

AFFINITY CALCULATIONS FOR LIPOPHILIC MODULATORS BINDING TO ISOLATED SITES ON GABA(A) RECEPTORS

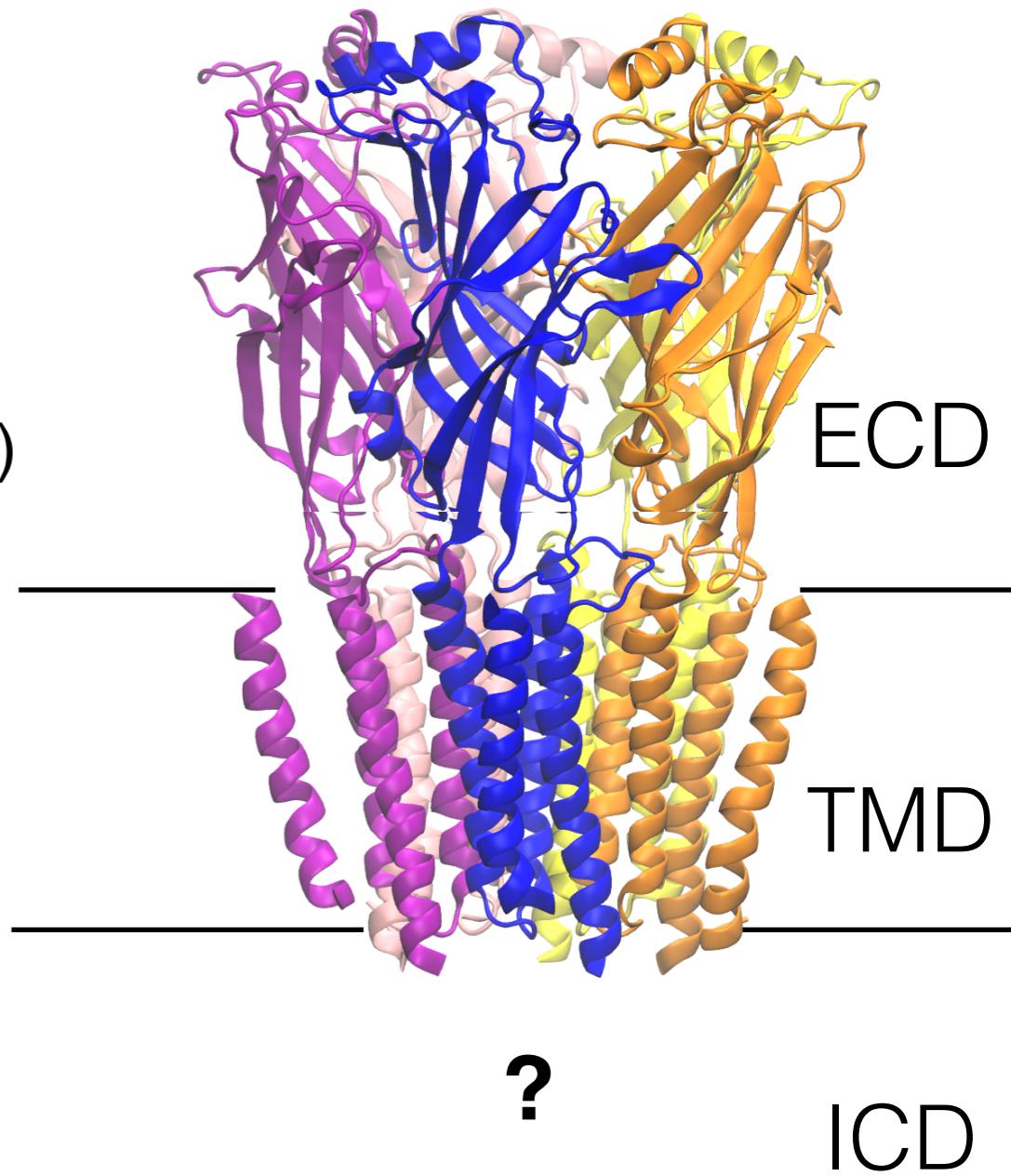
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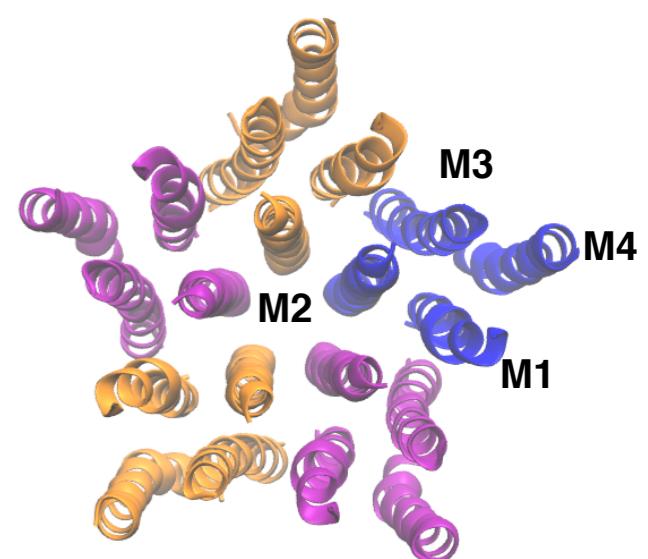
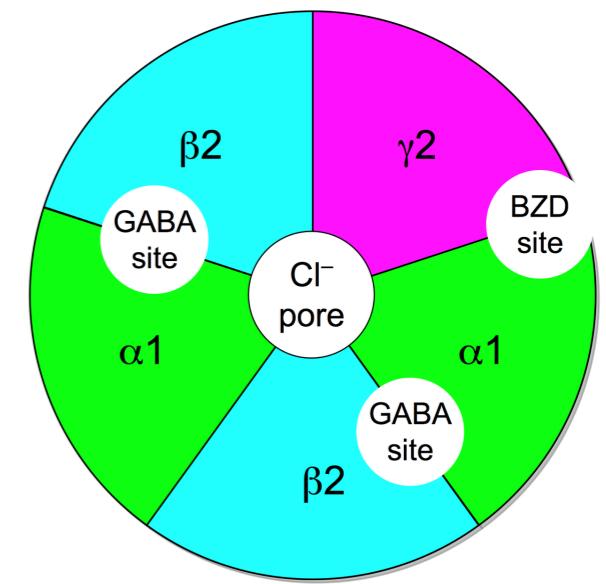
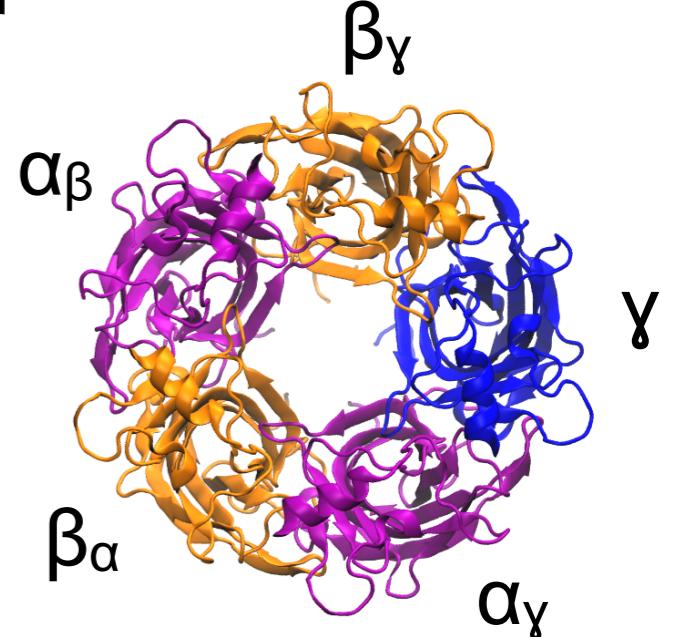
GABA_A Receptor

- Major inhibitory neurotransmitter receptor in mammalian CNS.
- Also found in liver, muscles of lungs and immune cells.
- Pentameric Ligand-Gated ion channel (**pLGIC**)
- Anionic - conducts chloride ions
- 3 domains:
 - extracellular domain(**ECD**)
 - transmembrane domain (**TMD**)
 - intracellular domain (**ICD**) - structure is unknown



GABA_A Receptor

- **Pentamer :**
 - 5 subunits arranged around pore
 - Here using common arrangement of $\alpha 1\beta 3\gamma 2$
(2 $\alpha 1$, 2 $\beta 3$, 1 $\gamma 2$ subunits)
- **Ligand-gated :**
 - primary agonist is γ -Aminobutyric acid (GABA),
 - binds between $\alpha-\beta$ subunits in ECD.
- **Transmembrane Domain:**
 - Each subunit has four helices : M1, M2, M3, M4
 - M2 helices line pore.



General Anesthetics

- Primary targets of anesthetics are ion channels, especially GABA_A Receptors. [Franks *et al* (1989);Krasowski *et al* (1999)]
- Most GAs potentiate or activate GABA_A receptors at clinical concentrations (μM - mM)
- Anesthetics classified under route of administrations:

inhalational (IN) → Isoflurane, Desflurane, Sevoflurane

intravenous (IV) → Propofol, Etomidate

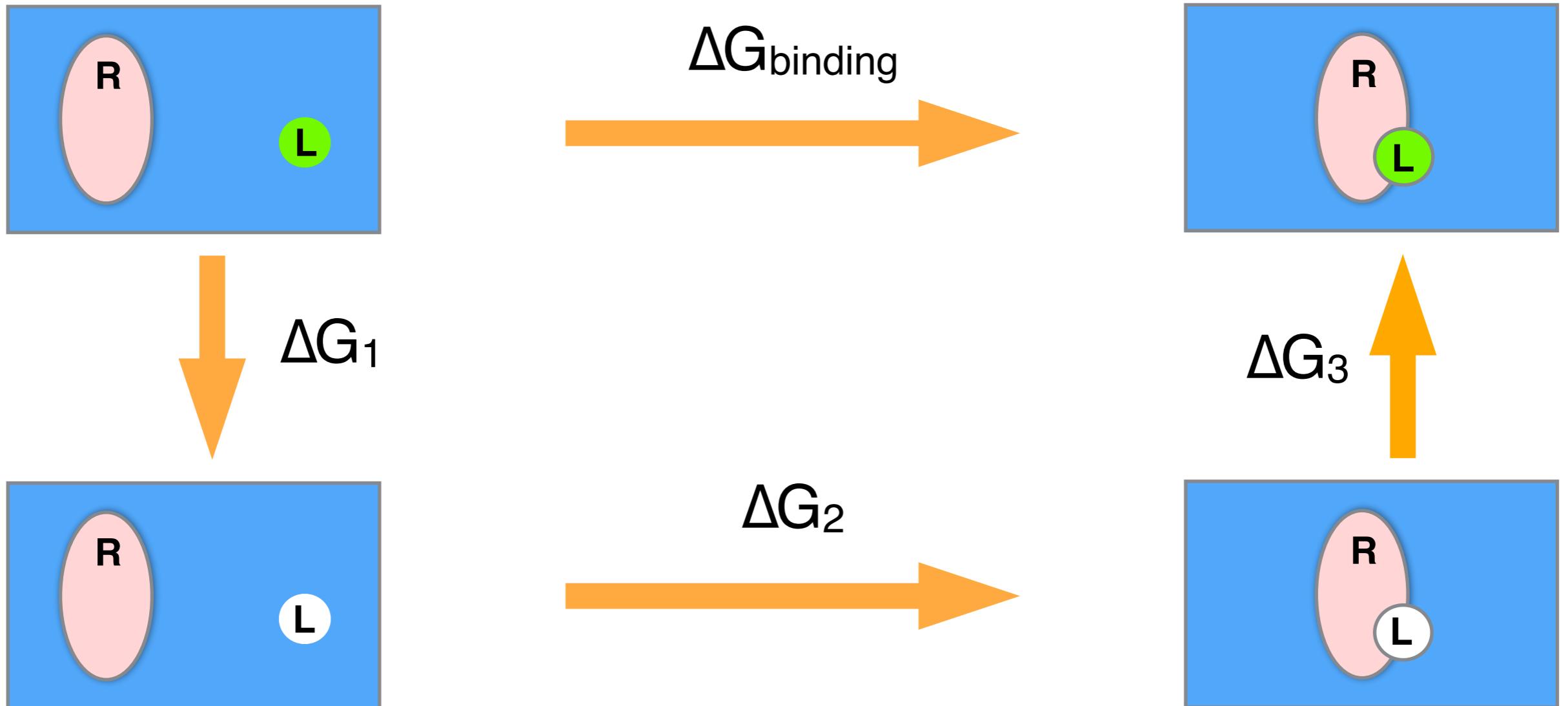
- Mutagenesis , Electrophysiology and Photolabeling - experimental techniques to identify binding site residues in the receptors.
- Drawbacks - difficulty in obtaining a crystal structure of the receptor with a bound anesthetic; low specificity of the anesthetics.

General anesthetics: some questions

Main Question: Where do GAs bind on GABA_A receptors?

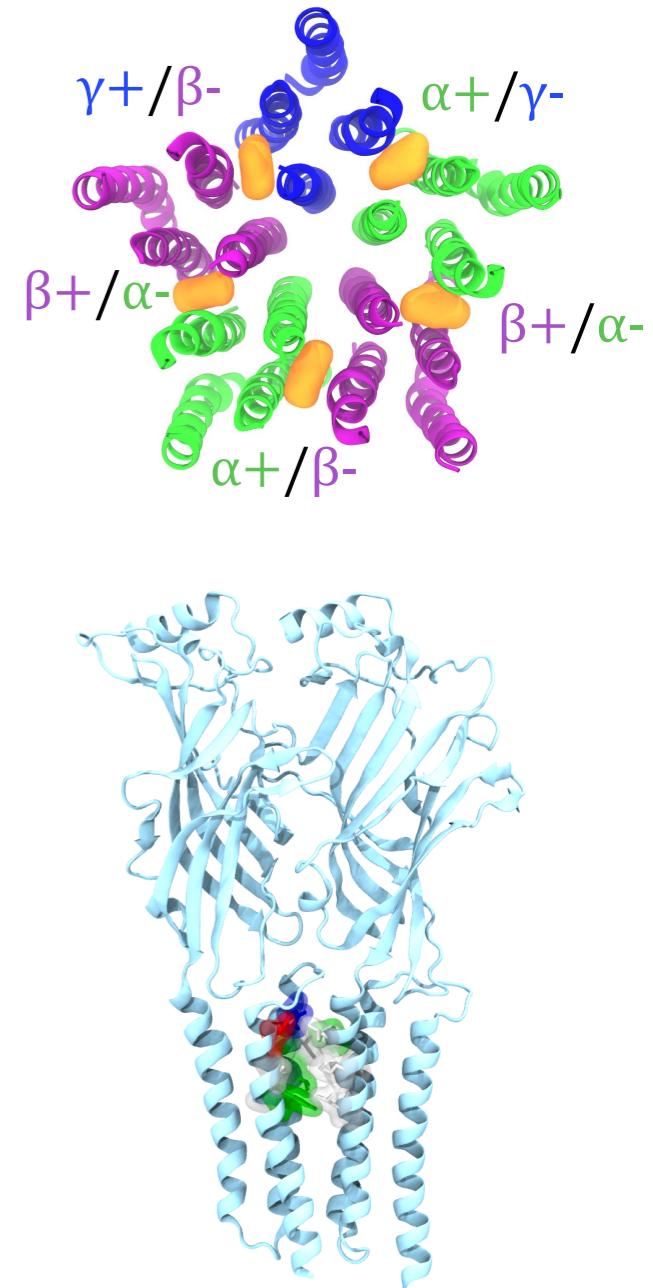
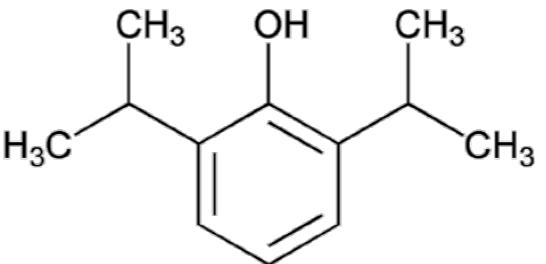
- 1) Which sites are occupied at clinical concentrations?
- 2) What are the affinities of two popular GAs for various proposed intersubunit sites?
- 3) What are the microscopic origins of affinity differences?

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

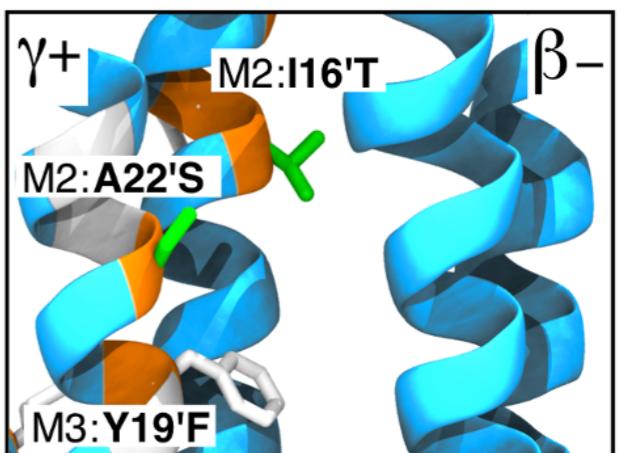
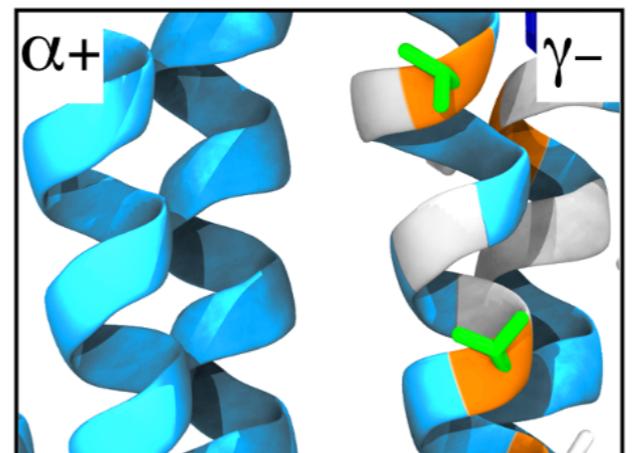
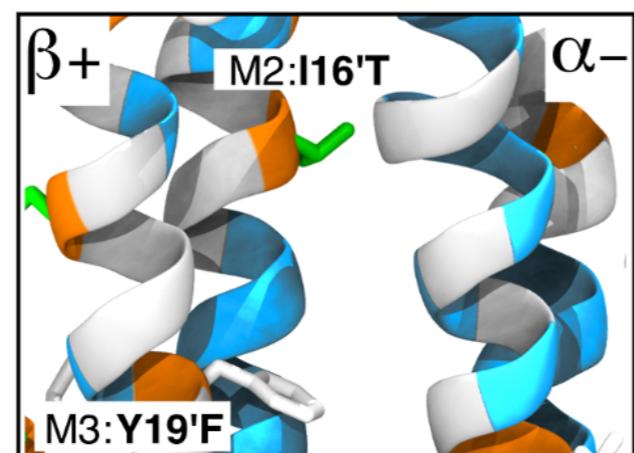
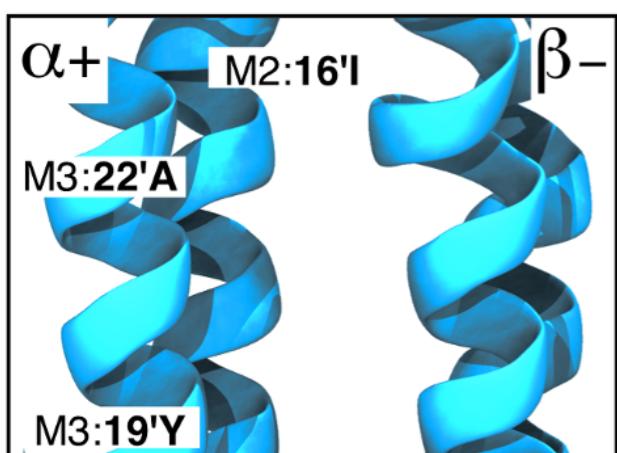


$$\Delta G_{\text{binding}} = \Delta G_1 + \Delta G_2 + \Delta G_3$$

PROPOFOL FEP calculations



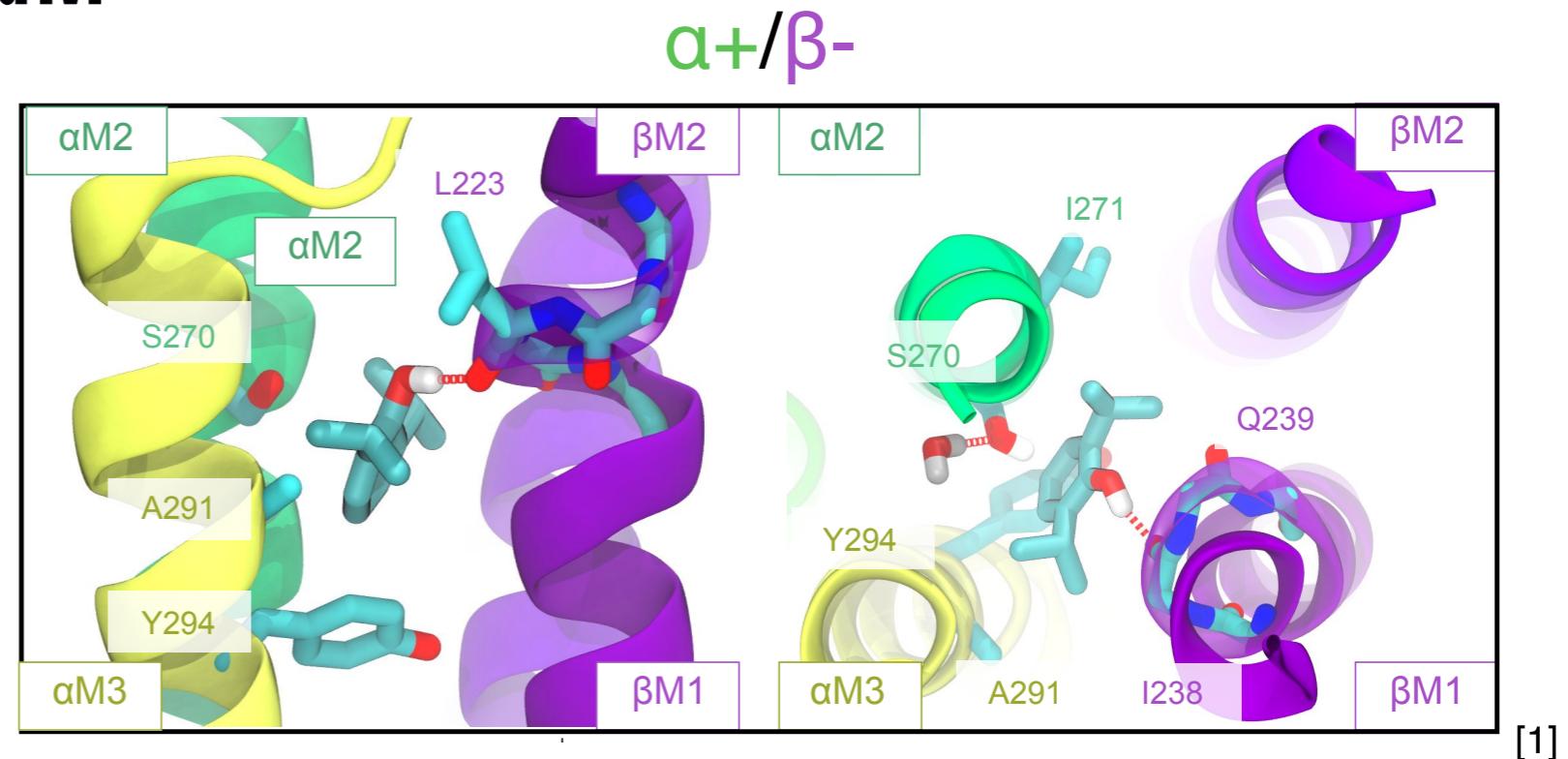
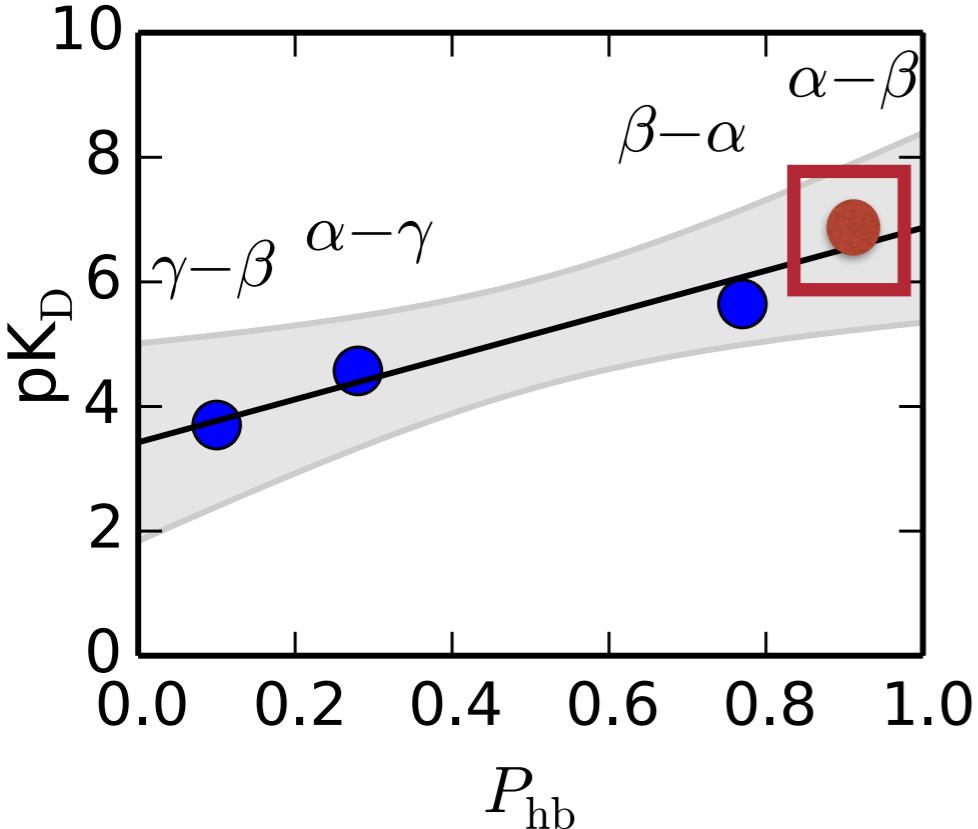
PFL EC₅₀ = 0.5-5 μM [1]



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

2. Woll, K. A. Murlidaran, S. et al.. J. Biol. Chem. (2016).

Site: $\alpha+$ / $\beta-$ $K_d = 0.1 \mu M$

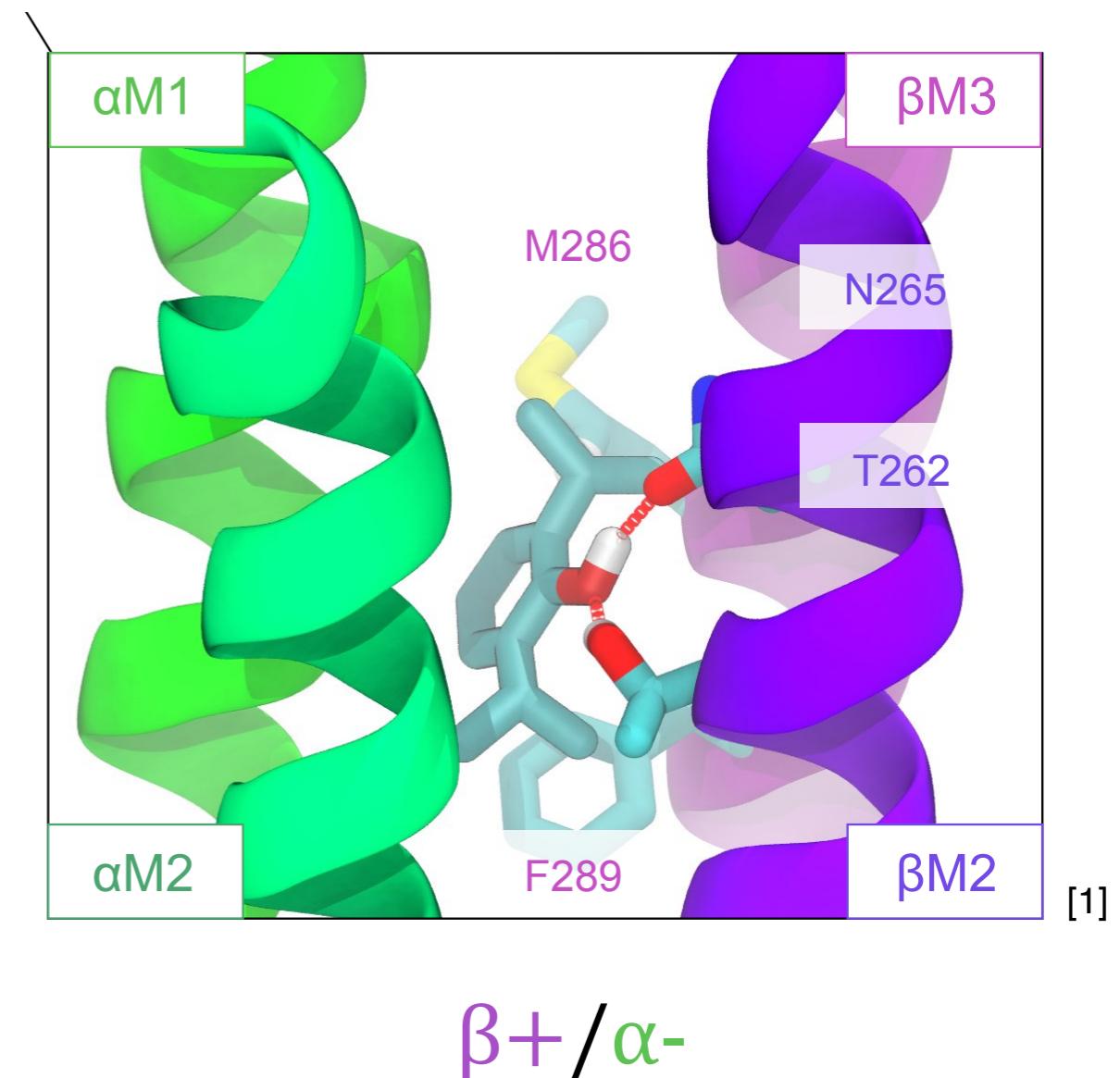
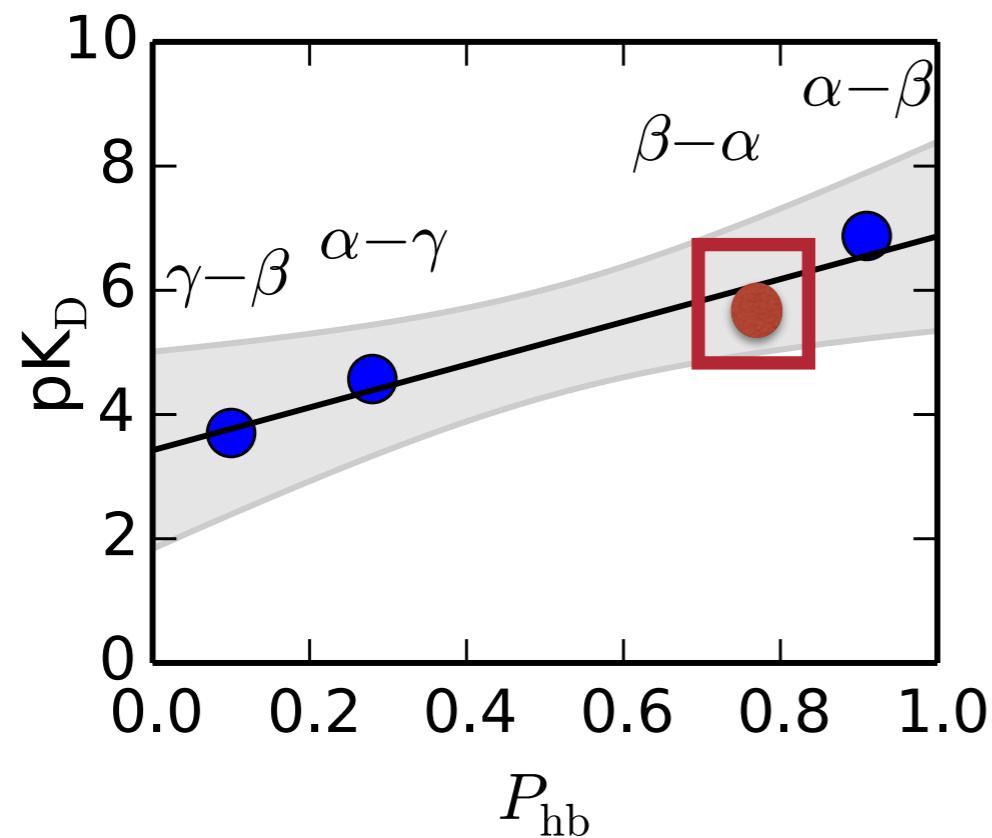


$\alpha+ / \beta-$ site :

- 7 polar residue side-chains facing the binding pocket.
- PFL forms persistent H-bond with backbone carbonyl of L223;
 - bulge in backbone is observed in crystal structures GluCl and GABA(β 3).
- K_d value consistent with higher P_{hb}

Site: β^+ / α^-

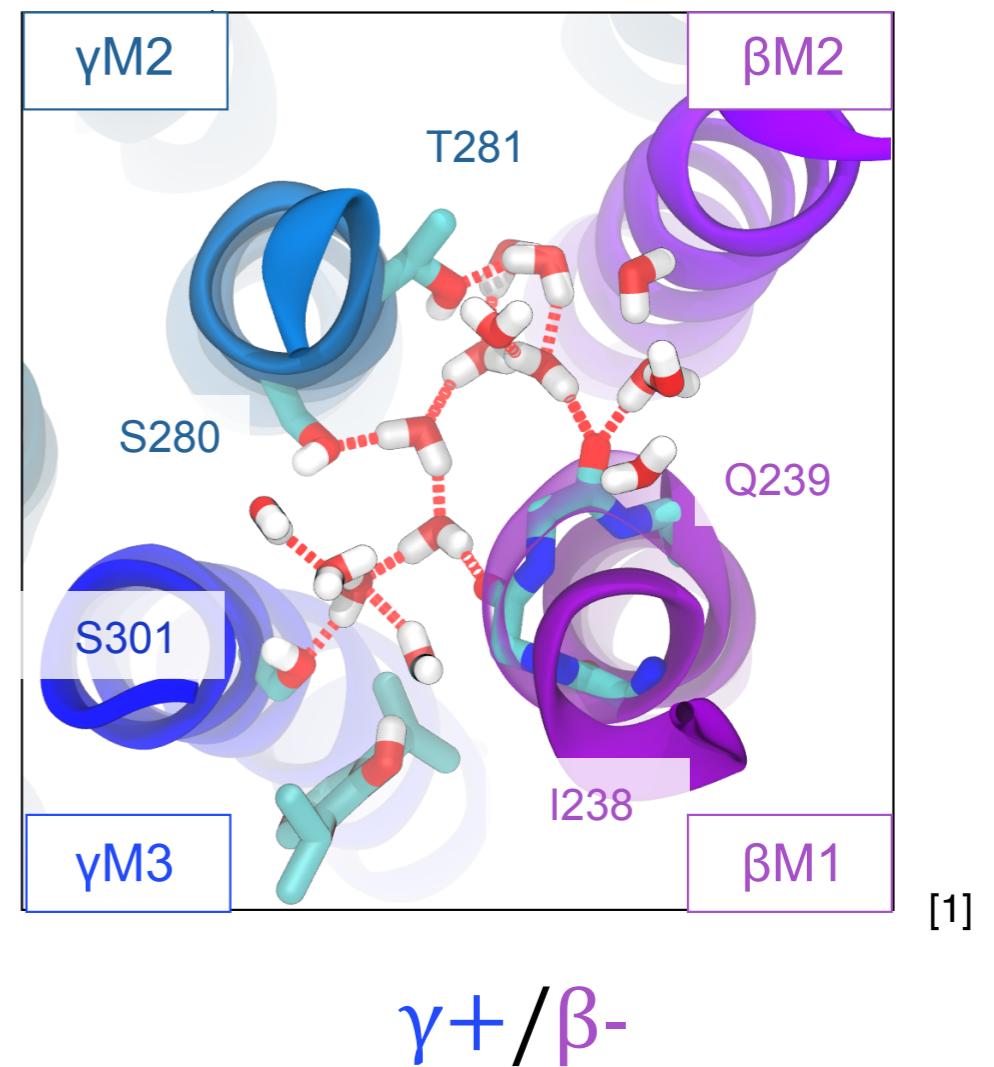
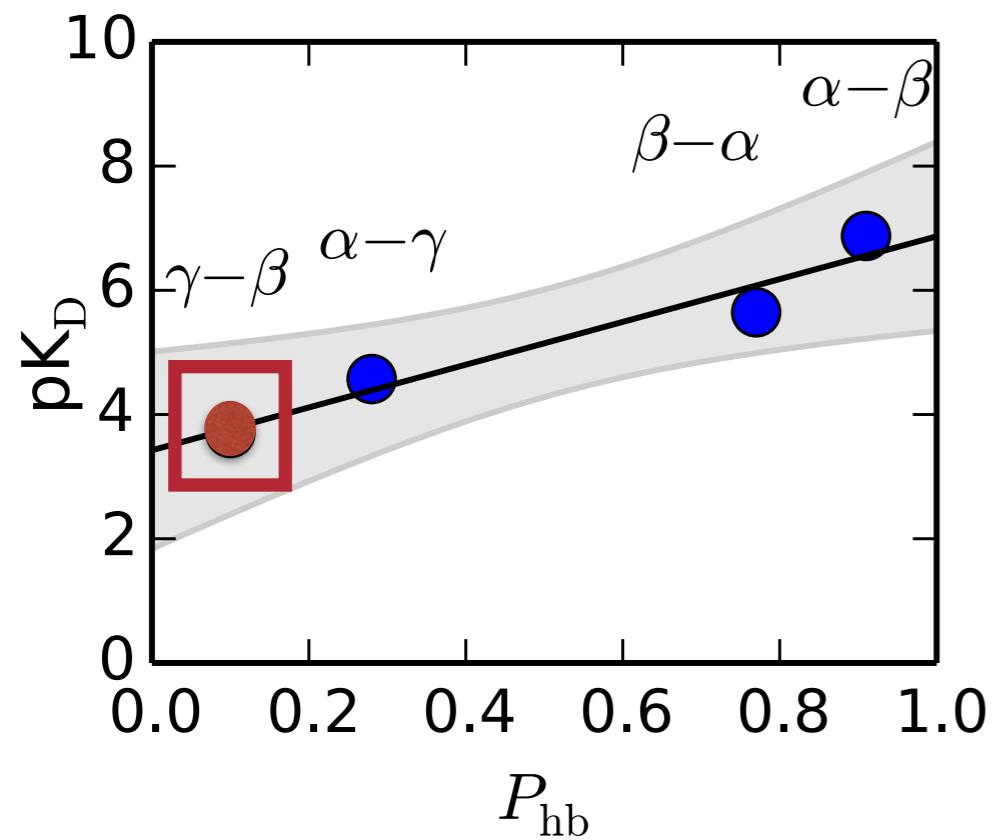
$K_d = 2\mu M$



β^+ / α^- site

- 7 polar residue side-chains facing the binding pocket.
- PFL forms alternate H-bonds between N265 and T262.
- slight reduction in pK_D is consistent with the slight reduction in P_{hb}

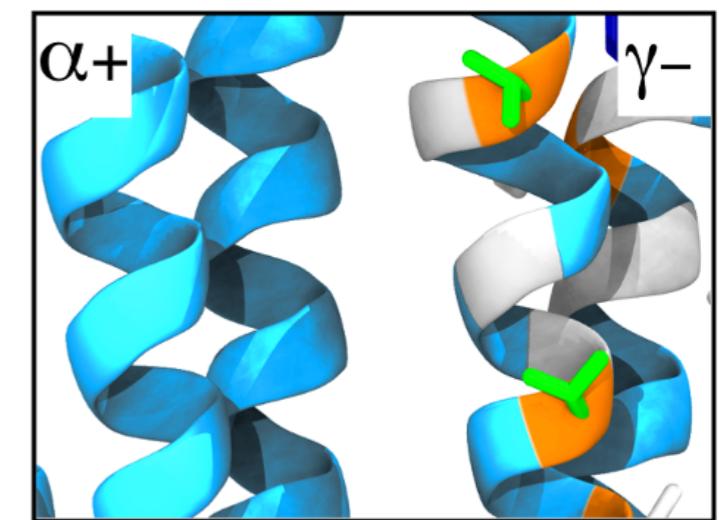
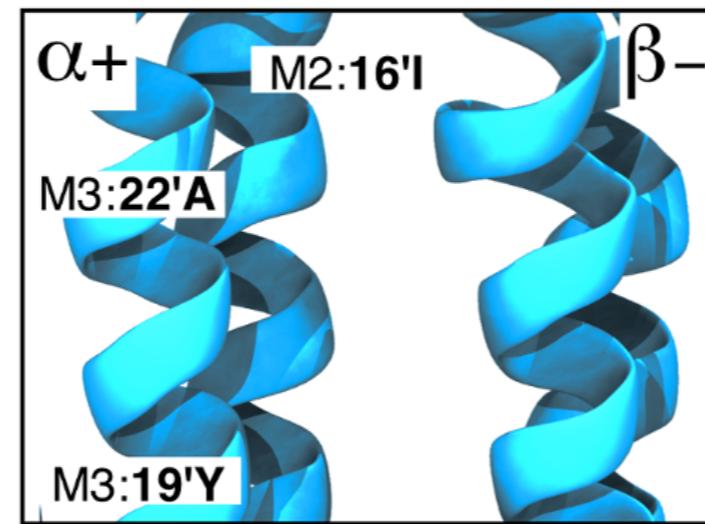
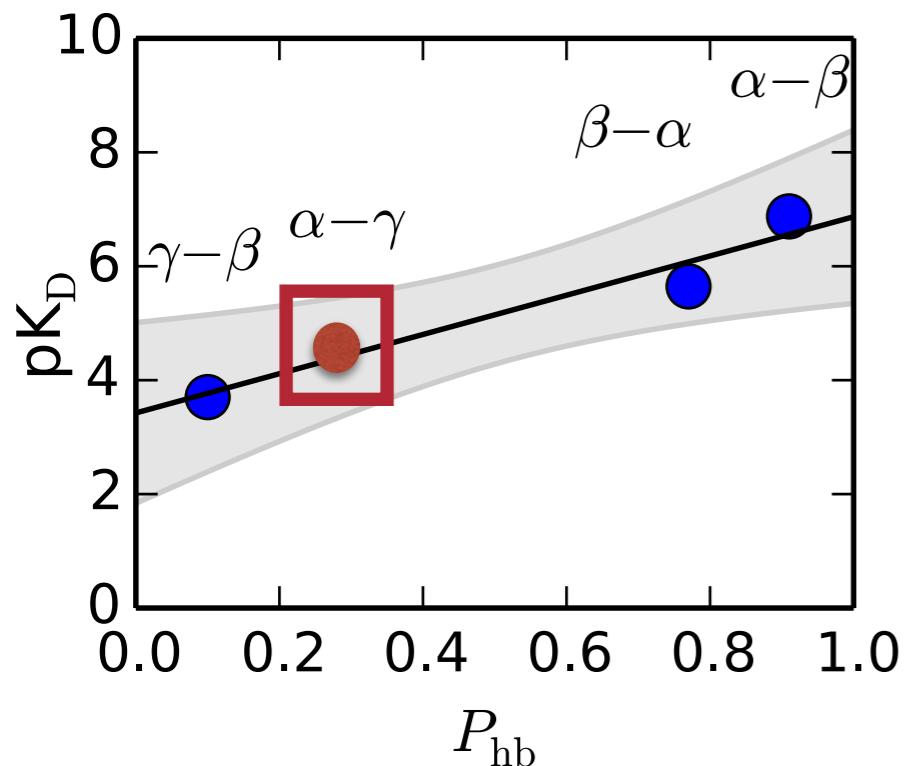
Site: $\gamma+$ / $\beta-$ $K_d = 20 \mu\text{M}$



$\gamma+$ / $\beta-$ site

- 8 polar residue side-chains facing the binding pocket.
- polar residues $\gamma+$ -T281 and $\gamma+$ -S301 - homologous to hydrophobic residues in α and β subunits.
- residues favor H-bond formation with water cluster.

Site: $\alpha+$ / $\gamma-$ $K_d = 200 \mu\text{M}$

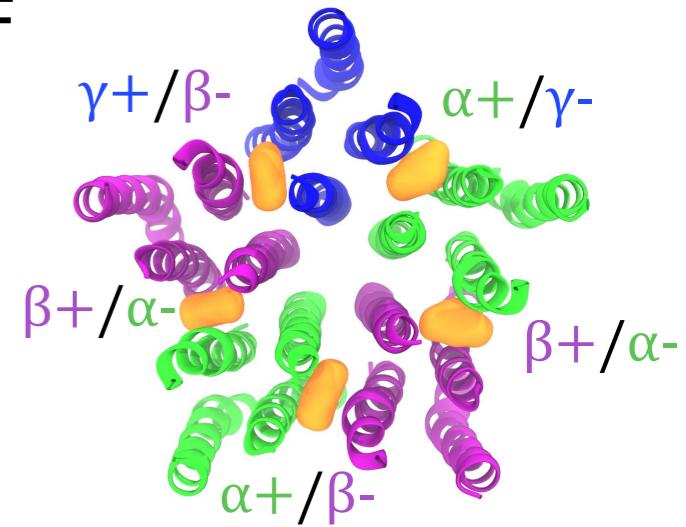
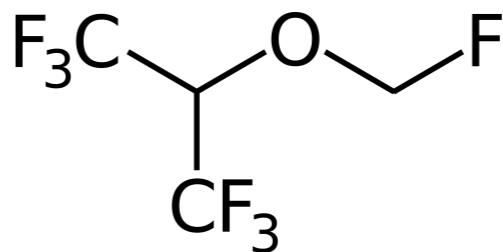


[1]

$\alpha+ / \gamma-$ site

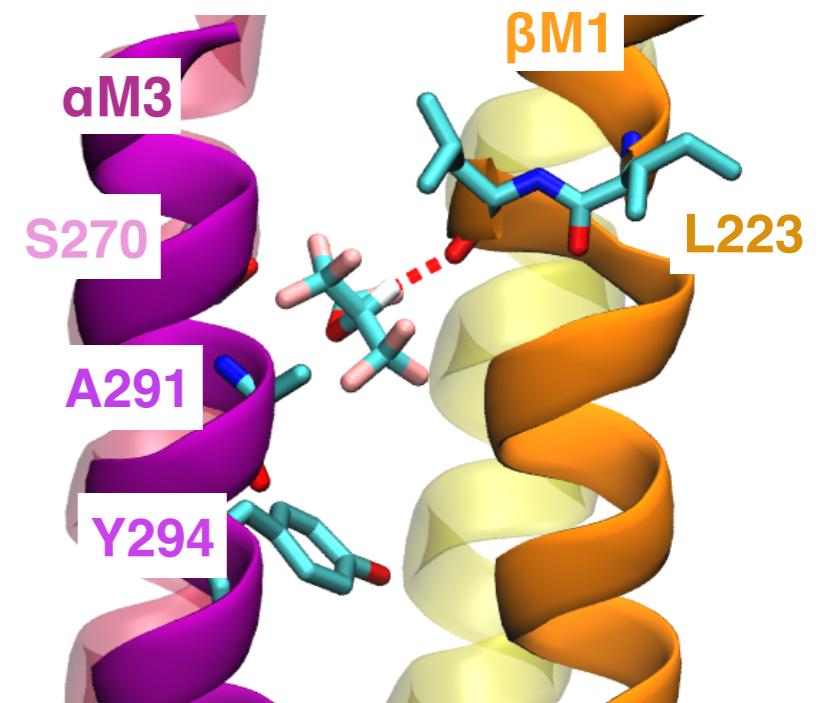
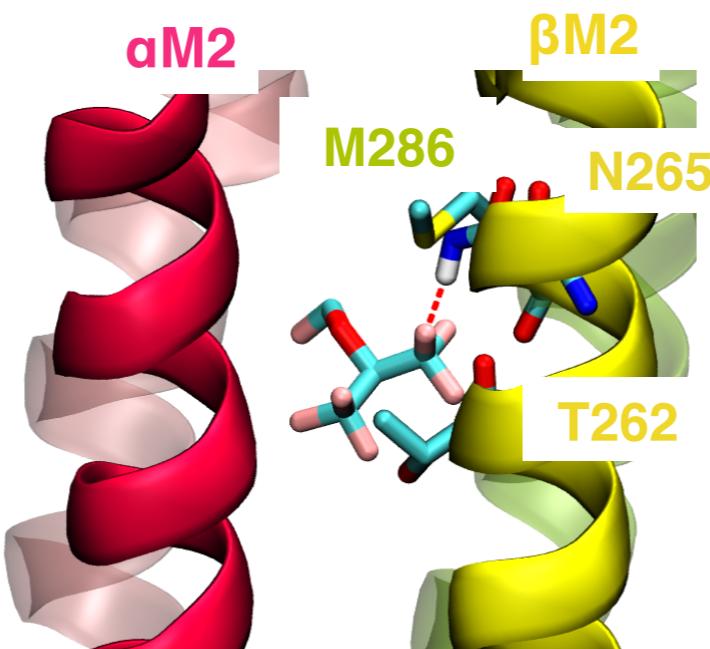
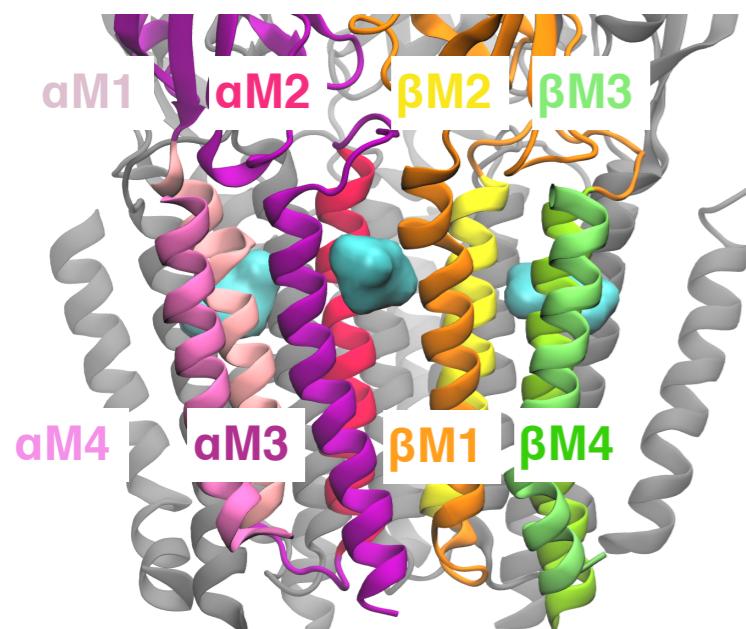
- side-chains are equivalent to those of the higher affinity $\alpha+/\beta-$ site.
- L223($\alpha+$ / $\beta-$) backbone more likely to serve as a H-bond acceptor than the homologous residue, I238.
- M1 backbone helicity depends on other subtle interactions with the helix.

Sevoflurane(SEV) FEP calculations

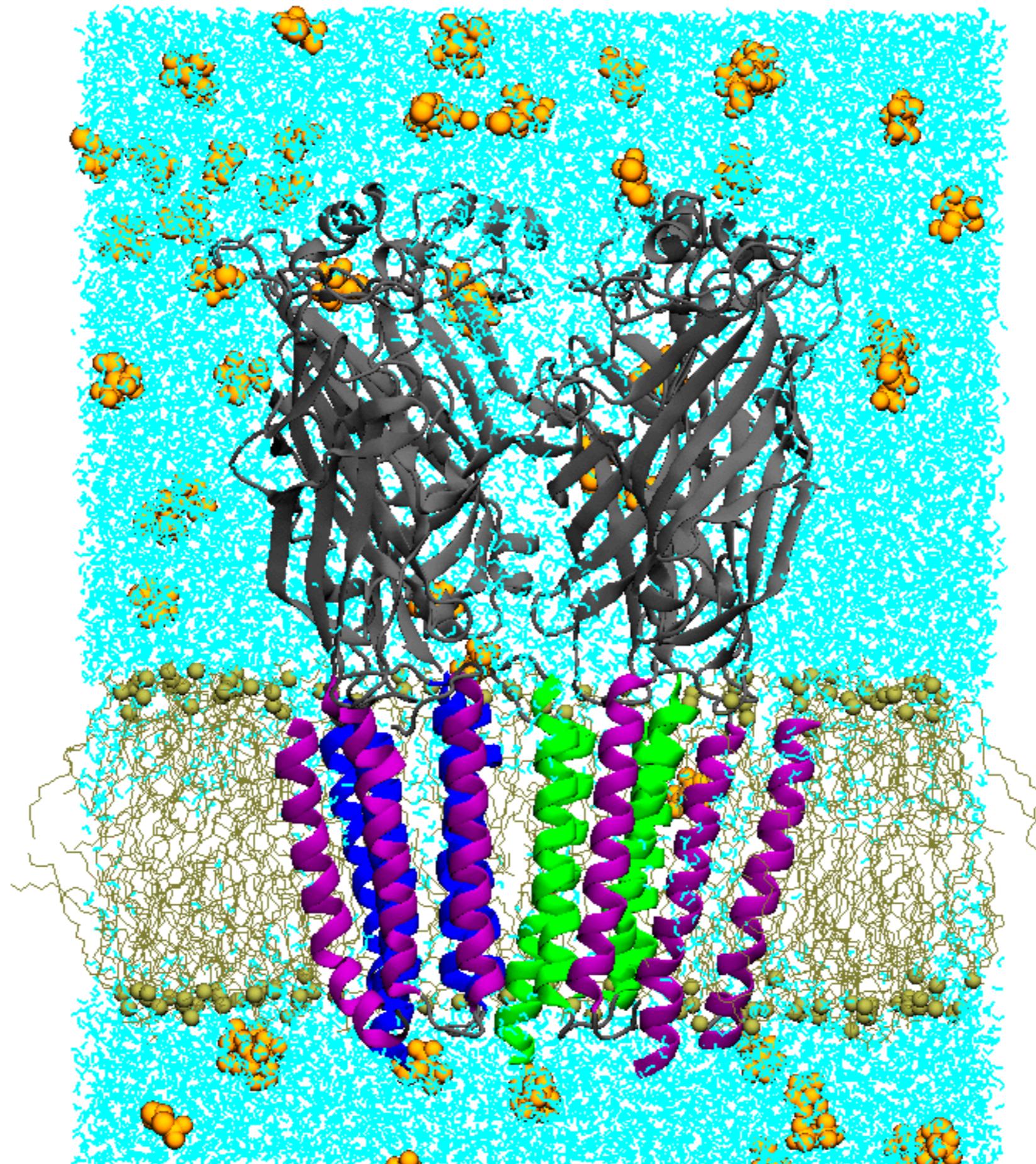


Binding site	β^+ / α^-	α^+ / β^-	β^+ / α^-	γ^+ / β^-	α^+ / γ^-
ΔG_{bind} (kcal/mol)	-7.5	-7	-6	-5	-5
Kd (μM)	3	7	4×10	2×10^2	2×10^2

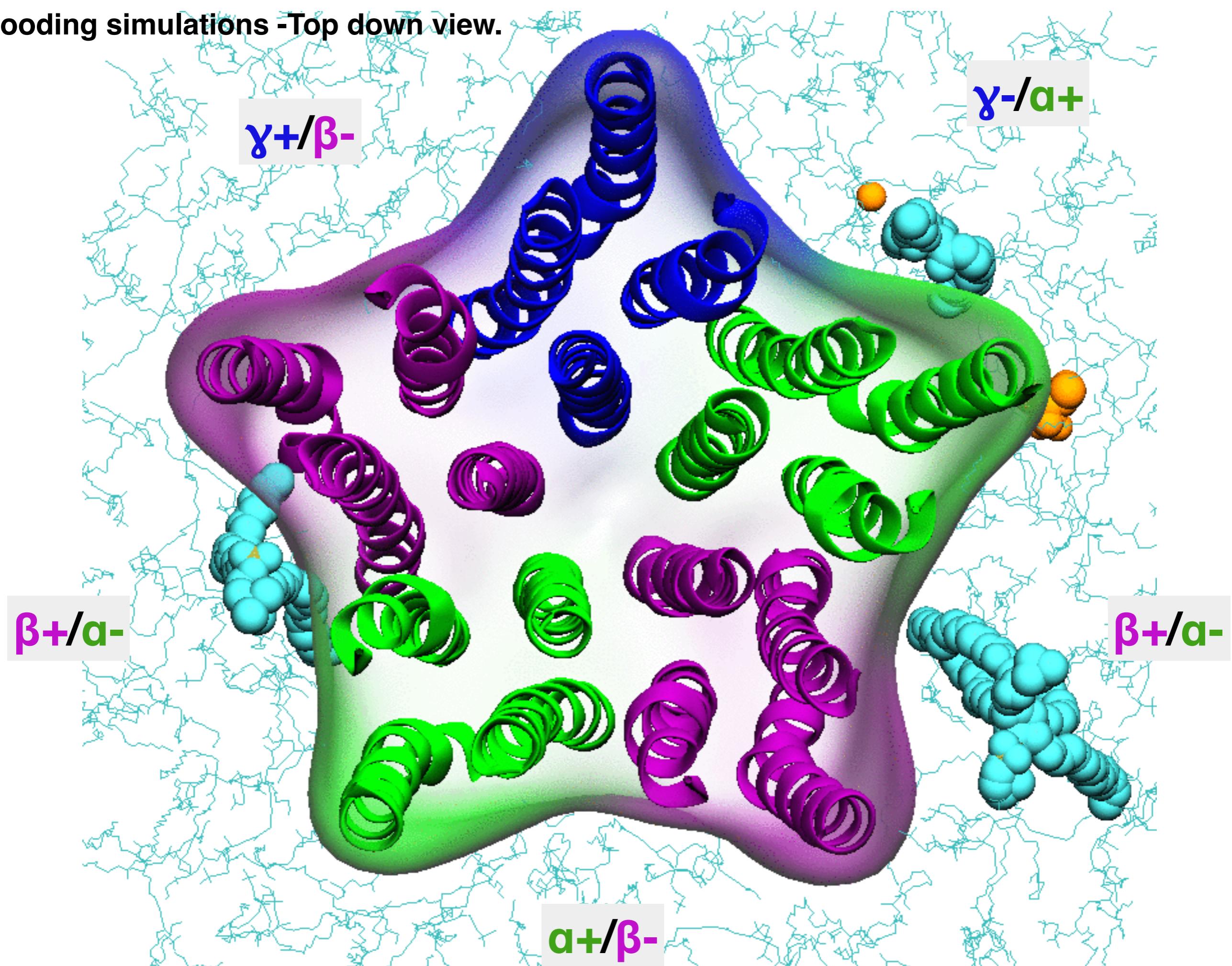
SEV EC₅₀ = 300-1000 μM [Jie Wu et al. Brit. J. Pharm., 1996.]



SEVOFLURANE Flooding simulations- 1.4μs

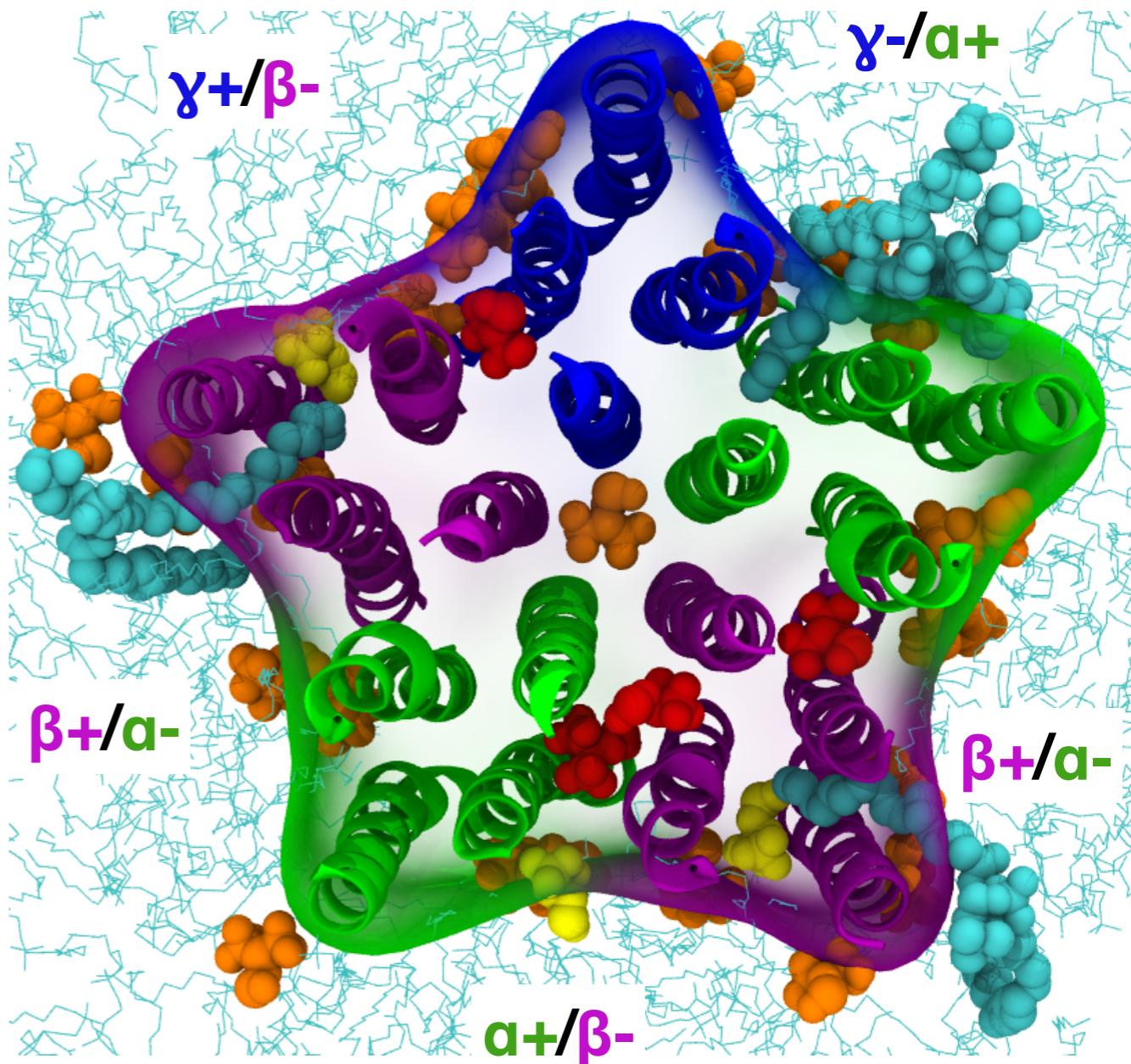


Flooding simulations -Top down view.



Key observations from flooding simulation

- $\alpha+/β-$ site: Highest affinity site, occupied by two SEV molecules.
- $\gamma+/β-$ site: Low affinity site; occurrence of binding and unbinding of ligand.
- $\gamma-/α+$ site; Low affinity site; lipids compete over SEV for binding.
- $β$ - Intra-subunit sites.
- Few SEV molecules stay in close proximity to the bound SEV ligand.
- Pore site: possible ligand binding pathway to enter intersubunit site.



Summary

AFEP predicts $\beta - \alpha$ & $\alpha - \beta$ as higher affinity sites for both PFL and SEV.

PROPOFOL:

- 1) Propofol : AFEP predicts **Kd = 0.01-200 μM** , in reasonable range for getting expt value for potentiation **EC₅₀ = 0.5-5 μM**
- 2) H-bonding favorability and polarity of the site confers affinity, but sites that are too hydrophilic result in lower affinities due to competition with water.

SEVOFLURANE:

- 1) Sevoflurane : AFEP predicts **Kd = 300-1000 μM** , in reasonable range for getting expt value for potentiation **EC₅₀ = 3-200 μM**
- 2) Flooding simulations results substantiates the location of the inter-subunits binding sites, reveals other binding sites and possible binding pathway.

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