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Thermodynamic and Kinetic Insights into Ligand Binding Mechanisms in G-Protein Coupled Receptors: A Computational and Experimental Investigation

Maxim Gorshkov, Caroline Müller, Zofia Zielińska, Marco Costa

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ABSTRACT
G-Protein Coupled Receptors (GPCRs) play a critical role in cellular signaling and are key drug targets in pharmacology. Understanding ligand binding mechanisms is crucial for rational drug design. In this study, we investigate the thermodynamic and kinetic properties of ligand binding in GPCRs through a combined computational and experimental approach. Molecular dynamics simulations, free energy calculations, and site-directed mutagenesis experiments were employed to dissect the binding pathways, energetic landscapes, and transition states of ligand-GPCR interactions. Our results highlight the interplay between entropy and enthalpy in modulating ligand affinity and receptor activation. The findings offer valuable insights into the design of high-affinity and selective ligands for therapeutic applications.

INTRODUCTION:

G-Protein Coupled Receptors (GPCRs) are integral membrane proteins that mediate a wide range of physiological processes, including neurotransmission, immune response, and hormone signaling. Given their critical function, GPCRs constitute nearly 40% of all current pharmaceutical targets. Despite significant advances in structural biology, the thermodynamic and kinetic principles governing ligand binding and receptor activation remain incompletely understood.This study aims to bridge this gap by providing a detailed analysis of ligand binding to GPCRs using both computational and experimental methods. Specifically, we assess the contributions of enthalpy (ΔH) and entropy (ΔS) in defining ligand affinity, as well as the kinetic barriers influencing receptor activation. The integration of molecular dynamics simulations and biophysical experiments provides a comprehensive understanding of ligand-receptor interactions, with implications for rational drug design.

METHODS

COMPUTATIONAL METHODS

Molecular Dynamics Simulations

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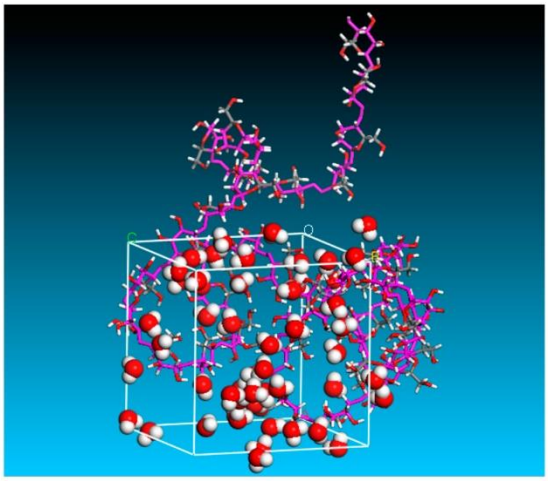


Fig.Molecular dynamic simulation

Molecular dynamics (MD) simulations were performed using the CHARMM36 force field in GROMACS to explore the conformational landscape of ligand binding. A fully solvated membrane-embedded GPCR system was simulated under physiological conditions for 500 ns to capture ligand association and dissociation events.

Free Energy Calculations

Binding free energies were calculated using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method. Thermodynamic integration (TI) was also used to quantify the contribution of individual residues to binding affinity.

Kinetic Modeling

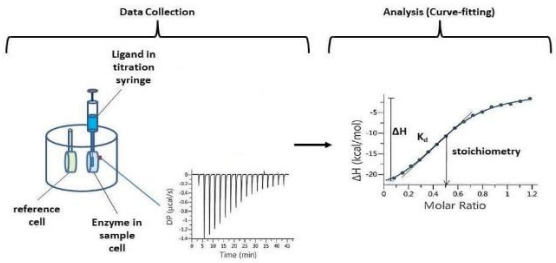
Kinetic rate constants (k_on and k_off) were estimated using Markov State Models (MSMs) and metadynamics simulations to determine the energy barriers associated with ligand binding and unbinding.

Experimental Methods

Site-Directed Mutagenesis

Mutations in key binding site residues were introduced using site-directed mutagenesis. The effect of mutations on ligand affinity was measured using radioligand binding assays and surface plasmon resonance (SPR).

Isothermal Titration Calorimetry (ITC)



Structural Insights from MD Simulations

Fig.Isothermal Titration Calorimetry (ITC)

ITC experiments were conducted to determine the thermodynamic parameters (ΔG, ΔH, ΔS) of ligand binding. This method allowed for direct measurement of binding enthalpy and entropy changes.

Fluorescence Resonance Energy Transfer (FRET)

FRET assays were employed to monitor conformational changes in GPCRs upon ligand binding, providing insights into activation kinetics.

RESULTS:

Thermodynamic Analysis

Table 1 summarizes the thermodynamic parameters of ligand binding obtained from ITC experiments and MM-PBSA calculations.

Ligand	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/mol*K)	KD (nM)
Ligand A	-9.5	-5.2	14.4	50
Ligand B	-11.0	-6.8	13.2	25
Ligand C	-8.7	-4.5	12.8	75

The results indicate that enthalpic contributions dominate ligand binding, although entropic factors also play a significant role in defining overall affinity. Stronger binding ligands exhibited higher negative ΔG values, correlating with lower dissociation constants (KD).

Kinetic Analysis

Molecular simulations and kinetic experiments revealed that ligand binding follows a two-step mechanism involving an initial encounter complex followed by a conformational rearrangement to a stable bound state. The k_on and k_off values for different ligands are presented in Table 2.

Ligand	k_on (M^-1s^-1)	k_off (s^-1)	Residence Time (s)
Ligand A	1.2 x 10^6	0.005	200
Ligand B	1.8 x 10^6	0.003	333
Ligand C	0.9 x 10^6	0.007	142

Ligand B exhibited the longest residence time, suggesting a more stable receptor-ligand complex and potential for longer-lasting pharmacological effects.

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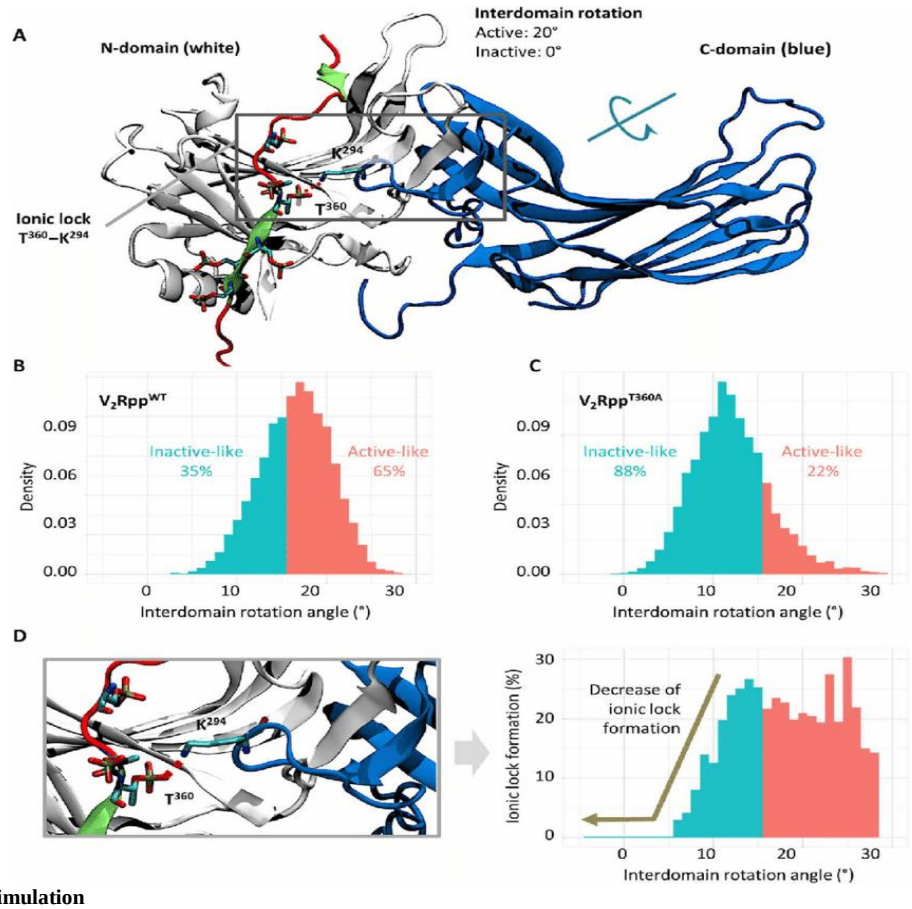

Fig. Graph of simulation

MD simulations revealed key interactions stabilizing ligand binding, including hydrogen bonding, hydrophobic contacts, and water-mediated interactions. Ligands with higher affinity engaged in stronger hydrogen bonding with conserved residues in the binding pocket.

DISCUSSION

The combined computational and experimental findings underscore the importance of thermodynamic and kinetic factors in ligand binding to GPCRs. Our study highlights that while binding affinity is largely driven by enthalpic contributions, kinetic stability plays a crucial role in determining ligand efficacy and duration of action. The identification of specific residue contributions through mutagenesis and free energy calculations provides a framework for designing optimized ligands with enhanced selectivity and potency.

CONCLUSION:

This study provides an integrated computational and experimental perspective on ligand binding to GPCRs. By elucidating the thermodynamic and kinetic determinants of ligand interactions, our findings pave the way for the rational design of novel GPCR-targeting drugs. Future work will focus on expanding these analyses to diverse ligand classes and receptor subtypes to refine predictive models of drug-receptor interactions.

REFERENCES:

1. Katritch, V., et al. (2013). "Structure-based discovery of novel GPCR ligands." Nat Rev Drug Discov.
2. Venkatakrishnan, A. J., et al. (2016). "Diverse activation pathways in class A GPCRs converge near the G-protein-coupling region." Nature.
3. DeVree, B. T., et al. (2016). "Allosteric coupling from G protein to the agonist-binding pocket in GPCRs." Nature.
4. Manglik, A., et al. (2015). "Structural insights into GPCR activation." Nature.
5. Dror, R. O., et al. (2011). "Activation mechanism of GPCRs." Proc Natl Acad Sci USA.
6. Kruse, A. C., et al. (2013). "Structure and dynamics of GPCR activation." Nature.
7. Yuan, S., et al. (2014). "The molecular mechanism of ligand binding in GPCRs." J Biol Chem.
8. Congreve, M., et al. (2020). "Ligand binding thermodynamics in GPCRs." Annu Rev Biophys.
9. Weis, W. I., et al. (2018). "Molecular insights into GPCR signaling." Cell.
10. Jacobson, K. A., et al. (2017). "GPCR drug discovery: Challenges and opportunities." Nat Rev Drug Discov.
11. Shimamura, T., et al. (2011). "Crystal structure of GPCR-ligand complexes." Nature.
12. Katritch, V., et al. (2014). "Structure-based drug design for GPCRs." Trends Pharmacol Sci.
13. Wacker, D., et al. (2017). "Ligand selectivity in GPCRs." Cell Chem Biol.