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Accurate Modeling of Receptor Functional Response: GPCRs and Beyond

Lingle Wang, Martin Vögele

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Today’s Speakers

Lingle Wang

Senior Vice President

Schrödinger

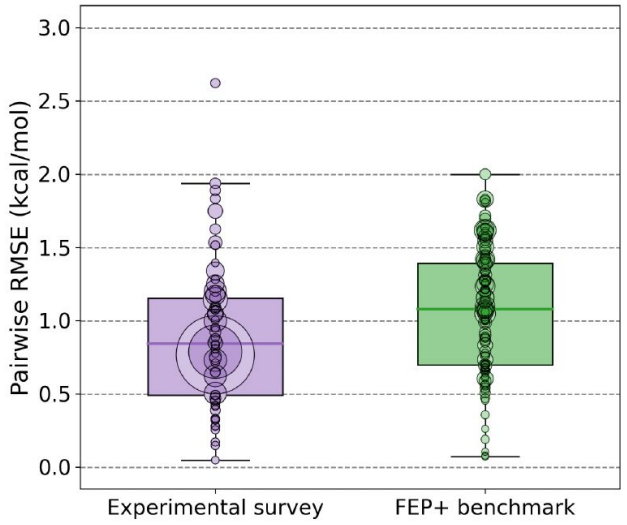
Martin Vögele

Senior Scientist

Schrödinger

Reliable Binding Potency Prediction with FEP+

FEP+ is reaching experimental accuracy in a recent large-scale validation on small molecule datasets.



Ross G., et. al. Commun. Chem. 6, 222, (2023)

Data set name	Proteins in data set	No. compounds
FEP+ R-group set[16]	BACE1, CDK2, JNK1, Mcl1, p38, PTP1B, thrombin, TYK2	199
FEP+ charge-change[54]	CDK2, DLK, EGFR, EPHX2, IRAK4, ITK, JAK1, JNK1, PTP1B, TYK2	53
OPLS stress set[27]	BACE1, CHK1, Factor Xa	114
OPLS drug discovery[27]	A, B, C, D, E	93
Water displacement[34]	BRD4(1), CHK1, Hsp90, scytalone dehydratase, TAF1(2), thrombin, urokinase	76
FEP+ Fragments[55]	T4 lysozyme, LigA, Mcl1, MUP-1, JAK-2, hsp90, p38	79
FEP+ macrocycles[31]	BACE1, CHK1, CK2, MHT1, HSP90	34
FEP+ scaffold-hopping[32]	BACE1, β-tryptase, CHK1, ERα, Factor Xa,	17
Merck sets[56]	CDK8, cMet, Eg5, HIF-2α, PFKFB3, SHP-2, SYK, TNKS2	264
GPCRs[57;58]	A2A, OX2, P2Y1	98
Bayer macrocycles[59]	Ftase, BRD4	8
Janssen BACE1[36;60]	BACE1	74
MCS docking[61]	HNE, Renin	49
Miscellaneous	CDK8[62], Galectin[10;63], BTK[64], HIV1 protease[65], FAAH[66]	79
	Total	1237

Accuracy metric	Experimental survey	FEP+ benchmark
Pairwise RMSE (kcal/mol)	0.91 [0.83, 1.11]	1.25 [1.17, 1.33]
Pairwise MUE (kcal/mol)	0.67 [0.61, 0.83]	0.98 [0.91, 1.05]
Edgewise RMSE (kcal/mol)	N/A	1.17 [1.08, 1.25]
Edgewise MUE (kcal/mol)	N/A	0.91 [0.84, 0.98]
R^2	0.79 [0.75, 0.82]	0.56 [0.51, 0.60]
Kendall τ	0.71 [0.65, 0.74]	0.51 [0.48, 0.55]

Schrodinger Online Course: Free energy calculations for drug design with FEP+

G-Protein–Coupled Receptor (GPCR) Activation

The binding potency of a ligand may not correlate with its efficacy.

- GPCRs transmit signals across lipid membranes.
  - ⇨ *“control panel of the cell”*
- Most important class of drug targets! ~34% of all FDA-approved drugs
- **Agonists** activate a receptor upon binding by stabilizing an **active** conformation.
- **Antagonists** bind to the receptor but then they stabilize an **inactive** conformation.

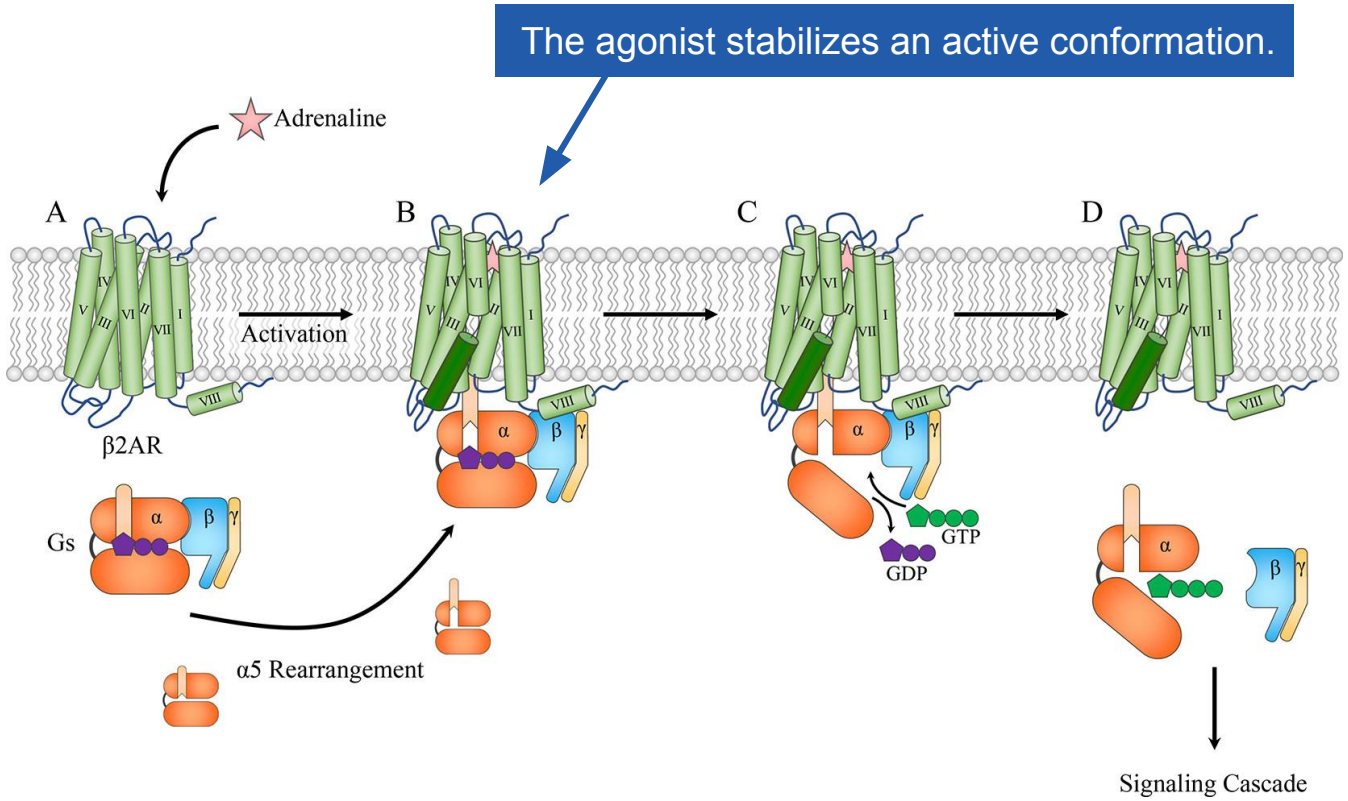
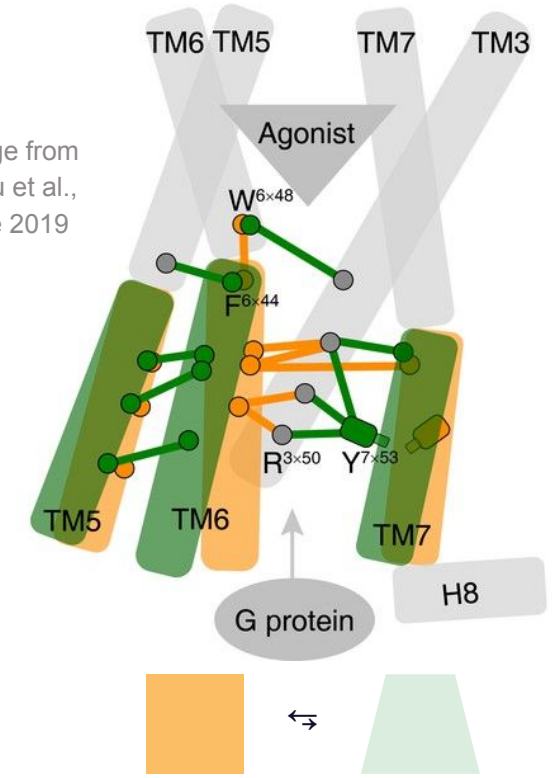


Figure from Bai et al., J. Am. Chem. Soc. 2021, 143, 29

G-Protein–Coupled Receptor (GPCR) Activation

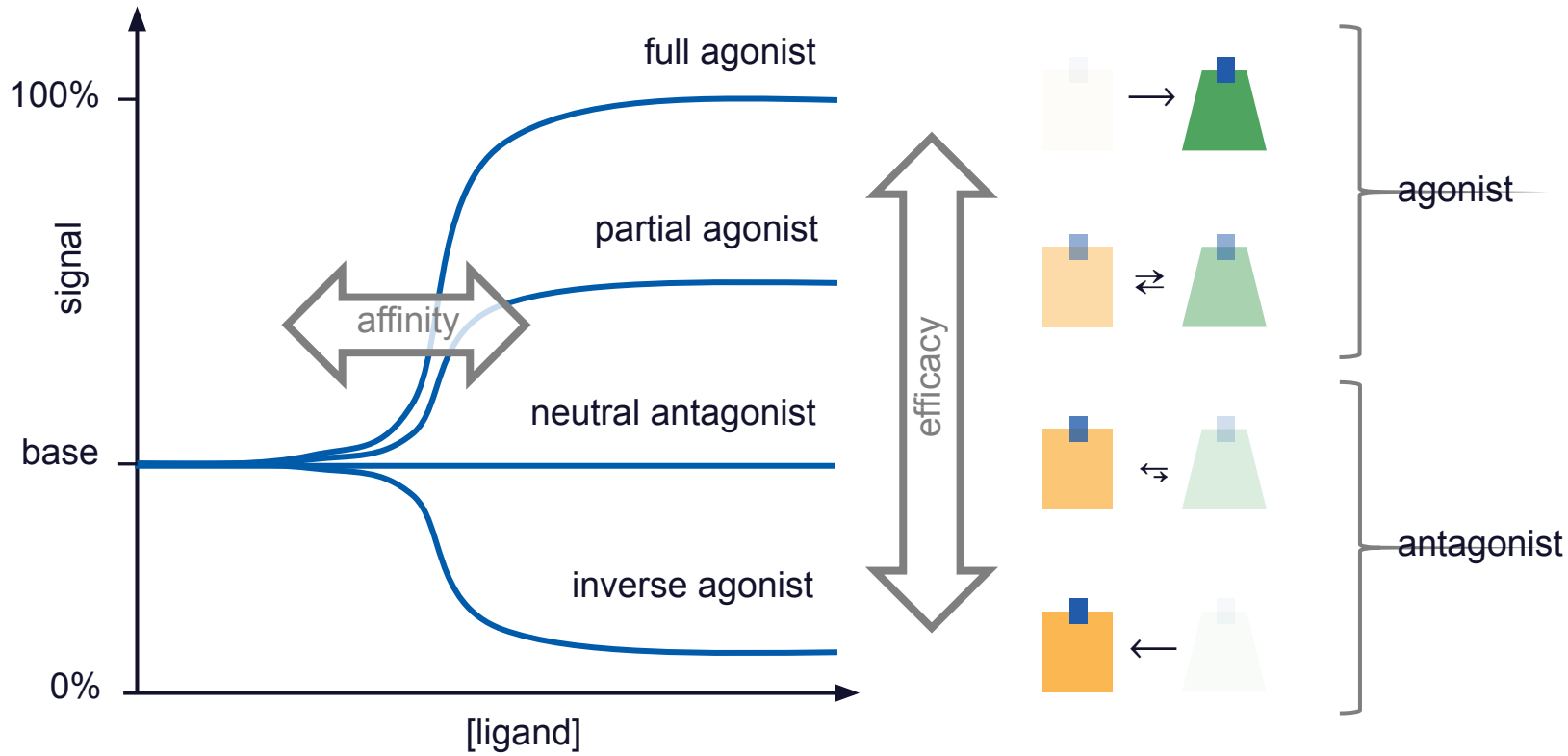
Efficacy depends on the structural equilibrium between active and inactive states.

Image from Zhou et al., eLife 2019



apo-state equilibrium

(receptor-dependent, base can be zero)



holo-state equilibrium

Efficacy Prediction by Comparing Conformations

The binding free energy difference between active and inactive states predicts efficacy with high accuracy.

- Study compares binding free energies on the active and inactive state for 180 target-ligand pairs.
- The binding free energy difference ΔΔG is calculated via **FEP+**.
- Ligands are classified via a target-specific threshold → **Accuracy: 98%**

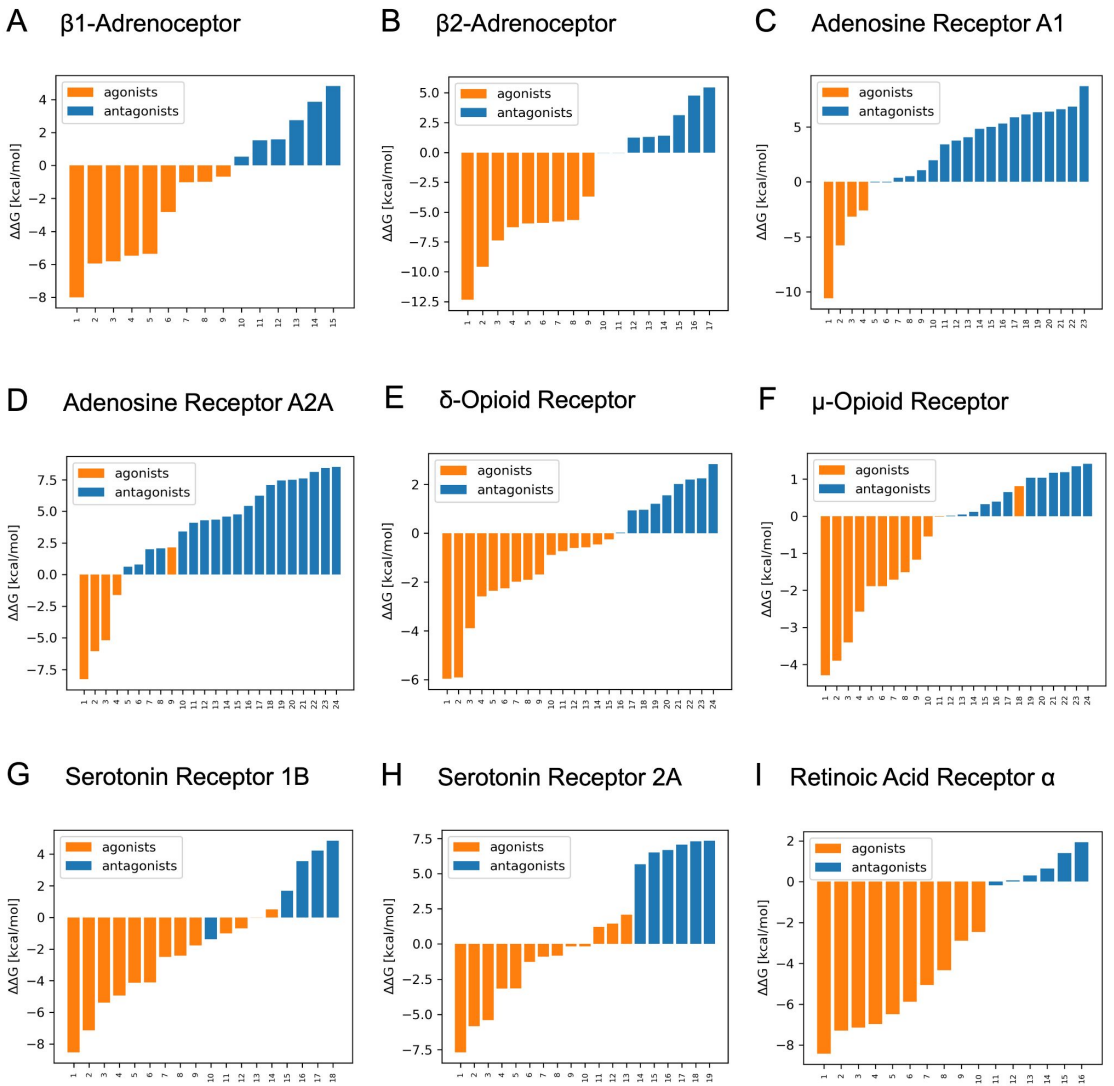


Figure: Comparison between ΔΔG calculated using FEP+ and experimentally determined efficacy (agonist/antagonist)

Accurate Modeling of Receptor Functional Response: GPCRs and Beyond

Outline

Methods Overview

- Example: β2-Adrenoceptor
- Thermodynamics of Ligand Efficacy
- Simulation protocols

Results Summary

- Results Overview
- Accuracy across Chemical Space
- Limitation: Receptor Conformations
- The Role of the Template
- The Role of the Ligand Poses
- Beyond GPCRs

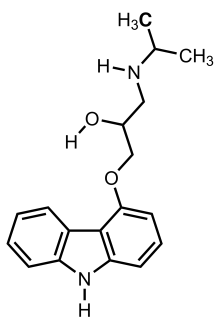
Conclusions

Outlook

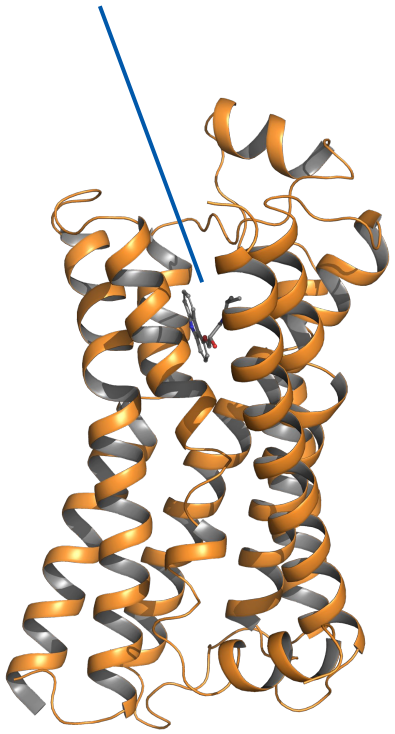
Methods Overview

Example Problem Statement

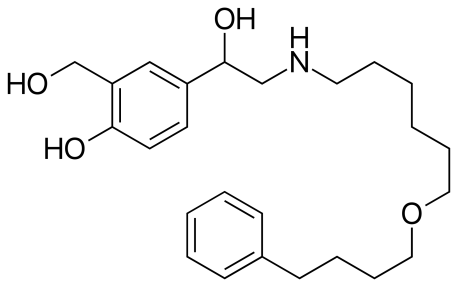
Is Salmeterol an agonist or an antagonist of the β2-Adrenoceptor?



Carazolol (antagonist)



Inactive, PDB: 2RH1

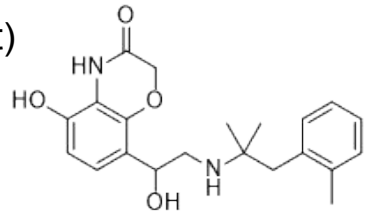


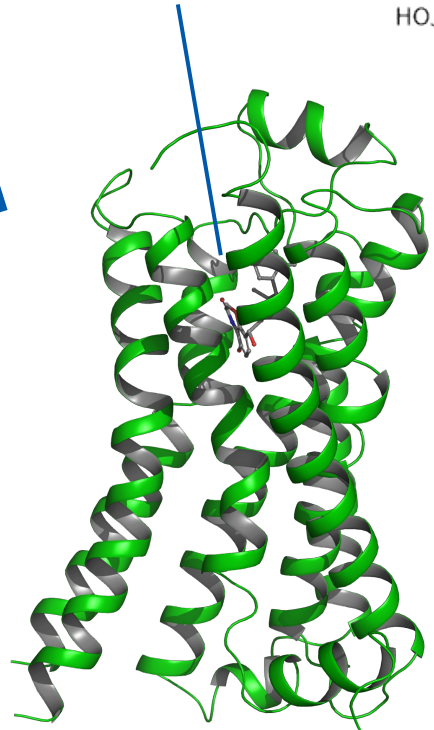
Salmeterol

?

⇄

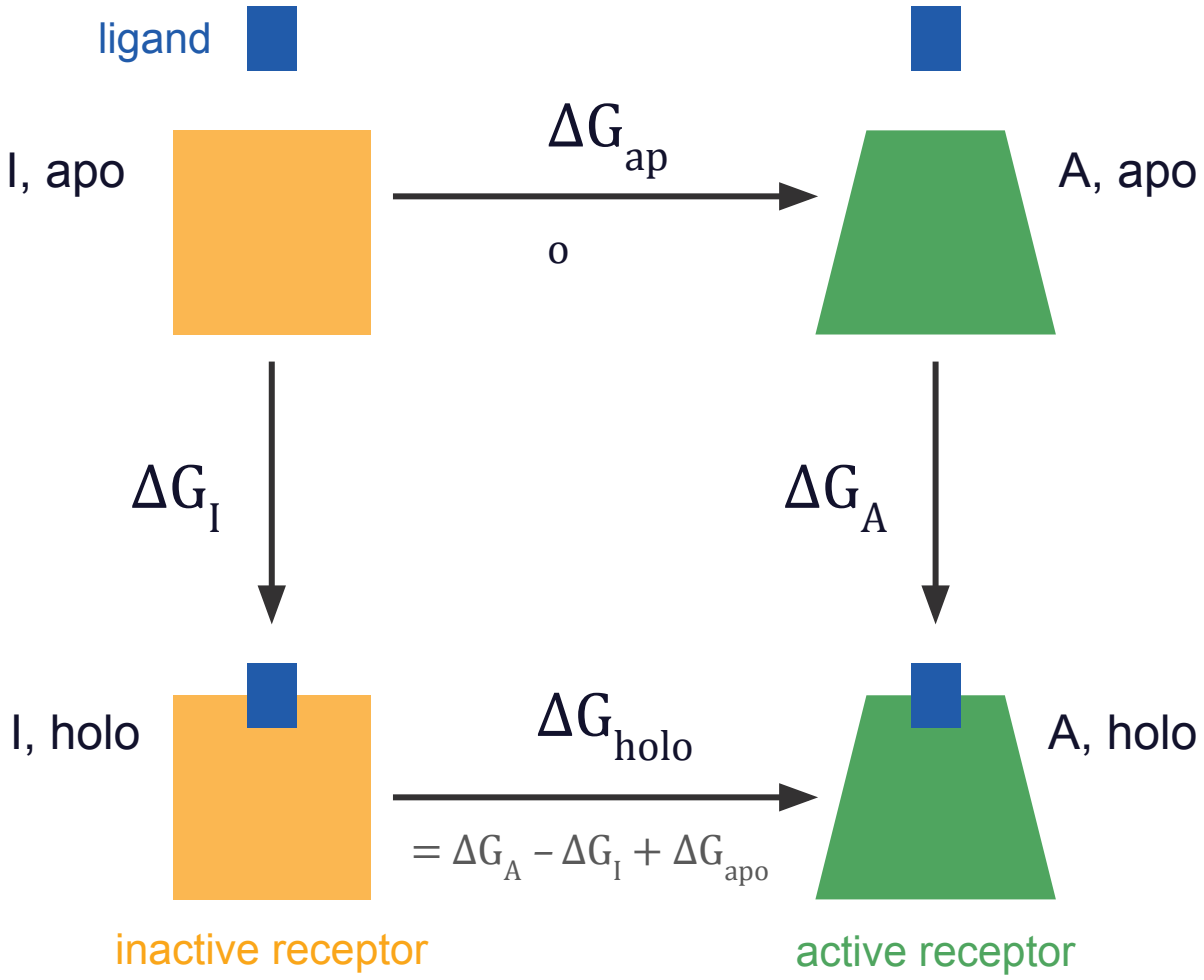
BI-167107 (agonist)





Active, PDB: 3P0G

Thermodynamics of Ligand Efficacy

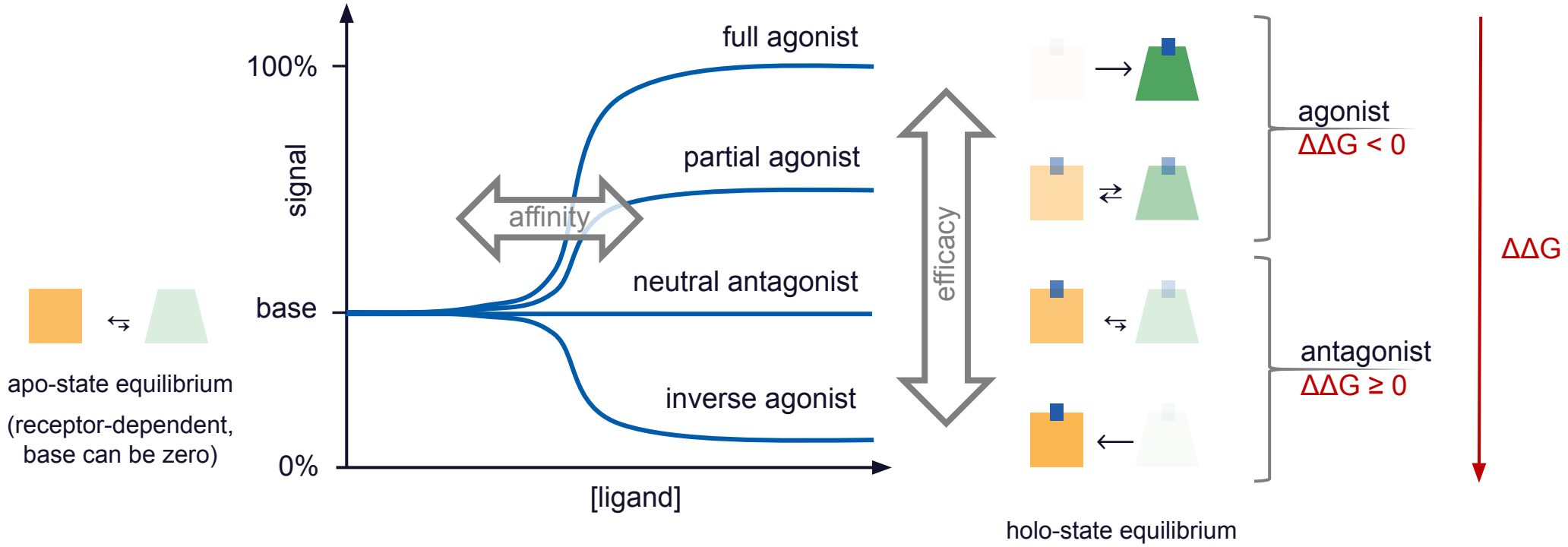


- The presence of a ligand changes the equilibrium between two receptor states.
  - Agonists: $\Delta\Delta G = \Delta G_{holo} - \Delta G_{apo} < 0$
  - Antagon.: $\Delta\Delta G = \Delta G_{holo} - \Delta G_{apo} \geq 0$
- ΔG_{apo} is unknown but ligand-independent.
- ΔG_{holo} can be written as $\Delta G_A - \Delta G_I + \Delta G_{apo}$.
- For the shift of the equilibrium caused by the ligand, we get $\Delta\Delta G = \Delta G_A - \Delta G_I$.

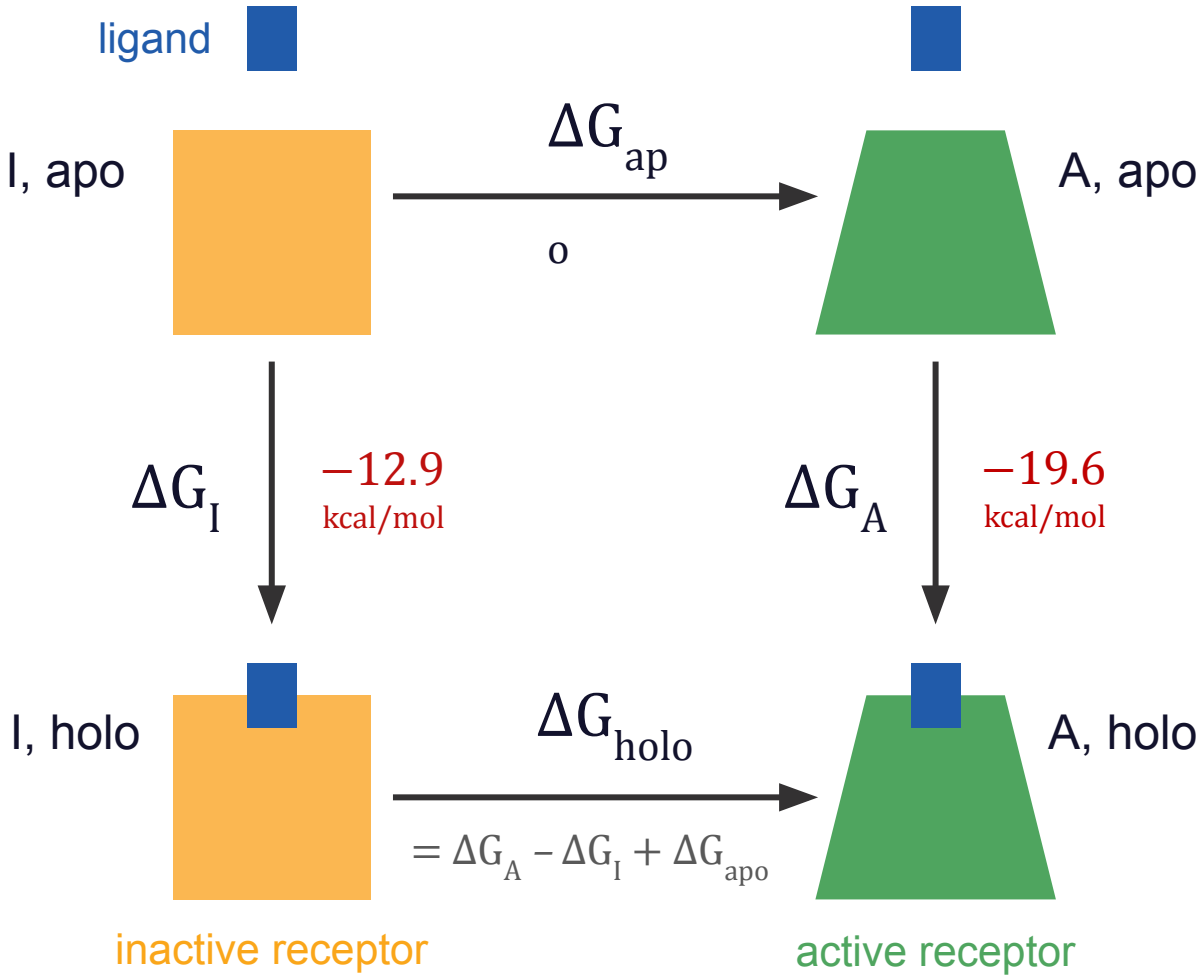
We only need two AB-FEP runs per ligand!
(ideally)

Thermodynamics of Ligand Efficacy

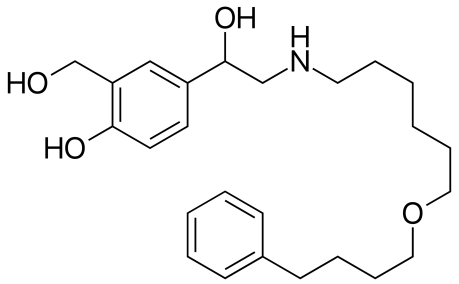
Agonists and antagonists should separate along $\Delta\Delta G = \Delta G_A - \Delta G_I$.



Thermodynamics of Ligand Efficacy



Salmeterol



$$\Delta\Delta G = \Delta G_A - \Delta G_I$$
$$= -19.6 \text{ kcal/mol} + 12.9 \text{ kcal/mol}$$
$$\Delta\Delta G = -6.7 \text{ kcal/mol} < 0$$

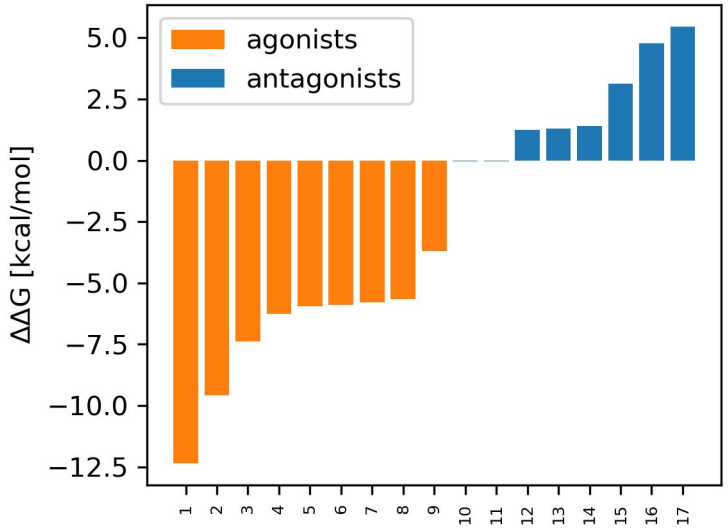
Prediction: Salmeterol favors the active state of the β2-adrenoceptor. It is likely an agonist.

Clinic: Salmeterol is indeed a β2AR agonist and used against asthma and COPD.

Example: β2-Adrenoceptor

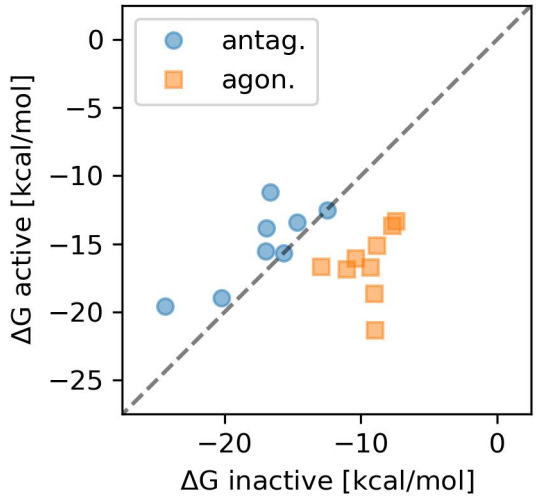
- Agonists and antagonists are well separated.
  - Areas under the curves (AUC) of receiver operating characteristic (ROC) and the precision recall curve (PRC) are 1.0 – the perfect score.

➔ $\Delta\Delta G = \Delta G_A - \Delta G_I$ is indeed an *excellent predictor* for agonism.



- The separation is not always exactly at $\Delta\Delta G = 0$.
  - Accuracy < 1.0, but still perfect predictability

➔ Can *calibrate offset* with a few known ligands for every study if needed!



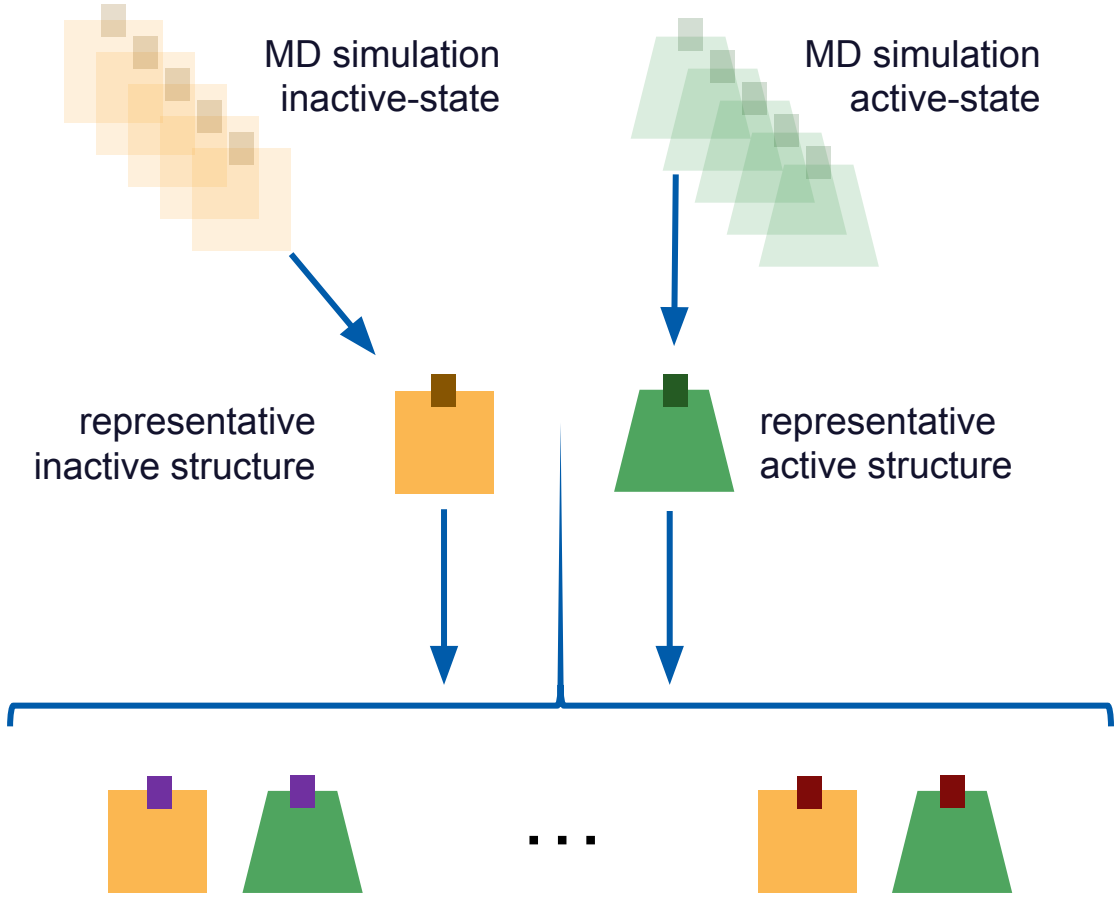
AUC-ROC
1.000

AUC-PRC
1.000

Accuracy
0.882

Simulation Protocol — Starting Poses

- *Pick frames* from template MD simulations that are *representative* of the respective state.
- Align the investigated ligands to each template and *replace the template ligand* with them.
  - Ligands from experimental structures:
    - ⇨ Align via the receptor structure.
  - Ligands congeneric to the template ligand:
    - ⇨ Align via the ligand.
  - Alternative options:
    - ⇨ MCS-docking (max. common substructure)
    - ⇨ IFD-MD (induced-fit docking + mol. dyn.)



each investigated ligand, replacing the template ligand in an inactive and in an active structure

Simulation Protocol — AB-FEP

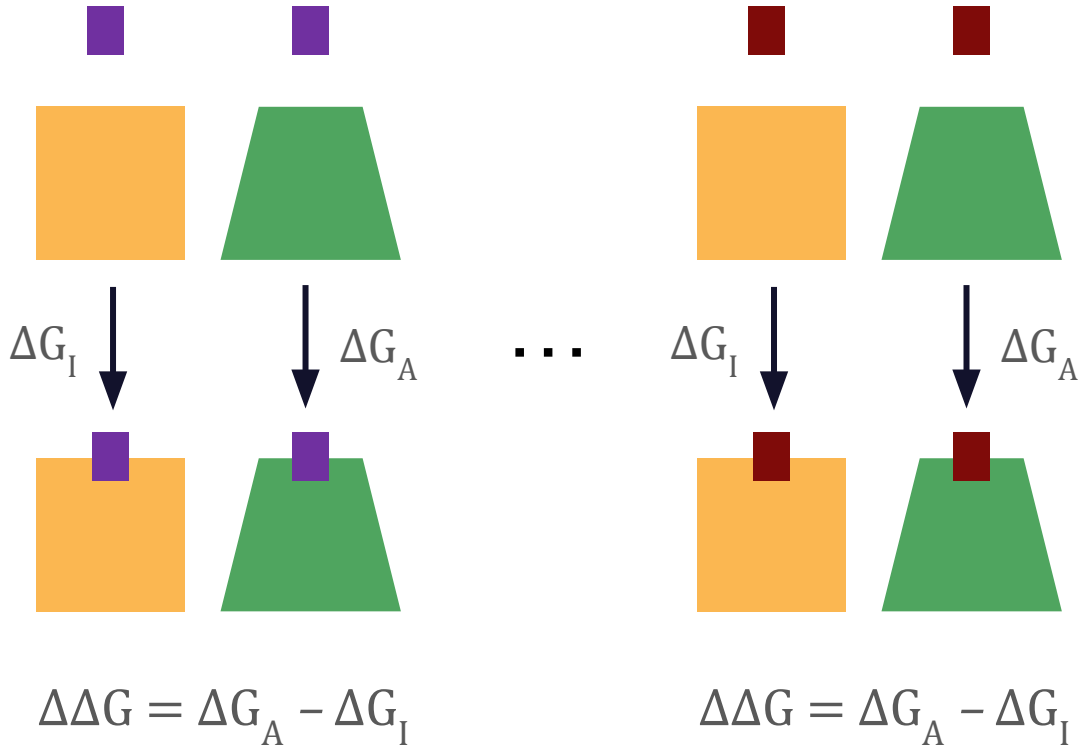
Absolute-Binding Free Energy Perturbation

- Calculate binding free energy of a ligand by perturbing the system's Hamiltonian.
- Fading the ligand in and out via λ parameter.
- Enhanced sampling via replica exchange.

Efficacy Prediction

- Run AB-FEP on the inactive and the active structures for each ligand.
- Rank them by the difference of the resulting binding free energies: $\Delta\Delta G = \Delta G_A - \Delta G_I$.

AB-FEP on each pair of receptor template and investigated ligand

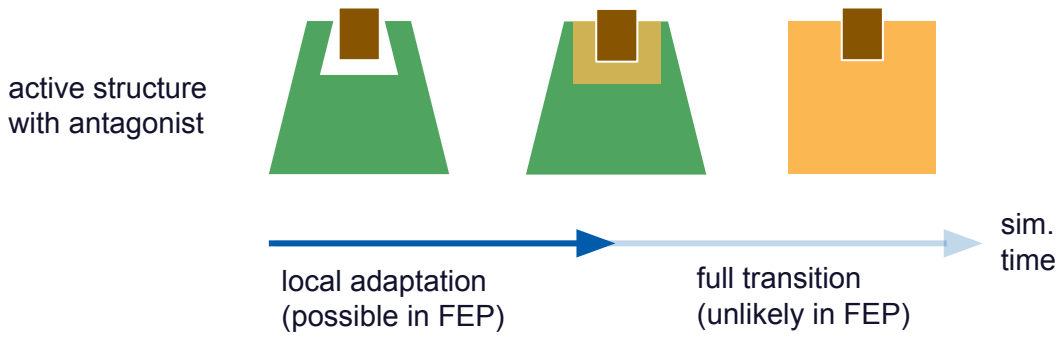


Difference of the binding free energies for each active-inactive pair

Simulation Protocol — Restrained AB-FEP

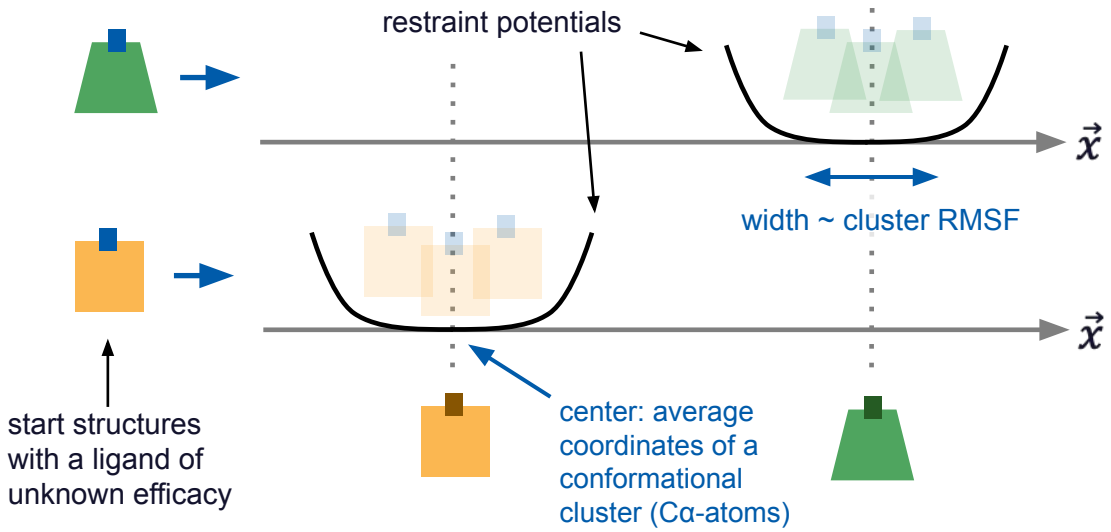
Problem

- The binding pocket partially adapts to the new ligand during AB-FEP.
- Not always clear whether it is still in an active or an inactive state.



Solution

- Use position restraints to prevent transition!
  - Reproduce conformational ensemble of each state.
  - Wide enough to allow for flexibility.
  - Just narrow enough to prevent transition.
- Flat-bottom harmonic restraints to conformation clusters from template MD simulations.



Results Summary

Results for GPCRs – Overview

High Accuracy across targets

With threshold ΔΔG = 0:

95%

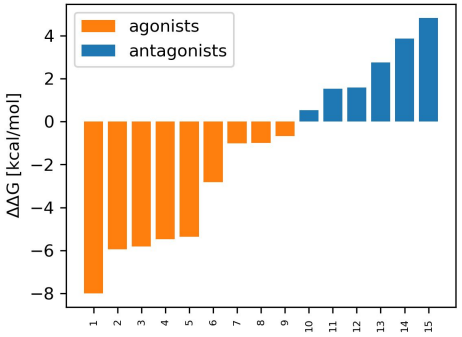
correct predictions.

With ΔΔG threshold adapted for each target:

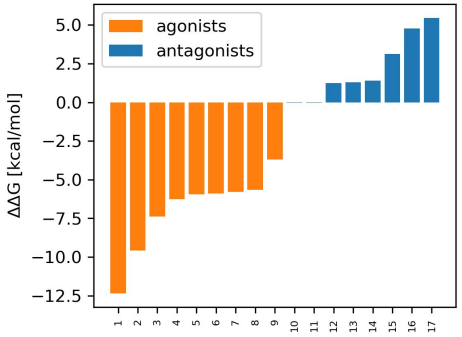
98%

correct predictions.

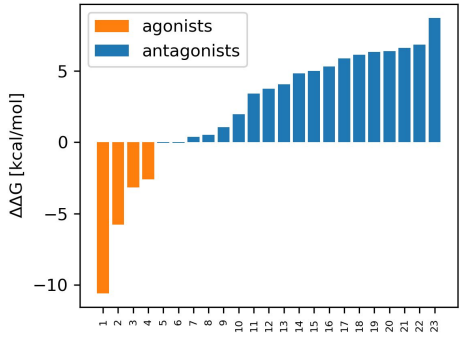
A β1-Adrenoceptor



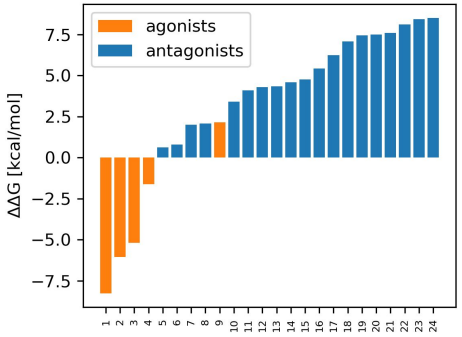
B β2-Adrenoceptor



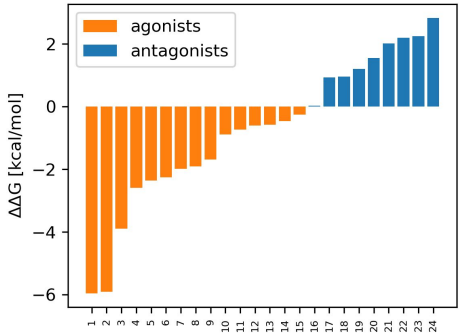
C Adenosine Receptor A1



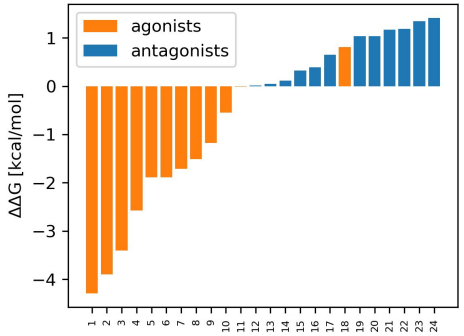
D Adenosine Receptor A2A



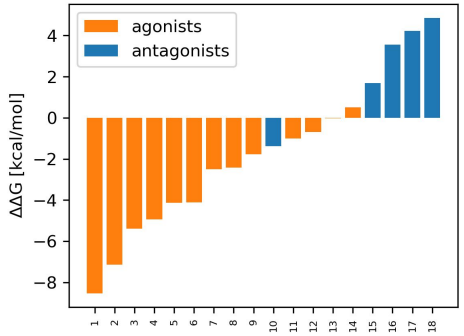
E δ-Opioid Receptor



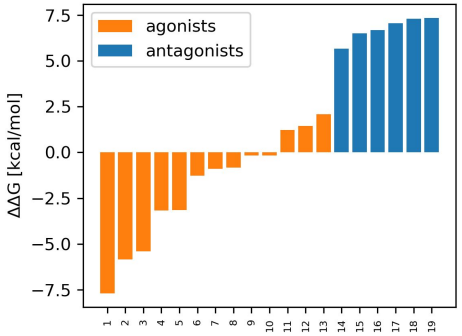
F μ-Opioid Receptor



G Serotonin Receptor 1B

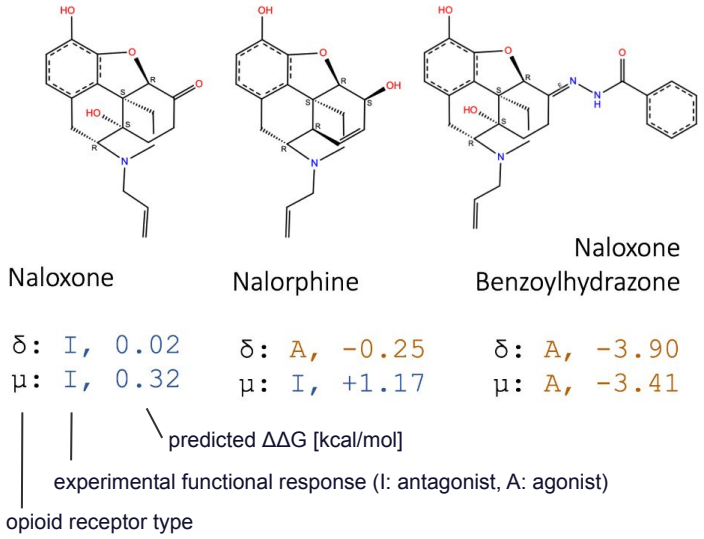


H Serotonin Receptor 2B

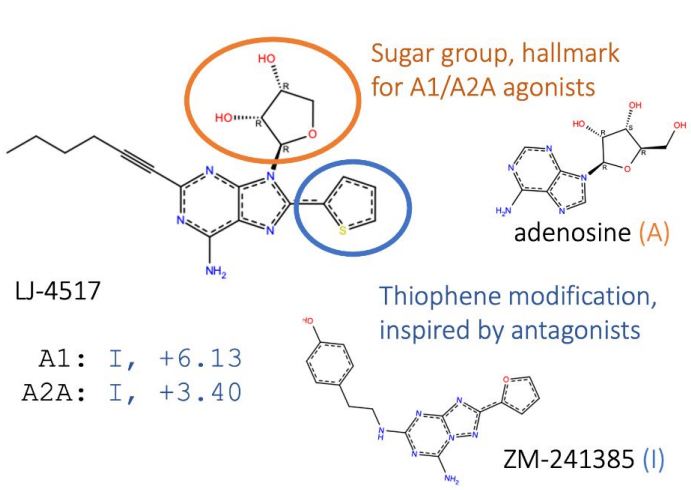


High Accuracy across Diverse Chemical Space

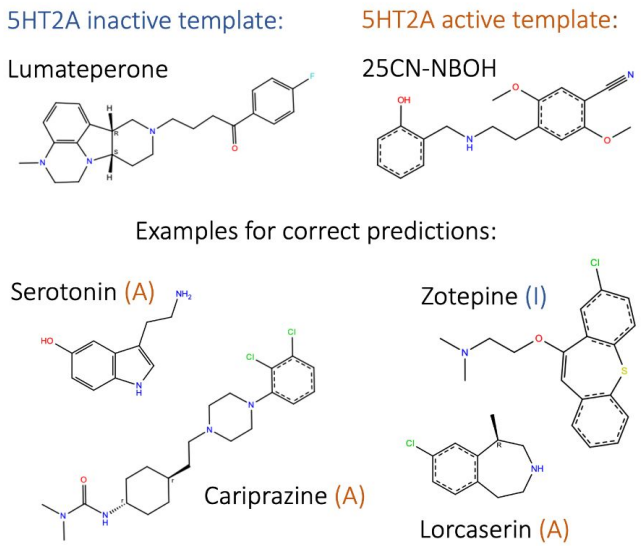
- Small changes in morphinan opioids can qualitatively change their function.
- These activity cliffs are predicted correctly.



- The adenosine receptor ligand LJ-4517 features hallmarks of agonists and of antagonists.
- We predicted it correctly as an antagonist.

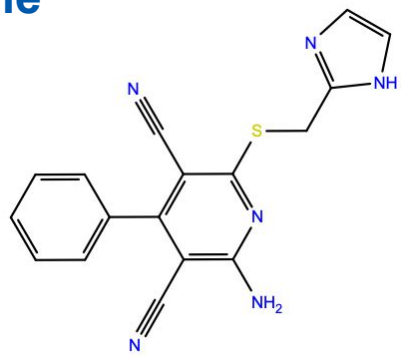


- We correctly predicted the efficacy of ligands that are chemically very different from the ligands in both template structures.



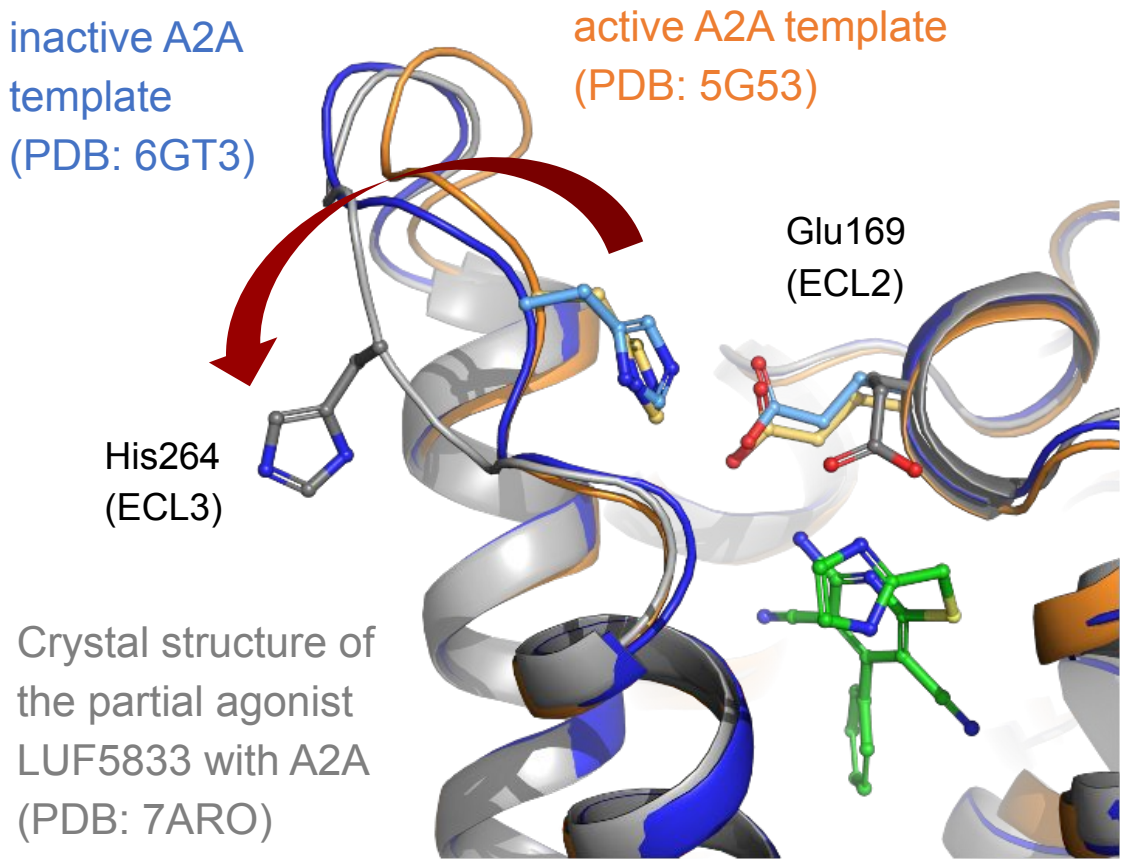
Large Receptor Conformational Changes may not be captured in short AB-FEP simulations

Example: LUF5833 on the Adenosine Receptor 2A



Predictions are inaccurate if a ligand's preferred receptor conformation differs too much from the templates.

⇒ Remedy: *Add additional templates* to capture the relevant conformational landscape.



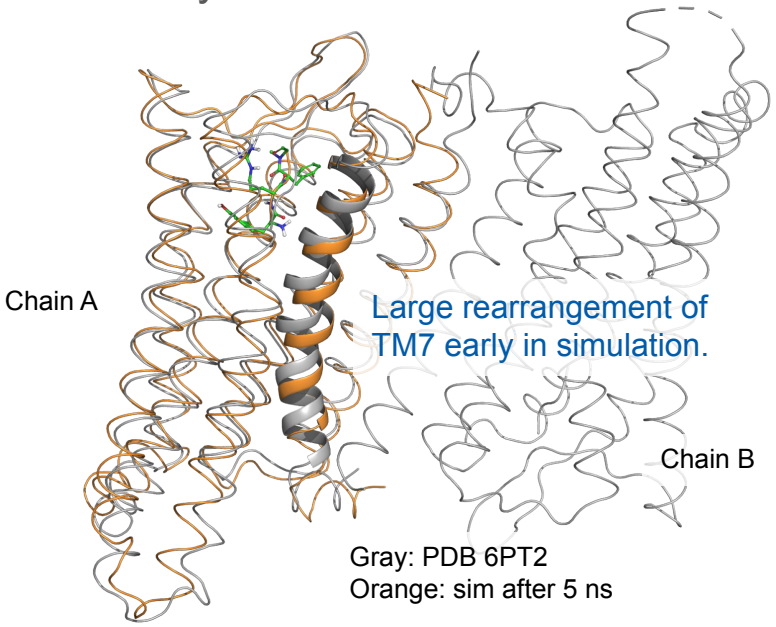
Crystal structure of the partial agonist LUF5833 with A2A (PDB: 7ARO)

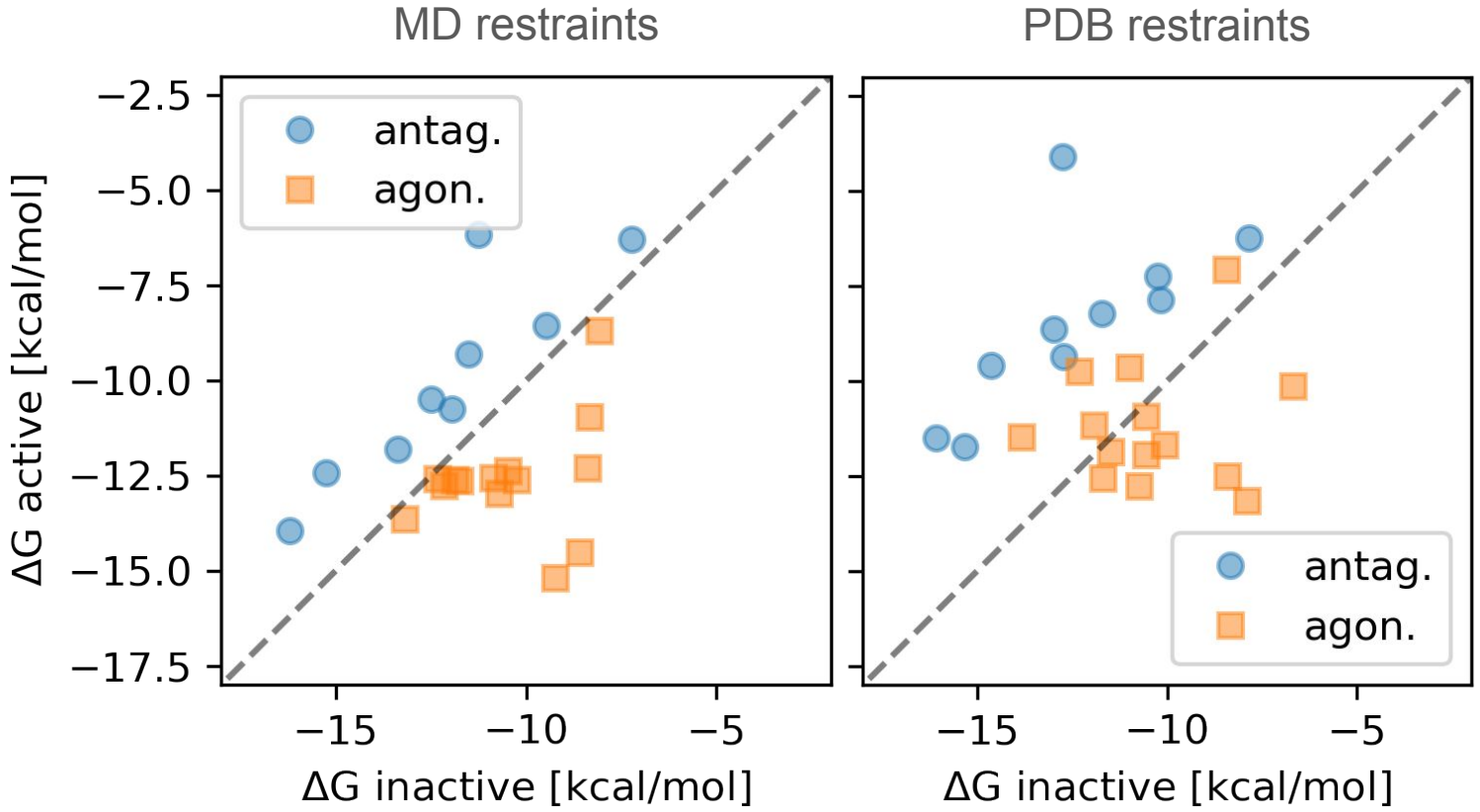
predicted as antagonist (+2.13)

MD Relaxation May Address Crystal Structure Artifacts

Example: δ-Opioid Receptor

- Instead of using cluster centers from MD, we restrained FEP to the original PDB structures.
- Prediction is worse because of crystal contact artifacts.





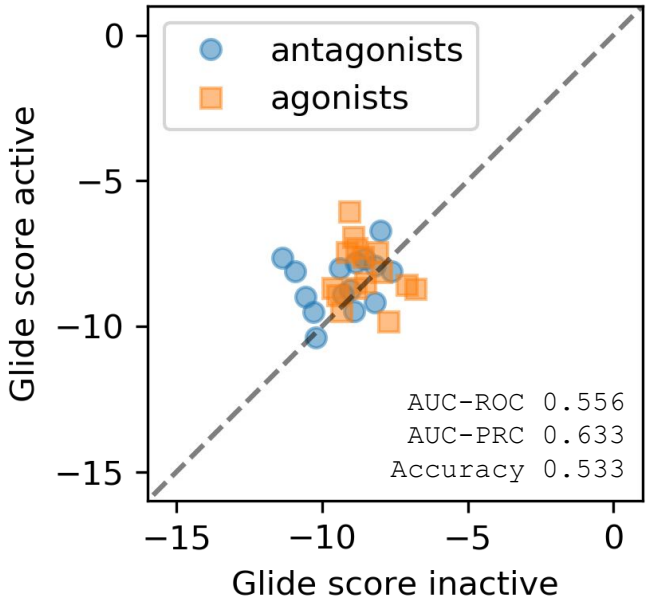
AUC-ROC: 1.000
AUC-PRC: 1.000
Accuracy: 1.000

AUC-ROC: 0.971
AUC-PRC: 0.982
Accuracy: 0.792

The Importance of Ligand Poses

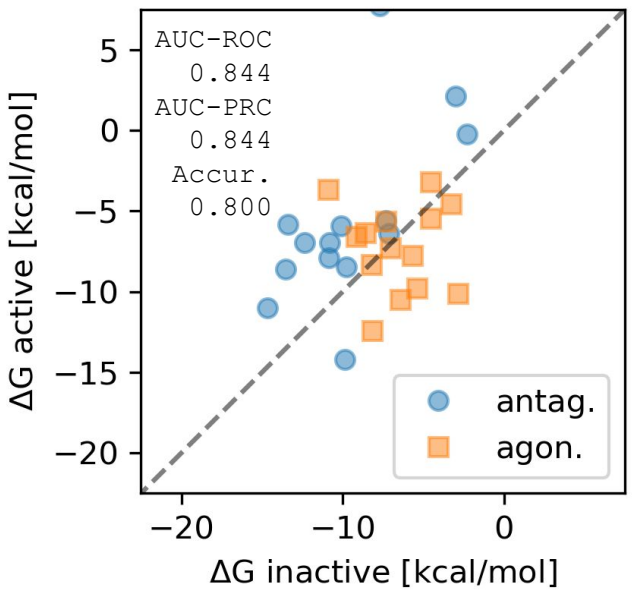
Example: Adenosine Receptor A1 from ATOM3D benchmark dataset.

Score of the best-ranked pose from unguided Glide docking



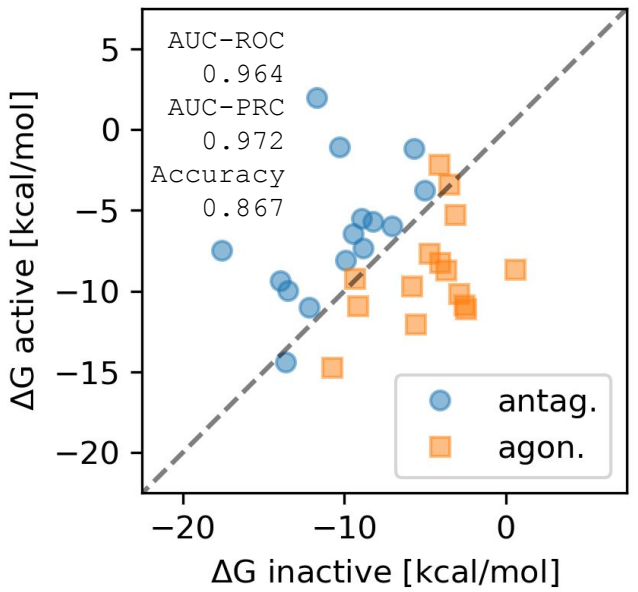
Best LEP method in ATOM3D[1].
Beat all tested ML models.

AB-FEP on the best-ranked pose from unguided Glide docking



Outliers due to some bad poses in the benchmark structures!

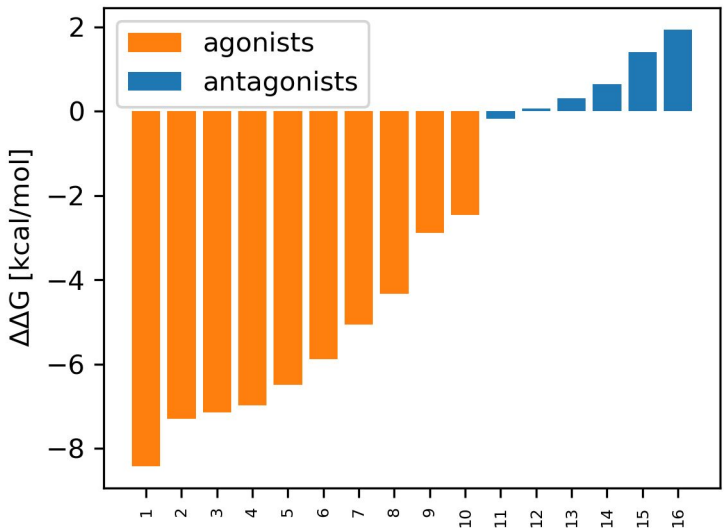
AB-FEP on poses from Glide guided by MCS to resolved ligands

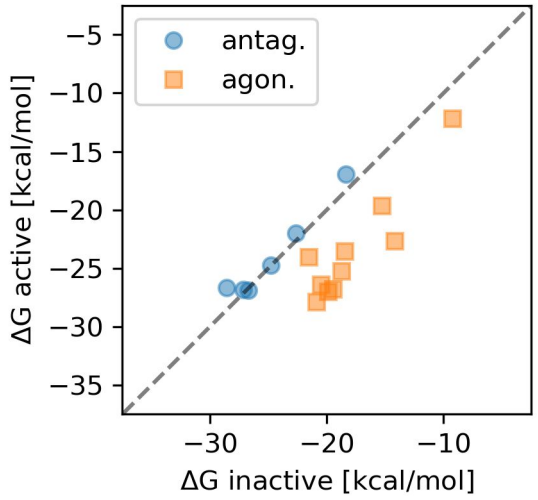


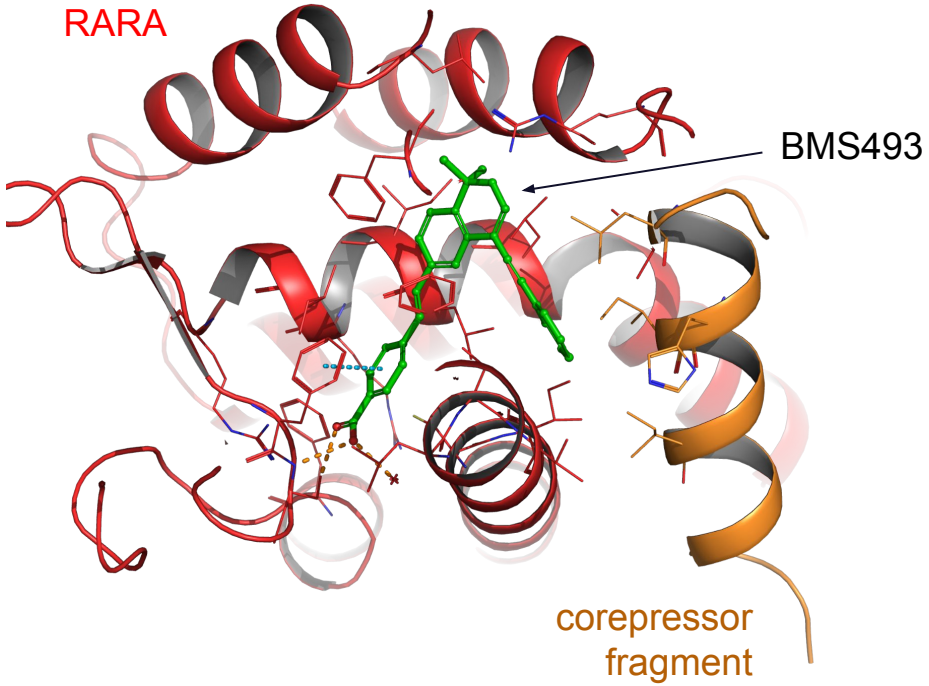
Even better predictions with good starting poses!

[1] Townshend et al., NeurIPS Datasets and Benchmarks 2021

Beyond GPCRs – Retinoic Acid Receptor α







Perfect separation, even without using restraints.

⇒ **Efficacy prediction via FEP+ works beyond GPCRs.**

PDB 3KMZ: RARA LBD with inverse agonist BMS493 and a fragment of nuclear receptor corepressor 1.

Conclusions and Outlook

Main Conclusions

Study Summary

- We can predict the functional response of a receptor via the ligands' free energy of binding to active and inactive structures
- Our workflow using Schrödinger FEP+ achieves excellent performance on several important drug targets.

FEP+ can be used to model ligand efficacy, not only to predict binding affinity.

Preconditions and Best Practices

- Knowing the relevant *conformational states* of the receptor.

→ experiment, predictions, enhanced sampling

- Good estimates for the *ligand poses* in each conformational receptor state.

→ experiment, alignment, docking, IFD-MD,...

- Accurate *modeling*. Poses, protonation states, ions,... might differ between states.

The *usual FEP+ best practices* and pitfalls apply.

Read more about it?

- Manuscript published in JCTC: https://doi.org/10.1021/acs.jctc.3c00899
- Preprint on ChemRxiv: https://doi.org/10.26434/chemrxiv-2023-p1507

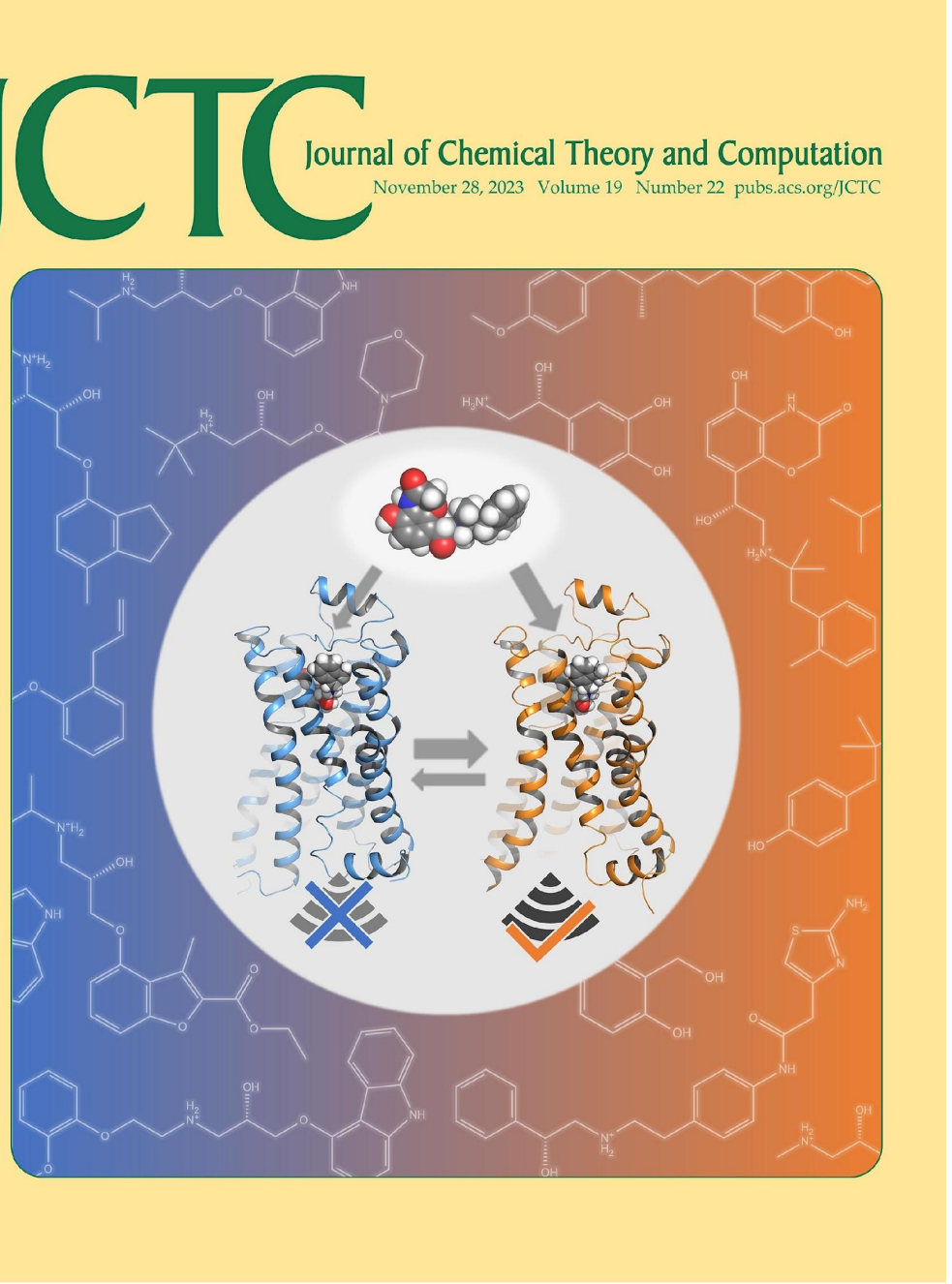
JCTC Journal of Chemical Theory and Computation

pubs.acs.org/JCTC Article

Is the Functional Response of a Receptor Determined by the Thermodynamics of Ligand Binding?

Martin Vögele, Bin W. Zhang, Jonas Kaindl, and Lingle Wang*

Cite This: https://doi.org/10.1021/acs.jctc.3c00899 Read Online



Outlook – Biased Signaling

- Arrestins inhibit G-protein signaling, but also invoke their own pathways.
- Some agonists can activate the receptor in a pathway-selective way, favoring signaling either via arrestin or via G proteins.
  - ⇨ *biased signaling*
- Activating only the desired pathway can help avoid serious side-effects.

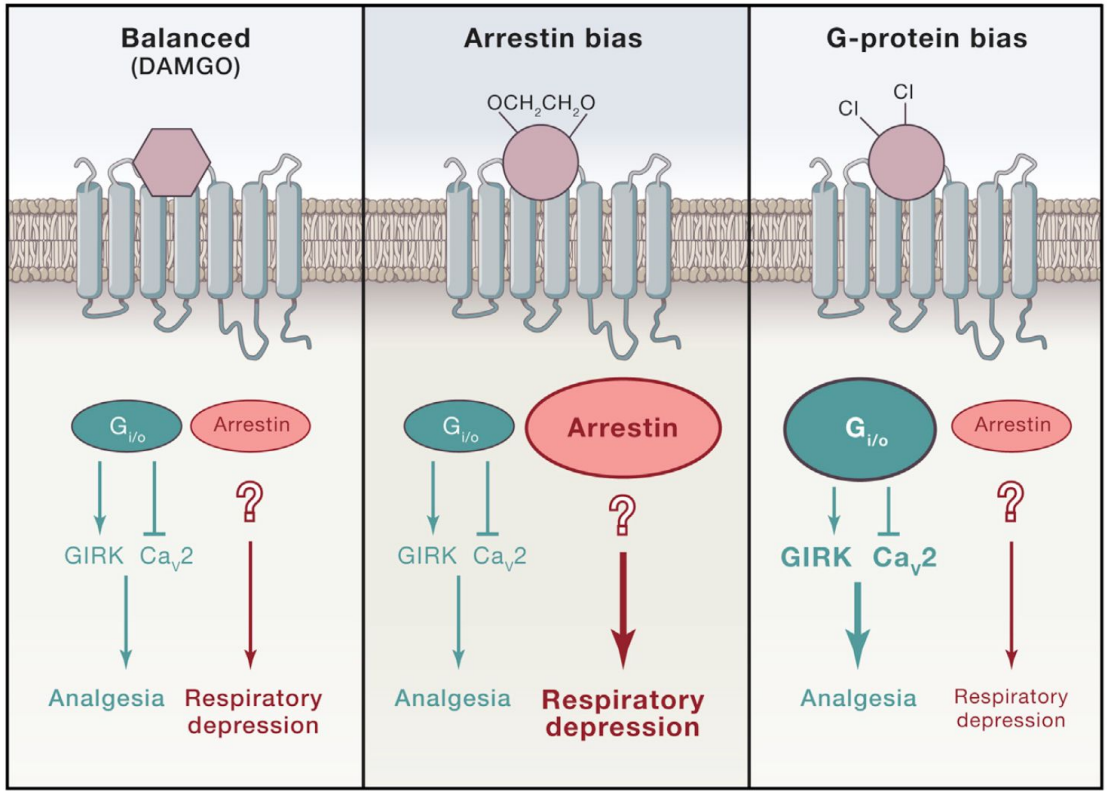
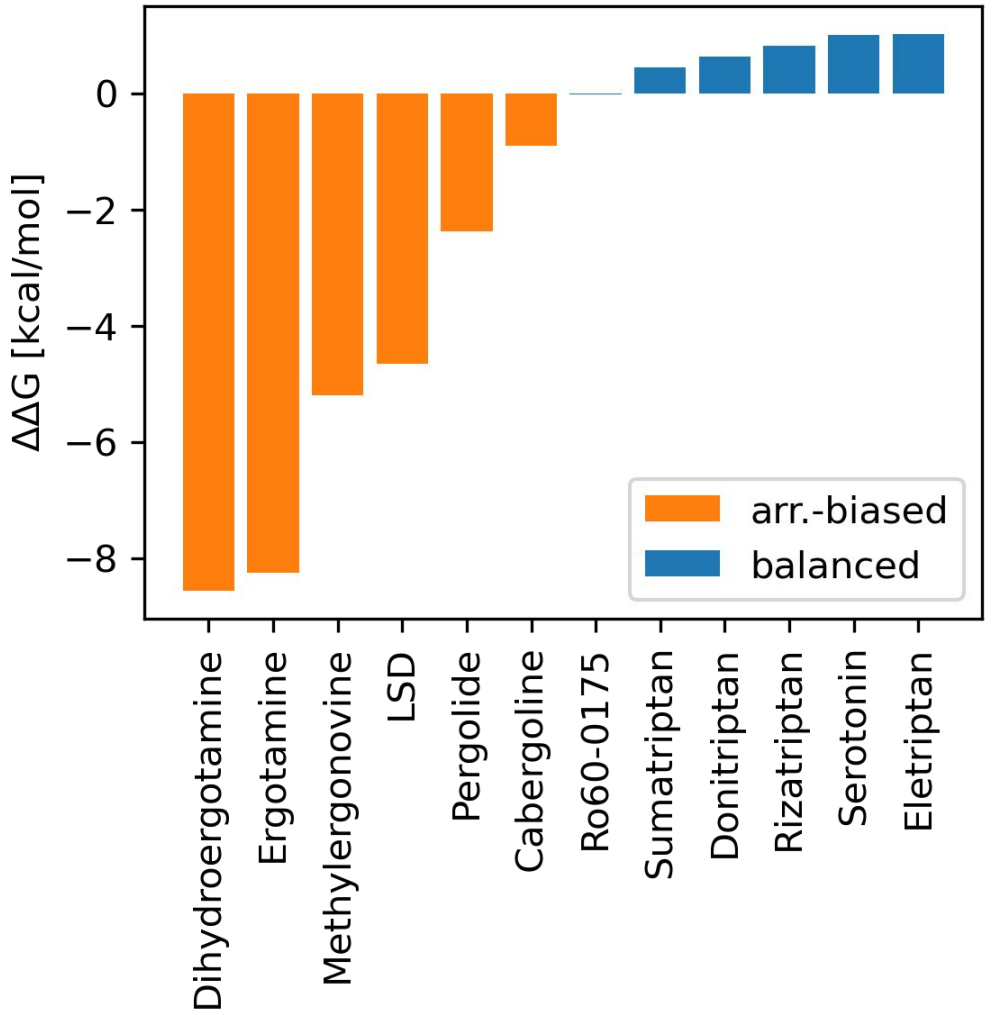


Figure: Biased signaling on the μ-opioid receptor.
Image from [Spangler & Bruchas, Cell 2017]

Serotonin Receptor 2B – Biased Signaling

- Compare the binding affinity in the balanced state to the binding affinity in the arrestin-biased receptor state.
- Template ensembles via two MD simulations from the same structure
  - one with a balanced agonist (serotonin)
  - one with an arrestin-biased agonist (ergotamine)
- Encouraging preliminary result:

  Good separation of balanced and arrestin-biased ligands.



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Schrödinger

Thank you!