

Mechanistic Analysis of Odorant Antagonism in *Drosophila* Or56a via Computational Docking and Structural Evaluation

Objective

This study aims to computationally elucidate the molecular mechanisms underlying odorant antagonism in the *Drosophila melanogaster* olfactory receptor Or56a. Specifically, I investigated the binding interactions of the known agonist geosmin and the antagonist linalool, integrating rigorous structural predictions, detailed docking analyses, and quantitative pocket evaluations to propose a robust mechanistic hypothesis.

Approach and Thought Process

My goal was not just to identify the best binding pose, but to understand the range of binding possibilities, how they relate to receptor activation or inhibition, and how these insights could inform a scalable, real-world discovery pipeline.

I approached the task by replicating a structure-based virtual screening workflow that could plausibly fit into Monarch's early-stage pipeline: combining structure prediction, ligand preparation, docking, and structural analysis.

I was particularly interested in whether linalool and geosmin bind the same pocket, and if not, whether their relative energies and spatial poses could support an allosteric or dual antagonism model. I also wanted to see how pose diversity could inform more robust computational strategies.

Additionally, I wanted to assess the plausibility of the Or56a AlphaFold-predicted pocket by comparing it to experimentally resolved insect olfactory receptors, namely 8V02 and 6C70. By aligning Or56a to these known structures, I hoped to evaluate the structural conservation of potential binding sites and further validate the biological interpretation of my docking results.

Methodological Framework and Rationale

The study emphasized structural diversity, receptor-ligand dynamics, and detailed binding-site characterization, aiming to:

Validate the AlphaFold2-predicted Or56a structure through alignment with experimentally resolved olfactory receptors (8V02 and 6C70).

Identify potential binding pockets using quantitative fpocket analyses.

Analyze ligand-receptor interactions using AutoDock Vina docking with enhanced sampling.

Address scalability for practical, high-throughput screening pipelines.

Comprehensive Workflow

1. Receptor Structure Preparation

Predicted Or56a structure from AlphaFold2 refined for docking analysis.

Validation by structural alignment with known experimental insect olfactory receptor structures (8V02 and 6C70).

2. Ligand Preparation

Optimized 3D structures of geosmin and linalool prepared using RDKit and UFF.

Structures converted to PDBQT format, preserving molecular geometry and applying Gasteiger charges via Open Babel.

3. Binding Pocket Identification (fpocket)

fpocket identified and ranked potential binding pockets:

Pocket 3 was ranked significantly lower in terms of druggability and hydrophobic character and was thus excluded from detailed docking analysis. Pocket 2 is more deep and non-polar. Ideal to bind small hydrophobic molecule like Geosmin, compared to more flexible Linalool.

4. Molecular Docking Analysis (AutoDock Vina)

Docking affinities analyzed for the two relevant pockets were:

5. Structural Validation

Structural alignments highlighted moderate alignment between Or56a and 8V02 (RMSD: 5.32 Å), while alignment with 6C70 showed considerable divergence (RMSD: 11.43 Å), supporting the predicted pocket location.

Downloaded experimental OR structures 8V02 (2.9 Å) (Magenta) and 6C70 (3.5 Å) (Yellow)

Aligned Or56a(Green) to both using PyMOL

Measured RMSD values:

Or56a - 8V02: 5.32

Or56a - 6C70: 11.43

8V02 - 6C70: 0.79

Overlayed all three structures to visualize fold similarity and pocket conservation

Results and Mechanistic Interpretation

Binding Pocket Analysis

Pocket 2 (ranked 2nd by fpocket): Exhibited highest hydrophobicity and lowest polar SASA, correlating with geosmin's strong binding affinity, thus likely representing the orthosteric activation site.

Pocket 1 (ranked 1st by fpocket): Moderate hydrophobicity and depth made it favorable for binding antagonistic ligands like linalool, suggesting potential for noncompetitive antagonism through conformational

modulation.

Docking Interpretation

Geosmin's significant preference for Pocket 2 reinforces this site as the orthosteric activation locus.

Linalool's higher relative affinity for Pocket 1 supports its role as a noncompetitive antagonist, potentially stabilizing inactive receptor conformations.

Structural Validation Using 8V02 and 6C70

Despite only moderate structural similarity between Or56a and 8V02 (RMSD = 5.32 Å), the close alignment between 8V02 and 6C70 (RMSD = 0.788 Å) indicates that insect olfactory receptors—at least their transmembrane domains—follow a conserved structural framework. This is particularly noteworthy considering that both 8V02 and 6C70 come from different insect species (*Apocrypta bakeri*), whereas Or56a is from *Drosophila melanogaster*. Given these species-level differences, some structural divergence is expected. The higher RMSD between Or56a and 6C70 (11.43 Å) likely reflects a combination of genetic distance and receptor subtype differences.

Among the two experimental structures, 8V02 stands out not just for its structural proximity to Or56a but also for its higher resolution (2.9 Å vs 3.5 Å for 6C70), making it a more suitable benchmark for binding site assessment. By overlaying Or56a with 8V02 and visualizing the predicted pocket location, we observed that the docking poses for geosmin and linalool fall within a transmembrane region that is spatially consistent with known odorant-binding cavities. This overlap reinforces the plausibility of the docking predictions and supports the idea that even in the absence of an experimentally solved Or56a structure, AlphaFold-based models can provide structurally reasonable starting points—especially when interpreted in the context of conserved fold families.

These structural comparisons allowed me to place my docking results into a broader biological framework. While some caution is warranted due to species and model limitations, the high conservation between 8V02 and 6C70 provides strong indirect support for using this framework to interpret the behavior of Or56a. It also highlights how reference-based validation can be a practical tool for increasing confidence in predicted pocket locations and guiding mechanistic hypotheses when experimental structures are unavailable.

Biological Realism and Future Directions

In real biological systems, explicit solvent molecules, especially water, influence receptor-ligand interactions significantly. Future computational modeling should include molecular dynamics (MD) simulations and quantum chemical refinements to incorporate these critical effects.

Scalability and Integration into High-Throughput Screening Pipelines

This project underscored how valuable it is to look beyond single docking scores and instead capture the full

landscape of ligand poses and binding site environments. In a realistic drug or odorant discovery pipeline, pose diversity and binding topology are often more informative than just choosing the "top-scoring" pose. The fact that geosmin and linalool exhibit multiple plausible binding modes - including ones that likely reflect orthosteric and allosteric interactions - suggests that a scalable pipeline must be designed to preserve and interpret this complexity.

In a real-world setting, I would envision a tiered workflow that starts with high-throughput docking and pose clustering to prioritize ligands not just by affinity, but by the diversity and consistency of their binding modes. These top candidates could then be analyzed for key residue contacts and binding-site overlap, followed by molecular dynamics (MD) simulations to understand conformational flexibility, receptor gating effects, and pose stability.

Finally, to bridge accuracy with scale, machine learning models could be trained not just on docking scores but on a combination of pose clustering outcomes, residue-level interaction fingerprints, and energetics from QM or MD-refined subsets. Such models could learn to recognize antagonistic behaviors based on structural and energetic patterns rather than simplistic thresholds.

From my perspective as a quantum chemist, I believe that even as we aim for scale, it is essential not to lose sight of accuracy and physical interpretability. My own research has focused on combining quantum chemical methods, fragmentation strategies, and machine learning to predict interaction energies in complex systems like water-ion clusters and property prediction in biomolecular systems. These same techniques could be extended to odorant-receptor modeling. While QM is computationally expensive, selective application on key complexes and the integration of delta-learning or hybrid ML/QM workflows could provide more reliable predictions. In the long run, I believe that scalable pipelines will need to reflect a thoughtful balance of physics-based accuracy, chemical intuition, and data-driven generalization - and this project gave me an opportunity to prototype exactly that kind of framework.

Conclusion

This comprehensive computational analysis robustly delineates the plausible mechanism of odorant antagonism in Or56a, highlighting distinct roles for geosmin and linalool across different pockets. Pocket evaluations confirm the orthosteric function of Pocket 2 and the potential allosteric modulation by Pocket 1, validating the predictive power of computational modeling and structural alignment techniques.

My computational docking and pocket analysis strongly suggests that linalool likely inhibits OSN activation through allosteric antagonism. Specifically, linalool preferentially binds to a distinct pocket (Pocket 1), separate from the primary orthosteric site occupied strongly by geosmin (Pocket 2). This binding could stabilize receptor conformations incompatible with channel opening or agonist recognition, thereby preventing neuron activation. Additionally, the possibility of linalool binding weakly at the orthosteric site suggests a

potential dual mechanism, combining competitive and allosteric antagonism to robustly inhibit Or56a activation.

Structural alignment with experimental OR structures (8V02 and 6C70) provide further confidence in the biological plausibility of the predicted binding site. The high similarity between 8V02 and 6C70, despite being from different species, emphasizes the robustness of using conserved architecture to guide computational predictions in under-characterized receptors like Or56a.

As a quantum chemist, I approach this protocol with a healthy level of skepticism - particularly around the use of AlphaFold structures and rigid docking as definitive predictors of receptor-ligand behavior. While AlphaFold provides an invaluable starting point, it is not explicitly trained on membrane-bound olfactory receptors or allosteric pocket geometries, and its predictions do not account for conformational flexibility or ligand-induced shifts. Similarly, docking relies on static snapshots of dynamic systems and often oversimplifies the energetic landscape. In a more rigorous setting, I would consider refining the structural model using molecular dynamics to explore relevant conformational ensembles, followed by higher-level quantum mechanical calculations - such as single-point energy evaluations using GFN2-xTB or fragmentation-based DFT - to better capture interaction energetics and polarization effects. While it's computationally impractical to apply QM across large libraries or full receptor systems, a hybrid, top-down approach that combines structure prediction, dynamics sampling, and quantum refinement on key poses could yield far more reliable and interpretable results.

In parallel, I see machine learning as a natural bridge between the scale of docking and the accuracy of quantum chemistry. Once a representative set of ligands-receptor complexes is characterized through MD and QM refinement, that data can be used to train ML models - whether through Delta-learning, interaction fingerprinting, or graph-based architectures - to generalize quantum accuracy across a broader chemical space. This enables a practical middle ground: QM-derived accuracy at ML speed. Ultimately, I see AlphaFold and docking not as end points, but as entry points to a layered framework where ML models serve as the glue between physical realism and real-world throughput.