Calculating binding free energies for G protein-coupled receptors (GPCRs): accurately capturing lipid exposed binding interactions in P2Y1

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Introduction

G protein-coupled receptors (GPCRs) are a large family of cell surface receptors that play a crucial role in mediating the effects of various signalling molecules, including hormones, neurotransmitters, and other ligands. These receptors transmit signals from the extracellular environment to the inside of the cell, triggering a variety of cellular responses.[1]

P2Y1 is a specific subtype of GPCRs which, given their involvement in critical physiological processes, are potential targets for drug development. Medications that selectively modulate P2Y1 receptor activity can be used to treat conditions associated with abnormal platelet function, vascular disorders, and thrombotic events.

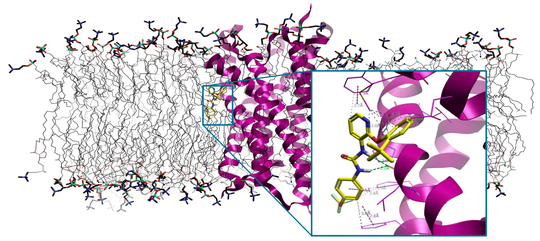


Figure 1: Representation of P2Y1 GPCR with the co-crystal ligand 'BPTU' in a lipid bilayer

P2Y1 is a challenging membrane protein:
- Class A 7 transmembrane GPCR
- Large system (93,255 atoms with water)
- Interface binding (important to get POPC/ ligand/ protein interactions correct)
- Allosteric

P2Y1 co-crystal ligand (A):
- Allosteric antagonist 'BPTU' from PDB:4XNV
- K_i = 16 nM / $\Delta G_{binding}$ = -11.2 kcal/mol)

In this study, we are starting with ligand (B):
- Five R-positions on the common substructure in the 30 ligand data series where modifications occur.[2]

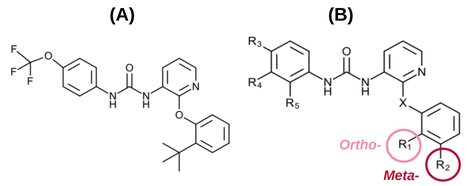


Figure 2: A: BPTU, the co-crystal ligand of P2Y1; B: Molecular scaffold that will be used in this study

Methods

Relative free energies of binding (ΔΔG) were obtained with Flare™ FEP3 by mutating the ligand in its intermediate states for both the protein−ligand complex in water and the unbound ligand.
- Small molecule forcefield: OpenFF 2.0.0
- Protein forcefield: AMBER FF14SB
- Charge method: AM1-BCC
- Solvent: Membrane POPC
- Initial simulation length per λ window: 4ns

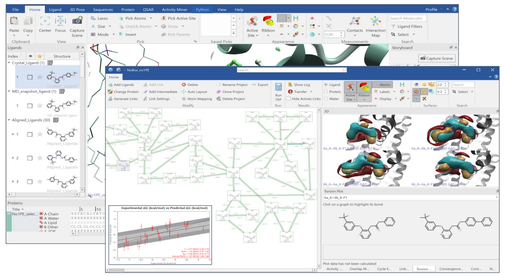


Figure 3: The user-friendly interface of Flare makes FEP calculations easy to run and troubleshoot

Results

Protein preparation:

The P2Y1 system (PDB: 4XNV) was built using Protein Preparation in Flare, with further exploration of electron density.

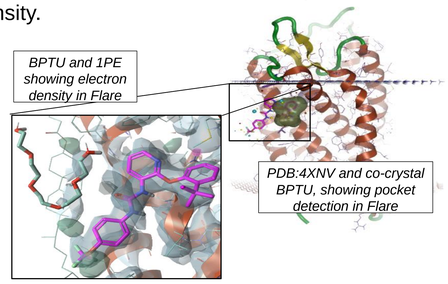


Figure 4: Electron density and pocket detection in Flare

Molecular Dynamics

A Dynamics simulation was run to select a snapshot, representative of the binding mode and pose.
Protocol:
- OpenFF 2.0.0, AMBERFF14SB
- POPC lipid membrane
- 298K, NPT ensemble, 4fs timestep, explicit TIP3P water
- 20ns simulation

The snapshot was used for water analysis and the rest of the ligands were realigned to the selected ligand conformation.

Water Analysis – 3D-RISM

3D-Reference Interaction Site Model (3D-RISM) is a modern approach to solvation. Conceptually, it is equivalent to running an infinite-time molecular dynamics simulation on the solvent (keeping the solute fixed), and then extracting the density of solvent particles.
- Investigates location and stability of water
- Uses Cresset's proprietary XED force field

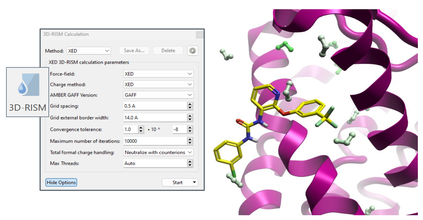


Figure 5: 3D-RISM in Flare can be used to identify bound water molecules which are not provided by crystallographic data

Why is 3D-RISM a solution?
- GPCRs are hydrophobic, but water, though it may be scarcer than in soluble protein systems, plays important roles[4]
- Impact on side chain ionization
- Key ligand/lipid/protein water interactions stabilizing the binding pose of ligand
- Structural waters: stabilizing active (or inactive) conformation of protein
- Timescales for water to arrange are practically too large
- Less certainty in experimental resolution of waters in membrane proteins

Ligand Alignment

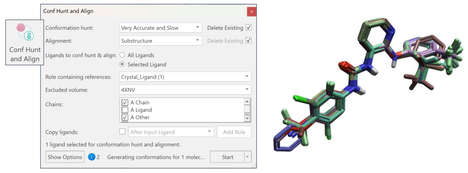


Figure 6: Conf Hunt & Align in Flare can be used pre-FEP with 'Very Accurate and Slow' and 'Substructure' matching to get a good alignment

Alignment and overlay affect how optimally the perturbation map is set up which defines the links between ligand pairs and 'maps' the transformations of atoms from one ligand to the other.

Flare FEP: benchmarking

'Out of the box' FEP graph (A):
- Use the carefully prepared input structures
- Aligned ligands are then 'mapped' together by similarity
- Use a 'normal' connected graph (creates cycles of links)
- Use automated intermediates

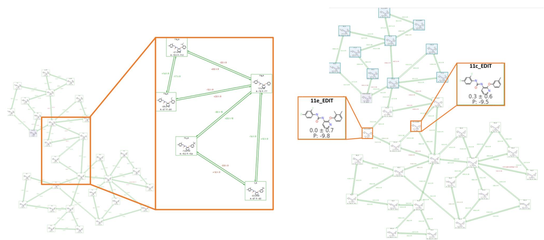


Figure 7: Left: 'Out of the box' normal FEP graph as generated by Flare FEP; Right: Same graph, but with the addition of manual intermediate molecules

Can the addition of 'manual' intermediates (B) strengthen the links? Intermediates smooth the transition between ligands and can help with the transformations of more dissimilar ligand pairs

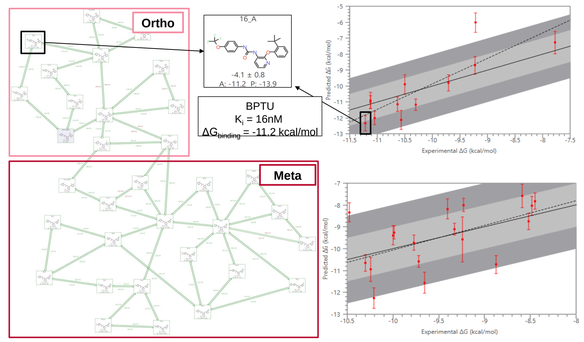
	R^2	MUE (kcal/mol)	Tau	$RMSD_{pw}$	No. of Ligands	No. of Perturb.
FEP+ [2,5]	0.48	-	-	1.41	30	76
Dickson et al. [2]	0.58	1.29	-	-	30	Approx. 70
Flare FEP ('out the box')	0.57	1.17	0.56	1.65	31	90
Flare FEP (with manual intermediates)	0.57	1.18	0.56	1.67	33	98

Table 1: Comparison of FEP benchmark results

The automated FEP setup in Flare produced very good results. Adding the manual intermediates did not in the end lead to improved statistics

Flare FEP: Analysis

Flare FEP subgraph analysis identifies two subgraphs, corresponding to the 'ortho' and the 'meta' substitutions of the ligand.



	R^2	MUE (kcal/mol)	Tau	$RMSD_{pw}$
Ortho	0.71	0.86	0.61	1.49
Meta	0.30	0.92	0.40	1.74

Figure 8: Highest correlation and lower error statistics are found in the 'Ortho' cluster, whereas less certainty in the 'Meta' cluster.

- Ligand 16_A is the strongest binder and is also the co-crystal ligand 'BPTU'
- The strongest binders appear to have substitutions at the R1 (ortho) and R3 positions

Conclusion

- After careful system preparation, Flare FEP run "out of the box" produced a very good correlation with experimental binding affinities, validating the use of Flare FEP on this GPCR system
- The benchmark run here, for a challenging GPCR, demonstrates accuracy and precision comparable, and in some cases, better than published results
- Flare FEP analysis tools help you pragmatically move on from the benchmark to production step
- Further Cresset tools, e.g. Hit Expander and Spark™ can drive a live project forward by easily creating new designs
- We show a workflow that can be extended generally to the class of membrane proteins (including GPCRs)

References

1. Heng et al., *Biotechnol. Adv.* **2013**, 31, 8
2. Dickson et al., *J. Chem. Inf. Model.* **2021**, 61, 12, 5923−5930
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