Unraveling the Puzzle: Linking Functional Activity to Binding Affinities in Complex Biological Systems

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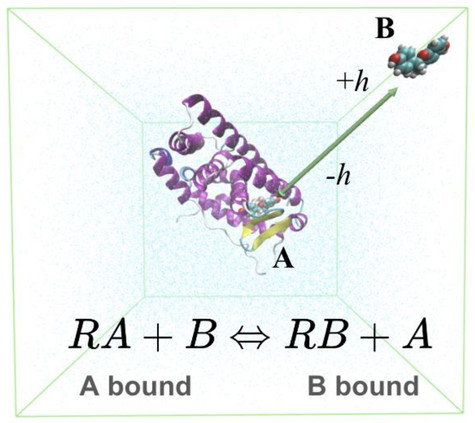
Correlating Binding Affinities to Functional Activity

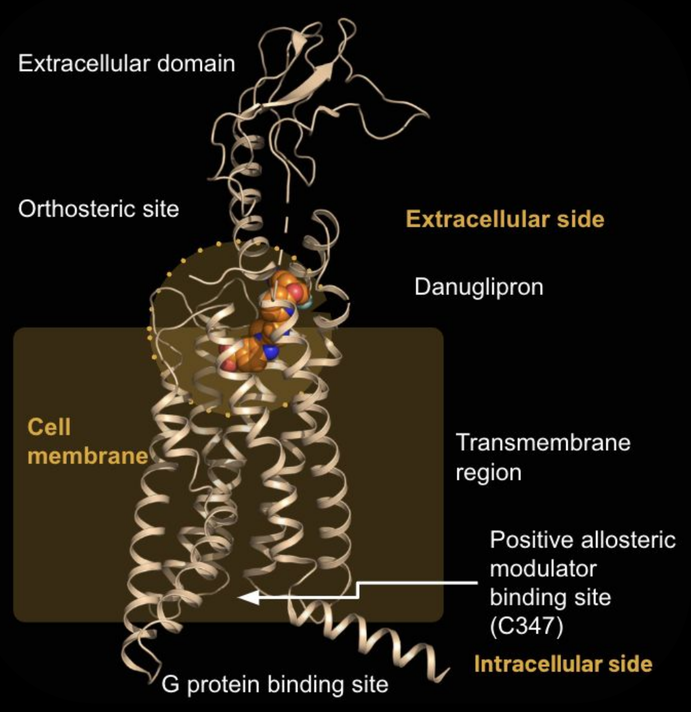
- Relative Free Energy Perturbation (RFEP) and similar methods are the gold standard for binding affinity prediction in drug discovery hit-to-lead and lead optimization phases
- EC_{50} and K_d values are not always directly interchangeable or directly proportional, as they measure different aspects of the drug-receptor interaction.
- In many cases, a lower EC_{50} corresponds to a higher affinity between the drug and its receptor, which correlates with a lower K_d value. This indicates that the drug binds more tightly to the receptor, requiring a lower concentration to produce its effect.
- Here, we evaluate the possibility to use *in silico* Relative (AToM-OpenMM) and Absolute (AQFEP) Binding Free Energy values for predicting functional activity of 2 challenging biological targets: agonists of GLP1R, a membrane receptor and Molecular Glues resulting in GSPT1 protein degradation.

AToM-OpenMM: Relative Binding Free Energy Calculations

- The RBFE protocol is based on a ligand swap perturbation in a dual-topology formulation
- Ligand B is displaced into the binding site and at the same time that A is displaced into the solvent.
- The energy of bound state is obtained by displacing the ligand from the solvent bulk to the binding site by a fixed displacement vector.

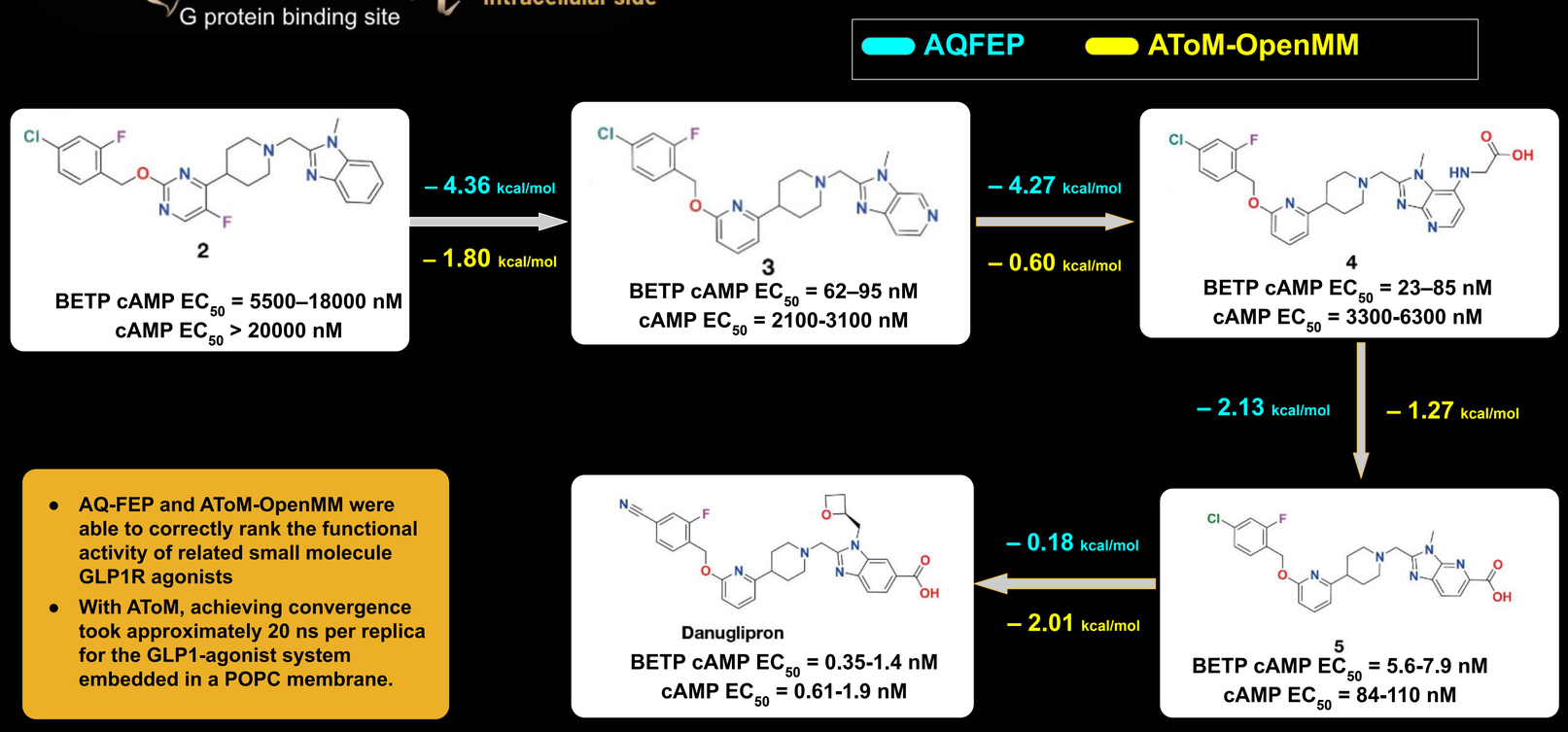
$$U_{RB+A}(x) = U_{RA+B}(x_A - h, x_B + h)$$





GLP1R Membrane Receptor Agonists

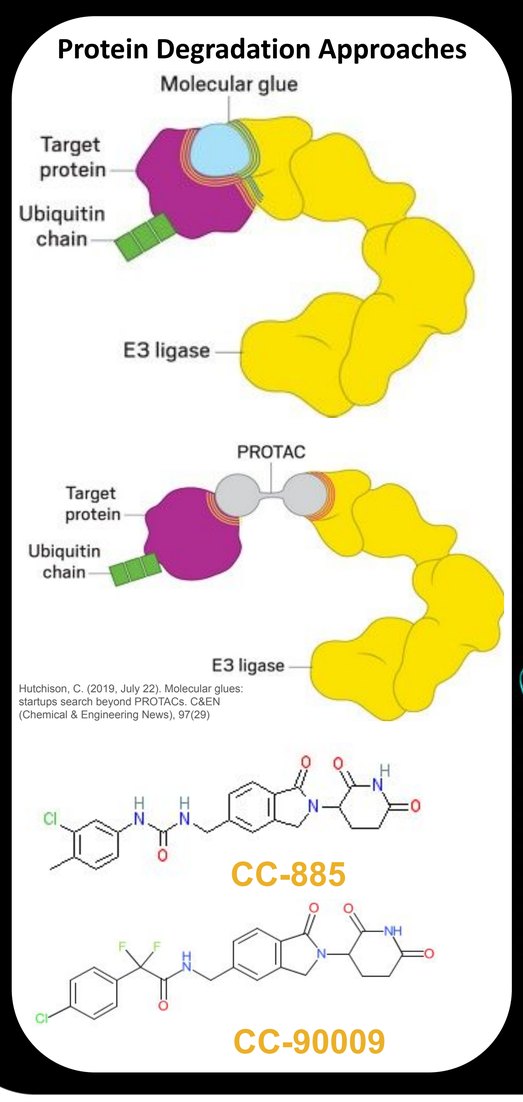
- Pfizer Danuglipron, is a small molecule agonist of the glucagon-like peptide 1 receptor (GLP1R), a membrane receptor part of the Family B GPCR.
- By binding to the orthosteric site of the GLP1R receptor, Danuglipron induces conformational changes, initiating intracellular signaling pathways involved in glucose metabolism regulation.
- Danuglipron's specific interactions with the GLP1R receptor offer potential therapeutic benefits for metabolic disorders like type 2 diabetes and weight loss, making it an exciting candidate for further investigation and development.
- The hit-to-lead and lead optimization resulting in the discovery of the clinical molecule Danuglipron started from a very weak small molecule agonist (represented by ligand **2** below) requiring the presence of a positive allosteric modulator (BETP) to result in detectable agonist activity.
- Examples of the reported medicinal chemistry optimization steps are shown below



- **AQ-FEP and AToM-OpenMM were able to correctly rank the functional activity of related small molecule GLP1R agonists**
- **With AToM, achieving convergence took approximately 20 ns per replica for the GLP1-agonist system embedded in a POPC membrane.**

- Danuglipron, met its primary target in a placebo-controlled Phase 2b trial, leading to a statistically significant amount of weight lost
- The weight reductions were smaller than those seen in trials of rival medicines targeting the same GLP-1 pathway, and a high rate of patients experienced side effects and dropped out of the trial
- Pfizer will not move twice-daily danuglipron into Phase 3 trials, but that instead it would focus on a once-daily formulation, which is currently undergoing pharmacokinetic studies. Data are expected in the first half of 2024.

GSPT1: Molecular Glues Driven Protein Degradation



- CELMoDs (cereblon E3 ligase modulators) are a class of molecular glues that recruit neosubstrates to the E3 ubiquitin ligase cereblon (CRBN) for downstream poly-Lys48-ubiquitination and proteasomal degradation.
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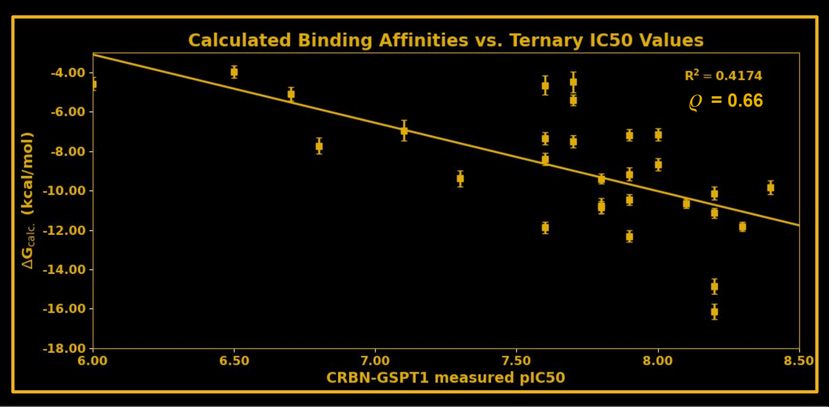
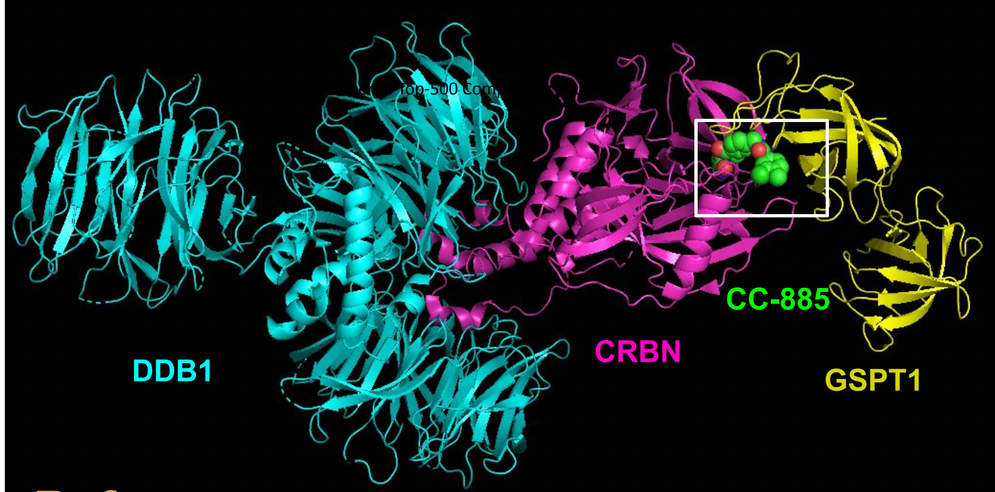
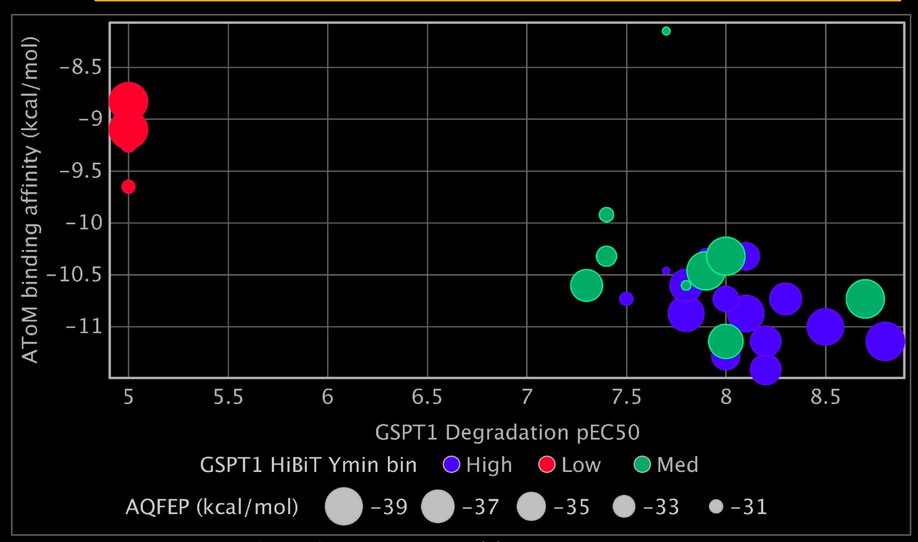
- Structure-based drug design can predict CELMoDs' binding affinity to protein-protein interfaces and correlate with cellular degradation of neosubstrates like GSPT1 and zinc finger Aiolos (IKZF3), aiding in the design of CELMoDs with desired degradation potency.







References

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Unraveling the Puzzle: Linking Functional Activity to Relative Binding Free Energy Calculations in Complex Biological Systems

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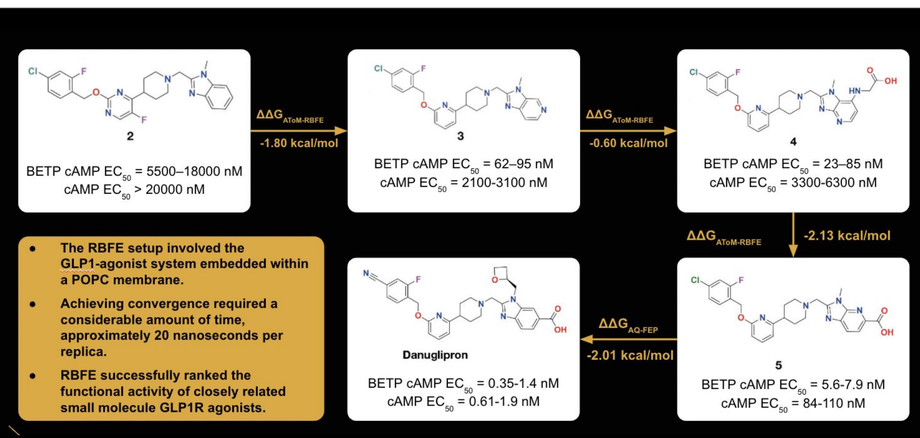
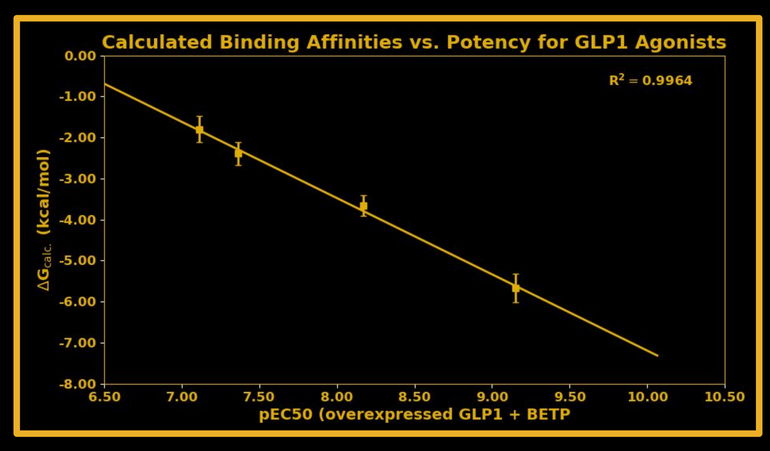
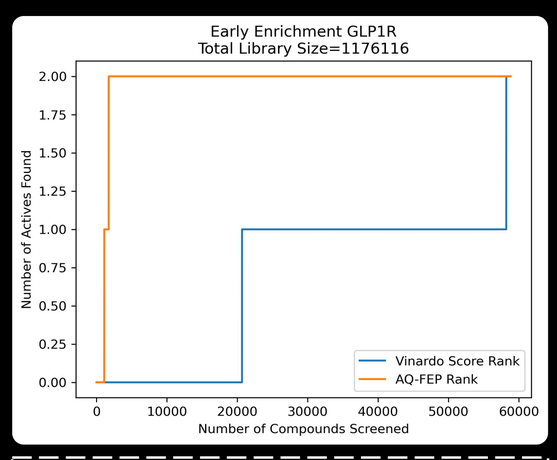
Correlating Binding Affinities for Protein Degradation and GLP1R Agonists

- Relative Free Energy Perturbation (RFEP) and similar methods are the gold standard for binding affinity prediction in drug discovery hit-to-lead and lead optimization phases
- Although EC50 and K_d values are not always directly interchangeable or directly proportional, as they measure different aspects of the drug-receptor interaction.
- In many cases, a lower EC50 corresponds to a higher affinity between the drug and its receptor, which correlates with a lower Kd value. This indicates that the drug binds more tightly to the receptor, requiring a lower concentration to produce its effect.
- Here, we present a correlation between the EC50 (potency) and In Silico Relative Binding Free Energy values (AToM-OpenMM) for challenging biological targets: GLP1R membrane system and Molecular Glues for Protein Degradation.

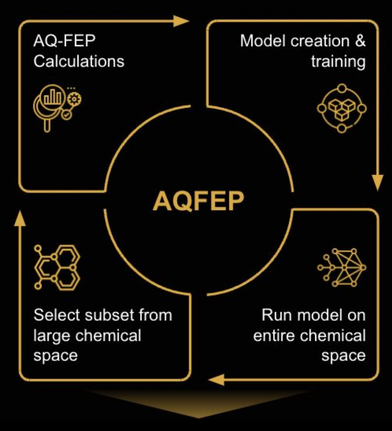




Combining AQFEP for VS + RBFE for Hit-to-Lead



Enrichment in Virtual screening using AQFEP and Vinardo scoring function. Molecule 2 and 3 were included as actives in the GLP1R library.



- AQFEP employs a hybrid Machine Learning and physics-based methodology to assess the binding free energy of various ligands to protein targets, surpassing traditional molecular docking scoring functions in its accuracy in predicting ligand binding free energy.
- Balances speed and ligand ranking accuracy.
- Doesn't need congeneric reference.
- Achieves early enrichment compared to scoring with semi-empirical scoring functions.
- By integrating a rapid and effective scoring function for hit identification with a reliable Relative Binding Free Energy Protocol, we're advancing toward a smoother hit-to-lead discovery process at Sandbox.

AToM-OpenMM: Relative Binding Free Energy Calculations

- The RBFE protocol is based on a ligand swap perturbation in a dual-topology formulation
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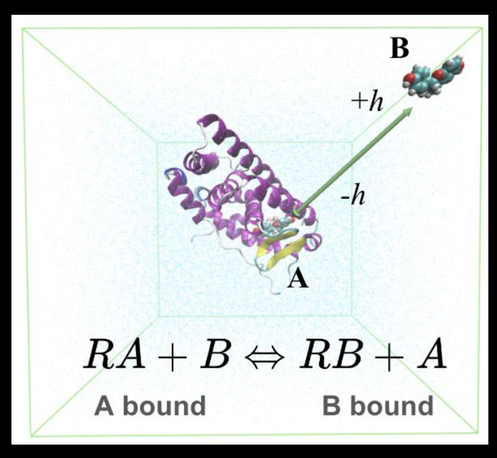
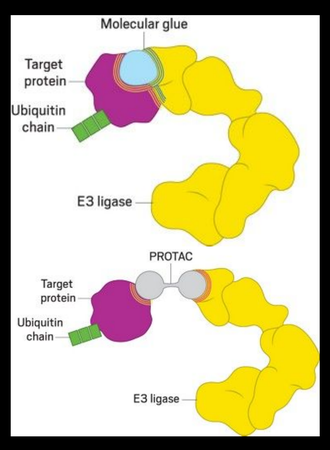


Molecular Glues for Protein Degradation



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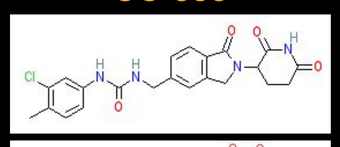

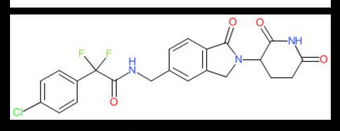

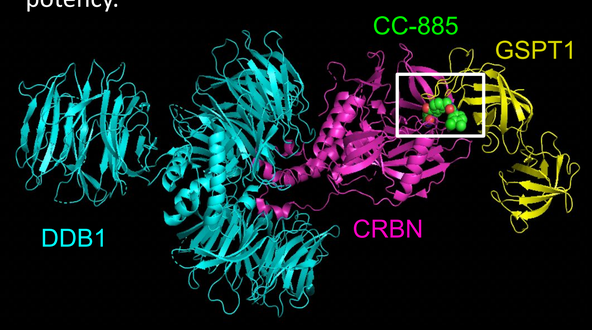

GLP1R membrane Agonists

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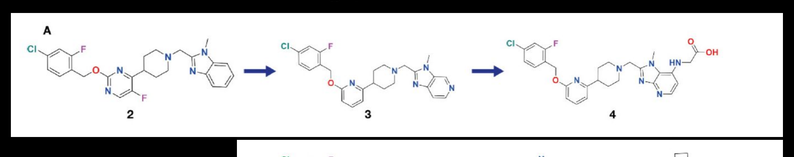
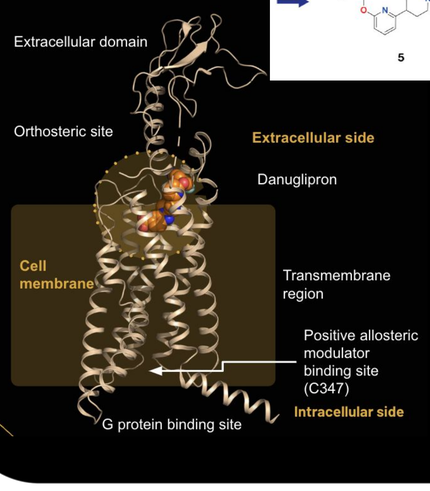
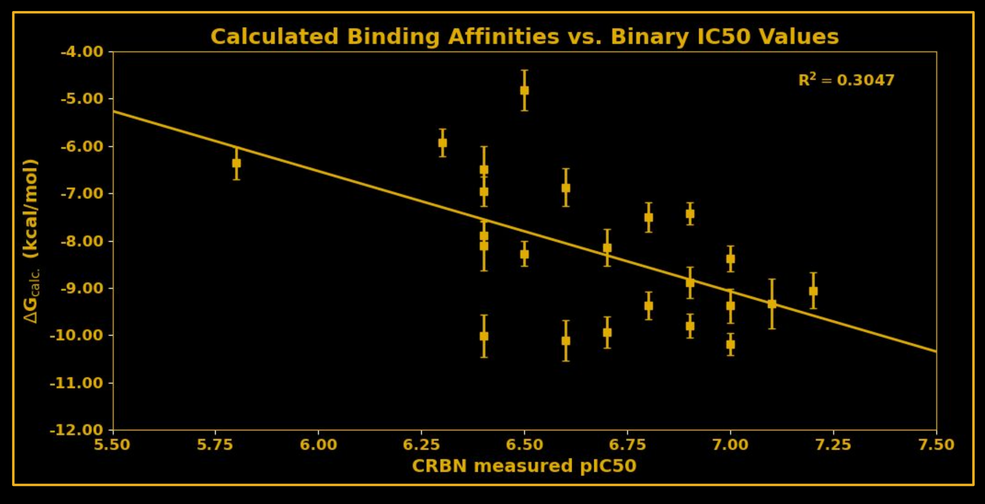
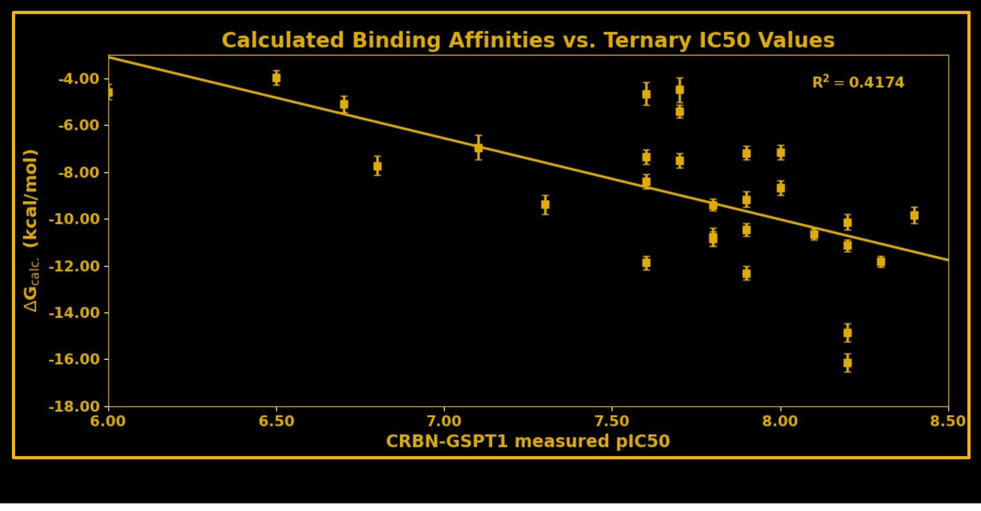
- Optimization of small molecule 2 culminating in the identification of the clinical candidate danuglipron.









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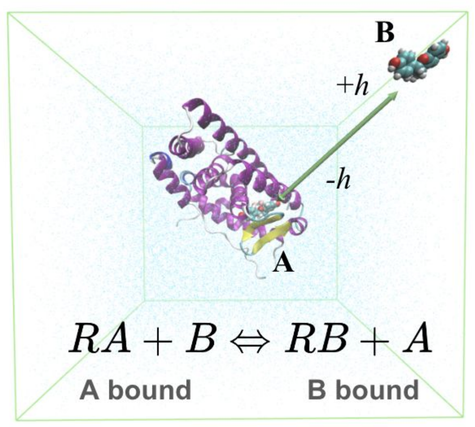

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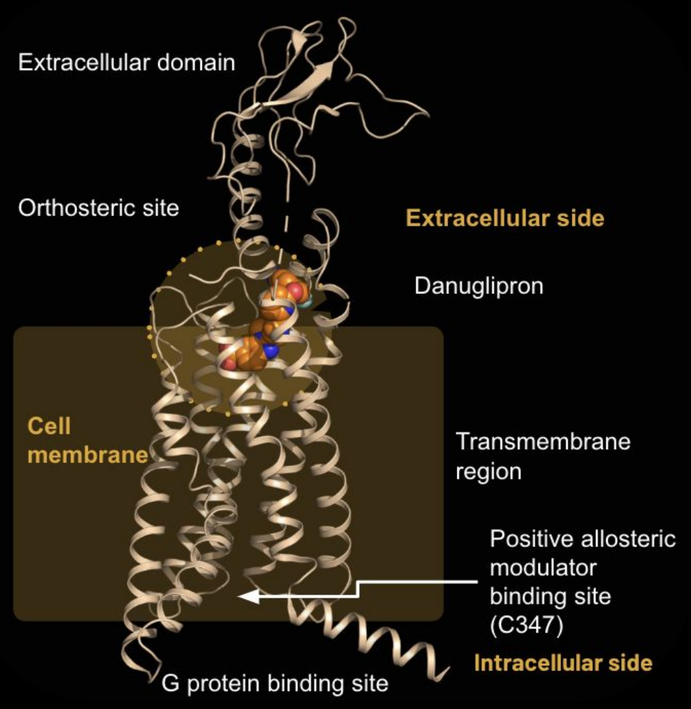

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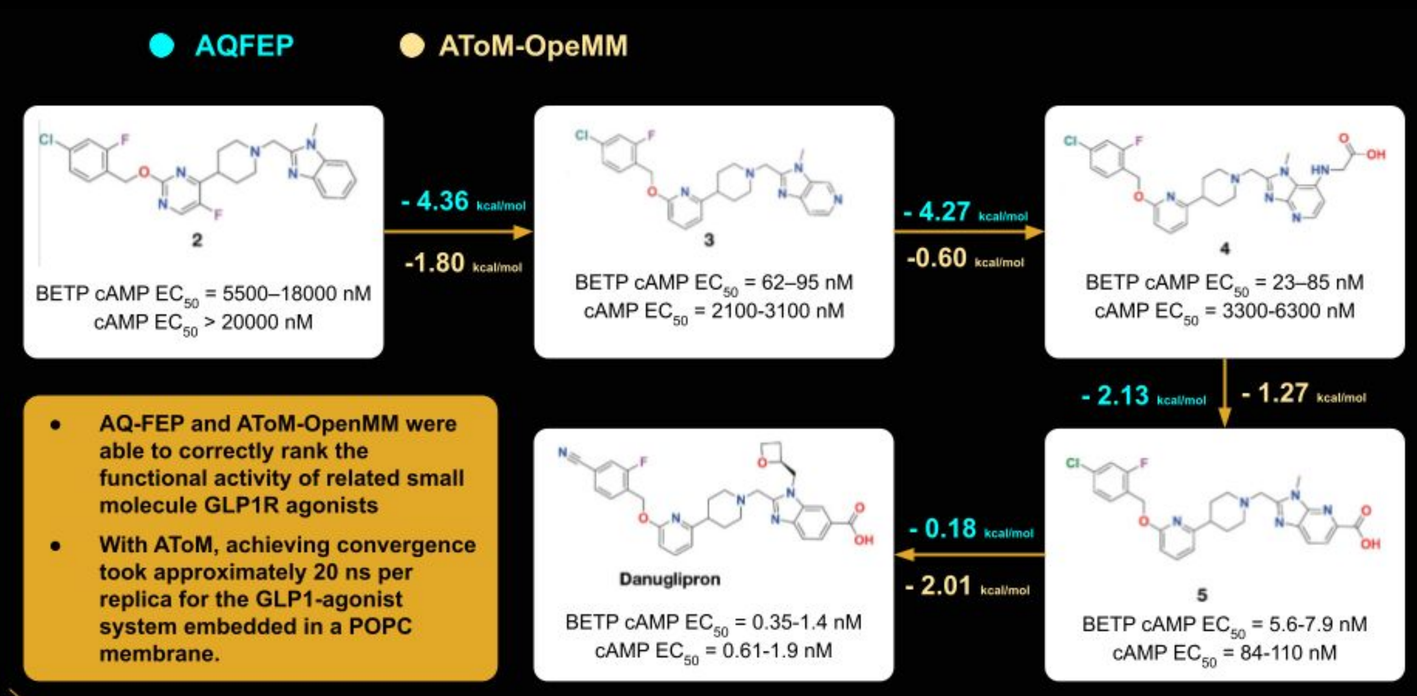

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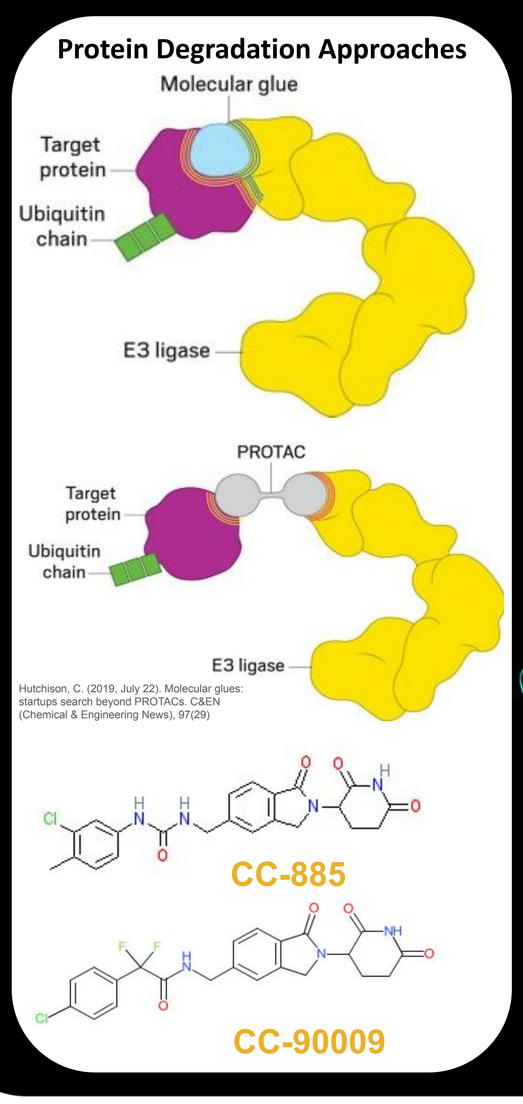

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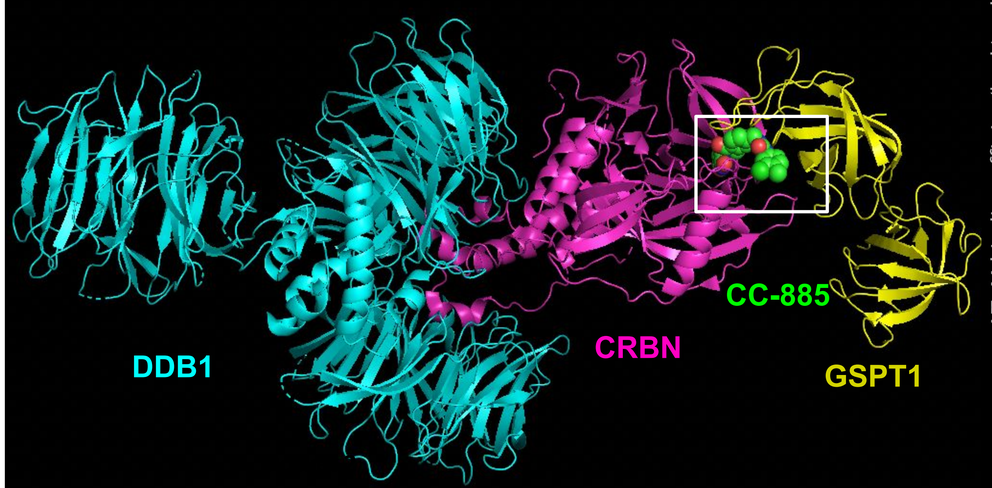

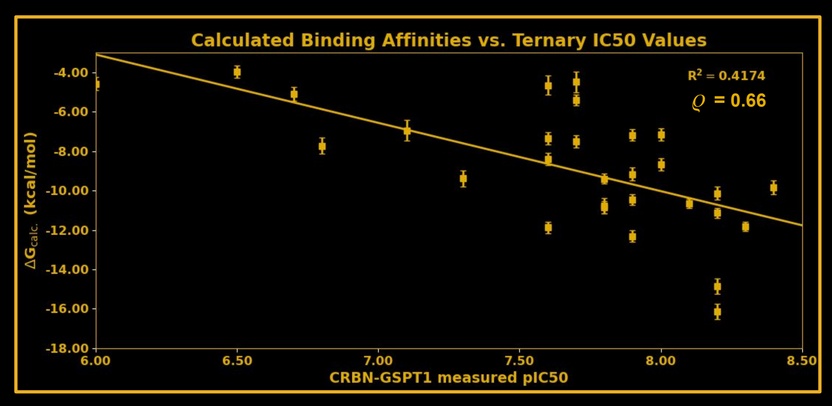

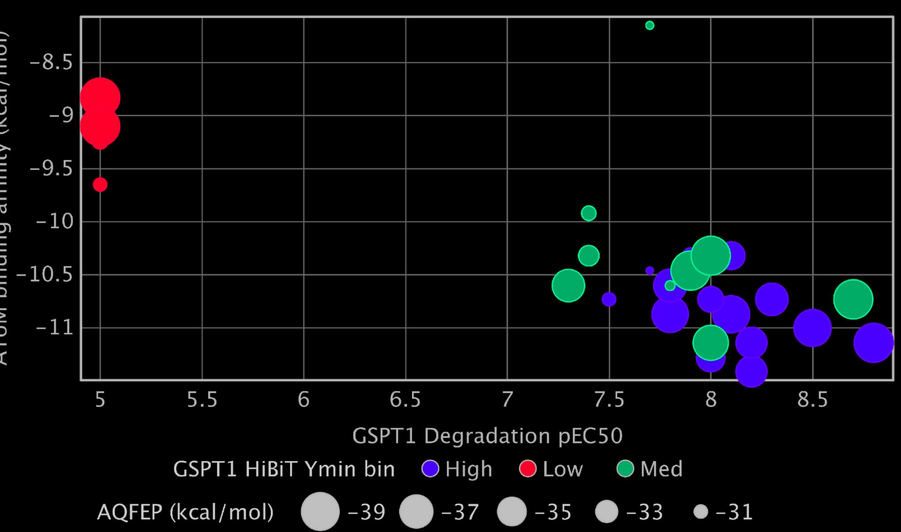

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