

# Mechanism Underlying Effects of Genetic Variance and General Anesthetics on Ligand Gated Ion Channels.

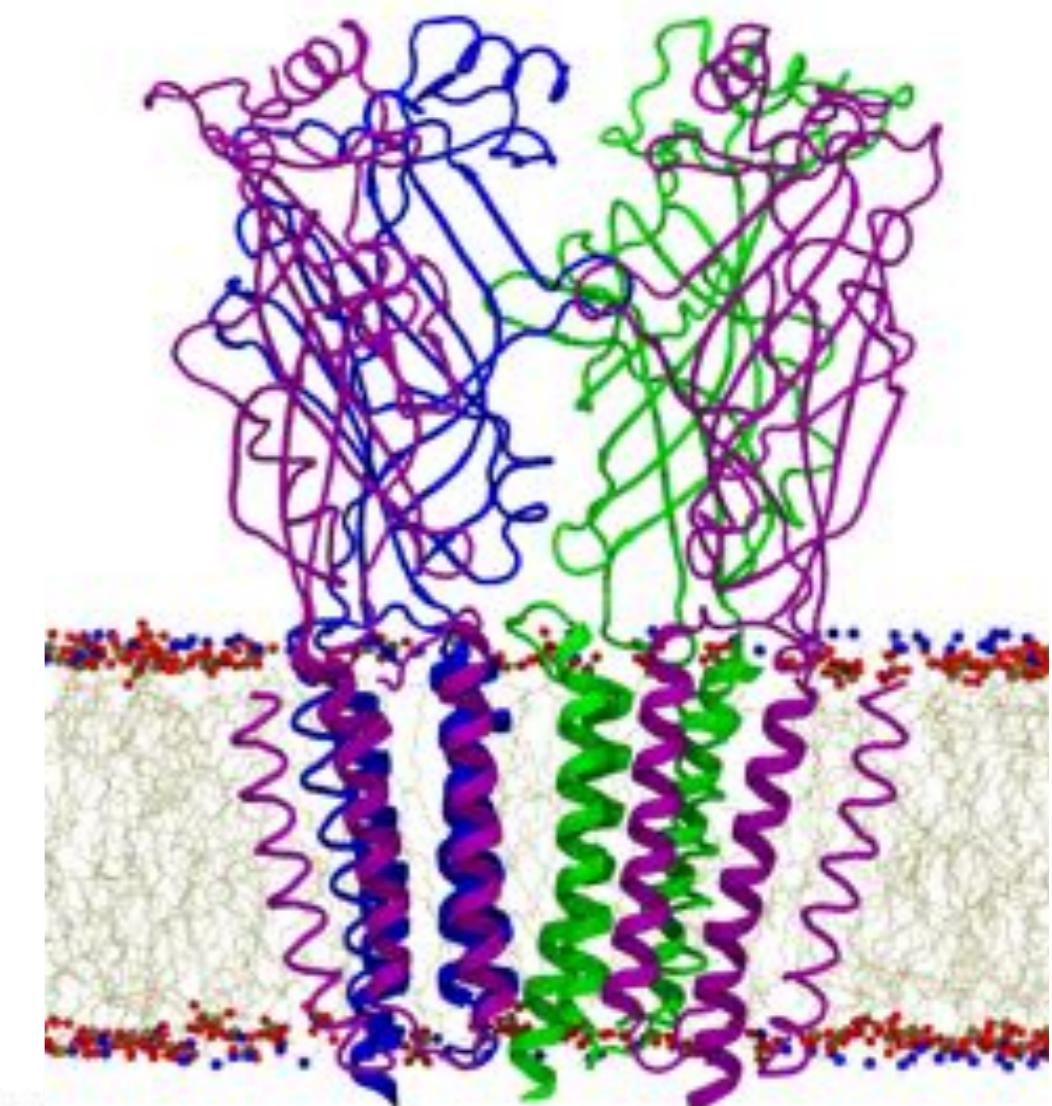
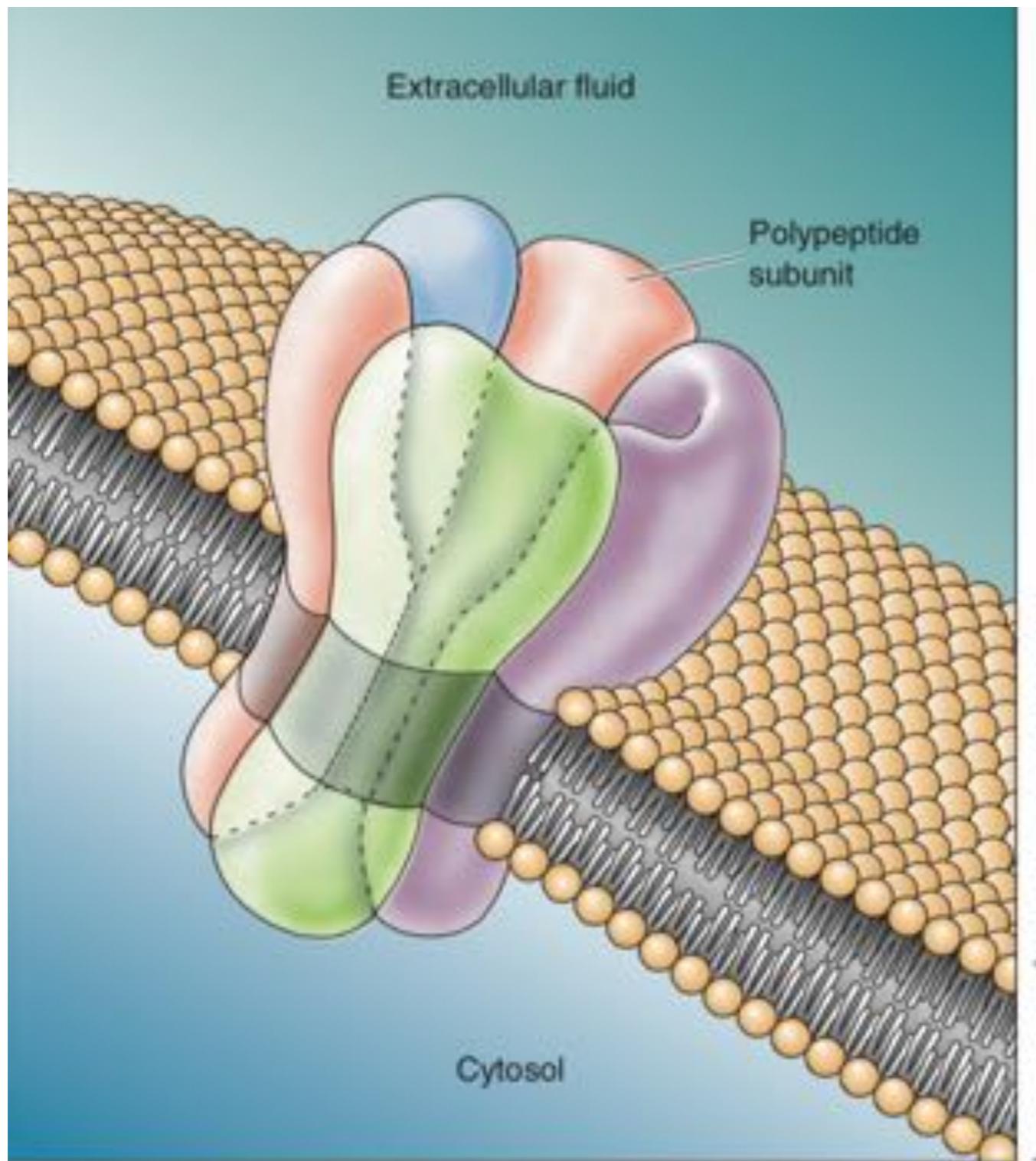
Sruthi Murlidaran  
Final PhD Defense

CENTER FOR COMPUTATIONAL  
AND INTEGRATIVE BIOLOGY

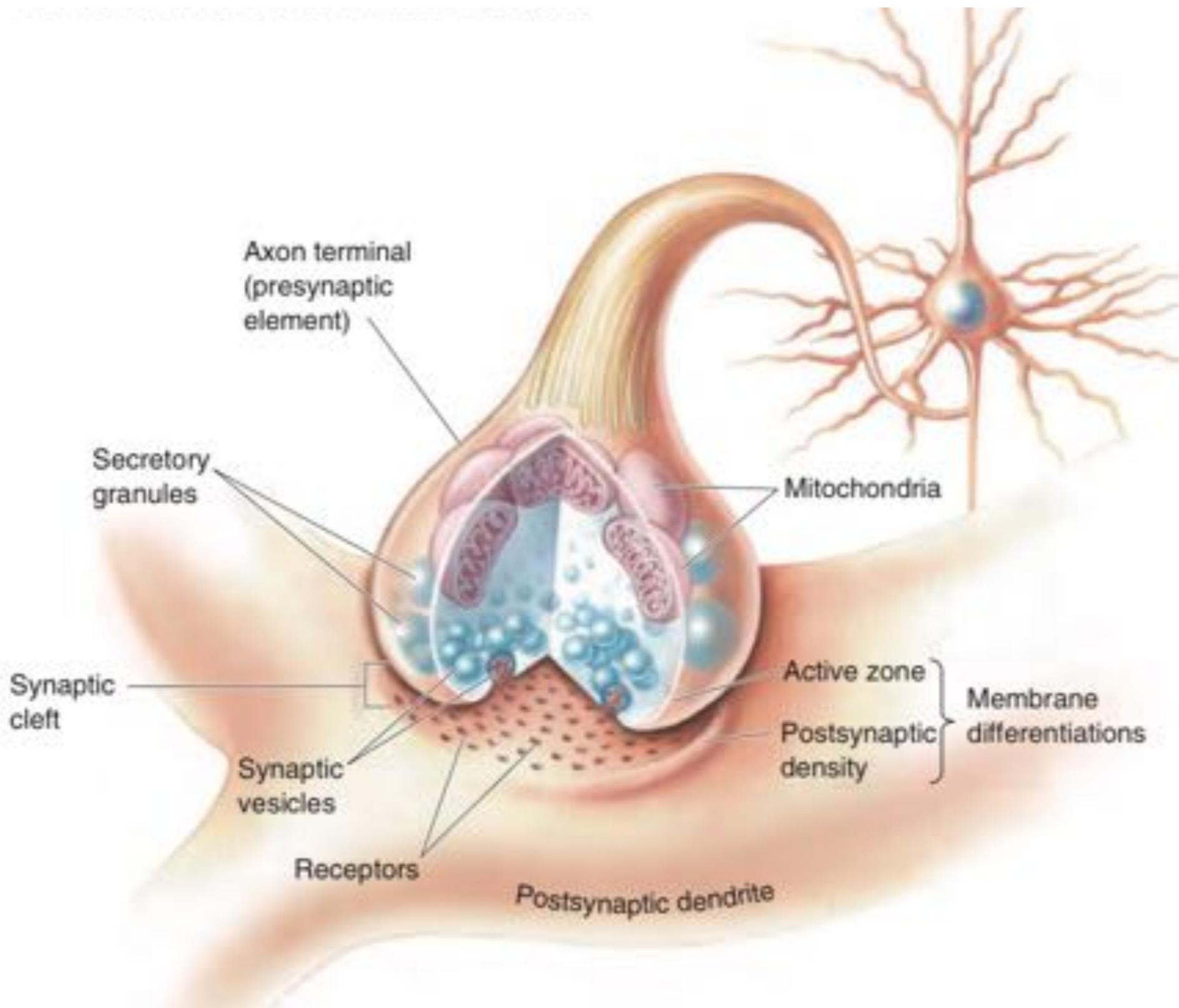


**RUTGERS**  
CAMDEN

# Pentameric Ligand gated ion channels(pLGICs)



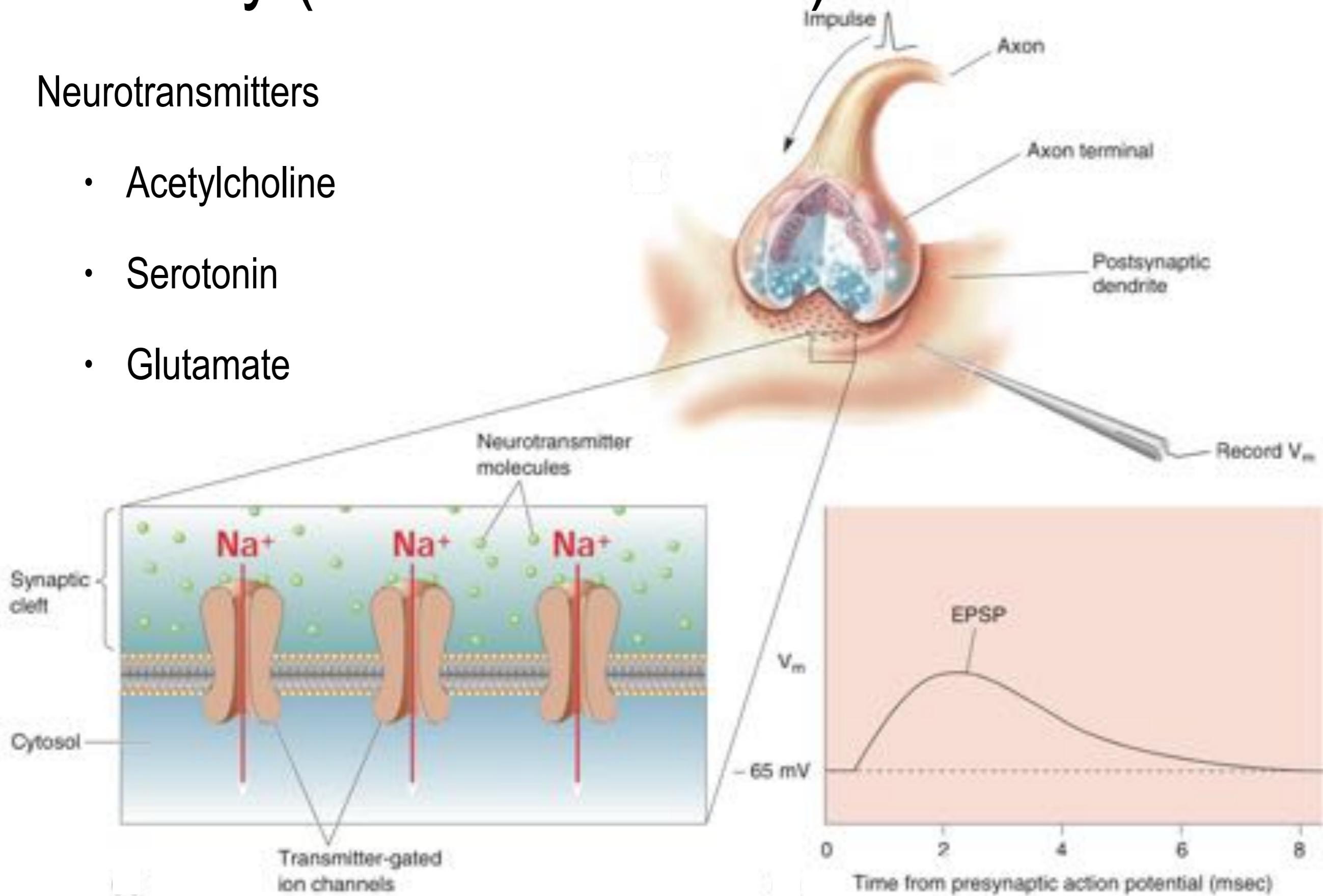
# Synaptic Transmission



# Excitatory (Cation Channels)

## Neurotransmitters

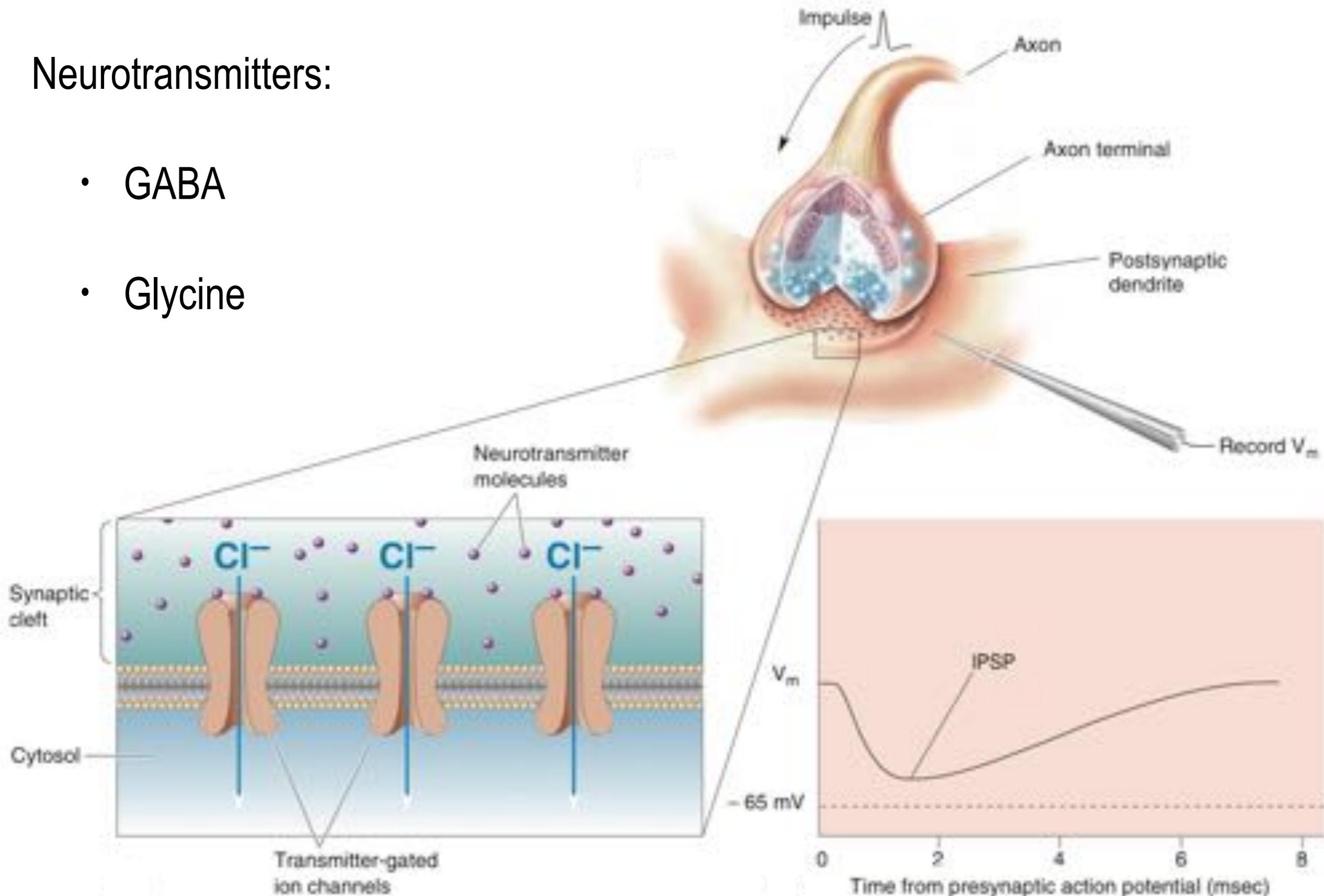
- Acetylcholine
- Serotonin
- Glutamate



# Inhibitory (Anionic Channels)

Neurotransmitters:

- GABA
- Glycine



# Conformations states of pLGICs

Ligand-gated ion channels are membrane proteins that are fundamental signaling molecules in neurons.

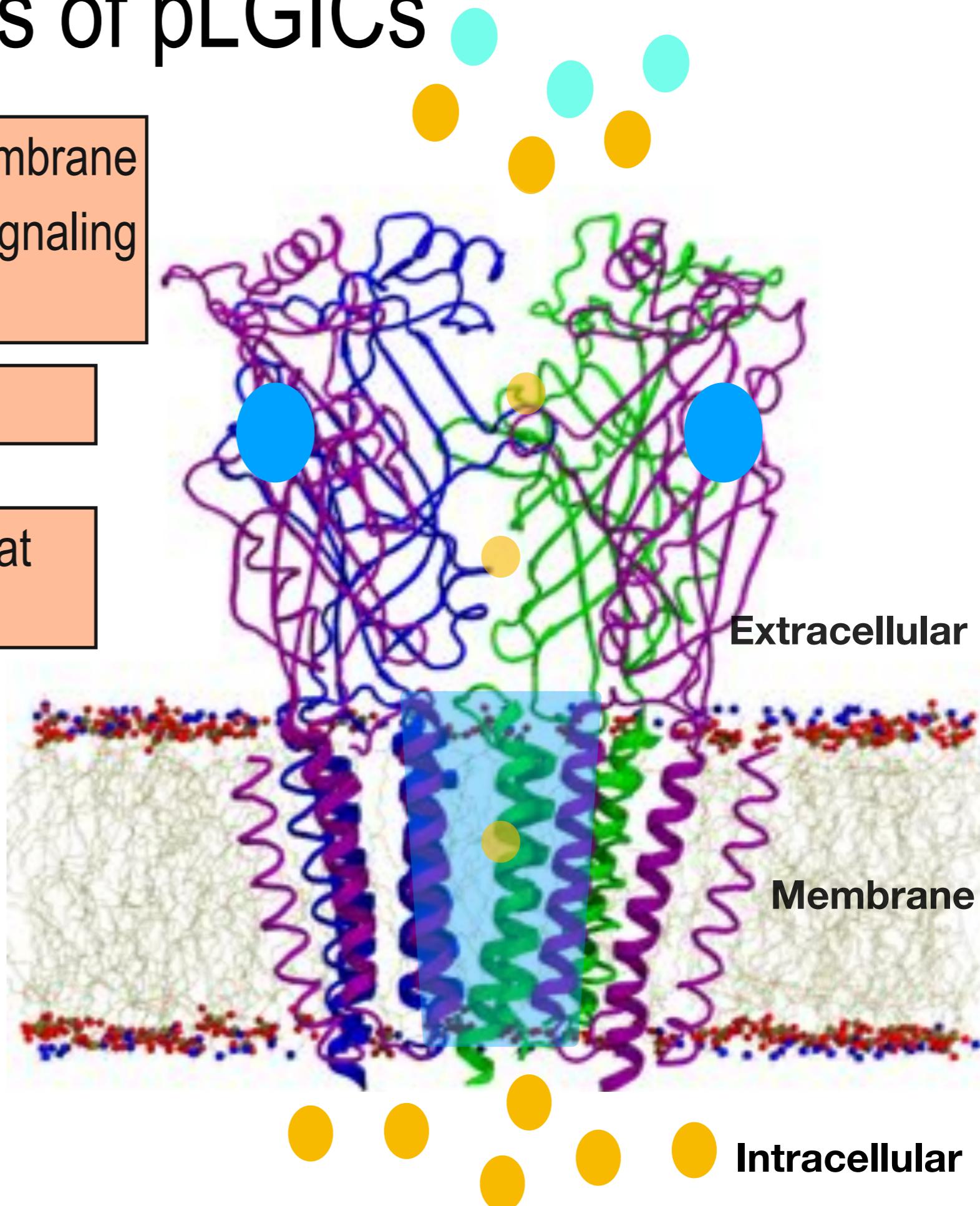
Ligands bind to the channel

Conformational changes occur that opens the pore - **OPEN state**

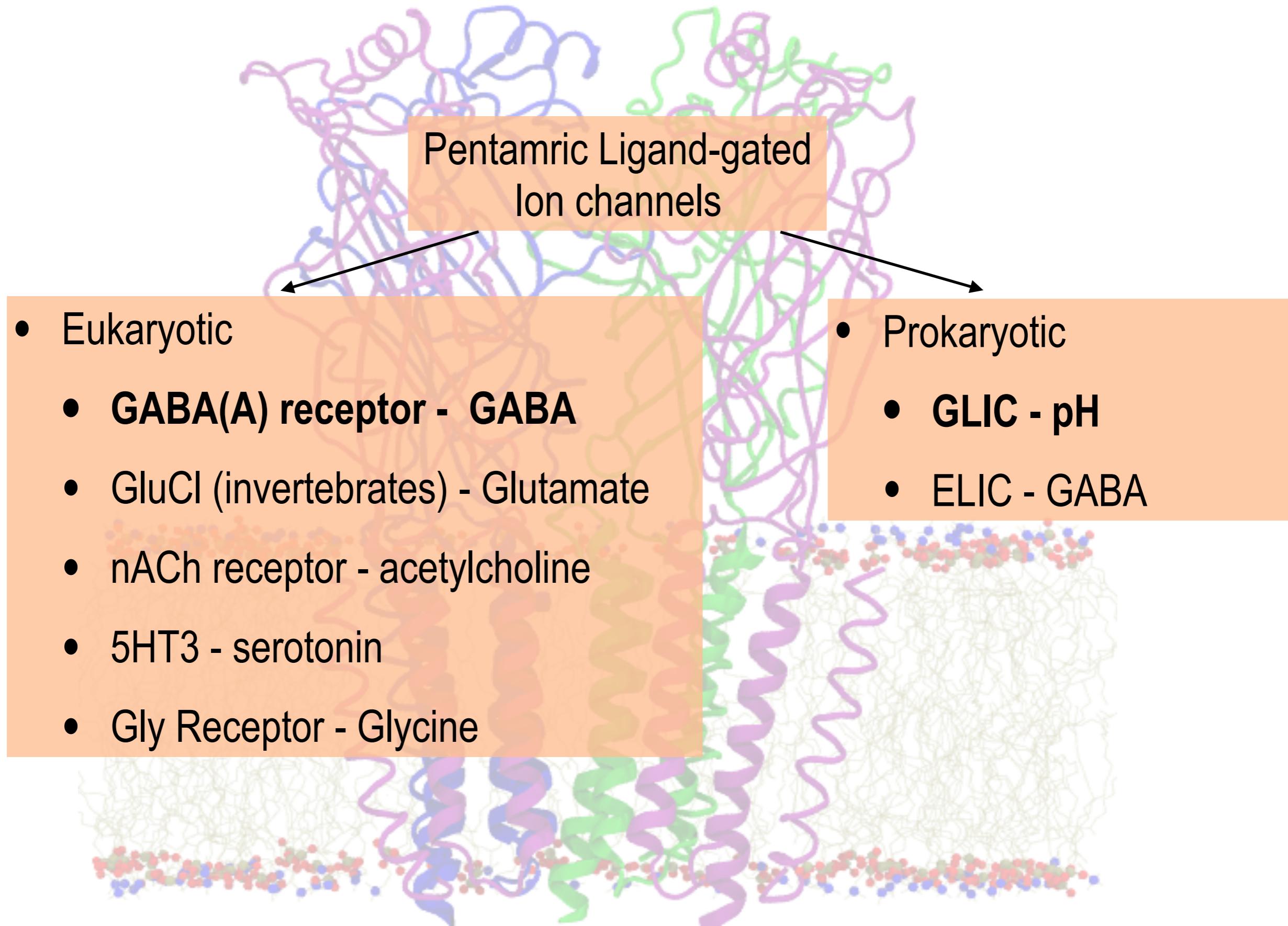
Conducts ions

Channel activity terminates -  
**DESENSITIZED State**

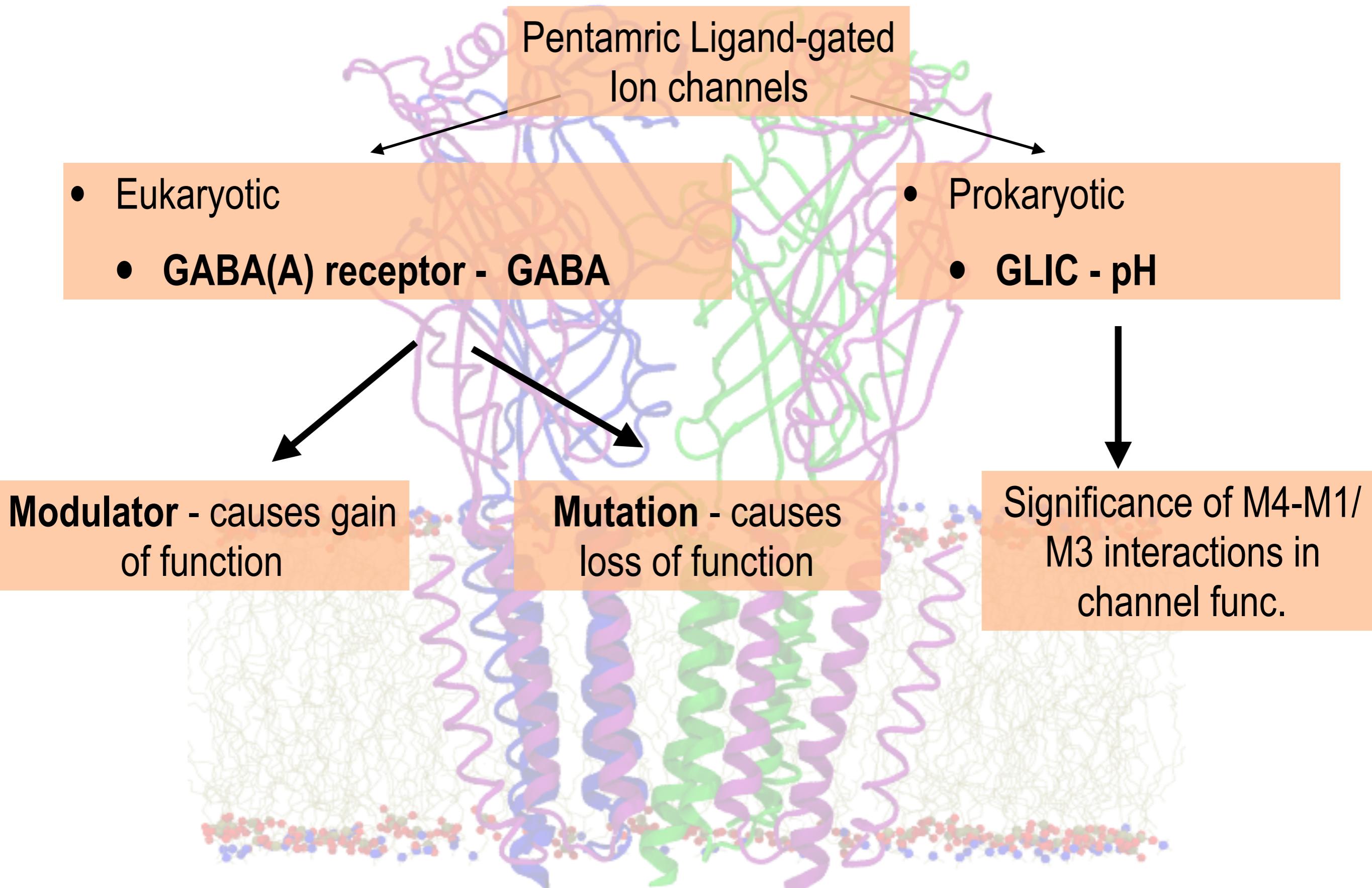
Ligand unbinds - **CLOSED State**



# Introduction



# OUTLINE



# GABA<sub>A</sub> receptor

# GABA<sub>A</sub> Receptors : Significance

**Major inhibitory neurotransmitter receptor**

- Gain of function causes
  - Anesthesia
  - amnesia
  - sedation
  - reduced anxiety
- Loss of function causes
  - Seizures
  - Anxiety
  - Insomnia

# GABA<sub>A</sub> Receptors : Significance

**Major inhibitory neurotransmitter receptor**

- Gain of function caused by
  - Anesthetics
  - Benzodiazepines
  - Barbiturates
  - Ethanol
  - Some neurosteroids
- Loss of function caused by
  - Mutation (epilepsy)
  - Thyroid hormone
  - Some neurosteroids

# GABA<sub>A</sub> Receptors : Significance

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**Modulator** - causes gain of function  
- Anesthesia

# Anesthesia

- Anesthetics induce immobilization, amnesia and unconsciousness by depressing neuronal signaling.

A world without Anesthesia!!



Amputation without anesthesia, 1775.

# Anesthesia



1846 - First public demonstration of the use of inhaled ether as a surgical anesthetic - ETHER DOME - Massachusetts General Hospital in Boston.

**~170 later... the mechanism of anesthesia is still not well understood!**

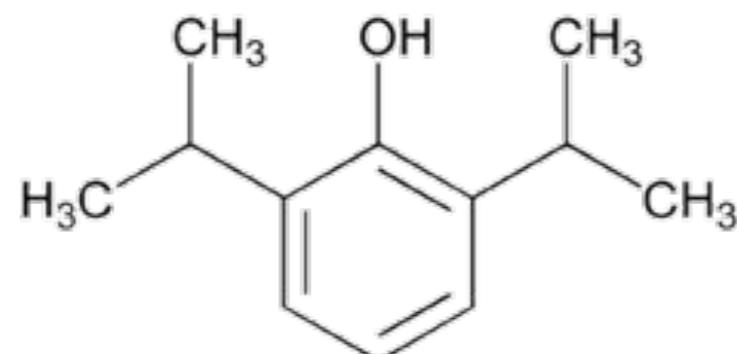
# General Anesthetics

- Primary targets of general anesthetics (GA) include ion channels, especially GABA<sub>A</sub> receptors.
- Most GAs potentiate or activate GABA<sub>A</sub> receptors at clinical concentrations ( $\mu\text{M}$ - $\text{mM}$ ).

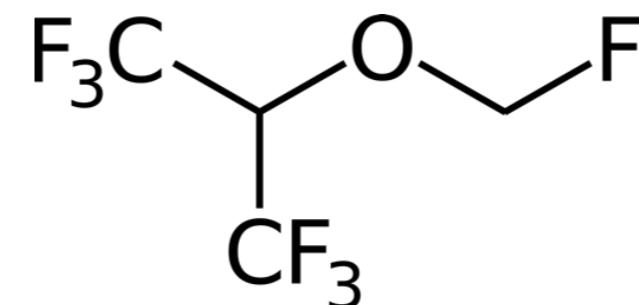
**Anesthetics classified under route of administrations:**

inhalational (IN) → Sevoflurane(SEV), Isoflurane, Desflurane,

intravenous (IV) → Propofol(PFL), Etomidate



**PFL**



**SEV**

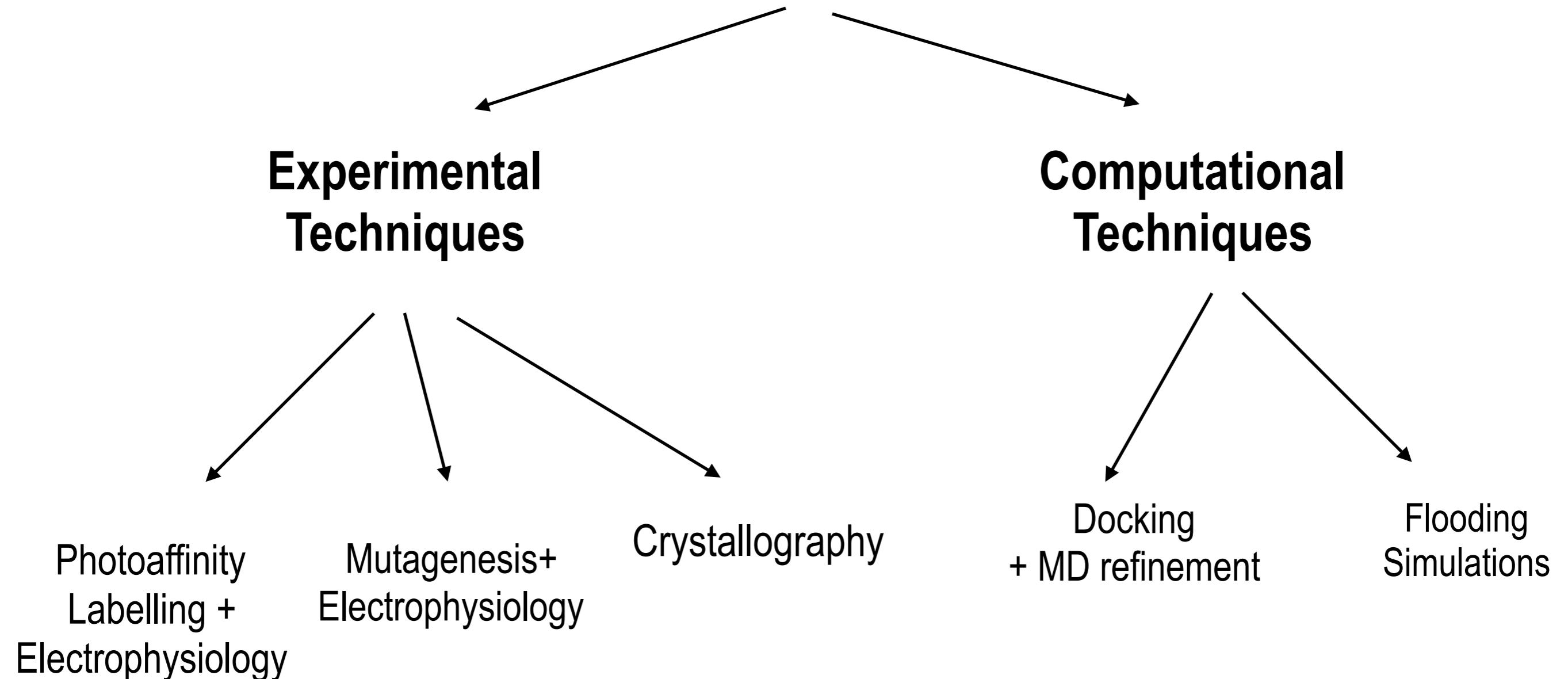
**Where do GAs bind on GABA<sub>A</sub>  
receptors?**

# General Anesthetics

Molecular mechanism is unclear:

- 1) **where** do GAs bind on GABA<sub>A</sub> receptors?
- 2) **which** sites are occupied at clinical concentrations?
- 3) **what** causes the affinity differences between different sites

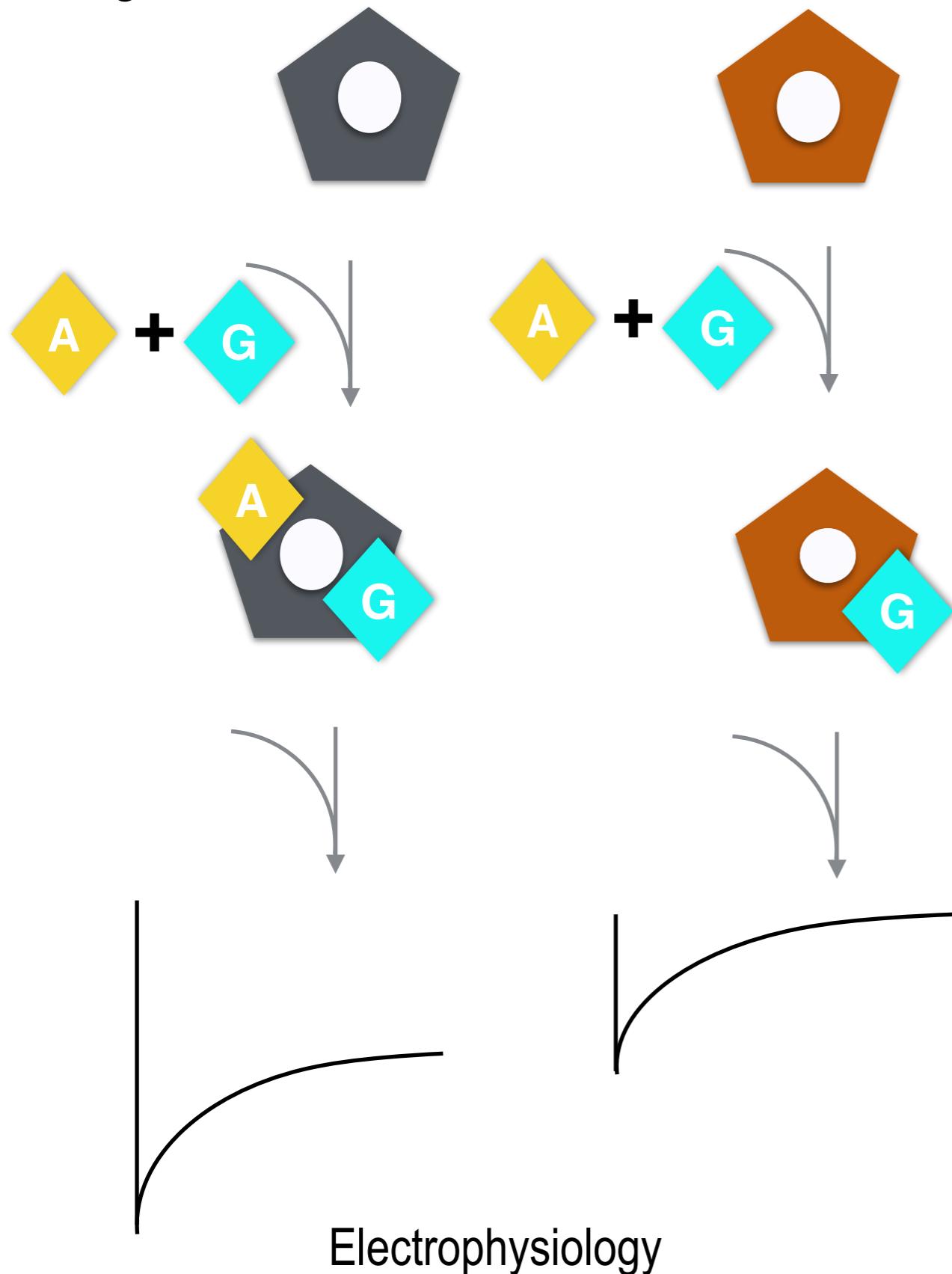
# Techniques



Our work involves utilizing **experimental insights** and **computational techniques** to identify binding sites

# Experimental Techniques

## Mutagenesis

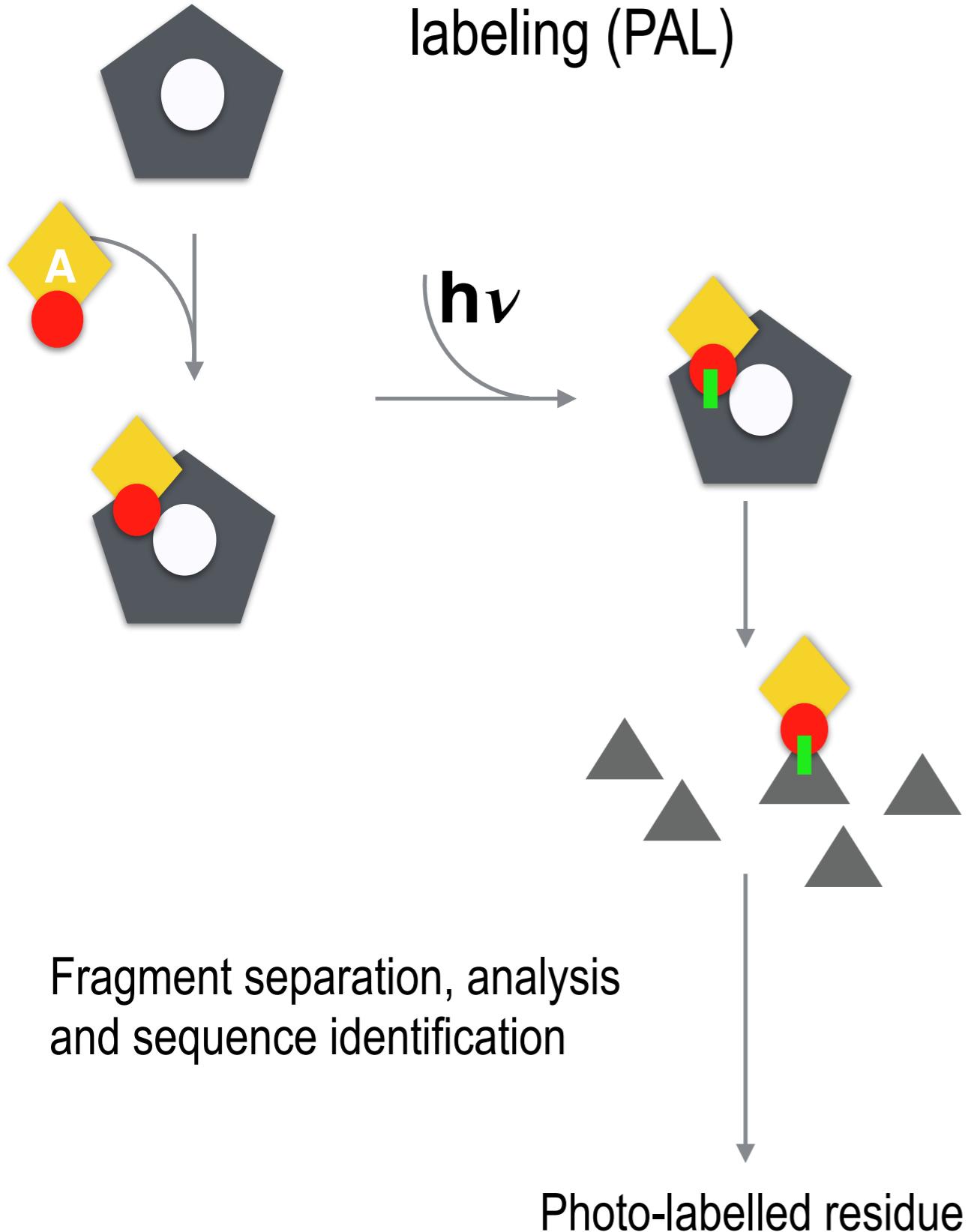


## Limitations

- undesired structural changes - loss of function.
- can mimic effects of bound anesthetic - misleading results
- In case of multiple binding sites - misleading results.

# Experimental Techniques

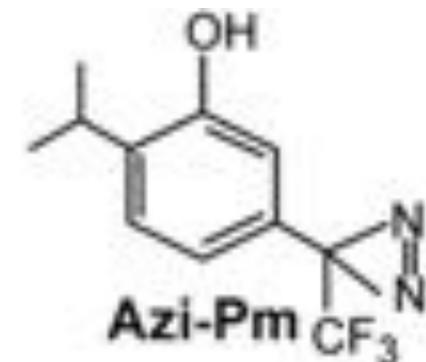
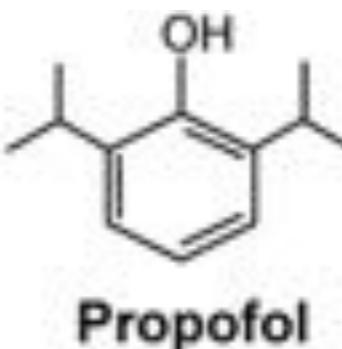
## Photoaffinity labeling (PAL)



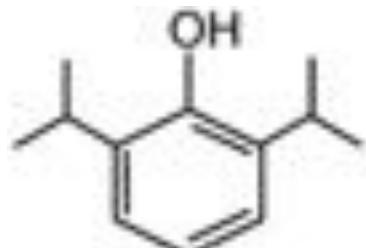
## Limitations

On comparison with the anesthetic molecule:

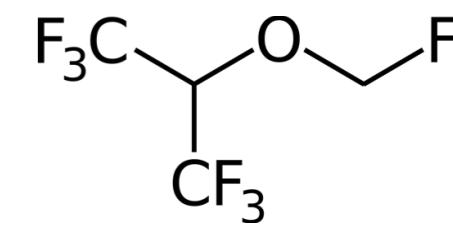
- photo-analogue can have different chemical properties.
- photo-analogue can be less potent
- show specificity to certain acids.



# Sites occupied at clinical concentrations: Experimental Insights



Propofol



SEV

PPTESEV<sup>1</sup> Mutated residues  
Photocatalyzed residue

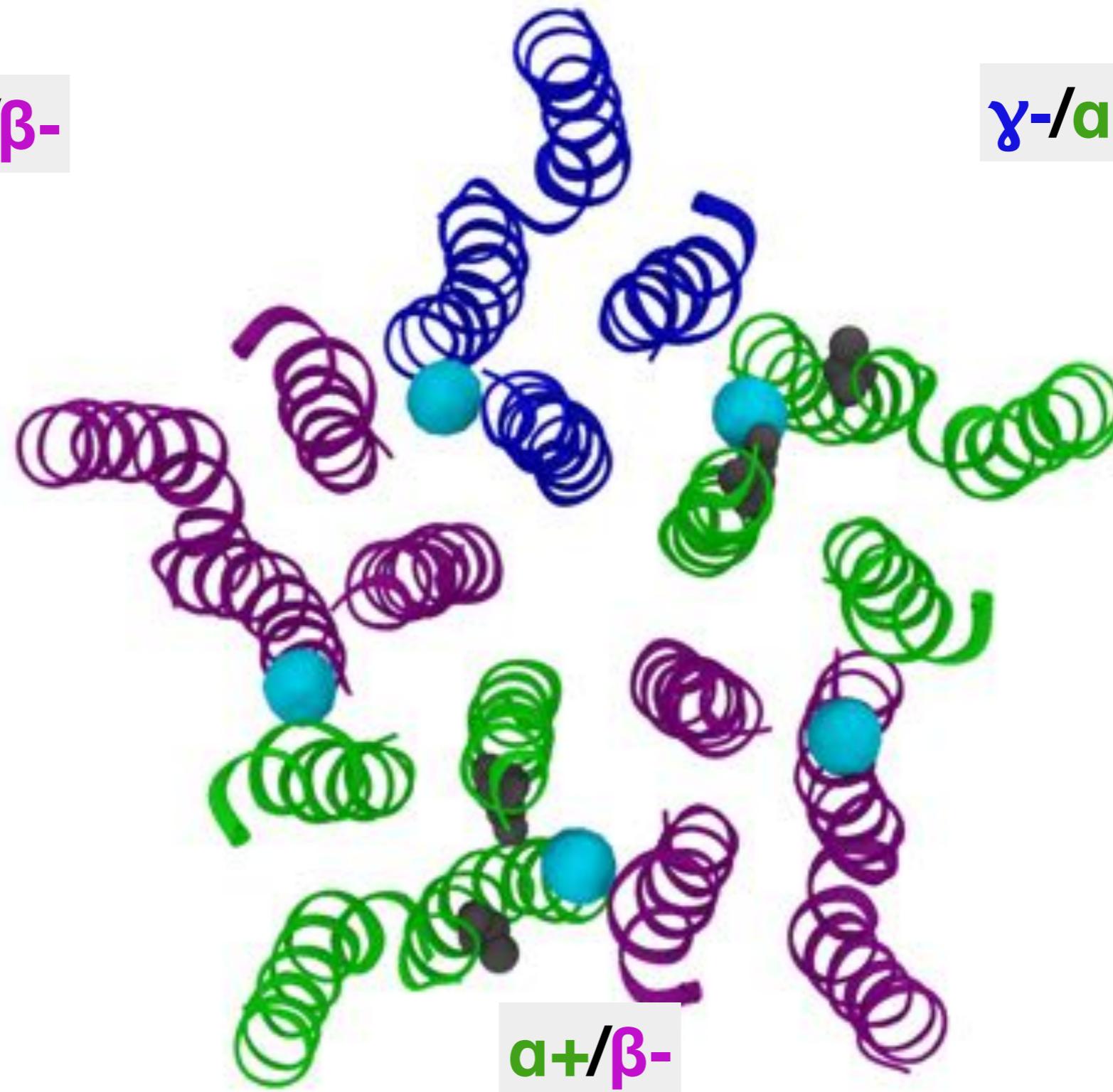
$\gamma+/ \beta-$

$\gamma-/ \alpha+$

$\beta+/ \alpha-$

$\beta+/ \alpha-$

$\alpha+/ \beta-$



**Which sites are occupied at clinical concentrations?**

# Binding sites identified through computational study

## General anesthetics

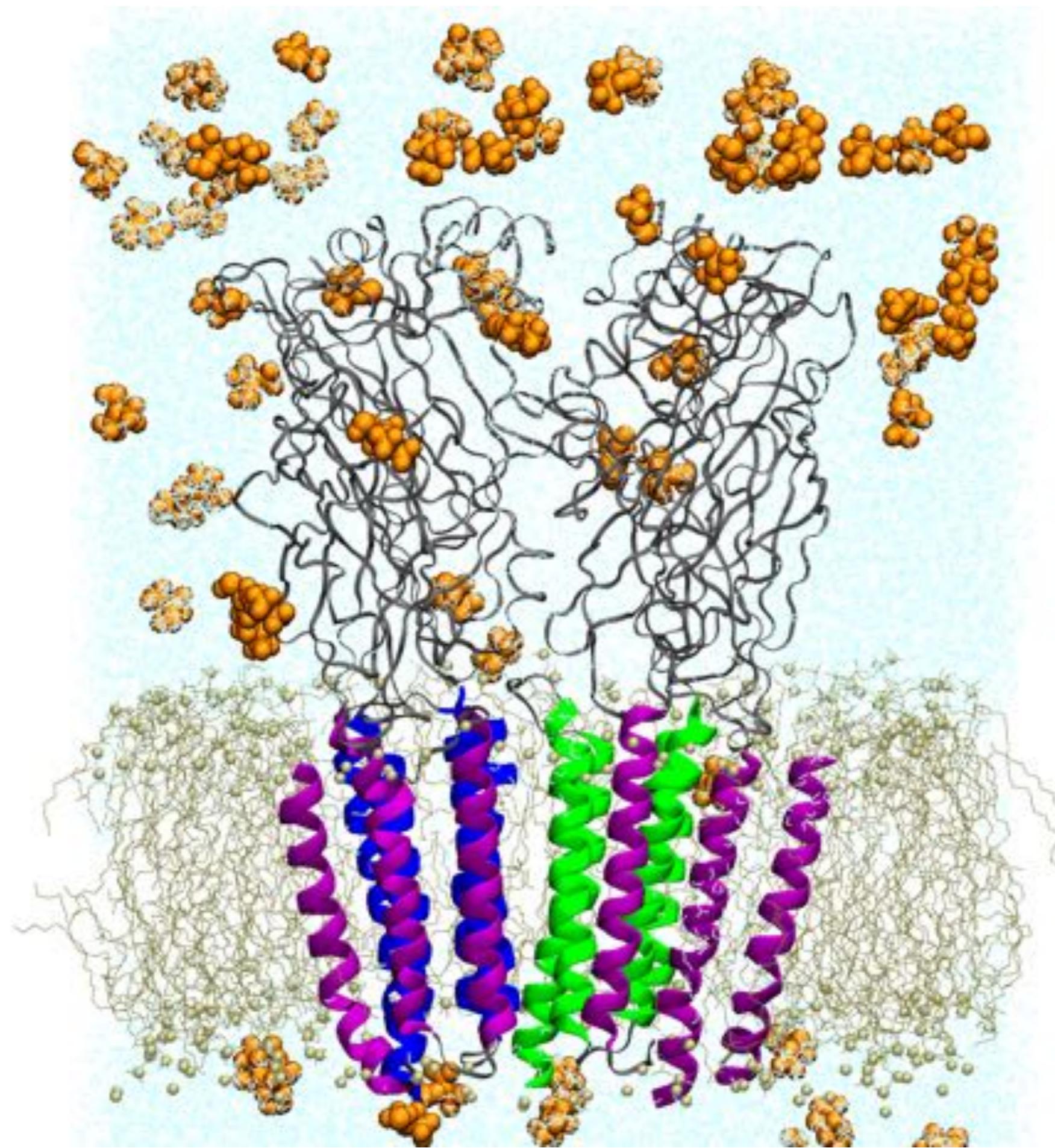
Propofol

Sevoflurane

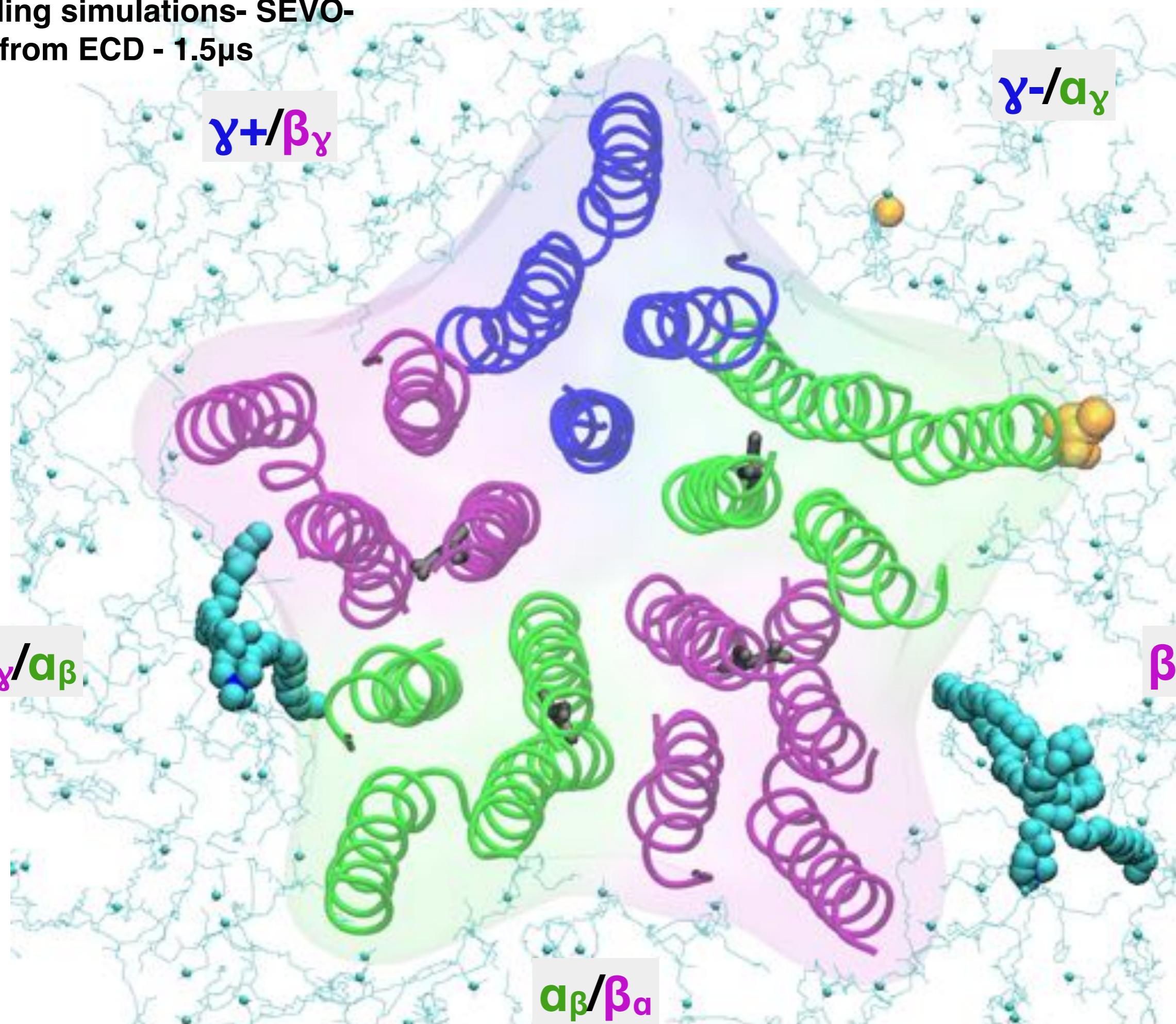
- **Docking** — generation of reasonably favorable binding modes in a particular region of a protein,
- **Traditional MD** simulation after docking - to refine protein-anesthetic complex
- **Flooding simulations** -
  - Unbiased simulations;
  - Let's anesthetic explore the protein system that mimics in-vivo environment.

**Alchemical Free Energy Perturbation** : Measure affinity by calculating free energy difference between two alternate states.

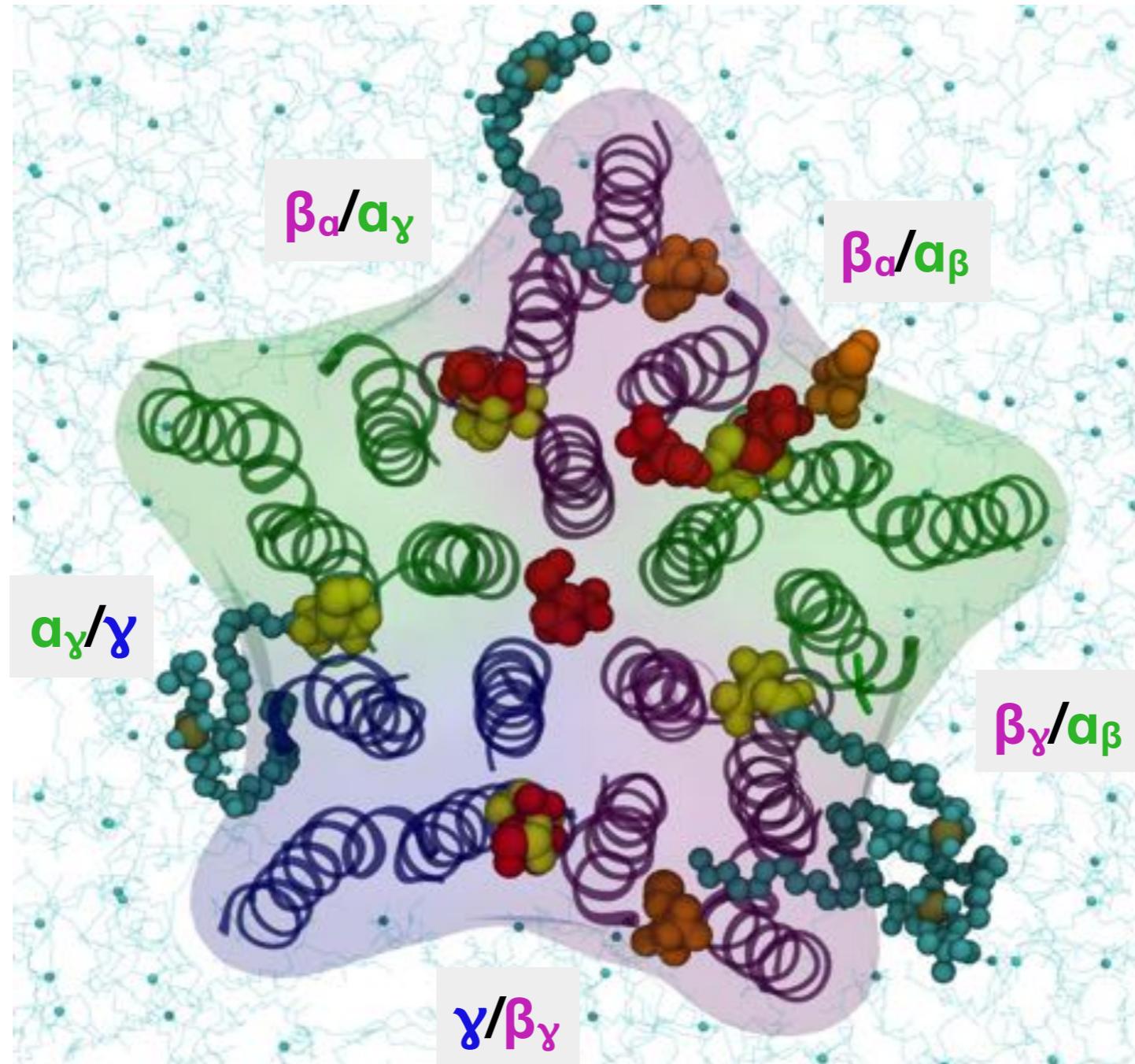
# SEVOFLURANE Flooding simulations- 1.5μs



# Flooding simulations- SEVO- View from ECD - 1.5μs

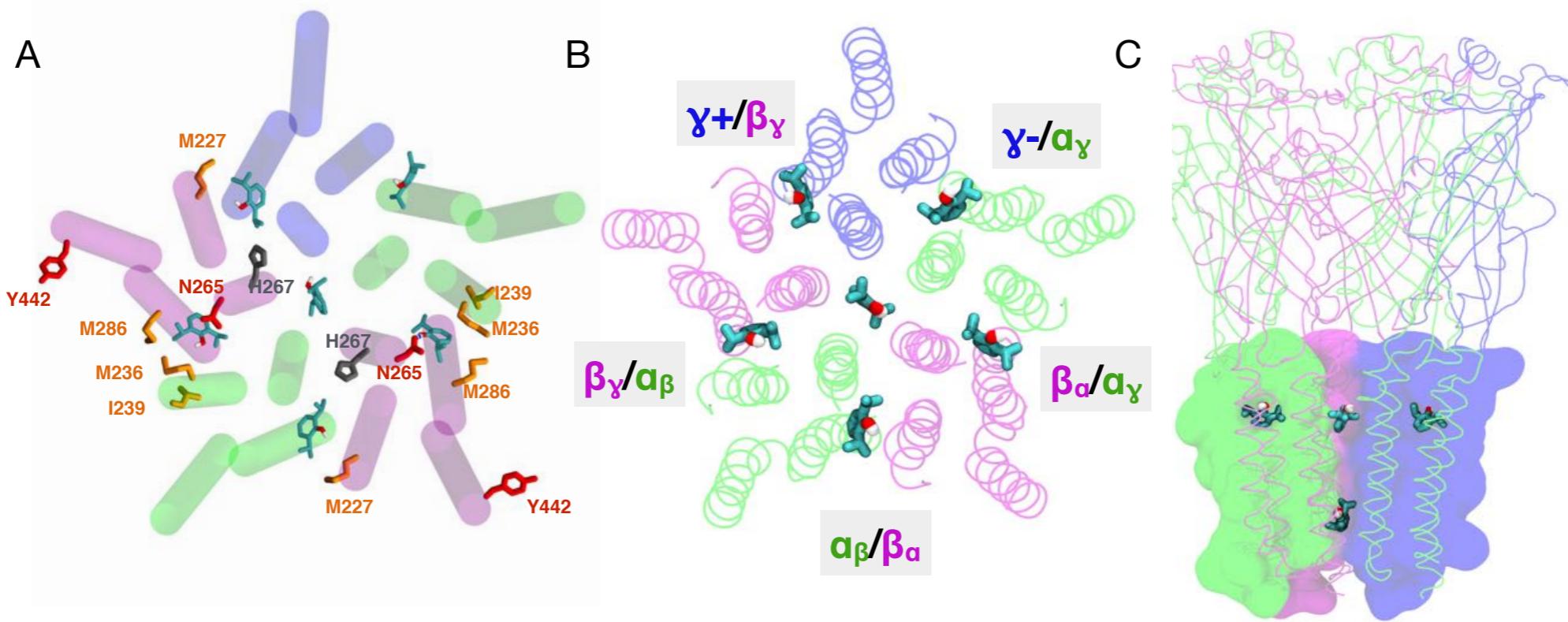


# SEV binding sites identified through flooding simulations

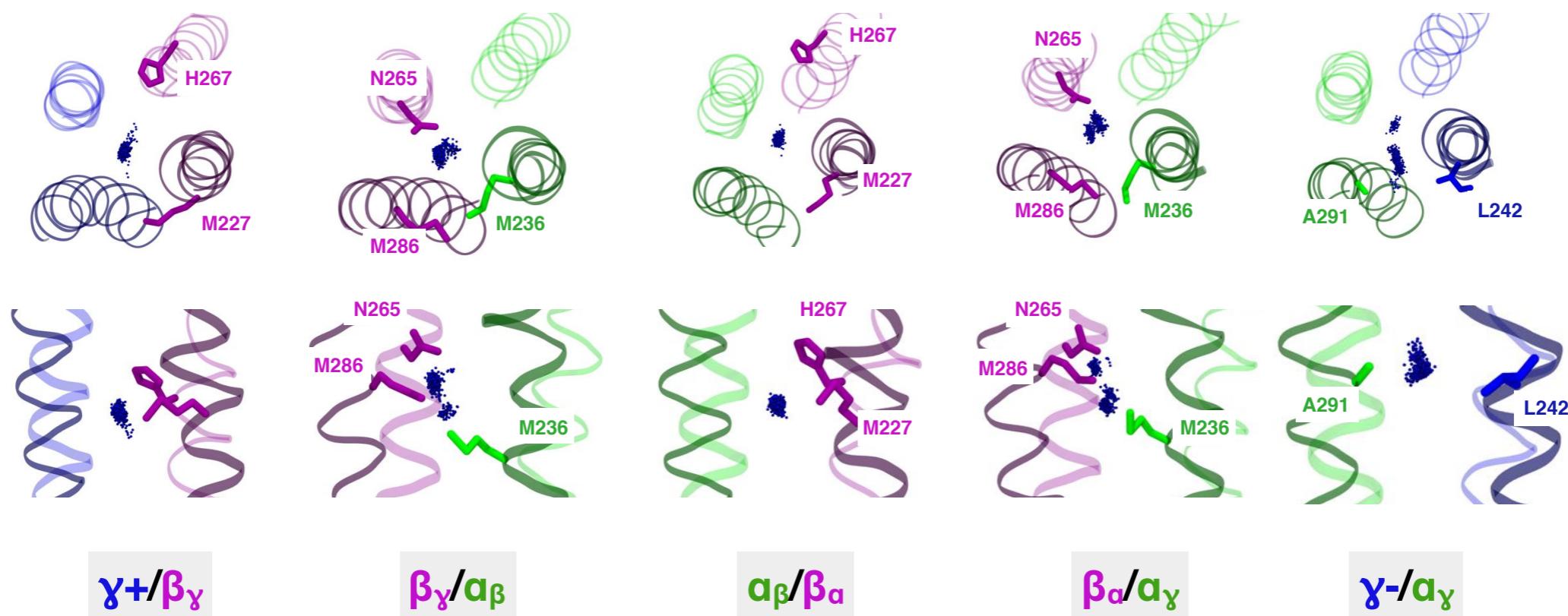


- Intersubunit sites -  $\alpha_\beta/\beta_\alpha$ ,  $\gamma/\beta_\gamma$ ,  $\beta_\alpha/\alpha_\gamma$
- $\beta$  - Intra-subunit sites - occupied by SEV
- Lipid interactions -  $\beta$  subunit and  $\gamma/\alpha_\gamma$  site.
- Multiple occupancy at  $\alpha_\beta/\beta_\alpha$  site

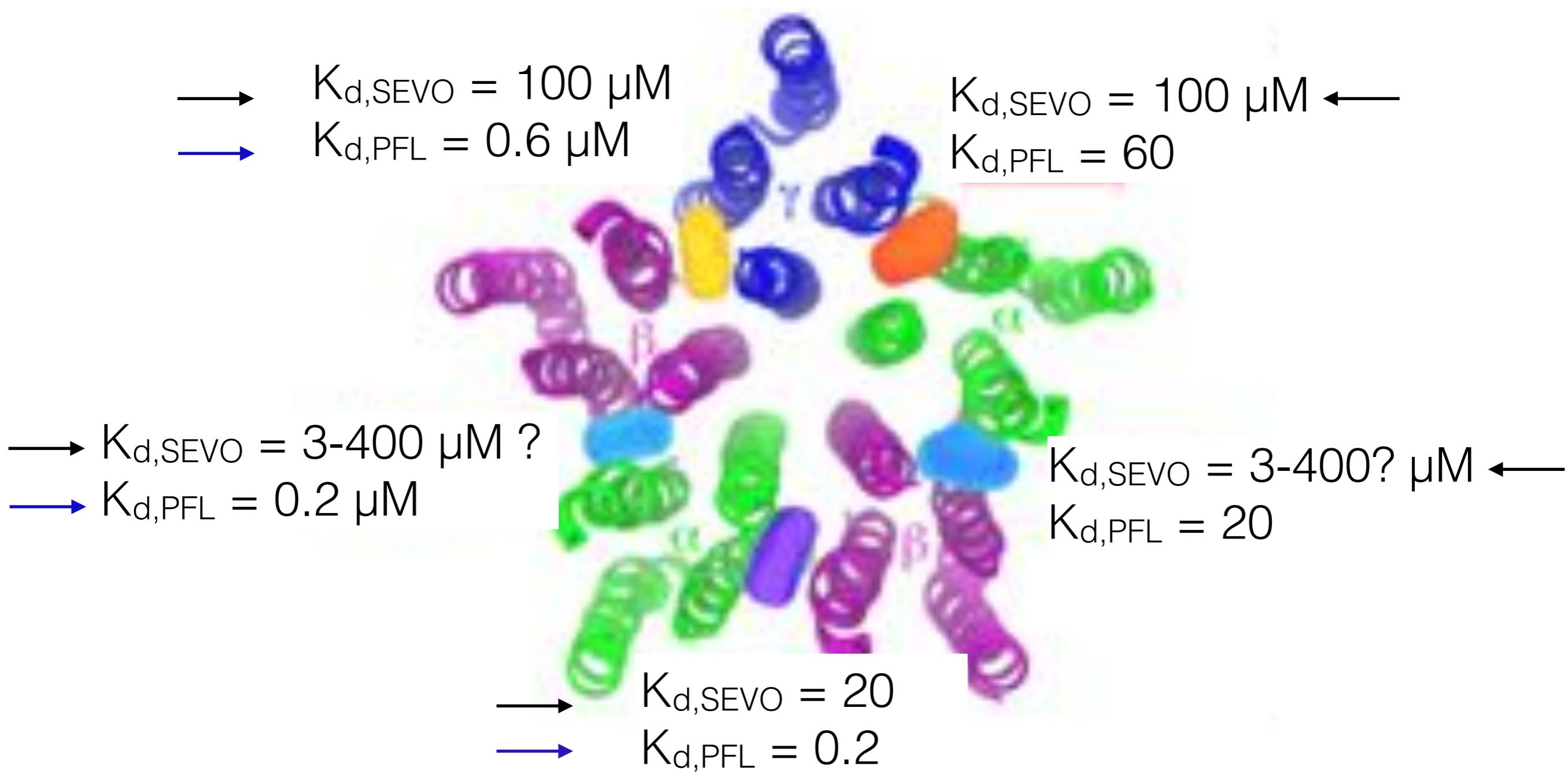
# PFL binding sites identified through docking



# PFL stabilized in binding sites through traditional MD



# Sites occupied at clinical concentrations: Computational Results



**SEV EC<sub>50</sub> = 300-1000 μM**

Adora et al. Brit. J. Pharm., 1995.

**PFL EC<sub>50</sub> = 0.5-5 μM**

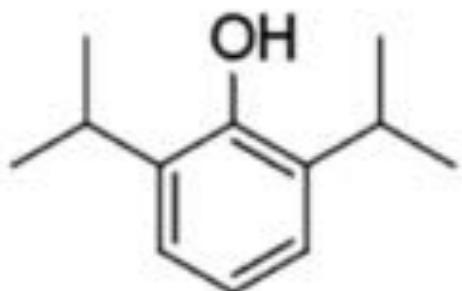
[Jie Wu et al. Brit. J. Pharm., 1996.]

- Knowledge regarding the specific interactions within the binding site is required to understand subunit specificity.

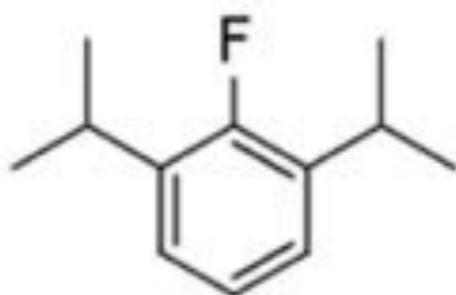
**What causes the affinity differences  
between different sites?**

# Microscopic origins of affinity differences: Experimental Insights

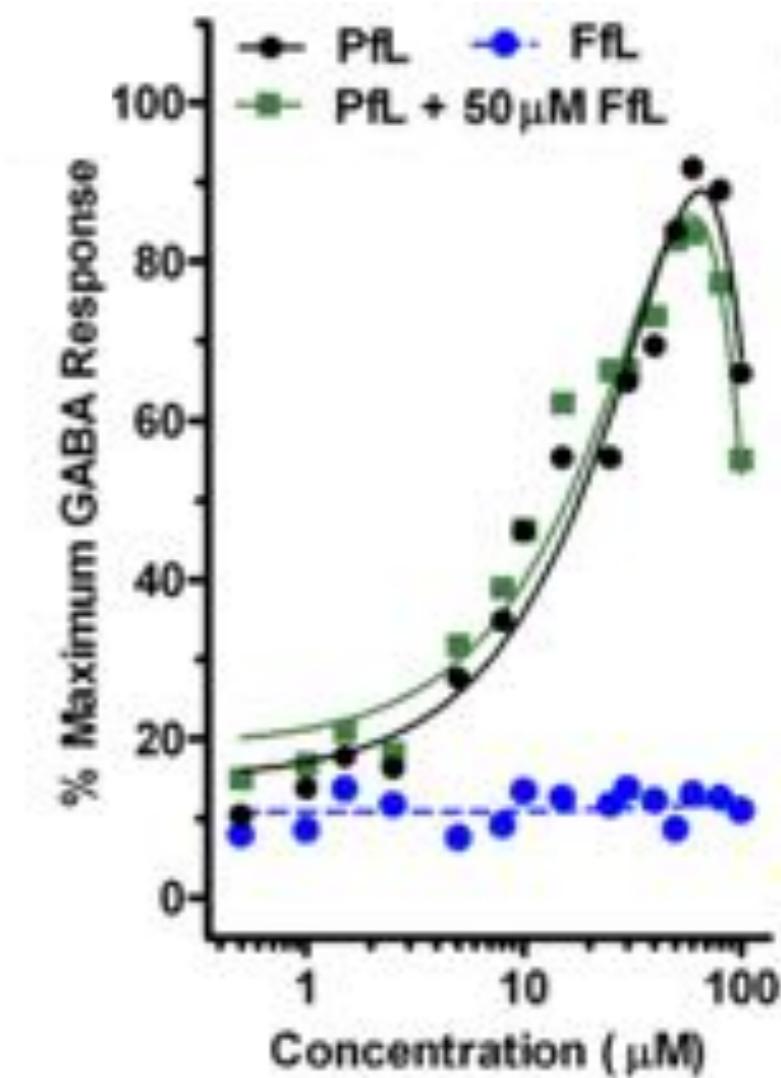
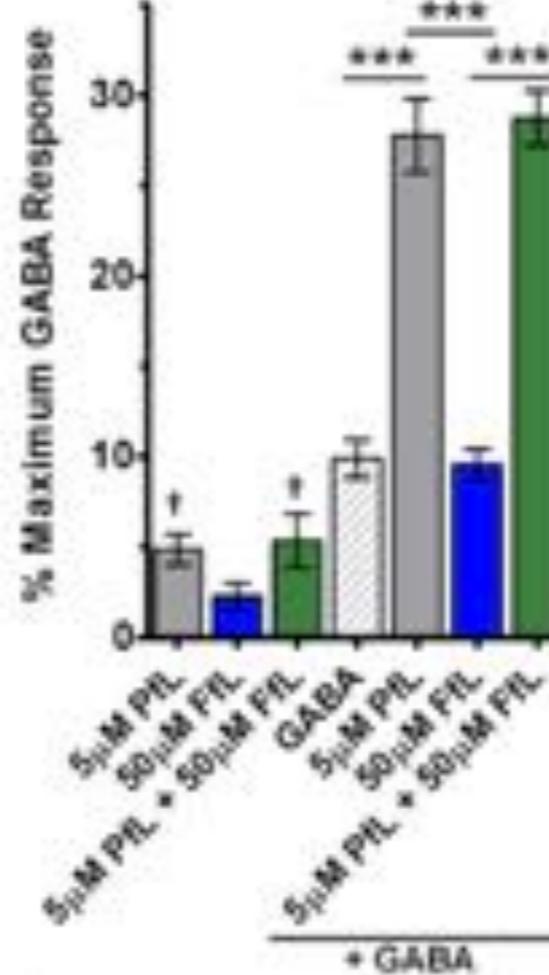
## Hydrogen Bonding



Propofol



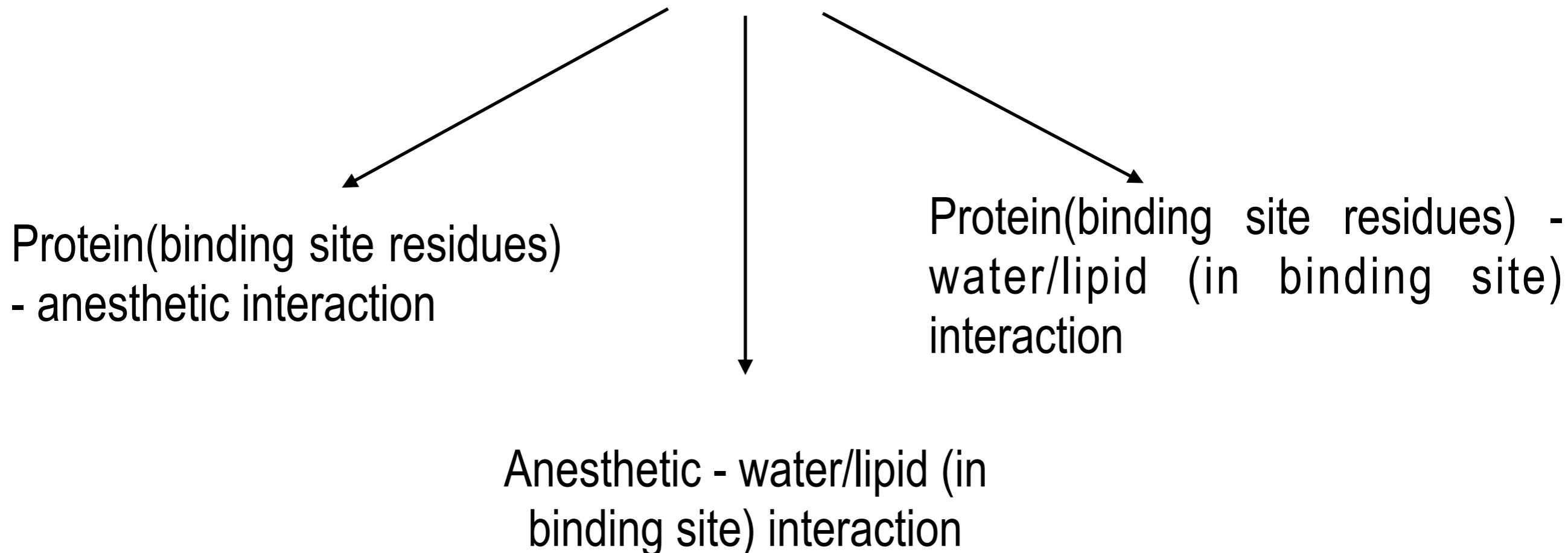
Fropofol



- Fropofol did not affect PFL positive modulation or direct activation of GABA(A) receptor currents.
- H-bond - critical molecular recognition feature for PFL binding sites.

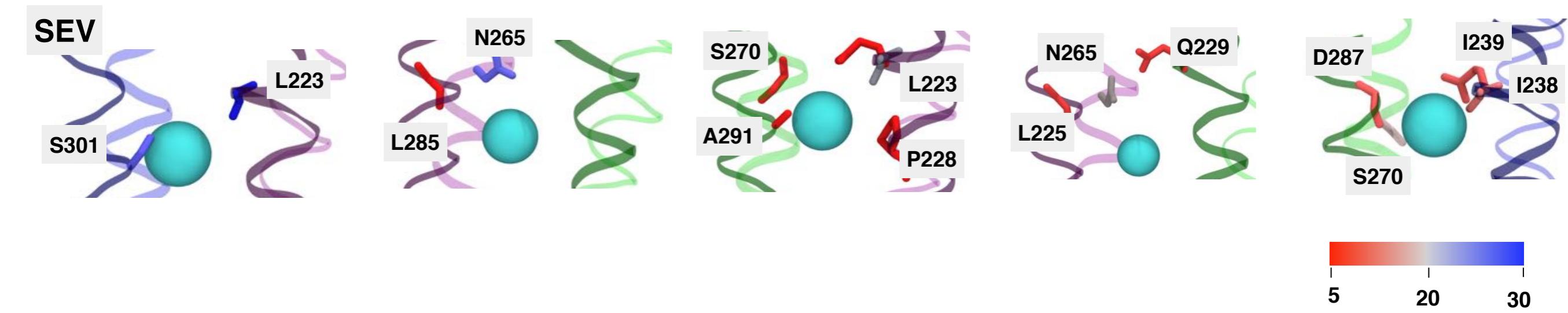
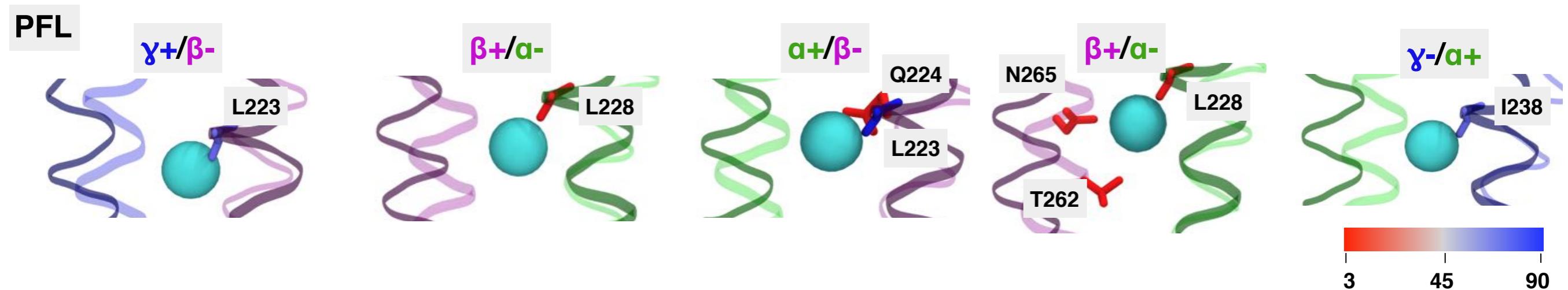
# Microscopic origins of affinity differences: Computational Results

Types of interactions that causes affinity differences



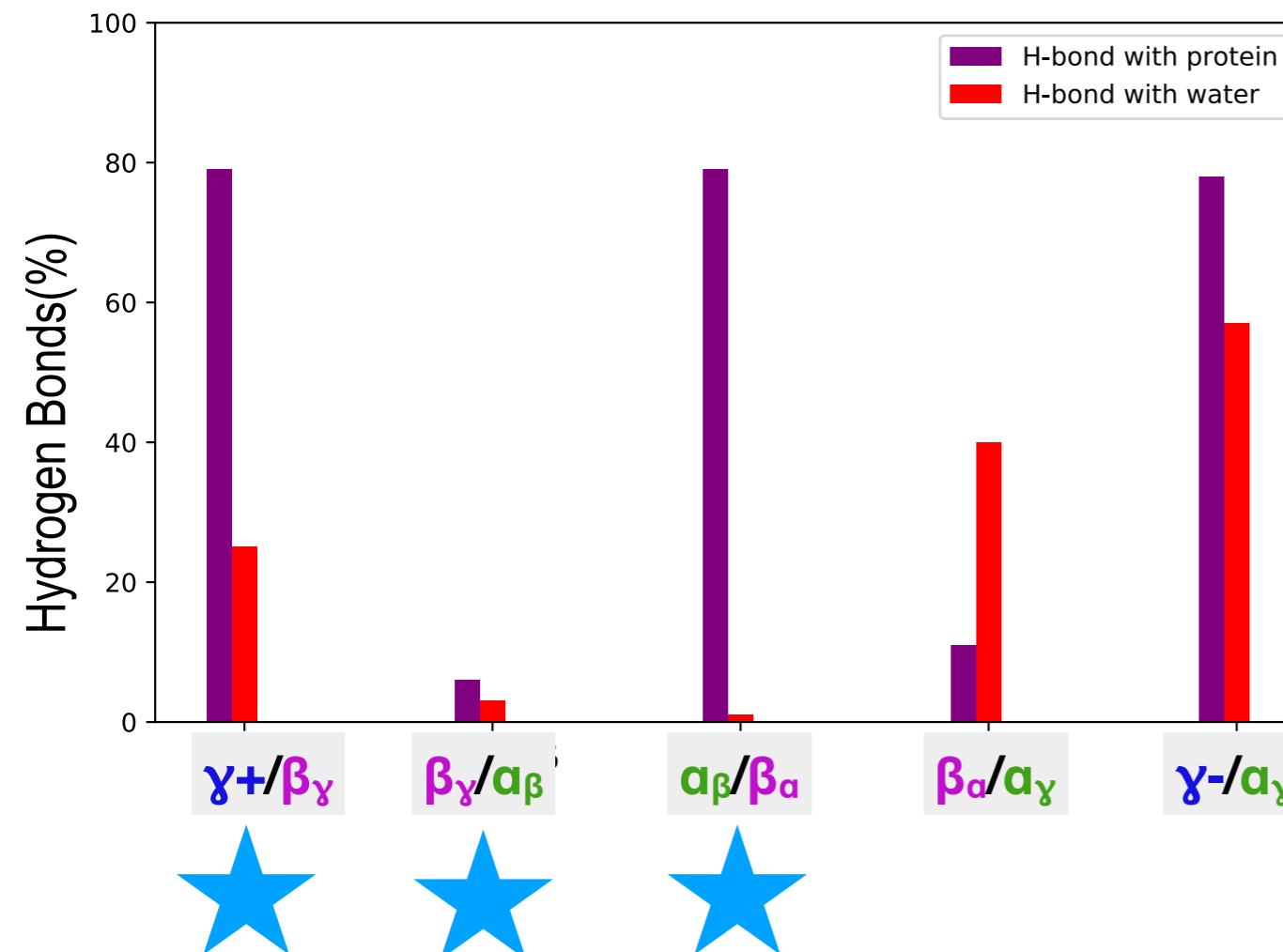
# Protein(binding site residues) - anesthetic interaction

## Hydrogen Bonding

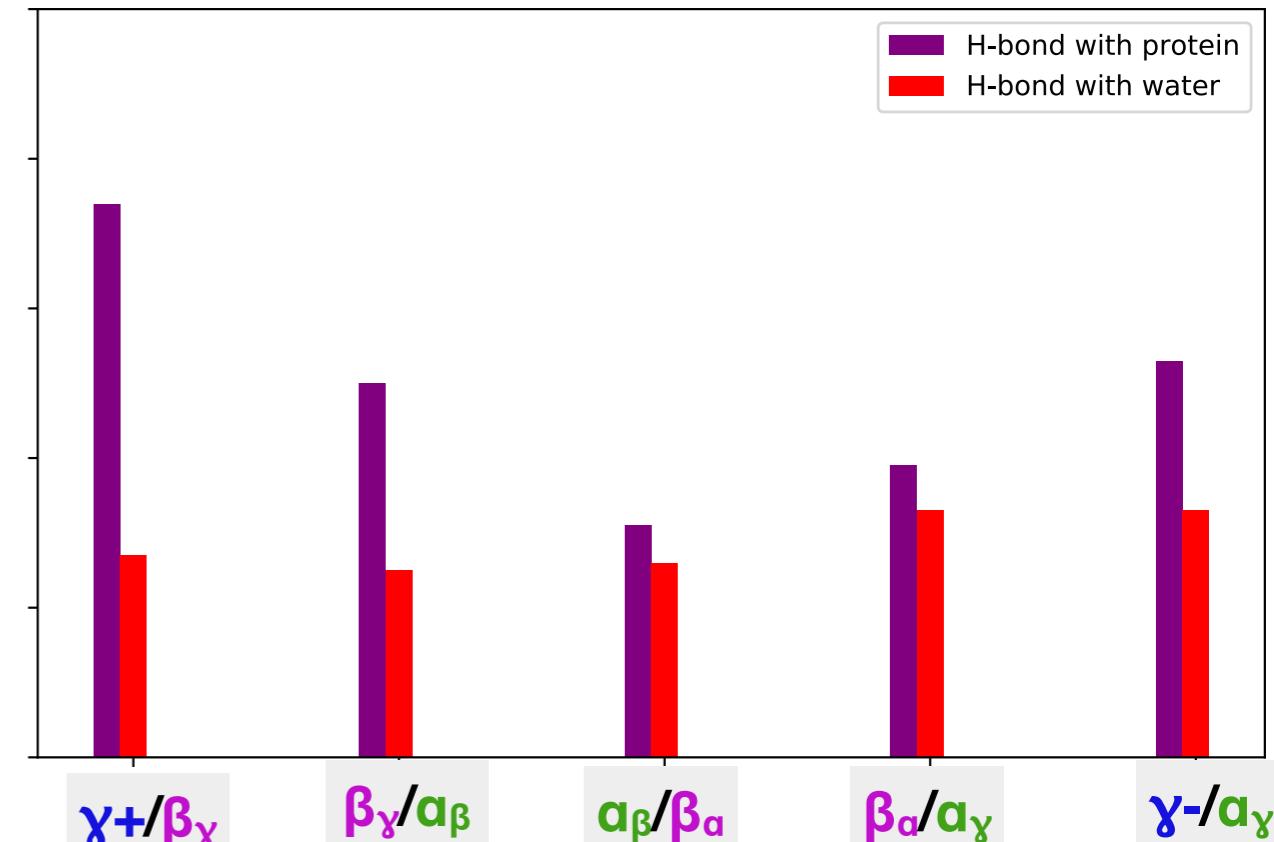


# Ligand - water/lipid (in binding site) interaction

PFL

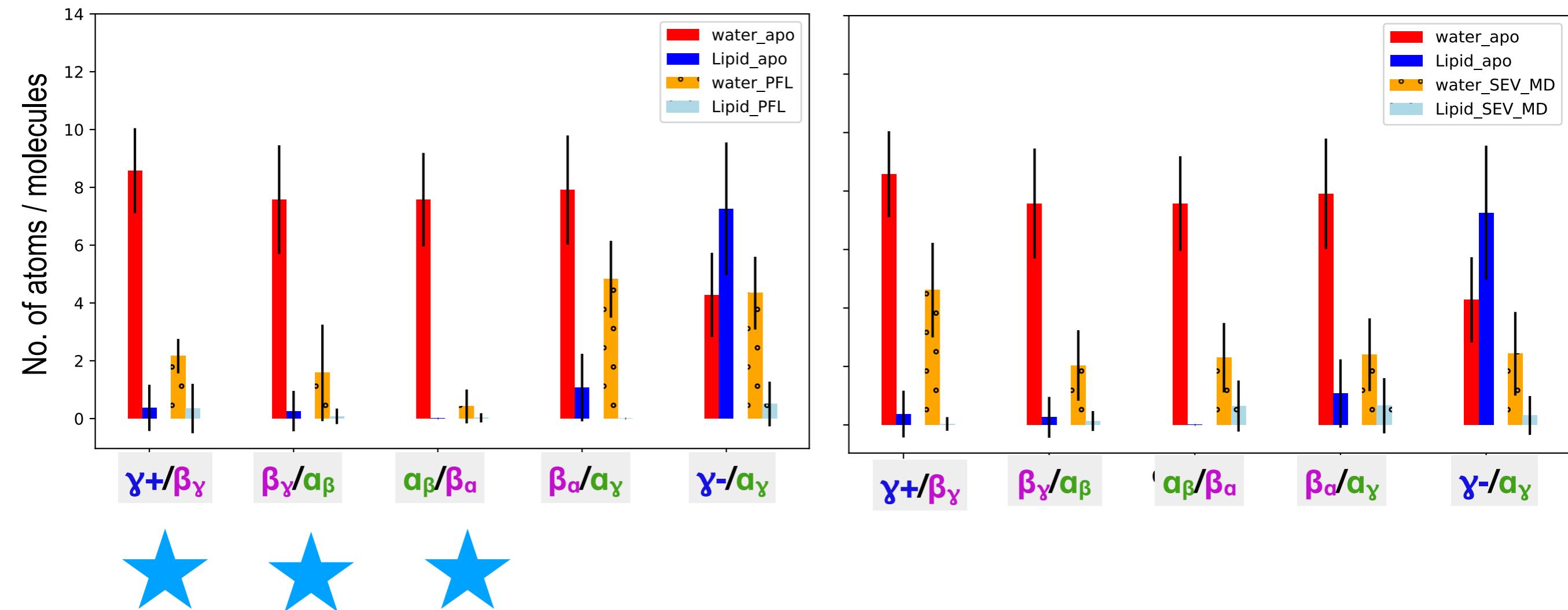


SEV



PFL - High affinity sites show lesser/no water interaction with propofol

# Protein(binding site residues) - water/lipid (in binding site) interaction



- PFL - 0-2 water molecules remain in high affinity sites
- SEV - 2-4 molecules remain in all sites.
- High lipid interference in  $\gamma^-/\alpha_\gamma$  site can cause its low affinity to both SEV and PFL.

# Conclusions

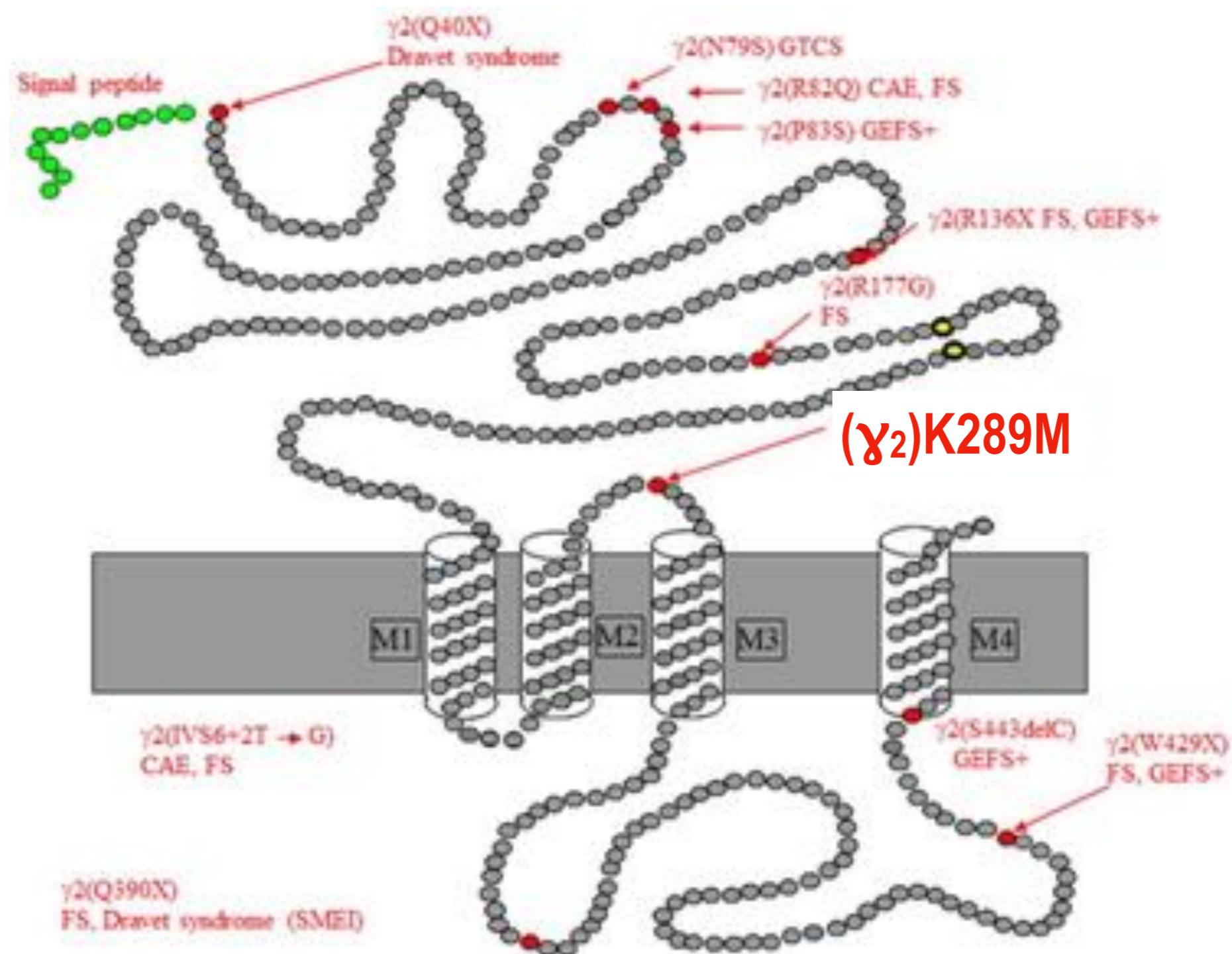
- Consistent with experimental results : GAs bind to upper TMD domain
- Sites occupied at clinical concentrations:
  - **3 intersubunit sites** being occupied by **PFL** at clinical conc.
  - **3-5 intersubunit sites** being occupied by **SEV** at clinical conc.
- Microscopic origins of affinity differences :
  - Anesthetic has to displace whatever molecules are already in the site, and affinity of those molecules (water or lipid) is also essential for determining affinity.
  - **PFL** — Contrary to experimental evidence, consistent hydrogen bonding is not required for PFL to have higher affinity to a site.
  - **SEV** — site specificity reflects what you have to displace, sevoflurane doesn't displace much -> less specificity.

# GABA<sub>A</sub> Receptors : Significance

**Major inhibitory neurotransmitter receptor**

- Gain of function caused by
  - Anesthetics
  - Benzodiazepines
  - Barbiturates
  - Ethanol
  - Some neurosteroids
- Loss of function caused by
  - Mutation (epilepsy) - K289M
  - Thyroid hormone
  - Some neurosteroids

**Mutation - causes loss of function**  
**- K289M**

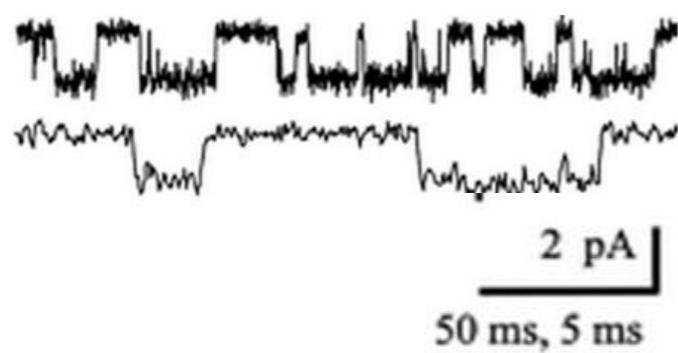


Schematic representation of a GABA<sub>A</sub> receptor subunit topology, showing the location of epilepsy mutations

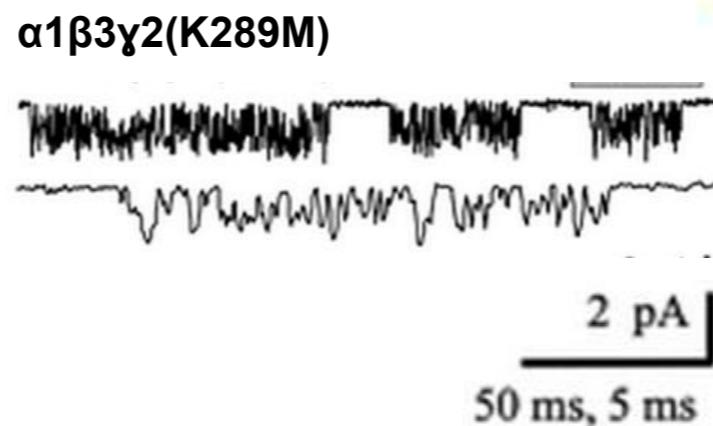
# Experimental Insights: K289M

- Present in human population < 0.4%
- Location : Between the ECD-TMD interface
- Phenotype : Febrile (Fever induced) seizures
- Molecular effect on  $\text{GABA}_A$  single-channel conductance: Mean open time four-fold shorted than WT.

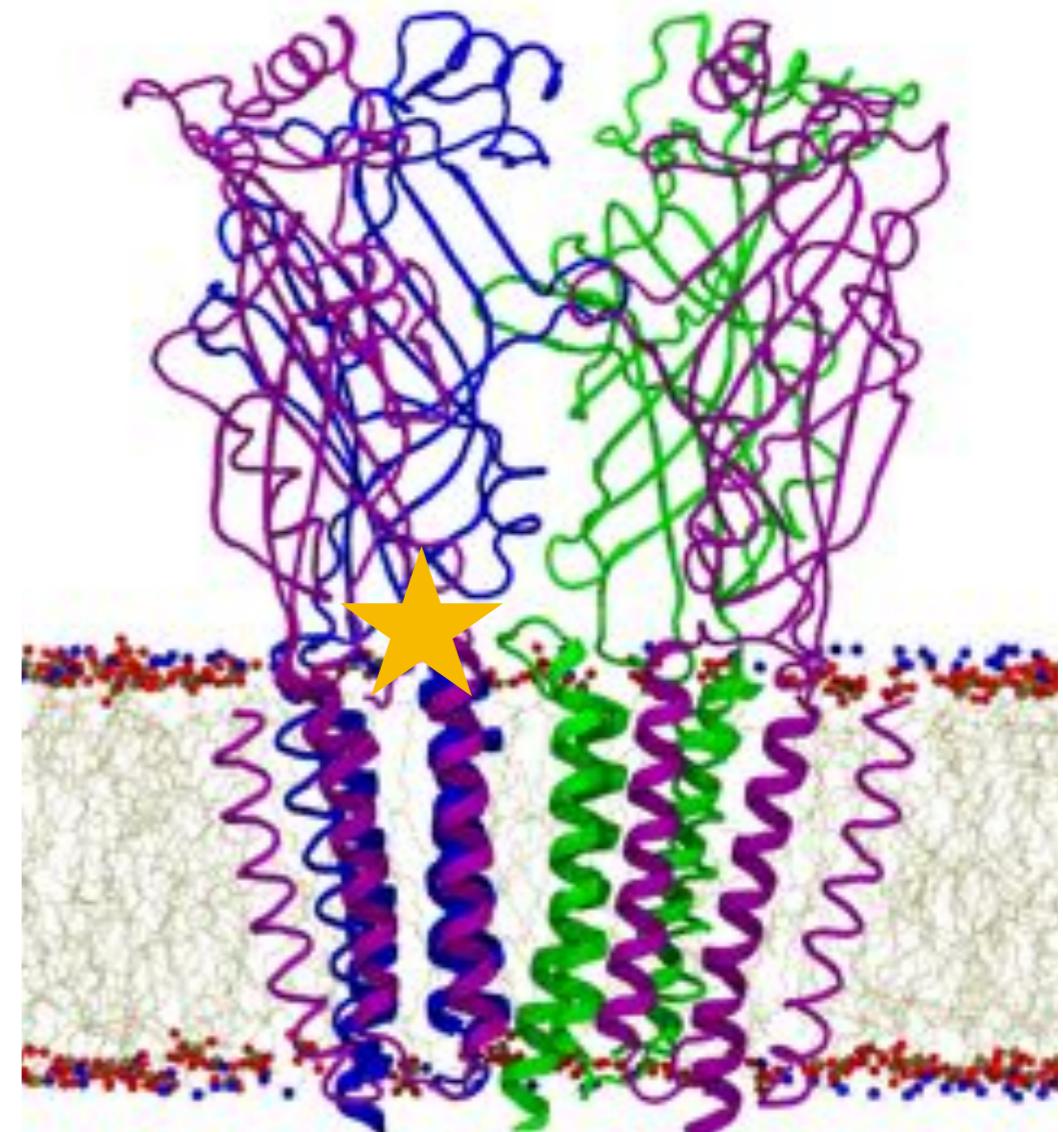
$\alpha 1\beta 3\gamma 2$



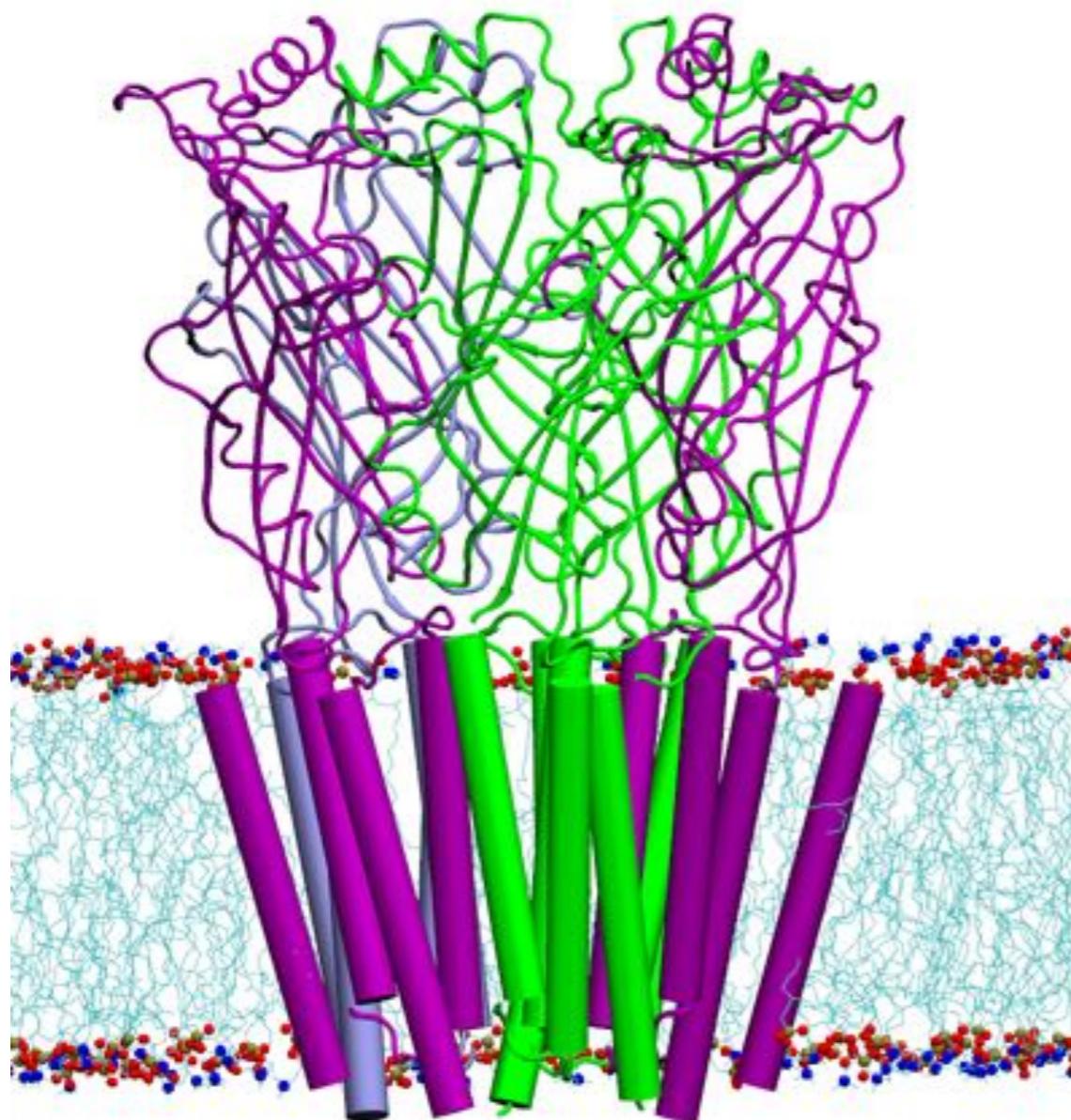
$\alpha 1\beta 3\gamma 2(\text{K289M})$



(Left) Single-channel analysis at -75 mV in the presence of 1 mM GABA from  $\alpha 1\beta 3\gamma 2\text{L}$ ; (Right) Single-channel conductance of mutant K289M. [Bianchi and Song, 2002]



# GABA(A) Receptor : Structure

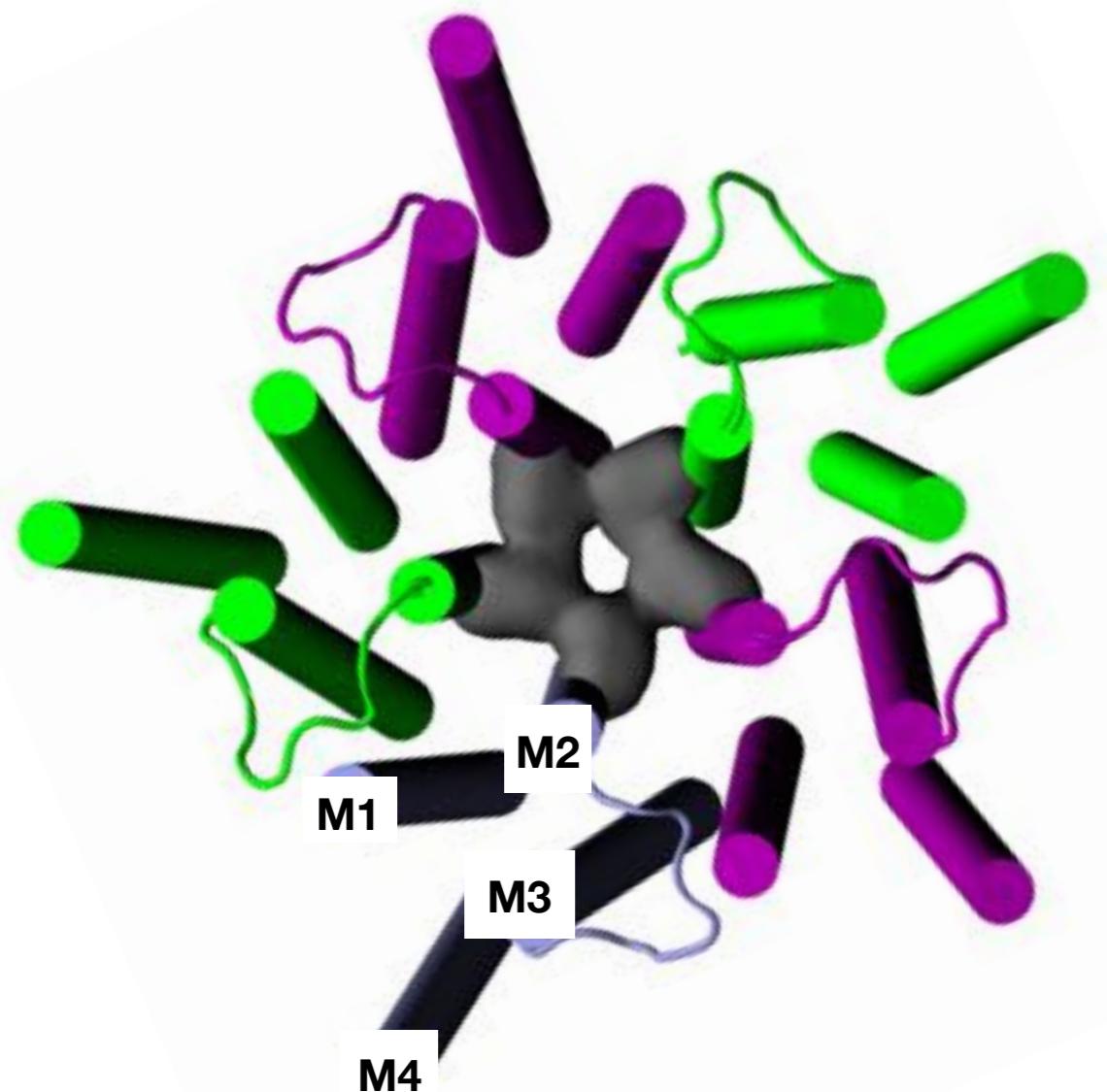


View of TMD from ECD

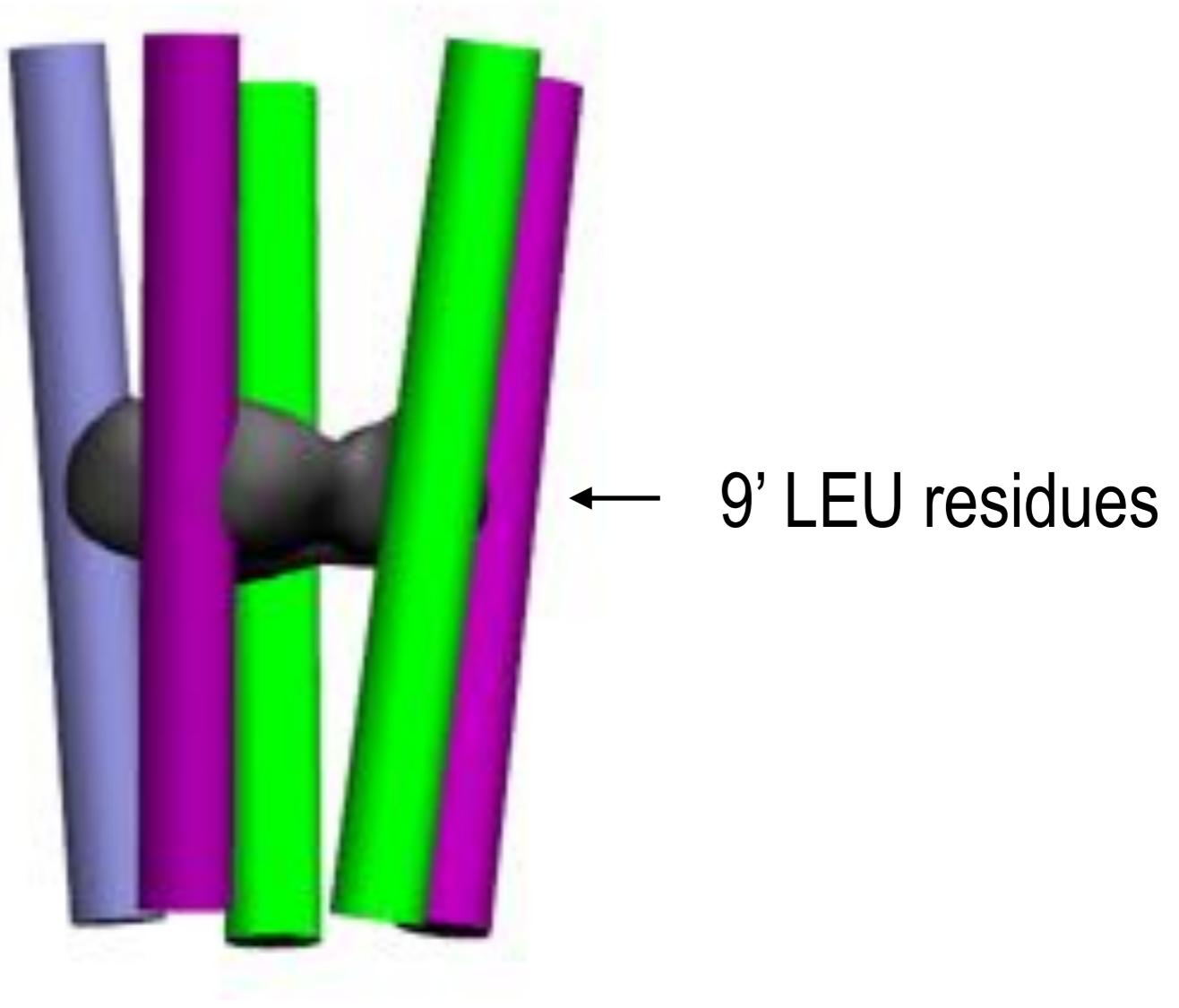


Minimum pore constriction

# GABA(A) Receptor : Structure



Minimum pore constriction

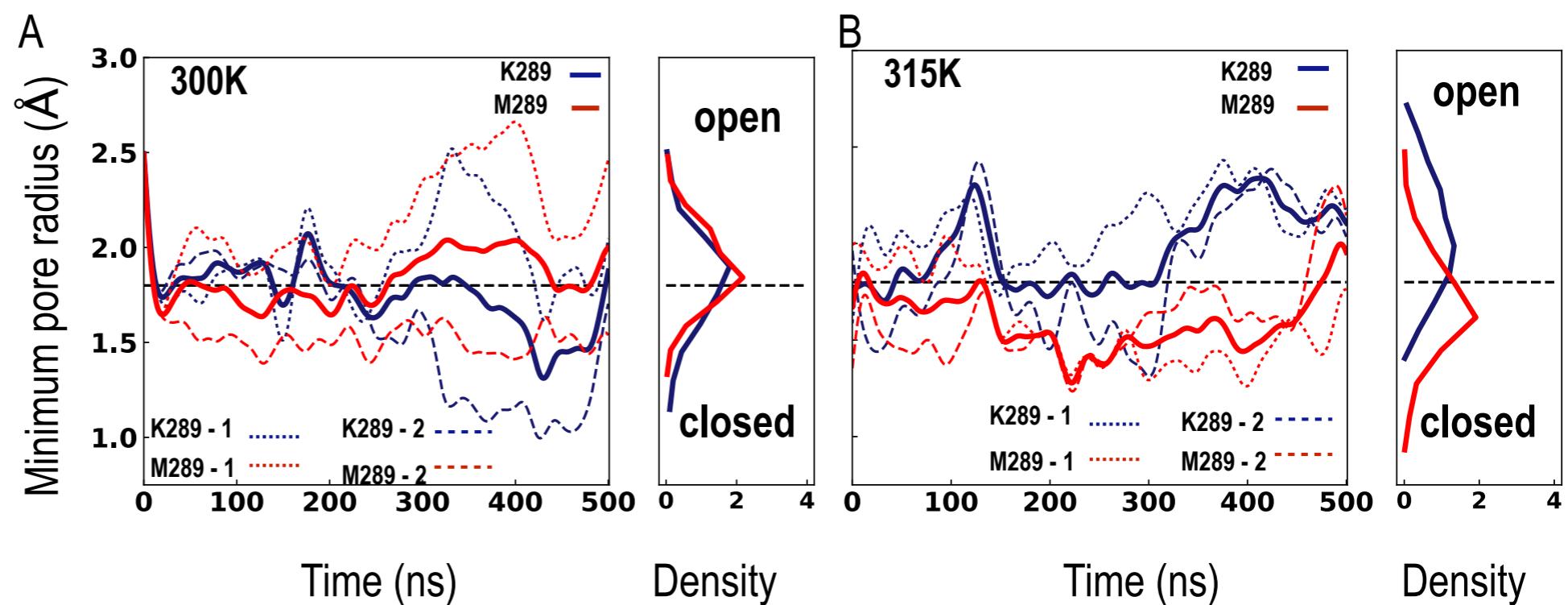
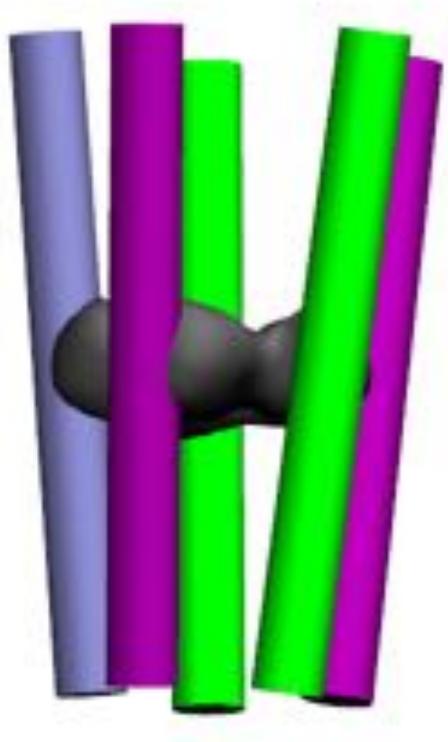


M2 helices

← 9' LEU residues

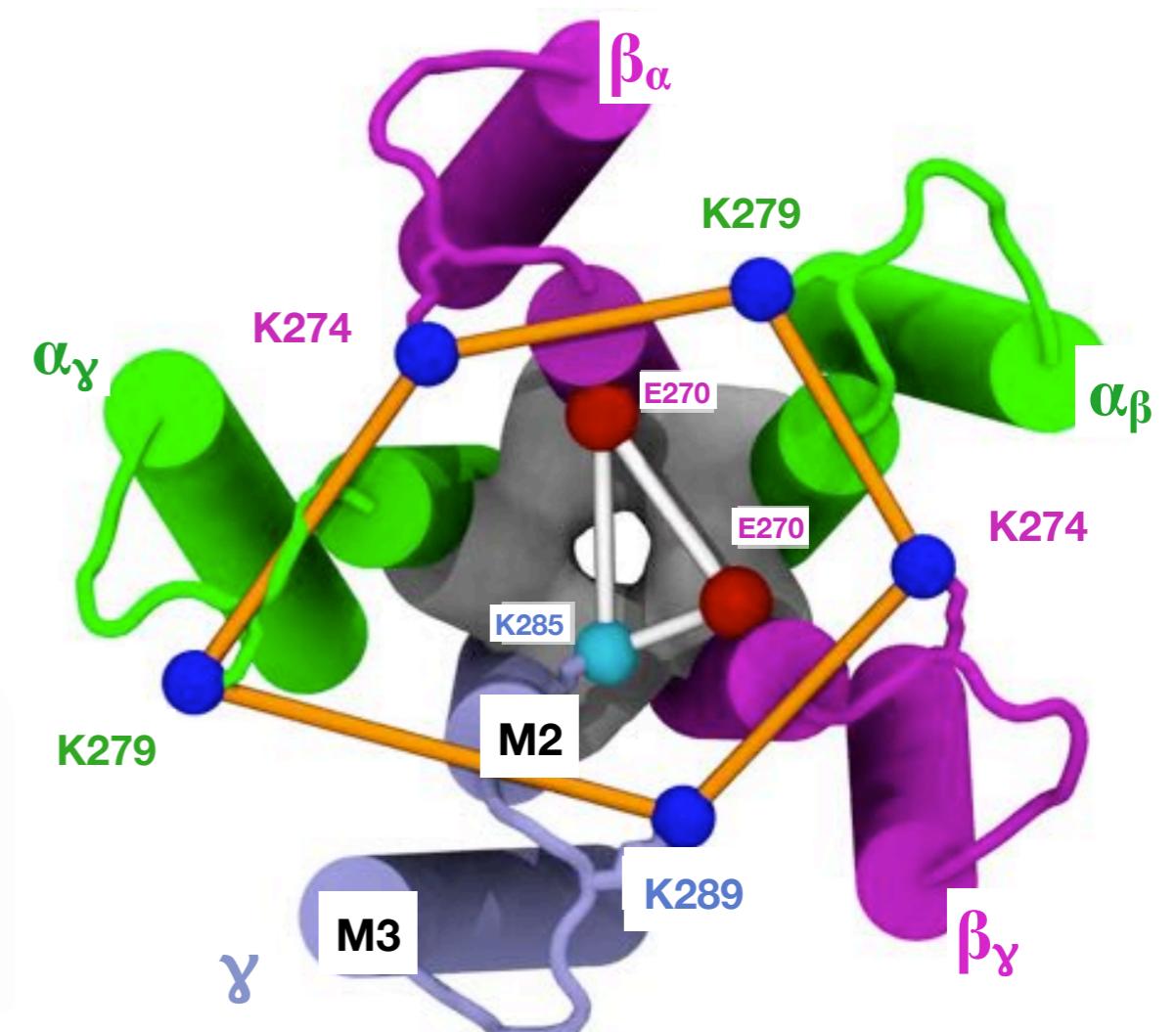
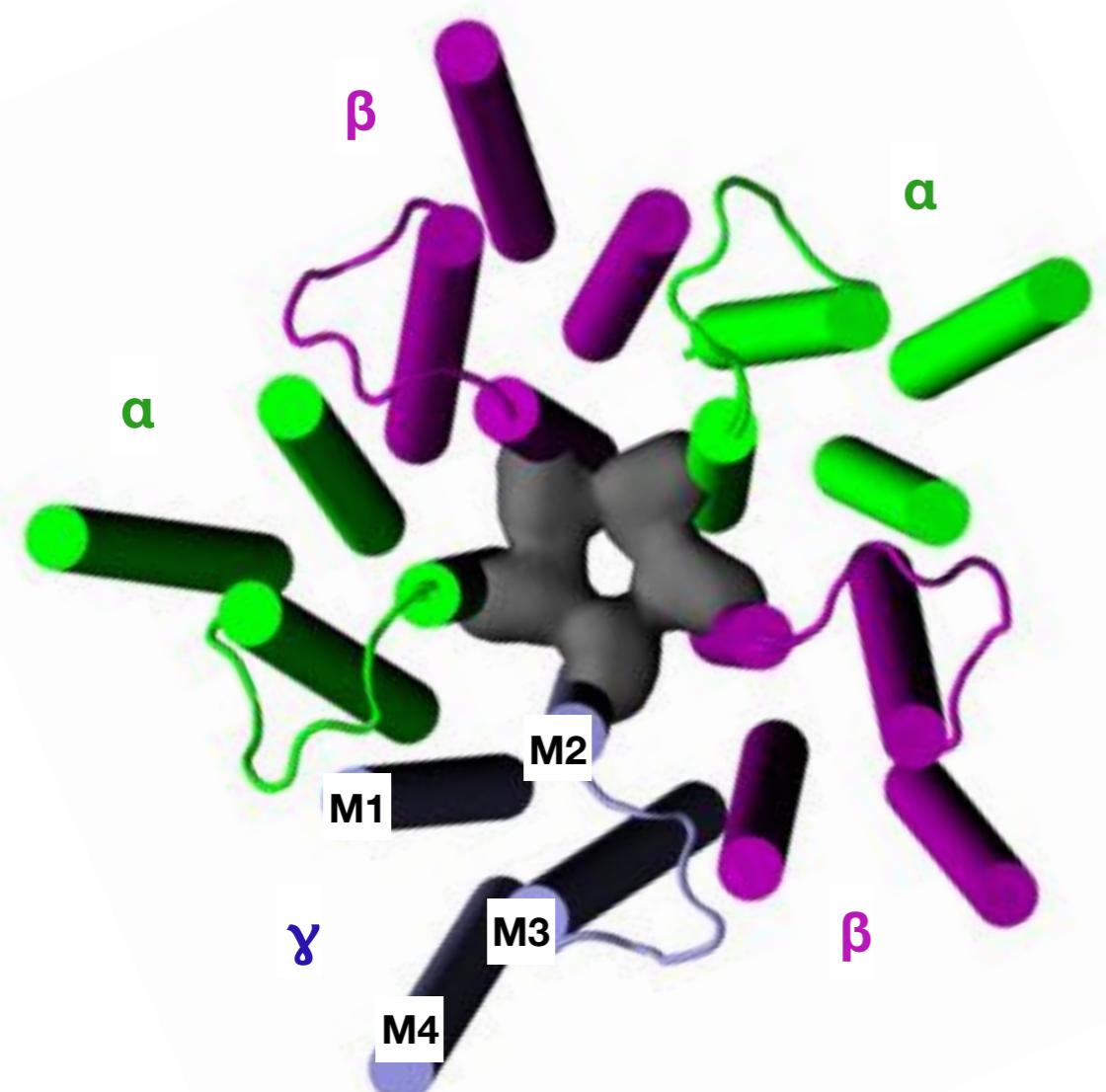
# Computational Results

## 1. Channel with K289M closes at 315K



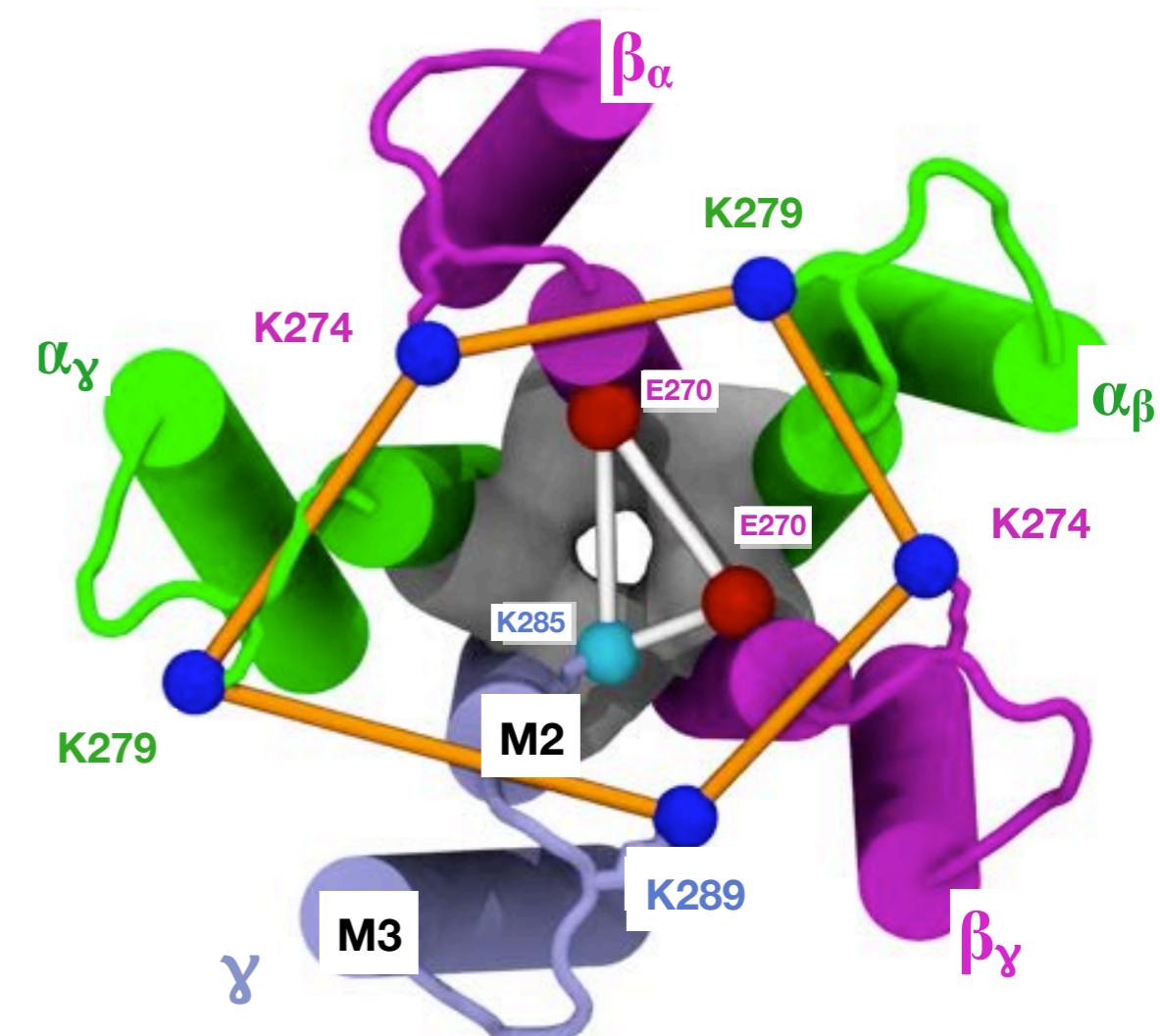
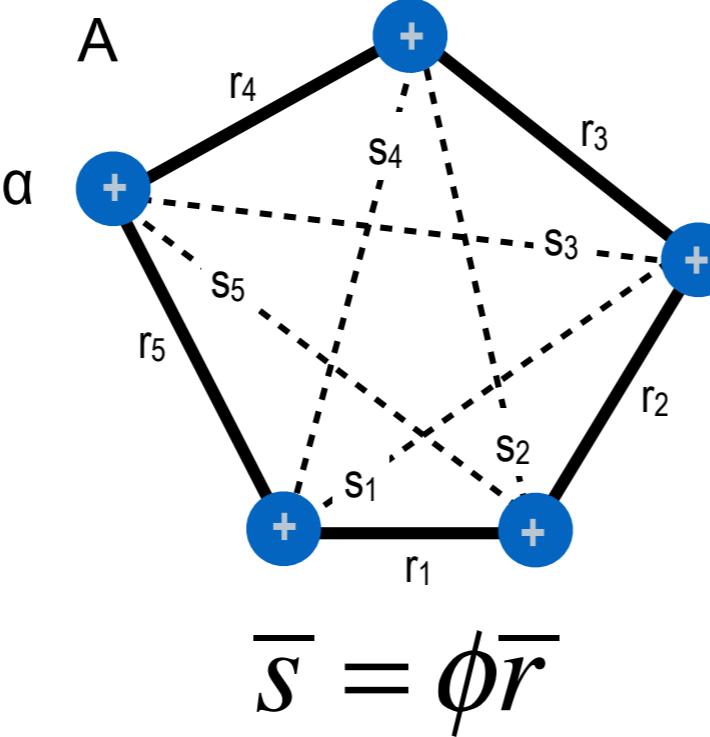
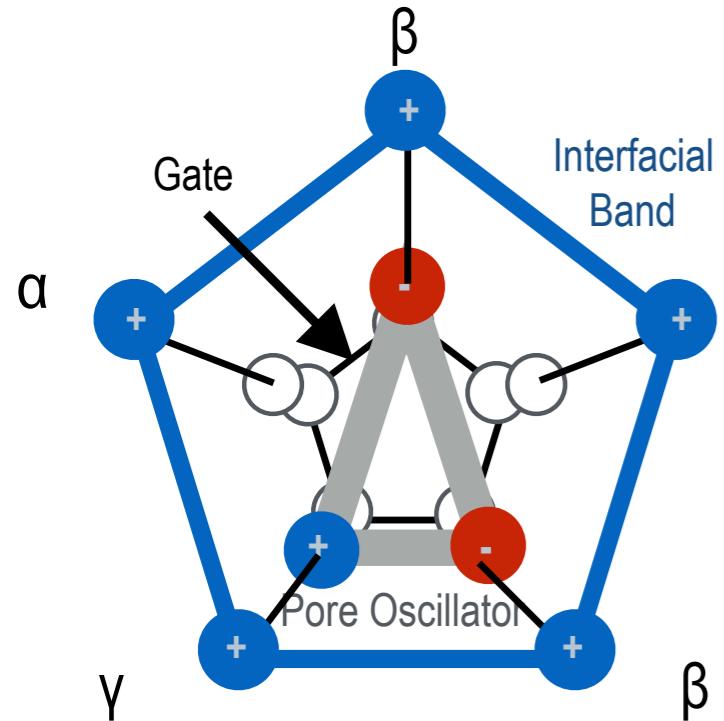
# Computational Results

2. WT channel has more stable open conformations at 315 K



### 3. Features of WT channel allows a more stable open conformations at 315 K

1. Interfacial band : This band of residues stabilizes the open state.

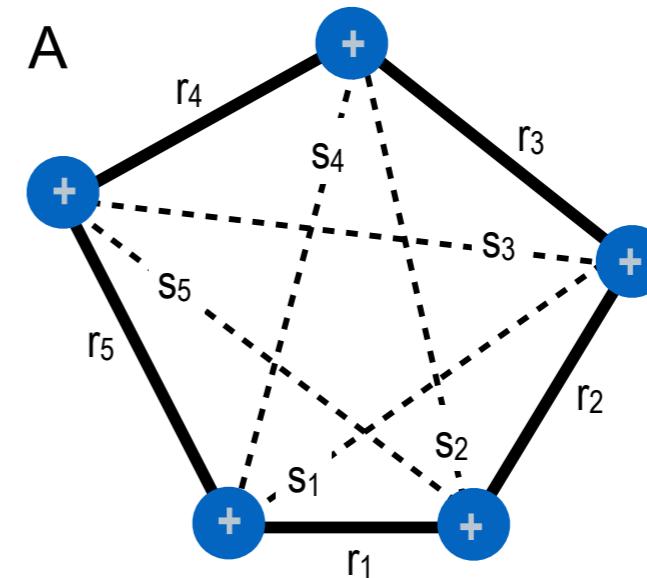
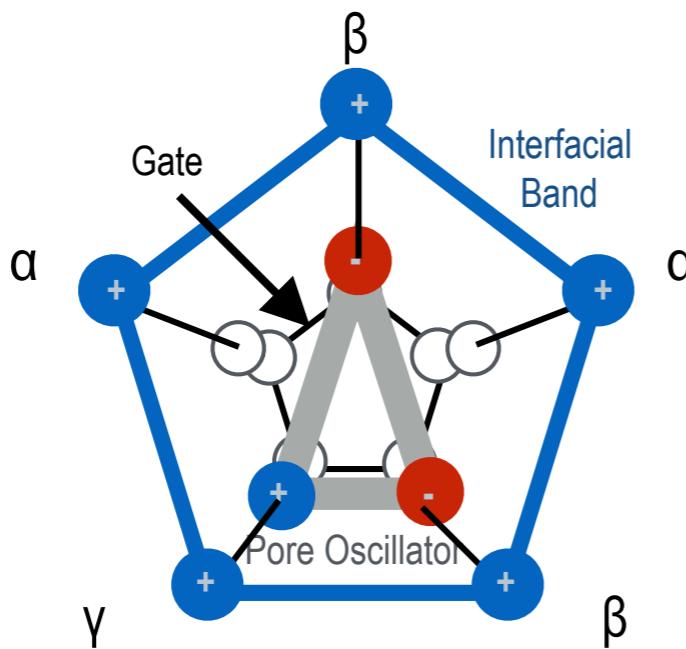


Regular Pentagon - geometric property — Golden ratio

$$\phi = (1 + \sqrt{5}) / 2 = 1.62$$

### 3. Features of WT channel allows a more stable open conformations at 315 K

1. Interfacial band : Is a PENTAMER of 5 charged residues



Regular Pentagon - geometric property — “Golden ratio”

$$\frac{r}{s} = \frac{1}{\phi}$$

$$\phi = (1 + \sqrt{5})/2 = 1.62$$

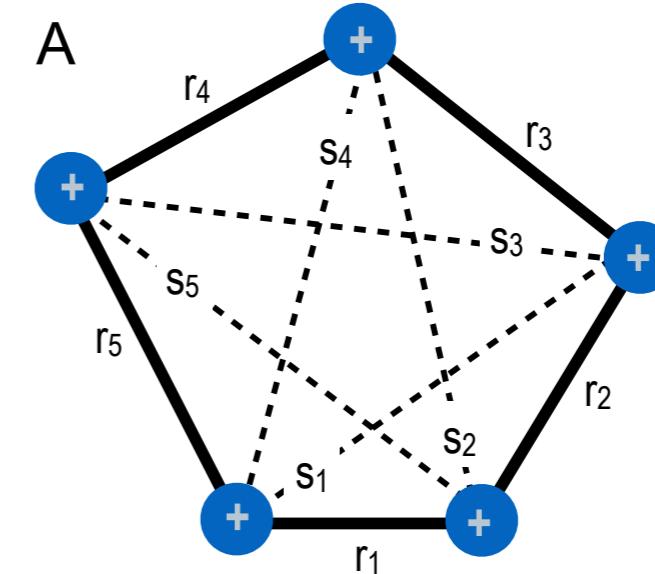
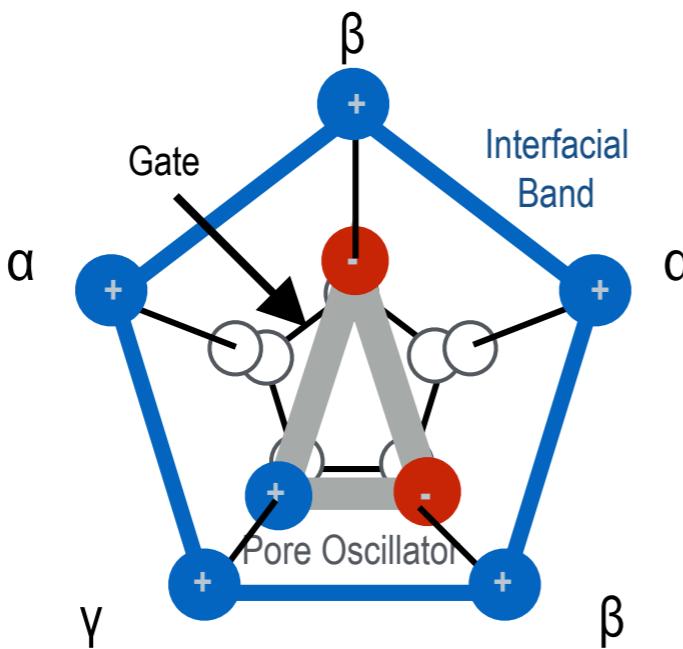
Coulomb energy of a PENTAMER

$$U_{+5}(r, \delta_\phi) = \frac{5k_e e^2 \phi}{r} \left(1 + \frac{\delta_\phi}{\phi}\right) + O(\bar{\delta} r^2) + O(\bar{\delta} s^2)$$

$$\delta_\phi \equiv \frac{\bar{r}}{\bar{s}} - \frac{1}{\phi}$$

### 3. Features of WT channel allows a more stable open conformation at 315 K

Interfacial band : Is a PENTAMER of 5 charged residues

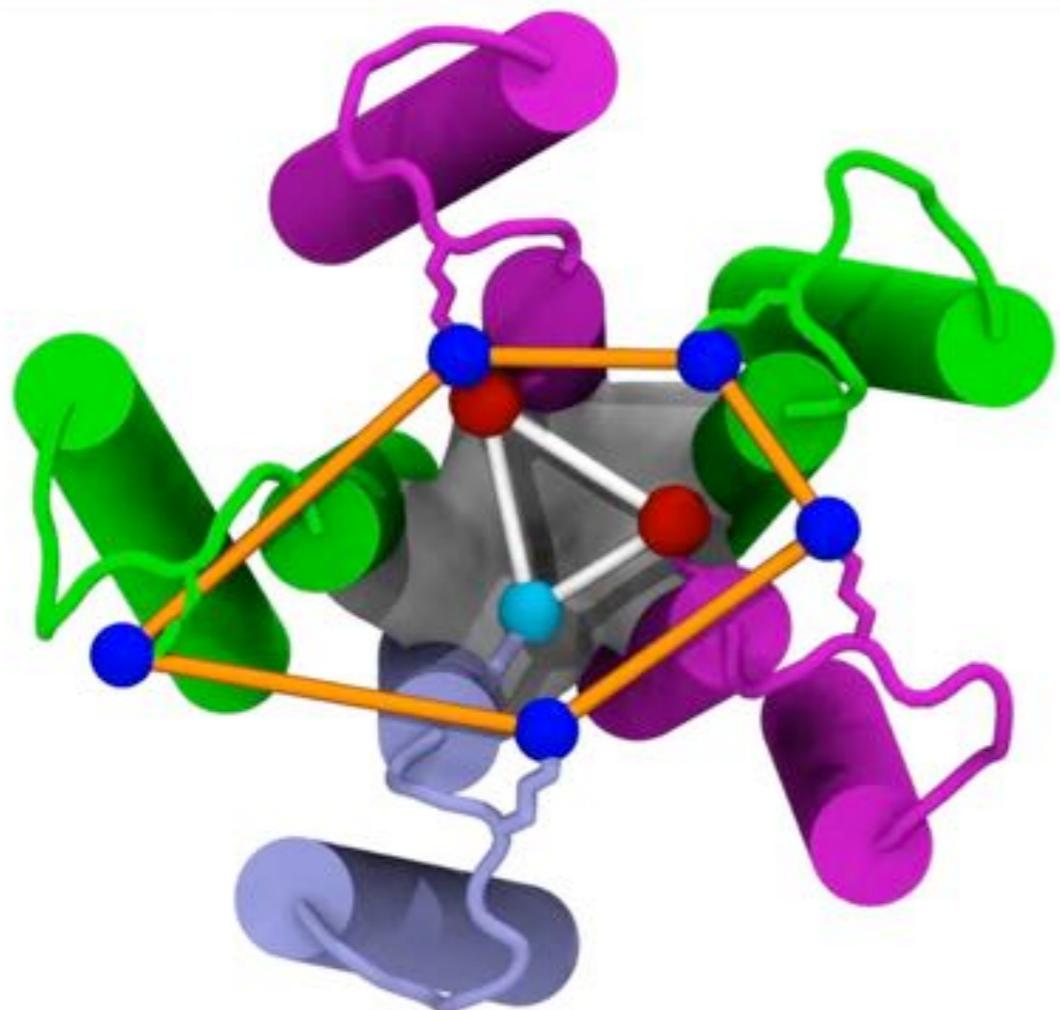


$$\delta_\phi \equiv \frac{\bar{r}}{\bar{s}} - \frac{1}{\phi}$$

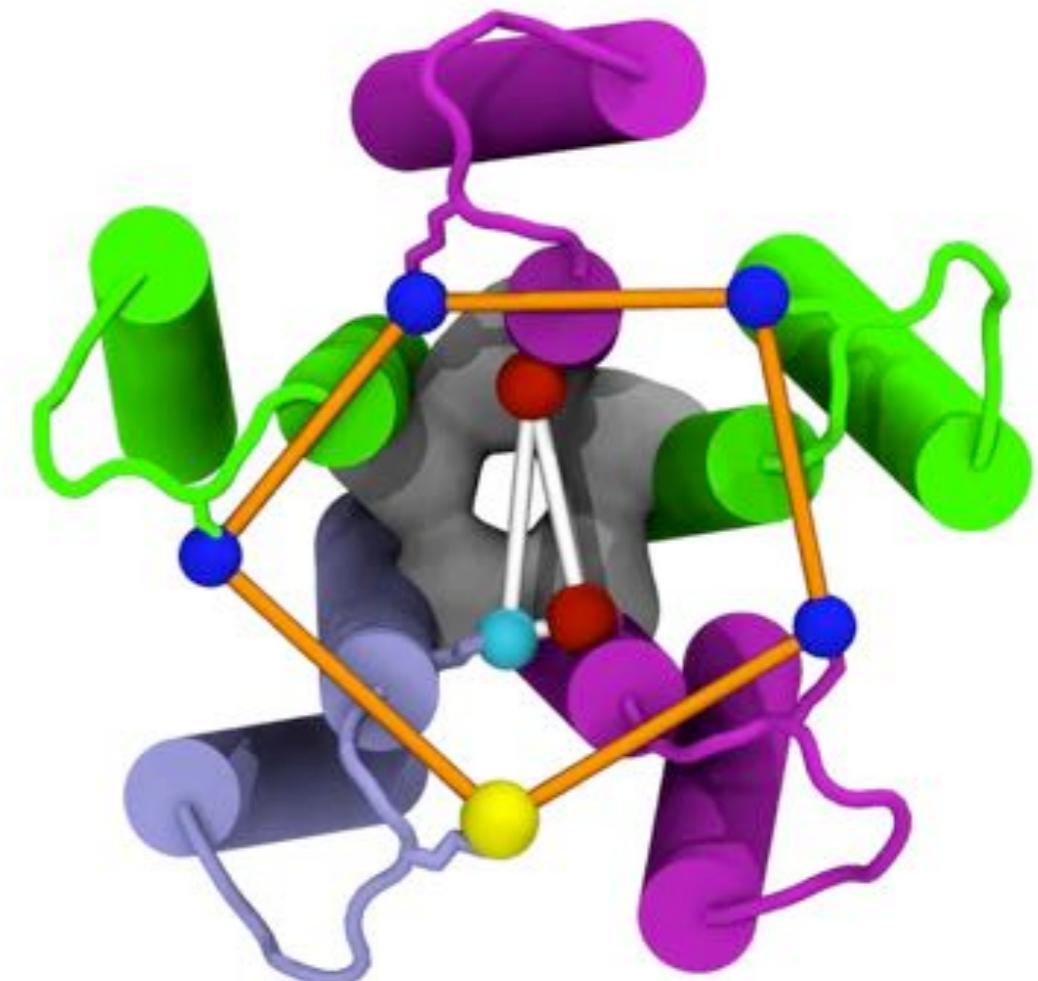
Diagonal (cross-pore) interactions keep the pore open!

#### 4. Mutant K289M visits more closed states at 315K

Interfacial band : Only 4 charges in K289M



WT



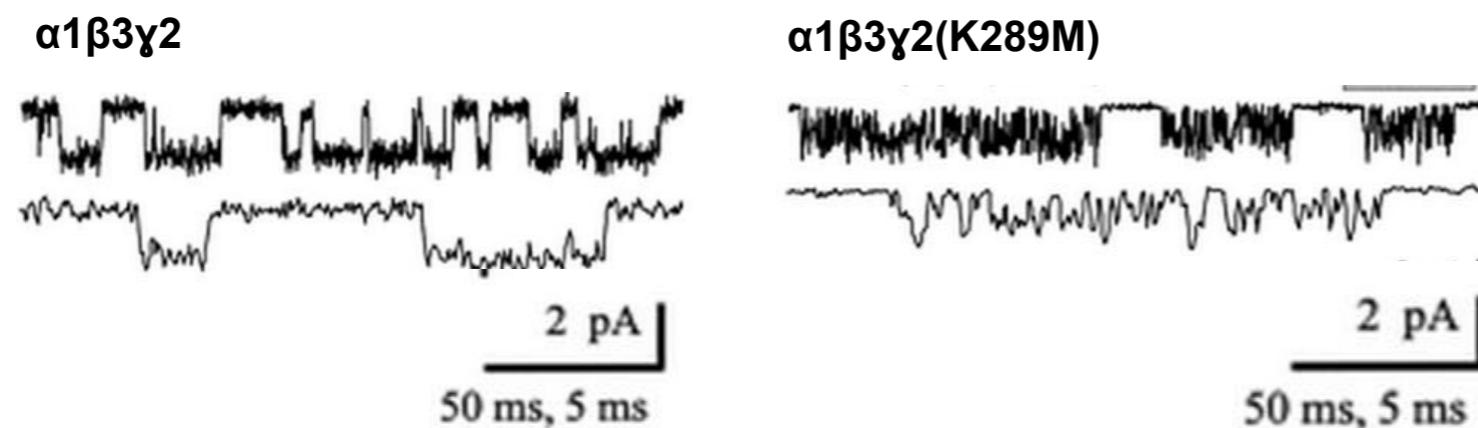
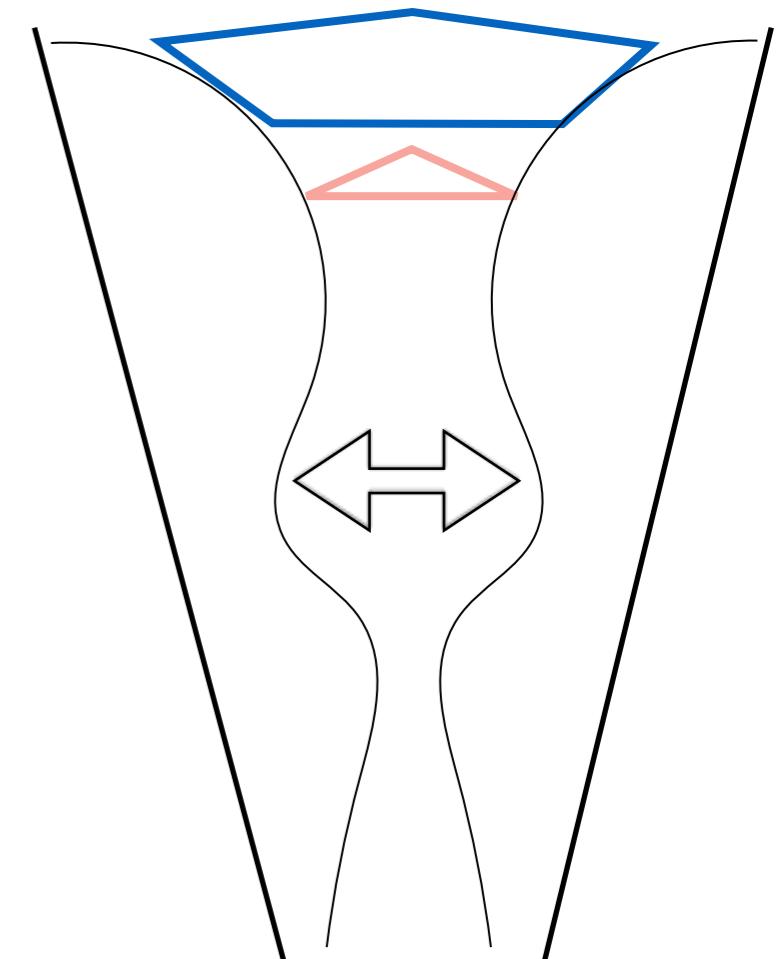
K289M

$$U_{+4}(r, \delta_\phi) = \frac{3}{5} U_{+5}(r, \delta_\phi)$$

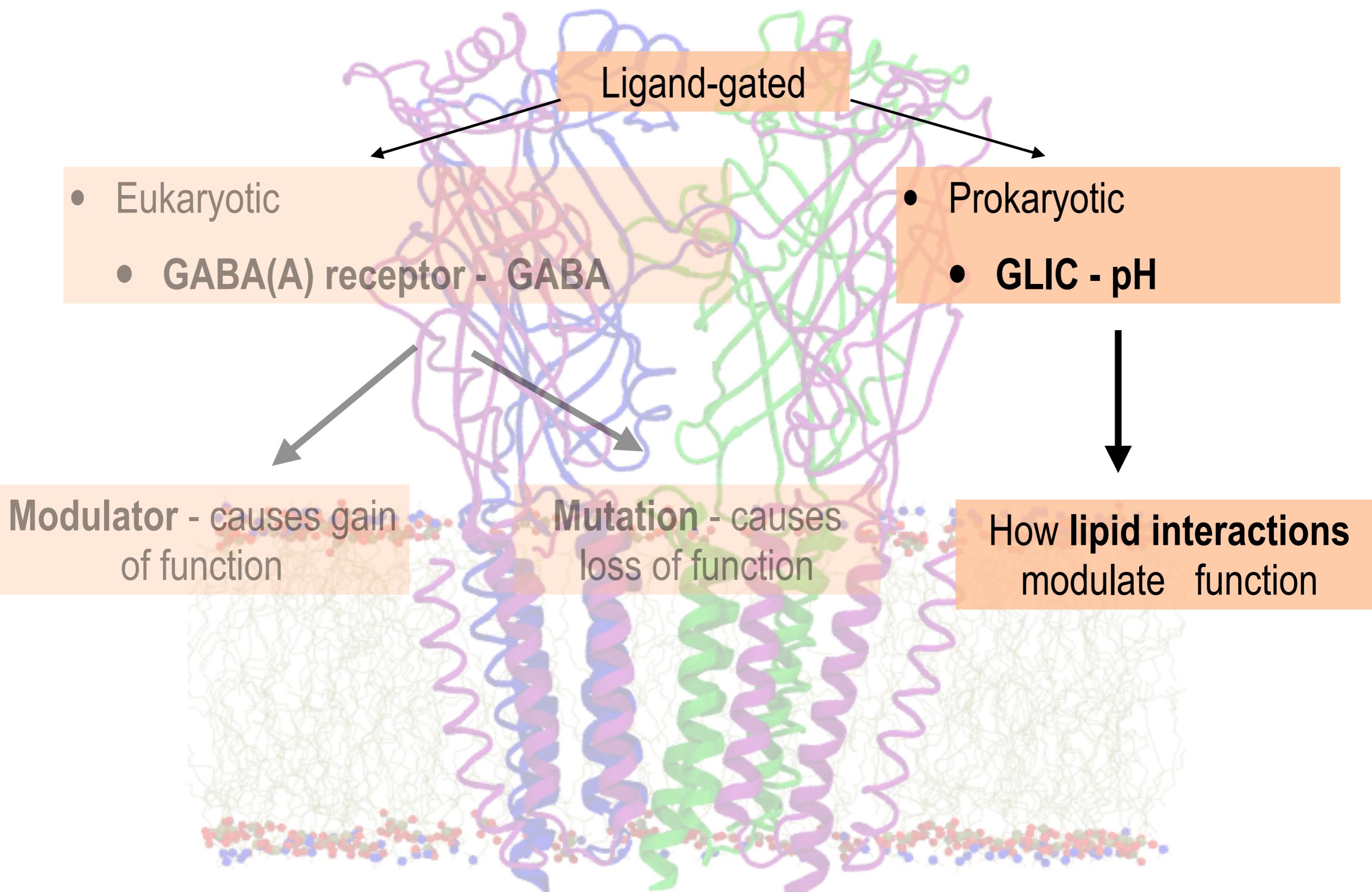
Reduced diagonal interactions destabilizes interfacial band.

# Conclusions

- WT - Interfacial band stabilizes open state.
- Only 4 charges: more likely to lose diagonal interactions; significantly exacerbated with high position dispersion.
- K289M - reduced cost of shrinking interfacial band at 315 K - higher population of closed states - consistent with the “flickering effect” observed in receptors, *in vitro*.



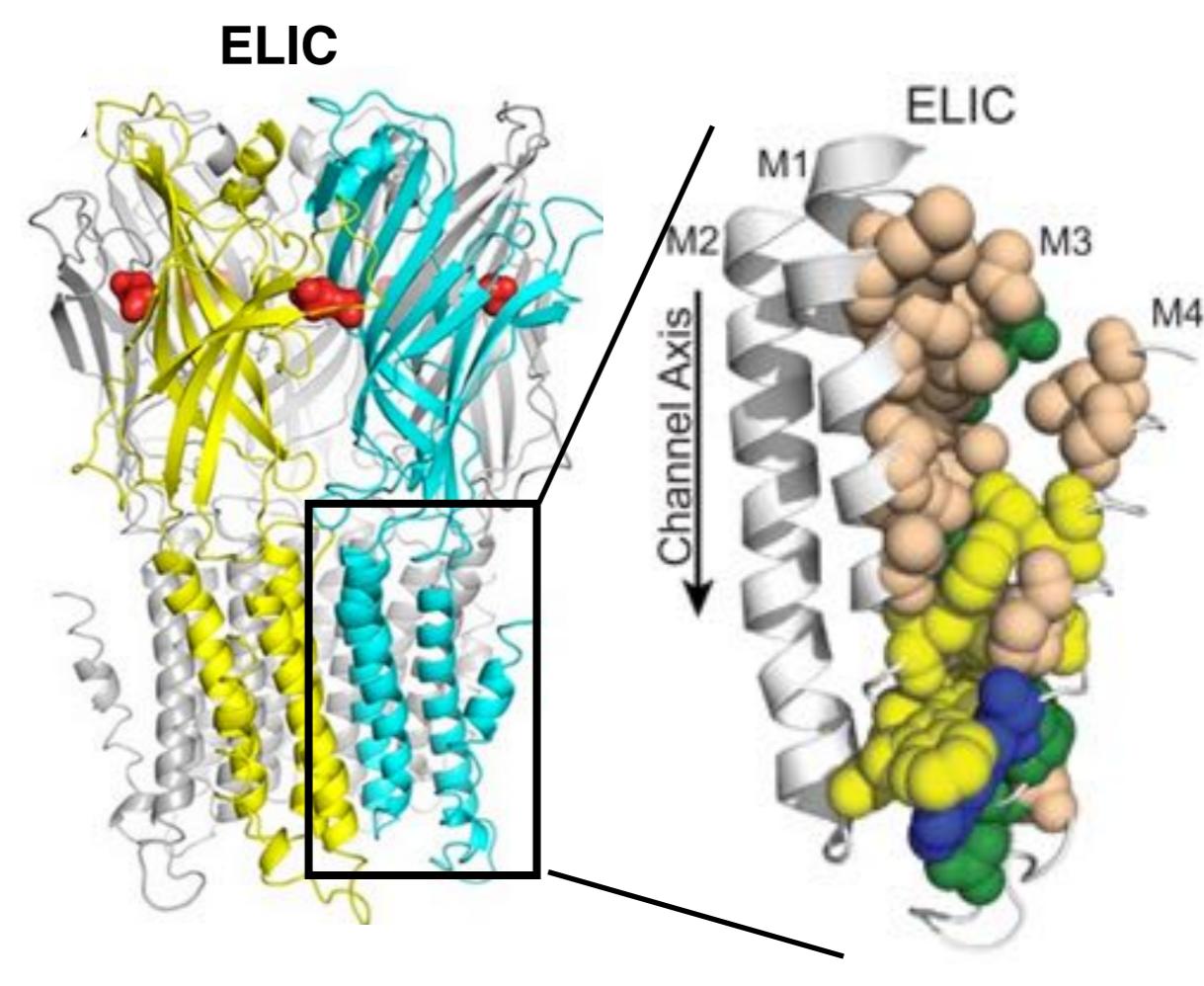
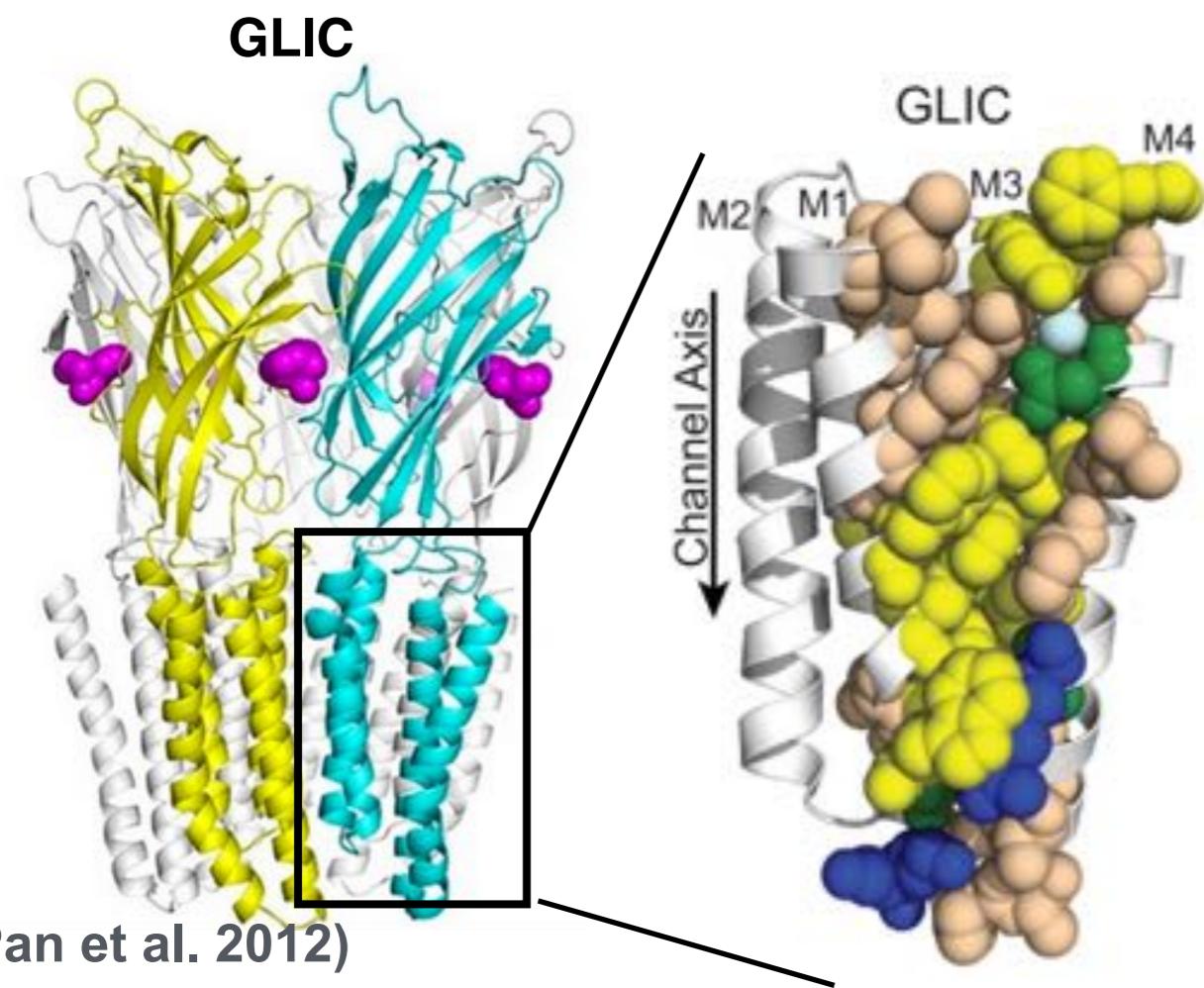
# OUTLINE



# GLIC receptor

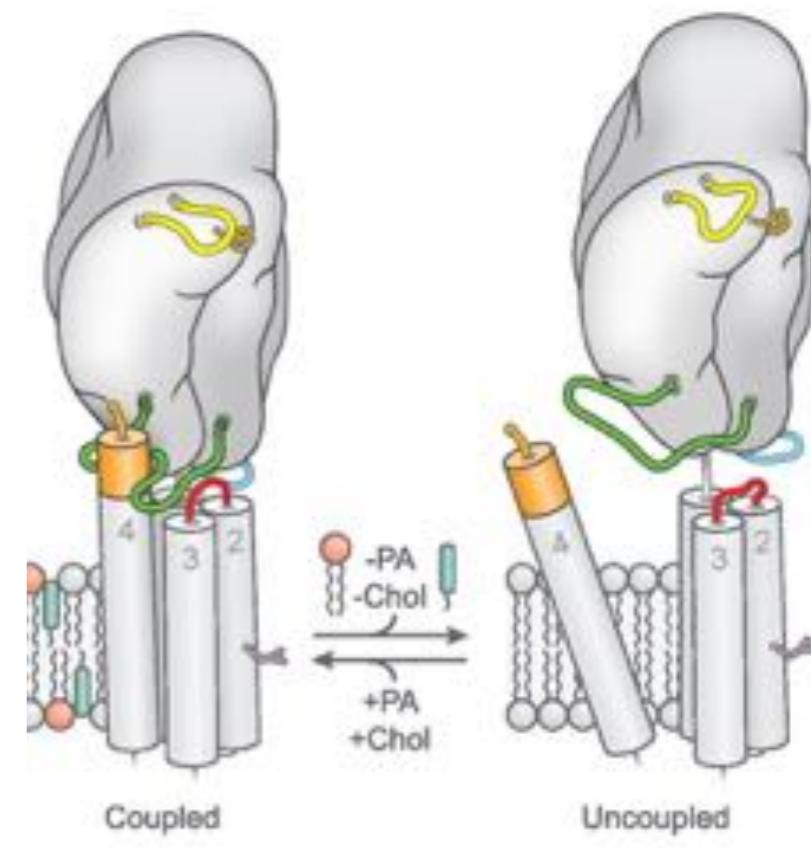
# GLIC & ELIC RECEPTORS

- (Gloeobacter) GLIC and (Erwinia chrysanthemi) ELIC pLGICs - homopentamer.
- Two structurally well-characterized prokaryotic pLGICs,
- Share structural similarity. But have distinct M4 conformations.

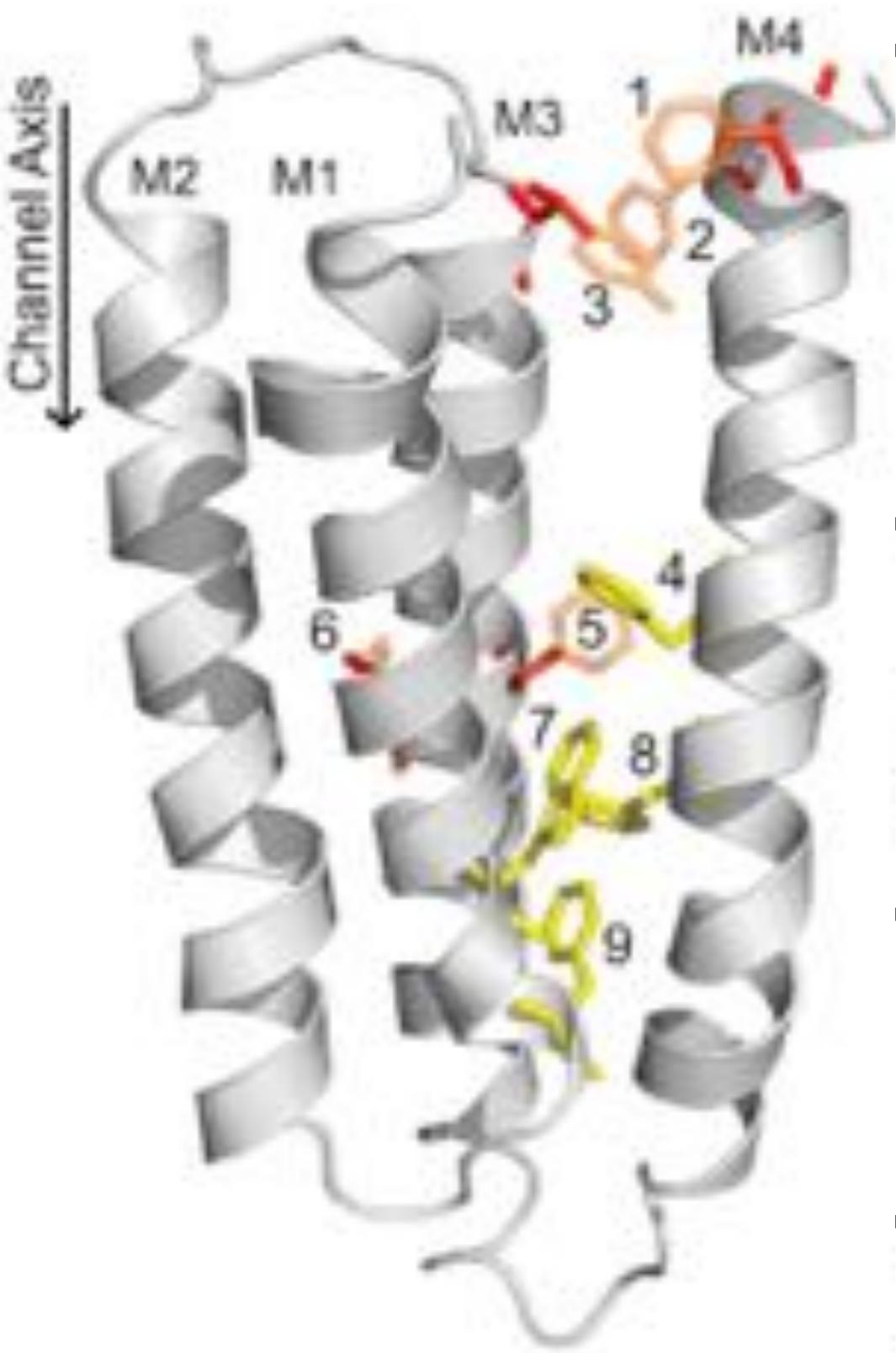


# Role of M4 - experimental evidence & hypothesis

- M4 extends beyond the bilayer to interact directly with the ‘cys-loop’, a key structure at the ECD/TMD interface that participates in channel gating.
- **Question :** How changes in M4 structure alter channel function.
- **Hypothesis :**
  - Effective M4-M1/M3 interaction - effective M4-cys-loop interaction ;
  - Ineffective M4-M1/M3 interaction abolish M4-cys-loop contact.
  - **π-π interactions within aromatic residues in M4-M1/M3 interface - causes effective interaction.**
  - **Mutation of aromatic residues to ALA - would lead to M4 tilting away.**

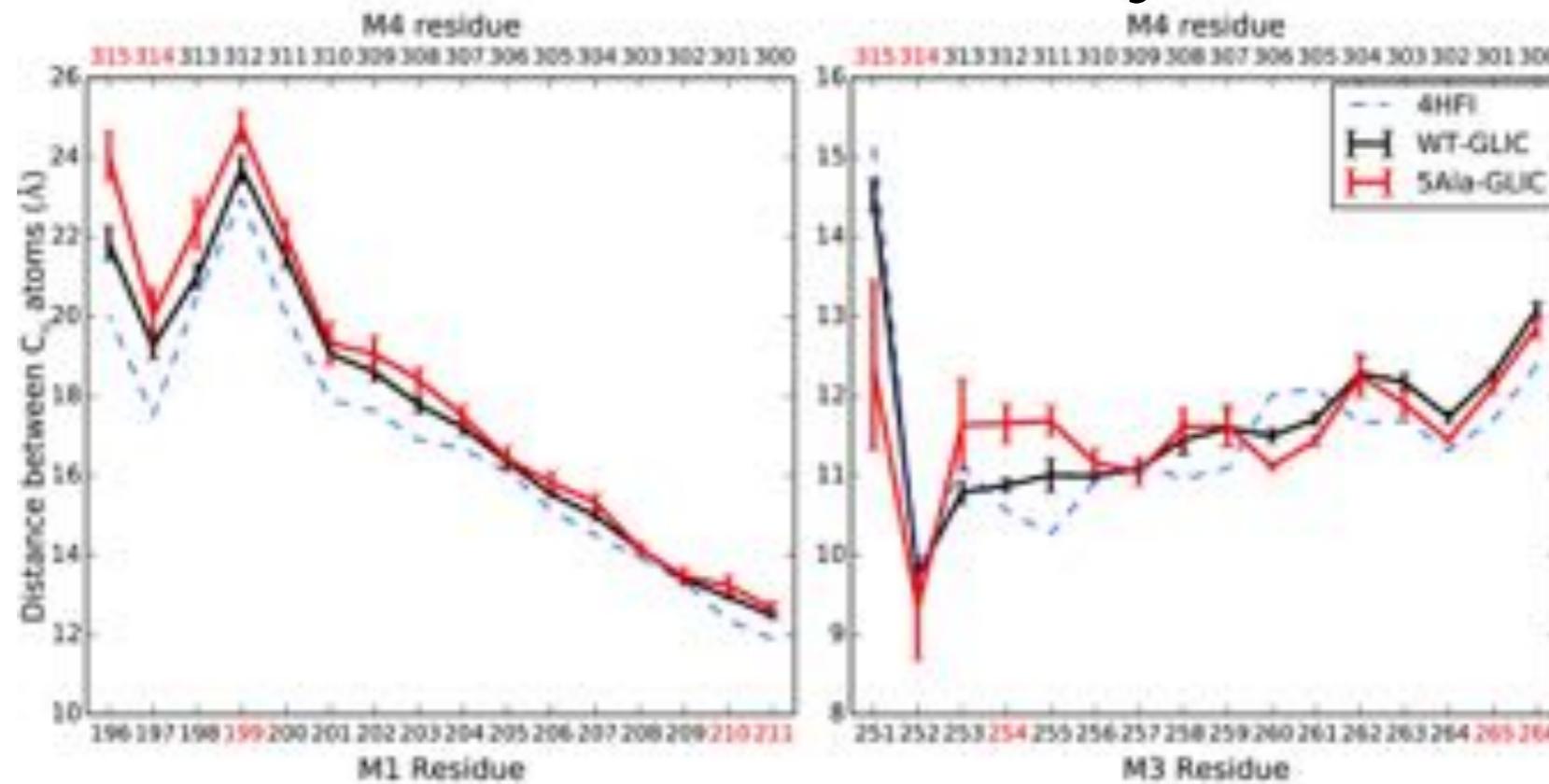


# Simulation details



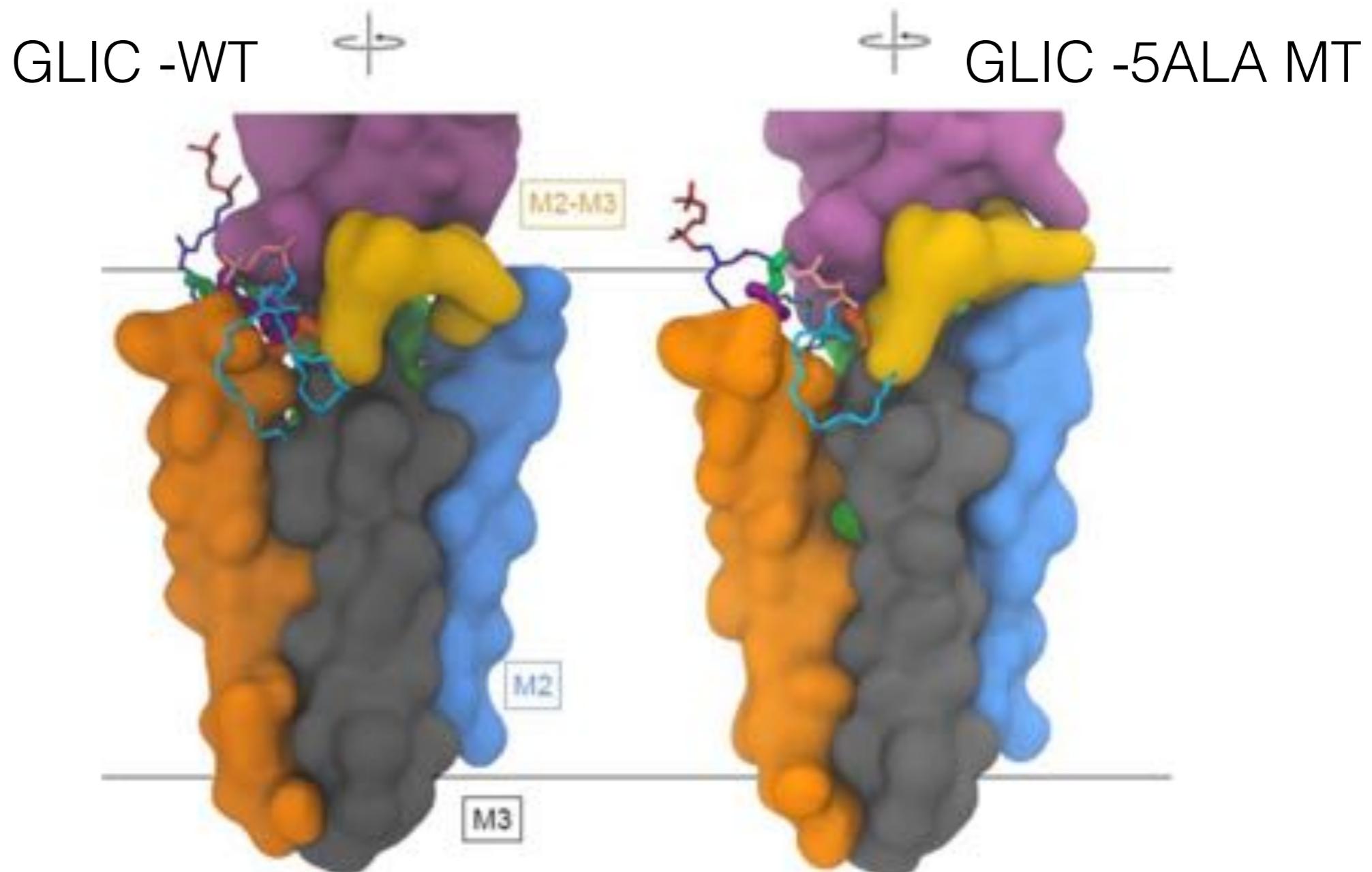
- Simulations were run for both wild-type GLIC(4HFI) (WT-GLIC) and a mutant using intact pentamers, as well as with a single-subunit-TMD.
- Five non-conserved aromatic residues were mutated to Ala (5Ala-GLIC: aromatic-to-Ala substitutions at positions 1, 2, 3, 5, and 6).
- Aromatic residues at the M4-M1/M3 interface promote strong M4-M1/M3 interactions.
- Elimination of these aromatic residues leads to increased Ca-Ca carbon atom separations between M4 and M1/M3

# Simulation analysis



- GLIC -WT - Displayed  $\pi$ - $\pi$  interactions within aromatic residues in M4-M1/M3 interface.
- 5ALA-GLIC - M4 tilts away from M1/M3, atom contacts increasing by roughly  $2\text{ \AA}^\circ$  - Not enough to decouple M4 from M1/M3 .
- **But MD simulations revealed what actually disrupts the M4-cys-loop interactions in case of Mutants! - LIPID interactions.**

# Conclusion



- Reduced side-chain volume in 5Ala-GLIC leads to a change in lipid binding; Observed across all the subunits.

# Summary

- MD simulations techniques have been utilized to visualize and predict
  - Conformational dynamics of pentameric ligand gated ion channel,
  - Binding sites for anesthetic in GABA(A) receptors.
  - Subunit specificity for anesthetics.

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