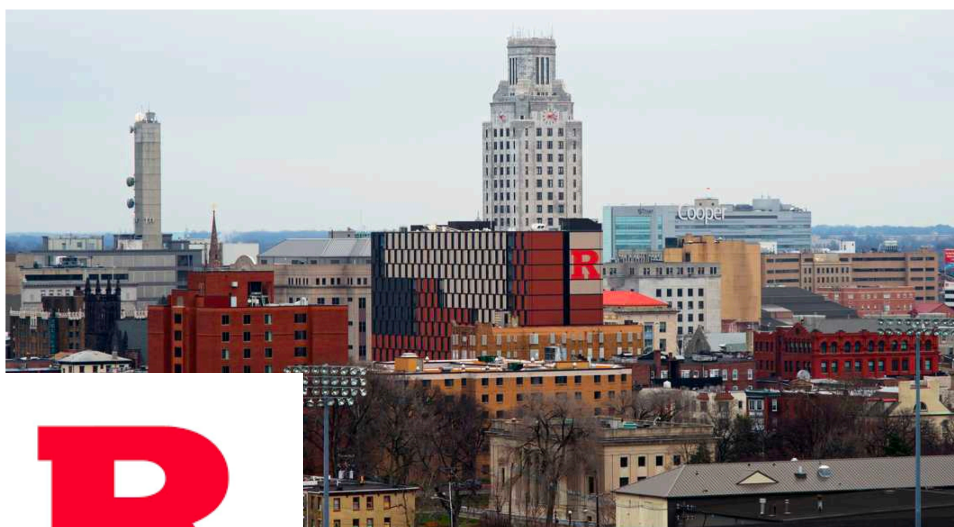
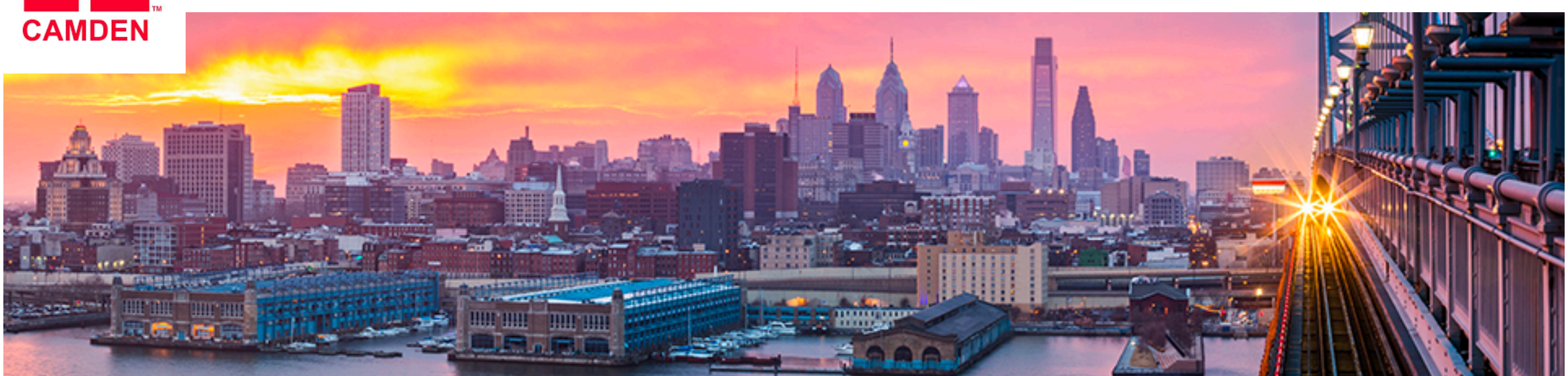
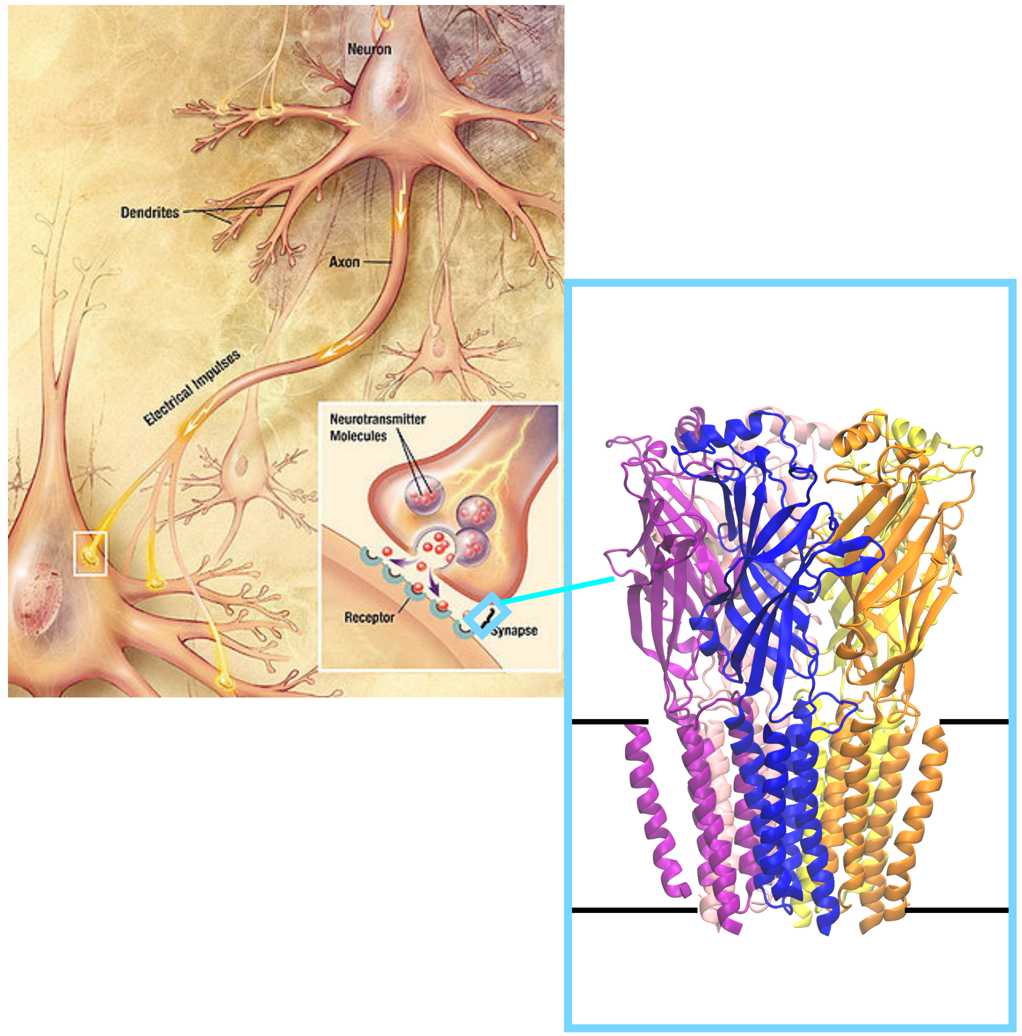
Alchemy-based predictions for binding affinities of lipophilic ligands and membrane proteins: *obstacles, solutions, and outcomes*

Grace Brannigan
Center for Computational & Integrative Biology
Rutgers University - Camden





main target: pentameric gated ligand channels (pLGICs)



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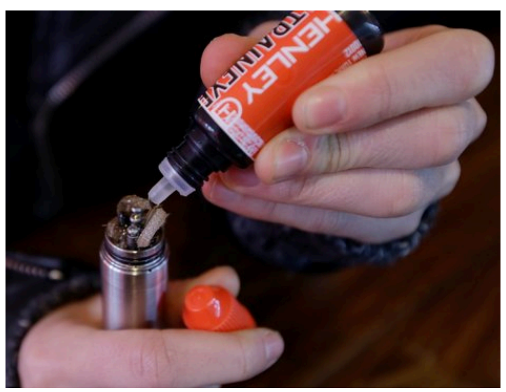
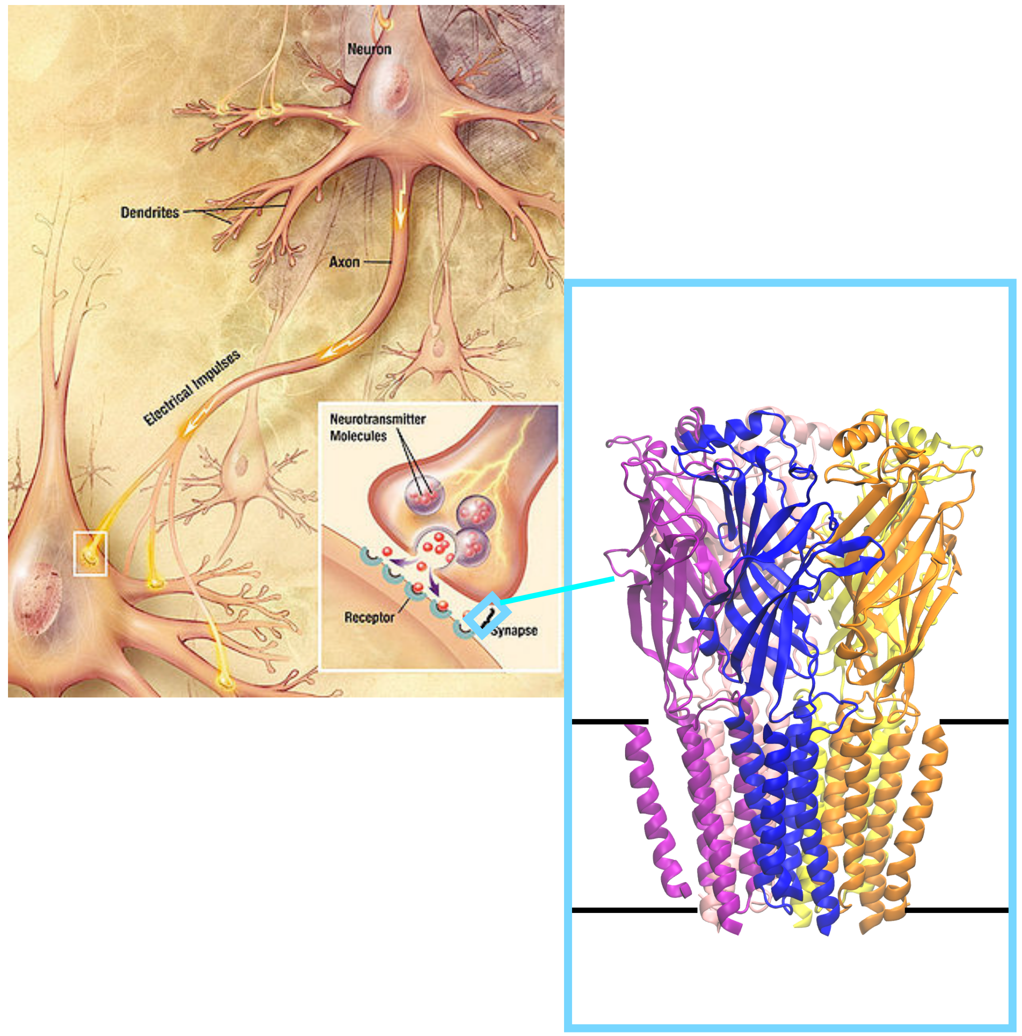
ethanol

recreation



nicotine



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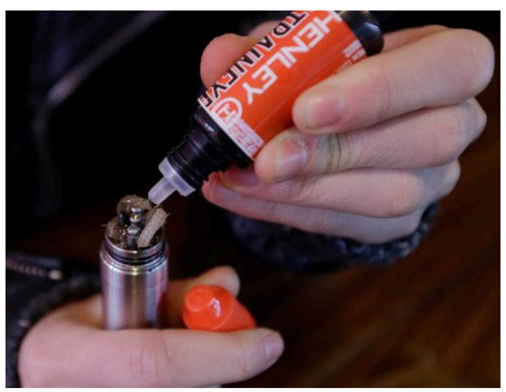

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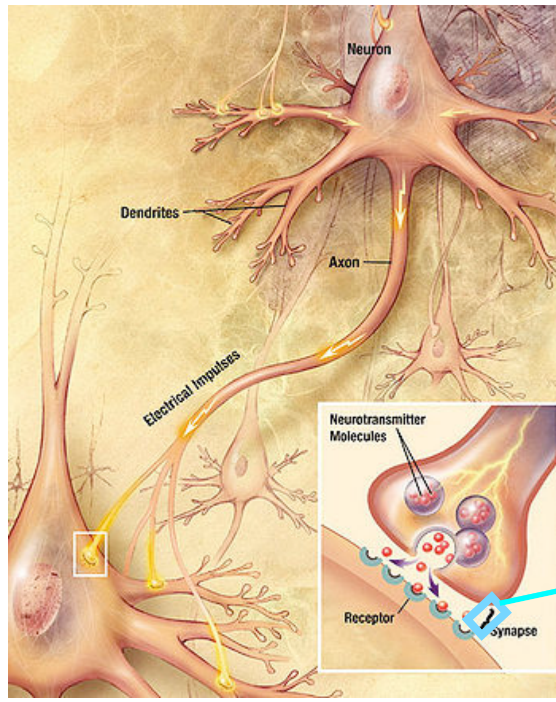

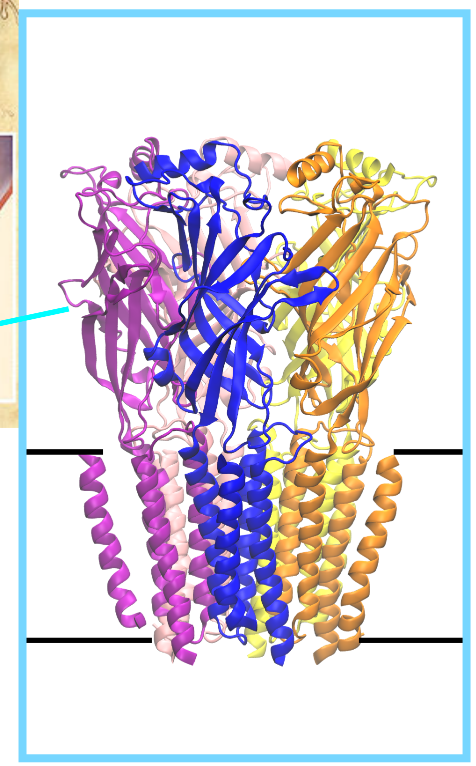

propofol

pentobarbital

chloroform

general anesthetics

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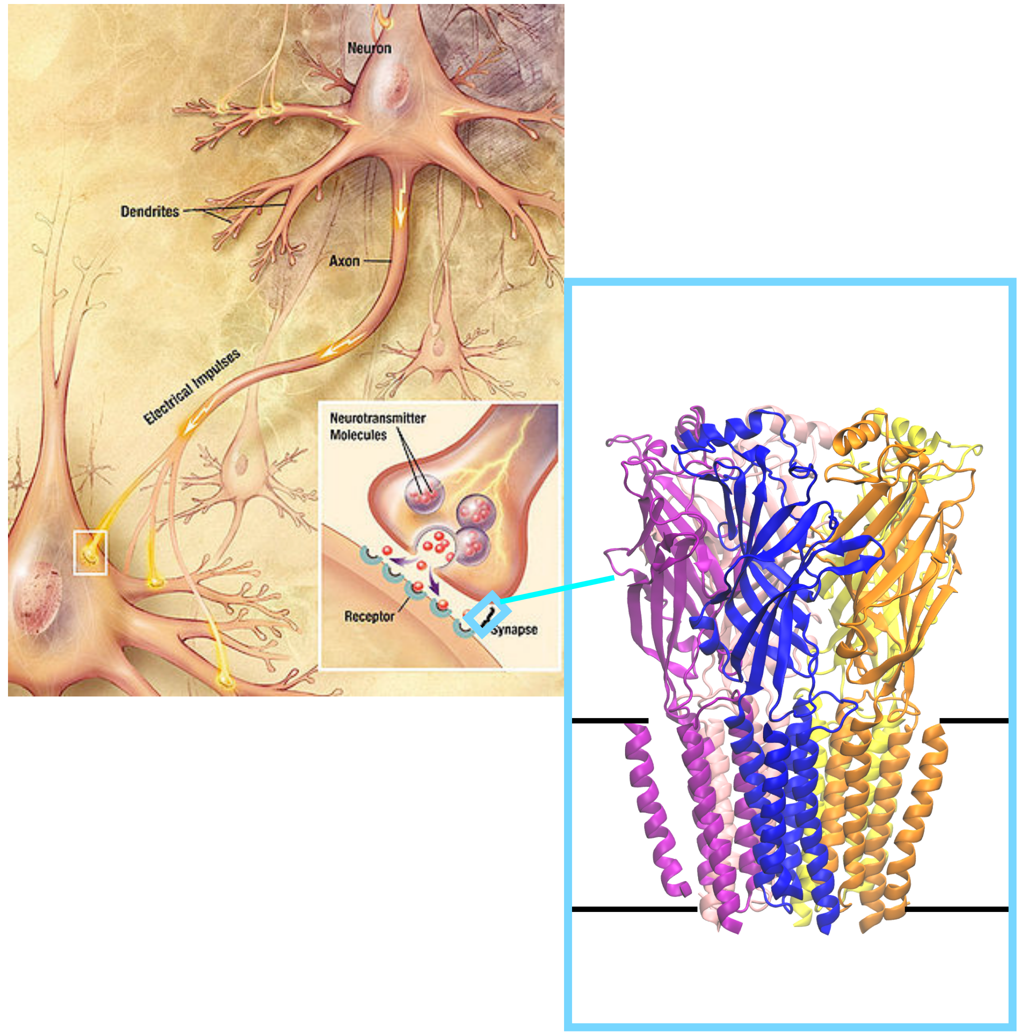

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poisons

rat poison (TETS)

strychnine

curare

Fishberry (picrotoxin)

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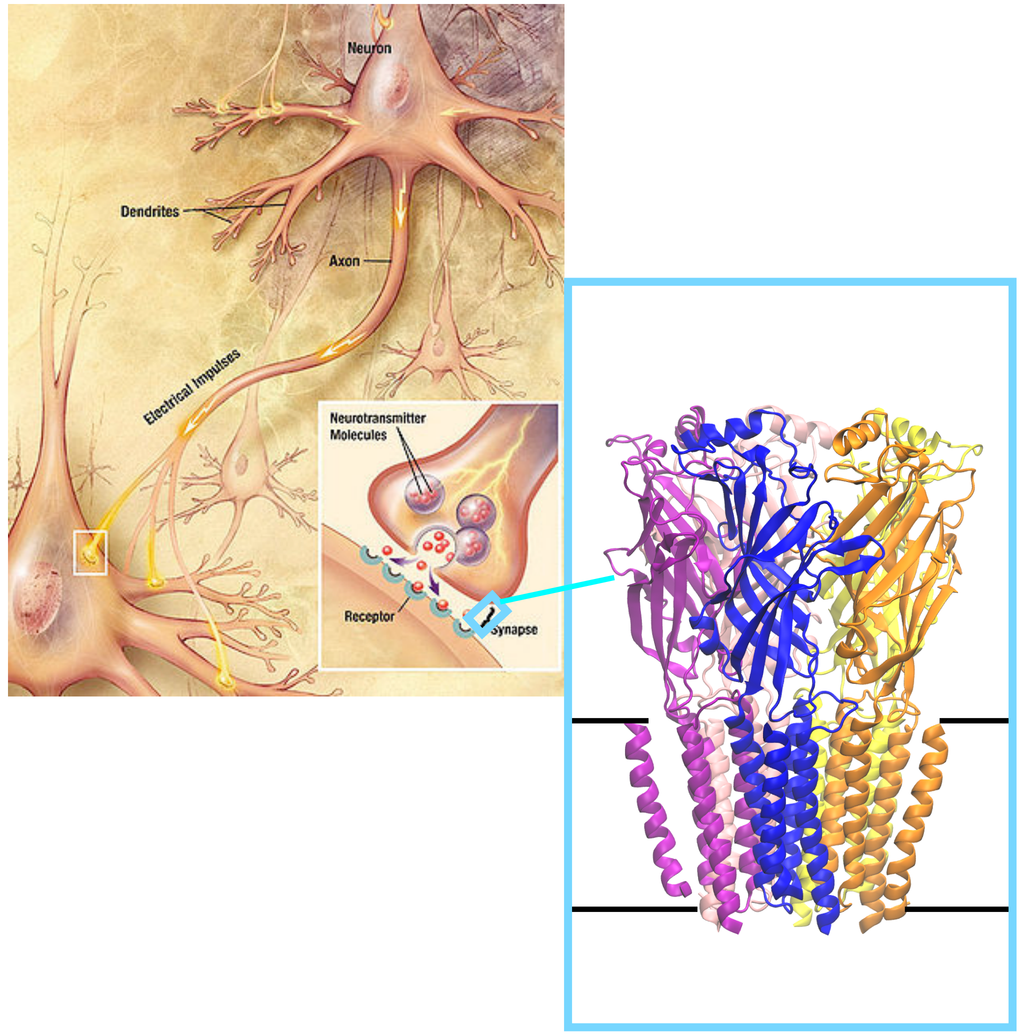

propofol

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general anesthetics

sedatives

benzodiazapenes

methaqualone

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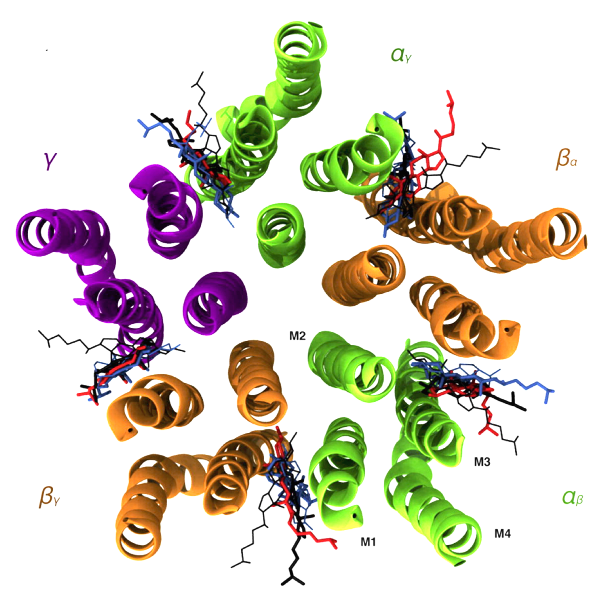

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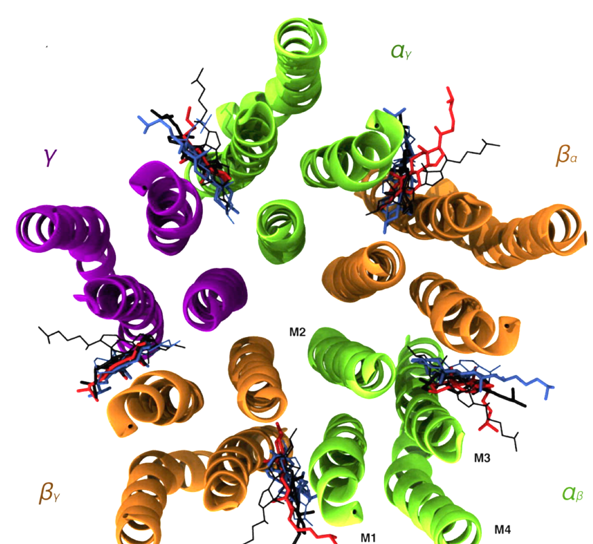

Henin...Brannigan, 2014, Biophys J

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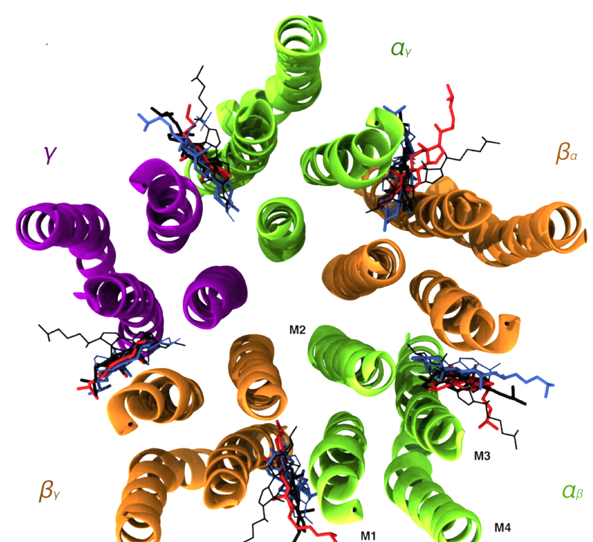

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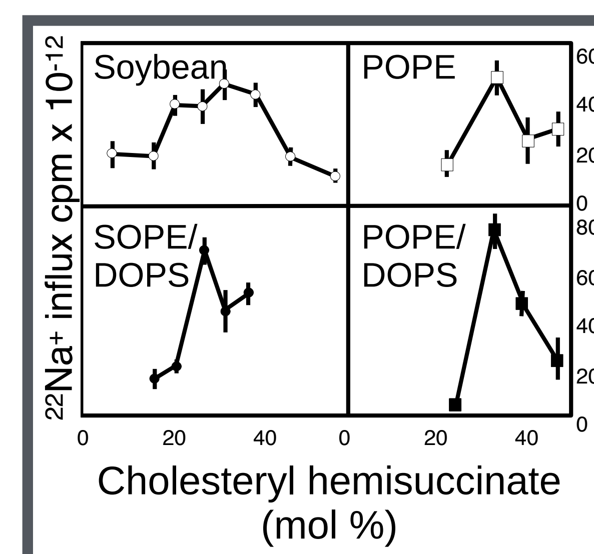

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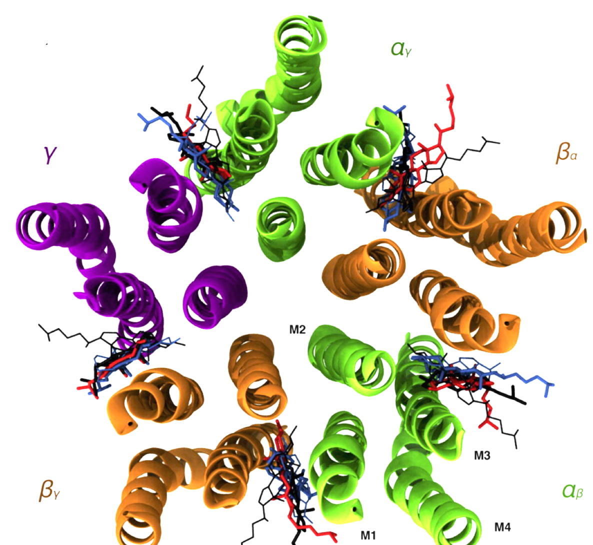

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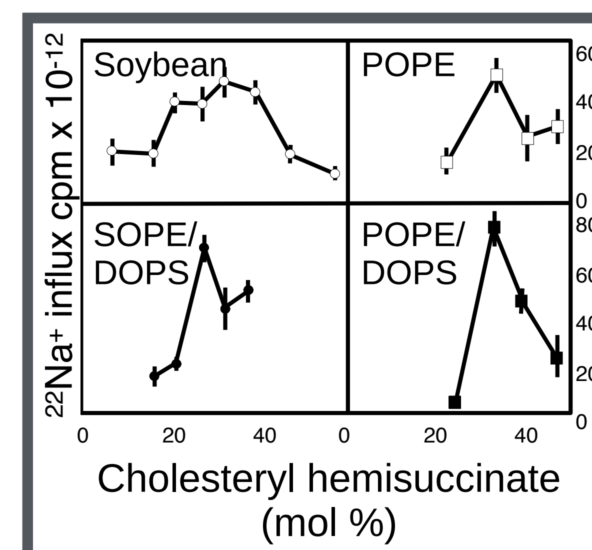
Criado...Barrantes, 1984, J.Biol.Chem.



General Motivation

1. Lipid-protein interactions: specific binding of

    - cholesterol

    - polyunsaturated fatty acids

    - anionic lipids

    to transmembrane receptors

2. Pharmacology of central nervous system proteins

    - general anesthetics

    - steroids

    - hormones

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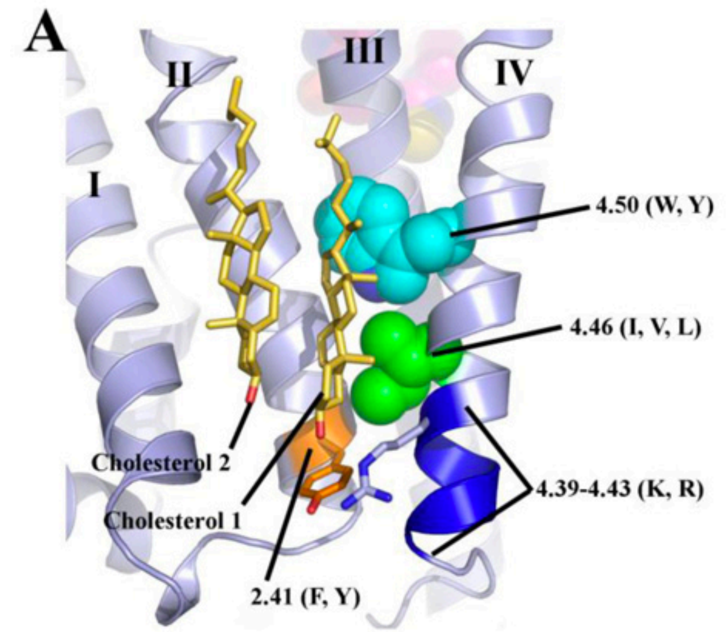

Hanson, ... Stevens, 2009, Structure

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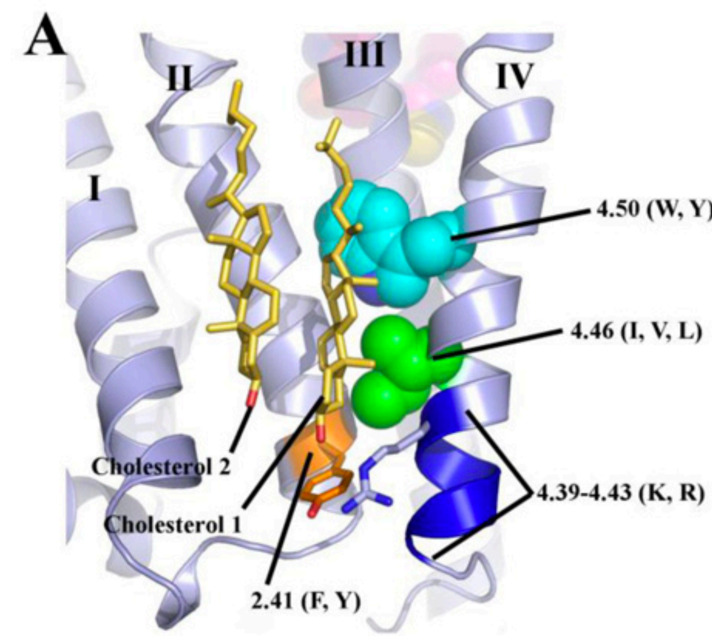

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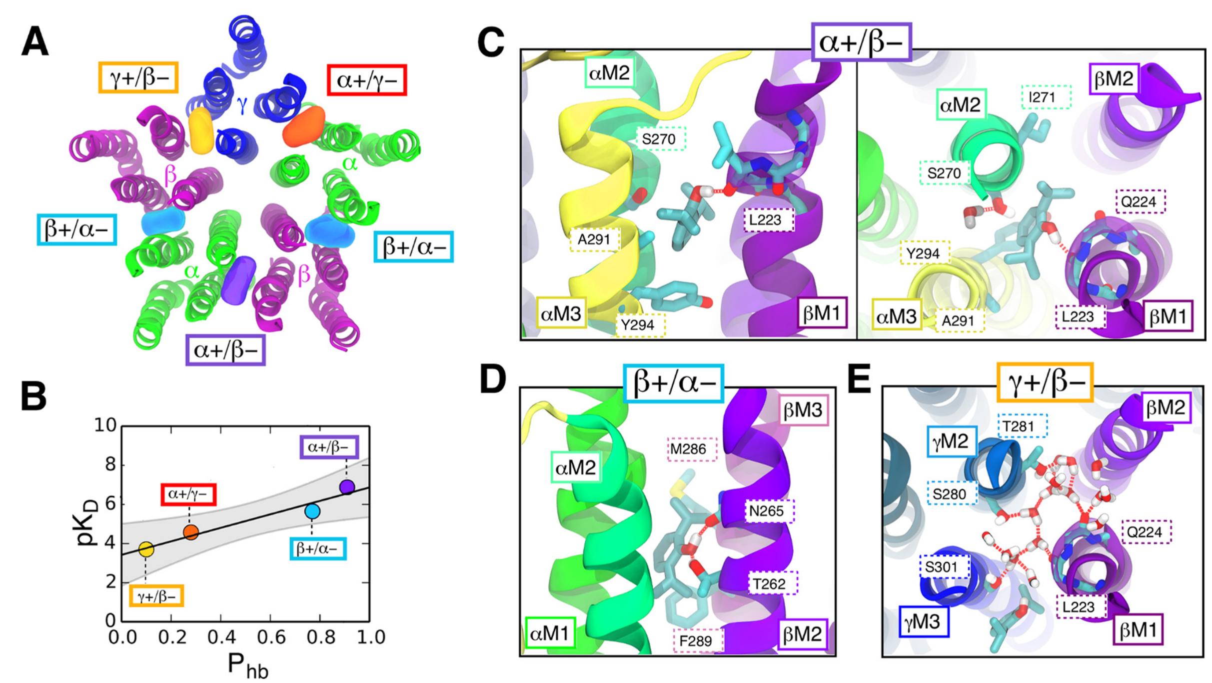
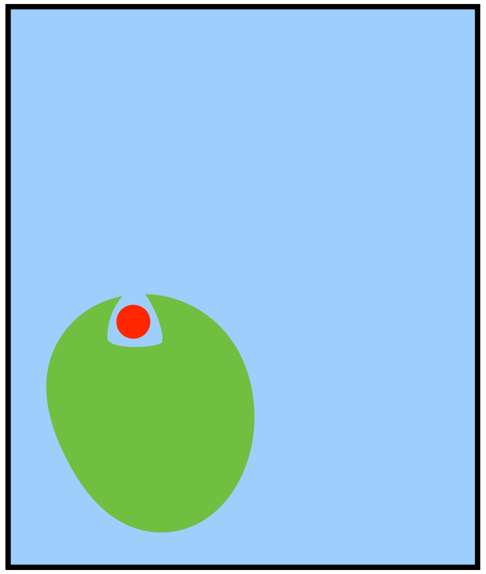


Woll, Murlidaran...Brannigan, Garcia, Eckenhoff, 2016, J Biol Chem

ligand-binding in a lamellar phase



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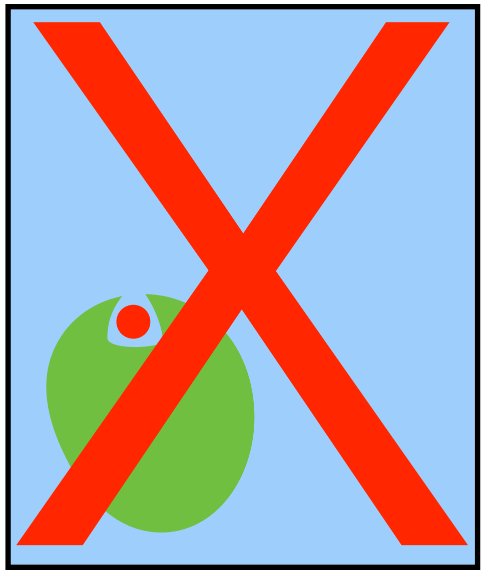

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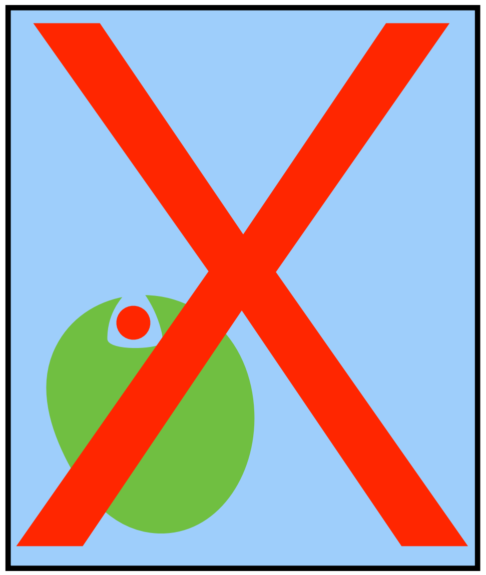

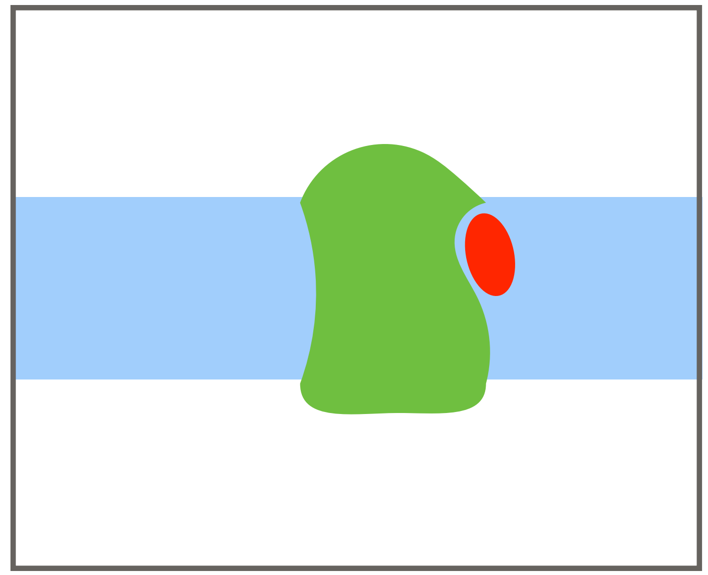

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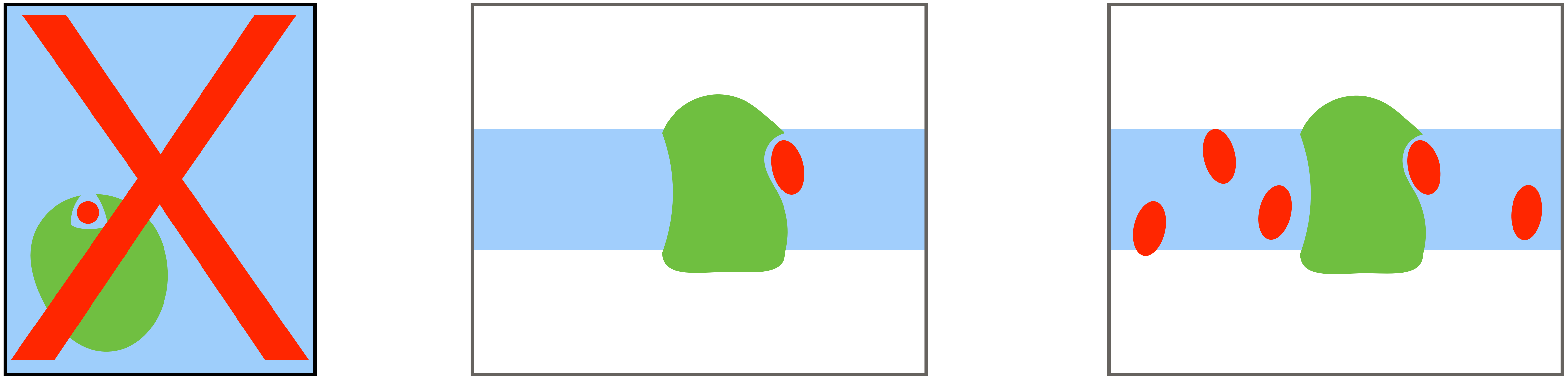

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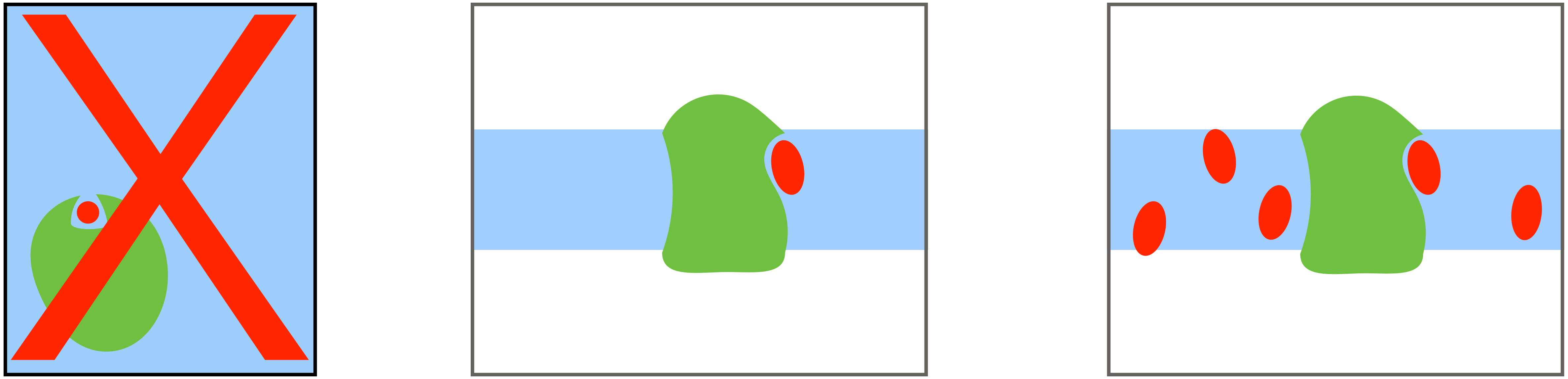

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(ABF/Umbrella Sampling/Metadynamics/Steered MD)

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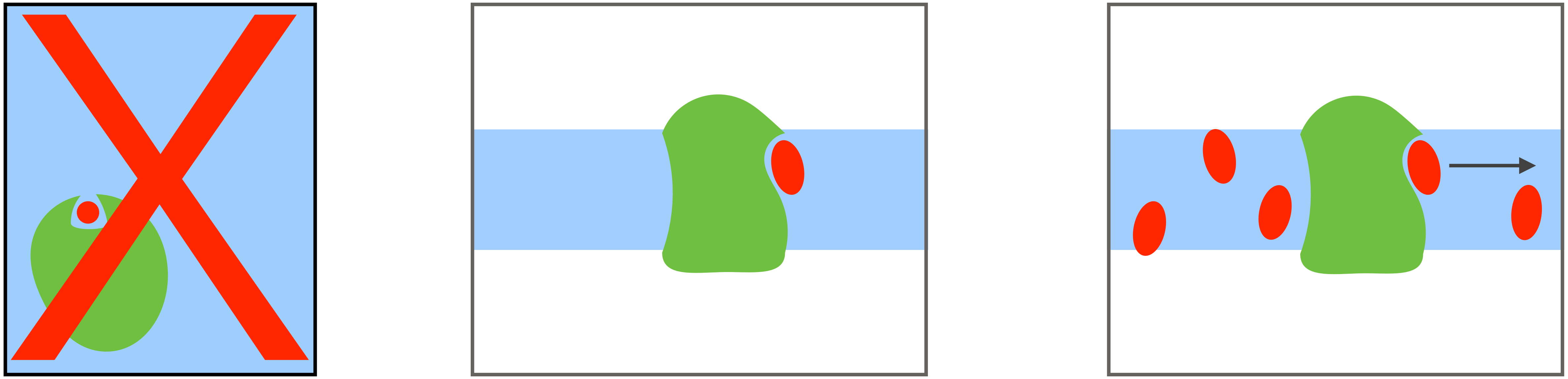

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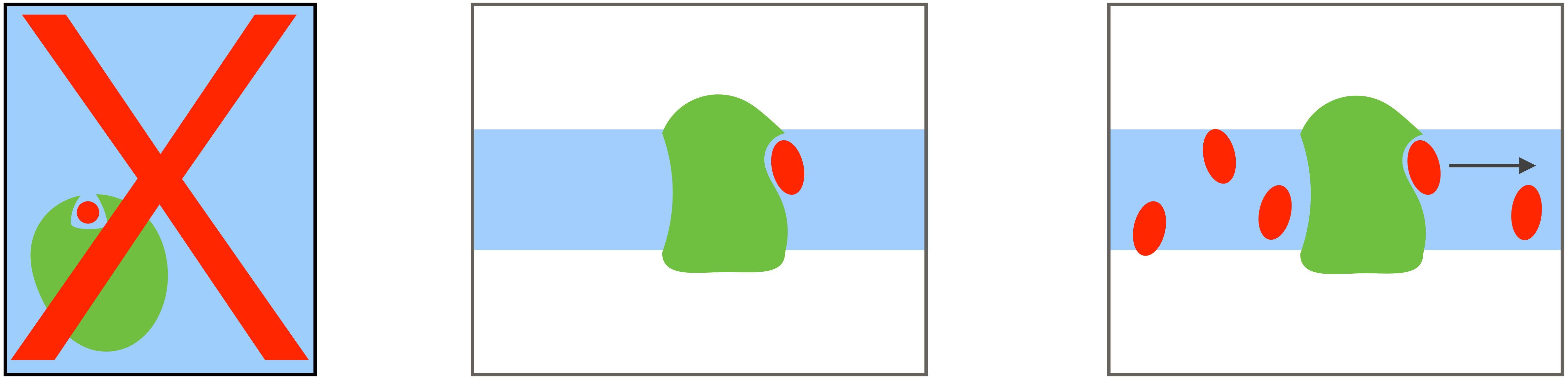

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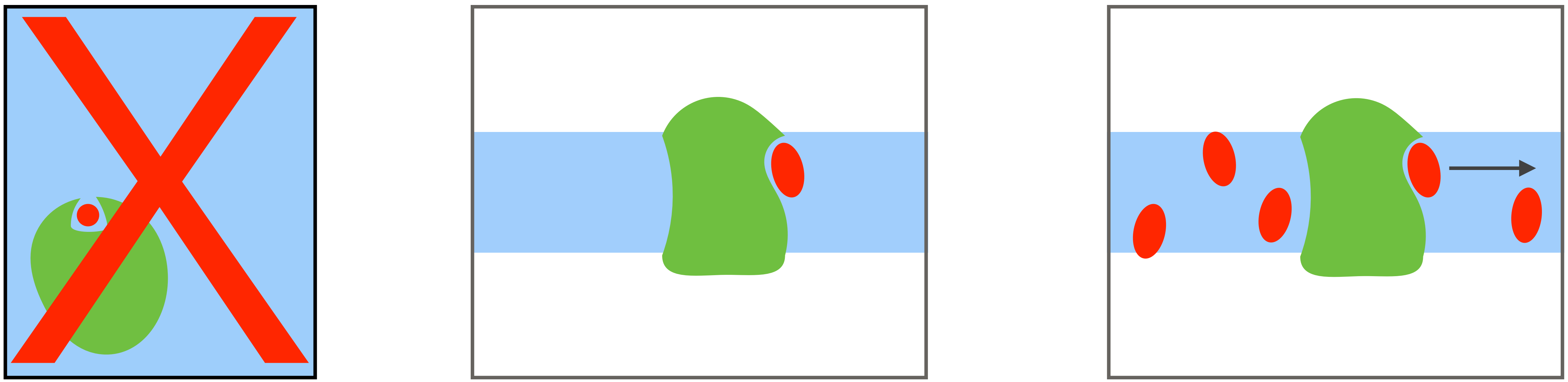

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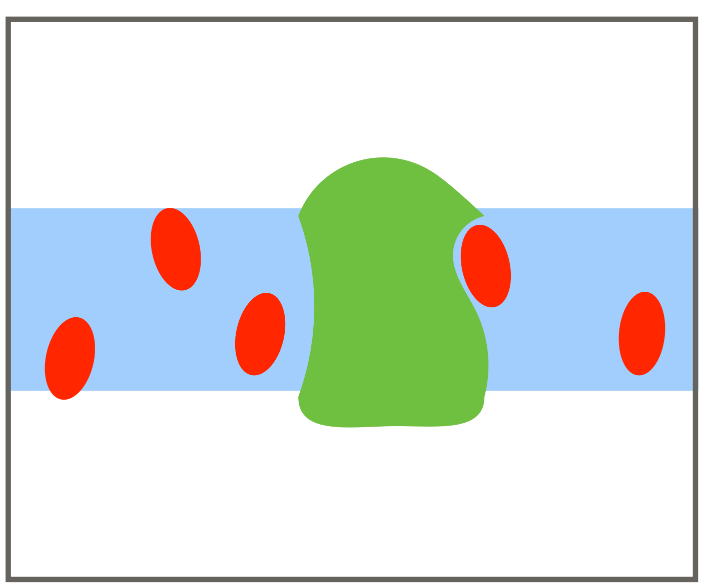

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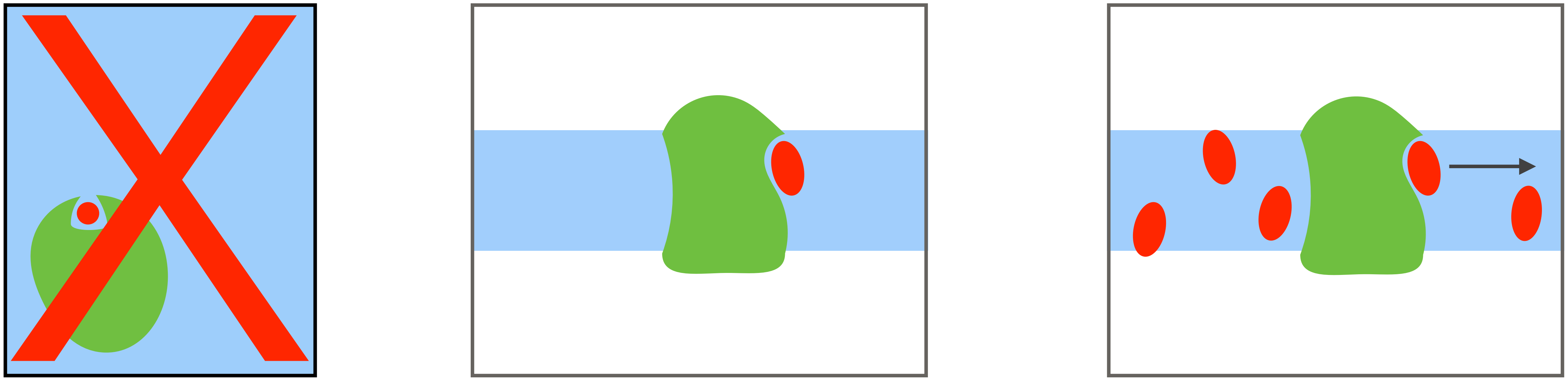

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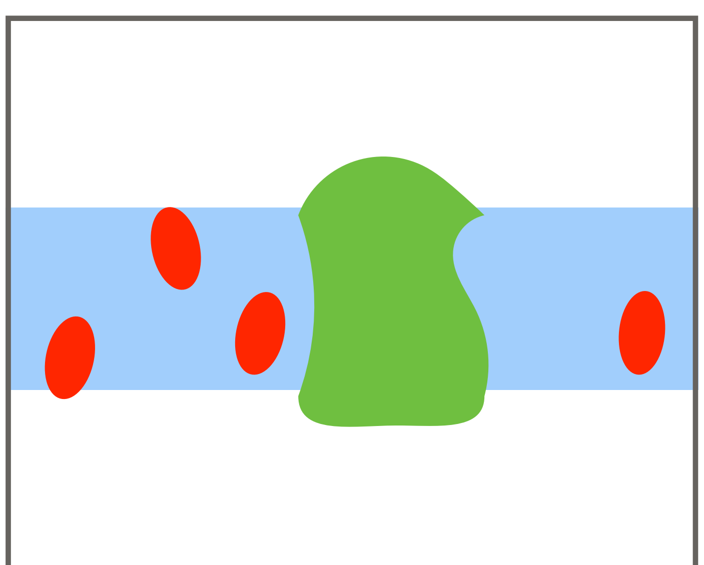

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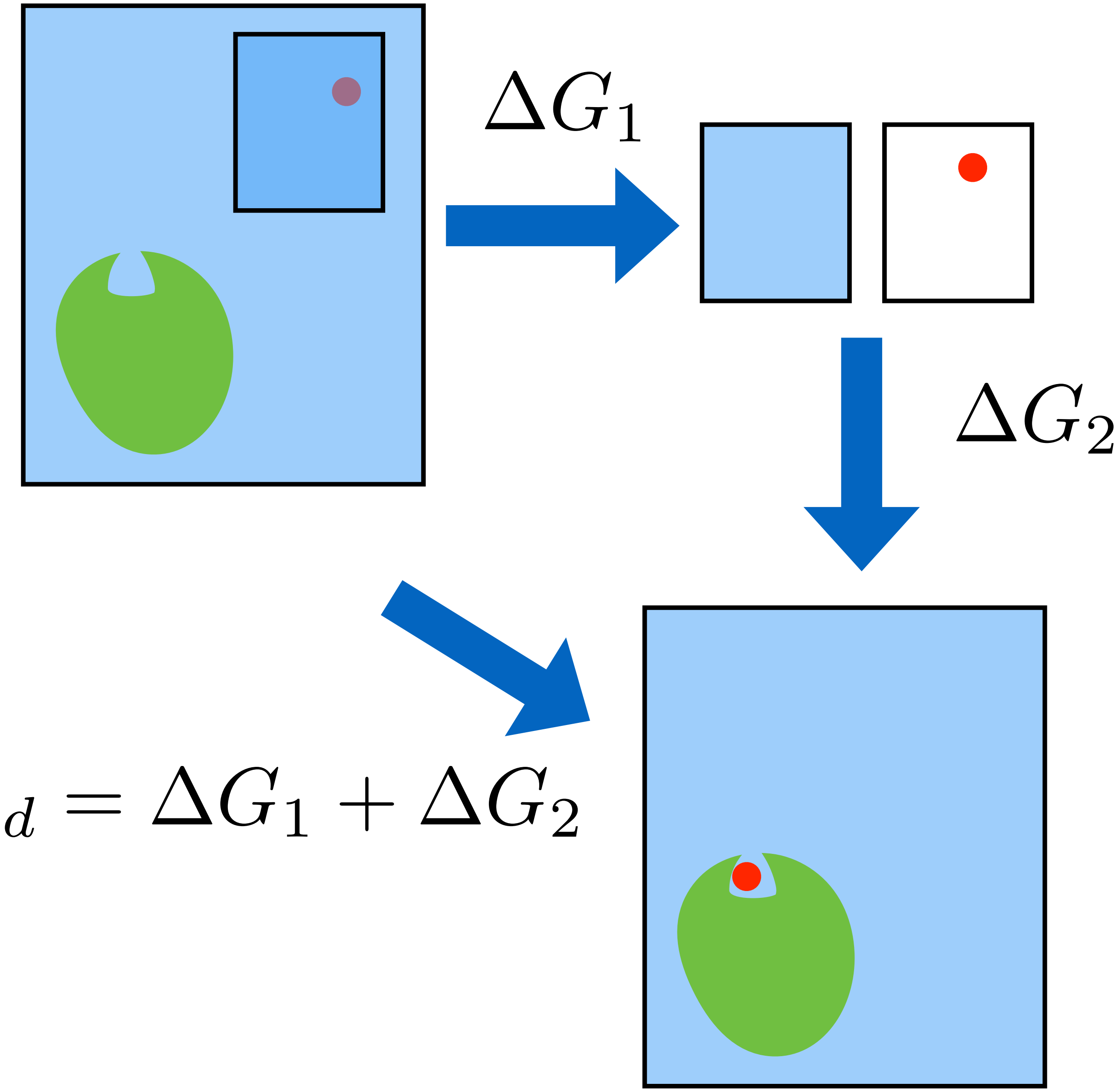
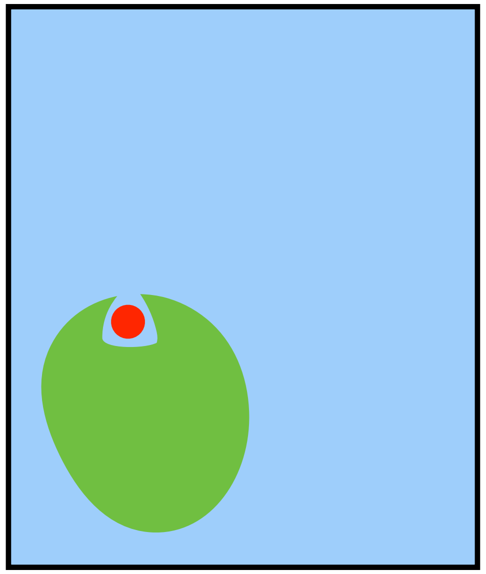
"Classic" Alchemical Free Energy Perturbation

Double Decoupling



$$\Delta G_{bind} = \Delta G_1 + \Delta G_2$$

Classical Binding Assumptions



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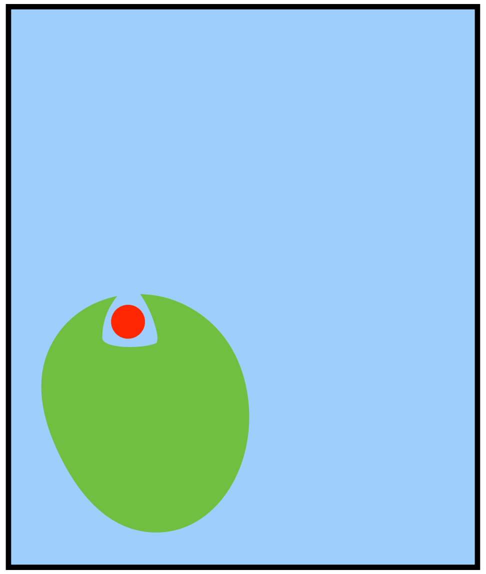

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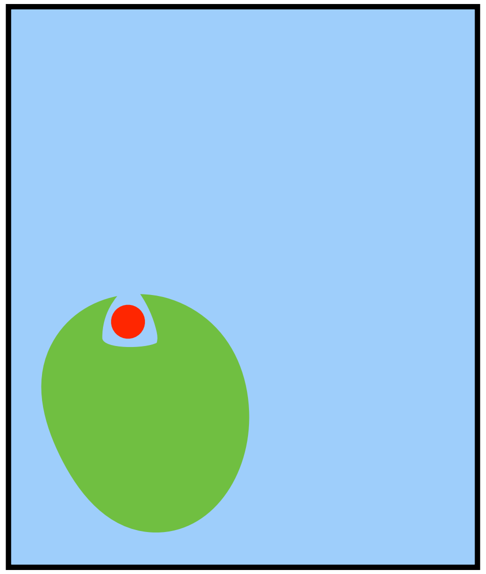

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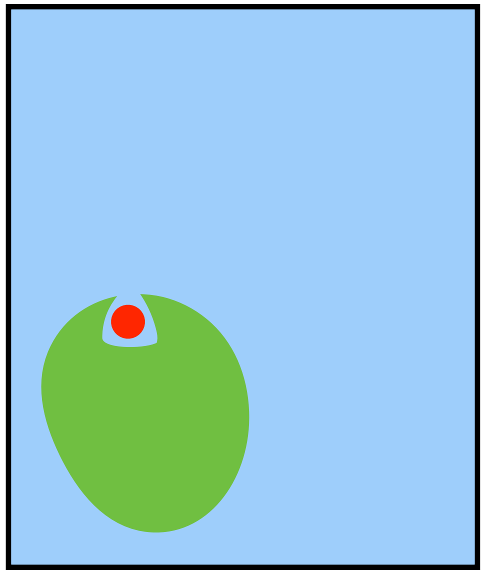

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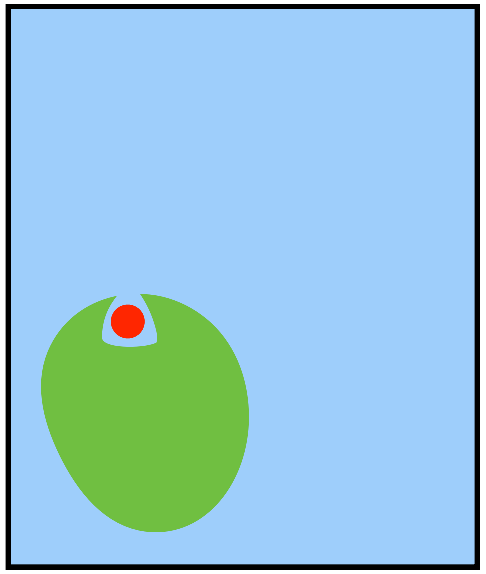

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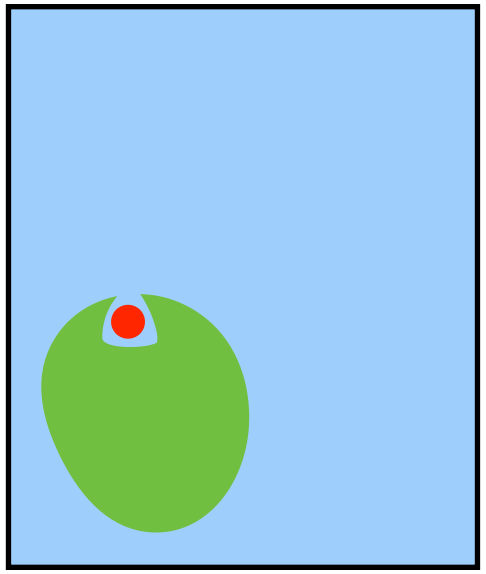

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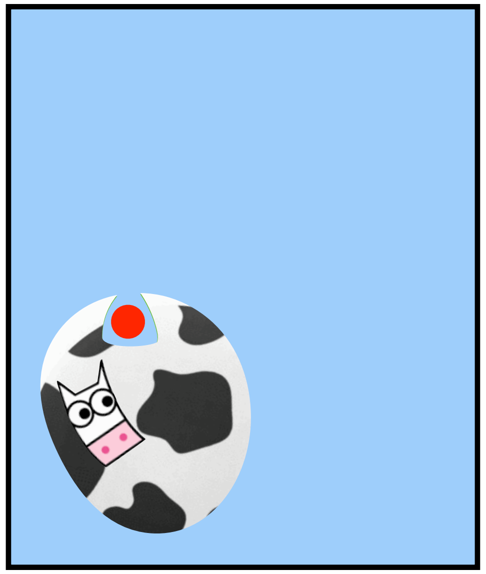

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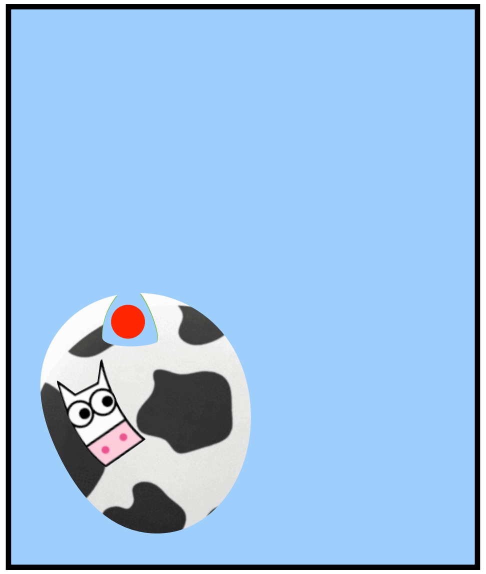

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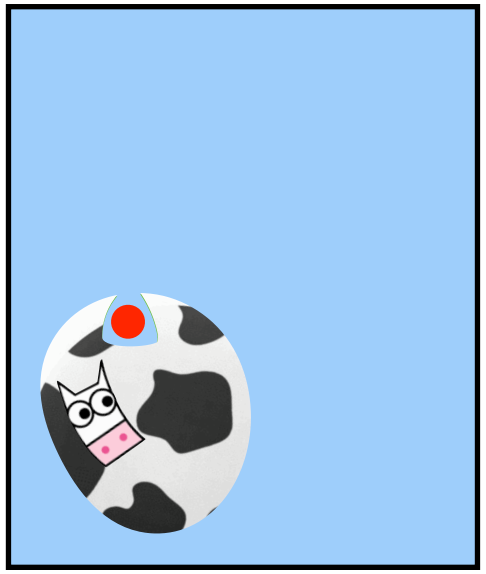

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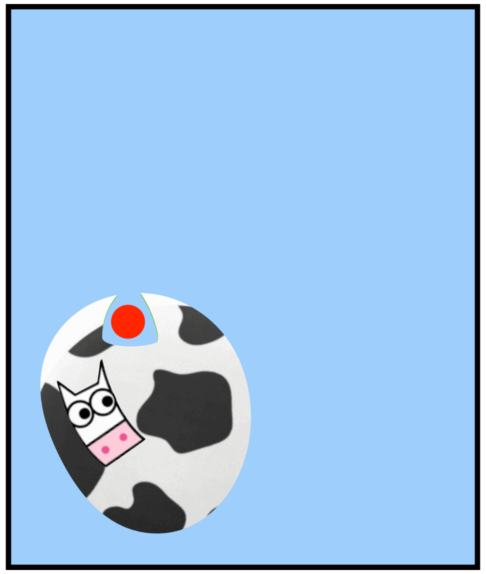

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5. Yields probabilities rather than constants of arbitrary dimension

Obstacles : Interpretation & Implementation

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Interpretation : predicting ligand titration given non-ideality and ambiguous concentration

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Implementation : Amplified ACRES obstacles
Accuracy **C**onvergence **R**estraints **E**ndpoint **S**olvation

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$$p_{occ} = \frac{1}{1 + \frac{1}{\kappa_L [L]_{tot}}}$$

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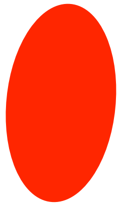
problem: What is the concentration of  in

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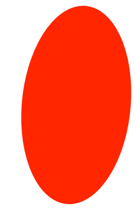
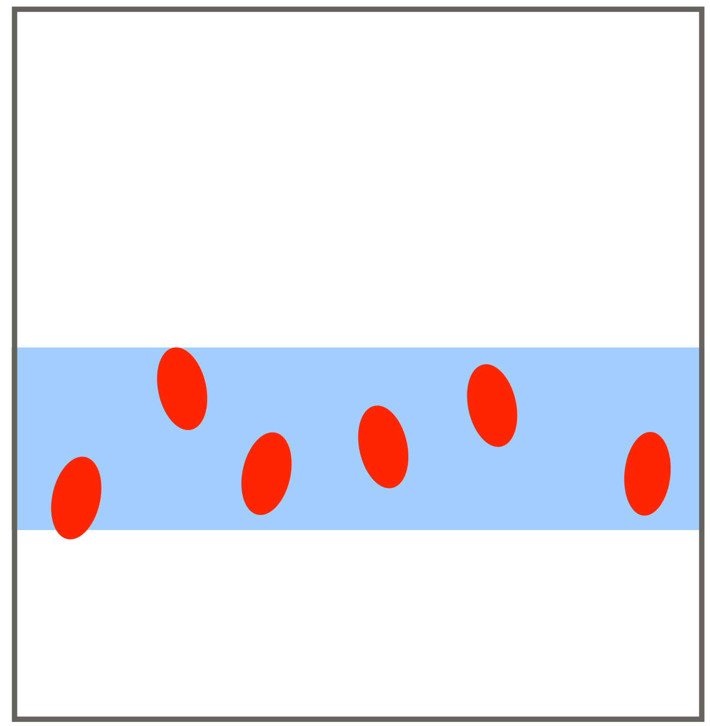
problem: What is the concentration of  in



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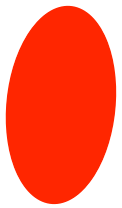
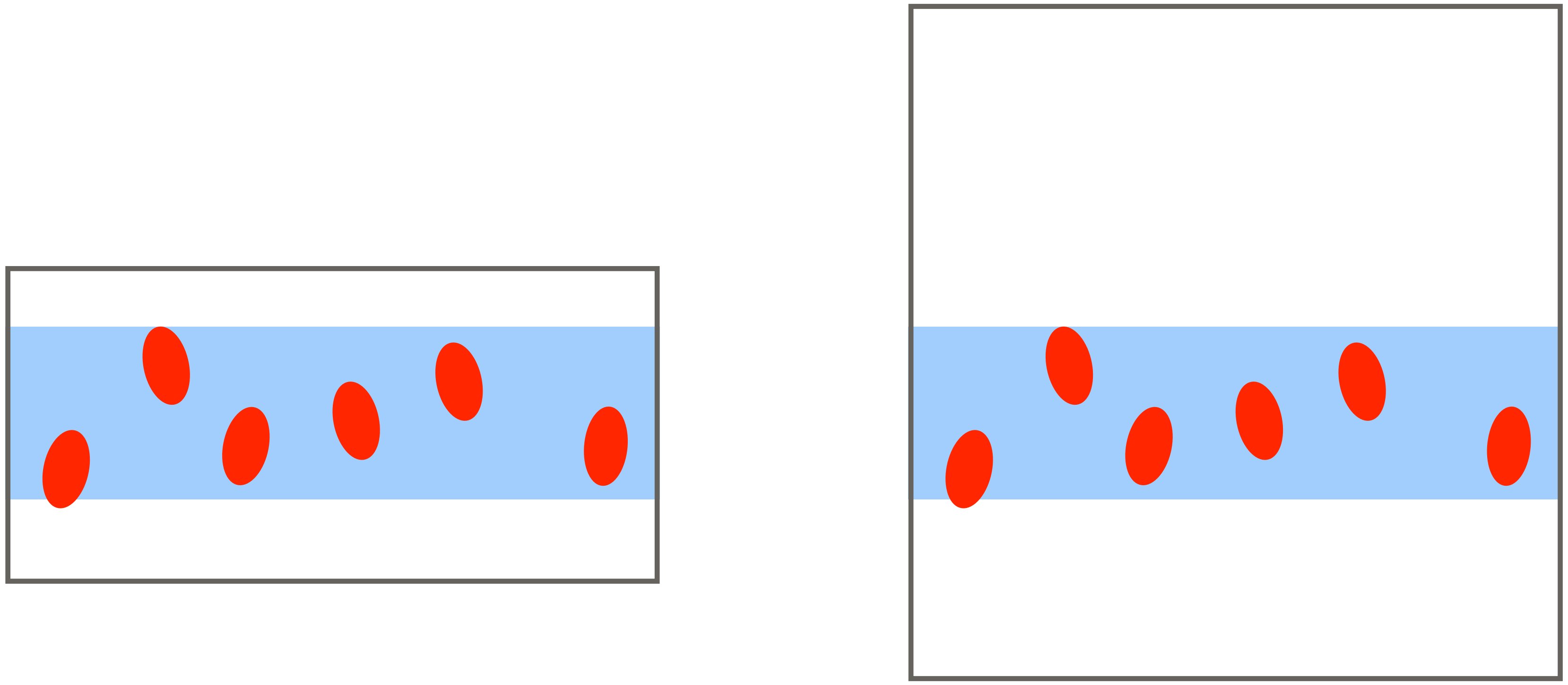
problem: What is the concentration of  in



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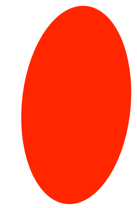
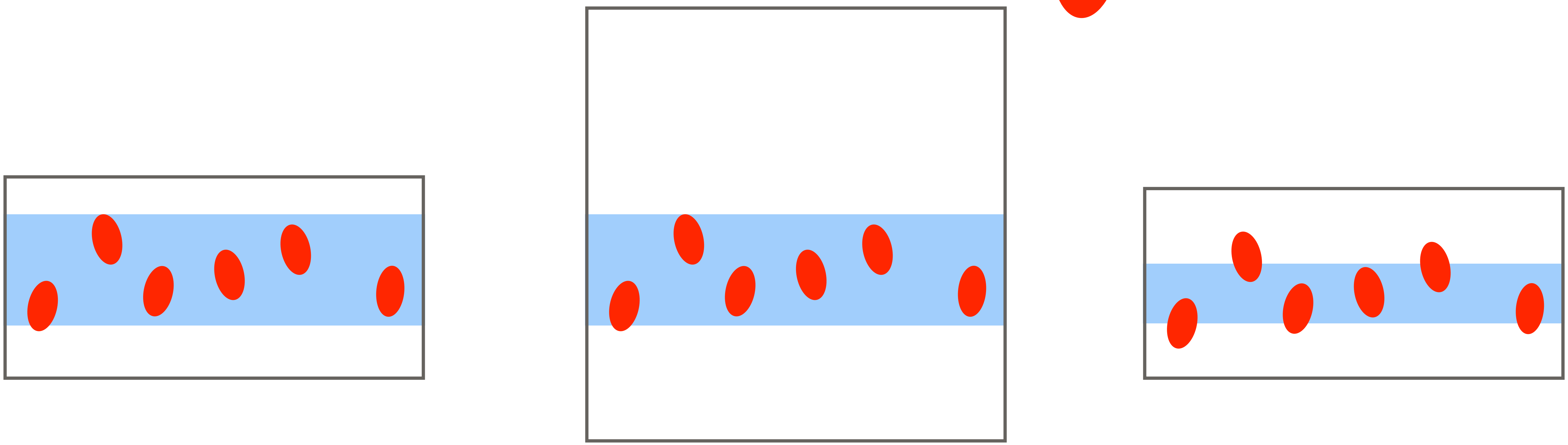
problem: What is the concentration of  in



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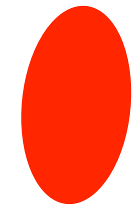
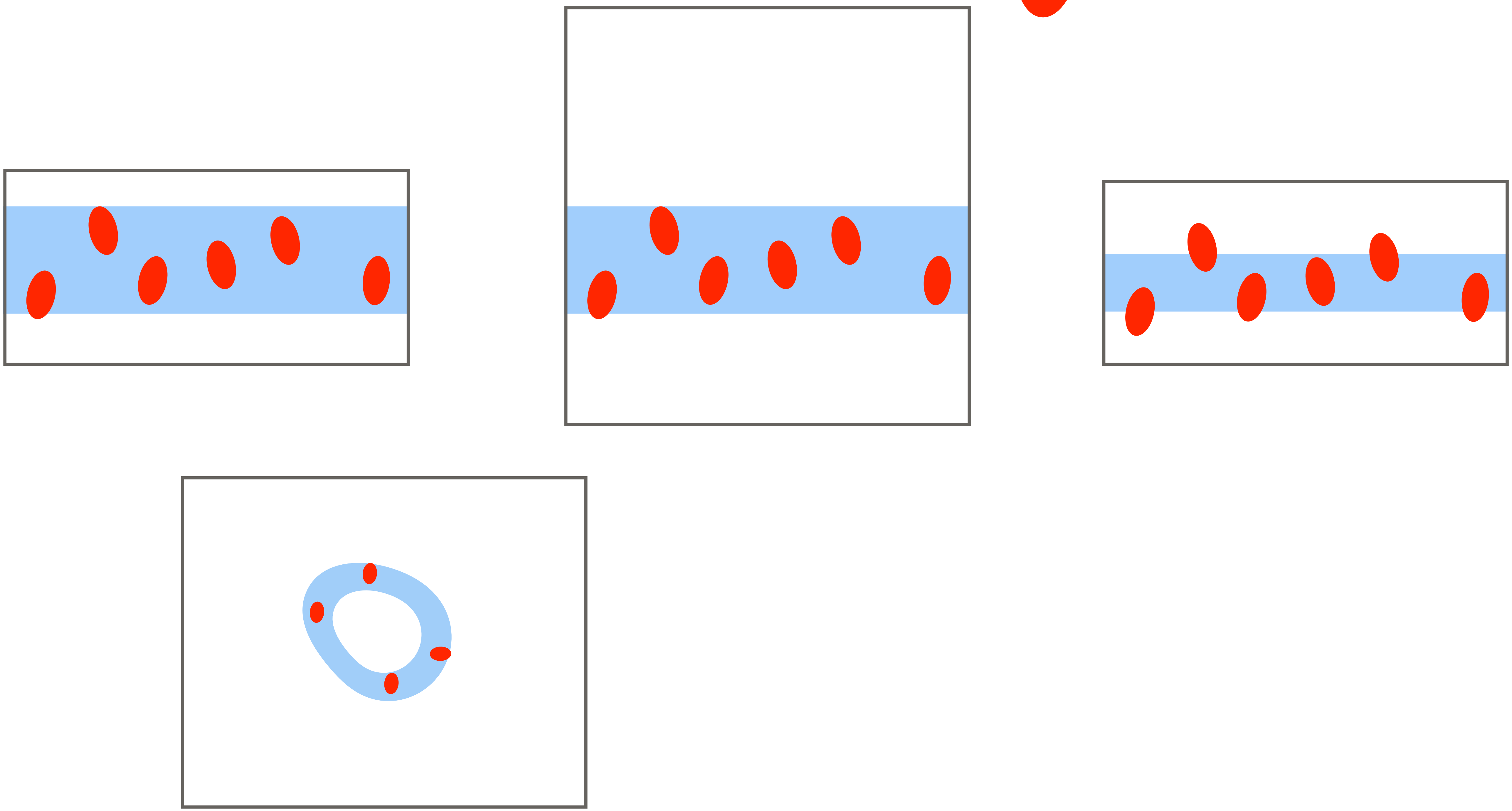
problem: What is the concentration of  in



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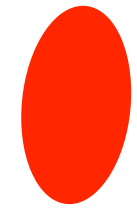
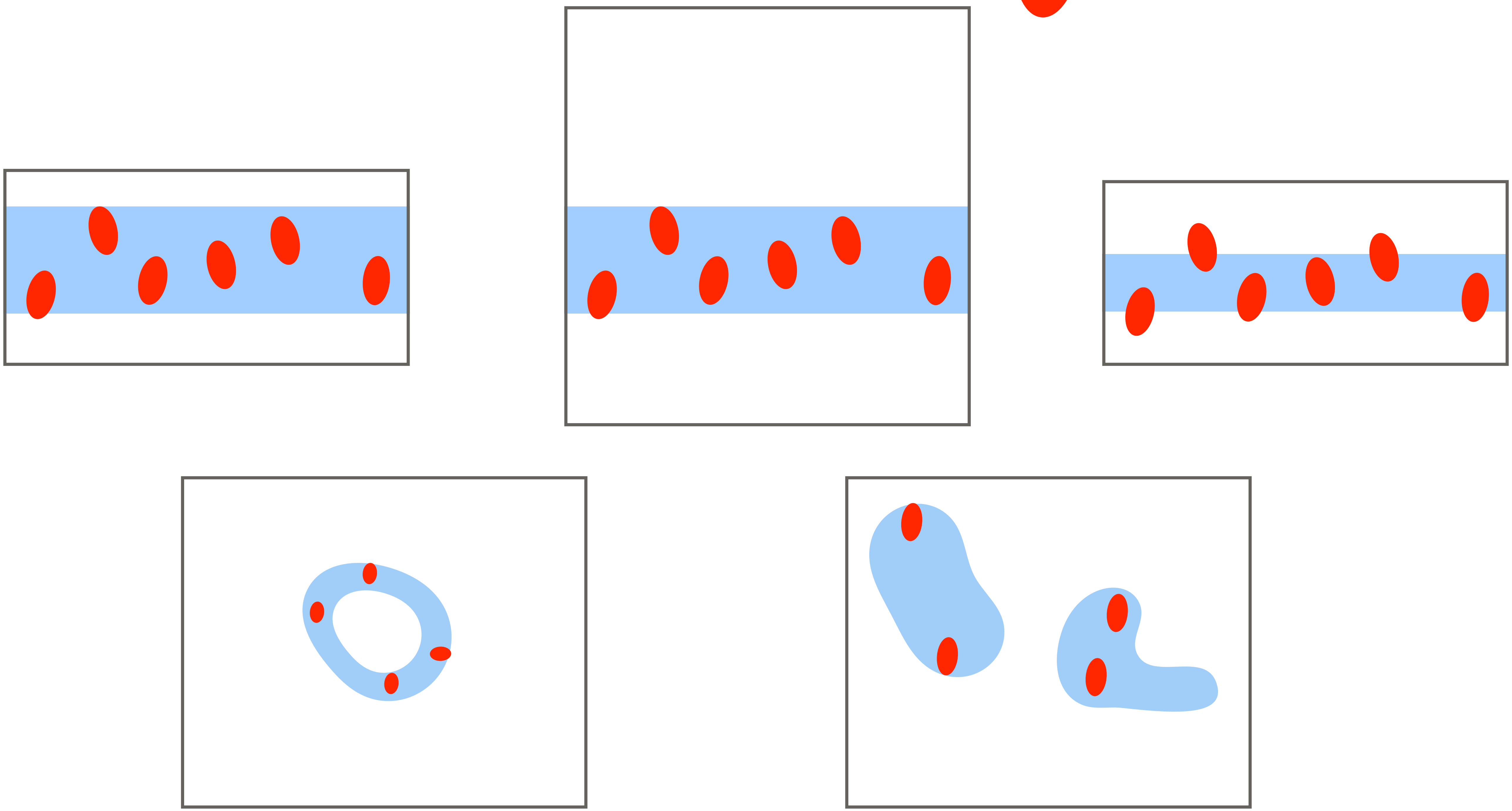
problem: What is the concentration of  in



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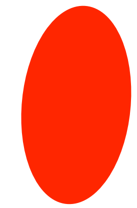
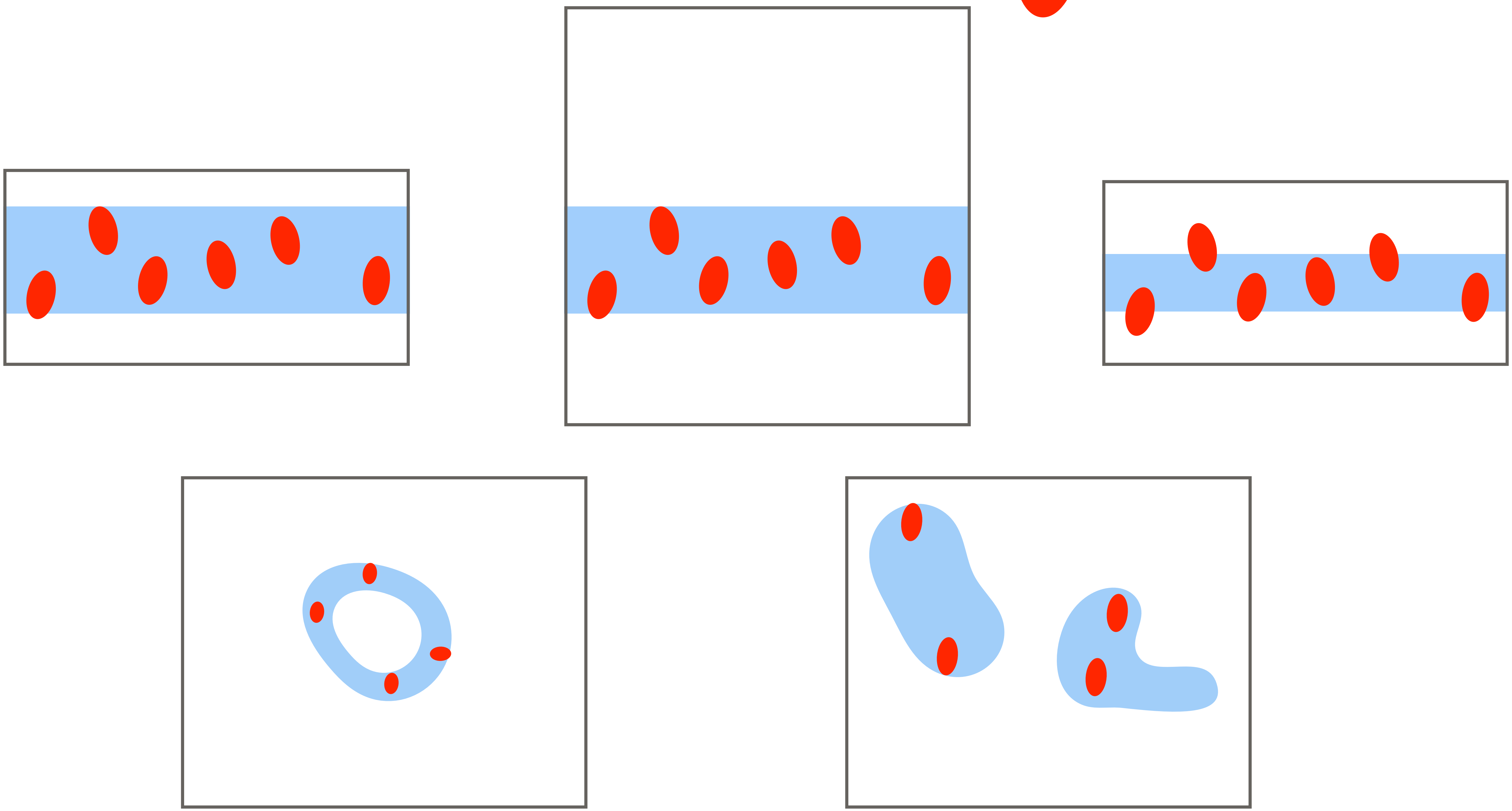
problem: What is the concentration of  in



What about standard state?

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but quantity $\kappa_L \, [L]_{\mathrm{tot}}$ remains **unambiguous**.

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Solution part 2: Represent intensive ligand abundance via "per receptor" units

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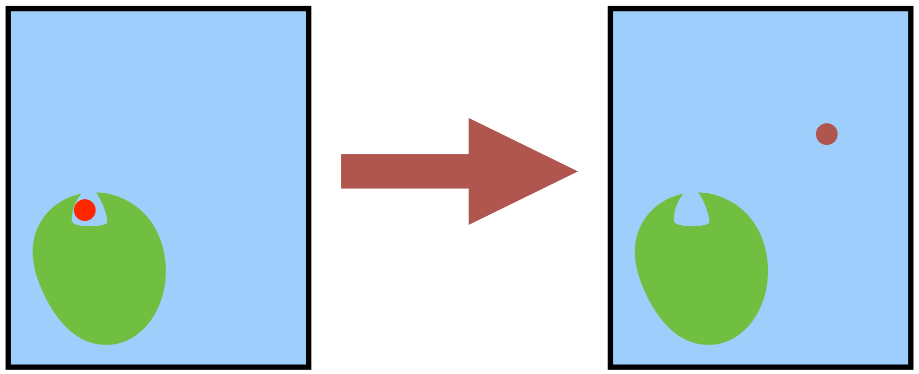

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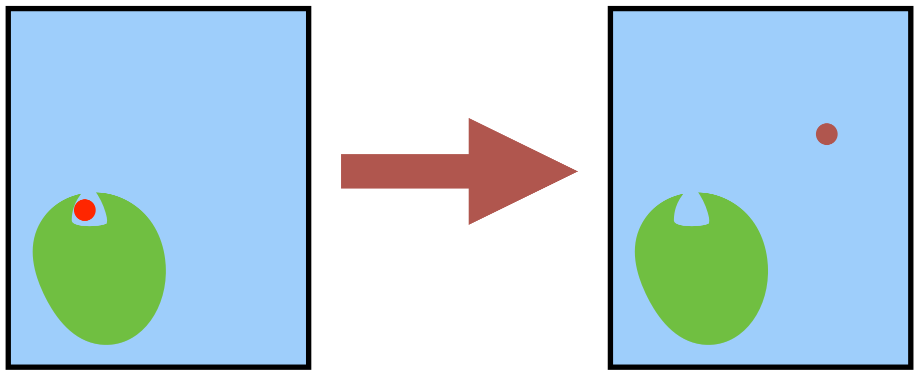

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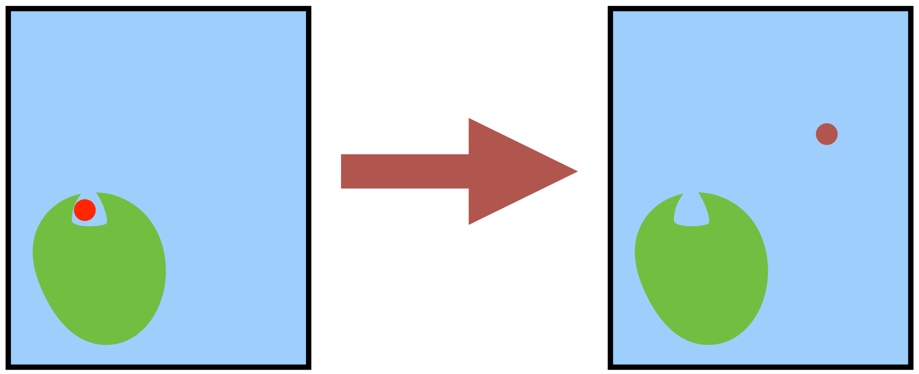

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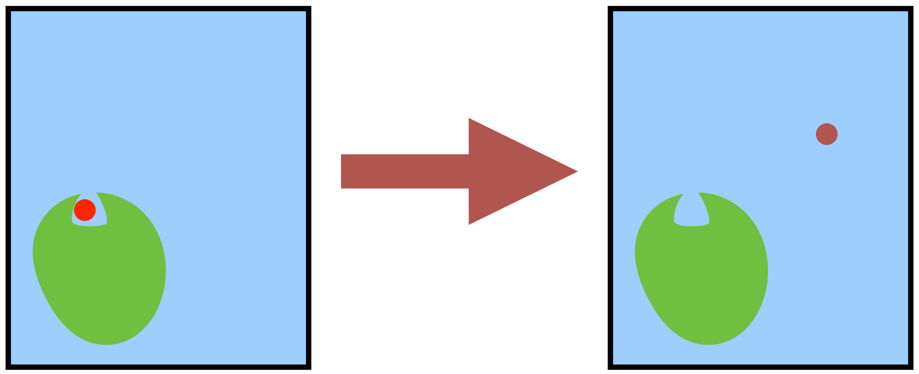

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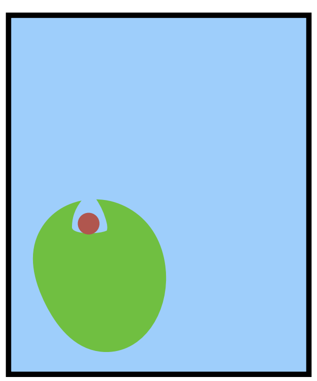

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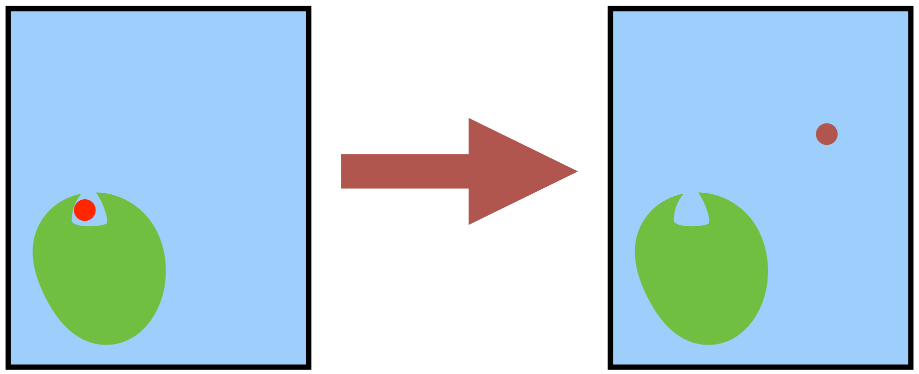

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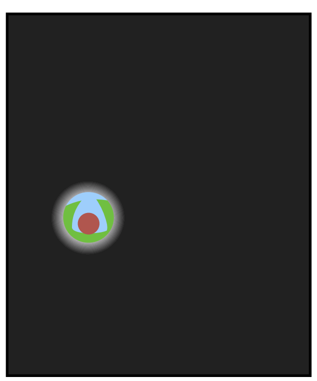

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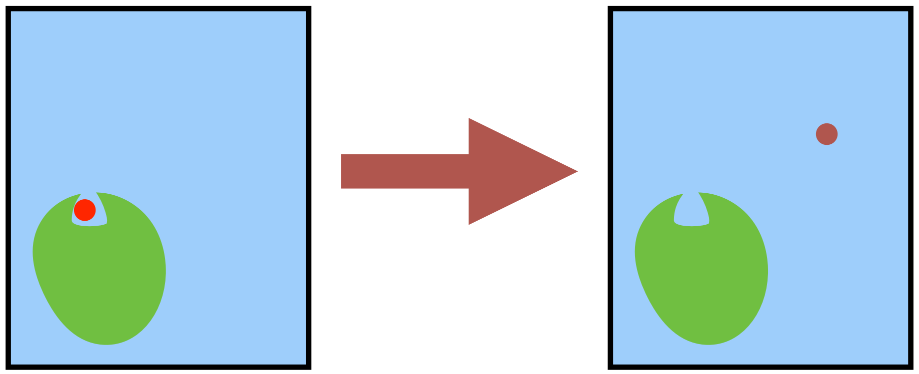

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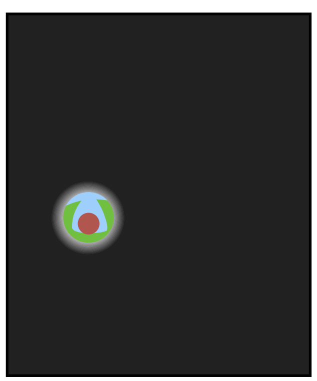

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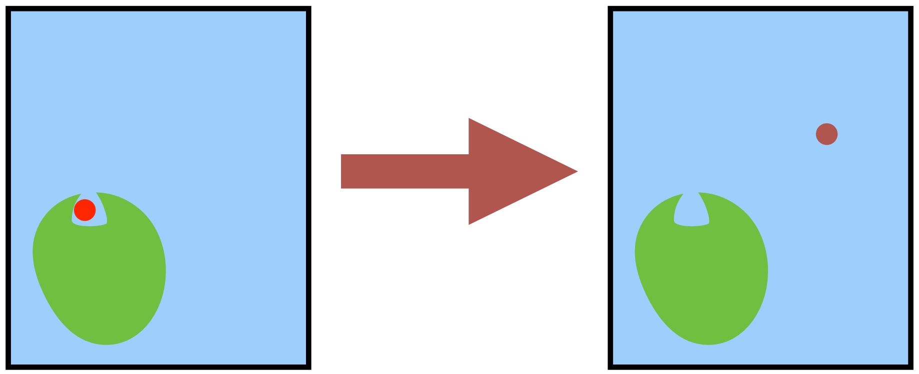

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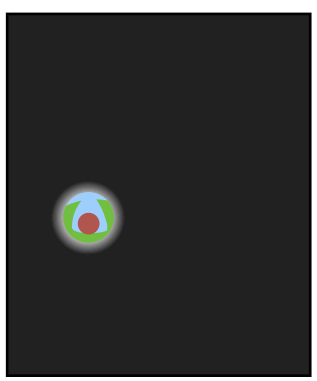

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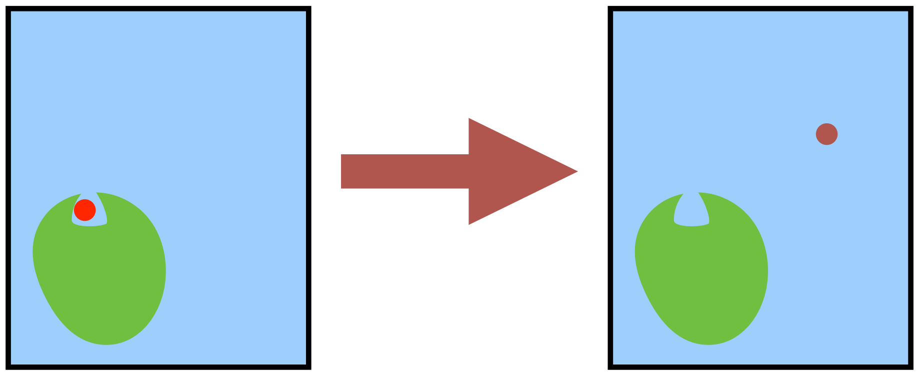

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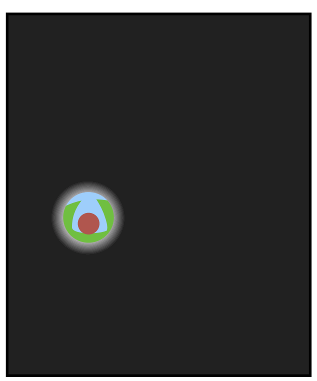

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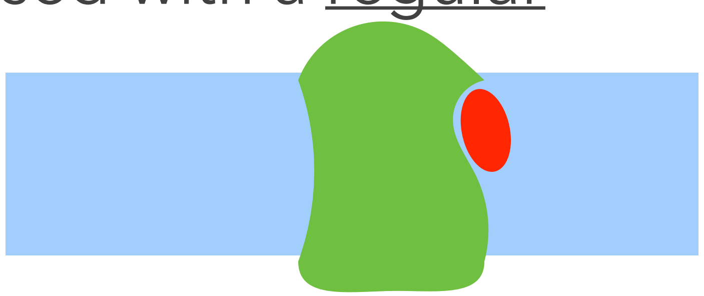

ACRES : Anisotropic Restraints

“Canonical” anisotropic approach: 7 restraints

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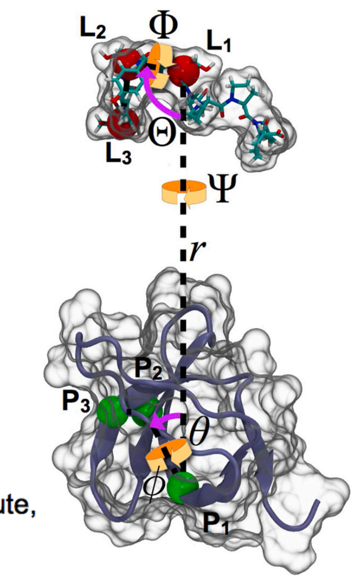

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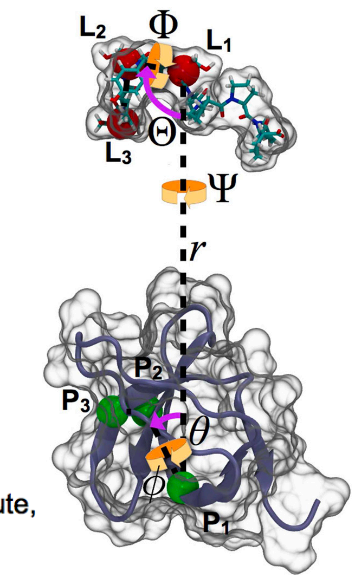

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Our **solution**: 1 anisotropic restraint

RMSD restraint in <u>protein reference frame</u>

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James C. Gumbart[†], **Benoît Roux**[†,¶,*], and **Christophe Chipot**[‡,§,*]
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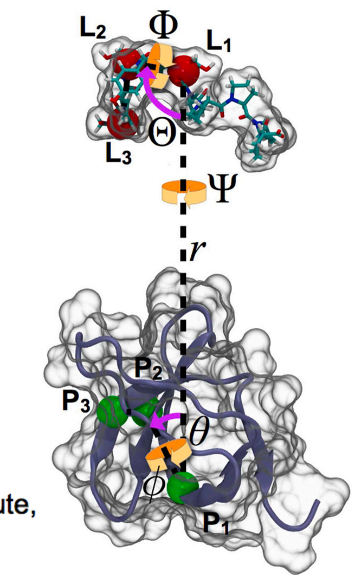


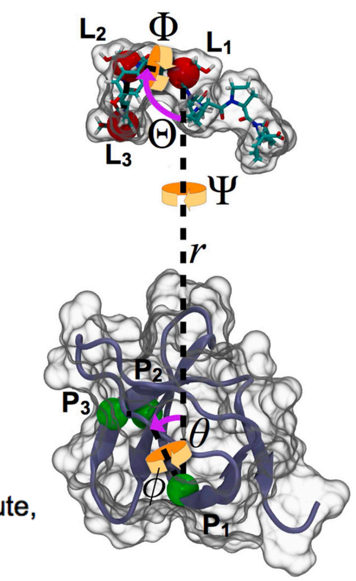
Our **solution**: 1 anisotropic restraint

RMSD restraint in protein reference frame

$$\mathbf{x}_i' = \rho(\mathbf{x}_i - \bar{\mathbf{x}}_R) + \bar{\mathbf{x}}_R^{\mathrm{ref}},$$

ACRES : Anisotropic Restraints

"Canonical" anisotropic approach: 7 restraints



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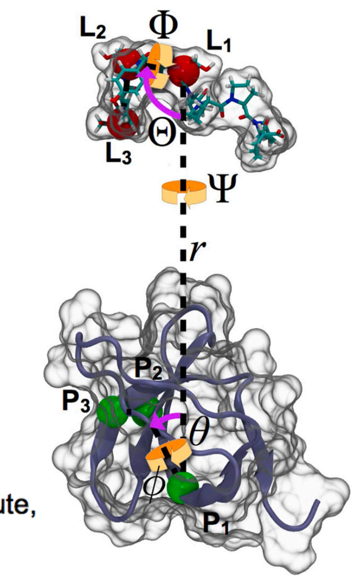

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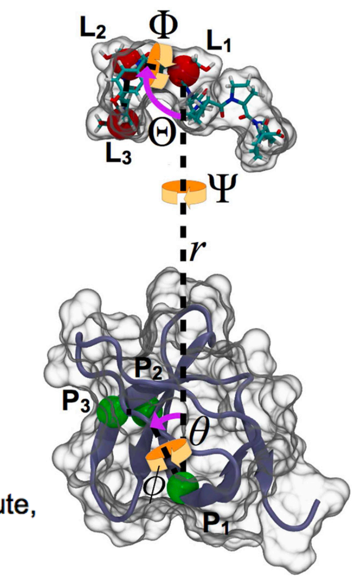

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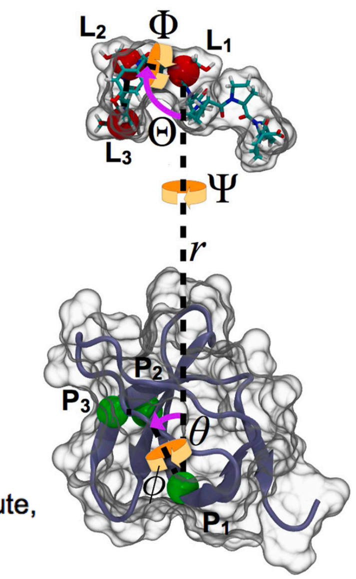

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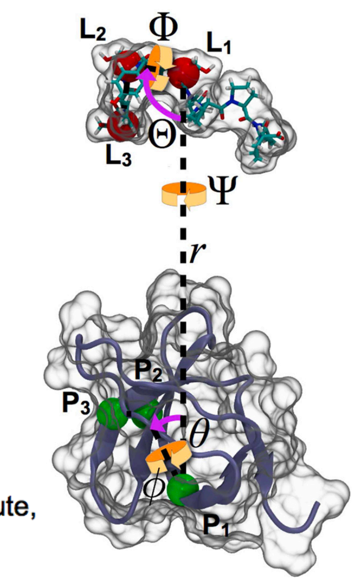

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Flat-well potential on DBC Collective Variable

ACRES: DBC Restraints

Salari, Joseph, Lohia, Henin, Brannigan,
JCTC 2018 submitted
https://arxiv.org/abs/1801.04901

calculated using blue protein atoms

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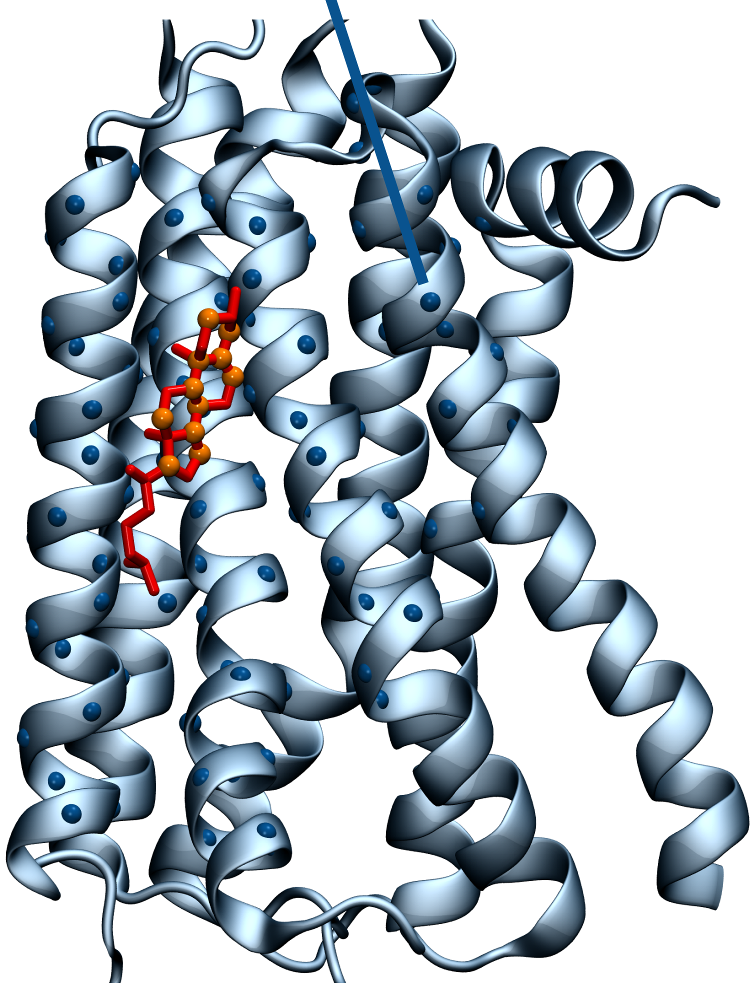

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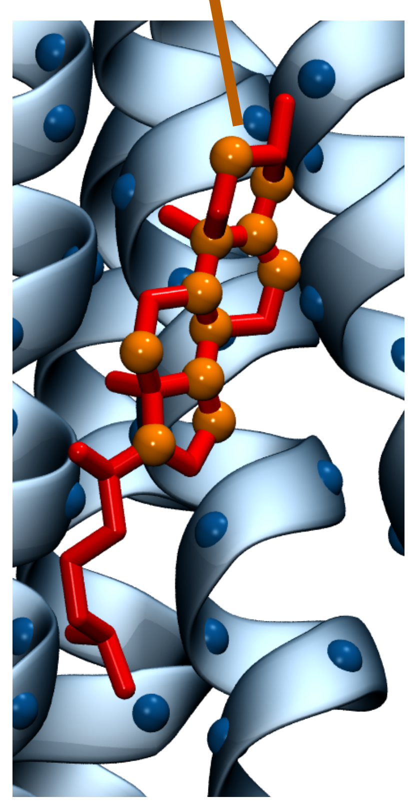

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restraint potential

d_{max} ~ 2A

k_d ~ 100 kcal/mol/A^2

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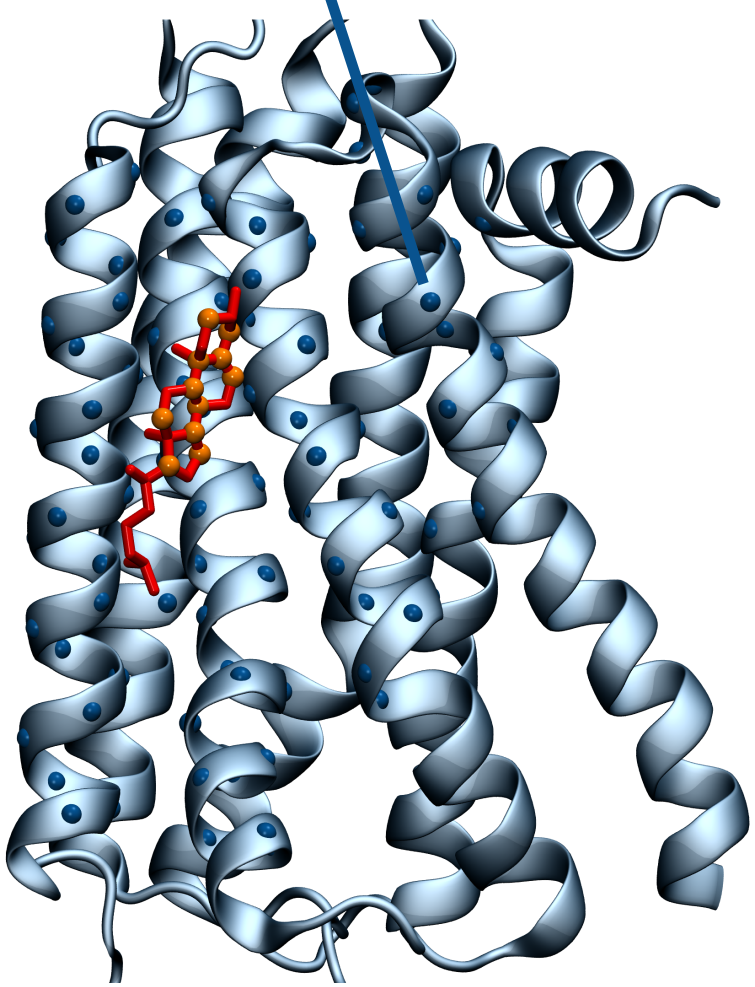

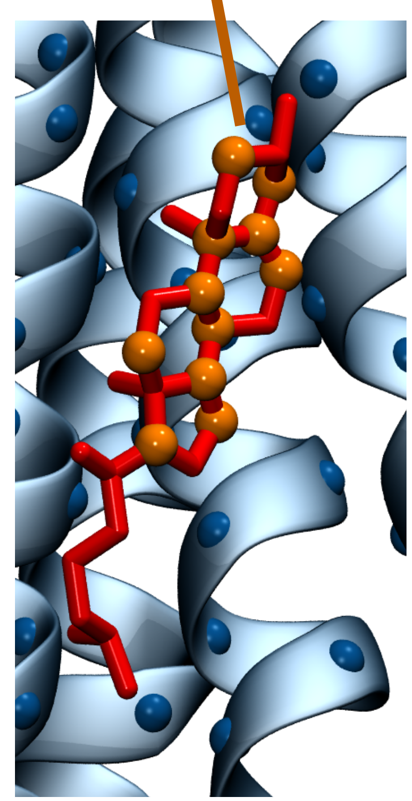

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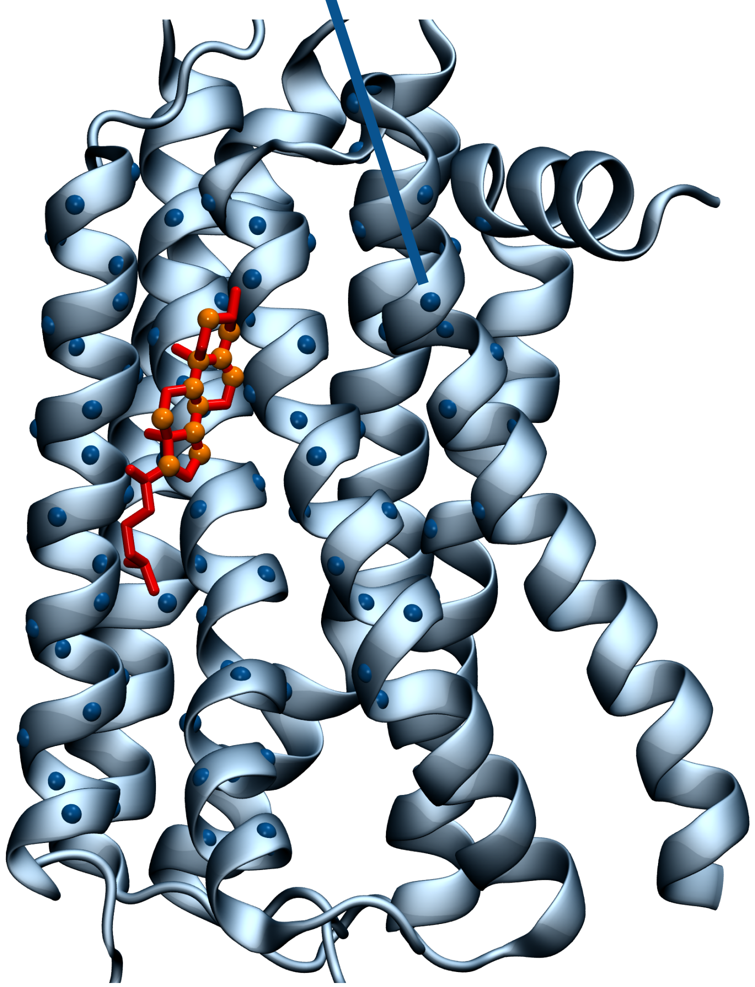

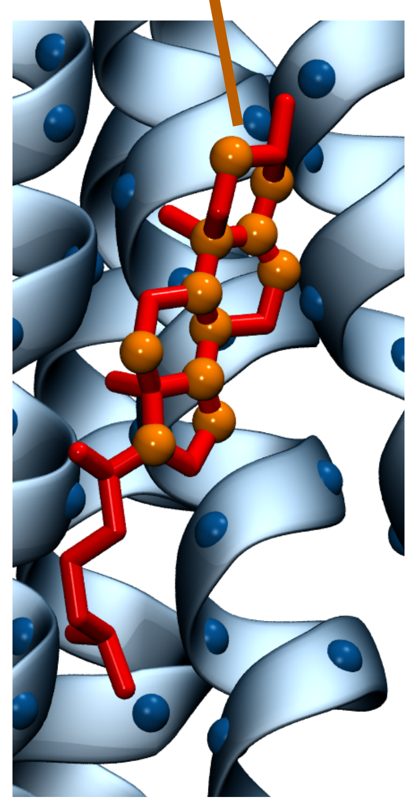

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Collective Variables Module

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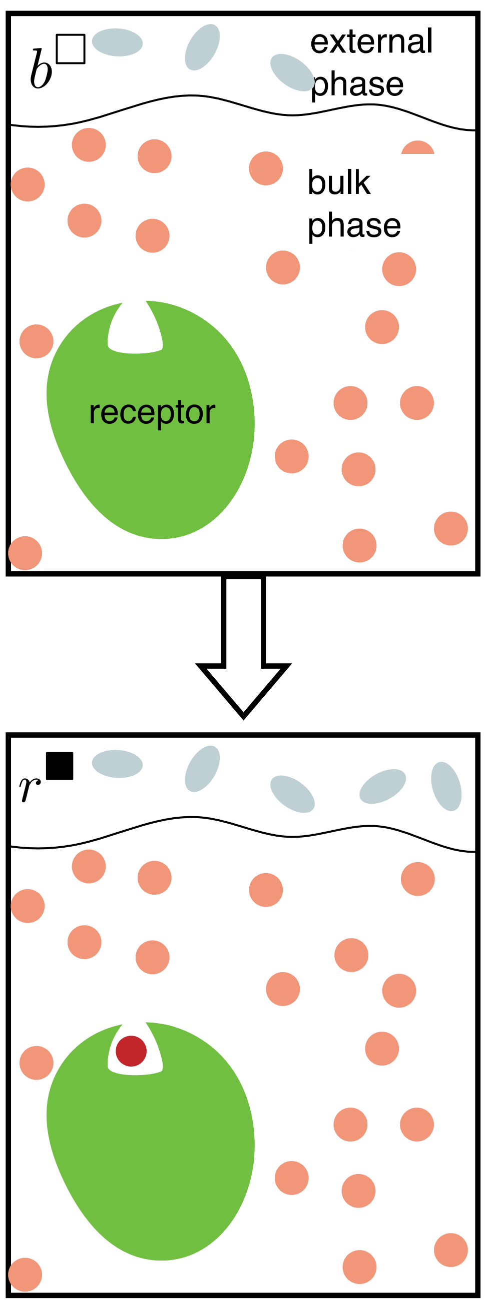
ACRES: Exclusion Restraints



problem high affinity bulk ligand replaces decoupled ligand

solution exclusion restraint potential (opposite of DBC restraint potential) on non-test ligand molecules

correction *none needed*: by definition, unoccupied state has no ligand bound

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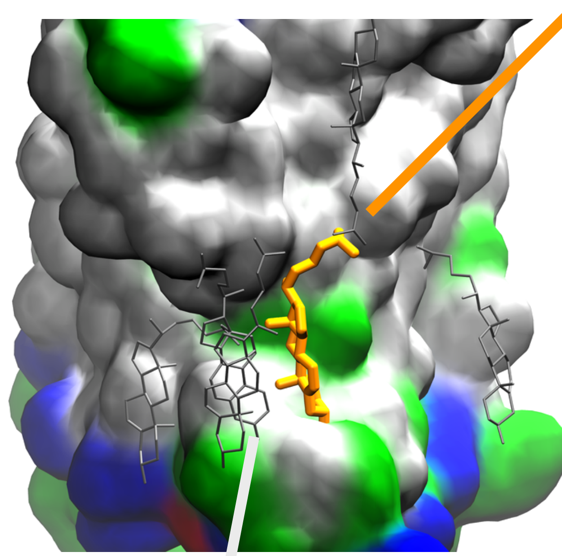

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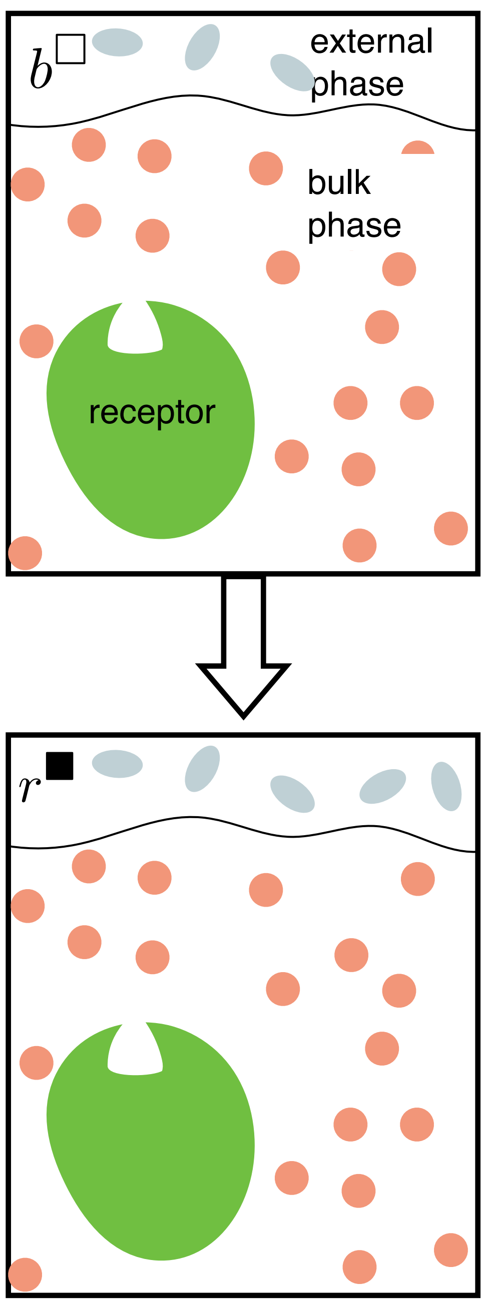

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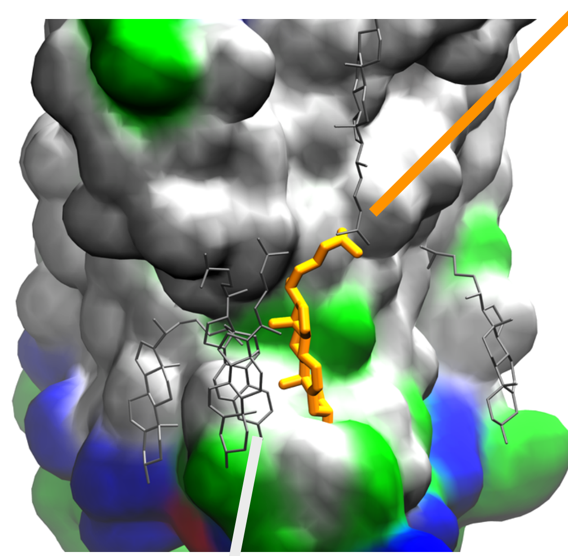

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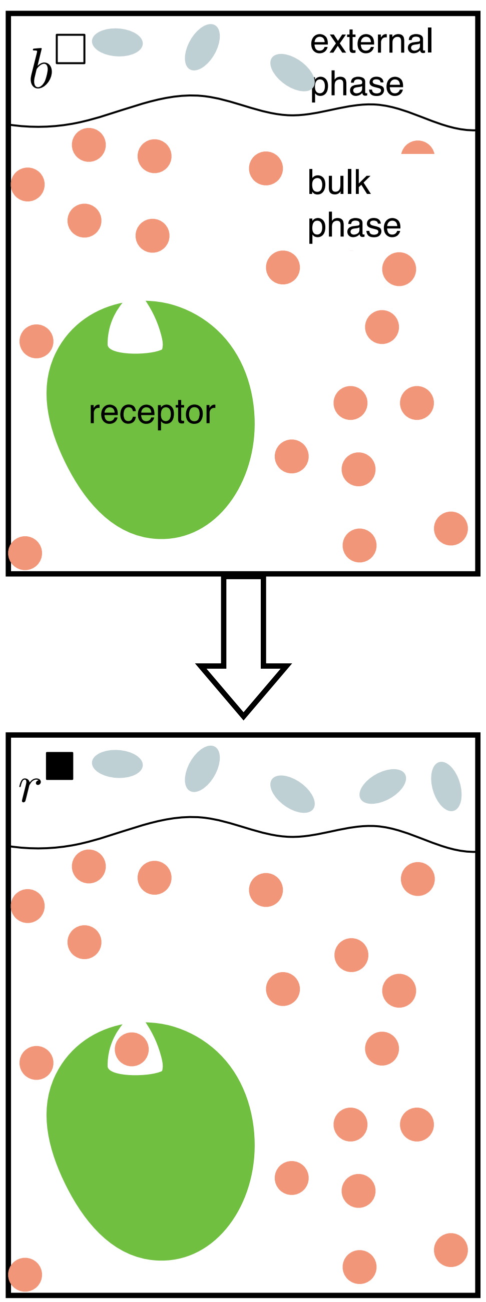

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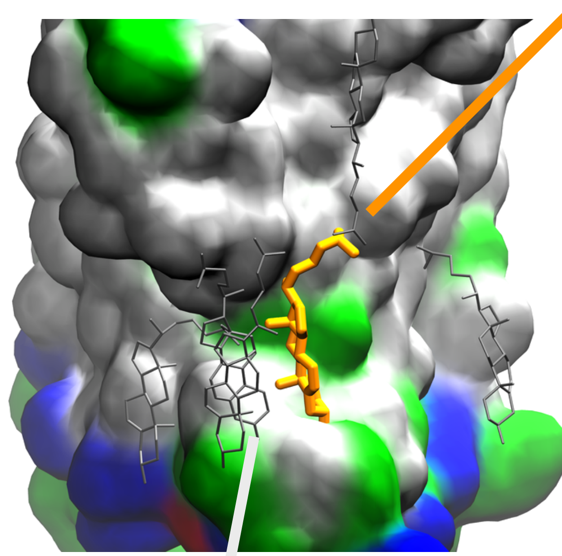

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Cycle for Lipophilic Ligand

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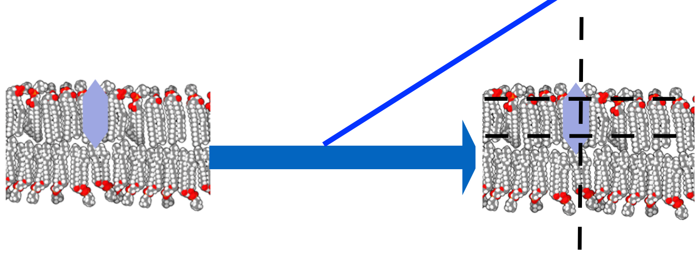

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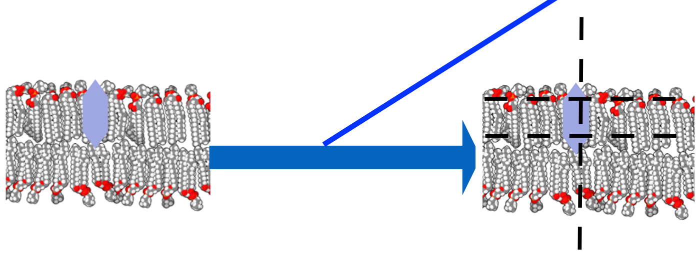

$n_L\alpha$

$\sim 10^2\, x_{\text{CHOL}}$

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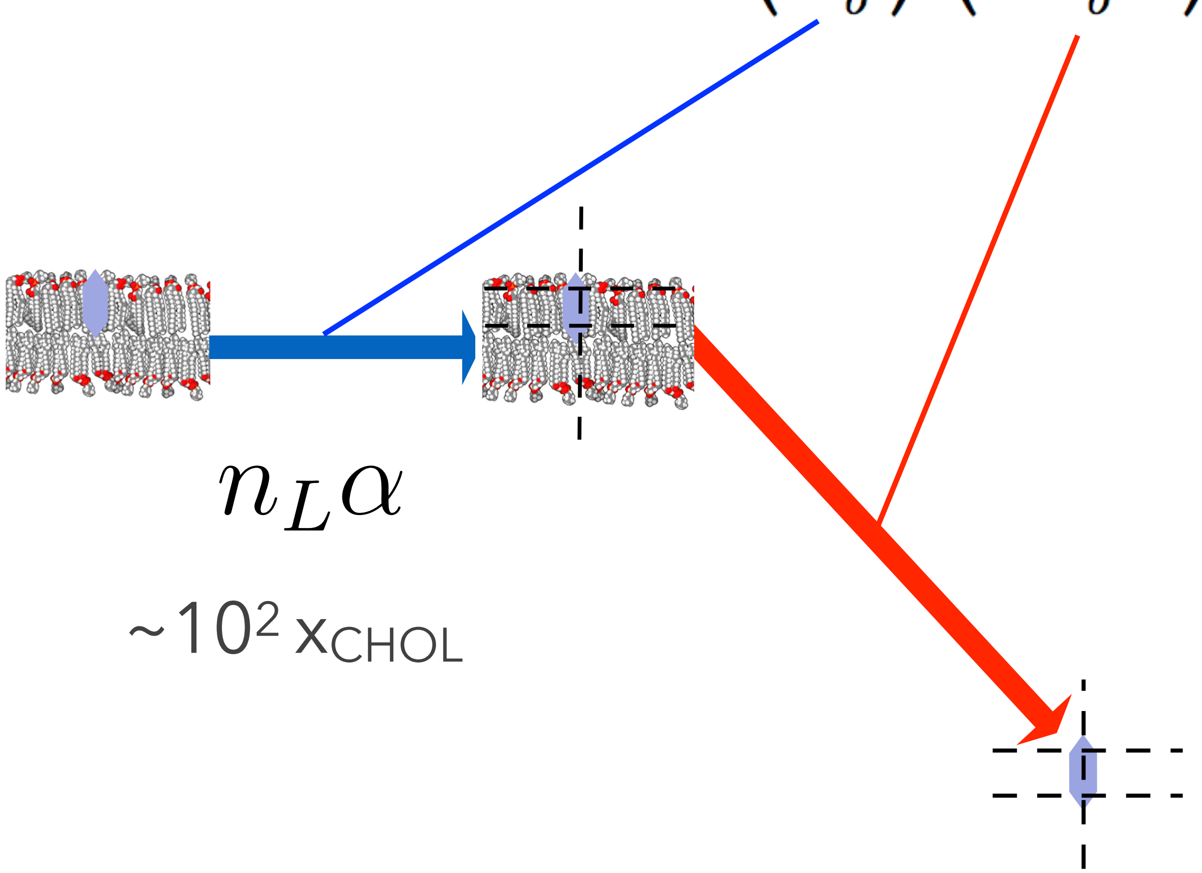

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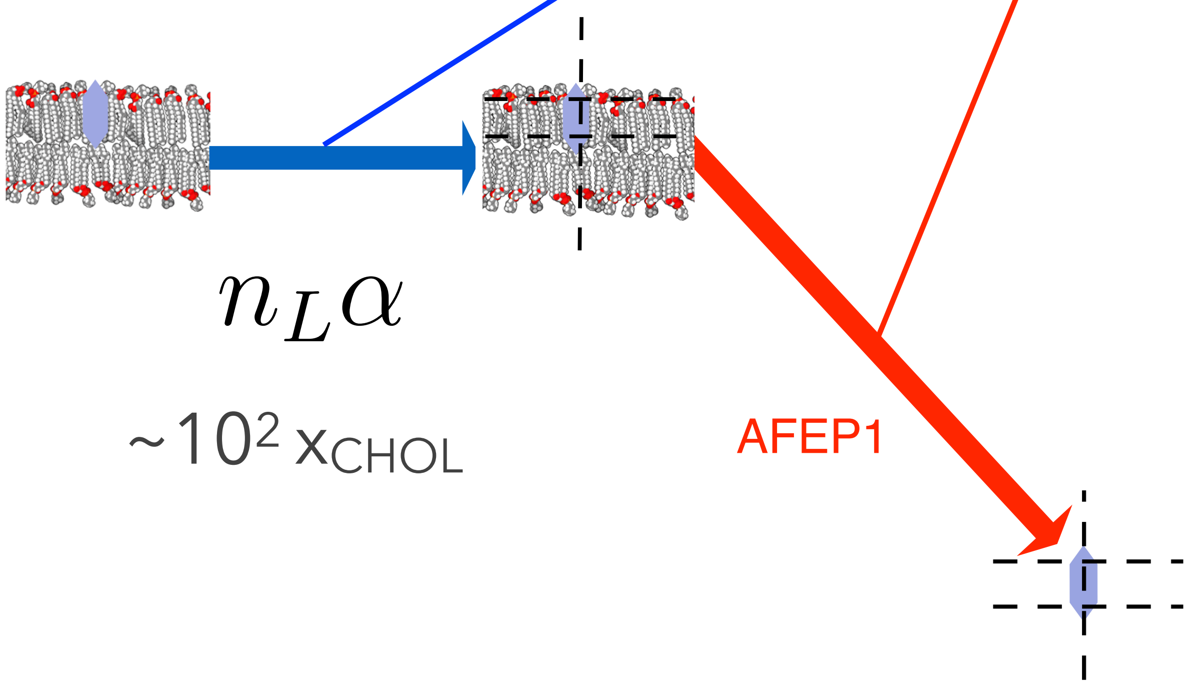

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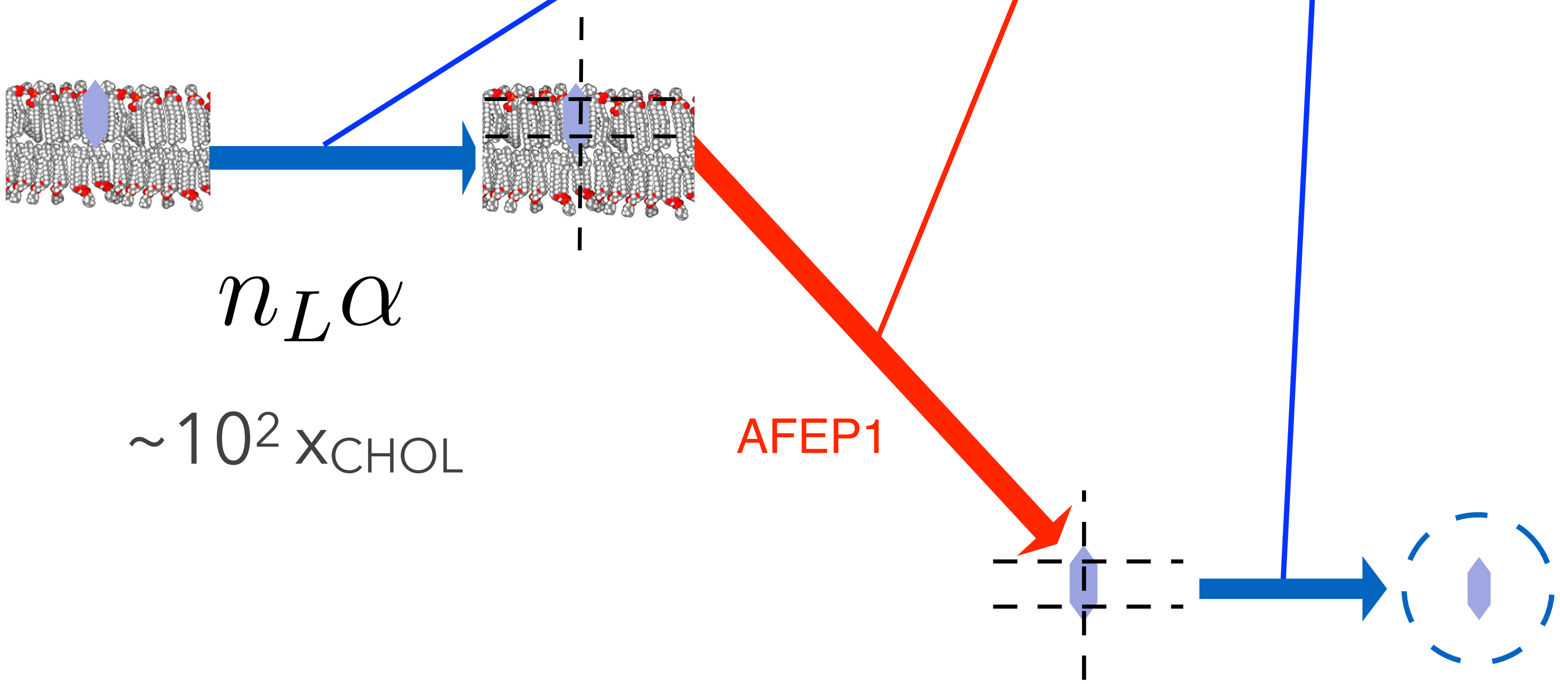

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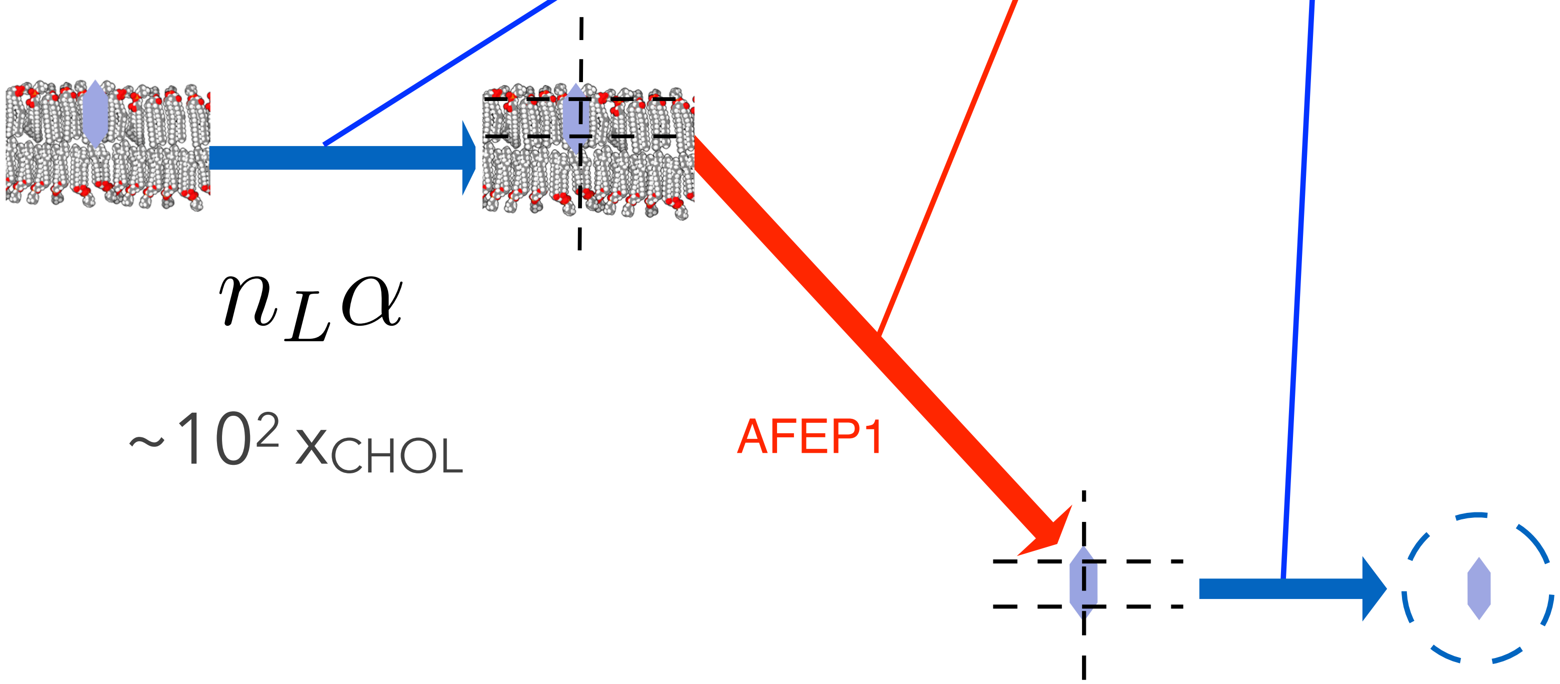

$n_L\alpha$

~10^2 x_{CHOL}

$$\frac{2\pi r_R^3}{3 z_R A^{=}}\,\frac{1}{1-\cos\theta_R} \quad \sim 10^{-1}$$

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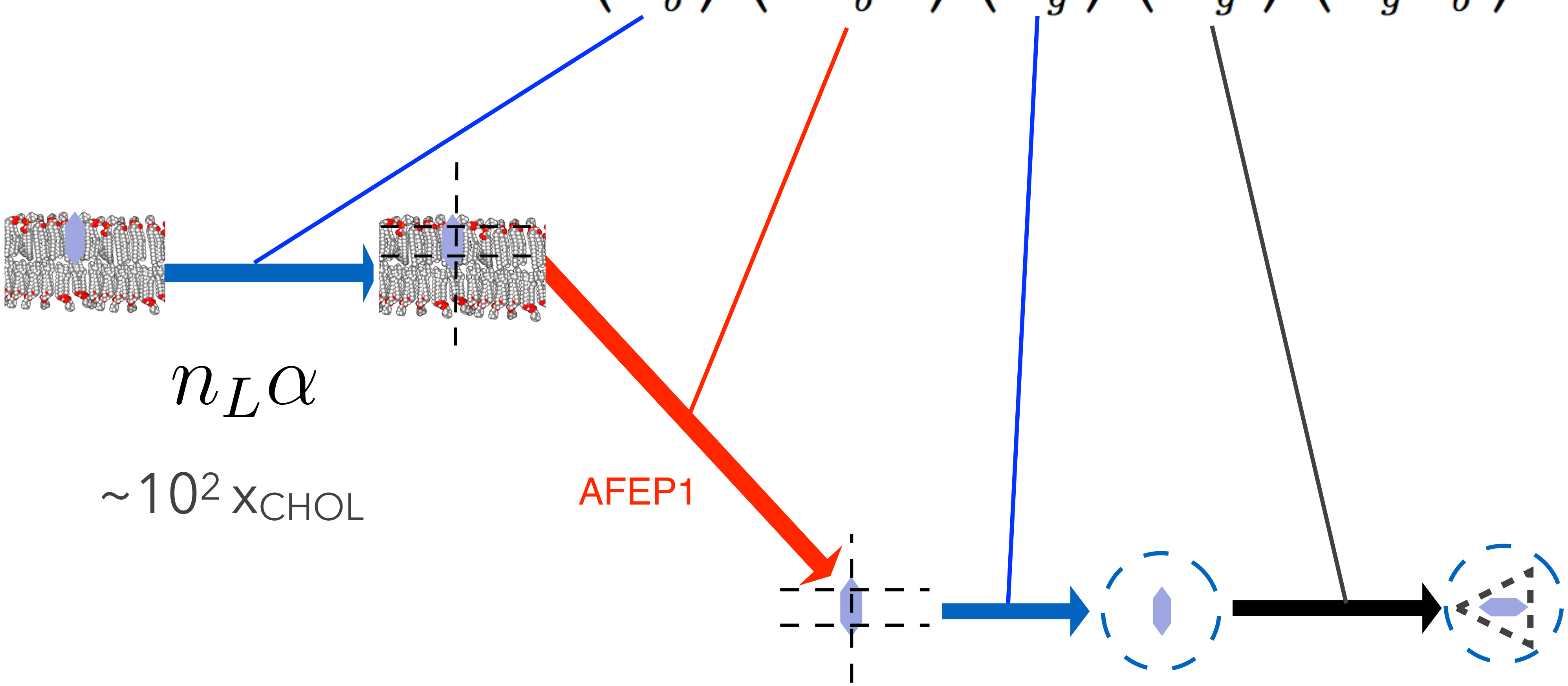

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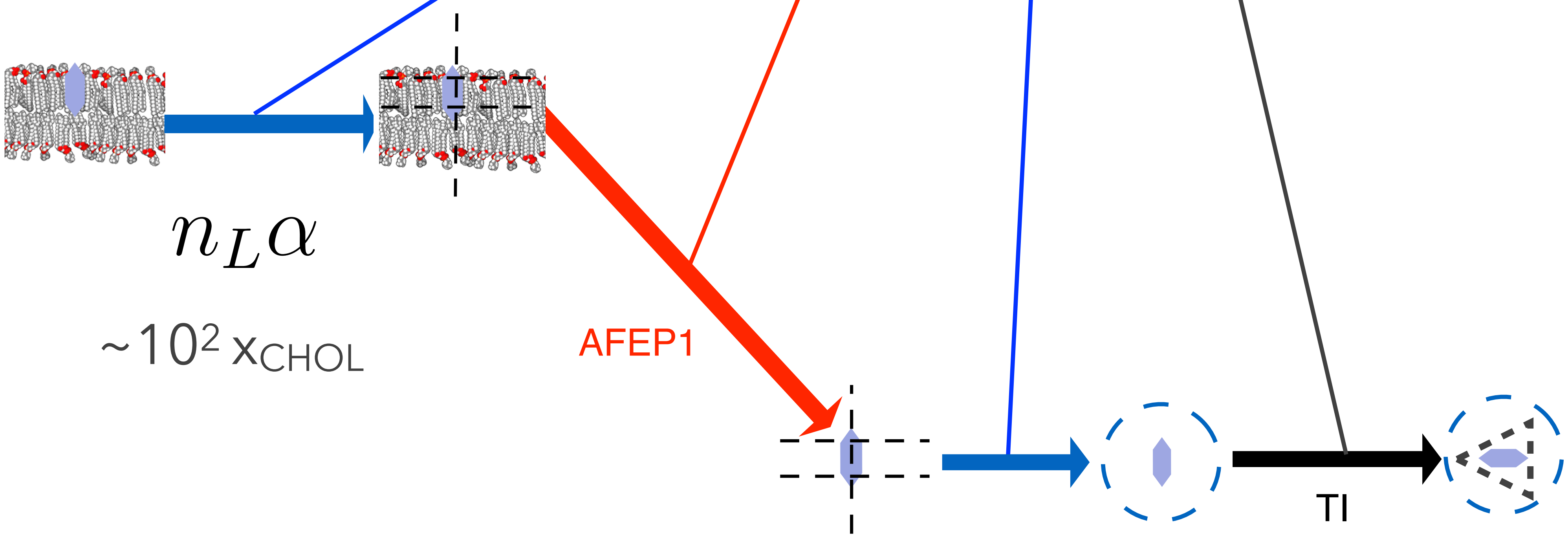

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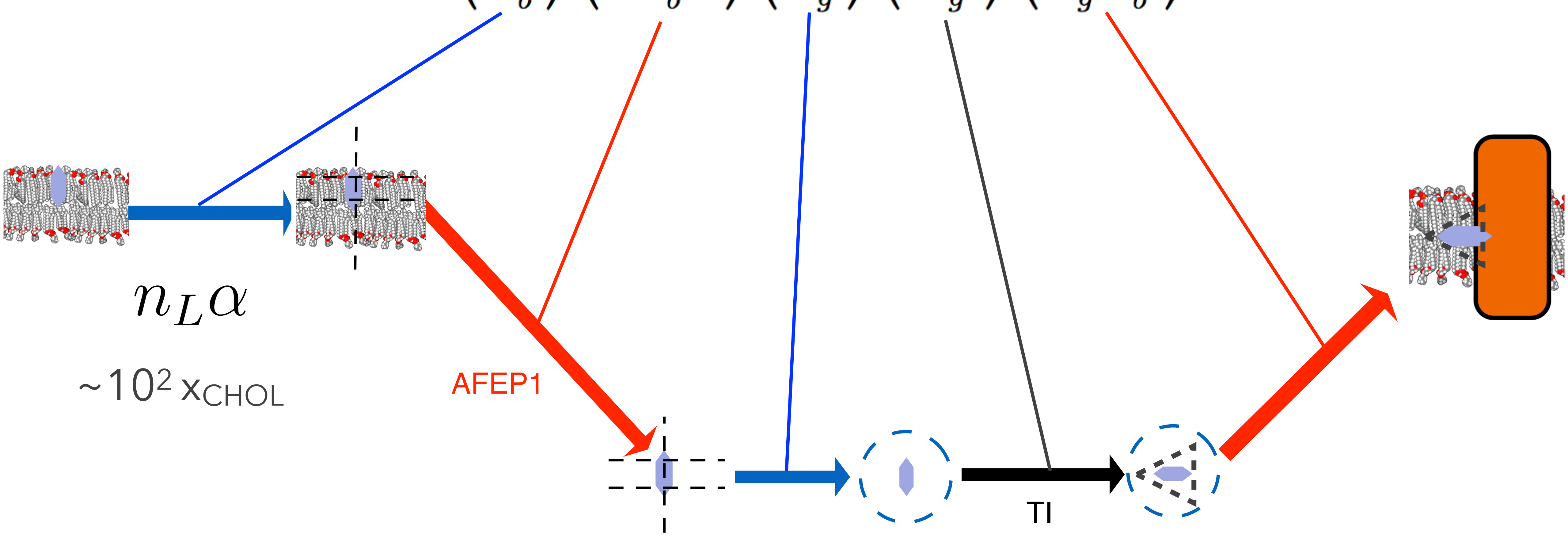

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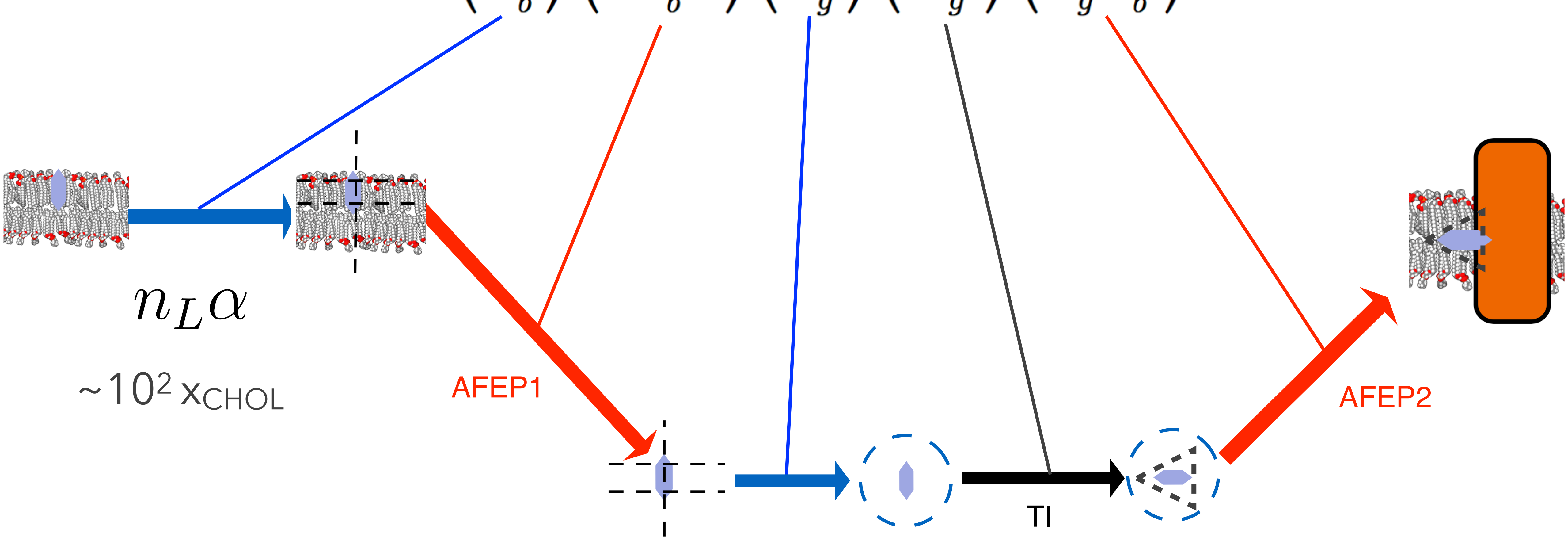

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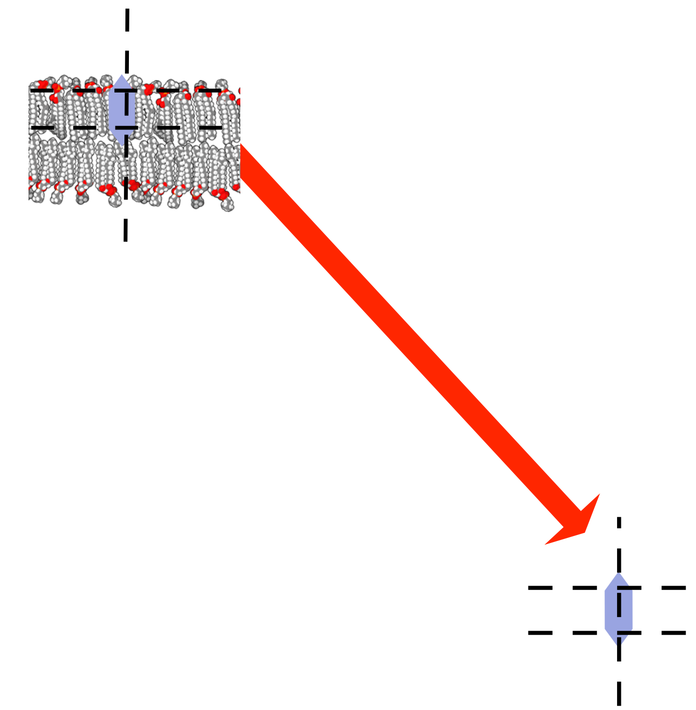
Application 1 : GPCR-Bound Cholesterol

bulk/gas partition coefficient

$$\frac{1}{P_x} \equiv \frac{Z_b^{-} Z_g^{=}}{Z_b^{=}} \quad \textcolor{red}{= \mathrm{AFEP1}}$$



Salari, Joseph, Lohia, Henin, Brannigan
JCTC 2018 submitted
https://arxiv.org/abs/1801.04901

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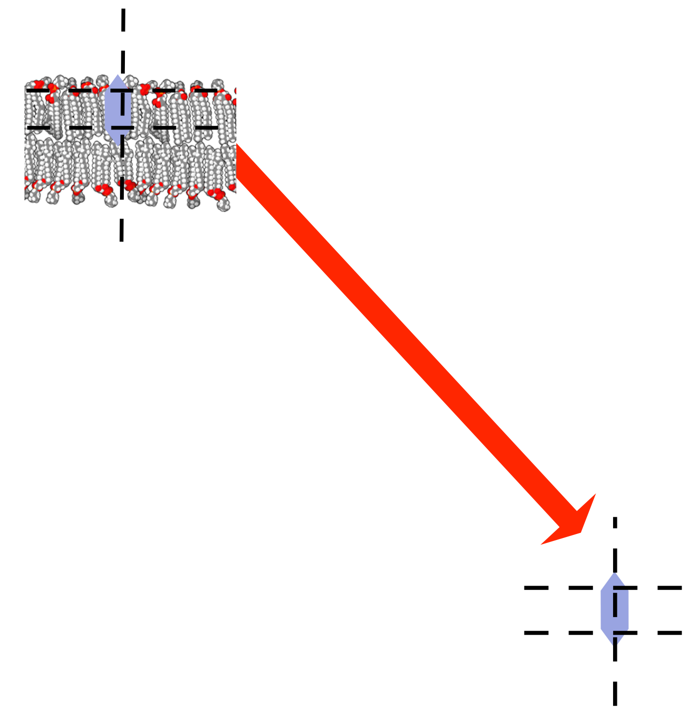

Quadratic Mixture Model

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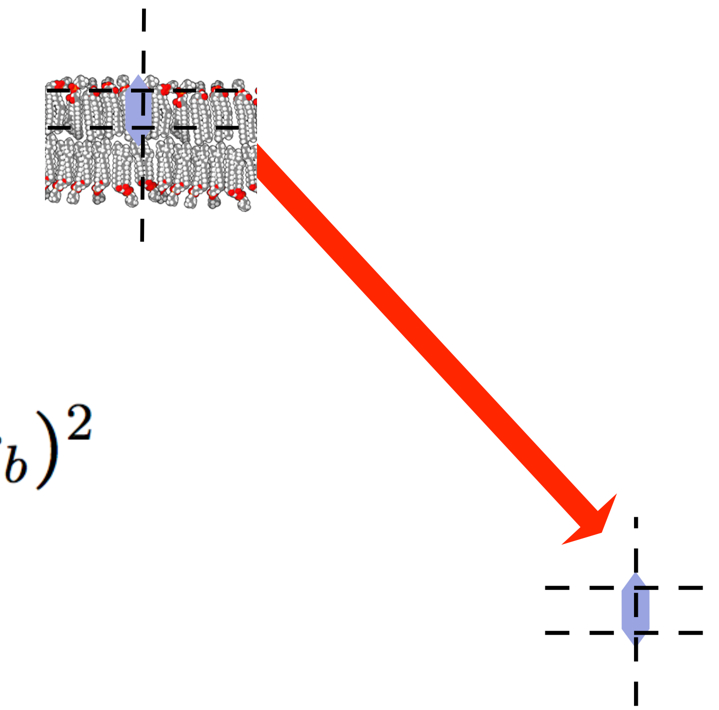

Quadratic Mixture Model

$$\mu_g^0 + RT \ln x_g \quad = \quad \mu_b^0 + RT \ln x_b + h^0 (1 - x_b)^2$$

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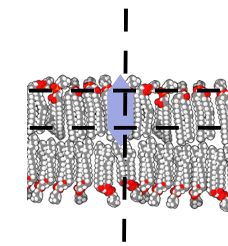

enthalpy of mixing

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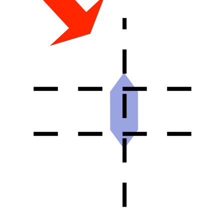

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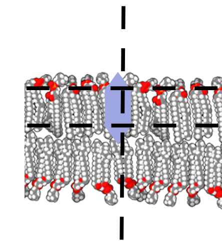

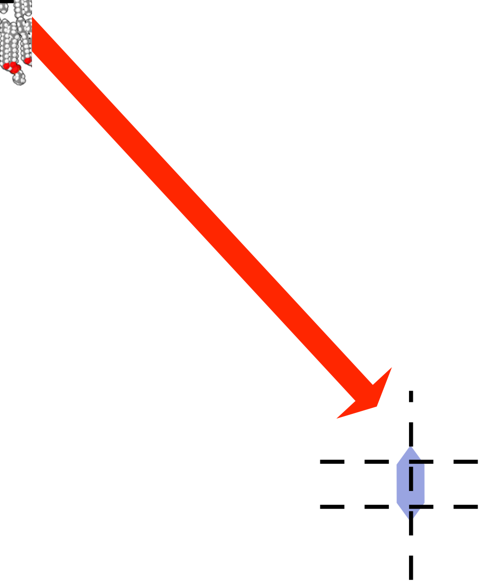

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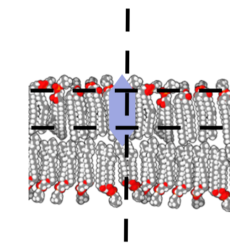

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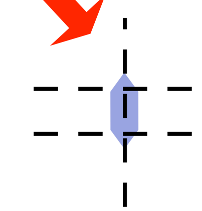

AFEP decouple single cholesterol molecule from cholesterol:POPC bilayer

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Application 1 : GPCR-Bound Cholesterol

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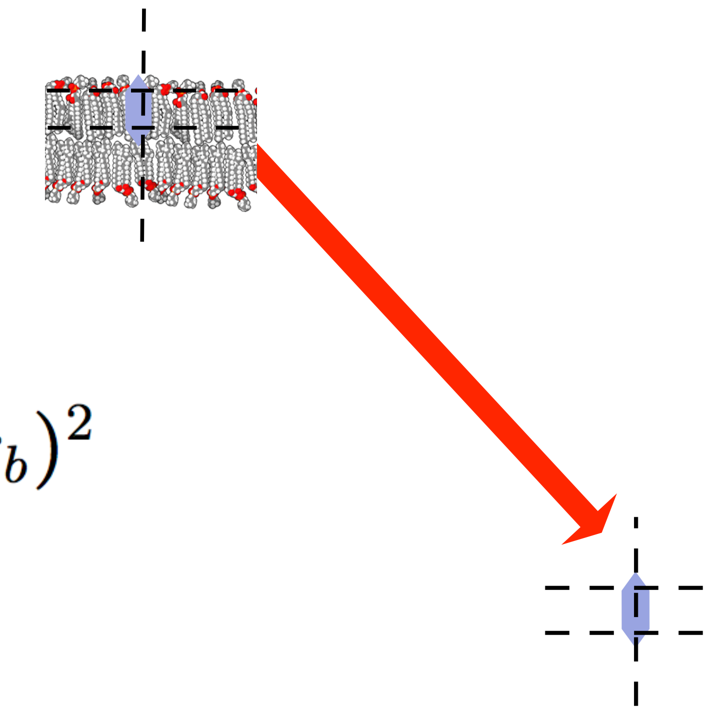

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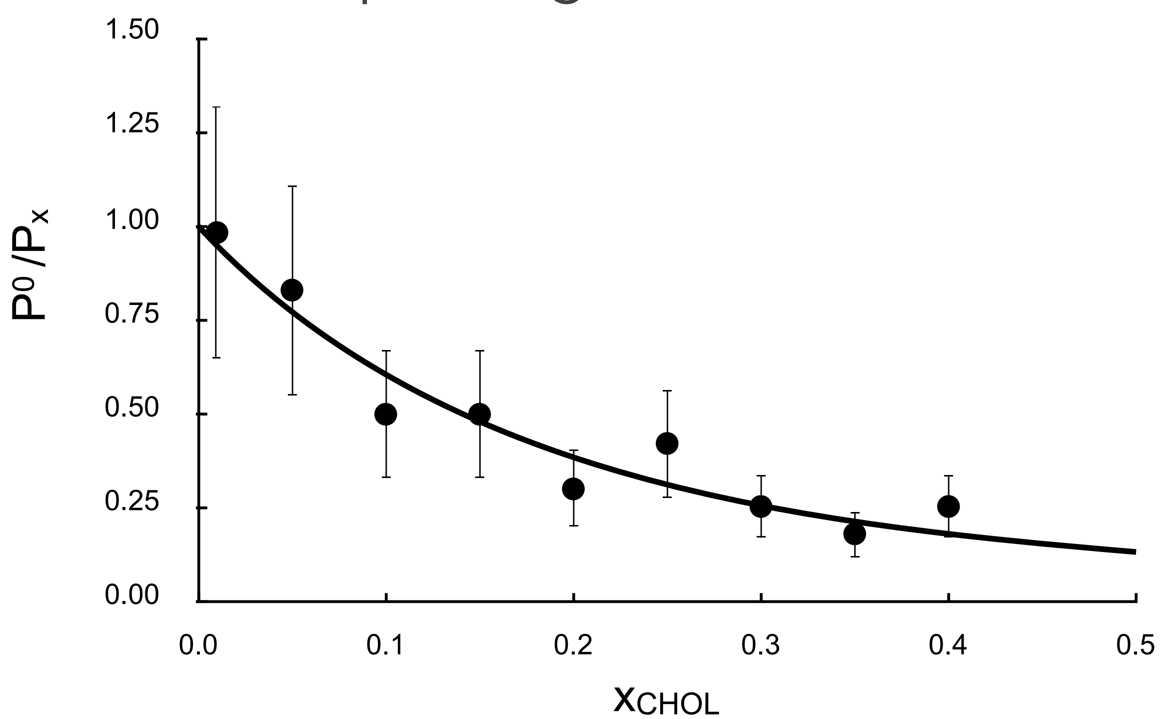

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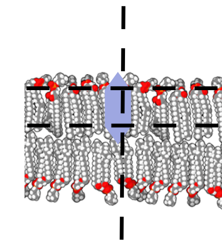

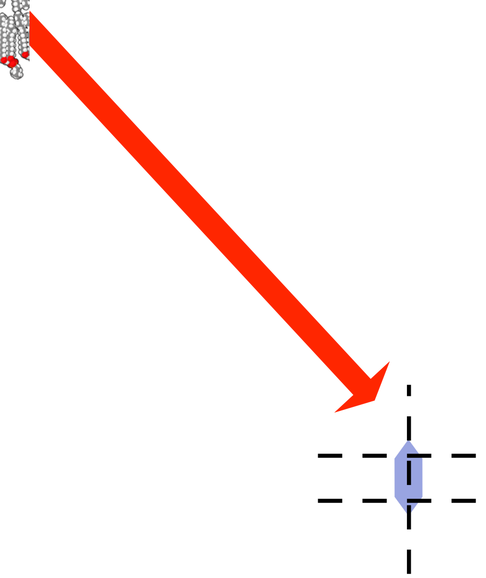

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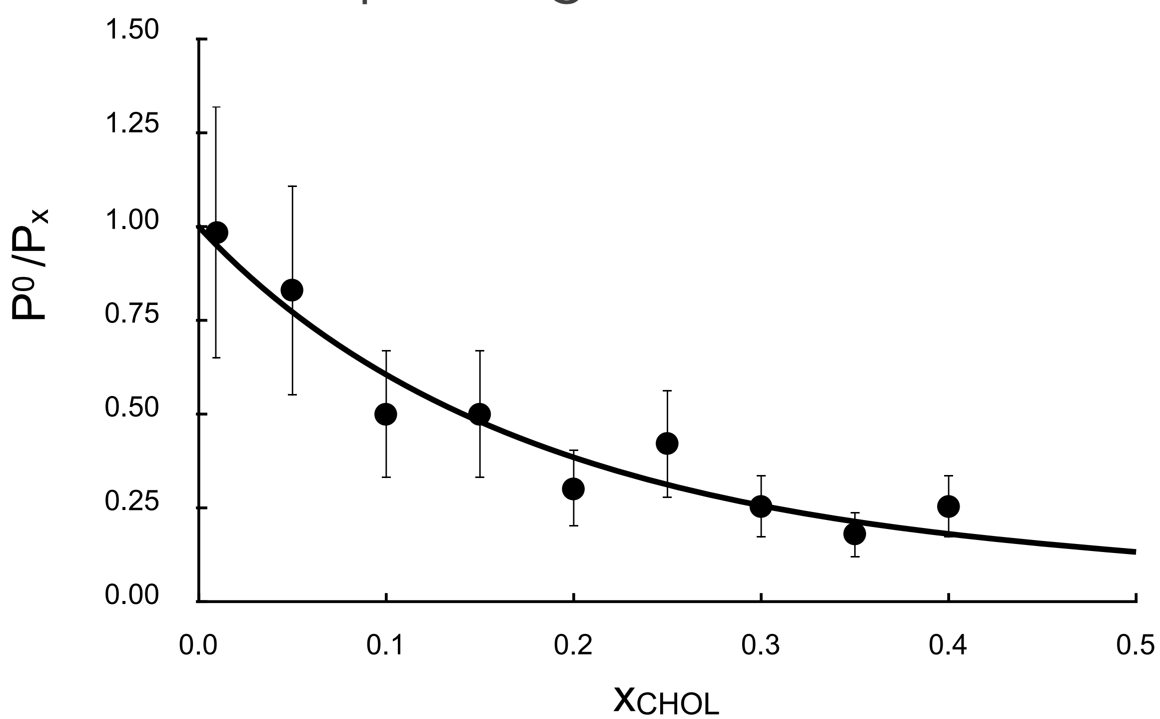

$P^0 = 3 \times 10^{14}$

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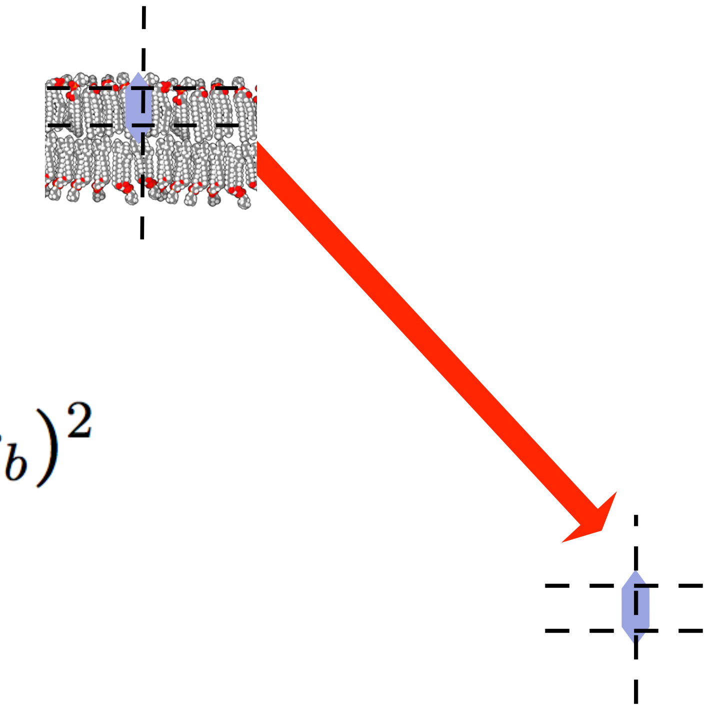

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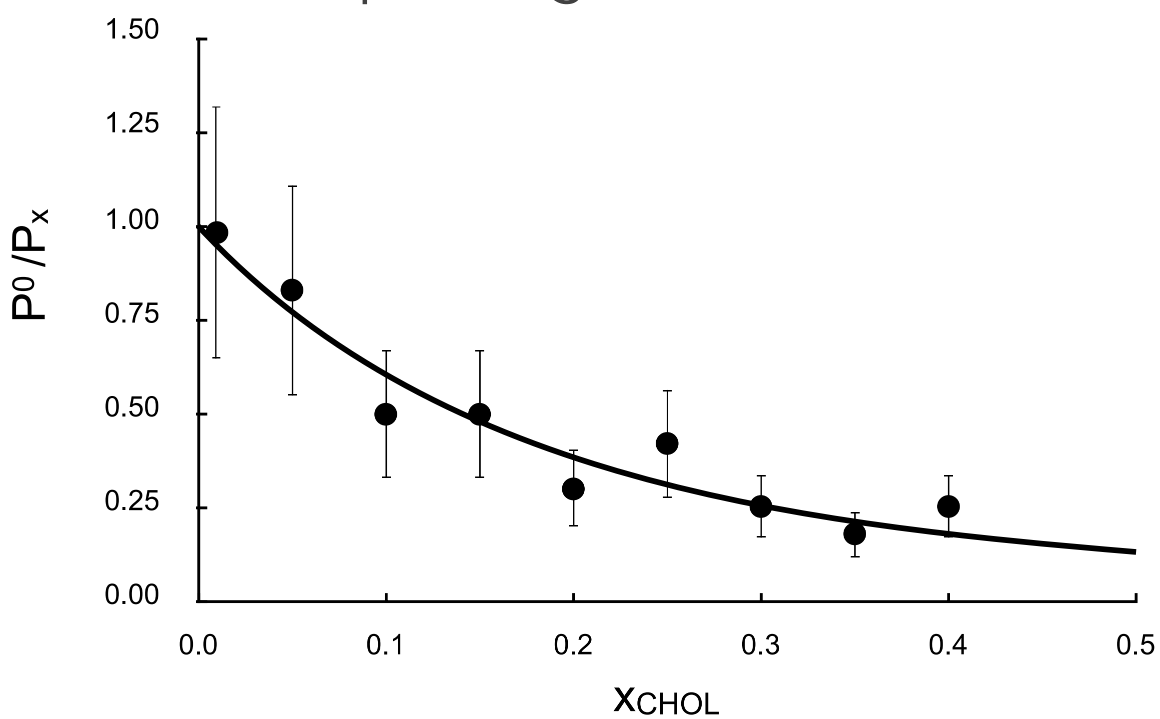

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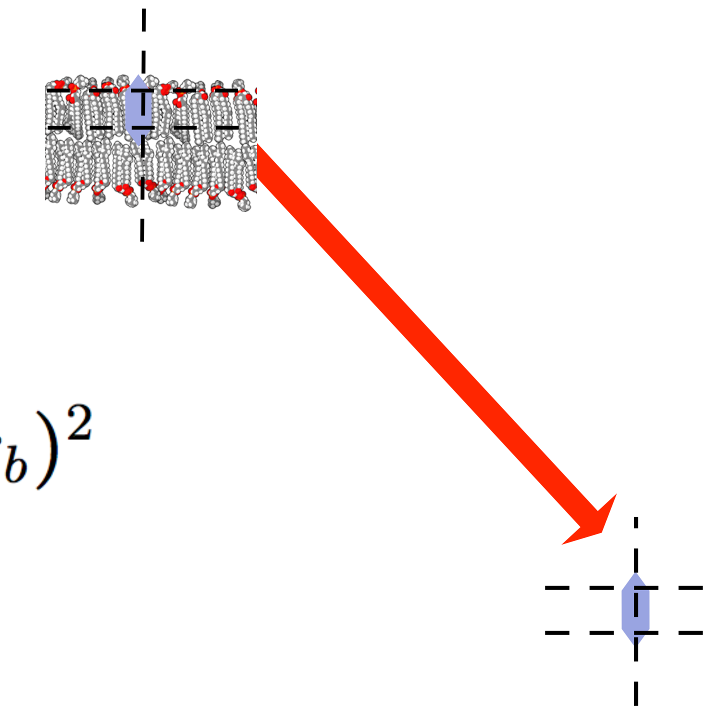

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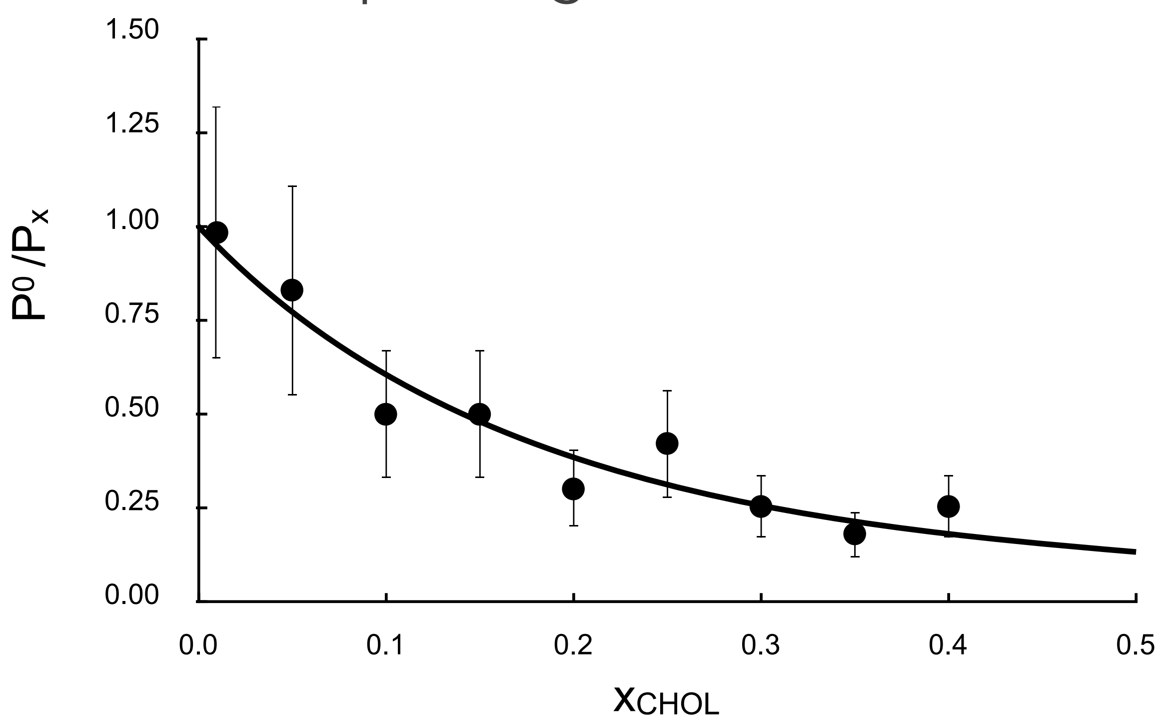

$P^0 = 3 \times 10^{14}$

$h^0 = 1.6$ kcal/mol

$h^0 \sim \langle u_{chol\text{-}popc} \rangle - \langle u_{chol\text{-}chol} + u_{popc\text{-}popc} \rangle / 2 > 0$

non-ideality

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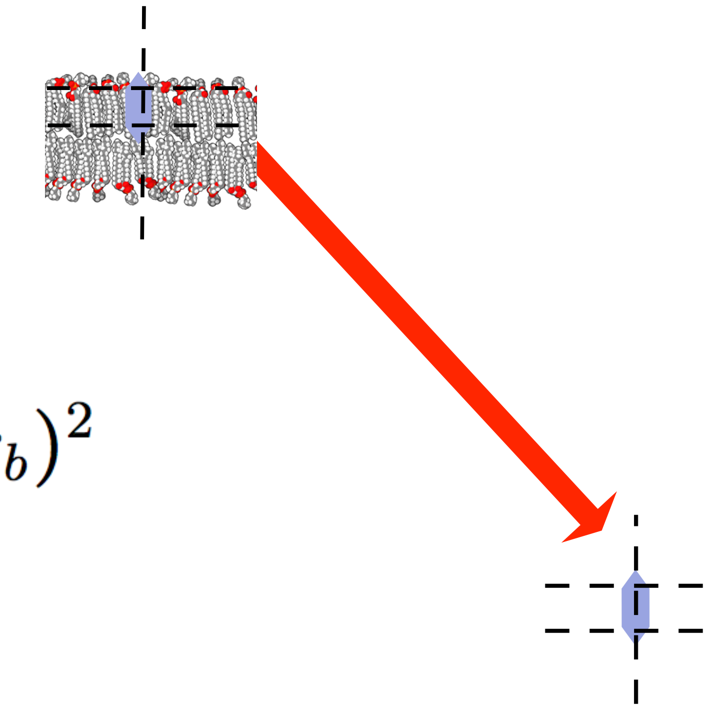

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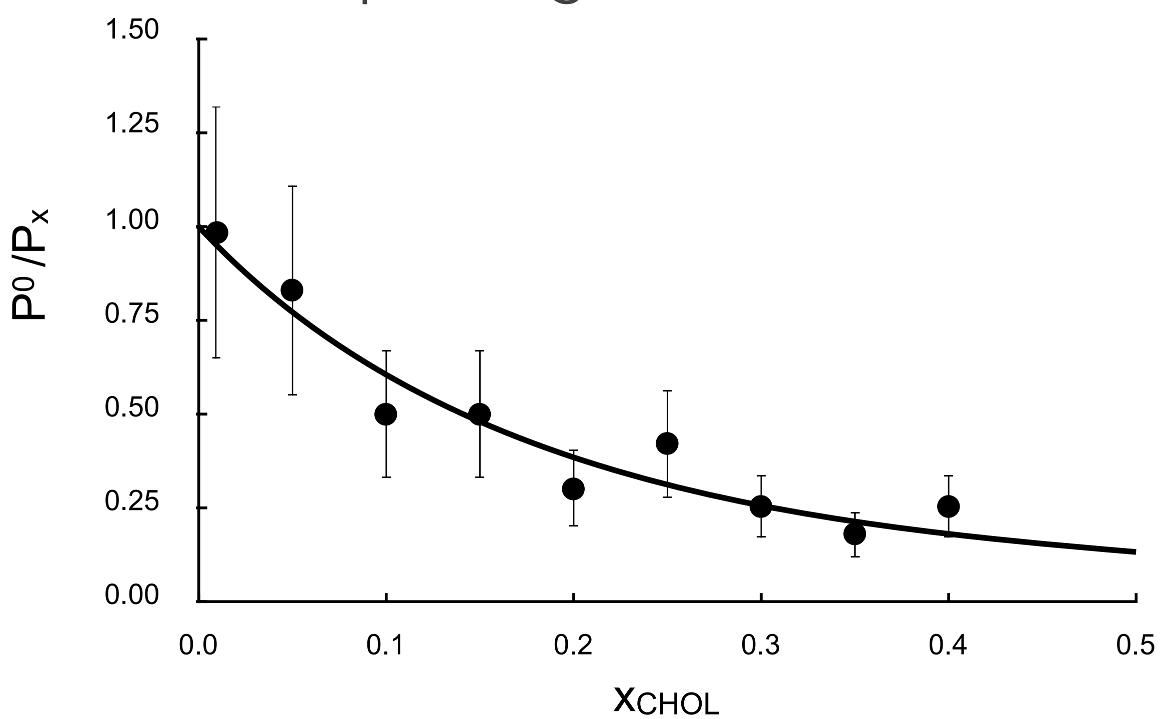

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non-ideality

AFEP benefit: non-ideality captured just by decoupling from bulk!

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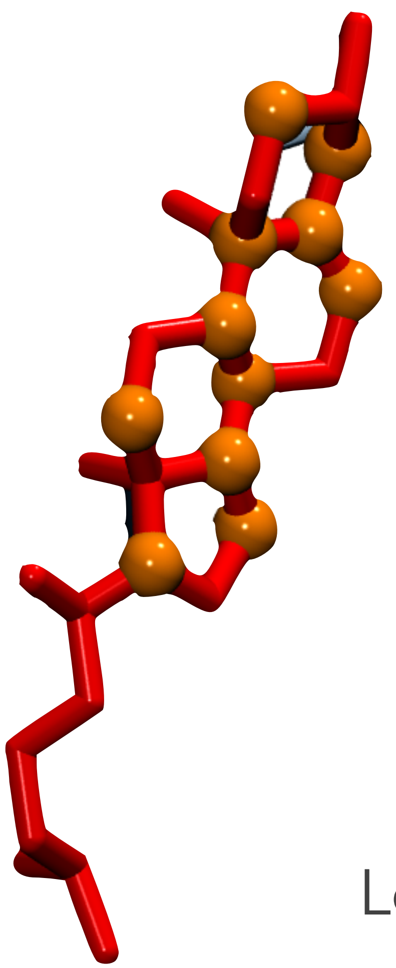
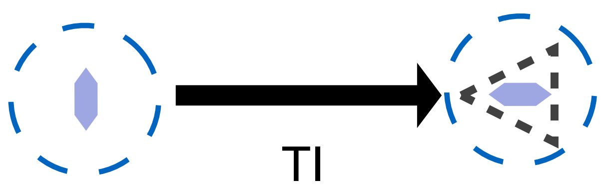
Application 1 : GPCR-Bound Cholesterol

One restraint correction calculation (TI/SOS) gradually decreasing restraint strength

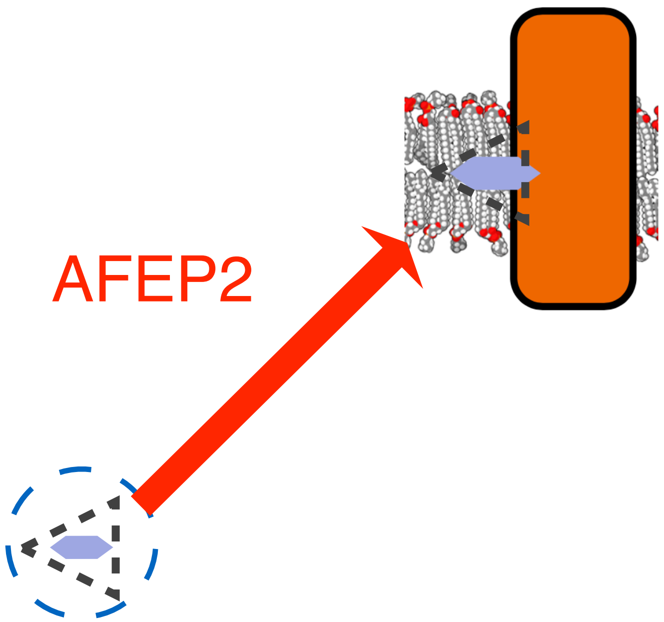


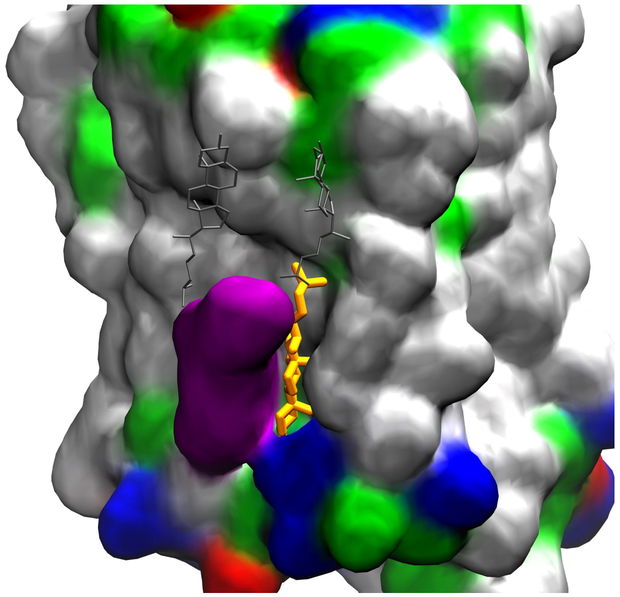


$$\frac{Z_g^{\circ\triangle}}{Z_g^{\circ}} \sim 10^{-3}$$

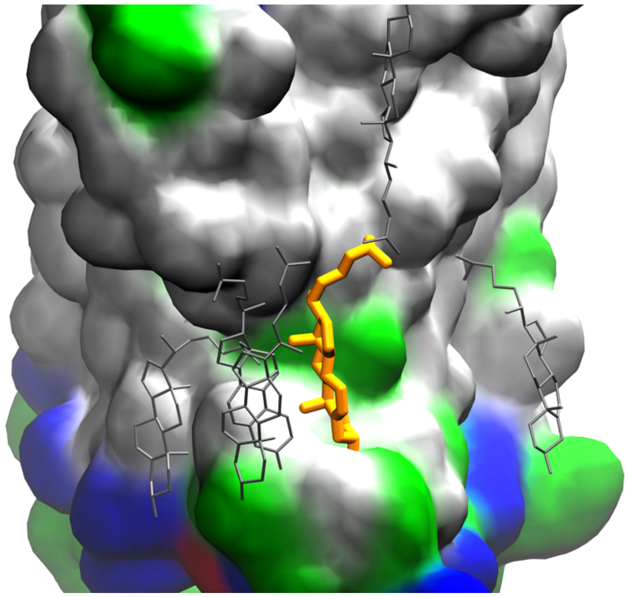
Less than 1 hour of calculation for single restraint correction

Application 1 : GPCR-Bound Cholesterol

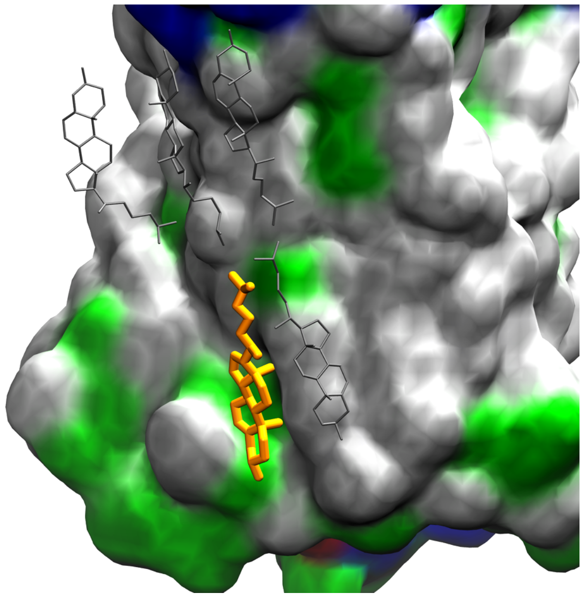




β2-Adrenergic
3D4S

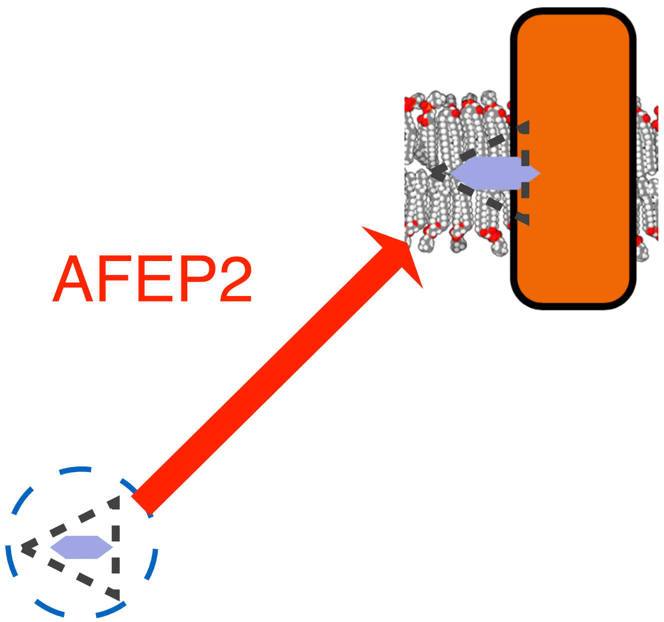


5-HT2B
4NC3



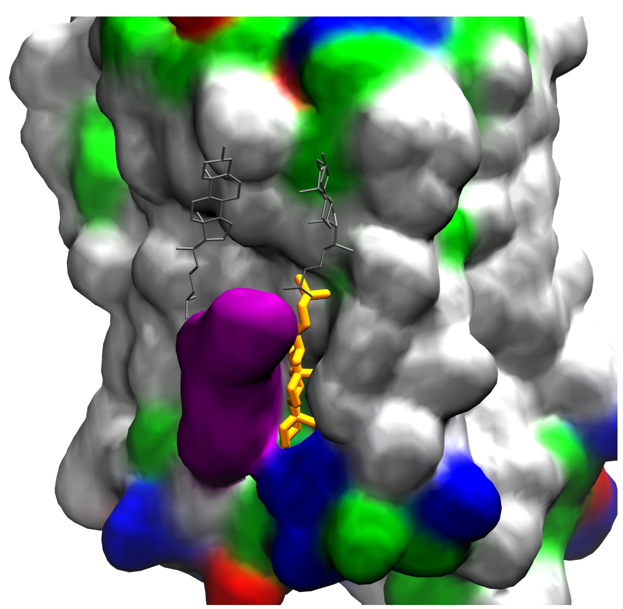
μ-Opioid
5C1M

Application 1 : GPCR-Bound Cholesterol



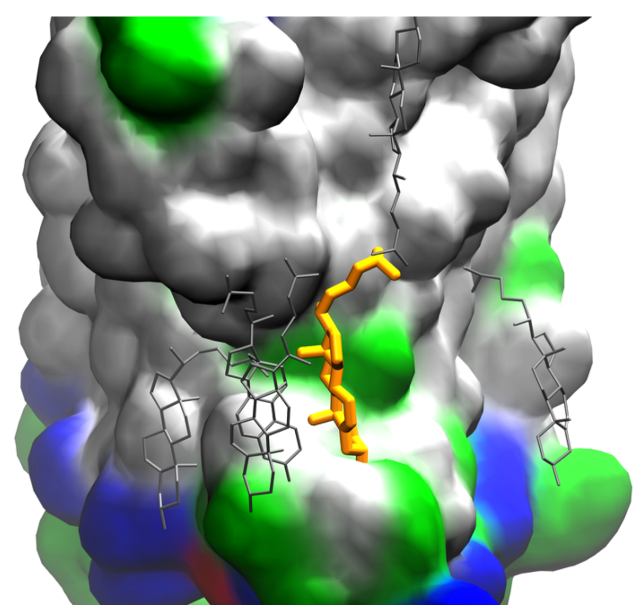
10^{16} x

10^{9}



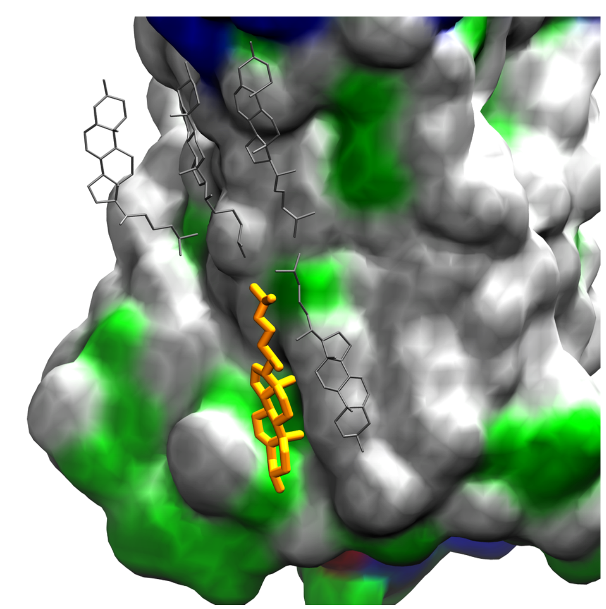
β2-Adrenergic
3D4S

10^{2}



5-HT2B
4NC3

1



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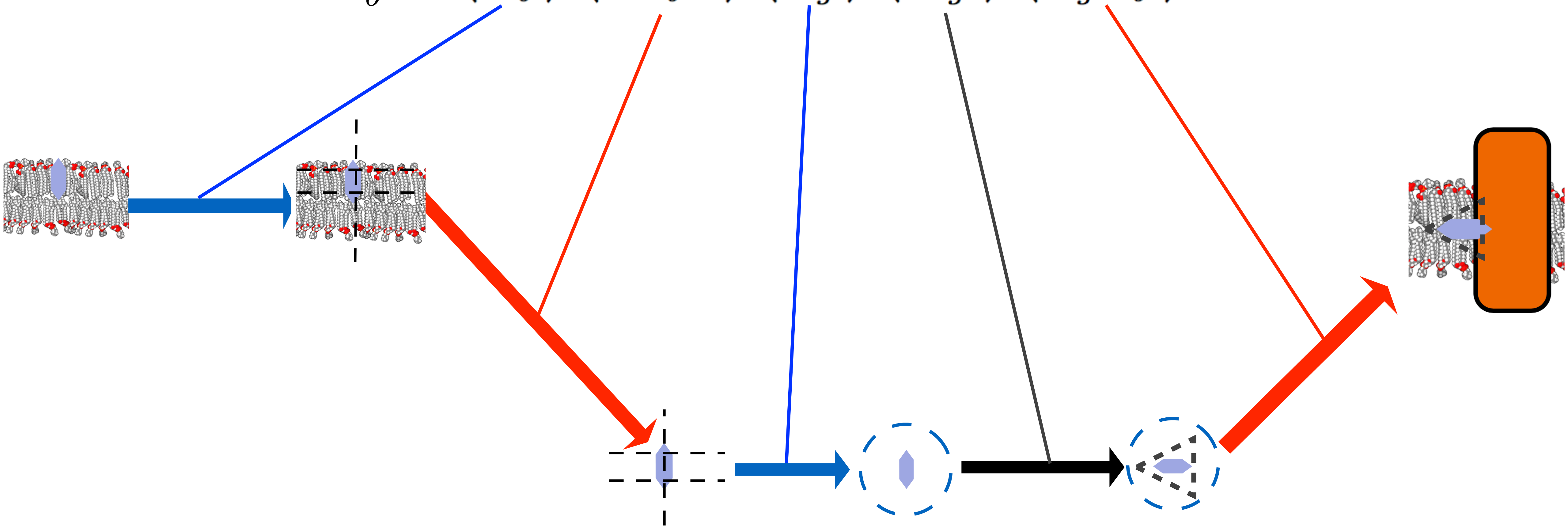

Salari, Joseph, Lohia, Henin, Brannigan
JCTC 2018 submitted
https://arxiv.org/abs/1801.04901

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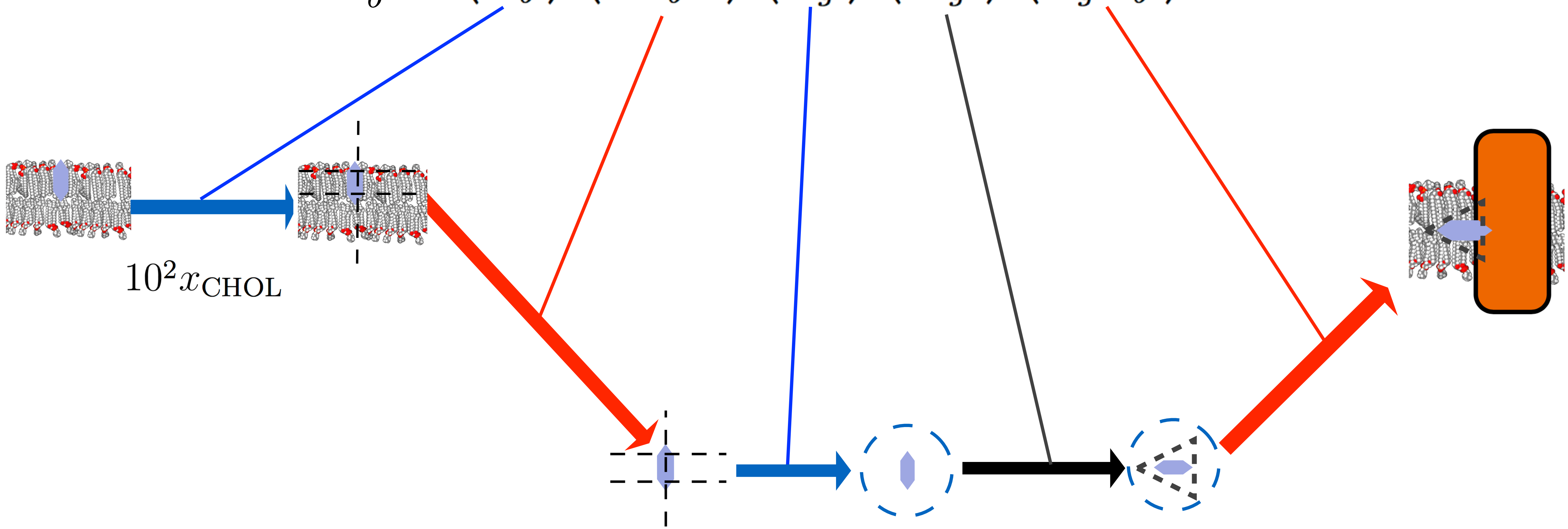

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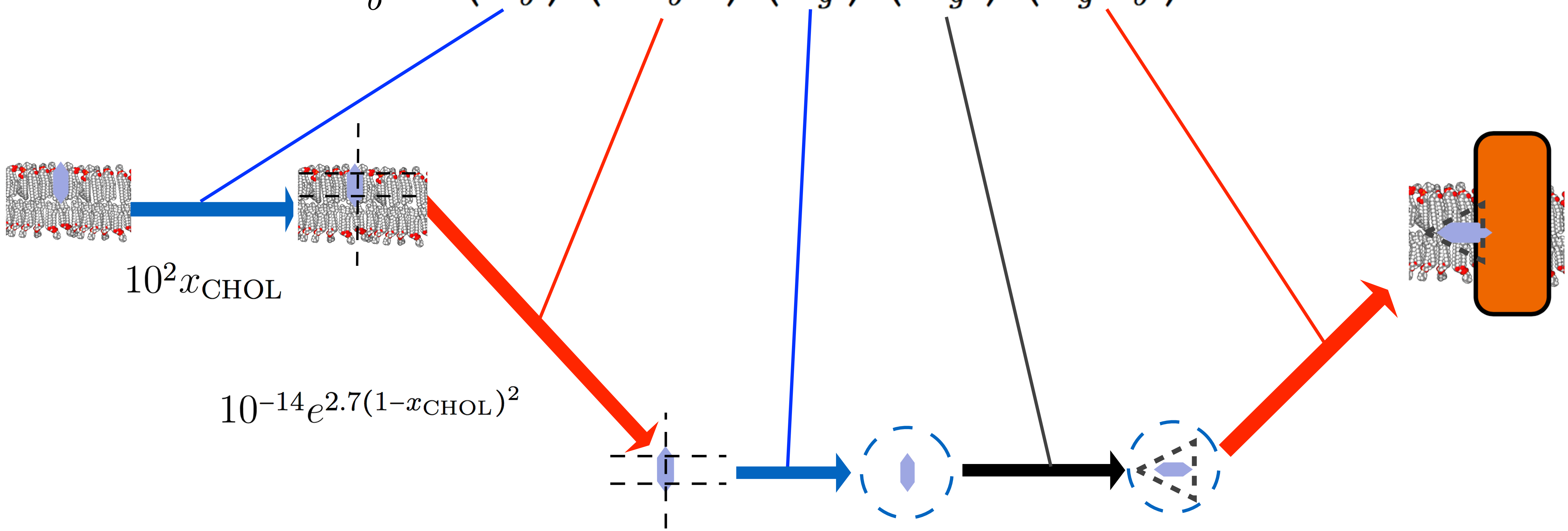

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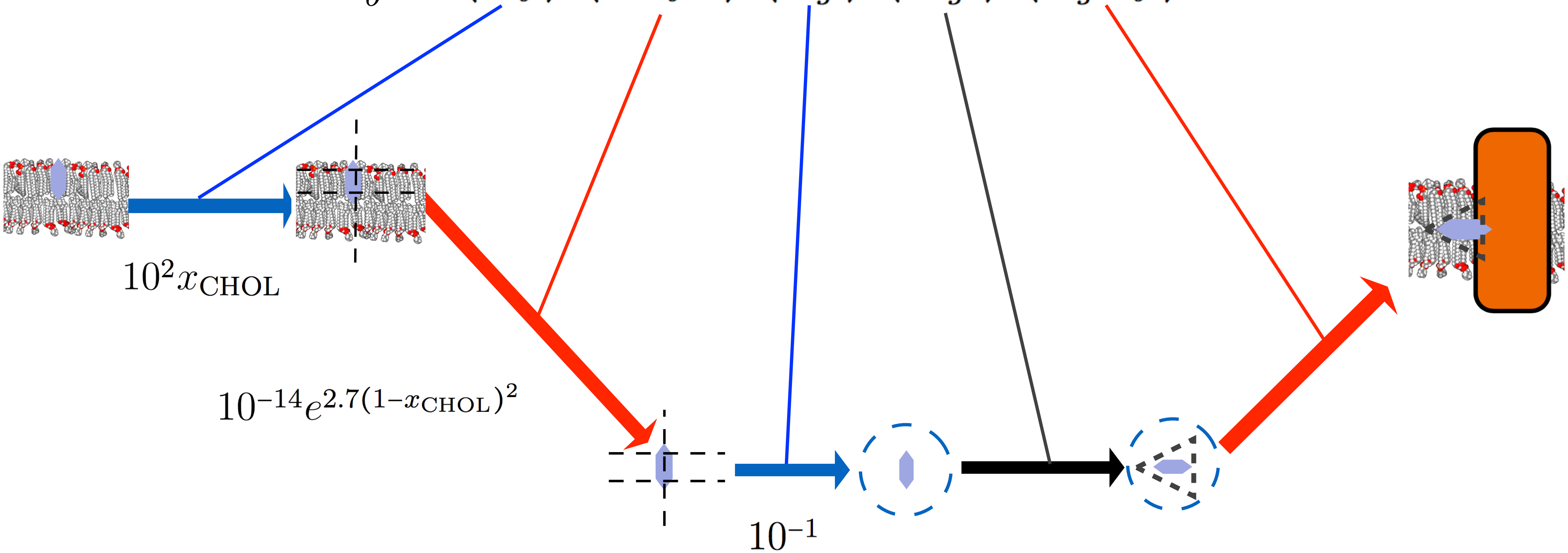

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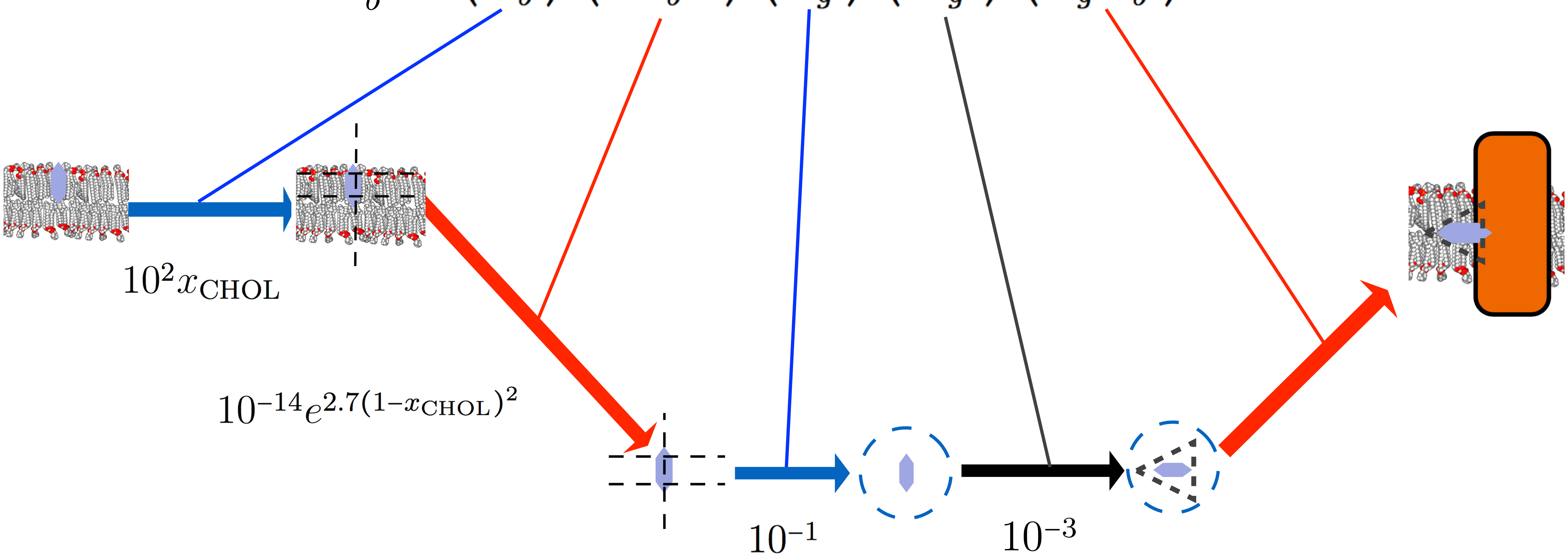

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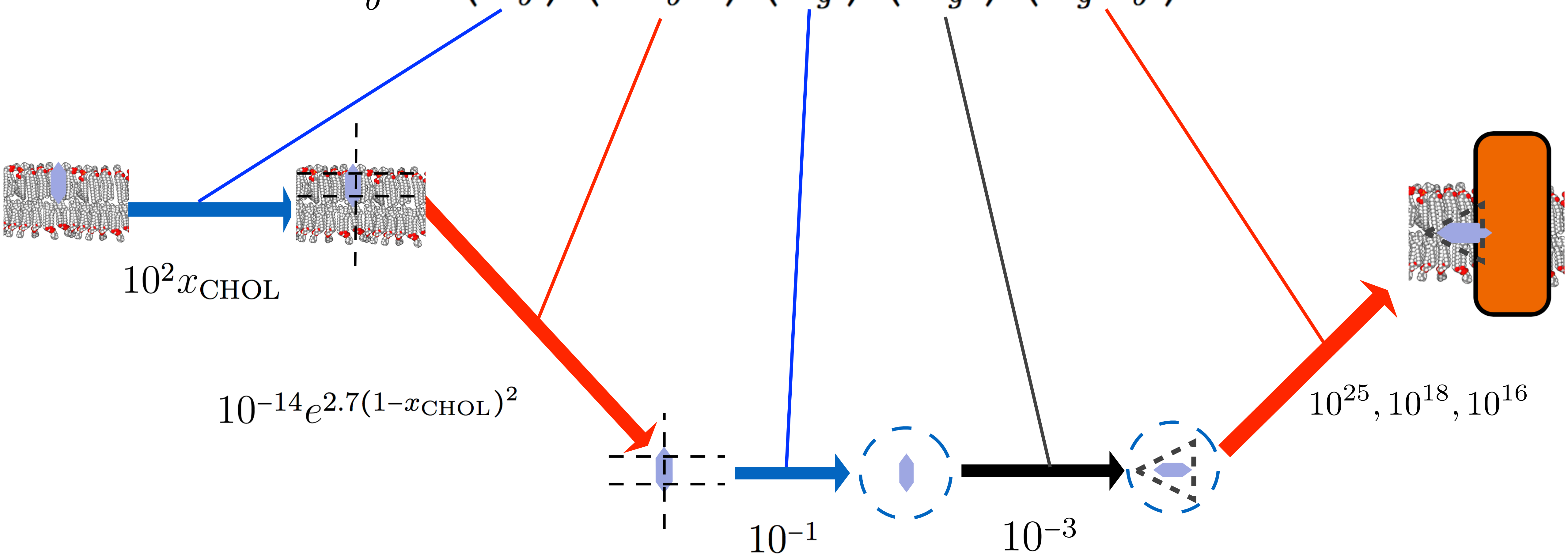

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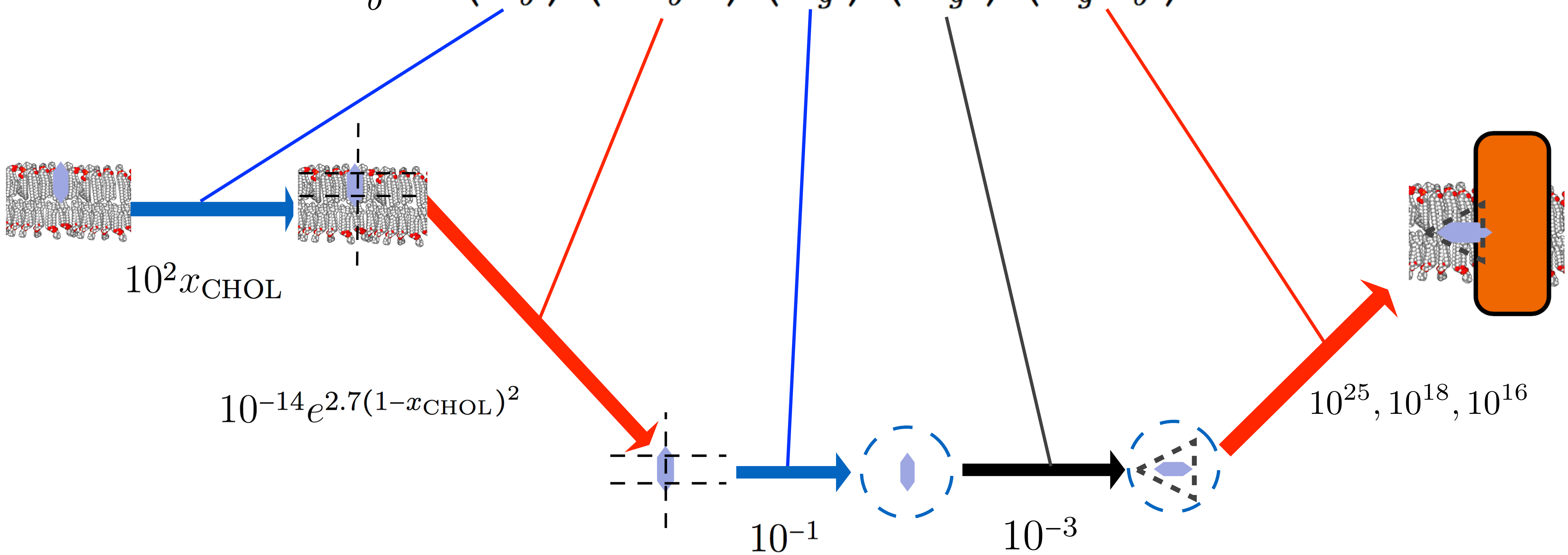

Ratio	Method	β2-adrenergic	5-HT2B	μ-opioid
κ_x	$\frac{Z_r^{\blacksquare}}{Z_b^{-} Z_g^{\circ}} \times \frac{Z_b^{-} Z_g^{\circ}}{Z_b^{\square}} \frac{1}{x_{\text{CHOL}}}$	$e^{2.7(1-x_{\text{CHOL}})^2}$ $\times$		
		10^9	10^3	10
x_{50}	x_{CHOL} for which $\kappa_x x_{\text{CHOL}} = 1$	10^{-9}	10^{-3}	–

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Application 1: Predicted ligand titration

$$p_{occ} = \frac{1}{1 + \frac{1}{\kappa_x x_{\mathrm{CHOL}}}}$$

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κ_x	$\frac{Z_r^{\blacksquare}}{Z_b^- Z_g^\circ} \times \frac{Z_b^- Z_g^\circ}{Z_b^\square} \frac{1}{x_{\mathrm{CHOL}}}$	$e^{2.7(1-x_{\mathrm{CHOL}})^2}$ $\times$		
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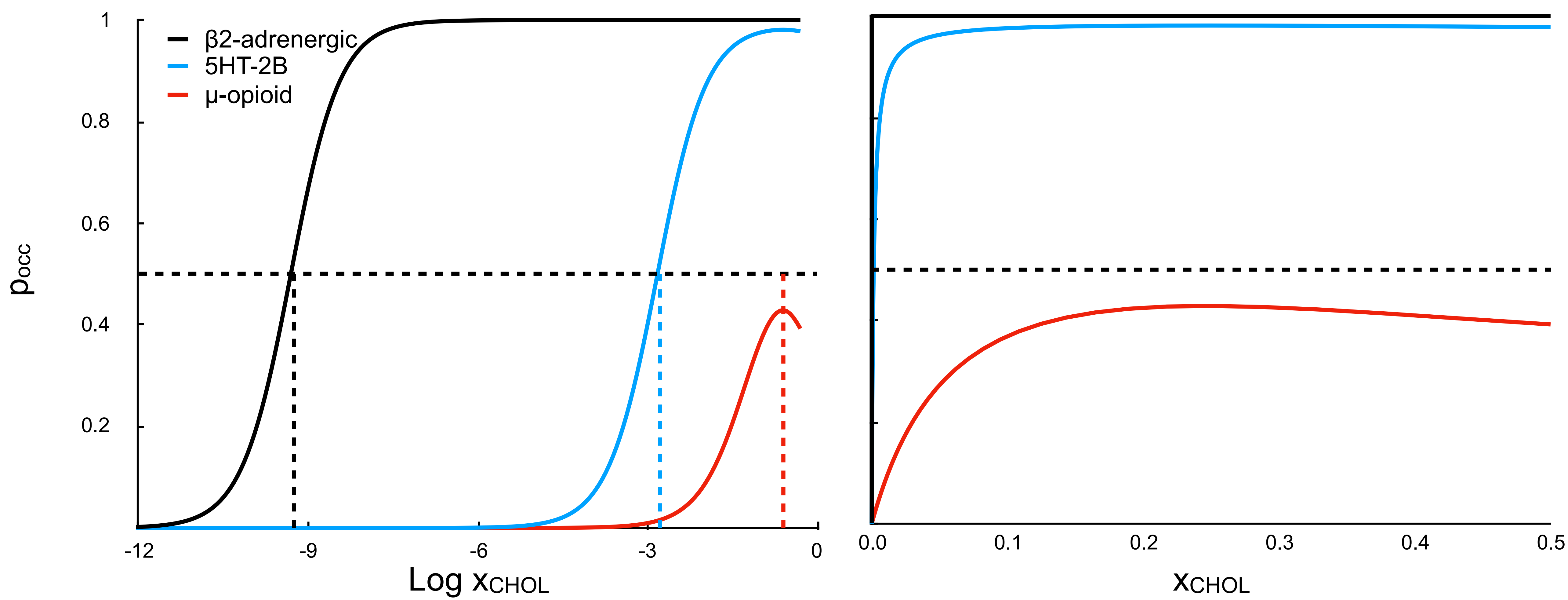

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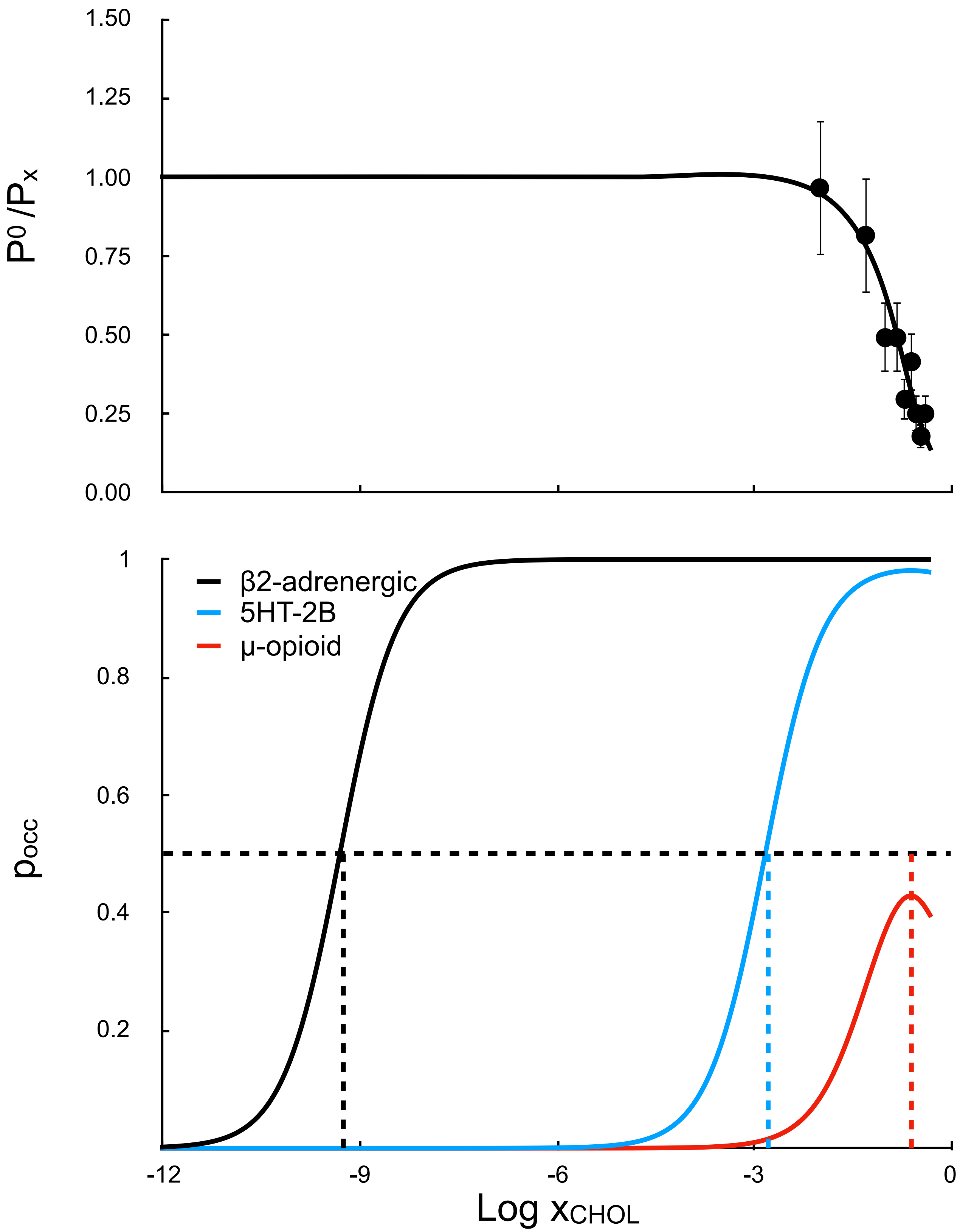
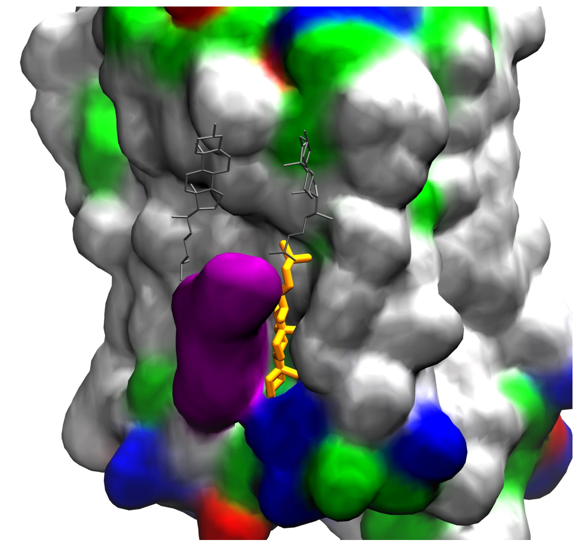
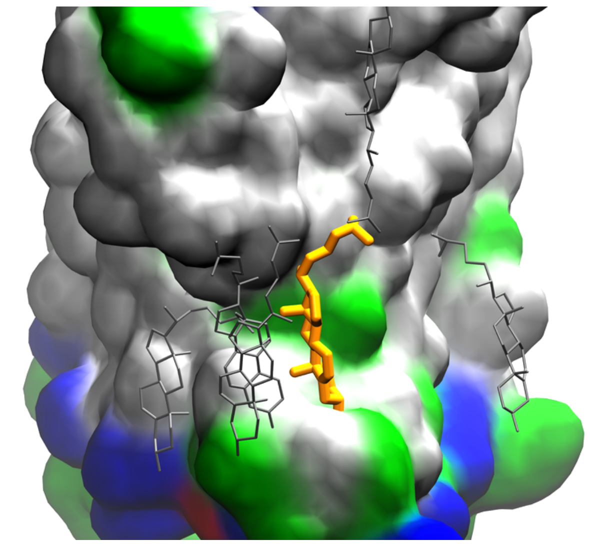




β2-Adrenergic
3D4S
$x_{50} = 10^{-9}$



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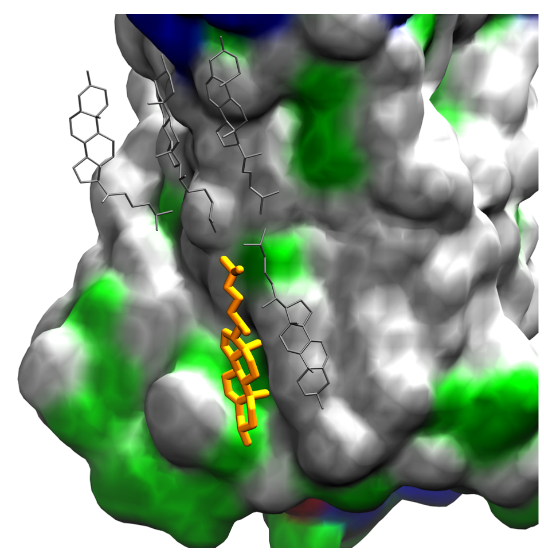

μ-Opioid
5C1M
no x_{50}

Natural x50 is in non-ideal range, probability of binding reduced because bulk has become so favorable

Salari, Joseph, Lohia, Henin, Brannigan
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ACRES : Endpoint Solvation

Simplest AFEP process: assumes decoupled/annihilated state is equivalent to apo state.

Obviously not true

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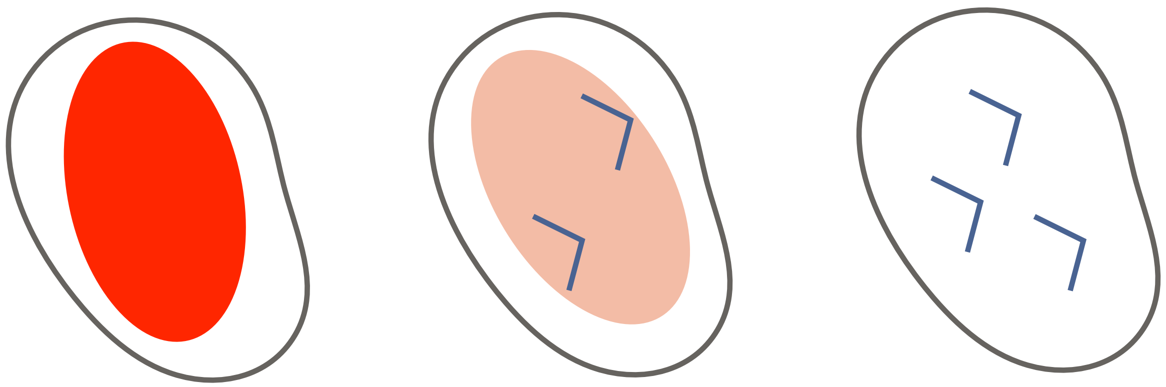
A robust calculation requires considering this - different ways to do so.

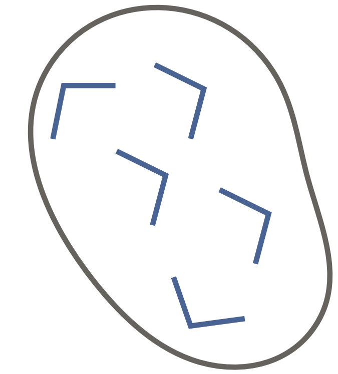
ACRES : Endpoint Solvation

Typical problem with gradual AFEP:



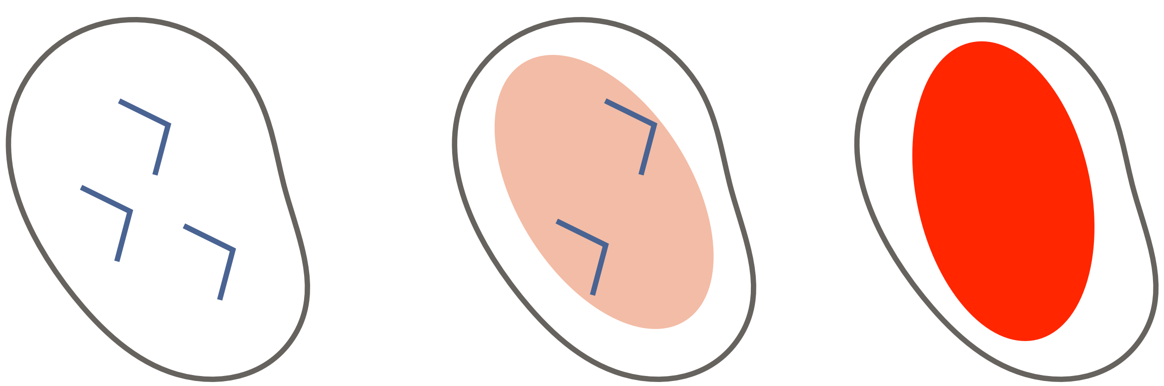
bound intermediate end-point

Overestimates affinity!



actual apo

Common remedy - reverse for recoupling, combine with overlap sampling



end-point intermediate bound

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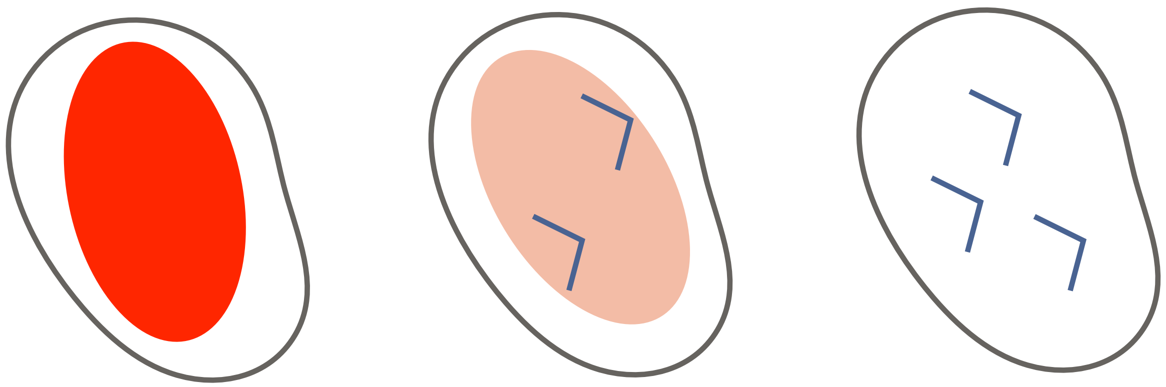

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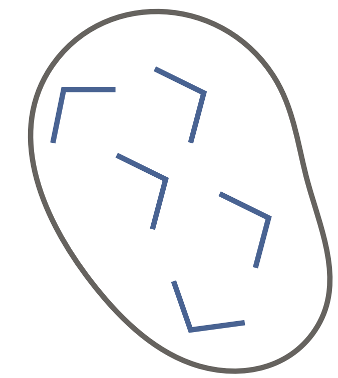

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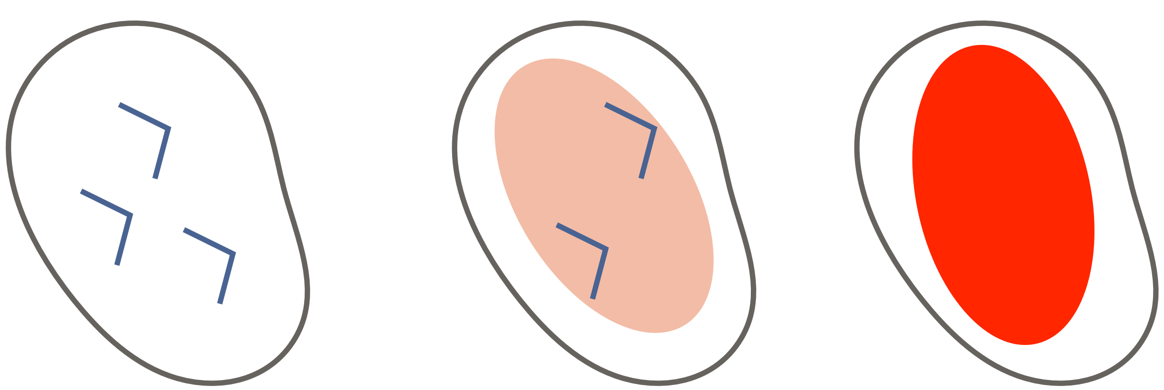

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They match! (Both are **wrong**… but they'll match!)

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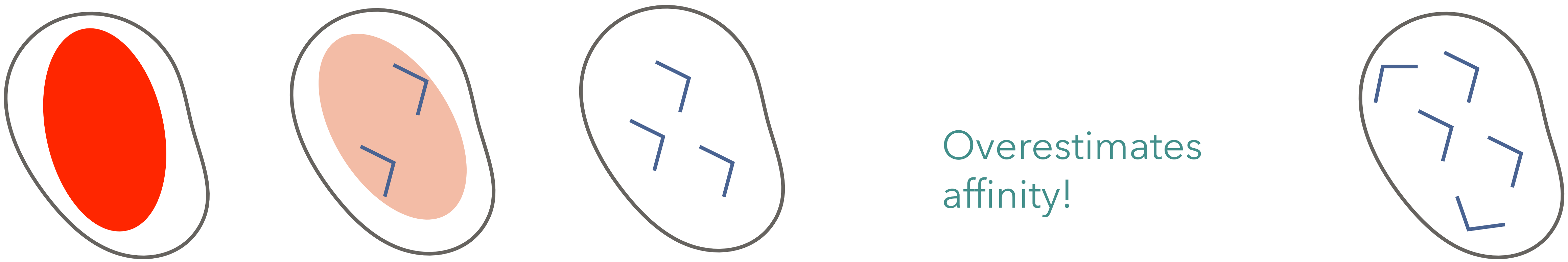

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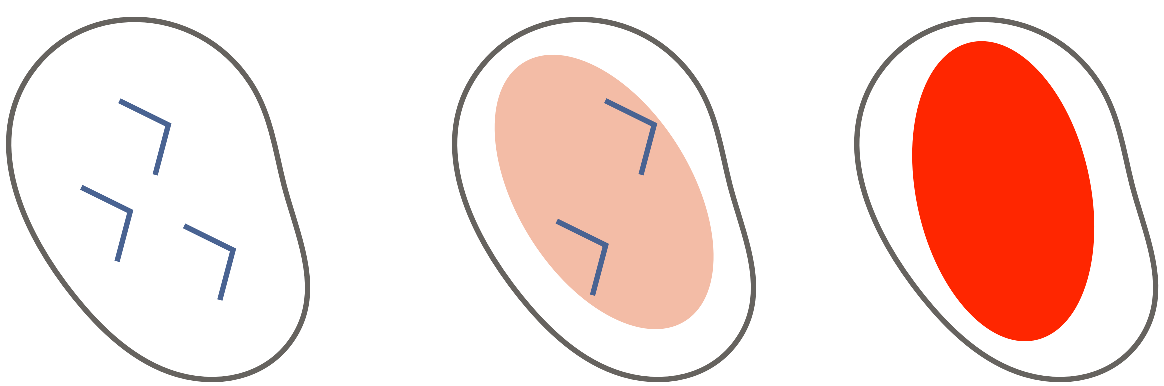

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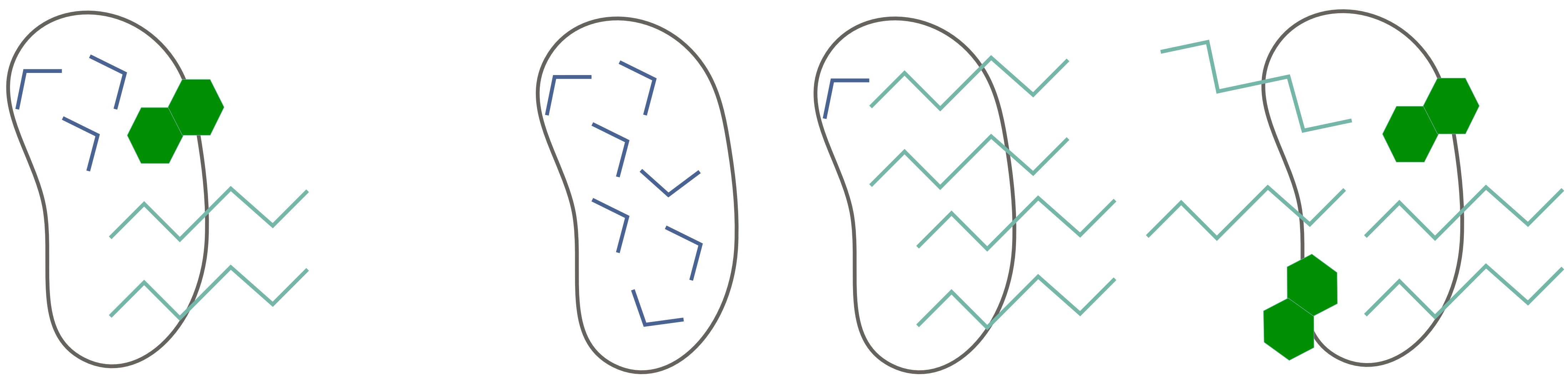
REST can help

ACRES : Endpoint Solvation

Problem is amplified in membranes due to heterogeneous solvation:

if proper membrane apo site looks like

many possible incorrect endpoints!



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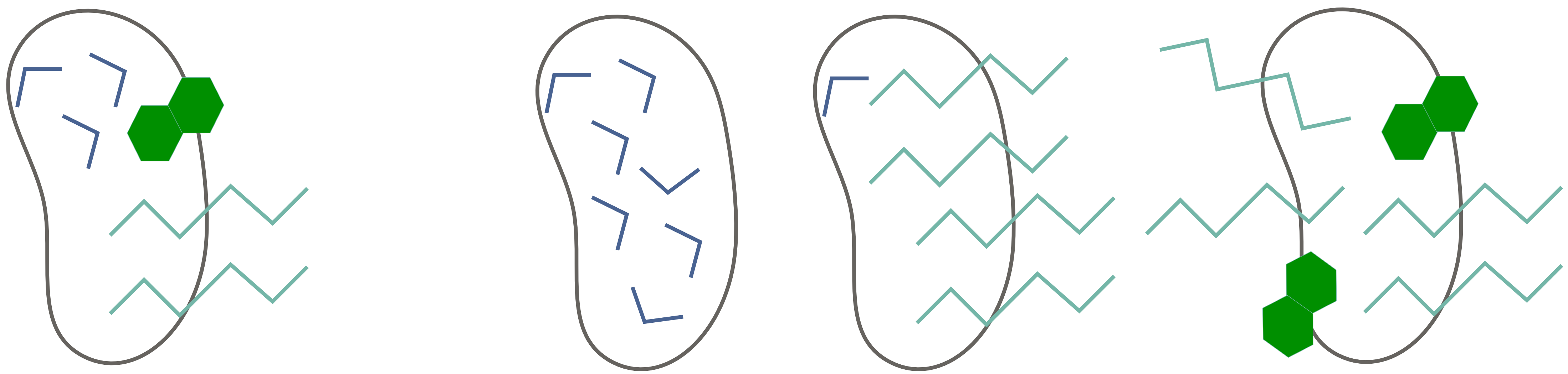

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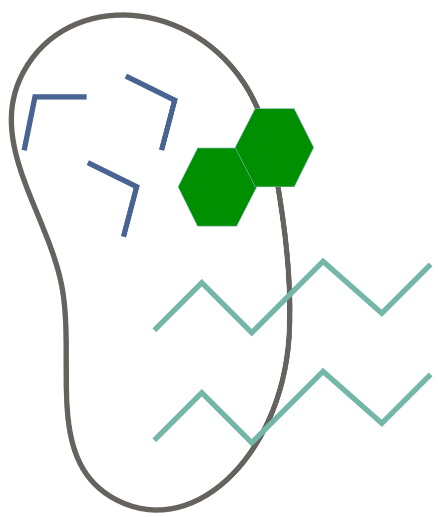

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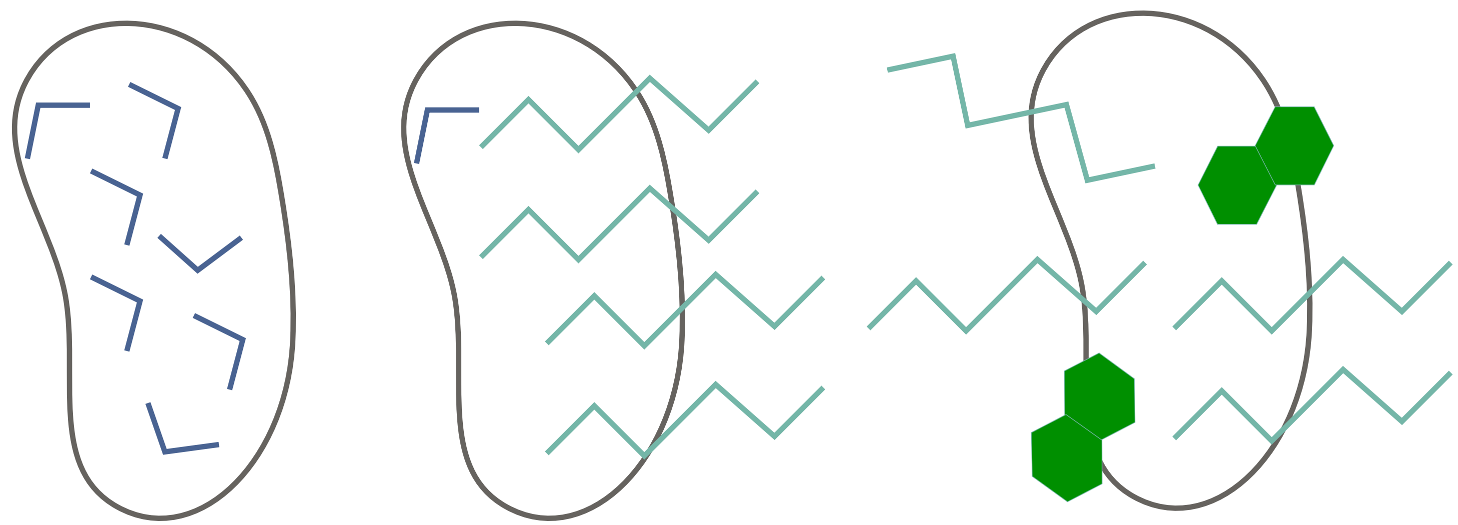

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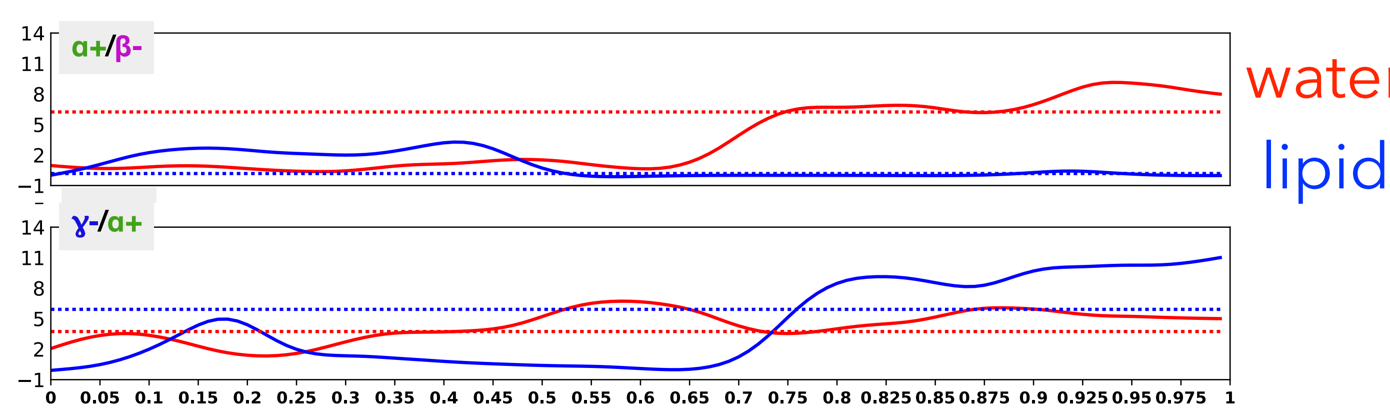

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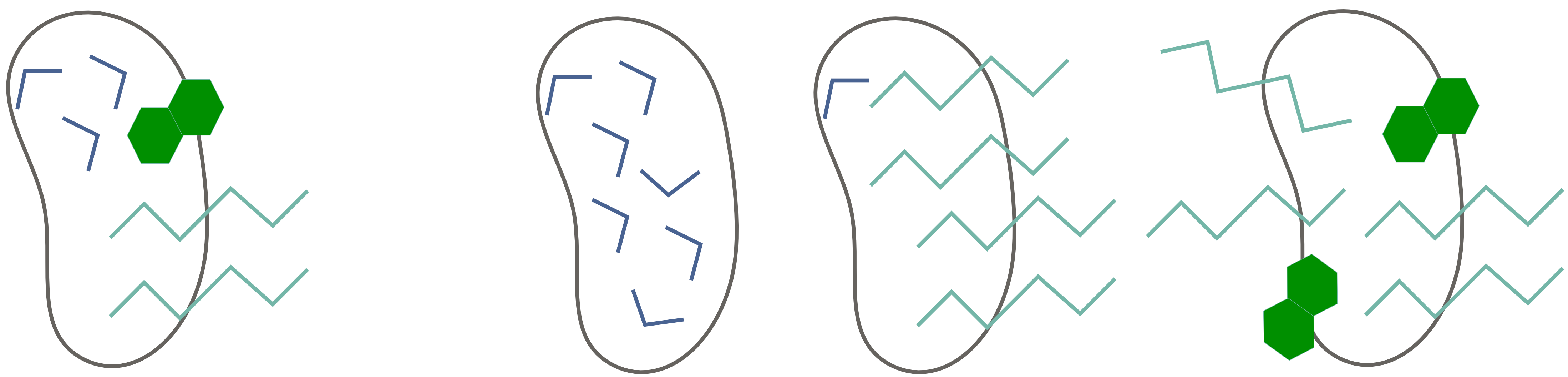

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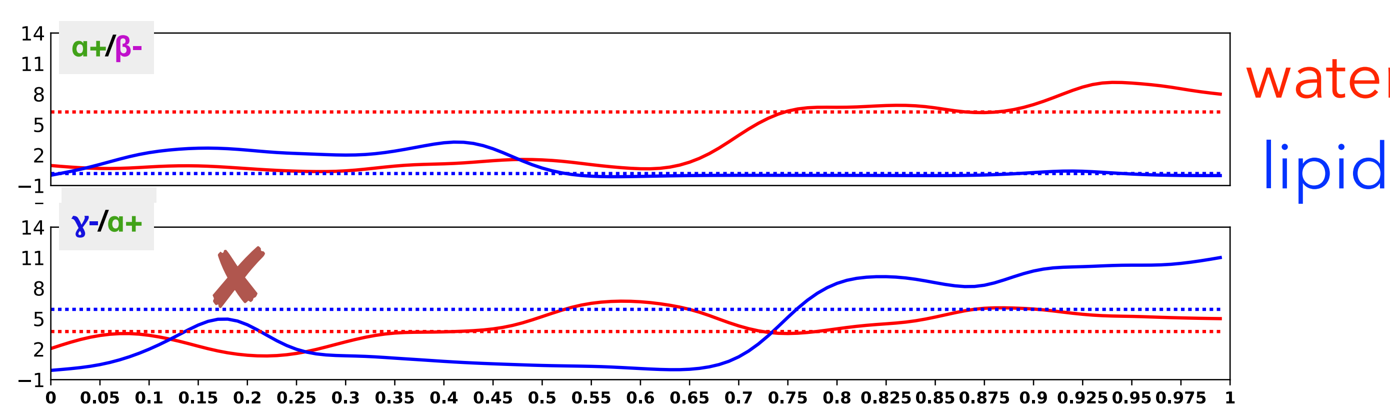

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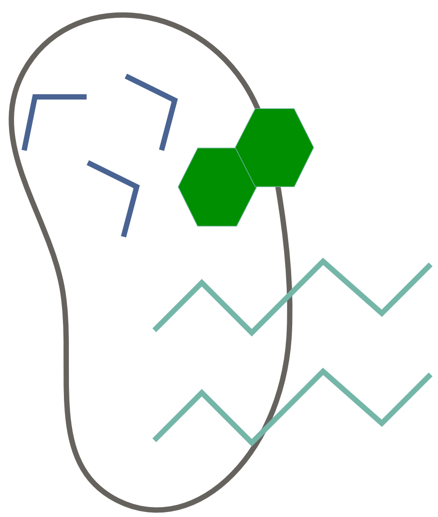

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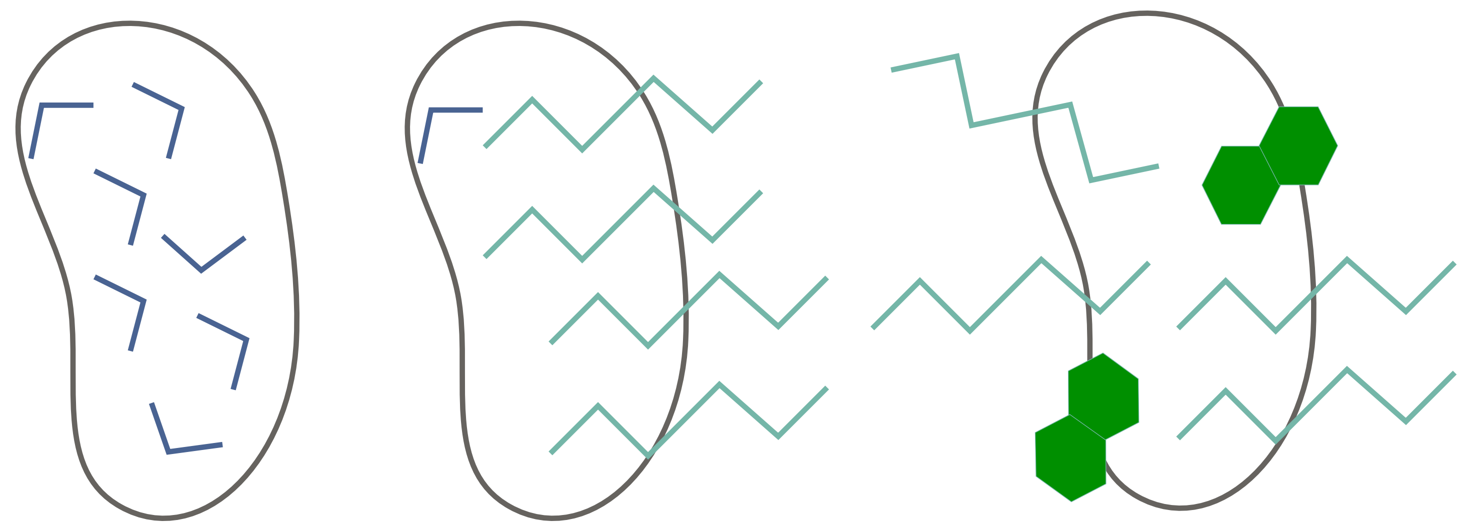

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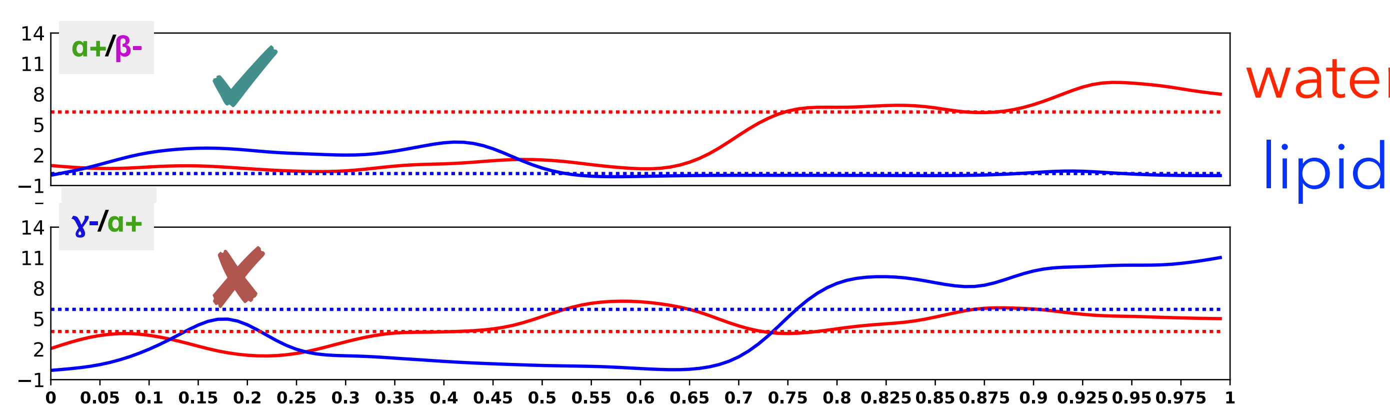
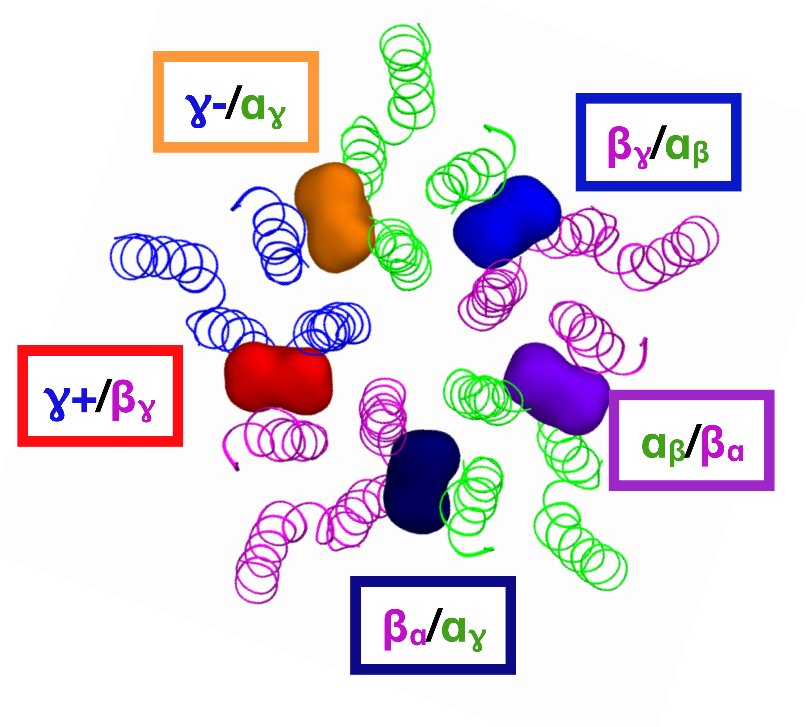
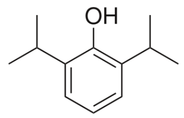
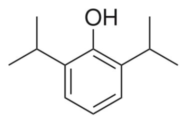


Application 2 : pseudosymmetric sites for propofol on GABA(A) receptors



Woll, Murlidaran…Brannigan, Garcia, Eckenhoff, 2016, J Biol Chem





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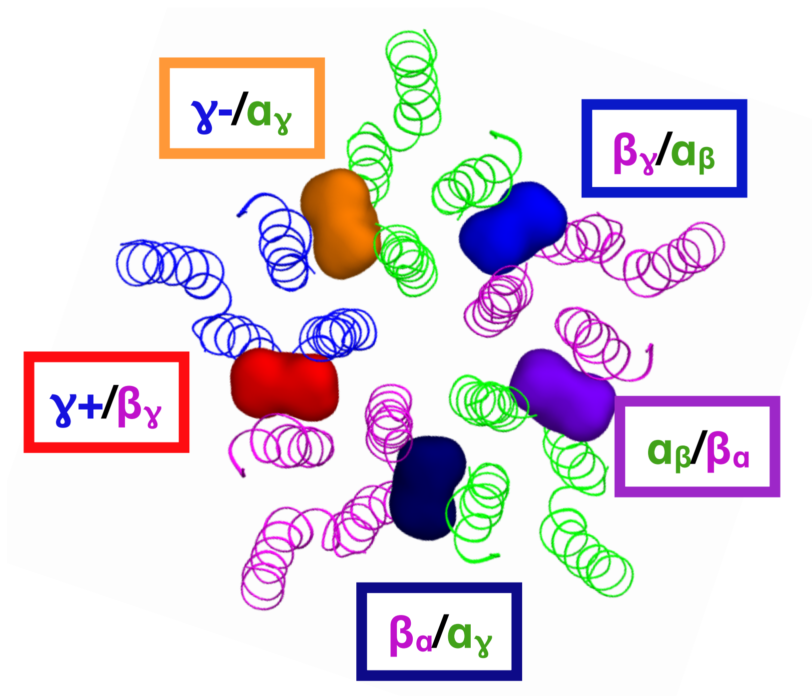

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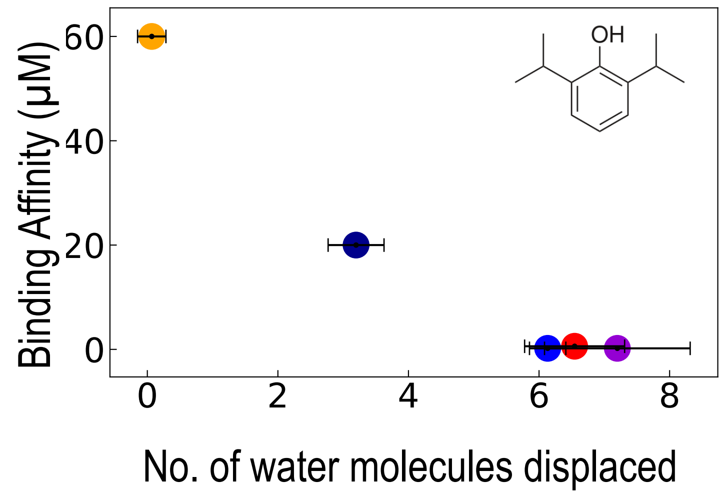

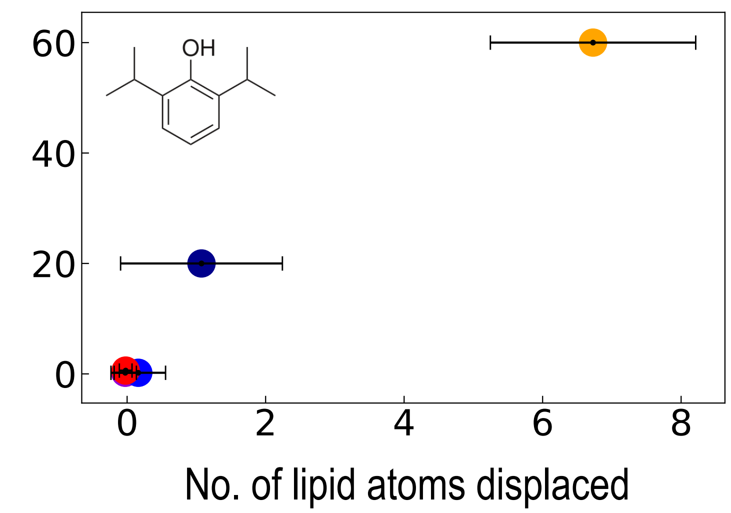

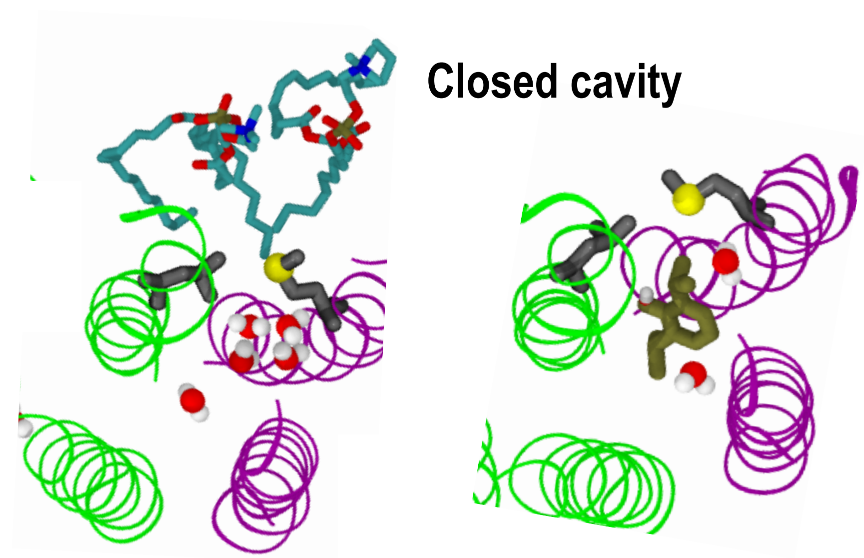

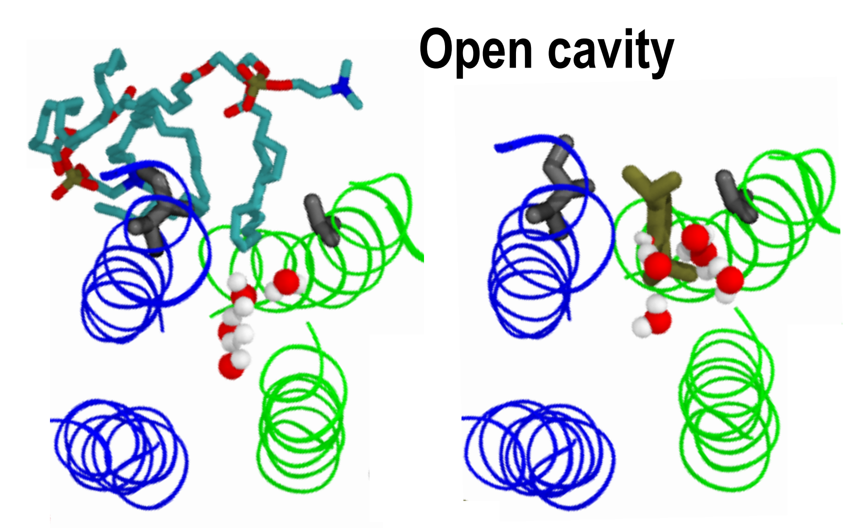

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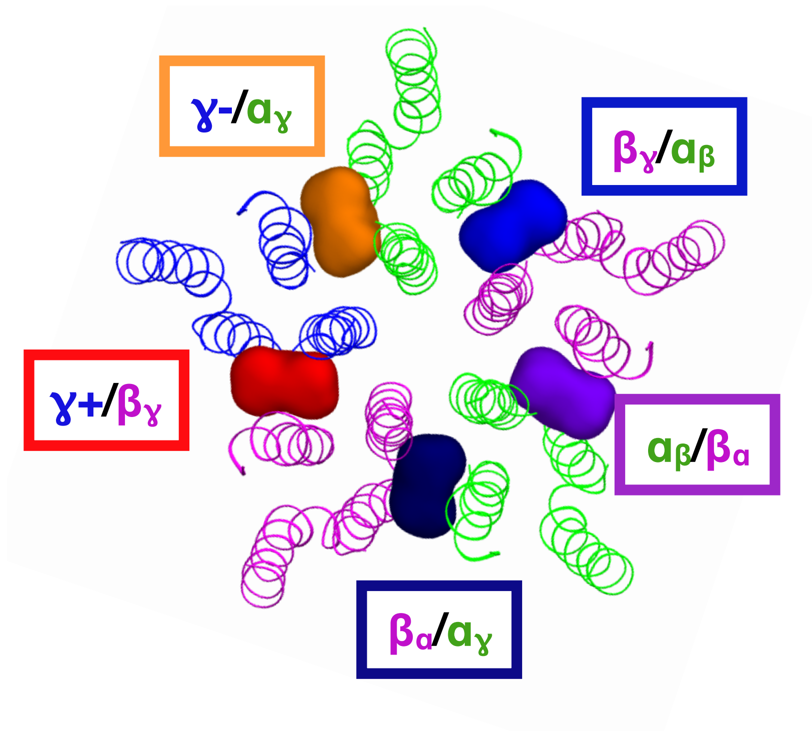

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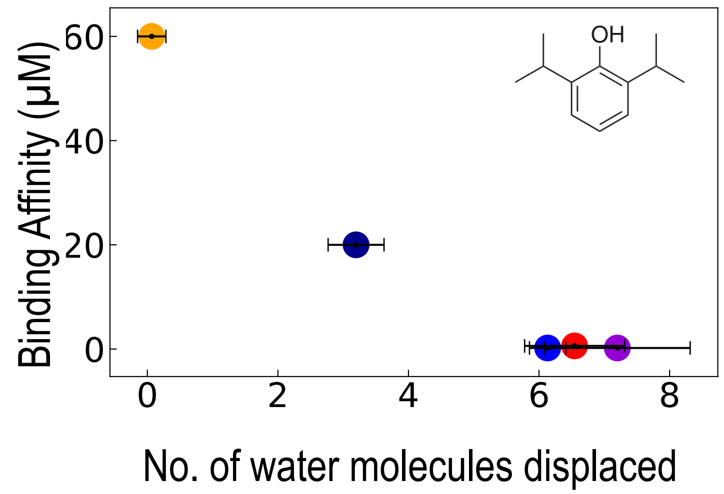

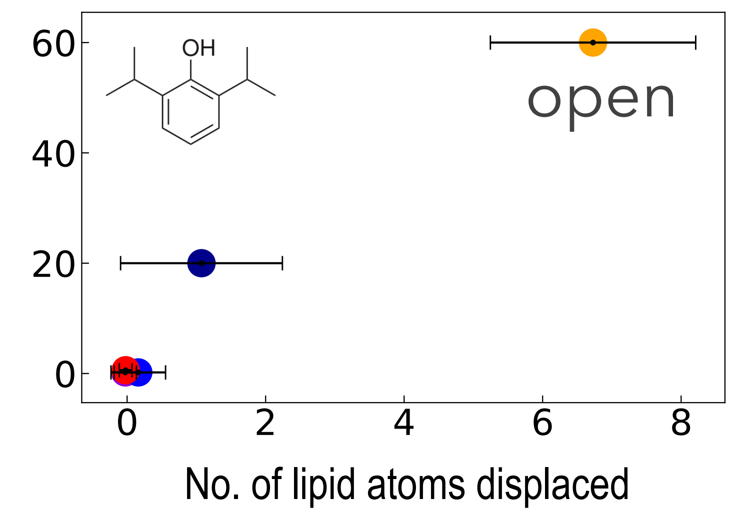

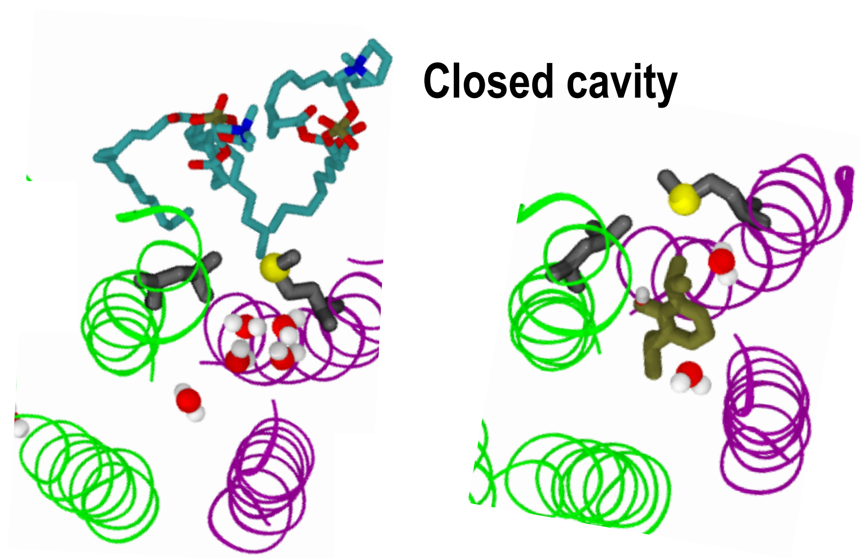

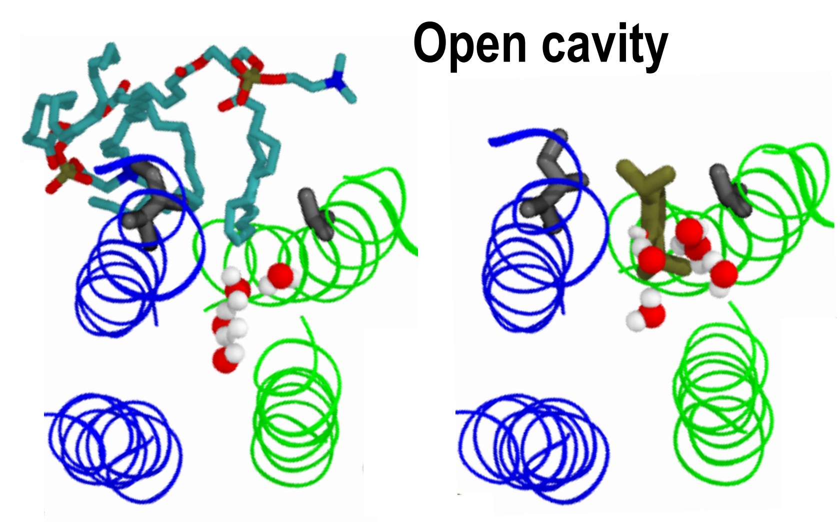

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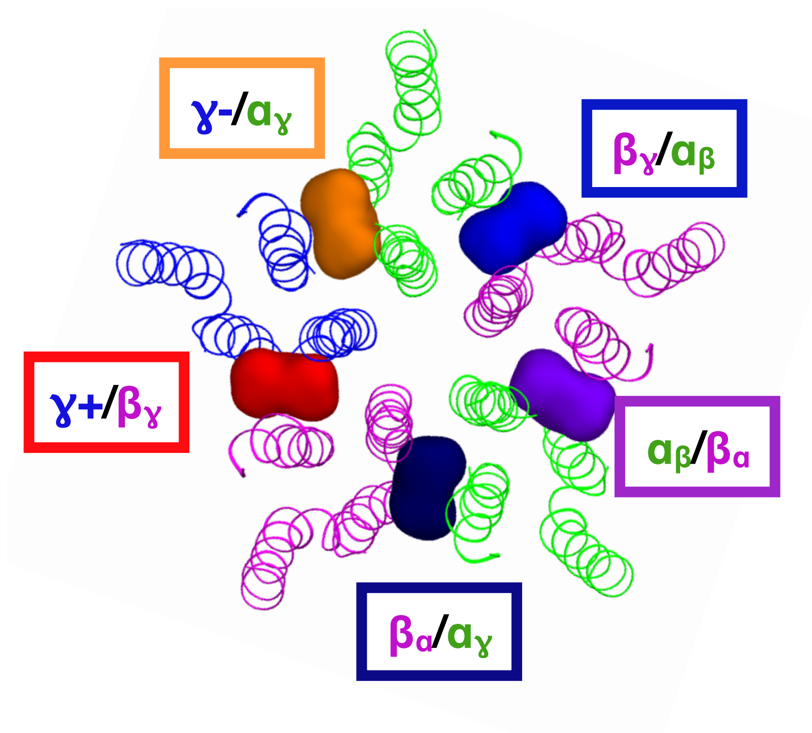

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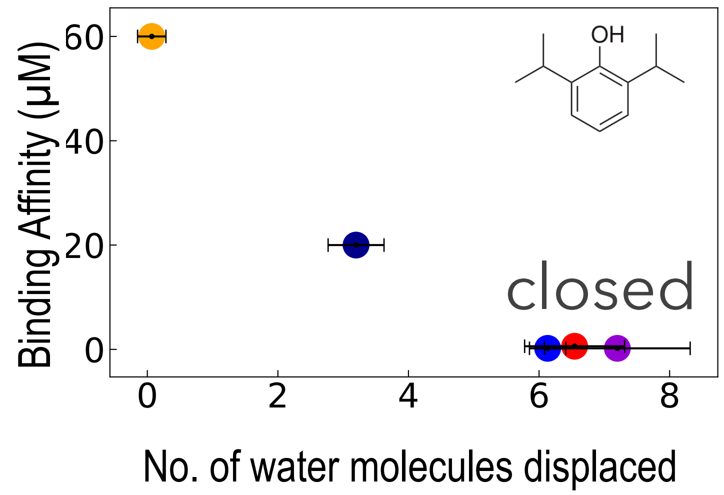
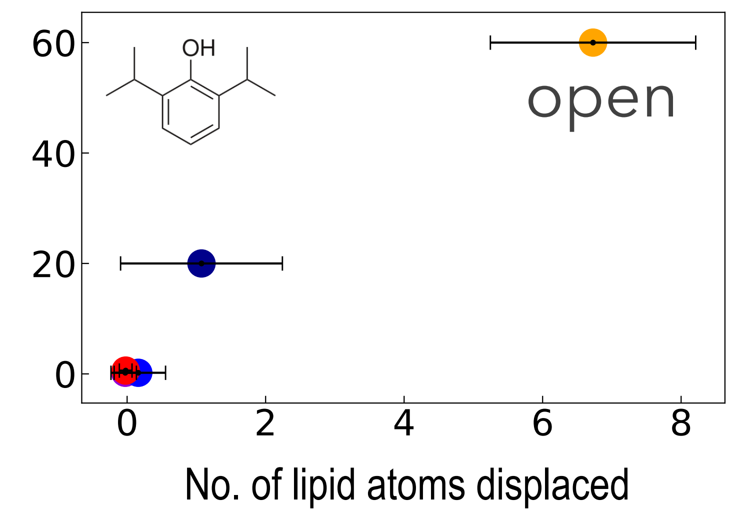
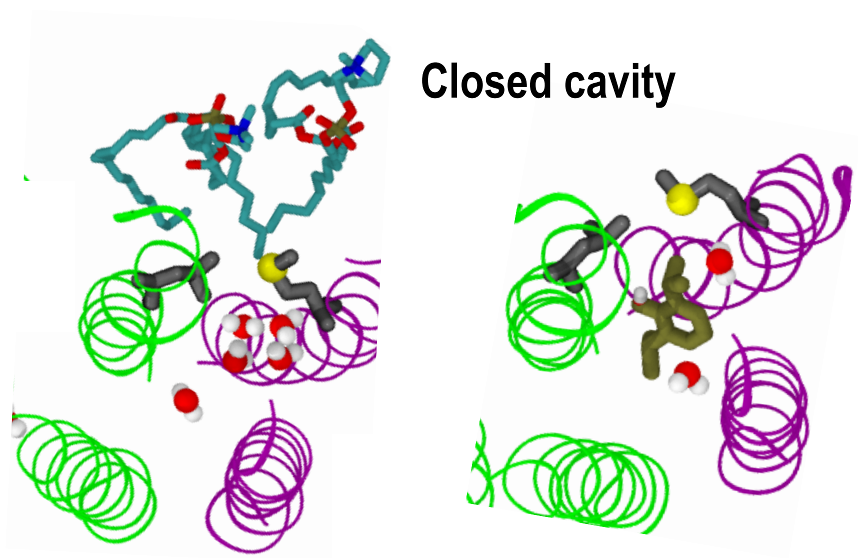
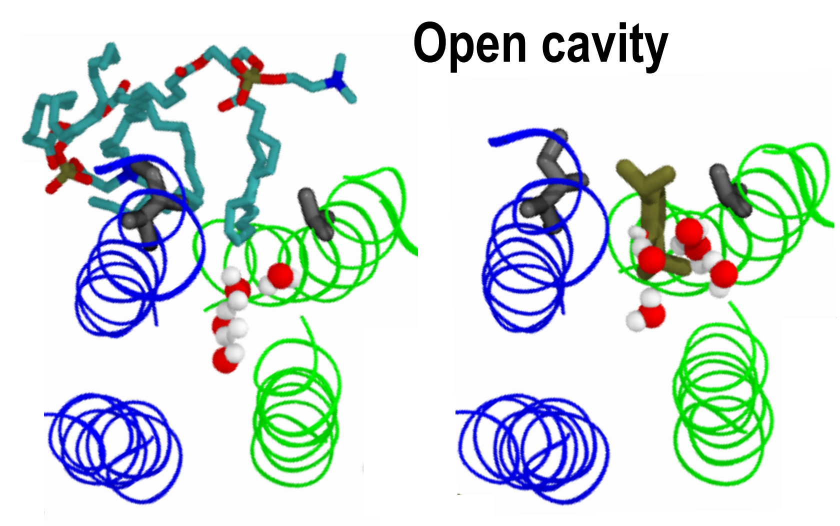
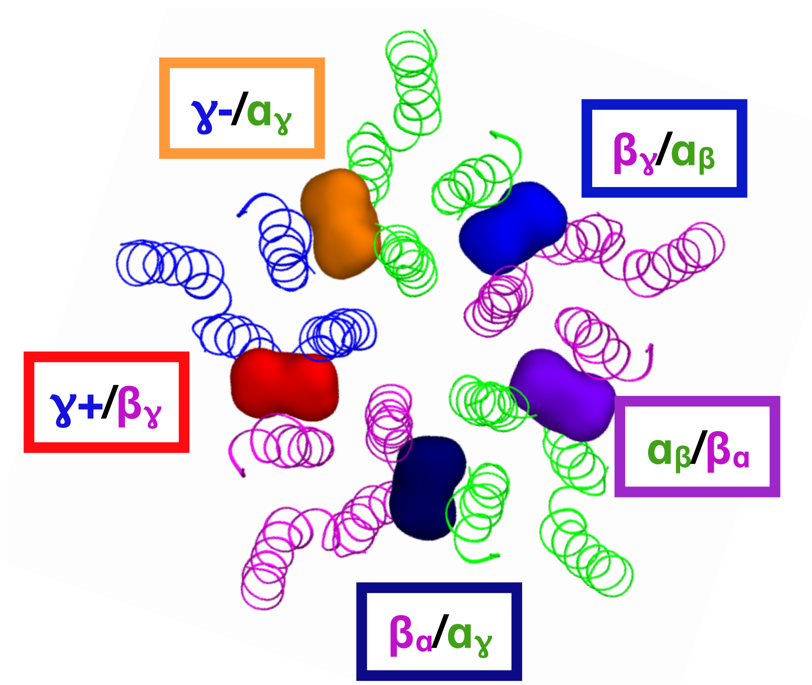




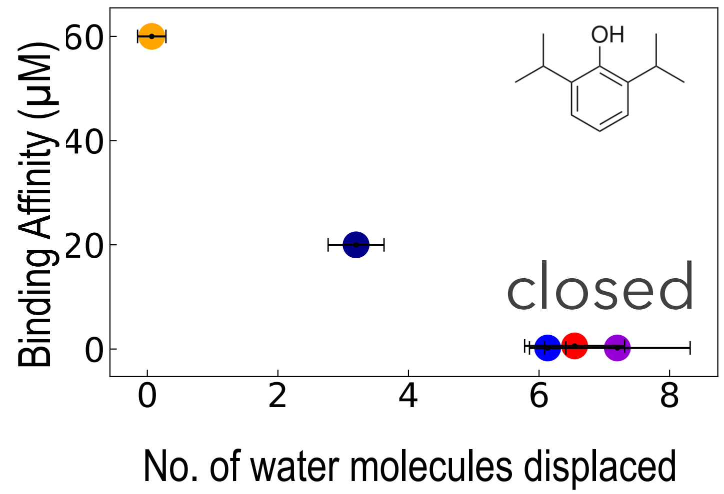


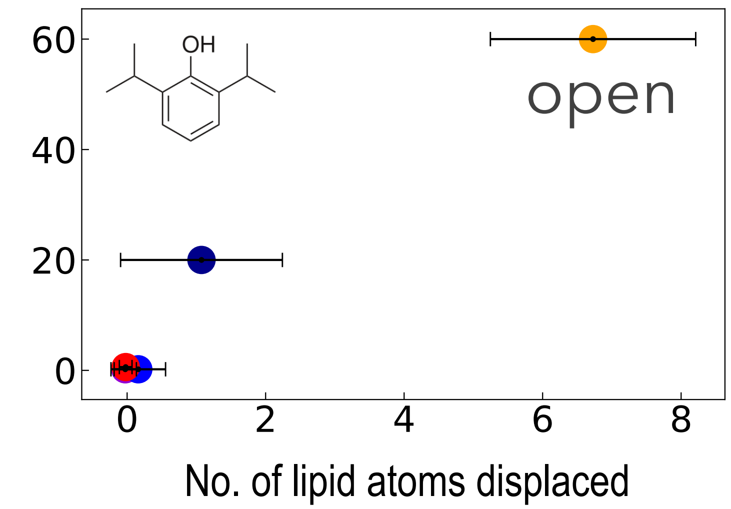


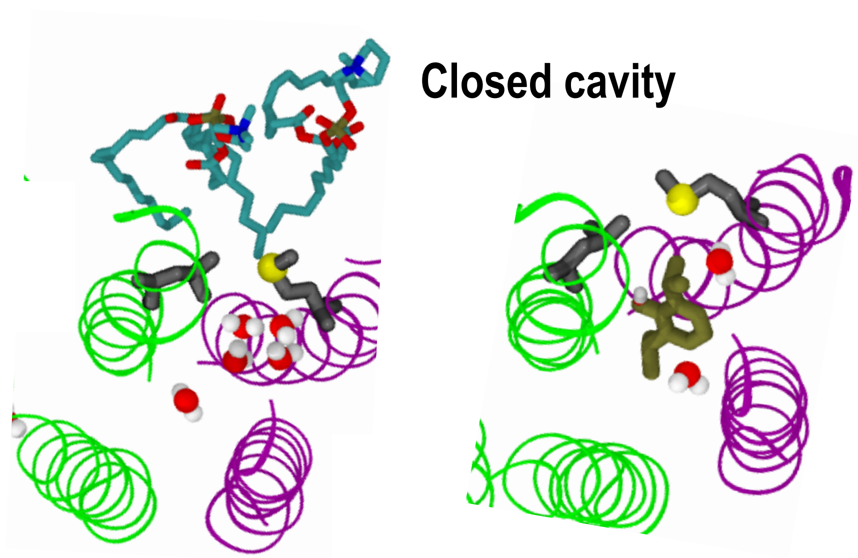
Application 2 : pseudosymmetric sites for propofol on GABA(A) receptors

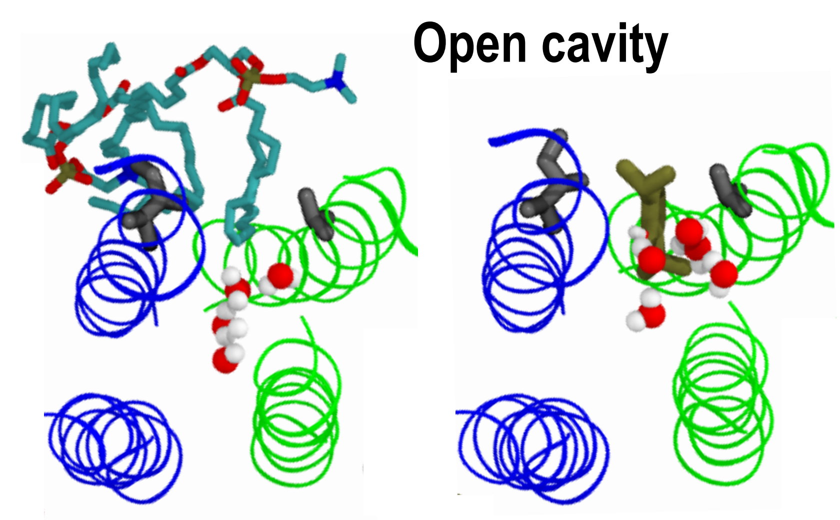


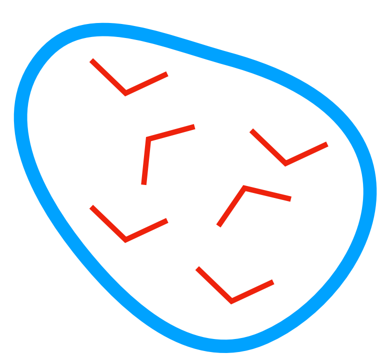
Woll, Murlidaran...Brannigan, Garcia, Eckenhoff, 2016, J Biol Chem



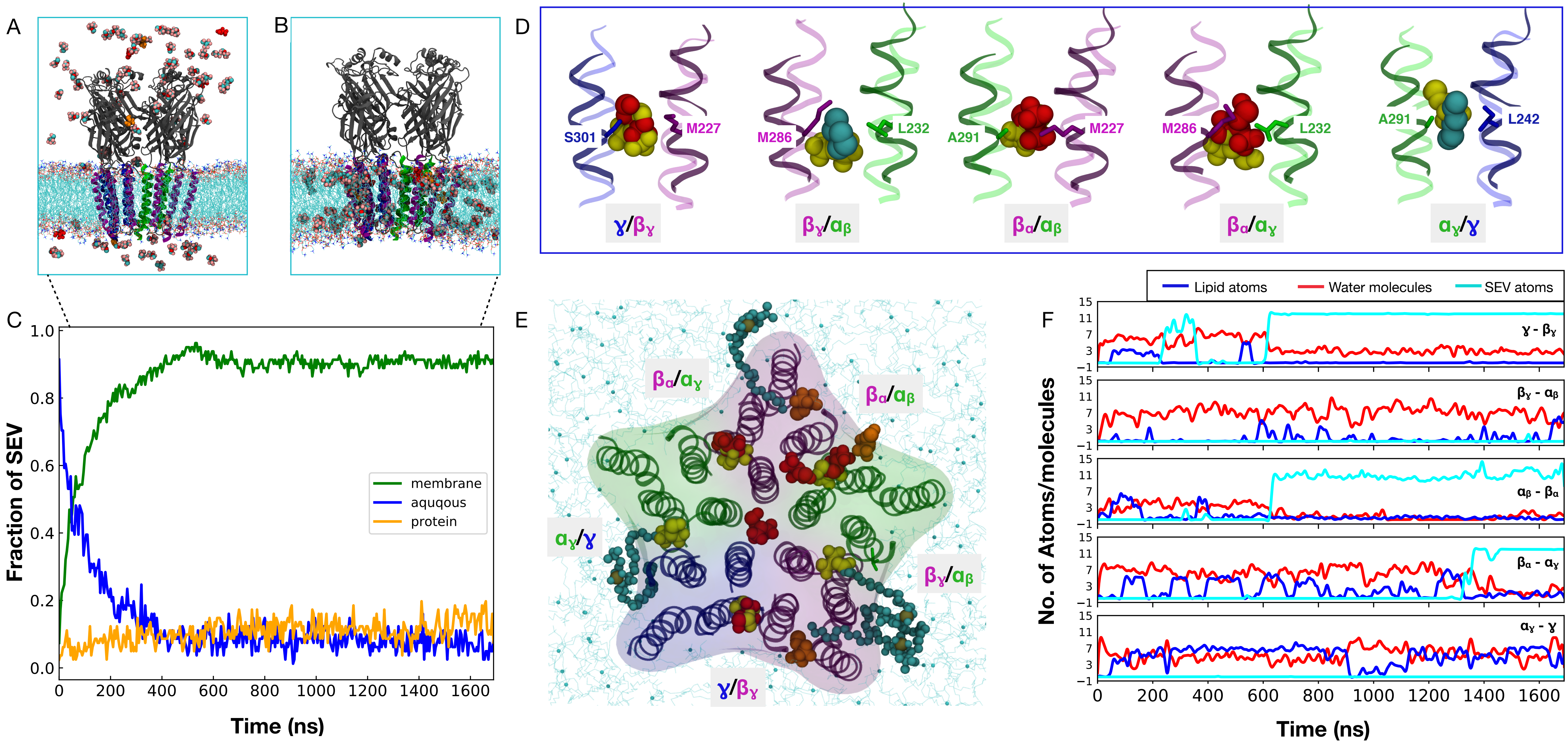








Application 2 : pseudosymmetric sites for sevoflurane on GABA(A) receptors



Murlidaran and Brannigan, in preparation

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Acknowledgments

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