

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

CCIB RETREAT

2016

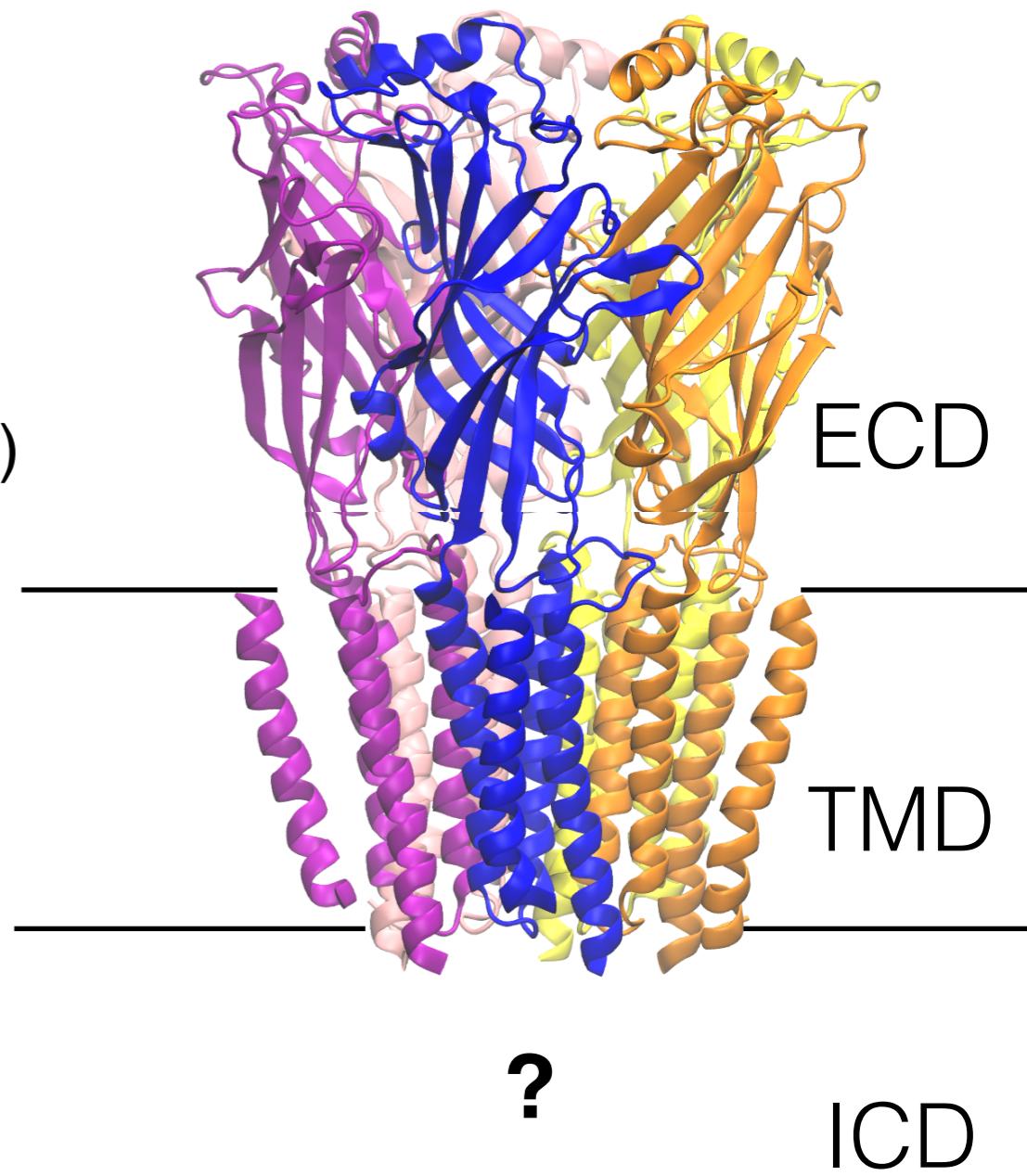
Sruthi Murlidaran



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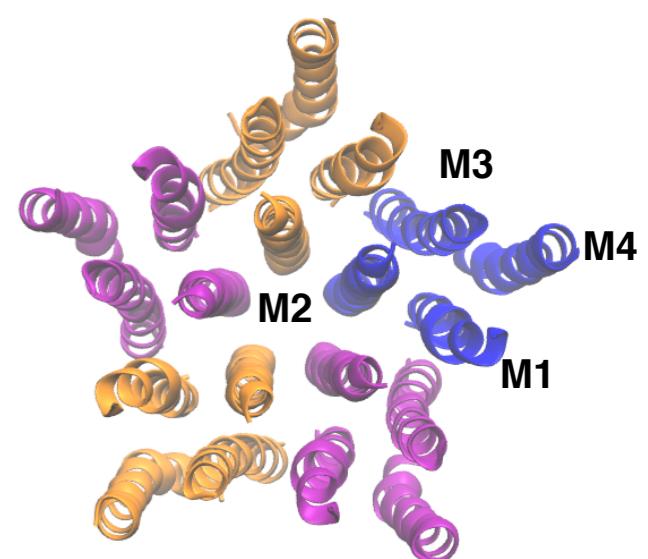
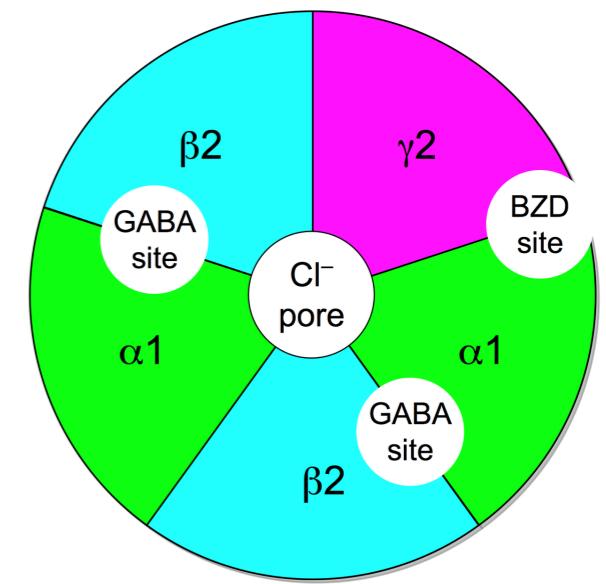
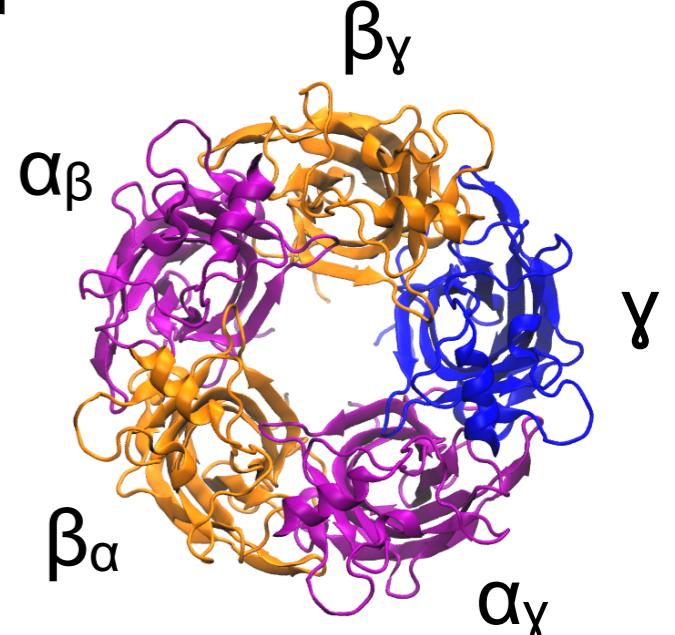
GABA_A Receptor

- 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.

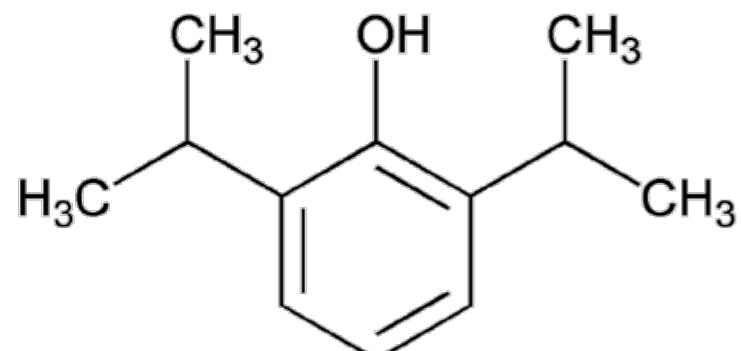


General Anesthetics

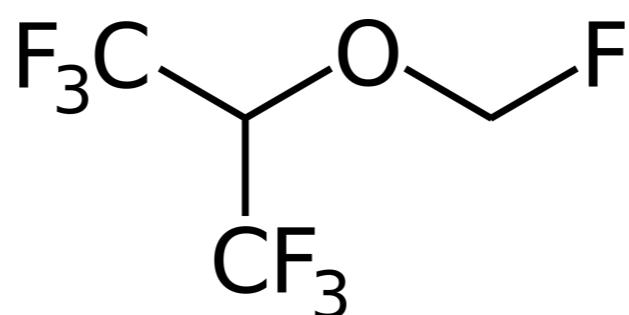
- Primary targets of anesthetics are ion channels, especially GABAA Receptors. [Franks *et al* (1989);Krasowski *et al* (1999)]
- Most GAs potentiate or activate GABAA receptors at clinical concentrations (μM - mM)
- Anesthetics classified under route of administrations:

inhalational (IN) → Isoflurane, Desflurane, Sevoflurane

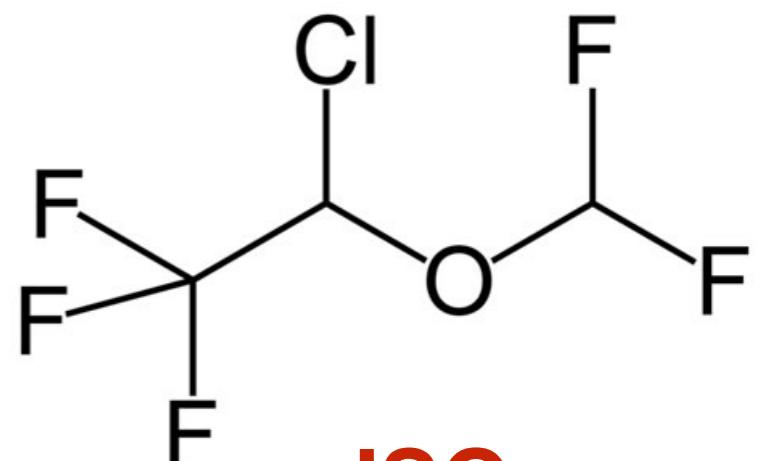
intravenous (IV) → Propofol, Etomidate



PFL



SEV



ISO

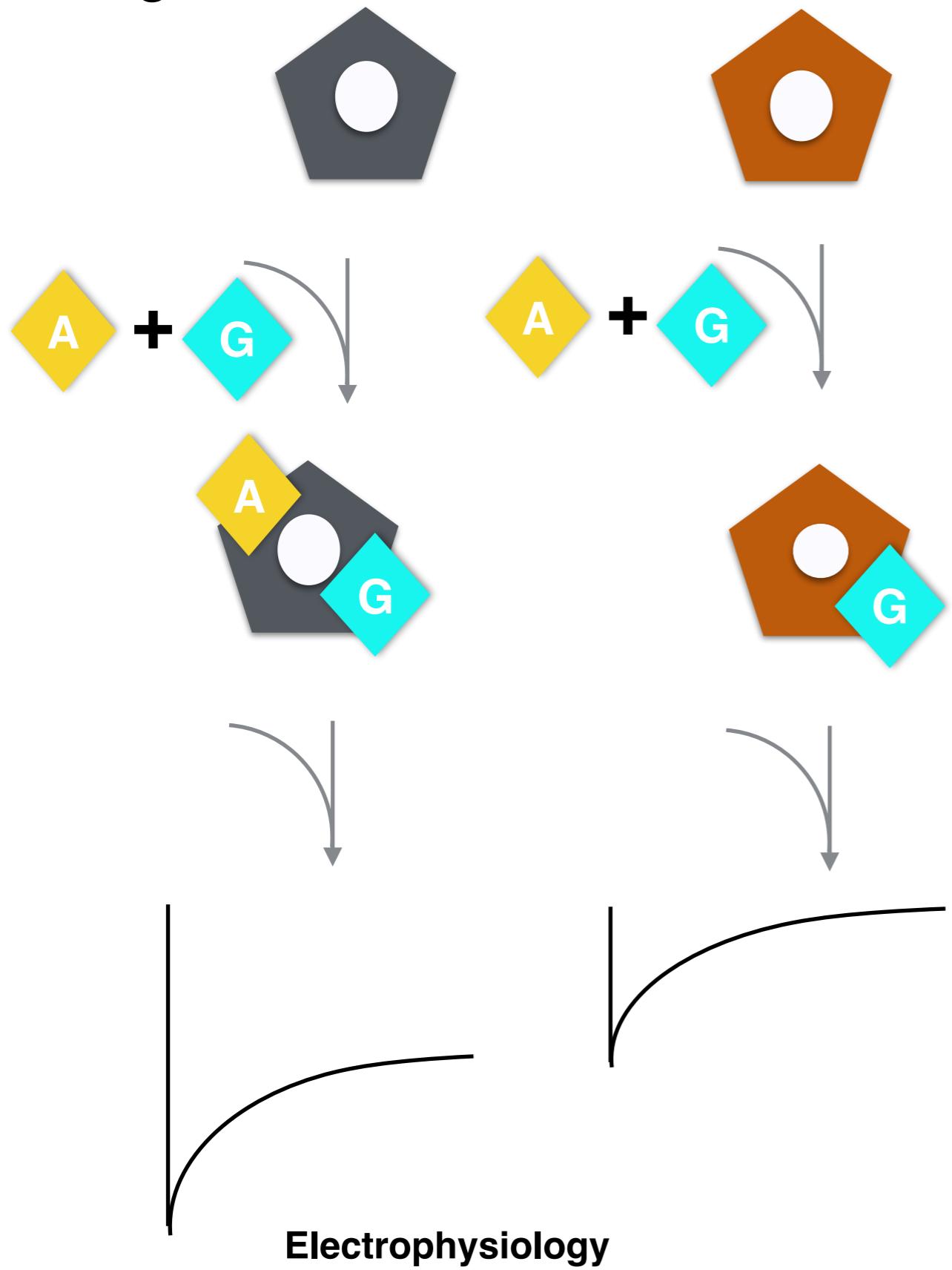
General anesthetics: Main question

Where do GAs bind on GABA_A receptors?

- 1) Which sites are occupied at clinical concentrations?
- 2) What are the microscopic origins of affinity differences?

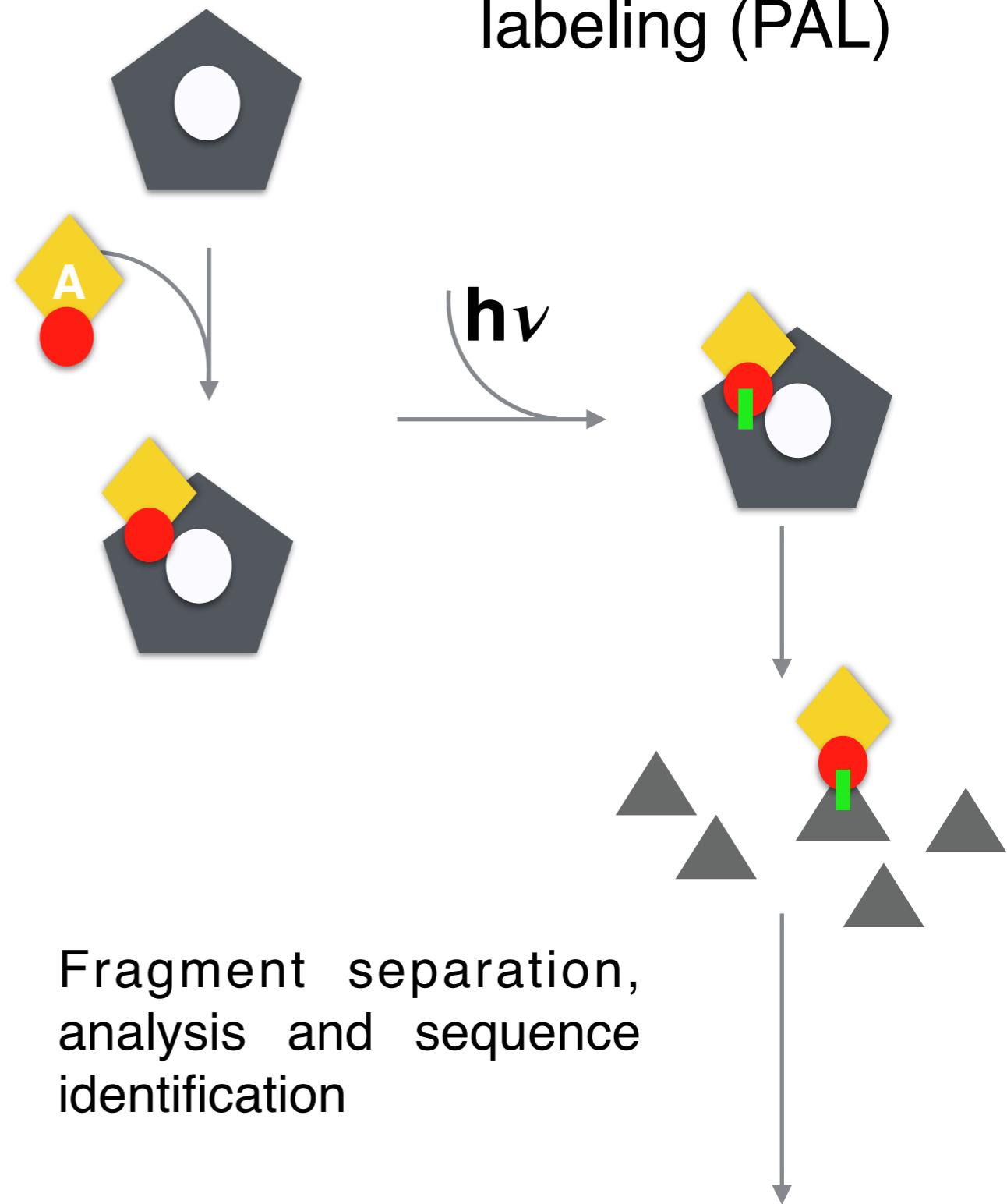
Experimental Techniques

Mutagenesis



Electrophysiology

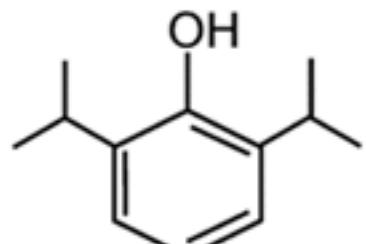
Photoaffinity labeling (PAL)



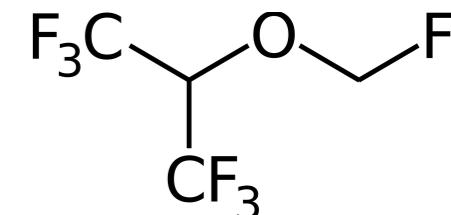
Fragment separation,
analysis and sequence
identification

Photo-labelled residue

Sites occupied at clinical concentrations: Experimental Insights

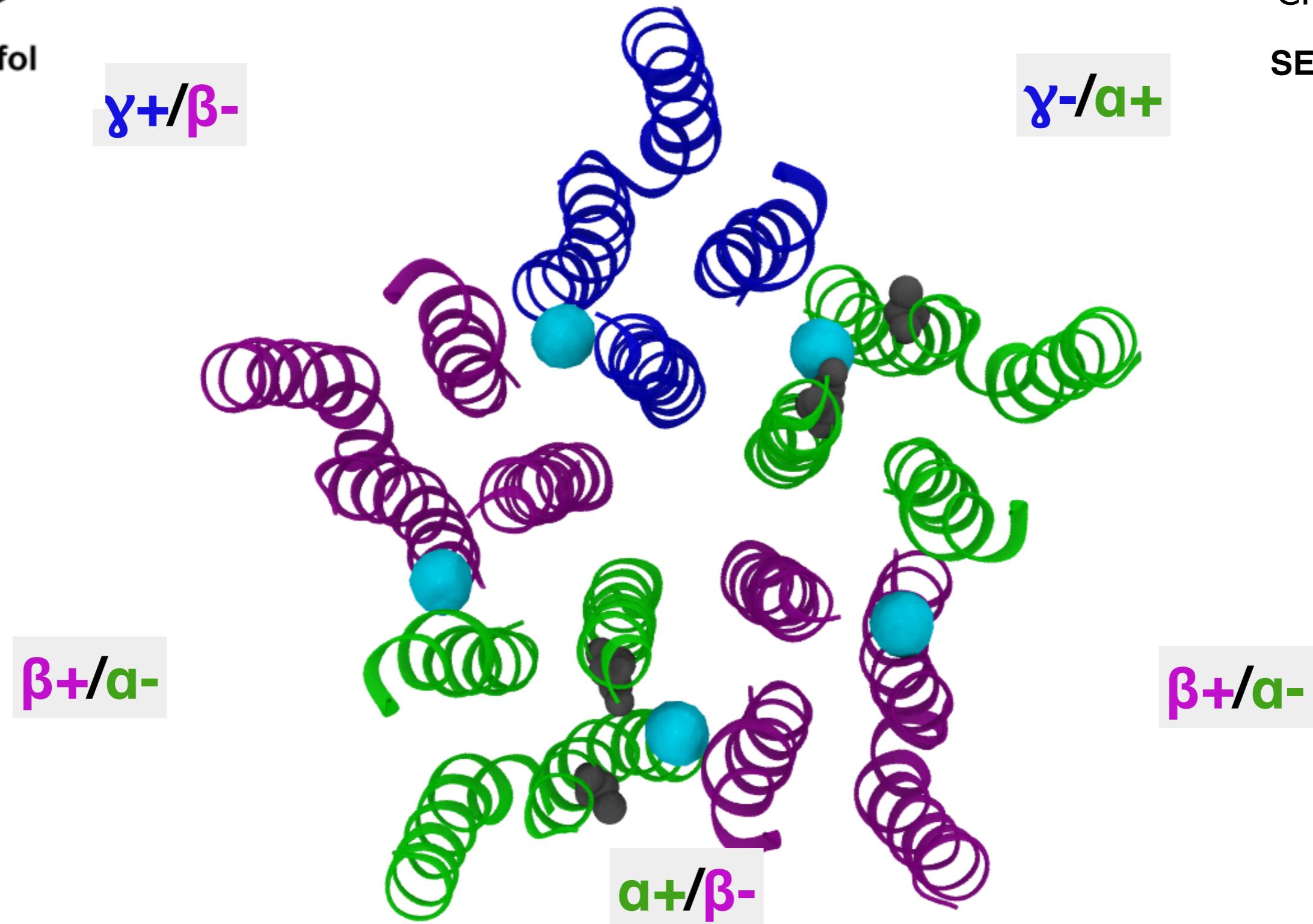


Propofol



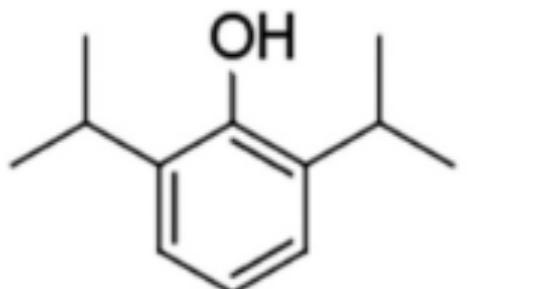
SEV

PPTESEV¹ Photocatalyzed residue²
Mutated residues³ Residues⁴

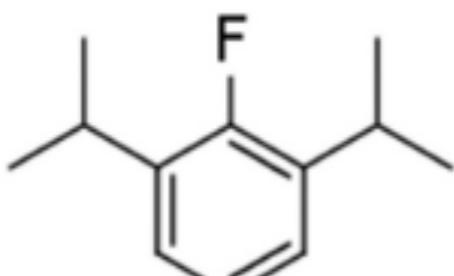


Microscopic origins of affinity differences: Experimental Insights

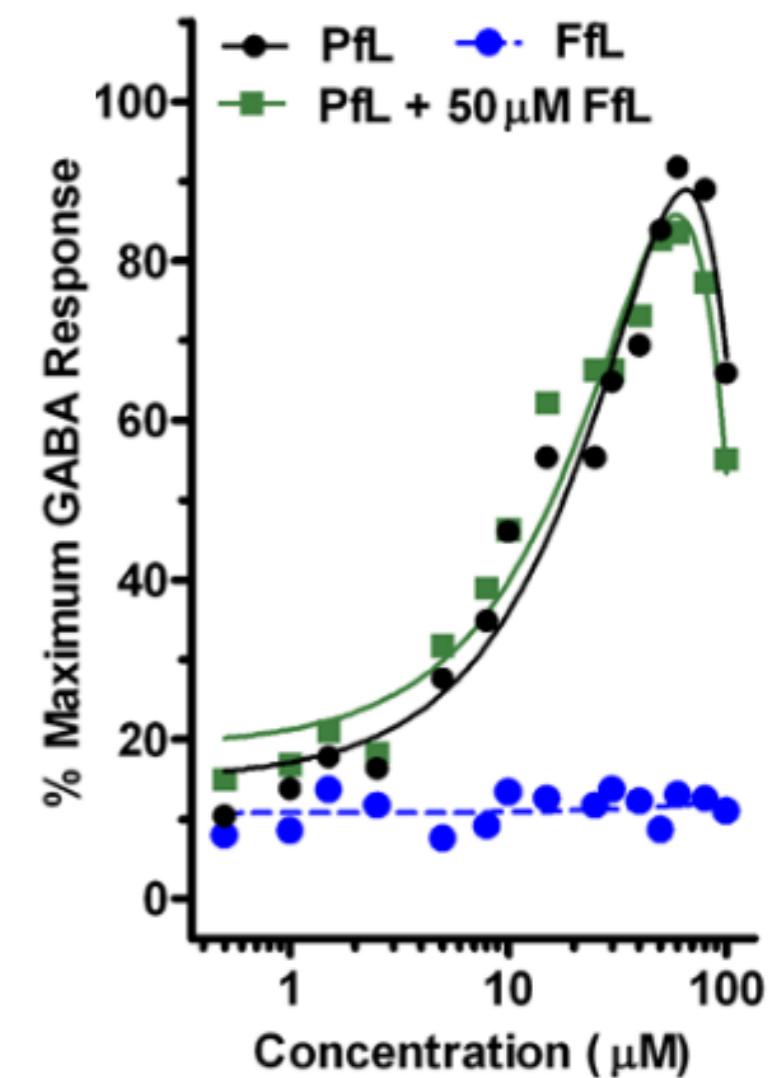
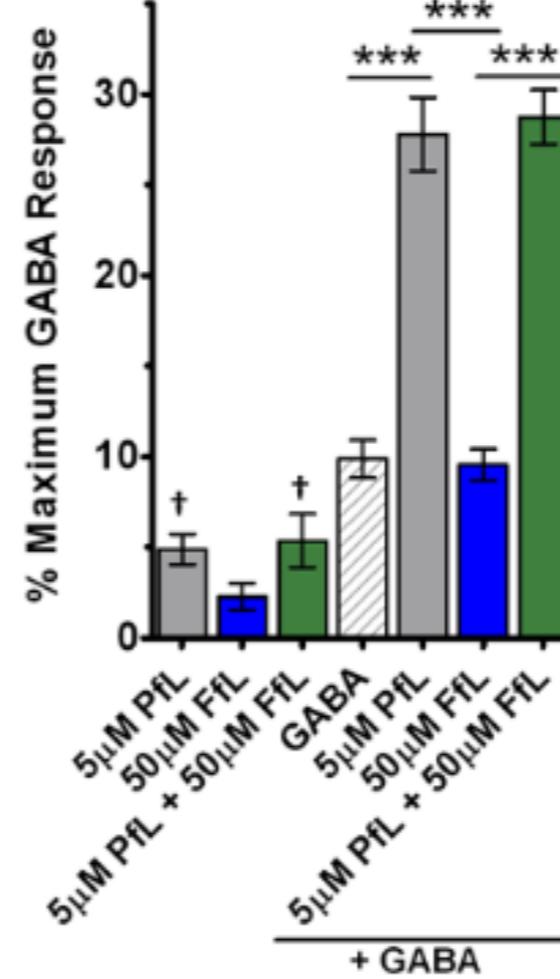
Hydrogen Bonding



Propofol



Fropofol

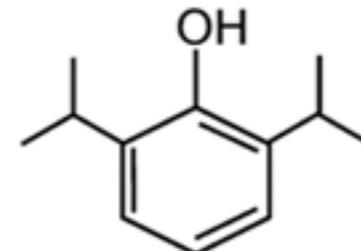


- Fropofol did not affect PFL positive modulation or direct activation of GABA(A) receptor currents.
- H-bond - critical molecular recognition feature for PFL binding sites.

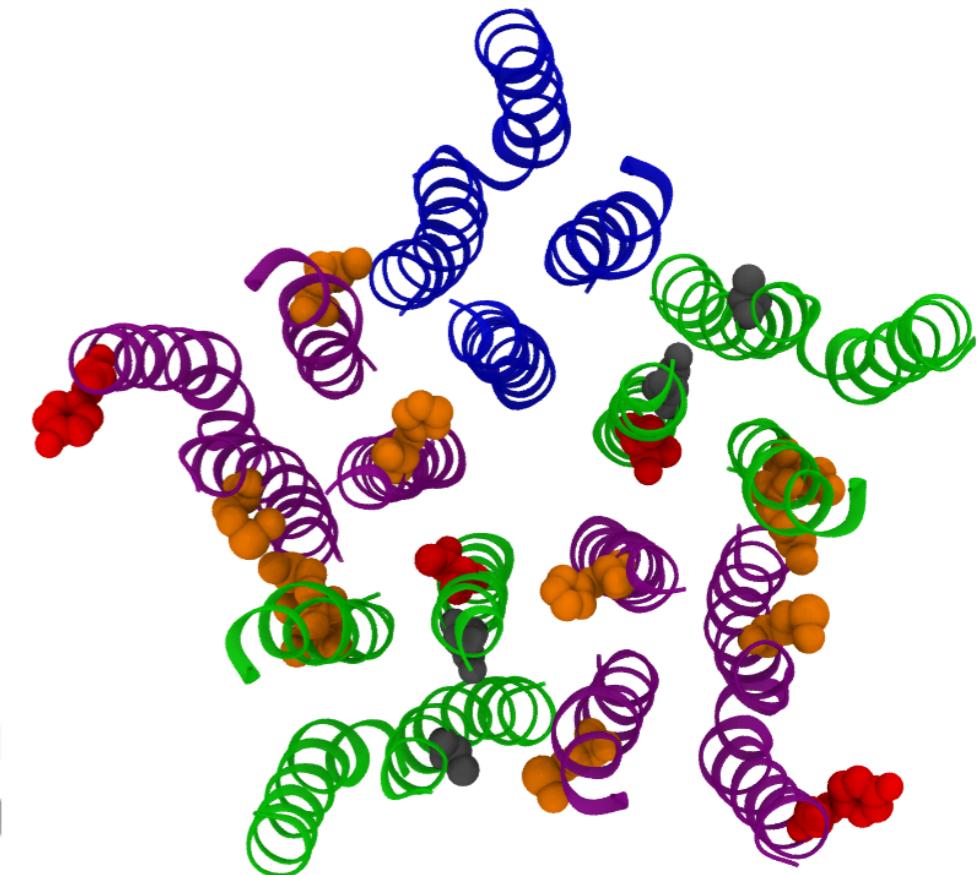
Experiments: Technical limitations

PAL

- Chemical probe - Change chemical properties of anesthetic analog.
- Anesthetic analog - specificity to certain am.acid residues.



Propofol



Mutagenesis

- can cause undesired structural changes - loss of function
- can mimic effects of bound anesthetic - misleading results
- Multiple binding sites - lead to misleading results.

Experiments: Fundamental limitations

PAL And Mutagenesis

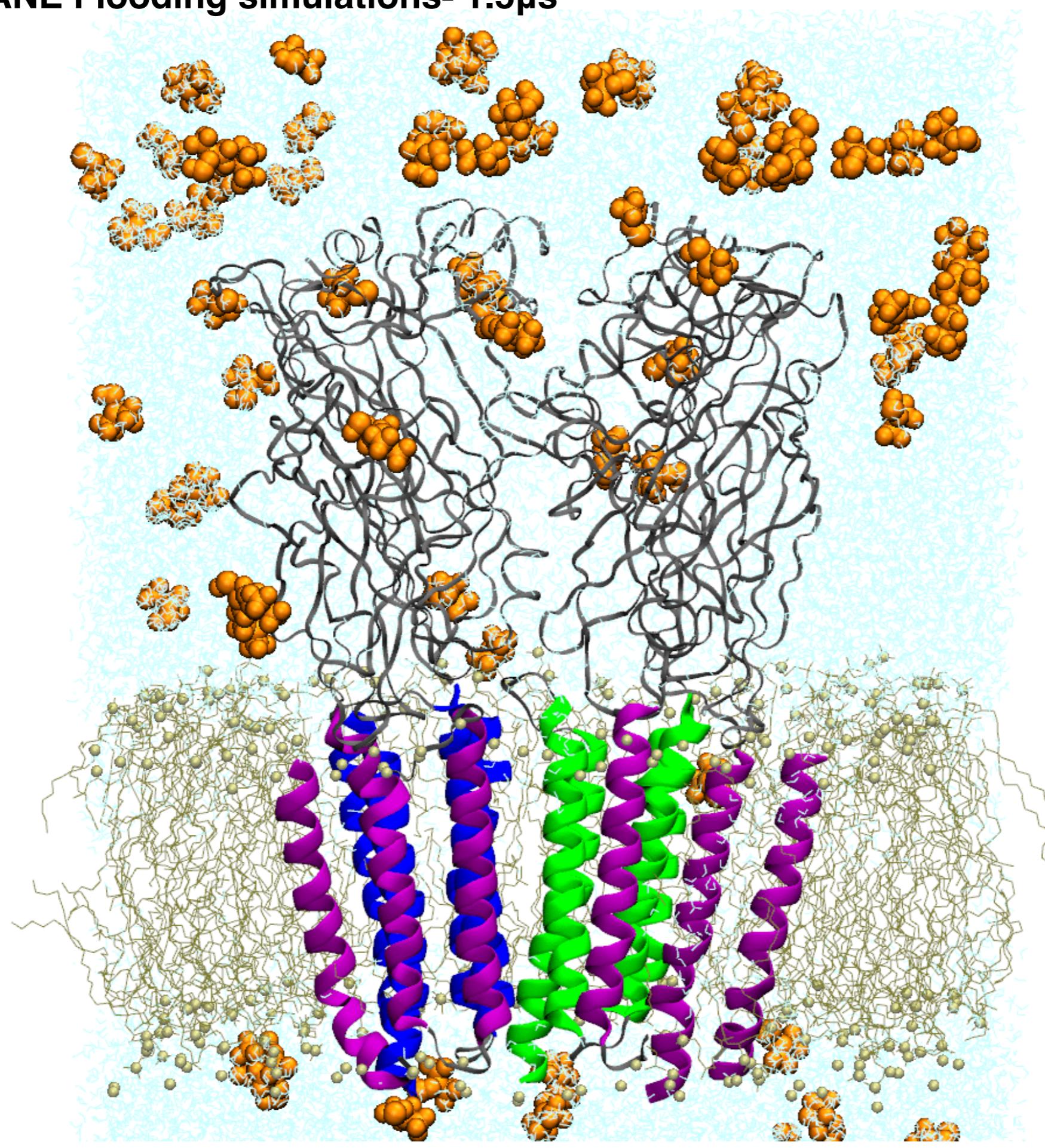
- cannot identify specific protein-anesthetics interactions
- Mutagenesis - cannot take into account protein-backbone-anesthetics interactions.
- cannot identify affinity of anes. for a specific site.

Computational study

Different types of simulations employed

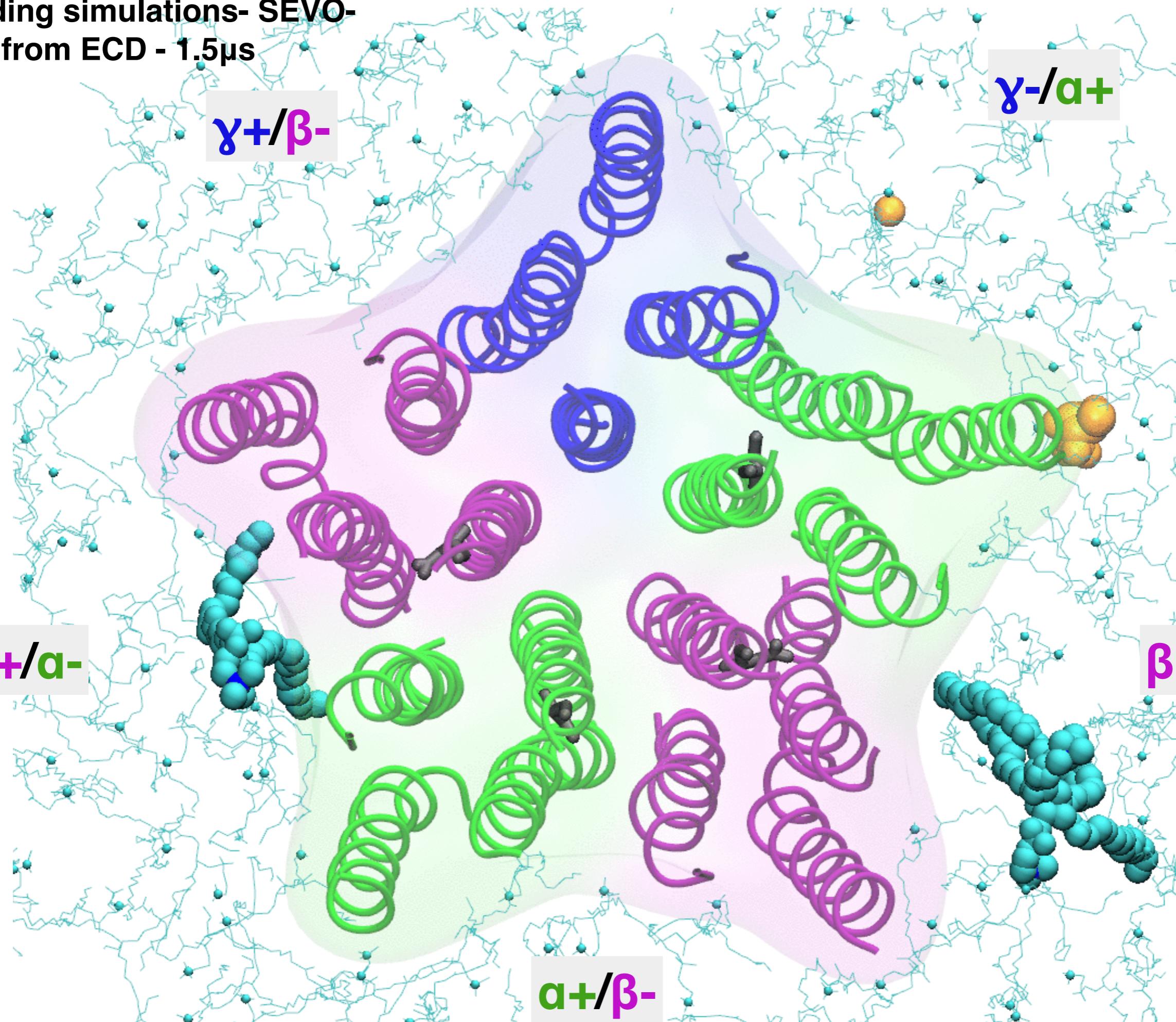
- **Flooding simulations** – Unbiased simulations; lets anesthetic explore the protein system that mimics in-vivo environment.
- Traditional MD of docked intersubunit conformations – obtain starting configuration of protein-ligand complex.
- **Alchemical Free Energy Perturbation(AFEP) simulations :** Measures affinity by calculating free energy difference between two alternate states.

SEVOFLURANE Flooding simulations- 1.5μs

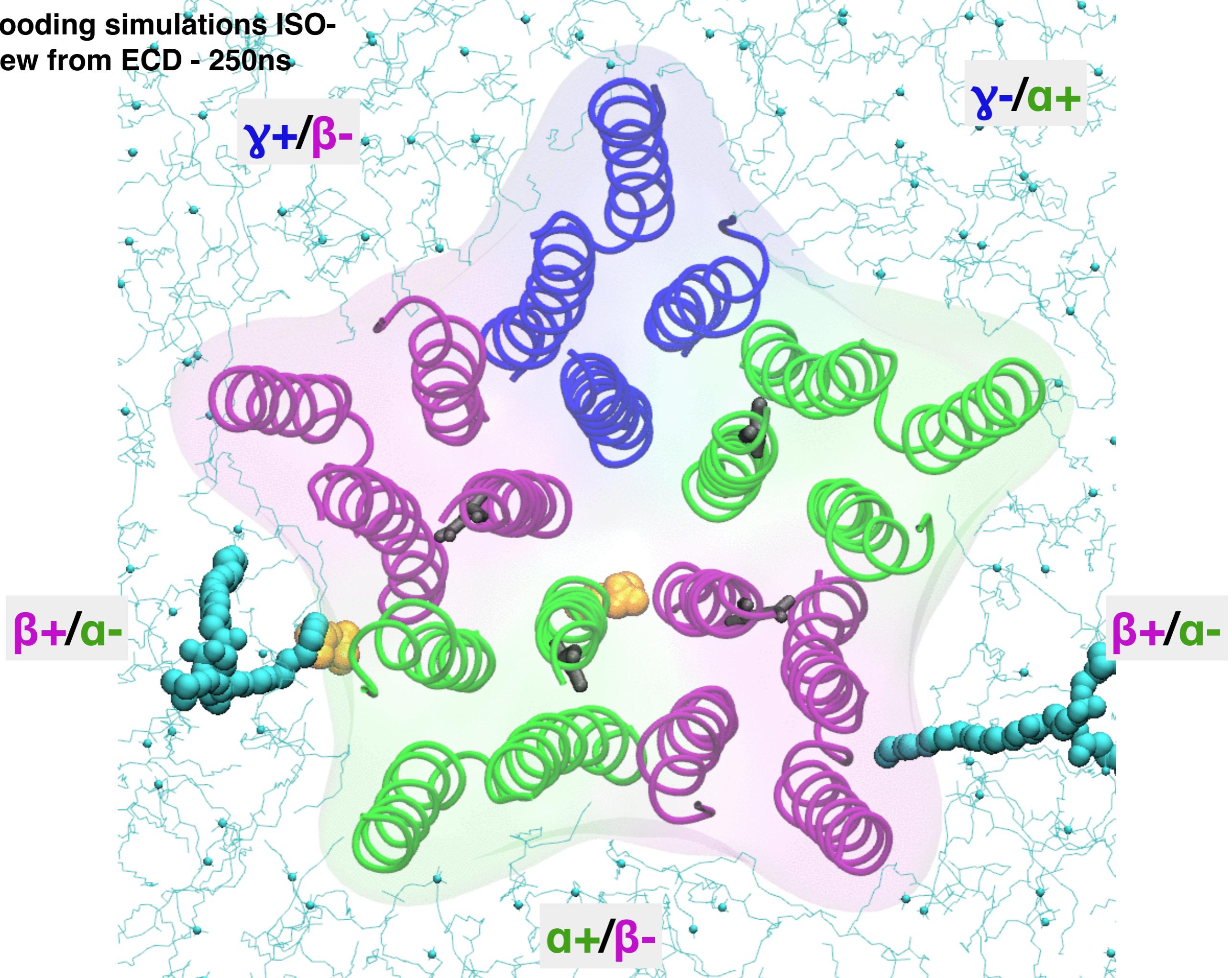


Flooding simulations- SEVO-

View from ECD - 1.5μs

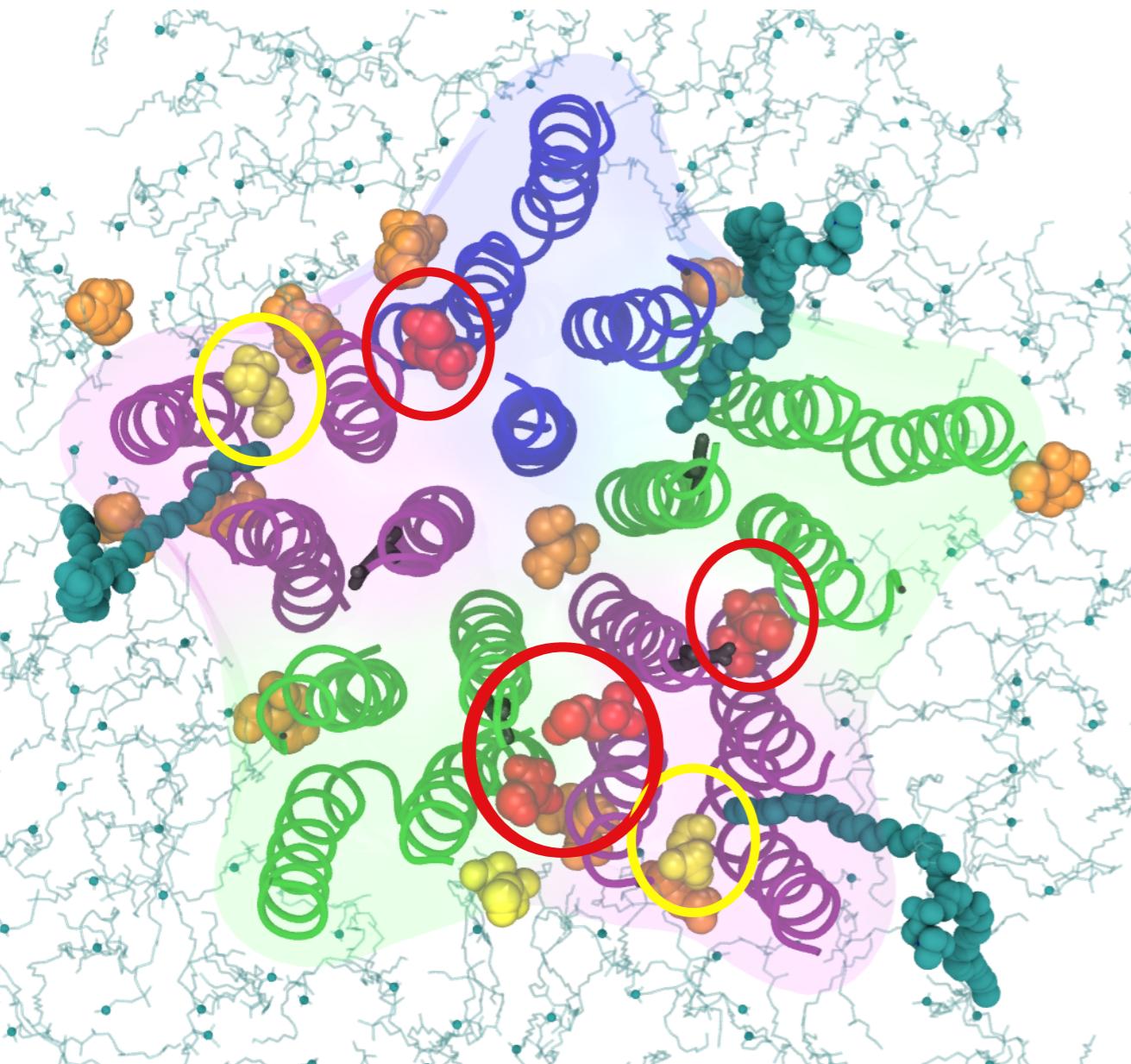


Flooding simulations ISO- View from ECD - 250ns

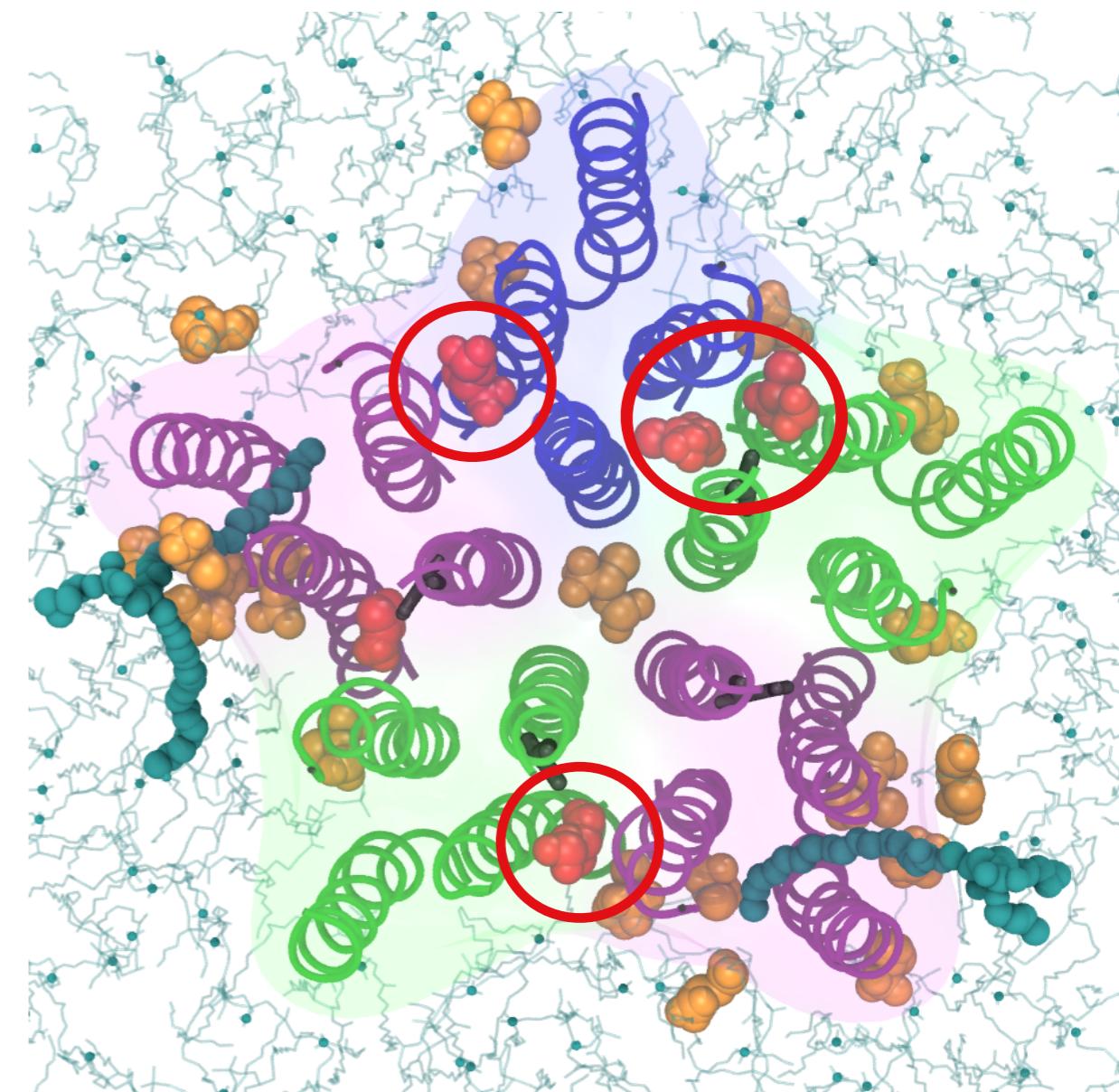


Possible sites for inhaled anesthetics from flooding MD

SEV

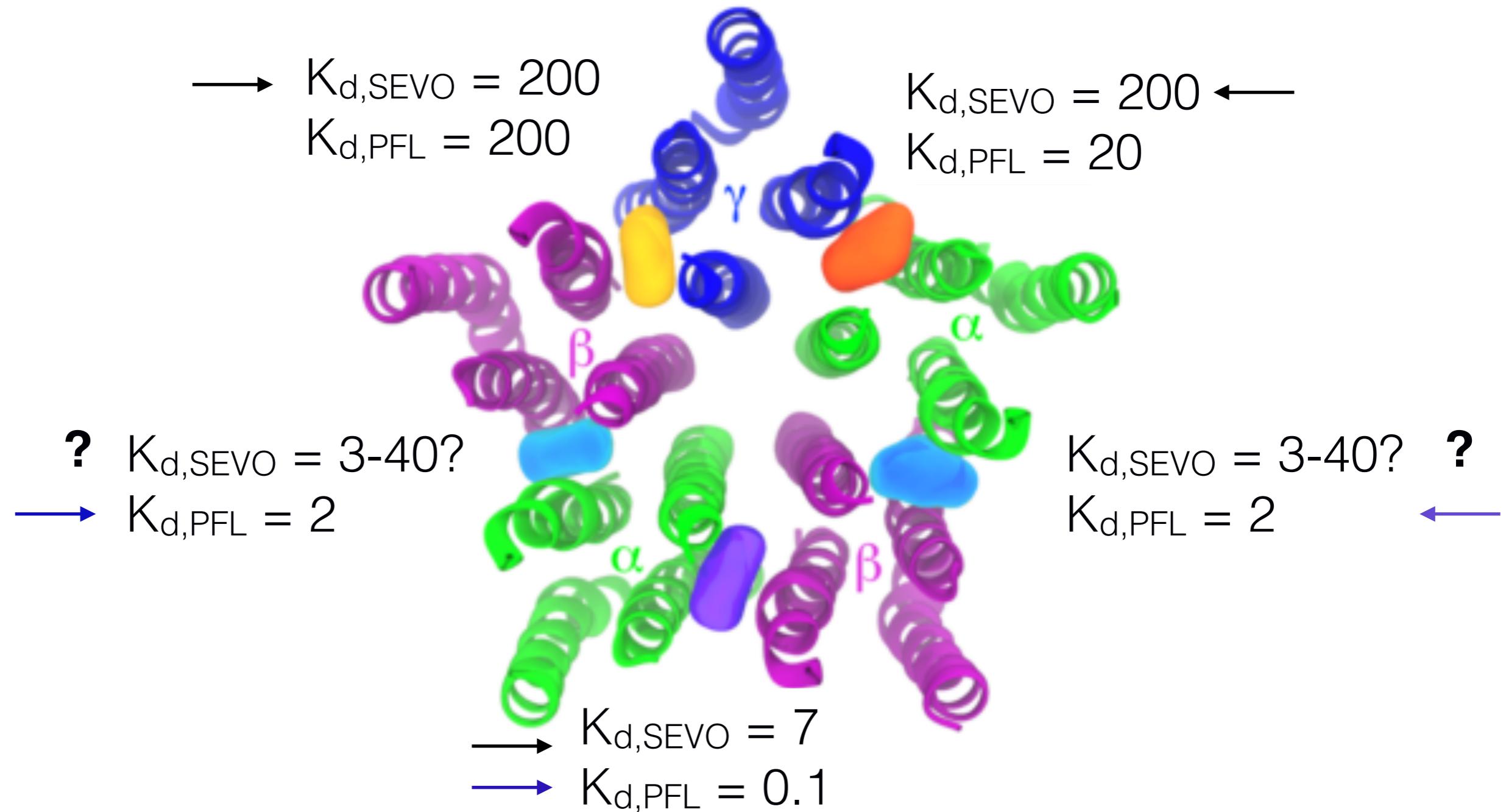


ISO



- Multiple occupancy -
- ~~B₁ + B₂ + B₃ + B₄ + B₅ + B₆ + B₇ + B₈ + B₉ + B₁₀ + B₁₁ + B₁₂ + B₁₃ + B₁₄ + B₁₅ + B₁₆ + B₁₇ + B₁₈ + B₁₉ + B₂₀ + B₂₁ + B₂₂ + B₂₃ + B₂₄ + B₂₅ + B₂₆ + B₂₇ + B₂₈ + B₂₉ + B₃₀ + B₃₁ + B₃₂ + B₃₃ + B₃₄ + B₃₅ + B₃₆ + B₃₇ + B₃₈ + B₃₉ + B₄₀ + B₄₁ + B₄₂ + B₄₃ + B₄₄ + B₄₅ + B₄₆ + B₄₇ + B₄₈ + B₄₉ + B₅₀ + B₅₁ + B₅₂ + B₅₃ + B₅₄ + B₅₅ + B₅₆ + B₅₇ + B₅₈ + B₅₉ + B₆₀ + B₆₁ + B₆₂ + B₆₃ + B₆₄ + B₆₅ + B₆₆ + B₆₇ + B₆₈ + B₆₉ + B₇₀ + B₇₁ + B₇₂ + B₇₃ + B₇₄ + B₇₅ + B₇₆ + B₇₇ + B₇₈ + B₇₉ + B₈₀ + B₈₁ + B₈₂ + B₈₃ + B₈₄ + B₈₅ + B₈₆ + B₈₇ + B₈₈ + B₈₉ + B₉₀ + B₉₁ + B₉₂ + B₉₃ + B₉₄ + B₉₅ + B₉₆ + B₉₇ + B₉₈ + B₉₉ + B₁₀₀ + B₁₀₁ + B₁₀₂ + B₁₀₃ + B₁₀₄ + B₁₀₅ + B₁₀₆ + B₁₀₇ + B₁₀₈ + B₁₀₉ + B₁₁₀ + B₁₁₁ + B₁₁₂ + B₁₁₃ + B₁₁₄ + B₁₁₅ + B₁₁₆ + B₁₁₇ + B₁₁₈ + B₁₁₉ + B₁₂₀ + B₁₂₁ + B₁₂₂ + B₁₂₃ + B₁₂₄ + B₁₂₅ + B₁₂₆ + B₁₂₇ + B₁₂₈ + B₁₂₉ + B₁₃₀ + B₁₃₁ + B₁₃₂ + B₁₃₃ + B₁₃₄ + B₁₃₅ + B₁₃₆ + B₁₃₇ + B₁₃₈ + B₁₃₉ + B₁₄₀ + B₁₄₁ + B₁₄₂ + B₁₄₃ + B₁₄₄ + B₁₄₅ + B₁₄₆ + B₁₄₇ + B₁₄₈ + B₁₄₉ + B₁₅₀ + B₁₅₁ + B₁₅₂ + B₁₅₃ + B₁₅₄ + B₁₅₅ + B₁₅₆ + B₁₅₇ + B₁₅₈ 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Binding affinities from FEP (μM)

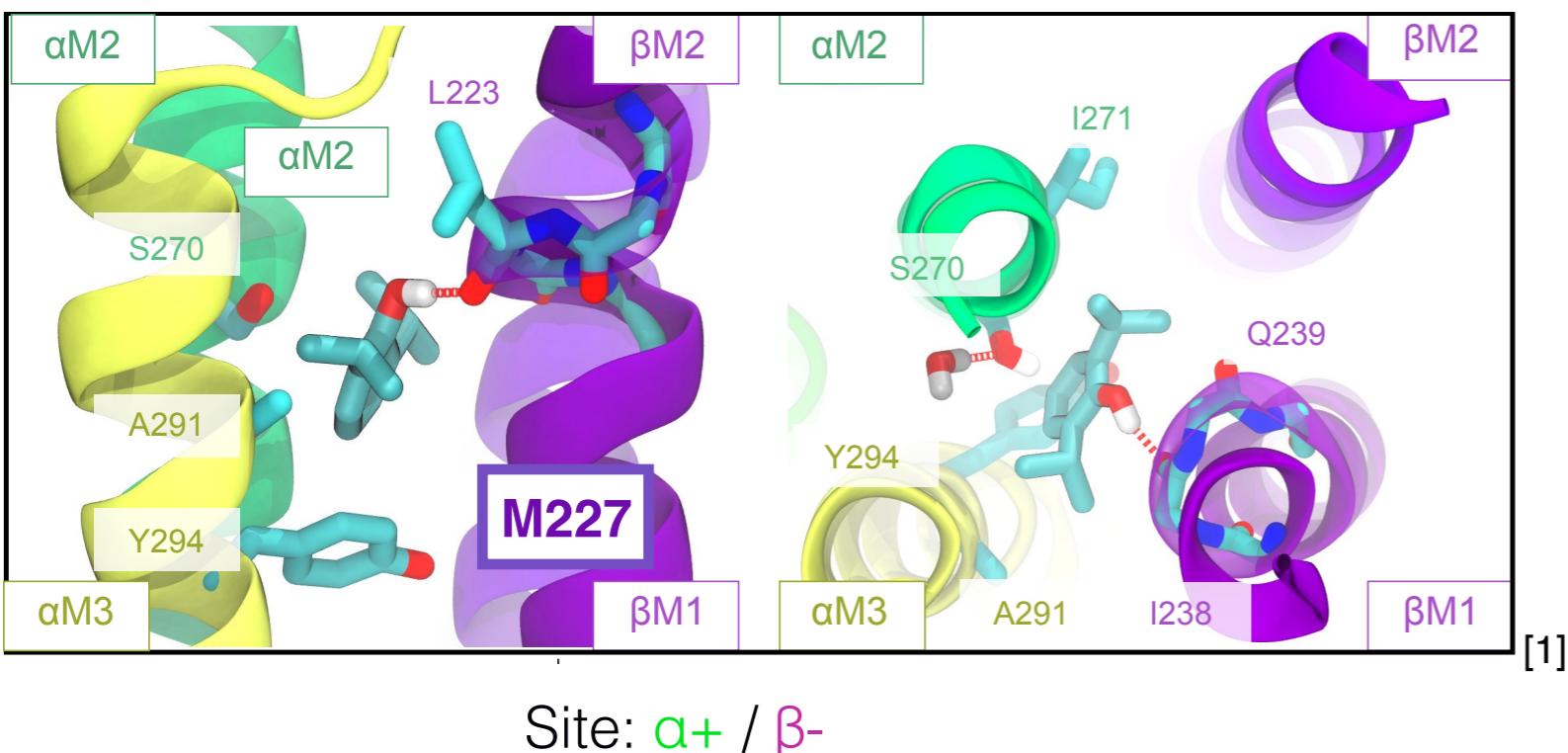


SEV EC₅₀ = 300-1000 μM [Jie Wu et al. Brit. J. Pharm., 1996.]

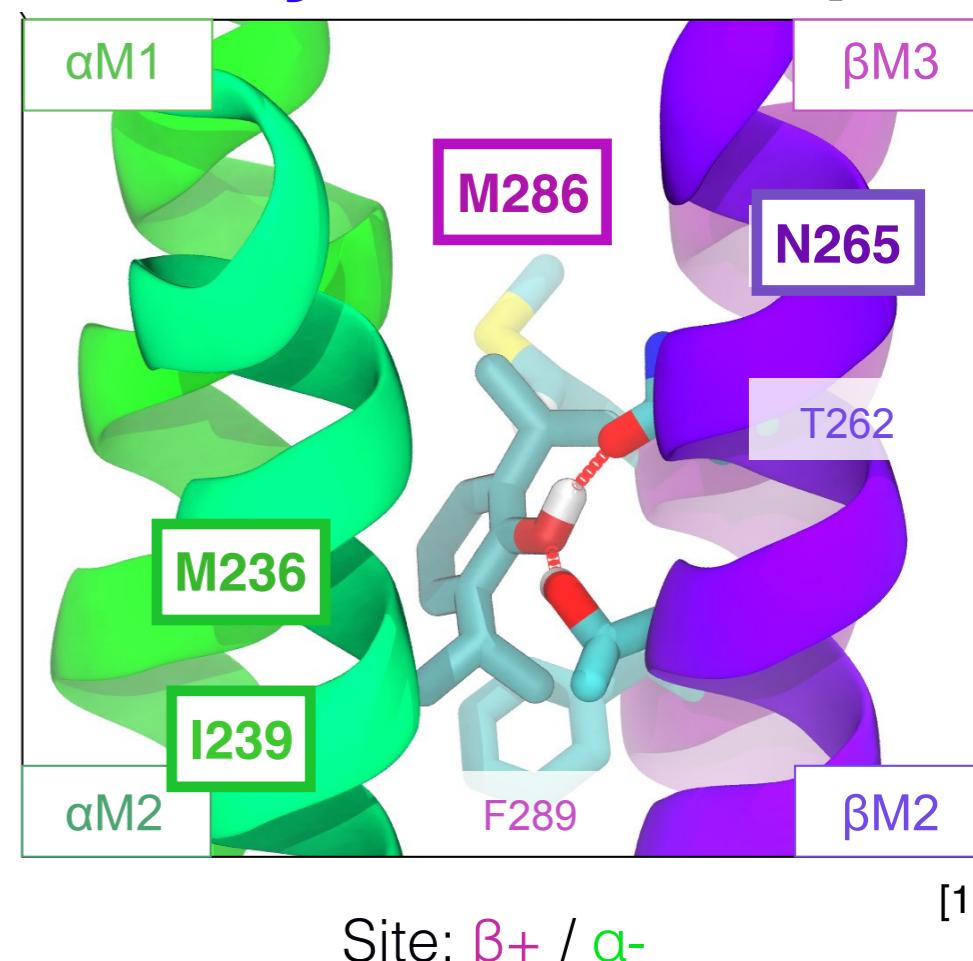
PFL EC₅₀ = 0.5-5 μM Adora et al. Brit. J. Pharm., 1995.

Comparison with photolabeling : propofol

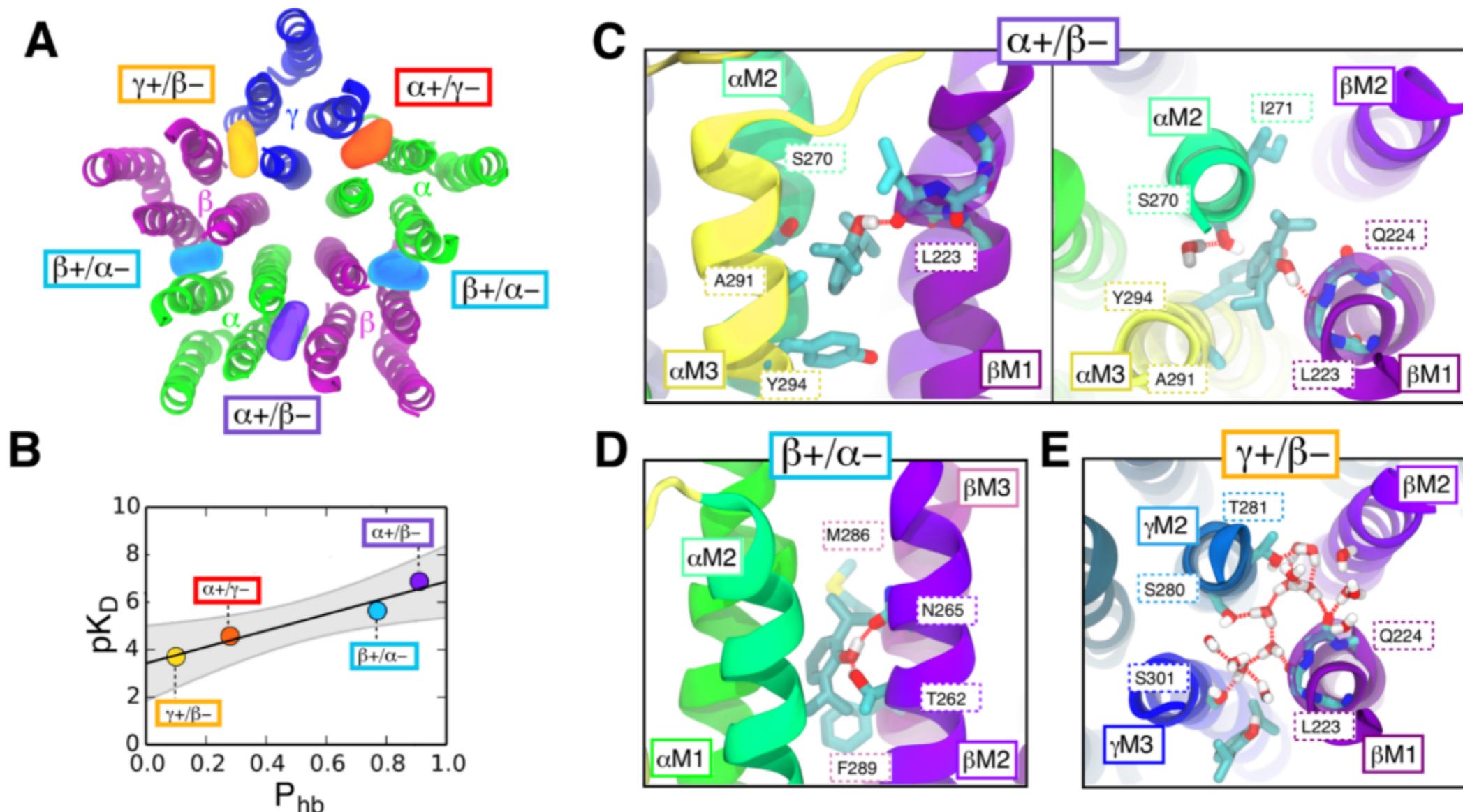
Highest affinity site: $K_d = 0.1 \mu\text{M}$



Second highest affinity site: $K_d = 2 \mu\text{M}$



Microscopic origins of affinity differences: simulation insights



- **$\alpha+$ / $\beta-$ site**: 7 polar residue sidechains; PFL forms persistent H-bond with backbone carbonyl group of Leu223.
- **$\beta+$ / $\alpha-$ site**: 7 polar residue sidechains; PFL forms alternate H-bonds between N265 and T262.
- **$\gamma+$ / $\beta-$ site**: 8 polar residue sidechains; favor H-bond formation with water cluster.

Summary

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

- Photolabelling and Mutagenesis studies have suggested sites on α/β interface.
 - PFL - N265 - reported in expts, identified as important h-bonding partner in the $\beta - \alpha$ site.
 - SEV - S270, A291 - reported in expts., also found in $\alpha - \beta$ binding site.
- AFEP determines -
 - **3 intersubunit sites** being occupied by **PFL** at clinical conc.
 - **3-5 intersubunit sites** being occupied by **SEV** at clinical conc.
- 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.

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