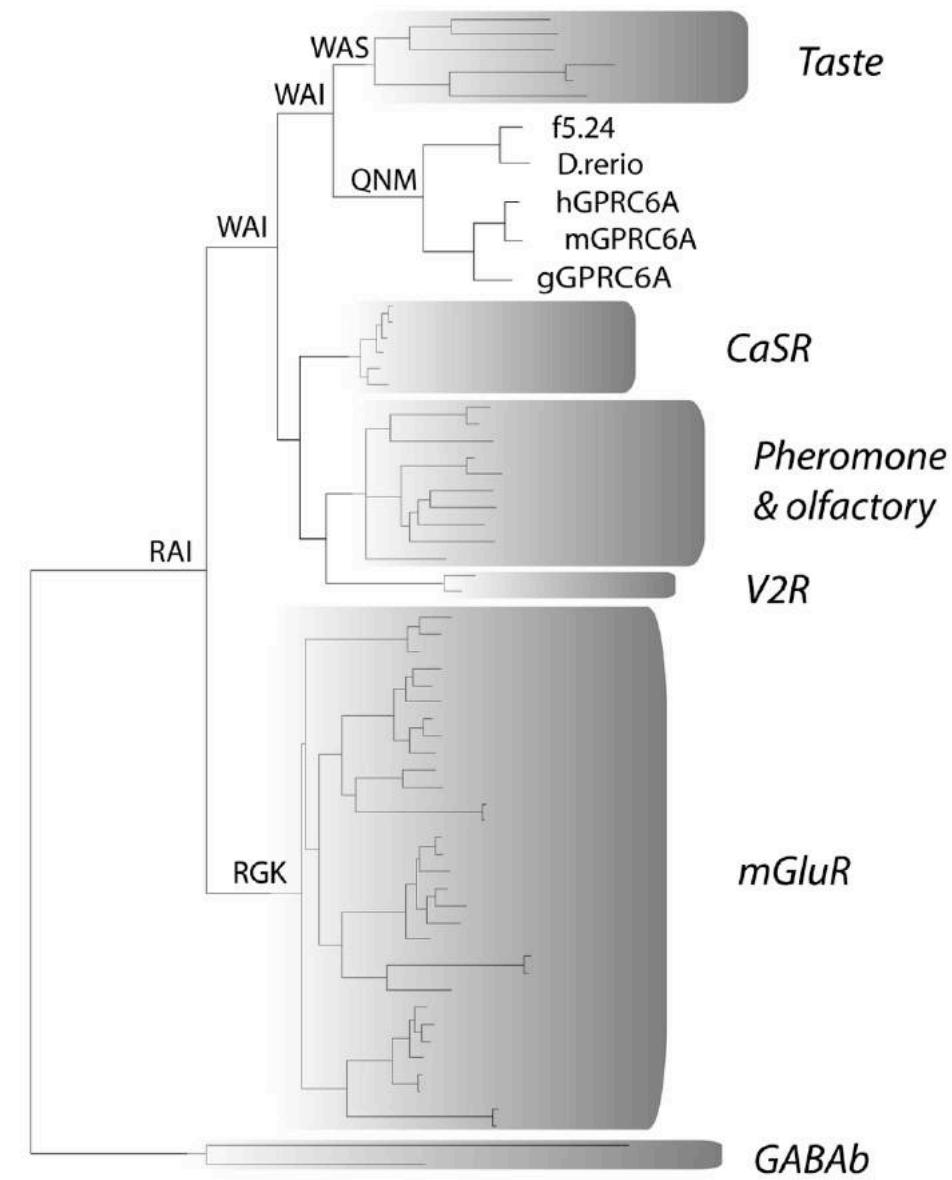
[47]



# Glutamate receptors: Excitatory synaptic neurotransmission

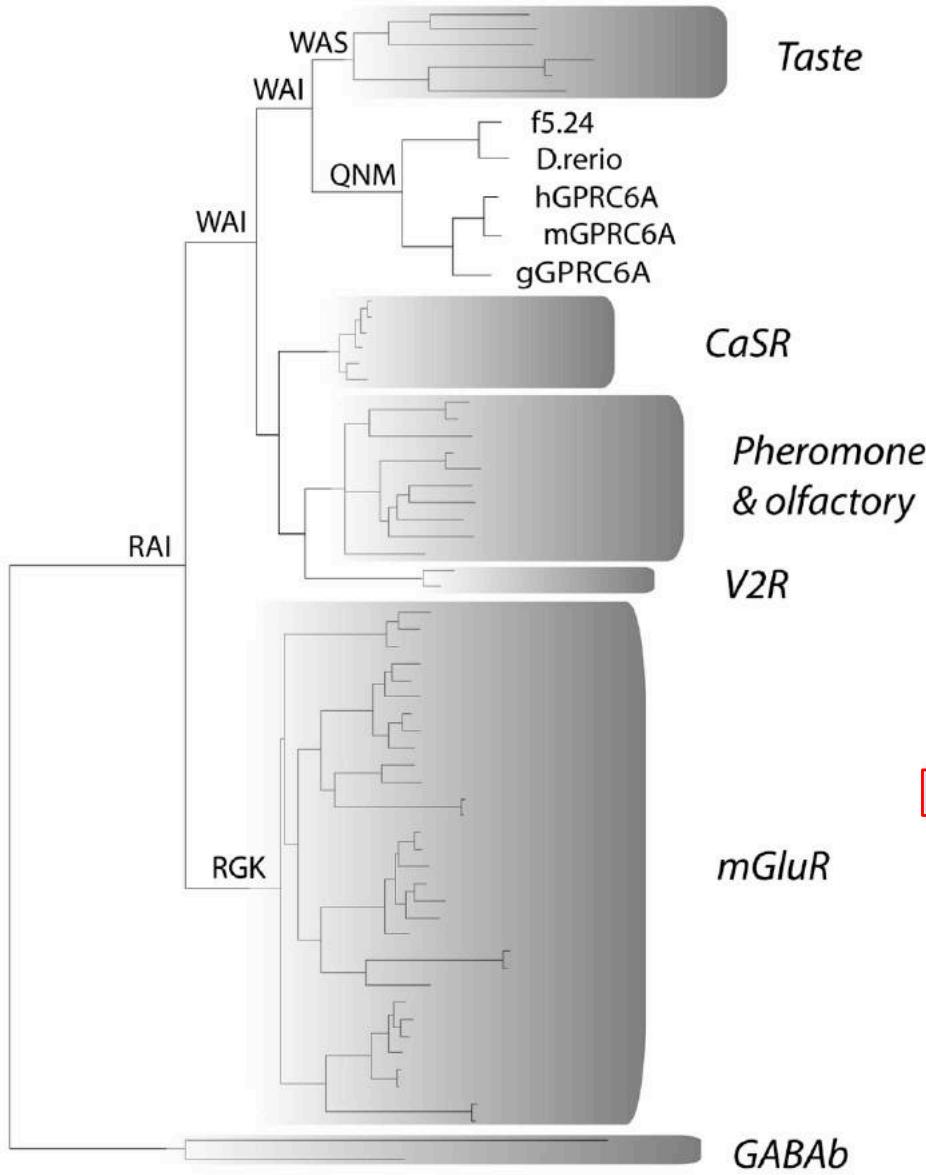


## Reconstructed ancestral AA-binding GPCR



Kuang et al., 2006  
PNAS (103):14050

# Reconstructed ancestral AA-binding GPCR

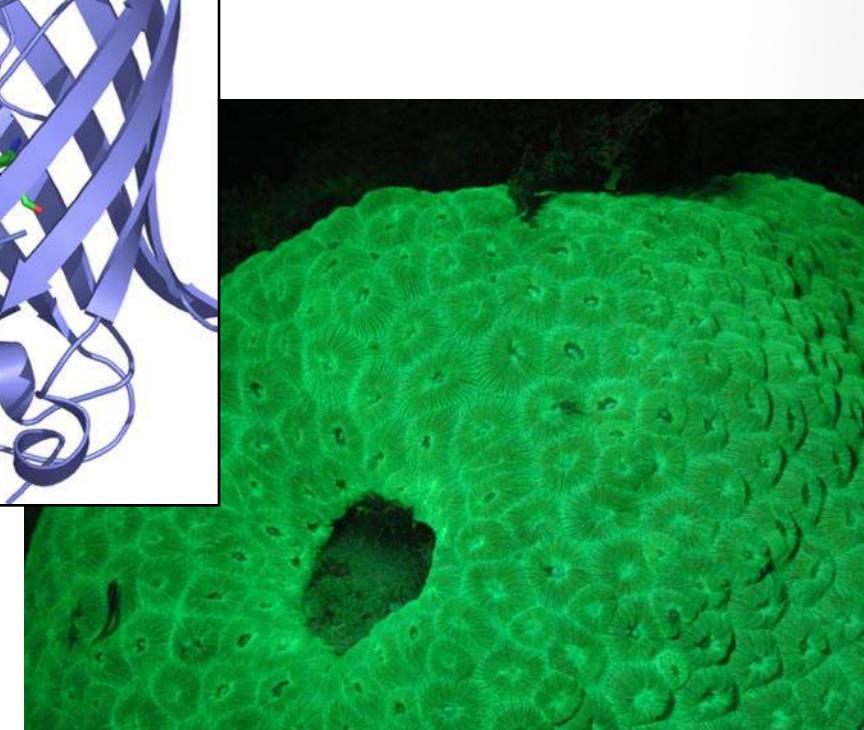
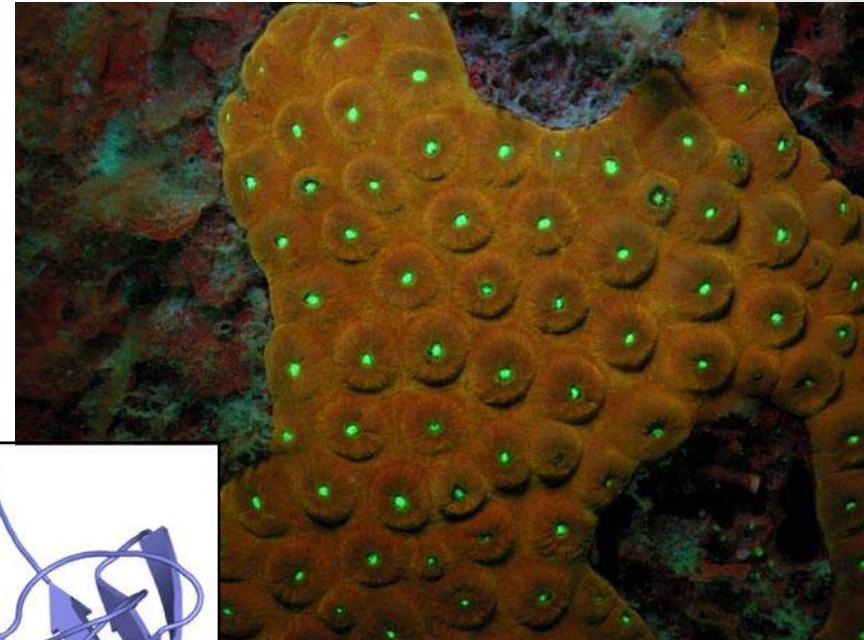
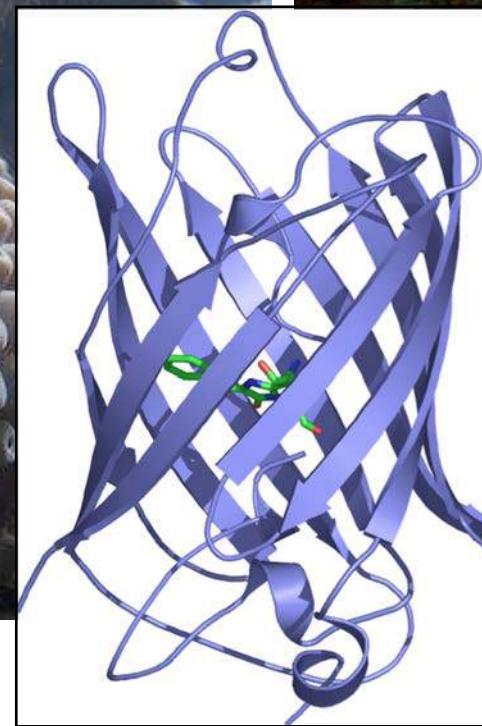


Comparison of potency (EC50)  
Glutamate R agonists

Agonists	5.24 receptor	Ancestral receptor	Q78R/ N310G	Q78R	mGluR1a
L-CCG-1	+	4.9 ± 1.2	3.5 ± 0.8	1.3 ± 0.1	24.7 ± 1.9
ibotenate	183.7 ± 22.5	0.8 ± 0.3	3.2 ± 0.4	6.4 ± 0.3	4.4 ± 0.9
quisqualate	230.0 ± 58.0	0.8 ± 0.2	3.5 ± 0.8	8.3 ± 0.4	0.4 ± 0.2
(S)-DHPG	-	2.4 ± 1.3	3.8 ± 1.1	+	3.3 ± 0.7
DCG-IV	-	-	-	-	-
L-SOP	-	-	-	-	-

Kuang et al., 2006  
PNAS (103):14050

# Coral pigments

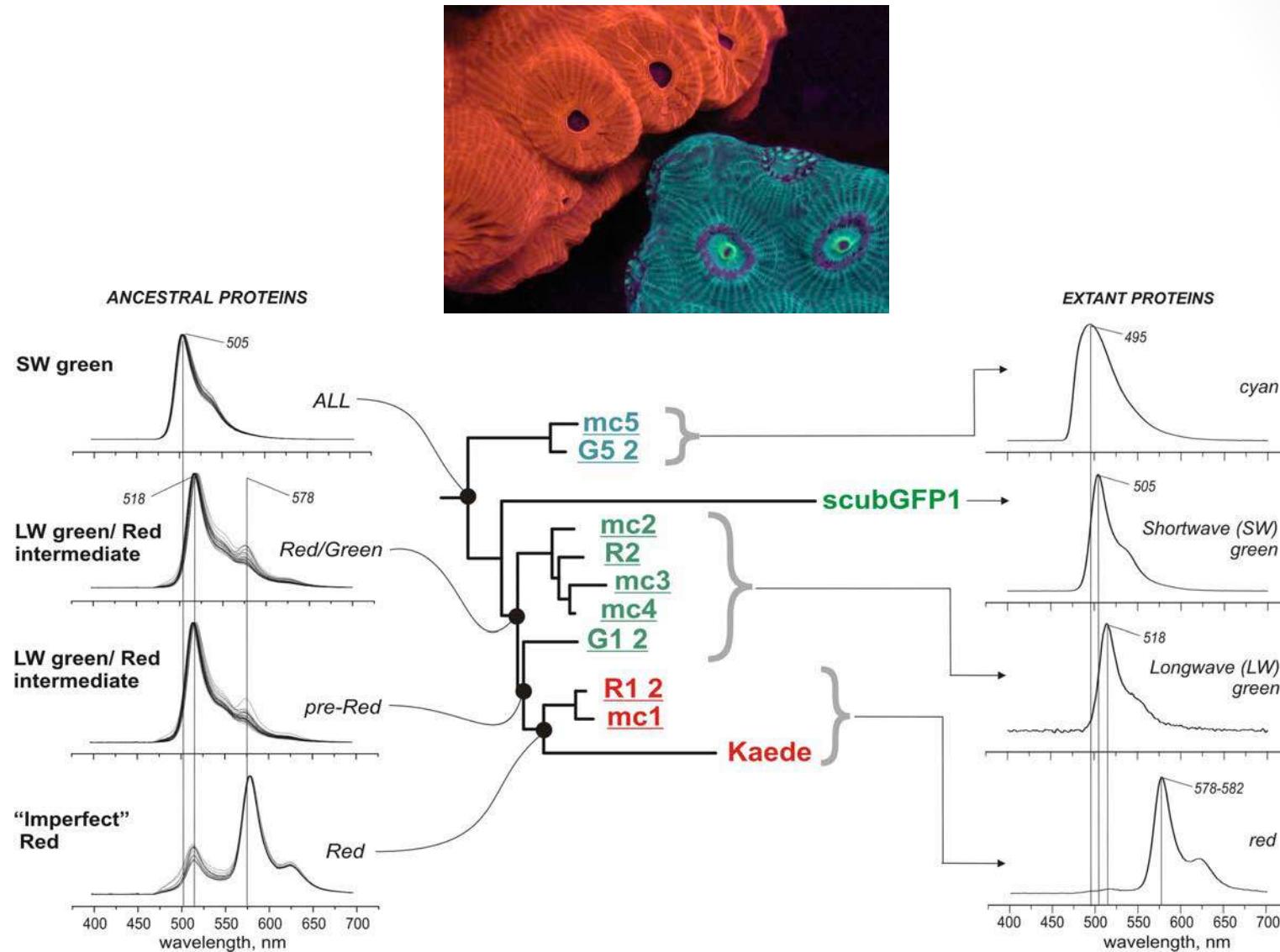


Adaptive protein evolution

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# Reconstructed GFP-like proteins from coral

great star coral (*Montastraea cavernosa*)



Ugalde et al., 2004  
Science (305):1433

## Adaptive protein evolution



(53)

## Conclusions: Ancestral reconstruction

Ancestral reconstruction approaches can offer a window into the past in studying ancient adaptive shifts in protein function

Computational analyses can be used to generate specific evolutionary hypotheses that can then be tested experimentally

Experimental approaches should not be viewed as applications of computational methods, instead serve to extend the hypothesis testing framework to study the evolution of protein function

Need for more interaction between computational and experimental methods in order to provide better insight into both approaches in the study of molecular evolution