Design of biased GPCR modulators by combining artificial intelligence and molecular simulation

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1. Context of the study

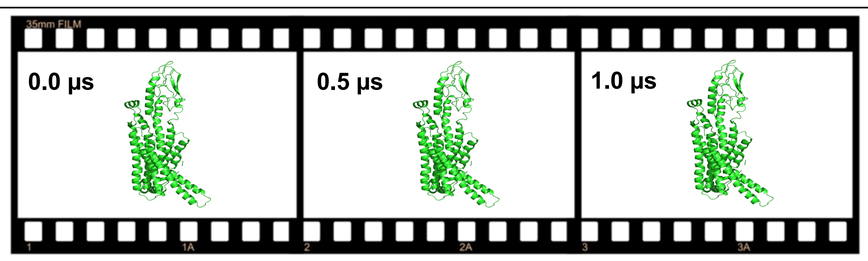
- G-protein coupled receptors (GPCR) are major drug targets involved in key physiological pathways.
- GPCRs Ligand bias: different ligands acting on the same receptor can selectively activate distinct intracellular signaling pathways.
- Parathyroid hormone receptor (PTH1R), a class B GPCR, is activated by two endogenous ligands PTH (parathyroid hormone) and PTHrP (parathyroid hormone-related peptide) **[1]**.

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                        10        20        30
PTHrP/1-34   1 AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTA
PTH/1-34     1 SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF
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Identity 32.35 %

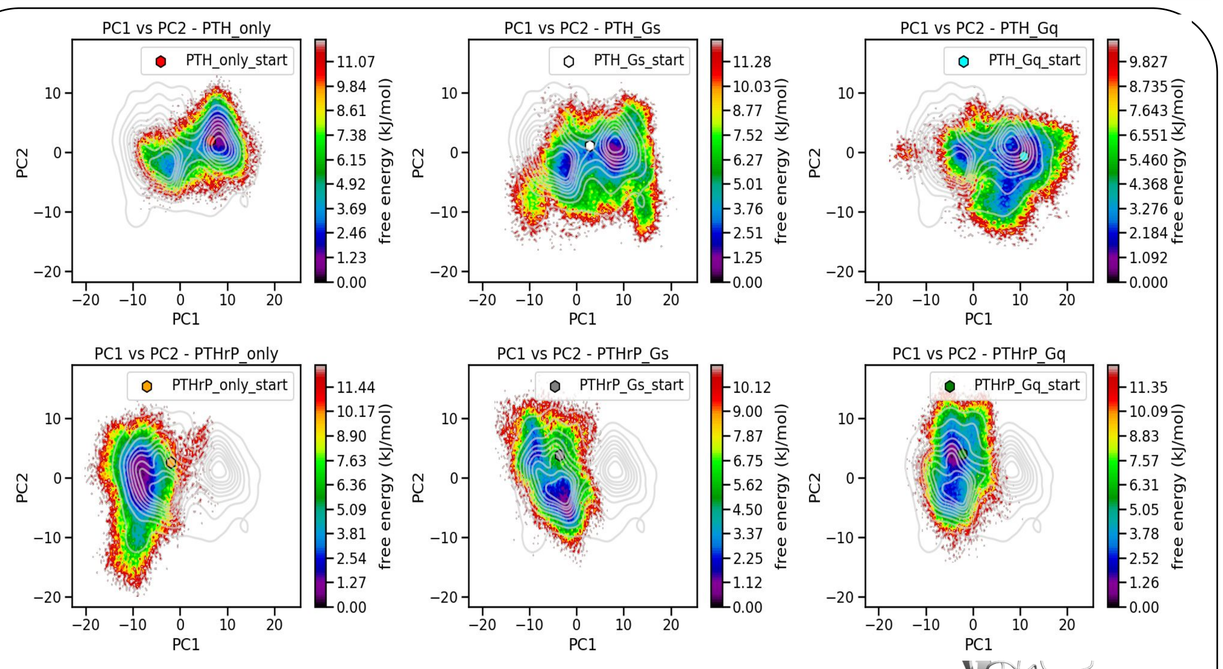
- They trigger distinct cellular responses , displaying distinct signaling profiles:
  - PTH → Gαs–cAMP–PKA
  - PTHrP → Gαq–PLCβ–PKC
- PTH1R is our study system as it showed evidences for ligand-biased signaling in the central nervous system **[2]**.
- **Objective**: To understand the molecular basis of PTH1R ligand bias using molecular dynamics simulations and identify strategies for designing biased GPCR modulators for potential therapeutic applications in metabolic disorders.

2. Molecular Dynamics: A Computational Microscope

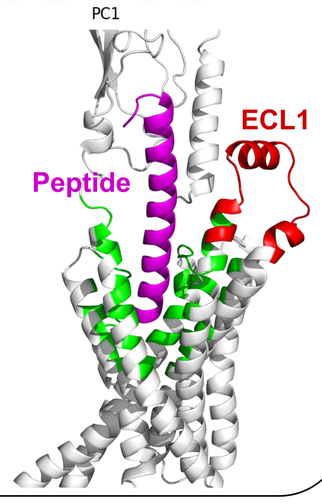


- Molecular dynamics (MD) is a computational technique that document the protein dynamics.
- The main output of MD simulation is a trajectory, which can be seen as a movie describing the evolution in time of a molecular system.
- This movie can capture every atomic movements and interactions in detail.
- These movie scenes can then be analysed using various techniques, including Principal Component Analysis (PCA), which captures the largest structural movements, or Time-lagged Independent Component Analysis (tICA), which identifies the slowest conformational transitions.
- For our simulated systems, these techniques are used to study the effect of ligands on receptor conformations, helping us understand the molecular basis of PTH1R ligand bias.

4. Peptide Binding Pocket Conformational Analysis



- PCA on binding pocket Cα residues (< 5 Å from ligand N-terminus; PC1 27%, PC2 10%) clearly separates PTH and PTHrP trajectories.
- PC1 loading analysis identifies Extracellular Loop 1 (ECL1) structural rearrangements as the primary driver of this conformational divergence.
- This dynamical divergence is strongly supported by recent literature **[4]**, showing that the long ECL1 acts as an electrostatic filter. Negatively charged ECL1 residues (E258–E260) repel acidic residues (E19, E22) in PTH while stabilizing the basic C-terminus of PTHrP, modulating peptide insertion and biasing downstream Gs *vs* Gq signaling.



3. Materials and Methods

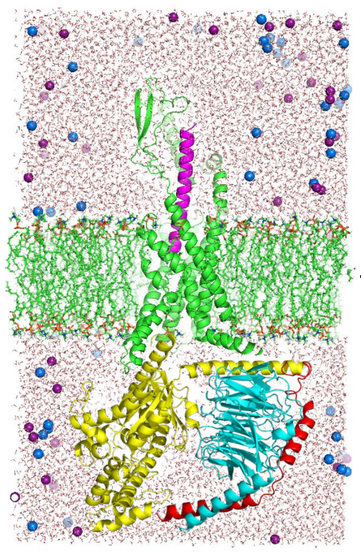
Structure Modelling (AlphaFold3)

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Membrane embedding POPC bilayer (CHARMM-GUI)

↓

All Atoms Molecular dynamics (OpenMM Program)



➔ AMBER14SB; PME, 1.0 nm cutoff; H-bond constraints; 4 fs timestep with HMR; Langevin thermostat, 300 K; semi-isotropic Monte Carlo barostat, 1bar

➔ Minimization (10,000 steps) + equilibration (2 NVT + 4 NPT) with 2.4 ns in total

↓

Conformational Analysis (PCA, tICA, Clustering, …)

↓

Peptide Design

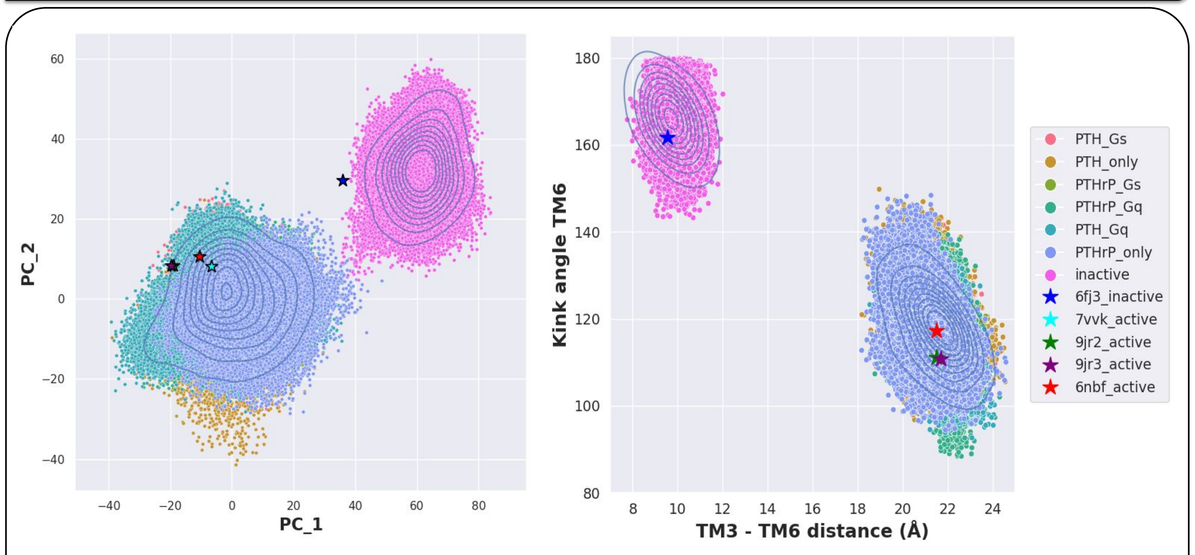
Simulated Systems

- PTH1R - **PTH** (10 rep *3 µs)
- PTH1R - **PTHrP** (10*3 µs)
- PTH1R - **PTH - Gs** (10*3 µs)
- PTH1R - **PTHrP - Gq :** (10*3 µs)
- PTH1R - **PTH - Gq** (10*2 µs)
- PTH1R - **PTHrP - Gs :** (10*2 µs)

[135,000 - 194,000 atoms]

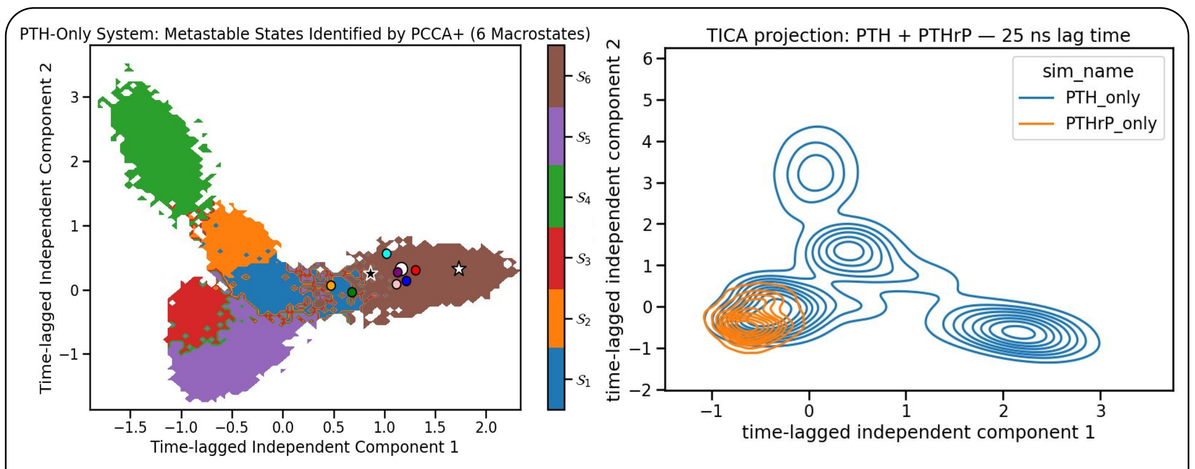
Total simulated time 160 µs

5. TMD Cα Backbone Conformational Analysis



- Key activation collective variables and PCA of the TMD Cα backbone confirm all simulated systems remain in an active conformation, separated from inactive (pink) **[3]**.
- No significant structural divergence between ligands, nor between Gs and Gq coupling.
- This suggests that signaling selectivity arises from subtler structural differences.

6. TMD tICA-Based Conformational Analysis



- Time-lagged Independent Component Analysis (tICA) based on TMD Residue-Residue Contact Scores (RRCS) was performed using a 25 ns lag time (validated via ITS plots), following the strategy of Zhou et al. **[5]**.
- PTH induces higher conformational flexibility, exploring multiple energy basins, whereas PTHrP remains confined to a single energy basin (also sampled by PTH).
- Preliminary results show that TIC1 is dominated by Arg2.60–Tyr6.53 interactions, and TIC2 by interactions between Phe5.51–Phe6.49 and His6.52–Phe6.56.
- Metastable Macrostates: PCCA + clustering performed on the PTH trajectory ensemble identified 6 metastable macrostates (validated by Chapman-Kolmogorov tests).
- Structural characterization of these macrostates is currently ongoing.

7. Conclusion

- Our findings highlight distinct pocket dynamics and conformational flexibility across ligands, resolving multiple metastable molecular states.
- These encouraging results pave the way for in-depth structural characterization to guide the rational design of biased PTH1R peptides.

References

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3. Bansal P, et al. bioRxiv. 2026.
4. McArthur ES, et al. Biochemistry. 2026.
5. Zhou Q, et al. eLife. 2019.

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