# Ancestral Sequence Reconstruction: What is it Good for?

Jeffrey Boucher

Theobald Laboratory

# Talk Outline

- Were Dinosaurs Afraid of the Dark?
- The Coral Red: Convergence or Divergence?
- Same Fold, Different Specificity: How'd That Happen?

# Rhodopsin

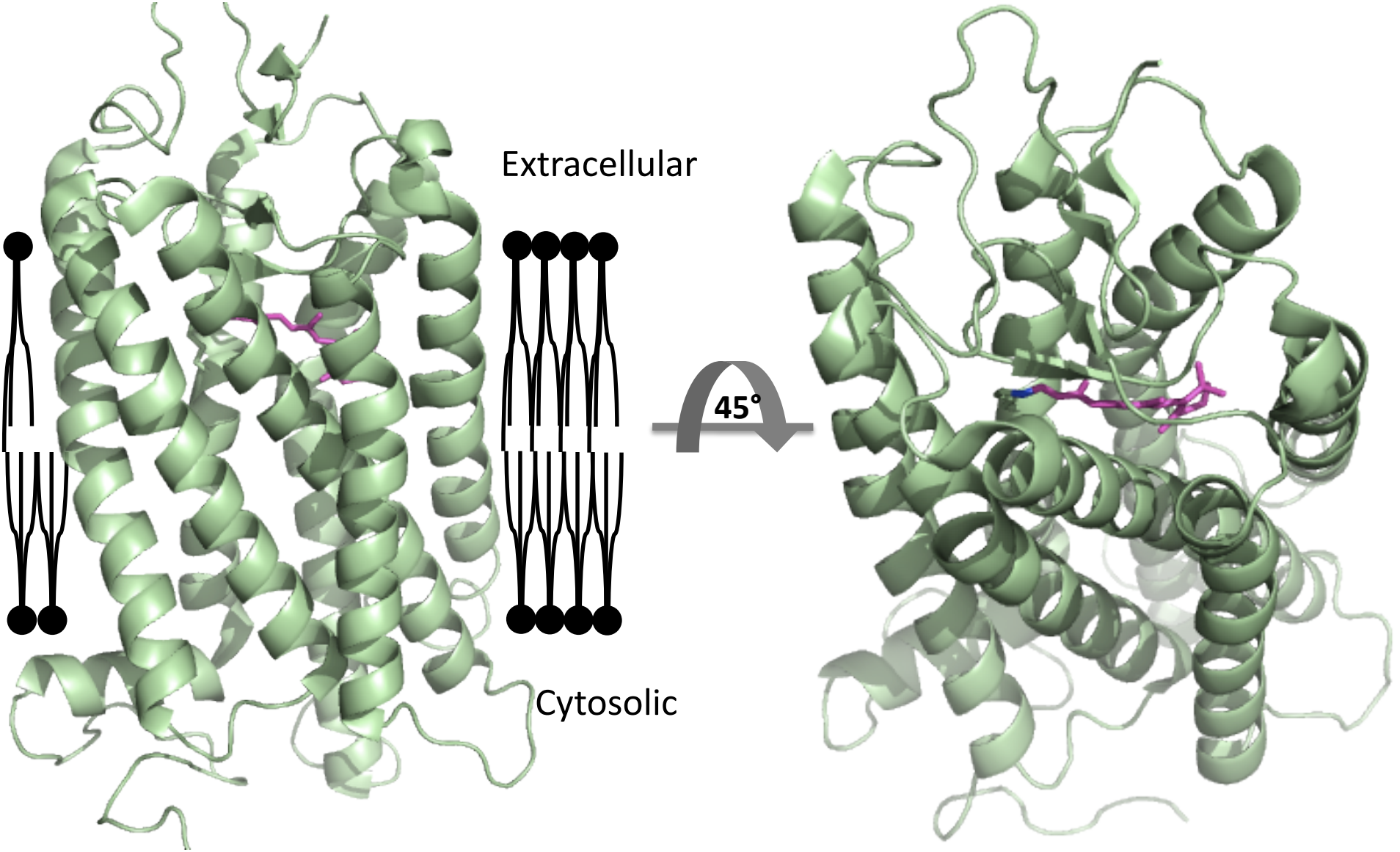
- G-Protein Coupled Receptor

- Responsible for low-light vision in vertebrates
  - Can detect a single photon
  - 100x more sensitive than opsins responsible for color vision

- Found in rod cells of the retina:

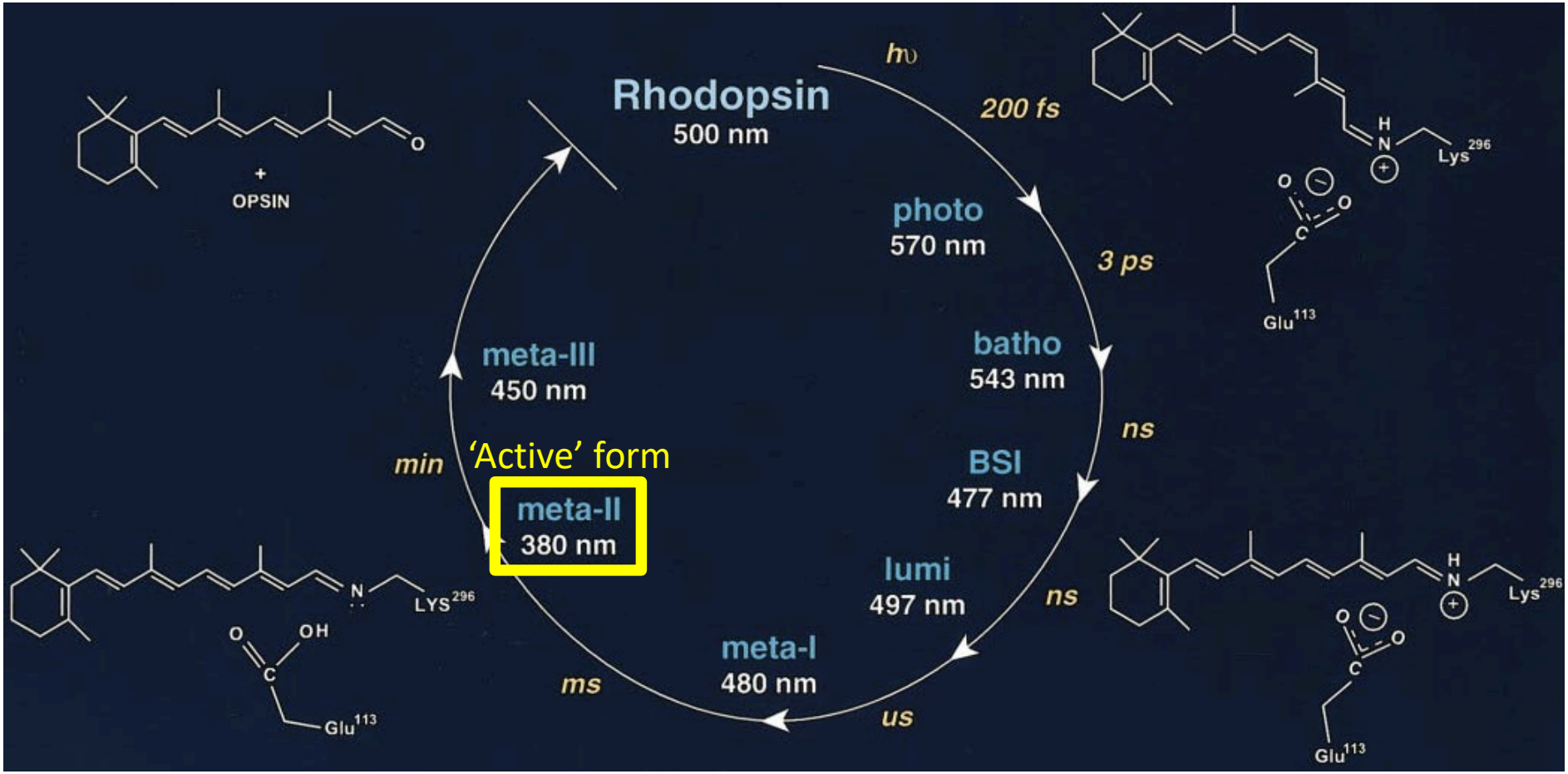
http://thebrain.mcgill.ca/flash/d/d_02/d_02_m/d_02_m_vis/d_02_m_vis.html

# Rhodopsin Structure




7 Transmembrane α-helices w/ chromophore in center

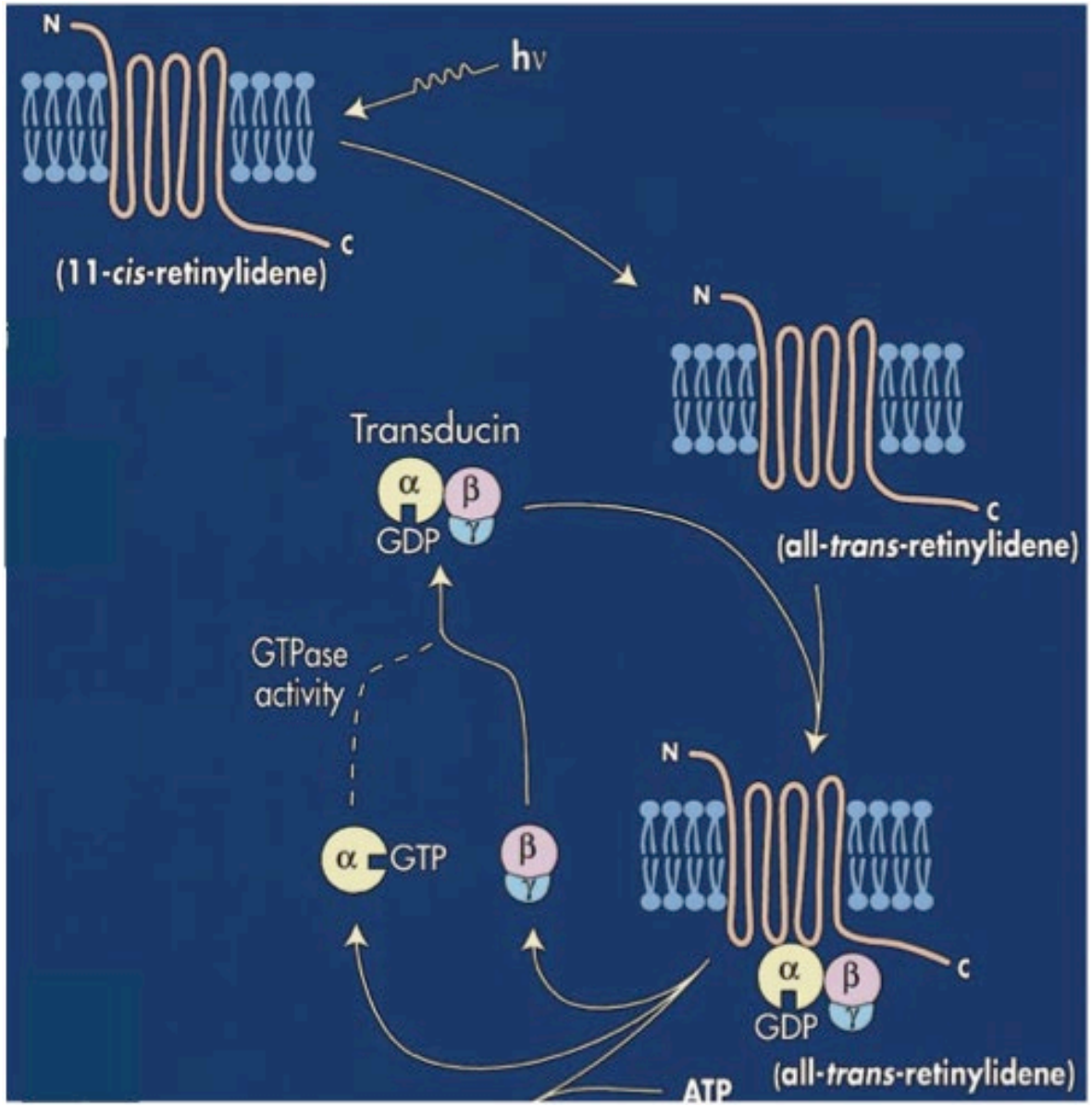
# Rhodopsin Photocycle




- 11-*cis*-retinal covalently attached to Lys$^{296}$
- *hv* absorption causes isomerization of 11-*cis*-retinal to all-*trans*-retinal

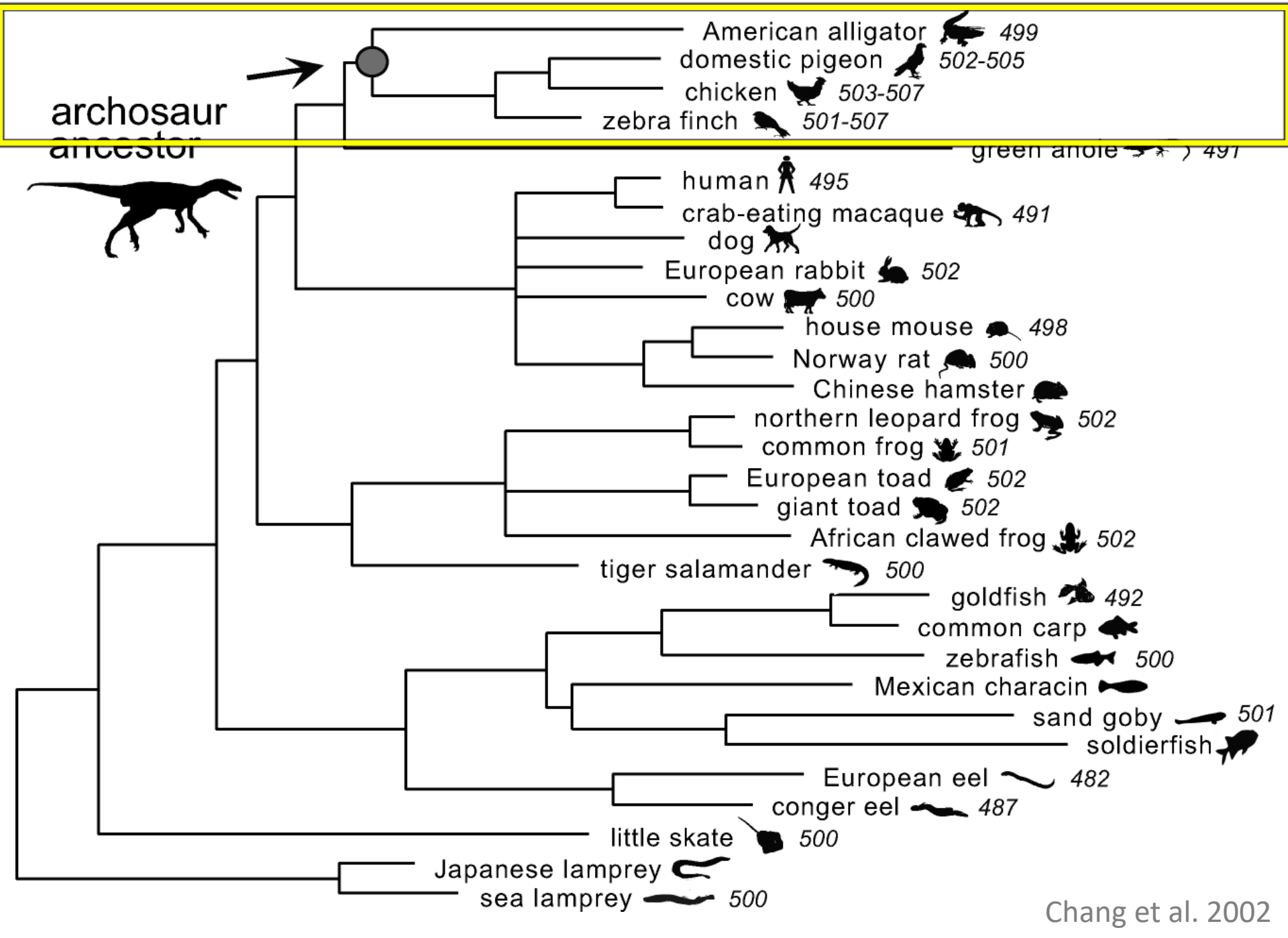
Menon, Han & Sakmar 2001

# MetarhodopsinII Activates Transducin



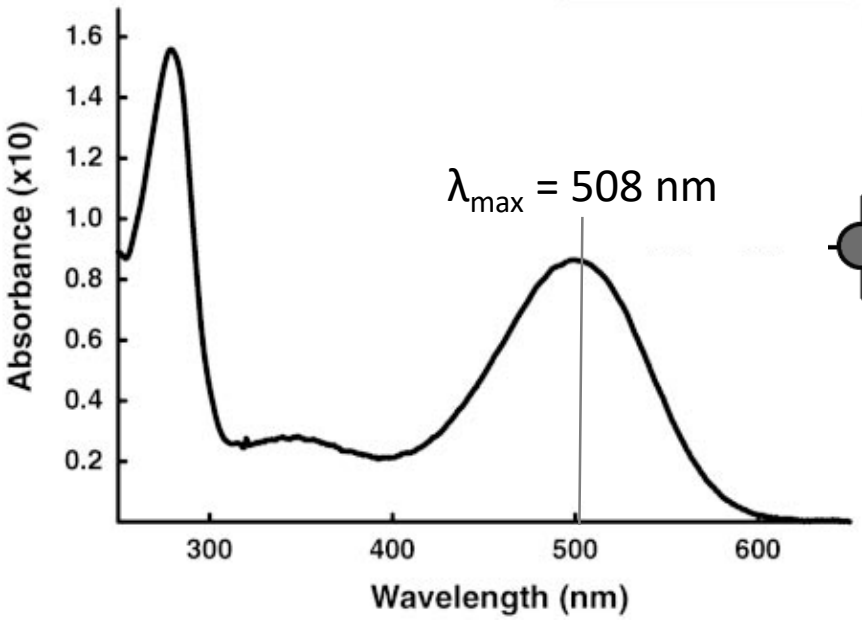

Menon, Han & Sakmar 2001

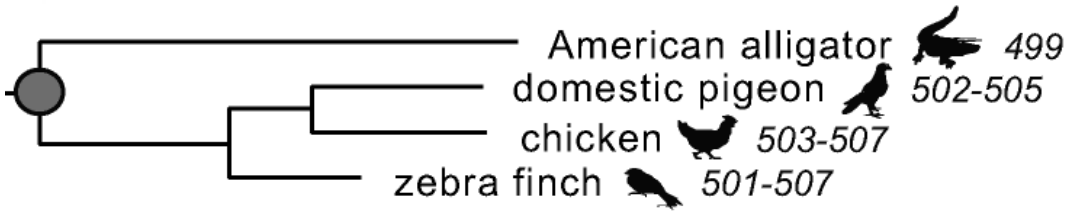

Bring On the Dinosaurs!



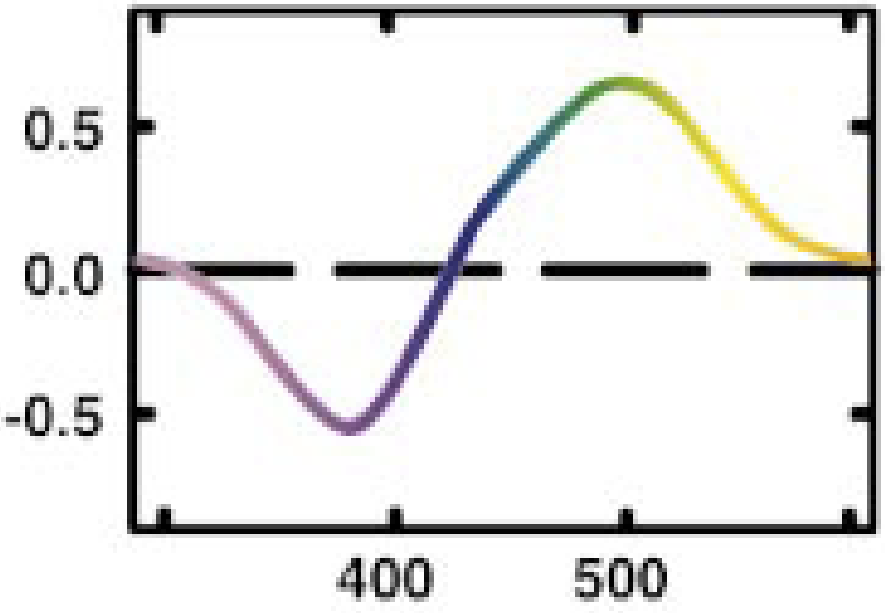

Chang et al. 2002

# AncRhodopsin Spectra



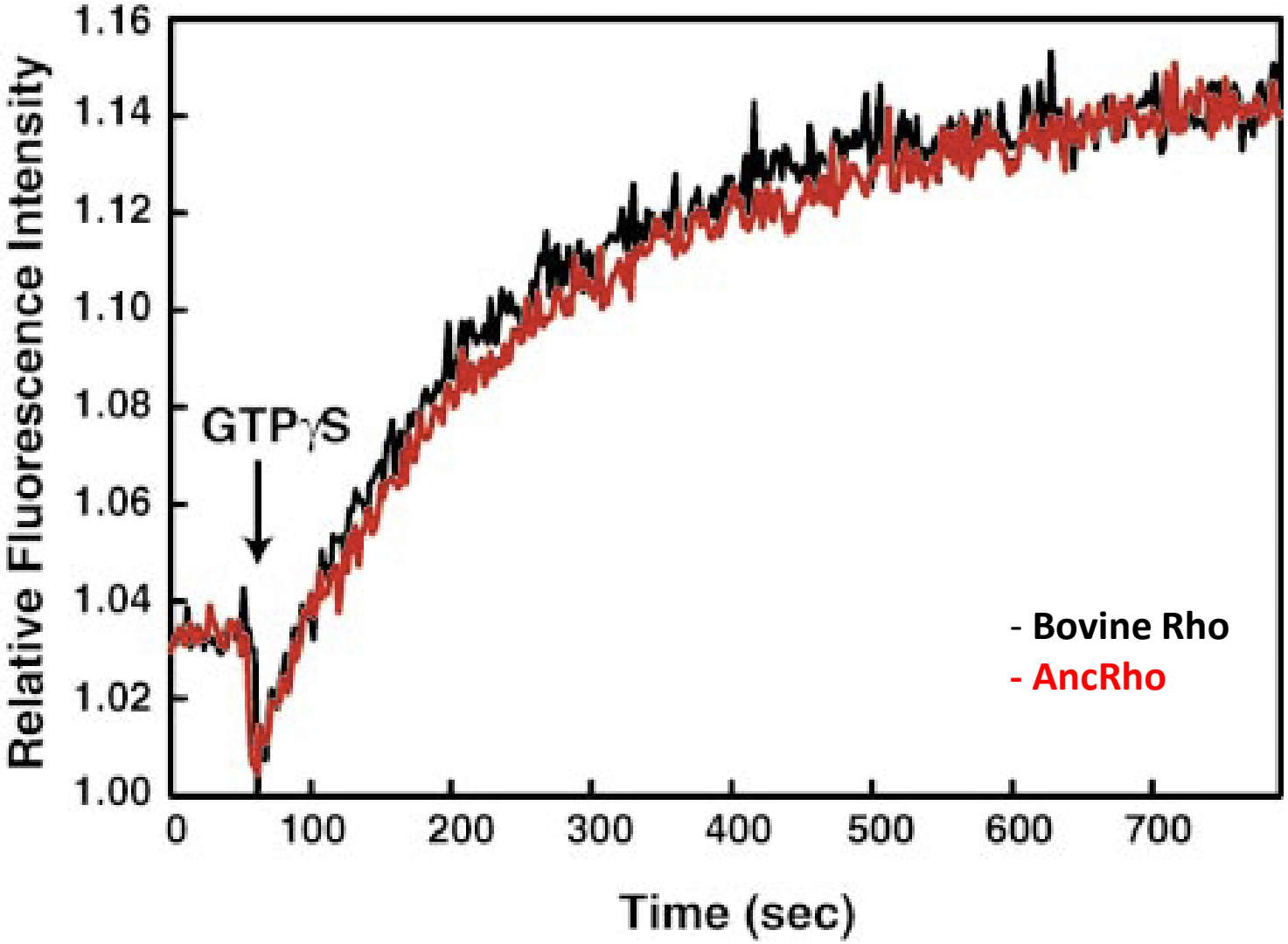



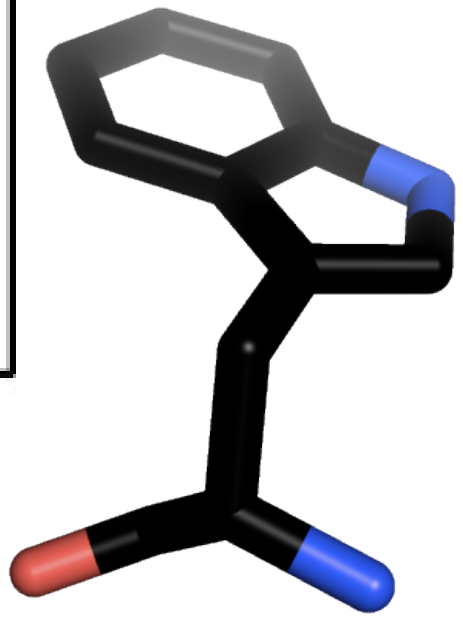

**MetarhodopsinII**




Chang et al. 2002

# AncRhodopsin Activates Transducin



Chang et al. 2002


You aren’t safe at night.

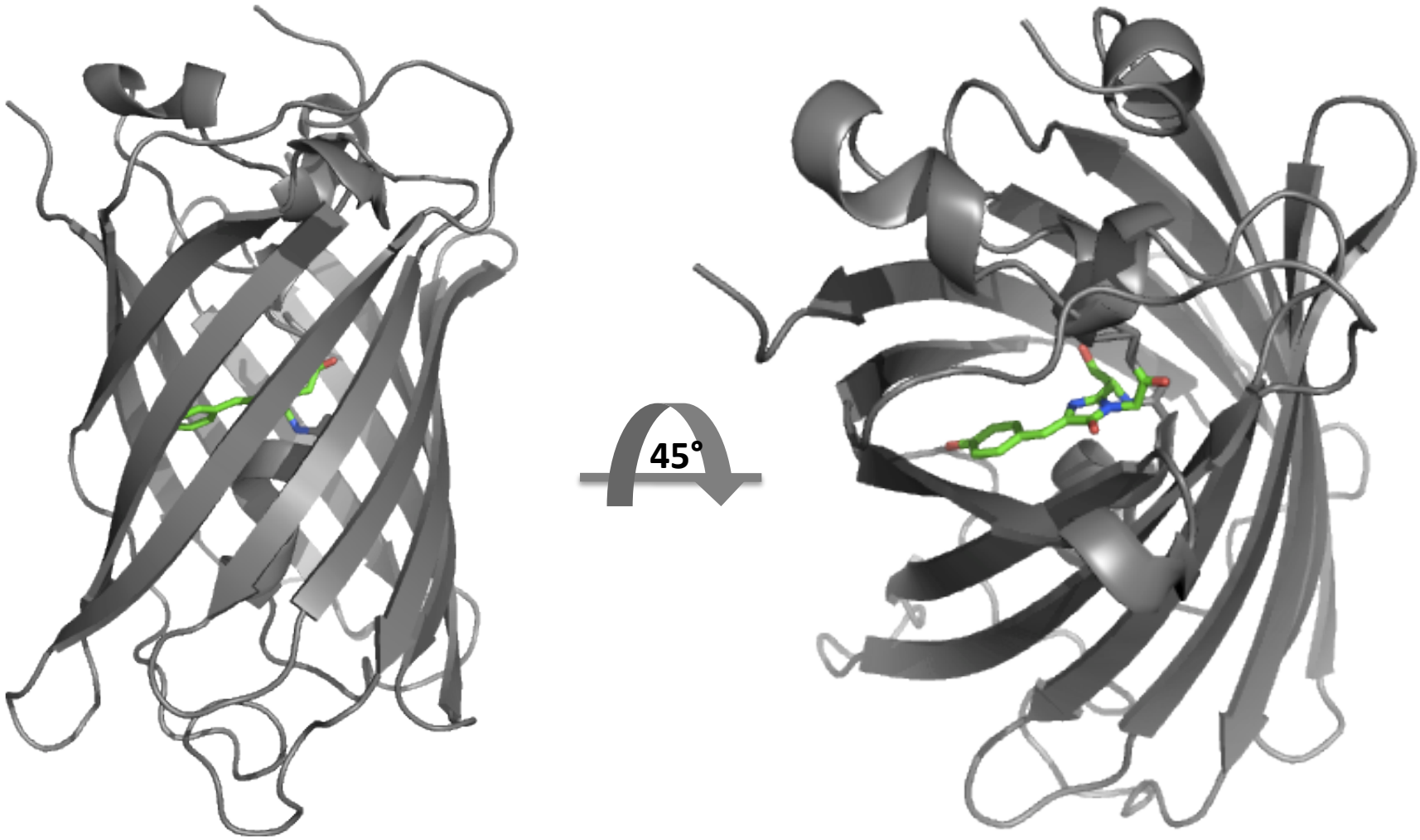
# Talk Outline

- Could Dinosaurs See at Night?
- The Coral Red: Convergence or Divergence?
- Same Fold, Different Specificity: How'd That Happen?

# Green Fluorescent Protein (GFP)

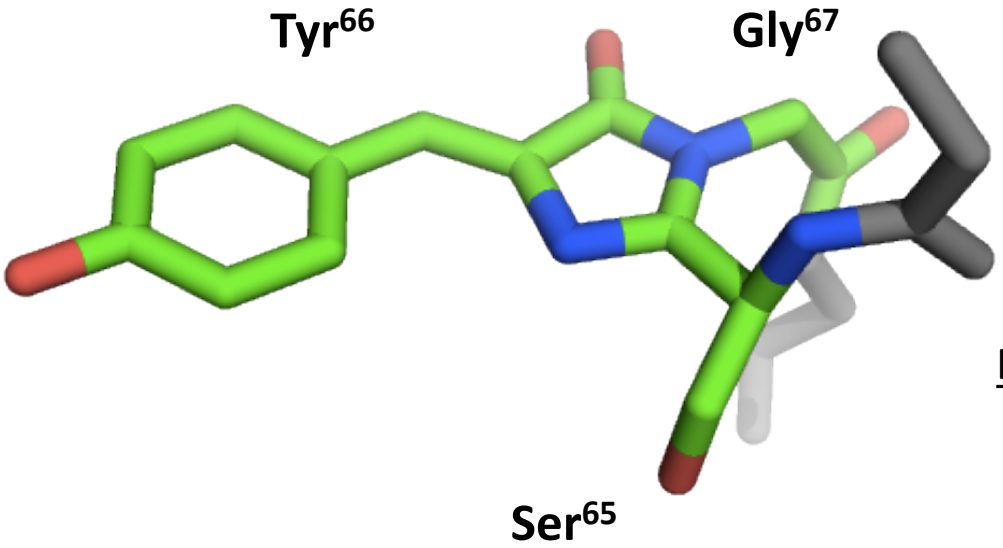
- Isolated from *Aequorea victoria* (jellyfish) in 1960s
  - Jellyfish from Friday Harbor, WA
  - Work done at the MBL in Woods Hole, MA
- Natural function is unknown
- In the lab, used as a reporter for expression
  - Nobel Prize awarded in 2008

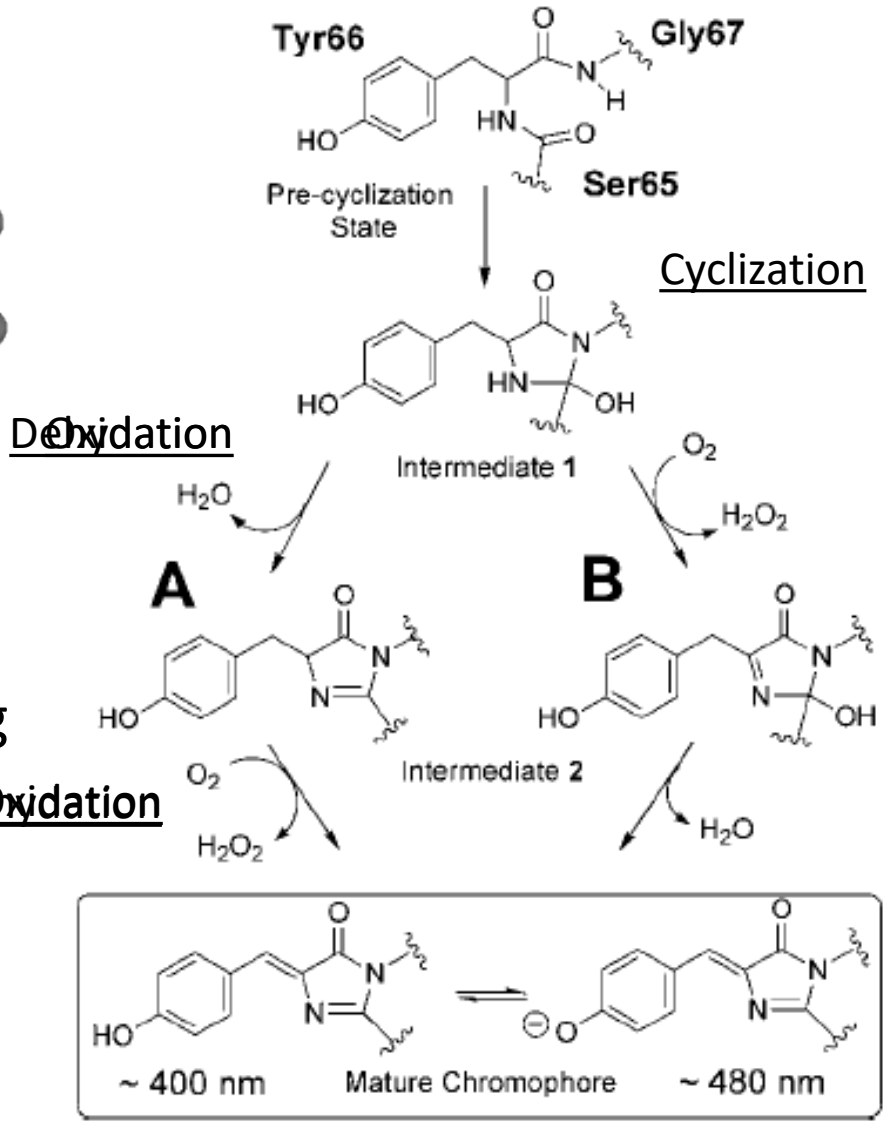
# GFP Structure




- 11-stranded β-barrel with chromophore positioned in center

# GFP Chromophore - Structure & Synthesis



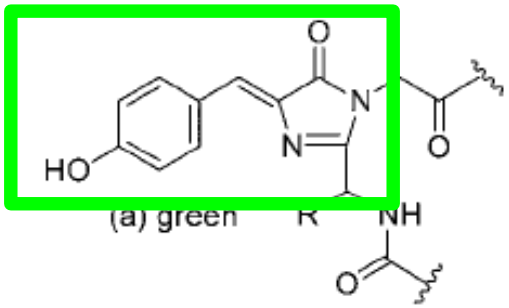

- Auto-catalyzation begins upon folding

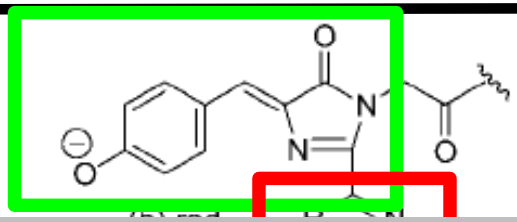
**Excitation - UV Blue** **Emission - Green**

Wachter 2006

# Colors of the Rainbow

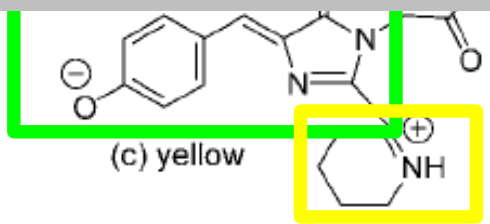
**2 Chemical Reactions**
**(Oxidation, Dehydration)**

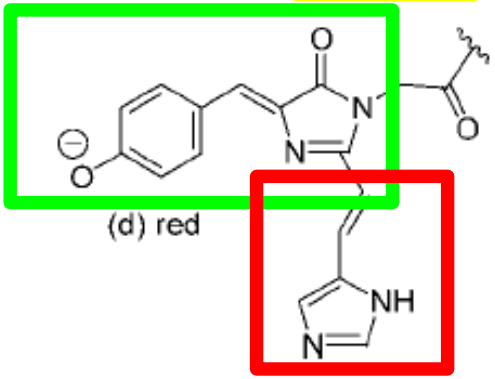



**Was complexity gained or lost?**

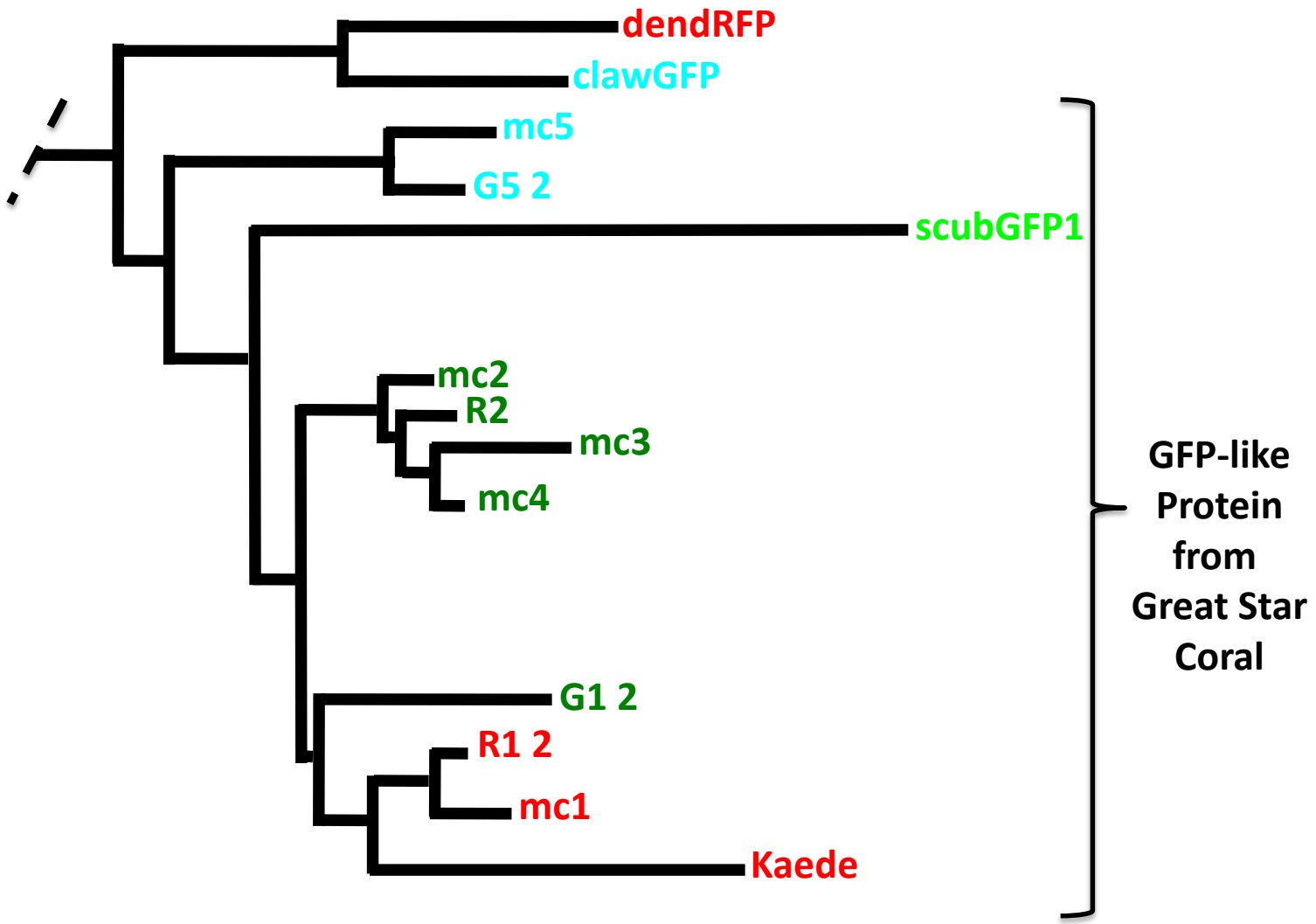
**3 Chemical Reactions**
**(Oxidation, Dehydration &**
**2nd Oxidation extends π-system)**



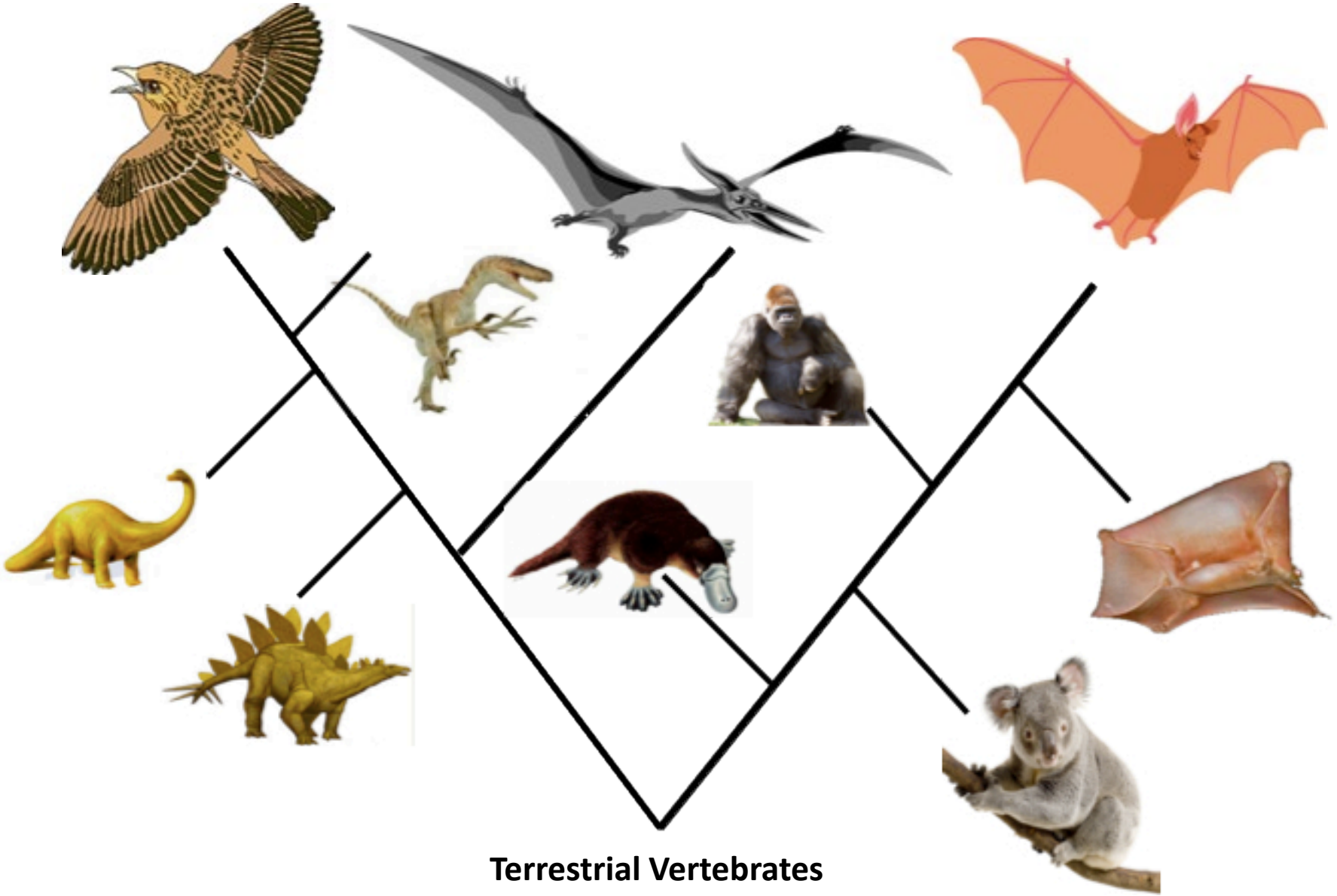

Wachter 2006

# GFP-Superfamily

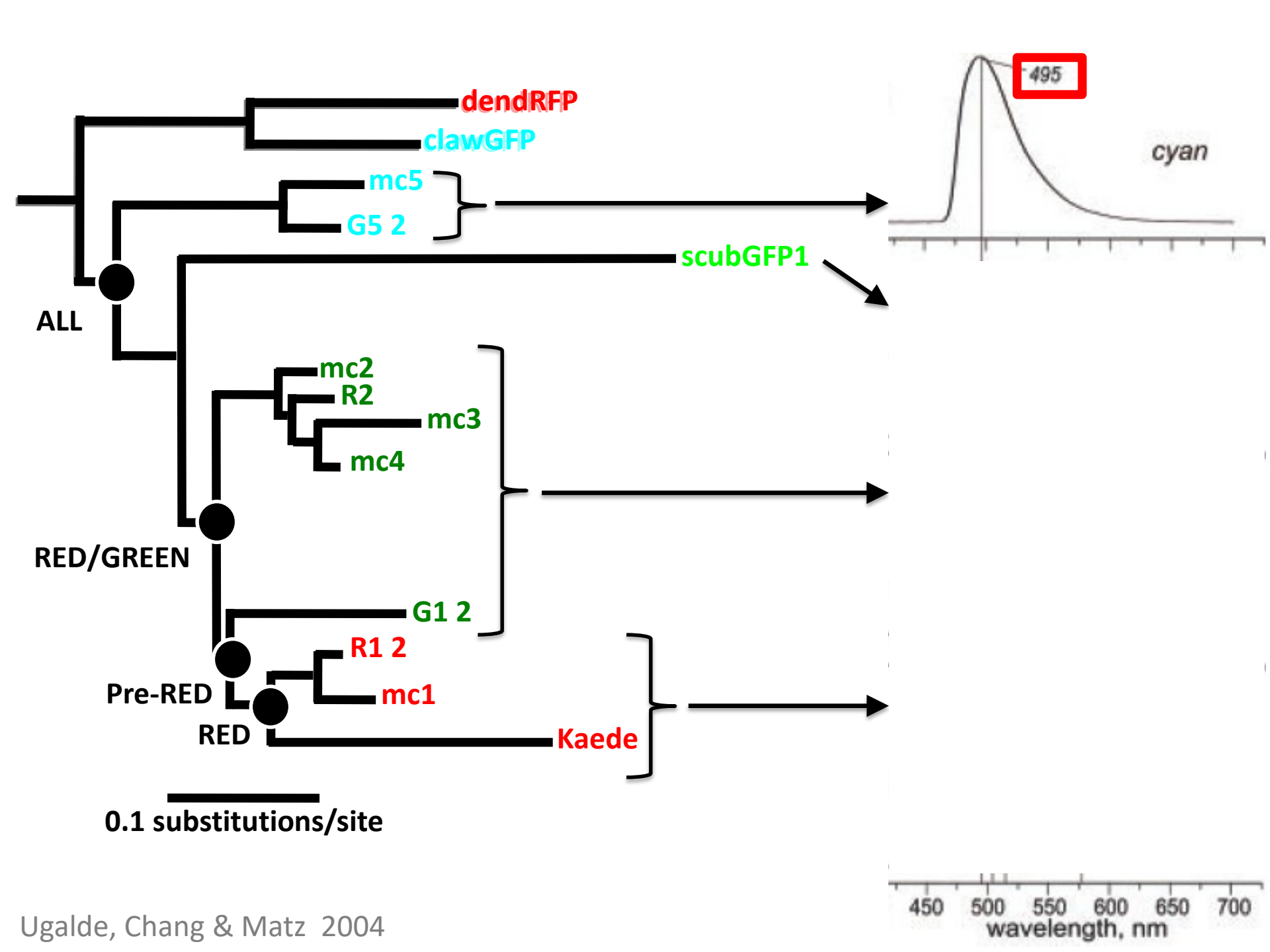



Ugalde, Chang & Matz 2004

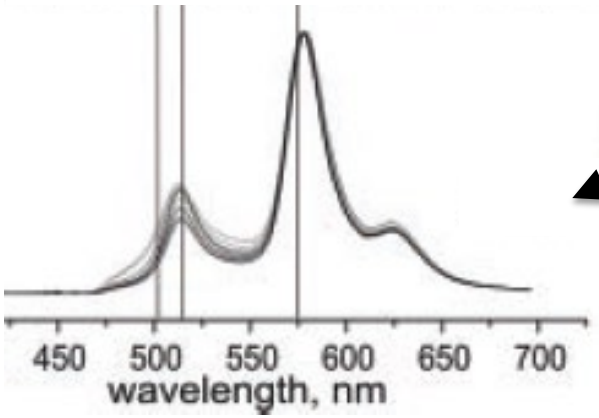
# Convergent vs. Divergent Evolution

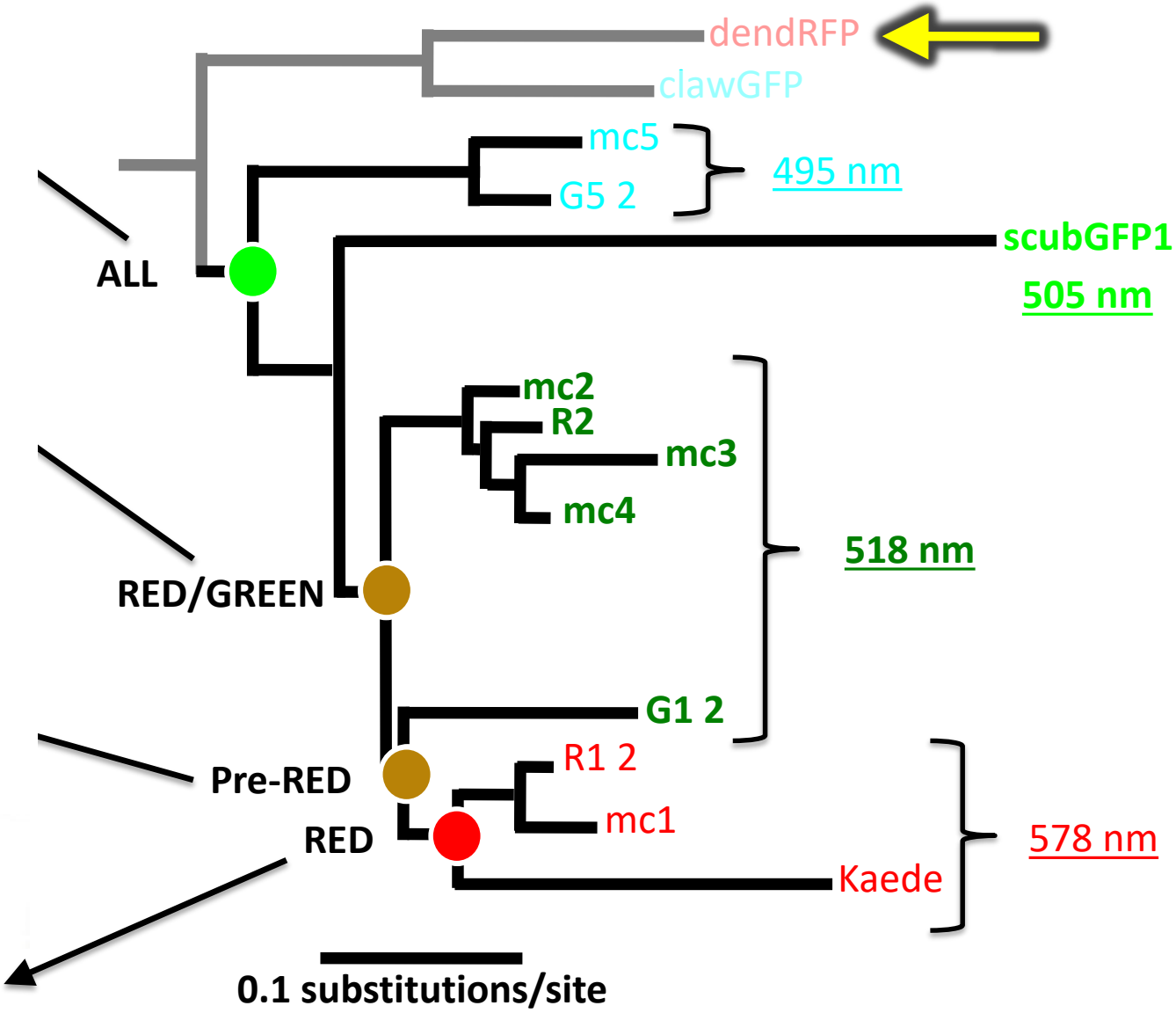


**Terrestrial Vertebrates**

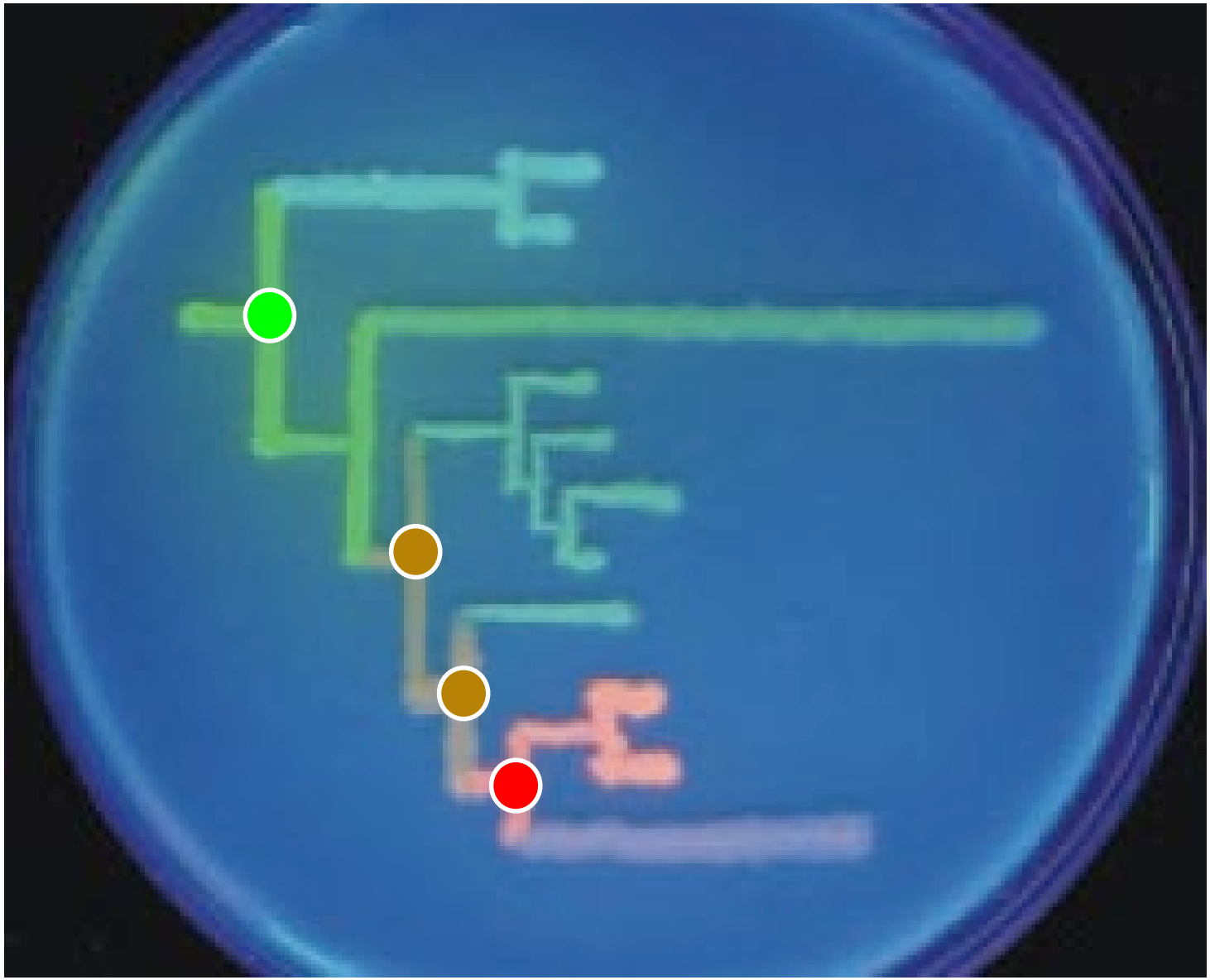



Ugalde, Chang & Matz 2004




Ugalde, Chang & Matz 2004



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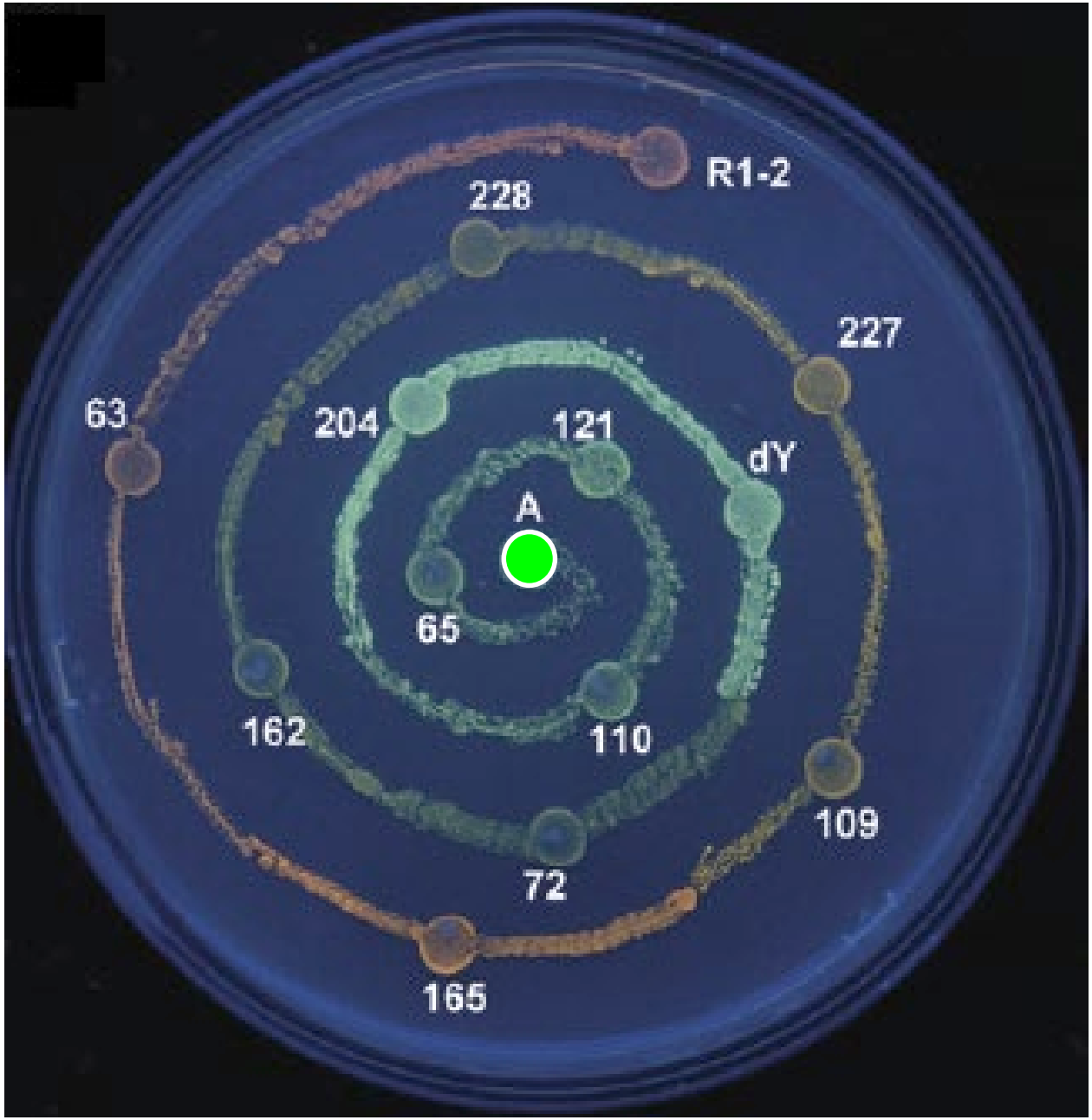


Field & Matz et al 2010

# Talk Outline

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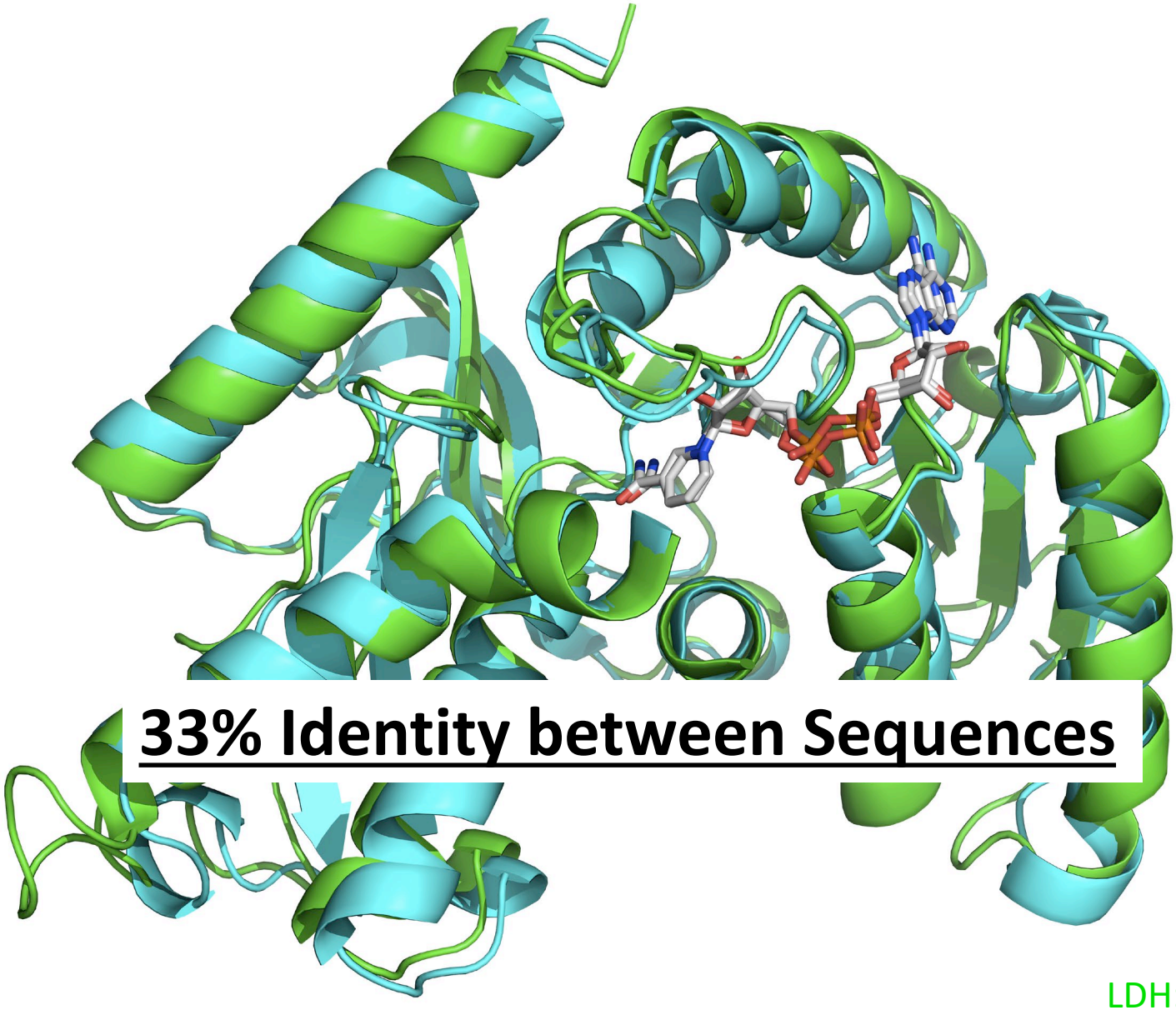
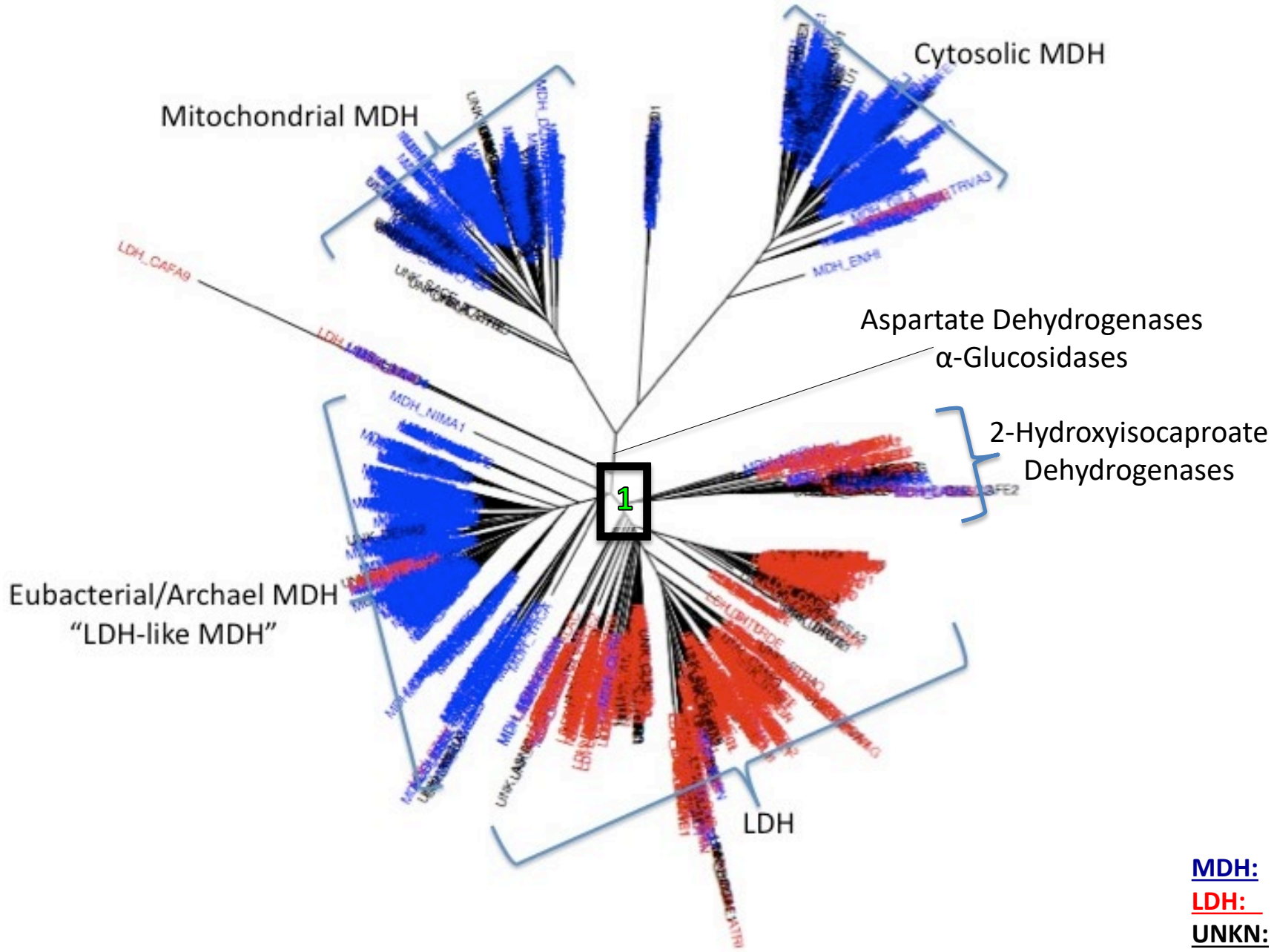
# Lactate & Malate Dehydrogenase Share a Fold



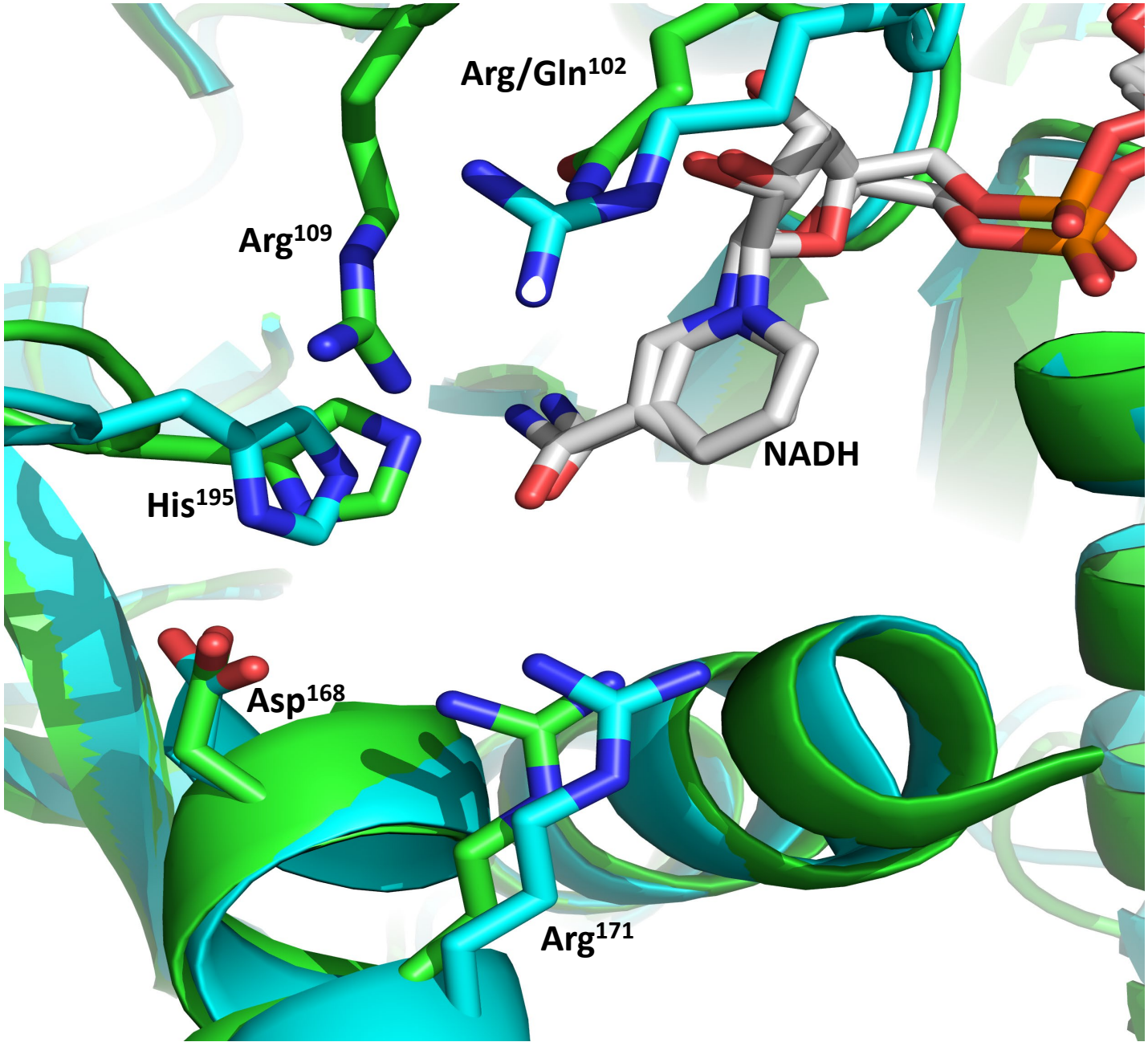
**33% Identity between Sequences**

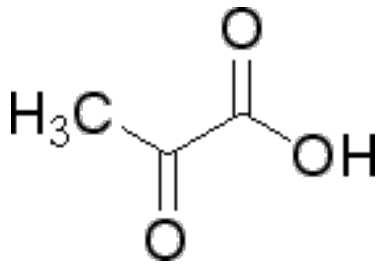
LDH: GREEN
MDH: CYAN

# LDH Has Evolved From MDH 4 Separate Times

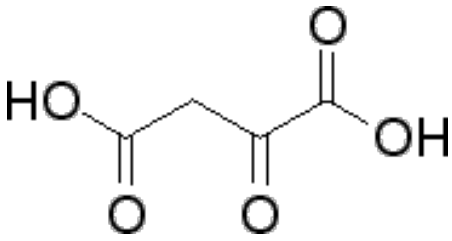


# Canonical Malate/Lactate Active Site




Pyruvate (LDH)




Oxaloacetate (MDH)

LDH: GREEN
MDH: CYAN

# Canonical Lactate & Malate Dehydrogenase

- Specificity switched through single point mutation (Gln ➔ Arg)

Wilks et al. (1988)
*B. stearothermophilus*

Cendrin et al. (1993)
*H. marismortui*

Nicholls et al. (1992)
*E. coli*

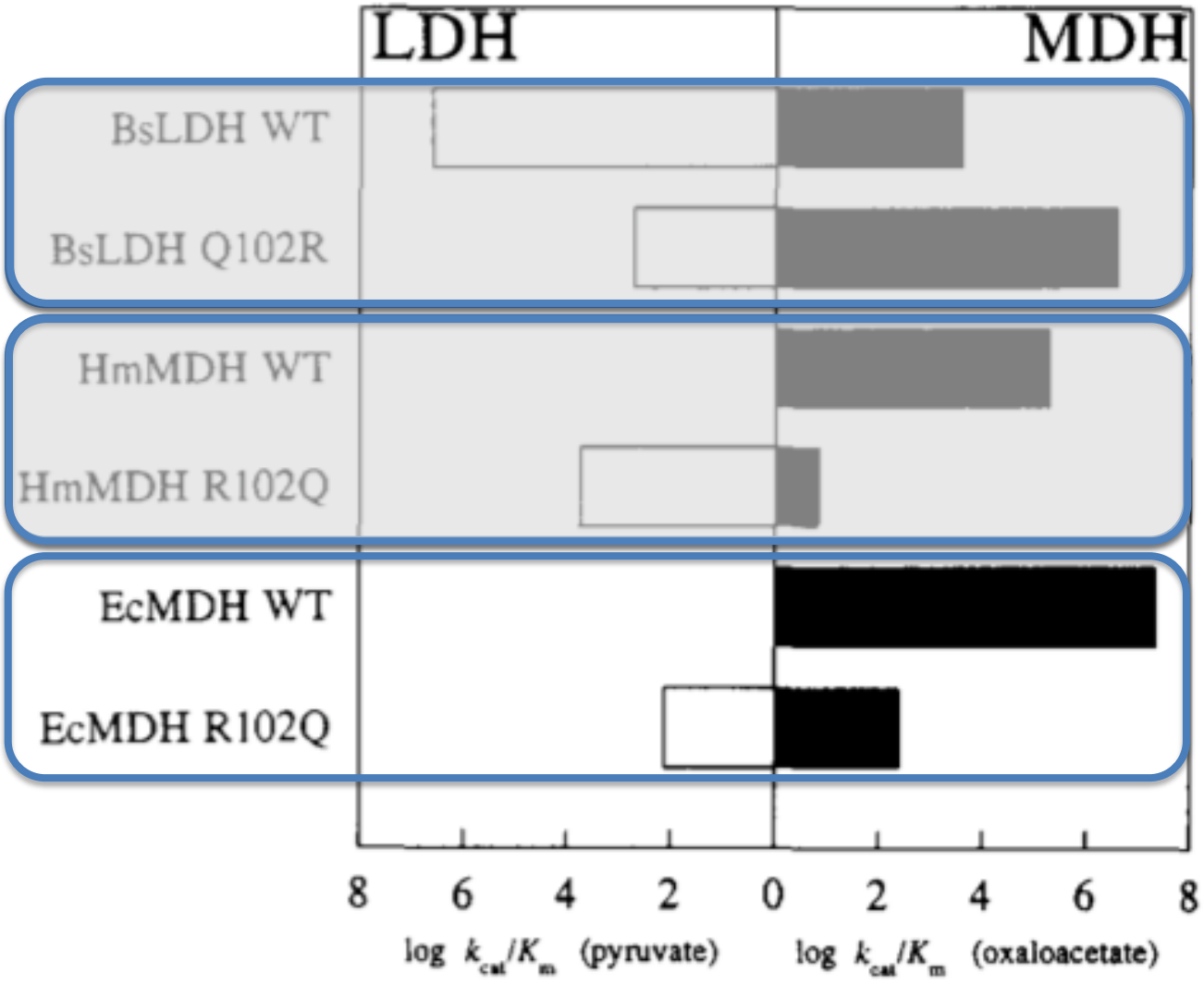
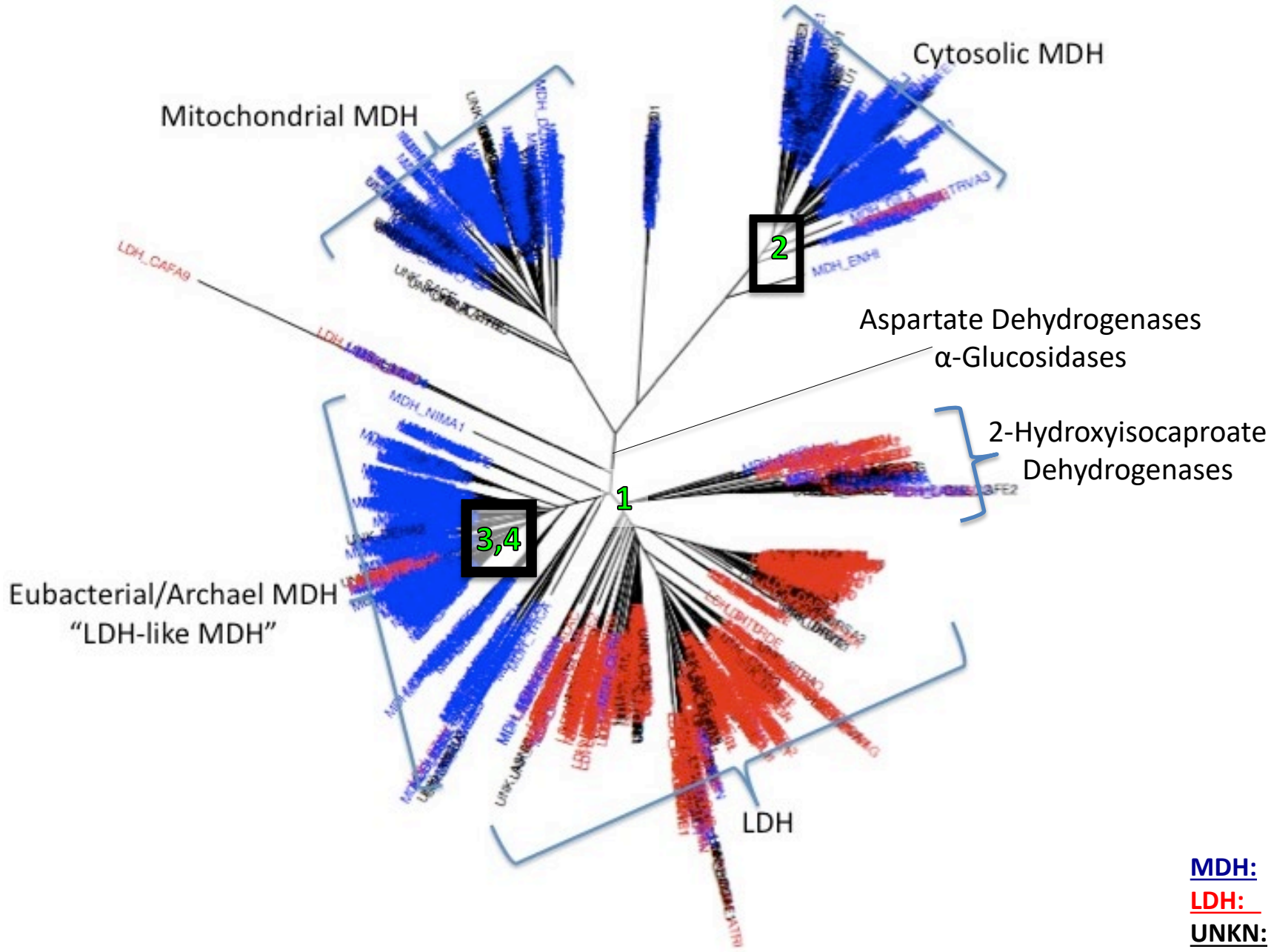


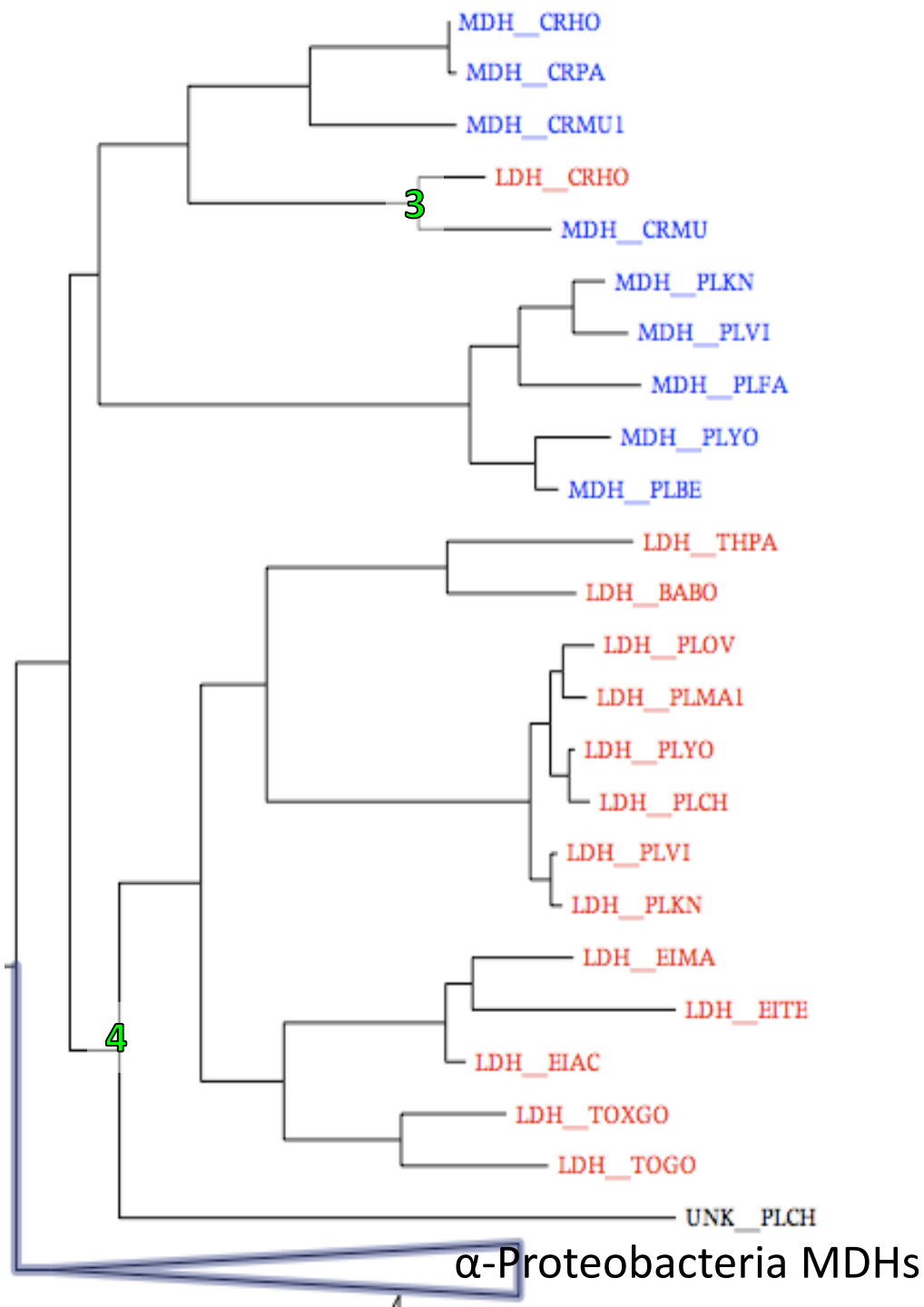

Image Goward & Nicholls 1994

# LDH Has Evolved From MDH 4 Separate Times

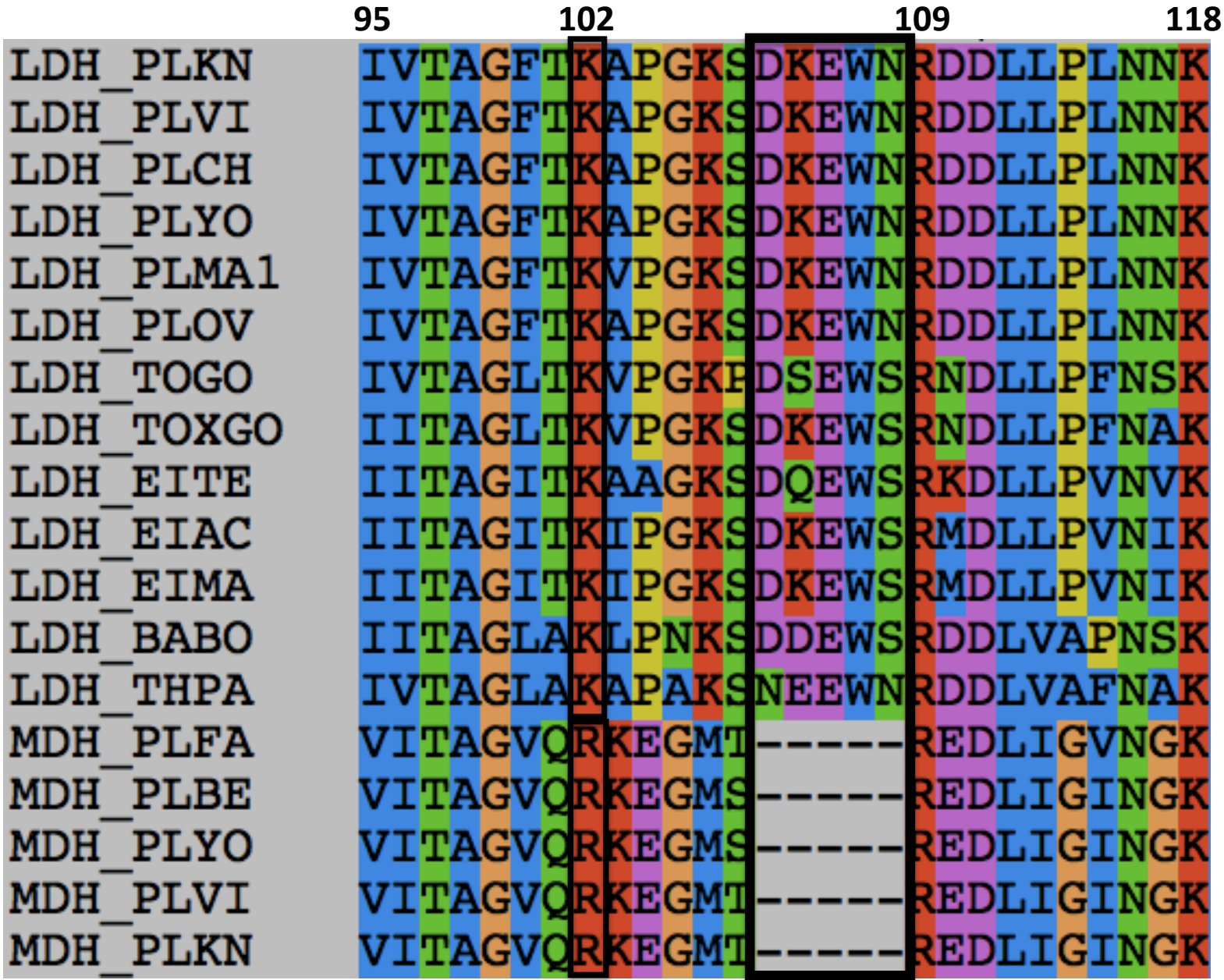


- Apicomplexan MDH & LDH evolved from α-Proteobacteria MDH through horizontal gene transfer

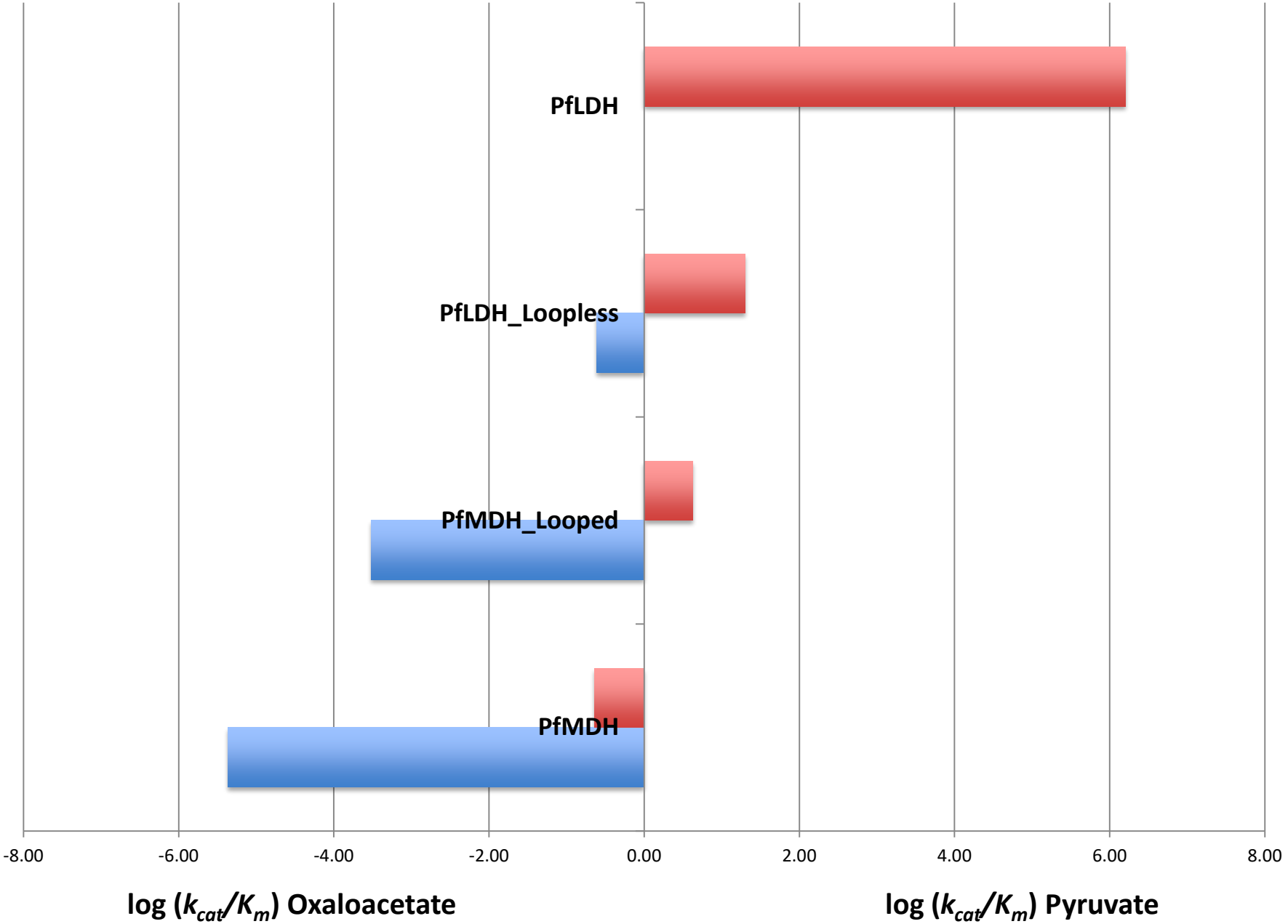
- Apicomplexan LDHs evolved from a gene duplication of transferred gene



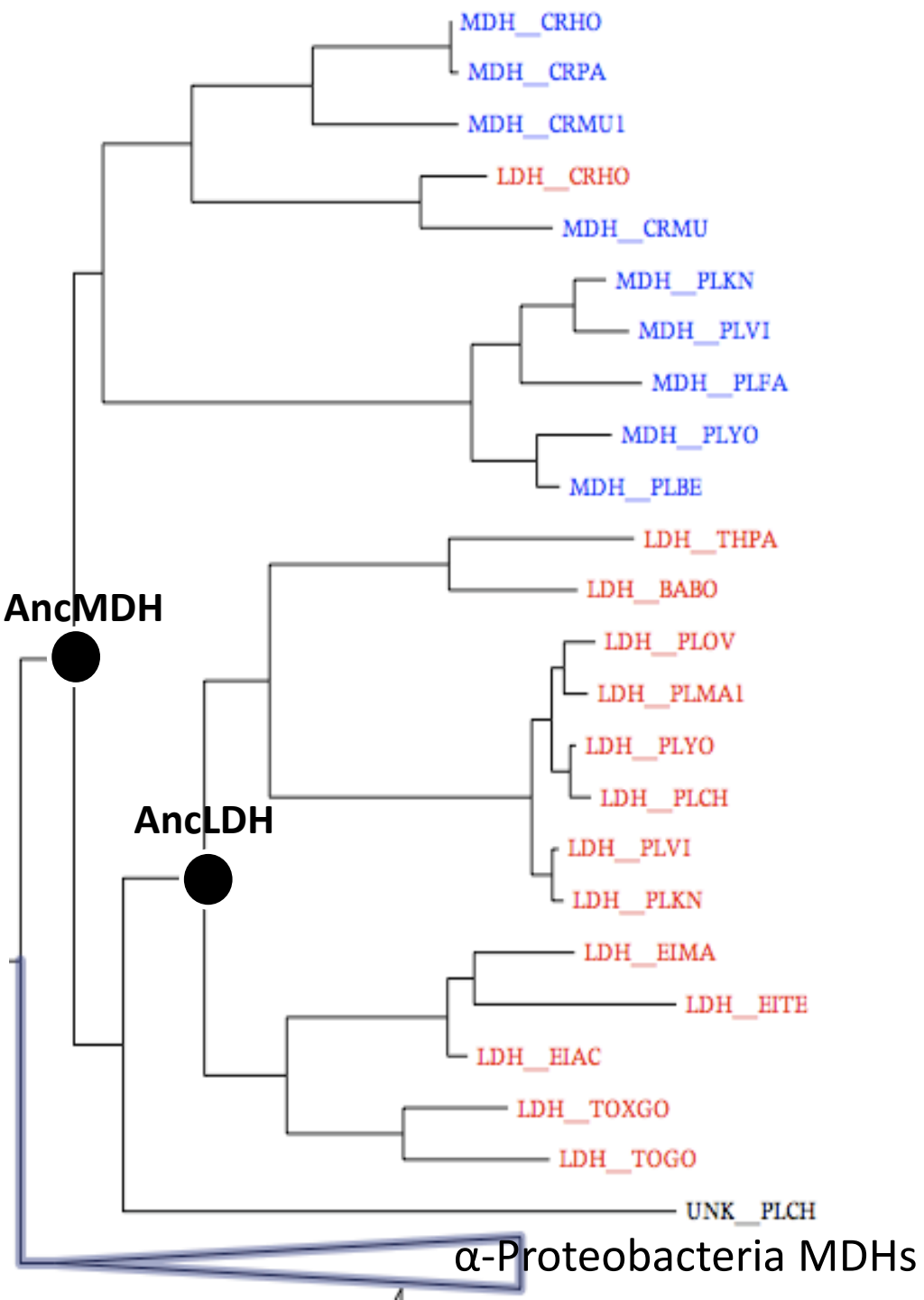
# Apicomplexan LDHs have a 5AA Insertion



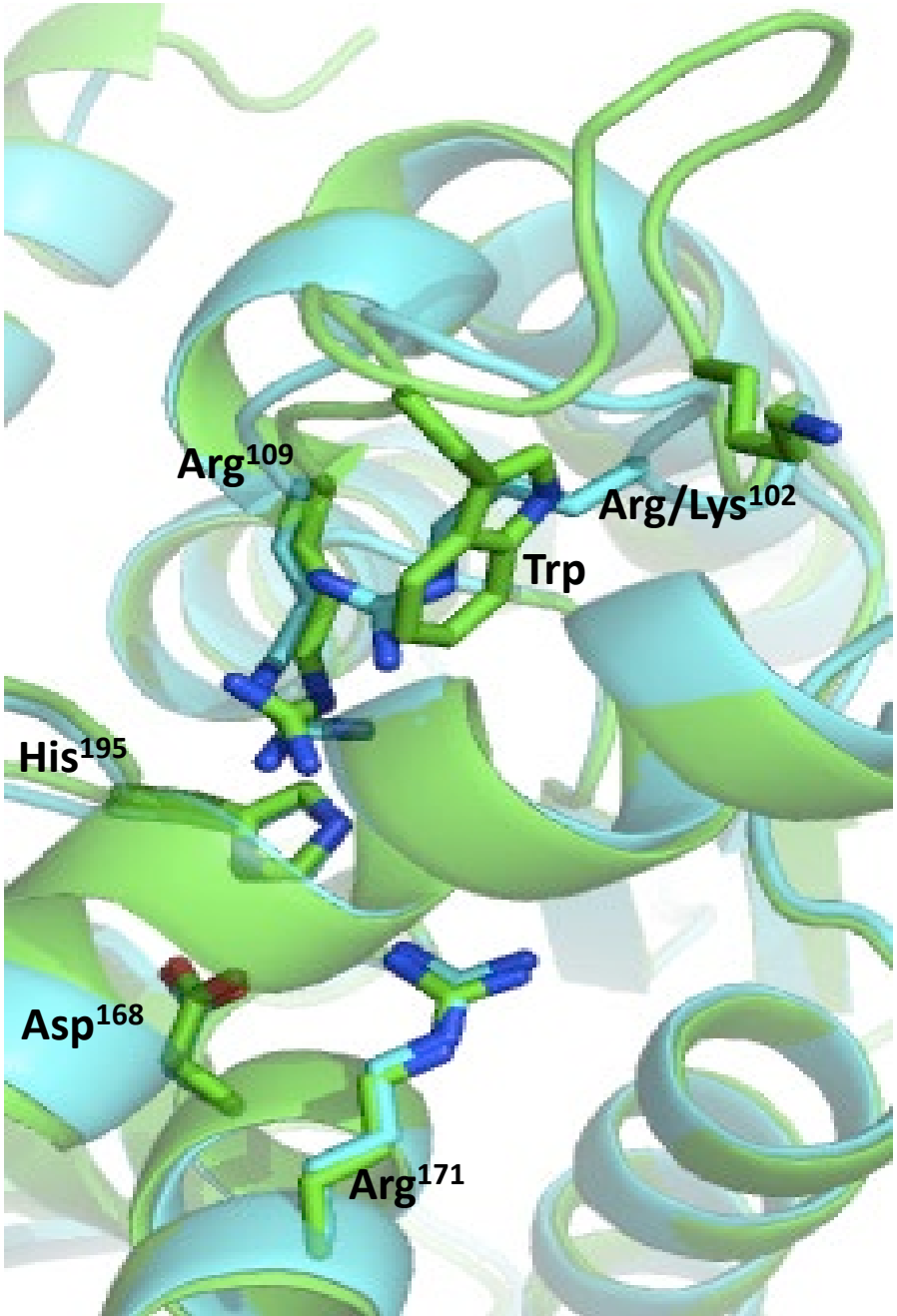
# Modern Enzymes Cannot Tolerate Loop Swap



- Sequence identity between PfLDH & PfMDH is ~40%
  - Difference of ~200 residues
- How can we reduce this to a more manageable number?



# AncMDH & AncLDH Homology Models - Active Site

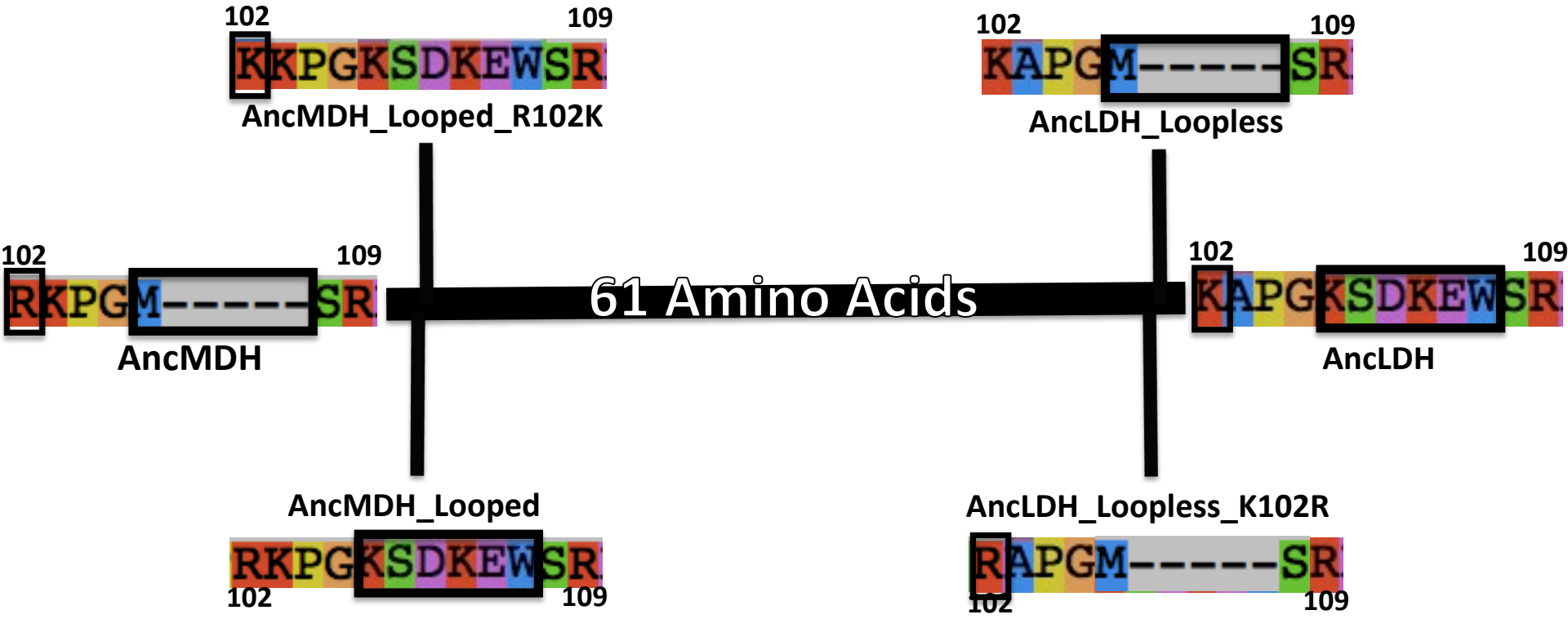




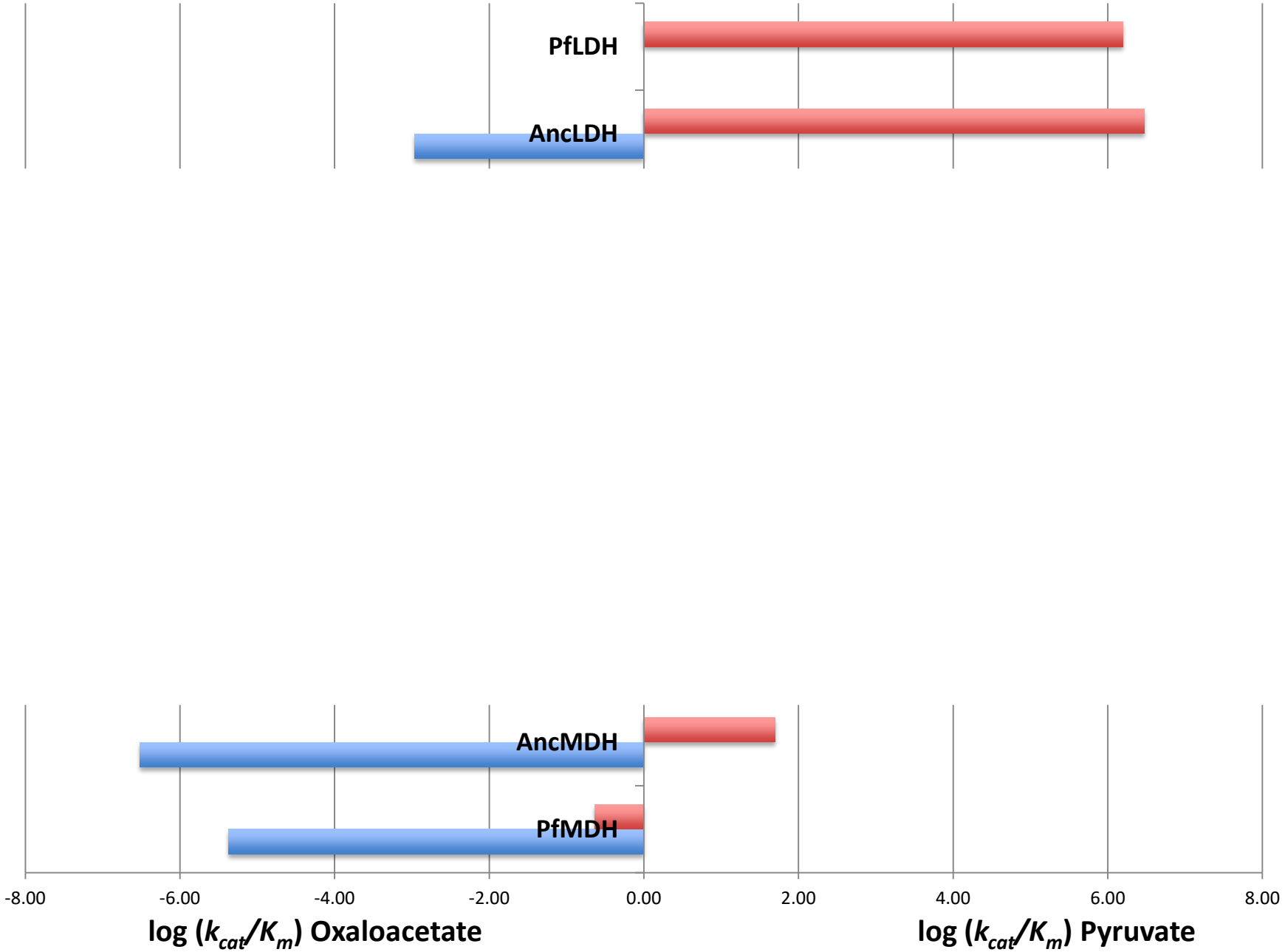
AncLDH: GREEN

AncMDH: CYAN

# Tracing the Switch in Specificity - The Plan

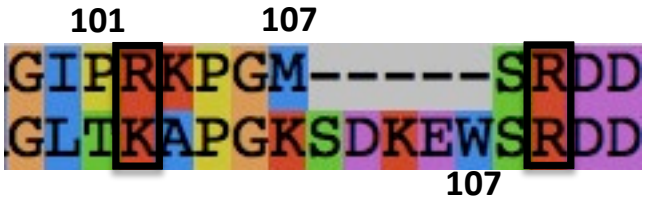


# Tracing the Switch in Specificity - Results



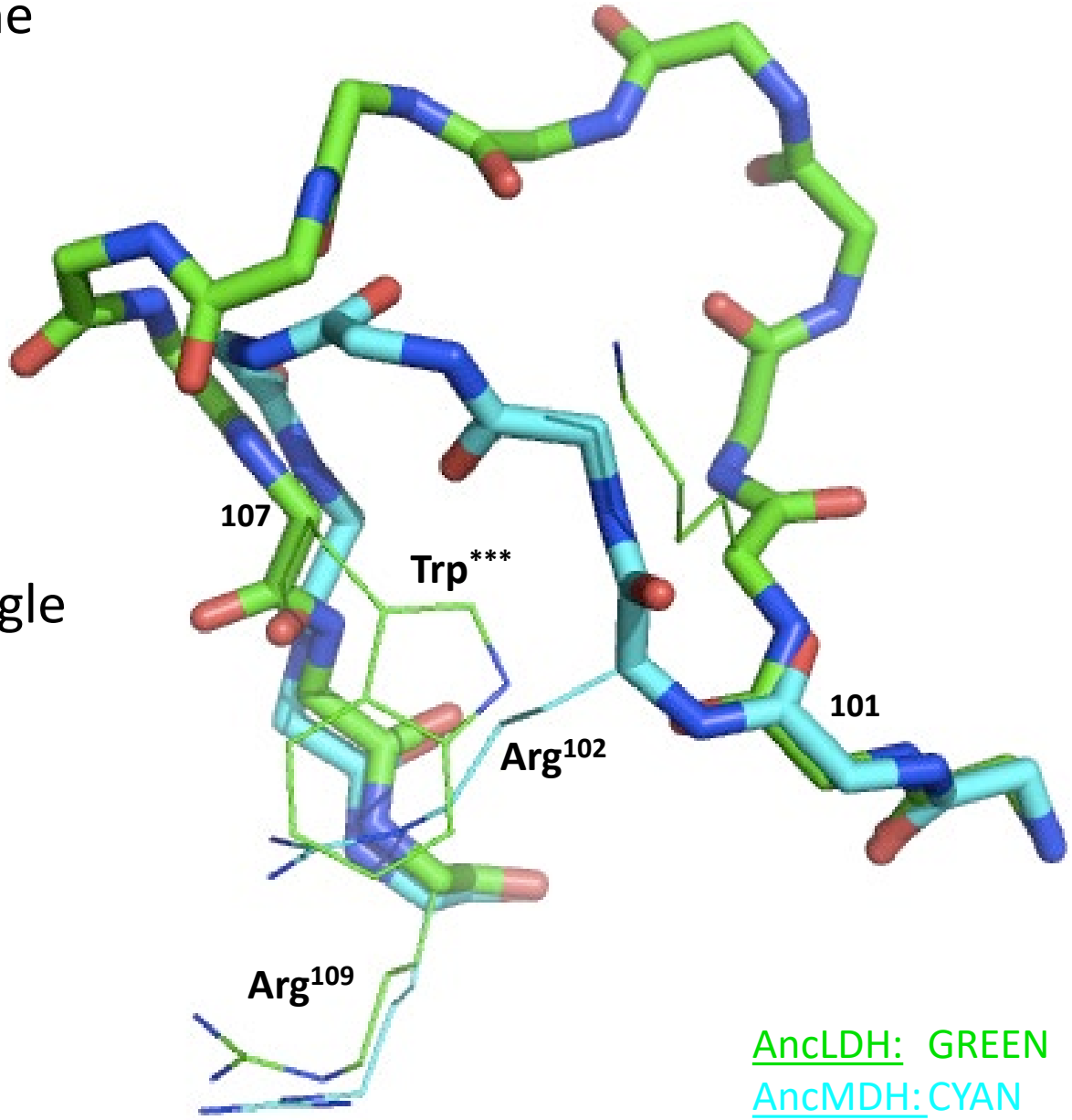
# One Residue to Rule Them All…

- Are all 5 amino acids of the insertion necessary?
  - Truncate loop insertion




  - 4 residues vs 9 residues

- Can we convert with a single point mutation?
  - AncMDH_R102W
  - AncLDH_W___R



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