

Evaluating the Predictive Contribution of Receptor-Specific Docking Interactions to Aminergic GPCR Selectivity

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Research Article

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Abstract

Molecular docking is often combined with ligand-based models to provide receptor-specific interaction information. In this study, we investigated whether such interactions improve the prediction of binding selectivity among aminergic G protein-coupled receptor (GPCR) anti-targets using a stepwise approach. Native redocking successfully reproduced the crystallographic binding poses of the 5-HT_{2B}, histamine H₁, and muscarinic M₂ receptors, but not those of the dopamine D₂ and α 1A-adrenergic receptors. In virtual screening against property-matched decoys, H₁ and M₂ showed moderate discrimination between active and decoy compounds, with ROC-AUC values of 0.717 and 0.704, respectively. In contrast, 5-HT_{2B} performed near chance (ROC-AUC = 0.525), despite highly accurate pose reconstruction (RMSD = 0.14 Å). Structural comparison revealed only four identical ligand-contacting residues across the three receptors: Asp3x32, Ser3x36, Trp6x48, and Tyr7x42. We then examined 35 compounds with experimentally measured binding affinities at H₁ and M₂, comprising 19 promiscuous and 16 H₁-selective compounds. The conserved Asp3x32 salt bridge was observed in 15/16 H₁-selective and 12/19 promiscuous compounds docked to H₁, compared with 3/16 and 5/19, respectively, at M₂. Despite these differences in receptor–ligand interactions, incorporating docking-derived features into Morgan fingerprint-based classifiers did not consistently improve predictive performance under scaffold-grouped cross-validation. Compared with the ligand-only baseline (AUROC = 0.661), adding anchor interactions yielded a modest improvement (Δ AUROC = +0.020; 95% bootstrap interval: –0.029 to 0.088), whereas shell and extended-pocket contacts reduced performance (Δ AUROC = –0.062; 95% bootstrap interval: –0.154 to –0.010). The complete interaction fingerprint also provided no clear improvement (Δ AUROC = –0.029; 95% bootstrap interval: –0.118 to 0.036). Overall, these findings highlight that accurate pose reconstruction and structurally interpretable receptor–ligand interactions do not necessarily translate into improved ligand-based predictions of binding selectivity. Validation using larger datasets and independent receptor systems will be needed to determine whether docking-derived interactions provide predictive benefits beyond ligand-based descriptors.

Introduction

G protein-coupled receptors (GPCRs) represent a major class of pharmacological targets and regulate numerous physiological processes (Lorente et al. 2025). Aminergic GPCRs, in particular, mediate signaling by neurotransmitters and biogenic amines such as serotonin, dopamine, histamine, and acetylcholine (Michino et al. 2015). Despite their therapeutic significance, structural and pharmacological similarities among these receptors can result in ligand cross-reactivity and unintended secondary pharmacological effects (Michino et al. 2015; Allen and Roth 2011). Elucidating the molecular determinants of receptor selectivity and promiscuity is therefore critical for drug discovery and early safety assessment (Allen and Roth 2011).

Aminergic GPCRs share a conserved seven-transmembrane architecture and several common features within their orthosteric binding pockets (Michino et al. 2015). A prominent example is the conserved Asp3x32 residue, which frequently forms an ionic interaction with the protonated amine of aminergic ligands (Kooistra et al. 2013). However, this interaction alone does not determine ligand recognition. Differences in surrounding aromatic, polar, and hydrophobic residues, as well as extracellular binding-pocket regions, can influence ligand accommodation and receptor selectivity (Egyed et al. 2021). Consequently, compounds with similar chemical structures may exhibit substantially different binding profiles across related receptors (Michino et al. 2015; Egyed et al. 2021).

Computational approaches increasingly investigate receptor cross-reactivity and predict secondary pharmacological interactions (Keiser et al. 2009, 2007). Ligand-based methods use molecular descriptors and structural fingerprints to link chemical structure to biological activity (Keiser et al. 2007). Although effective at capturing similarities among known compounds, these representations do not explicitly account for the receptor-specific interactions that underlie molecular recognition. Structure-based approaches, particularly molecular docking, complement these representations

by predicting ligand orientations and identifying interactions in receptor-binding pockets (Cookson et al. 1984). Docking-derived interaction fingerprints can encode ionic, hydrogen-bonding, aromatic, and hydrophobic contacts and may provide information beyond ligand molecular fingerprints (D. D. Wang et al. 2021; Li et al. 2024).

Nevertheless, the predictive utility of docking-derived interactions remains uncertain. Accurately reproducing an experimental binding pose does not necessarily ensure reliable discrimination between active compounds and decoys (Su et al. 2019; Mysinger et al. 2012). Likewise, structurally interpretable ligand–receptor contacts may not enhance predictions for compounds with novel molecular scaffolds. Docking performance should therefore be evaluated at multiple levels, including pose recovery, virtual-screening enrichment, interaction characterization, and predictive generalization (Su et al. 2019; Mysinger et al. 2012; Xu and Zou 2026). Additionally, receptor-specific interaction features may already be represented by ligand descriptors, potentially increasing model complexity without improving predictive performance (Wójcikowski et al. 2017).

This study evaluated whether receptor-specific docking interactions provide predictive information beyond ligand molecular fingerprints for distinguishing promiscuous from selective binding among aminergic GPCRs. Five receptors, namely 5-HT2B, H1, D2, α 1A, and M2, were initially examined through native-ligand redocking, followed by active–decoy enrichment and structural comparison of validated binding pockets. Subsequent analyses focused on experimentally characterized compounds exhibiting promiscuous or selective binding within the H1–M2 receptor pair. Docking-derived interaction fingerprints were assessed alongside ligand-based molecular fingerprints using scaffold-grouped cross-validation. By systematically separating structural validation, interaction interpretation, and predictive assessment, the study investigated whether molecular docking provides measurable complementary information for predicting aminergic GPCR binding selectivity.

Materials and Methods

Pharmacological data collection and classification

Experimental human receptor binding affinities (K_i) were obtained from the NIMH Psychoactive Drug Screening Program (PDSP) (Roth et al. 2000) (RRID:SCR_003281) and ChEMBL (Zdrazil et al. 2024) databases. Only binding affinity (K_i) measurements were included, while functional EC_{50} and IC_{50} values were excluded. When measurements were available from both databases, PDSP data were given priority. For compounds with multiple uncensored K_i measurements in the selected database, the median value was used. Censored measurements were retained with their original inequality constraints and were not replaced with measurements from the other database. Compounds were classified as potent binders ($K_i \leq 100$ nM) or weak binders ($K_i \geq 1$ μ M). A higher weak-binding threshold of 10 μ M was also evaluated in a sensitivity analysis.

The initial panel included five aminergic GPCRs: 5-HT2B, H1, D2, α 1A, and M2. Following receptor validation, the analysis was progressively restricted to receptors that met the predefined validation criteria. In the three-receptor panel (5-HT2B, H1, and M2), compounds were classified as promiscuous if they showed potent binding at all three receptors, or as selective if they were potent at one receptor and weak at the other two. The final predictive analysis focused on H1 and M2, with promiscuous compounds defined as potent binders at both receptors and selective compounds as potent at one receptor but weak at the other. Compounds without binding affinity measurements for both receptors, or those that did not meet either classification criterion, were excluded from the classification analysis..

Receptor preparation and molecular docking

Crystal structures of 5-HT2B (PDB: 4IB4) (Wacker et al. 2013), H1 (3RZE) (Shimamura et al. 2011), D2 (6CM4) (Sheng Wang et al. 2018), α 1A (7YMJ) (Toyoda et al. 2023), and M2 (3UON) (Haga et al. 2012) were retrieved from the Protein

Data Bank (Berman et al. 2000). Receptors were prepared using PDBFixer at pH 7.4 without reconstructing extensive missing loop (PDBFixer, n.d.). Crystal ligands were reconstructed from experimental coordinates and chemical component information. Aliphatic amines meeting the predefined protonation rule were protonated, whereas aromatic nitrogens, amides, and anilines were maintained in neutral forms (Westbrook et al. 2015).

Ligand conformers were generated using the ETKDv3 algorithm implemented in RDKit (Shuzhe Wang et al. 2020), subsequently energy-minimized using the MMFF94 force field (Halgren 1996). PDBQT files were prepared using Meeko (Meeko: Interface for AutoDock, Molecule Parameterization, n.d.). Molecular docking was performed with AutoDock Vina 1.2.5 using an exhaustiveness of 16, a maximum of nine output poses, an energy range of 3 kcal/mol, and a fixed random seed of 42 (Eberhardt et al. 2021). Docking boxes were centered on the crystallographic ligands and extended 6 Å beyond their coordinates along each Cartesian axis, with a minimum box dimension of 18 Å.

Docking protocols were validated by native-ligand redocking. Successful validation required the top-ranked pose to reproduce the crystallographic conformation with a symmetry-corrected heavy-atom RMSD ≤ 2 Å and to retain the Asp3.32 interaction. For $\alpha 1A$, rank 1 failed this cutoff while a crystal-like pose was present among lower-ranked modes. So, docking was therefore repeated once at exhaustiveness 32, as specified in the predefined protocol, to test whether increased search improved rank-1 placement. The rank-1 pose was unchanged. Receptors that failed validation were excluded from subsequent analyses without relaxing the established thresholds.

Virtual-screening enrichment

Virtual-screening enrichment was assessed for 5-HT_{2B}, H1, and M2 using DUD-E datasets containing 50 cationic active compounds (formal charge $\geq +1$) and 50 property-matched decoys per active (Mysinger et al. 2012). Decoys were selected using physicochemical matching criteria adapted from DUDE-Z (Stein et al. 2021). These required identical formal charges and maximum differences of 125 Da in molecular weight, 3.6 in logP, five rotatable bonds, four hydrogen-bond acceptors, and three hydrogen-bond donors relative to the corresponding active compound. A maximum Morgan fingerprint Tanimoto similarity of 0.35 between each decoy and its corresponding active was also imposed. Crystallographic ligands and decoys with known potent binding affinity ($K_i \leq 100$ nM) were excluded.

Actives and decoys underwent identical conformer generation, force-field optimization, and docking procedures. When MMFF94 optimization failed to converge, the Universal Force Field (UFF) was used as an alternative (Rappe et al. 1992). Compounds that failed ligand preparation were excluded without replacement, and failure of an active compound also resulted in the removal of its associated decoys. Compounds were ranked according to their best Vina docking scores, with ligand identifiers used to resolve ties. Virtual-screening performance was evaluated using ROC-AUC, logAUC, enrichment factors at 1% and 5% (EF1% and EF5%), and Boltzmann-enhanced discrimination of ROC (BEDROC; $\alpha = 20$) (Truchon and Bayly 2007). Confidence intervals (95%) were estimated using 1,000 active-level bootstrap resamples. To avoid overlap between virtual-screening validation and predictive modeling, enrichment actives were excluded from subsequent model development.

Structural comparison of receptor-binding pockets

Binding-pocket residues were identified from experimentally determined receptor–ligand complexes using a heavy-atom distance cutoff of 5 Å and mapped to the GPCRdb generic numbering scheme to enable comparisons across receptors (Isberg et al. 2015). Contacts were grouped into four predefined structural regions: the conserved Asp3x32 anchor, the surrounding TM5–TM7 shell, the extracellular and vestibular extended regions, and adjacent TM1–TM4 contacts. To examine whether the extensive extracellular contacts observed in the 5-HT_{2B}–ergotamine complex were influenced by ligand size and structure, we additionally analyzed the 5-HT_{2B} receptor bound to the smaller, non-ergoline ligand LY266097 (McCorvy et al. 2018), applying the same 5 Å contact criterion.

Docking of pharmacologically characterized compounds and interaction fingerprints

Compounds classified as promiscuous or selective at H1 and M2 were docked against both receptors using the validated docking protocols. Compounds included in the enrichment benchmarks, crystallographic ligands, and those with intermediate binding affinities were excluded. Protein–ligand interaction fingerprints were generated from the top-ranked docking poses. Salt bridges were identified using a distance cutoff of ≤ 4.0 Å between a positively charged ligand amine and a carboxylate oxygen of Asp3x32. Hydrogen bonds were defined by a donor–acceptor heavy-atom distance of ≤ 3.5 Å and a hydrogen–acceptor distance of ≤ 2.5 Å. Aromatic and hydrophobic interactions were identified using distance cutoffs of 4.5 Å and 4.0 Å, respectively, between the corresponding ligand and receptor atoms. Asp3x32 salt bridges were not counted separately as hydrogen bonds. The primary analysis compared promiscuous and H1-selective compounds, while M2-selective compounds were examined descriptively.

Predictive modeling and feature ablation

Predictive modeling was performed on 35 compounds, comprising 19 promiscuous and 16 H1-selective ligands. Four feature representations were compared using L2-regularized logistic regression ($C = 1$, balanced class weights, and an unpenalized intercept), without hyperparameter optimization. The ligand-only baseline consisted of 2,048-bit Morgan fingerprints (radius 2) (Rogers and Hahn 2010). Three additional models combined these fingerprints with (i) Asp3x32 salt-bridge indicators for H1 and M2, (ii) hydrogen-bonding, aromatic, and hydrophobic contacts within the shell and extended binding-pocket regions, or (iii) a complete interaction fingerprint covering anchor, adjacent, shell, and extended contacts. Receptor-specific interaction features were retained in separate H1 and M2 blocks. Docking scores were excluded so that the models tested contact patterns rather than Vina energy.

Model performance was evaluated using Bemis–Murcko scaffold-grouped cross-validation (Yang et al. 2019; Bemis and Murcko 1996). The largest feasible number of folds among five, four, and three was selected, ensuring that both classes were represented in every test fold. Identical cross-validation partitions were used across all models. Predictive performance was assessed using pooled out-of-fold ROC-AUC and average precision, while the contribution of structural features was quantified as the change in AUROC (Δ AUROC) relative to the ligand-only baseline. Uncertainty was estimated using 1,000 paired scaffold-level bootstrap resamples of the out-of-fold predictions, with a fixed random seed of 42. Models were not refitted during bootstrapping. Accordingly, the analysis represents internal validation with scaffold-held-out predictions rather than independent external validation. Progression to prospective virtual screening was contingent on demonstrating an improvement over the ligand-only baseline according to the predefined evaluation criteria.

Results

Validation of receptor docking protocols and virtual-screening performance

We initially validated molecular docking protocols using native-ligand redocking across five aminergic GPCRs (**Figure 1 and Table 1**). Successful recovery of the crystallographic ligand conformation was observed for three receptors: 5-HT2B, H1, and M2. The top-ranked docking poses exhibited symmetry-corrected heavy-atom RMSD values of 0.14 Å for ergotamine bound to 5-HT2B, 0.42 Å for E-doxepin bound to H1, and 0.58 Å for QNB bound to M2. All three poses reproduced the characteristic interaction with the conserved Asp3x32 residue (**Figure 1B**).

In contrast, the docking protocols did not satisfy the predefined validation criteria for D2 and α 1A. Although we generated a near-native risperidone pose for D2 (RMSD = 0.34 Å), it ranked second, with a predicted affinity 0.19 kcal/mol less favorable than the top-ranked pose (RMSD = 2.33 Å). Similarly, docking of tamsulosin to α 1A generated a near-native conformation (RMSD = 1.51 Å), but the top-ranked pose deviated substantially from the experimental

structure (RMSD = 8.84 Å). Increasing docking exhaustiveness from 16 to 32 did not alter the top-ranked α1A pose. Consequently, D2 and α1A were excluded from subsequent analyses. We evaluated virtual-screening performance for the three receptors that passed native-redocking validation by property-matched active and decoy datasets. We evaluated each receptor using 50 active compounds and approximately 2,500 decoys; we excluded one H1 decoy because conformer generation failed. Despite successful native-pose recovery, receptors showed substantial differences in enrichment performance.

Table 1. Receptor structures, redocking and enrichment outcomes, and final role in the study.

Receptor	PDB	Native ligand	Rank-1 RMSD (Å)	Crystal-like pose	Asp3.32 at rank 1	ROC-AUC (95% CI)	Early enrichment	Final role
5-HT2B	4IB4	ergotamine	0.14	rank 1	yes	0.525 (0.443–0.604)	EF 1% = 0	structural comparison only
H1	3RZE	E-doxepin	0.42	rank 1	yes	0.717 (0.630–0.795)	EF 1% = 9.8 (3.9–17.7)	primary predictive receptor
M2	3UON	QNB	0.58	rank 1	yes	0.704 (0.635–0.775)	EF 1% = 5.9 (0–11.8); EF 5% = 1.2	moderate-confidence predictive receptor
D2	6CM4	risperidone	2.33	mode 2 at 0.34 Å	rank 1 failed the 2 Å cutoff	not tested	not tested	excluded
α1A	7YMJ	tamsulosin	8.84	mode 3 at 1.51 Å; unchanged after exhaustiveness 32	rank 1 failed the 2 Å cutoff	not tested	not tested	excluded

The 5-HT2B receptor showed limited discrimination between active compounds and decoys, with a ROC-AUC of 0.525 (95% CI: 0.443–0.604). No enrichment was observed within the top 1% of ranked compounds (EF1% = 0), and the BEDROC value (0.052) was below the random-ranking reference of 0.060. In contrast, H1 exhibited stronger screening performance, with a ROC-AUC of 0.717 (95% CI: 0.630–0.795), an EF1% of 9.8 (95% CI: 3.9–17.7), and a BEDROC value of 0.266. M2 also demonstrated global discrimination between active and decoy compounds (ROC-AUC = 0.704; 95% CI: 0.635–0.775), although early enrichment was comparatively weaker (EF1% = 5.9, with a confidence interval including zero; EF5% = 1.2) (**Figure 1C**). Based on these results, we selected H1 as the primary receptor for predictive analysis, while retaining M2 with lower confidence in its early-enrichment performance. Although 5-HT2B satisfied the native-pose recovery criterion, its poor enrichment performance restricted its subsequent use to structural comparison. These results show that accurately reproducing crystallographic binding poses does not necessarily translate into reliable virtual-screening performance.

Conserved and receptor-specific features of aminergic GPCR binding pockets

Structural comparison of the validated 5-HT2B, H1, and M2 receptor complexes revealed both conserved and receptor-specific features within their ligand-binding pockets (**Figure 2**). Despite their common aminergic recognition mechanism, only four ligand-contacting positions contained identical amino acids across all three receptors: Asp3x32, Ser3x36, Trp6x48, and Tyr7x42 (**Figure 2A-B**).

Greater structural diversity was observed among residues surrounding this conserved core, particularly at positions 3x33, 5x43, 5x44, 5x461, 6x51, 6x52, 6x55, and 7x38. H1 and M2 shared ligand contacts involving Tyr3x33 and Trp4x57, whereas the corresponding residues in 5-HT2B were Val3x33 and Ala4x57, respectively. Additional receptor-specific differences included a contact with Phe6x44 in H1 and Cys7x41 in M2, which were not observed in the other two crystallographic complexes. The extracellular regions also displayed definite interaction patterns. The 5-HT2B–ergotamine complex exhibited 16 contacts within the extended binding-pocket region, compared with only two contacts each for the H1–E-doxepin and M2–QNB complexes (**Figure 2C**). To examine whether the extensive extracellular interactions observed for 5-HT2B were primarily associated with the relatively large ergotamine ligand, an additional crystal structure containing the smaller non-ergoline antagonist LY266097 (PDB ID: 6DS0) was analyzed (**Figure 2D**).

The LY266097-bound structure retained an extensive extracellular interaction pattern, with 11 contacts involving the extended binding-pocket region, including residues from extracellular loop 2 (ECL2) and the TM7 vestibule. The amino acid identities of the 5-HT2B binding-pocket shell remained unchanged between the two structures. Thus, the extensive extracellular contact footprint observed for 5-HT2B was not exclusive to the ergotamine-bound complex. Although this analysis provided further structural context for the receptor, it did not resolve its poor virtual-screening enrichment, and 5-HT2B was omitted from subsequent predictive modeling.

Docking-derived interaction patterns of promiscuous and selective compounds

The pharmacological dataset included 496 compounds with binding-affinity measurements for both H1 and M2 receptors. Based on the predefined affinity thresholds, we classified 26 compounds as promiscuous, 28 as selective, and 442 as intermediate. Among the selective compounds, 26 were H1-selective, and two were M2-selective. After excluding compounds lacking suitable structures, enrichment actives, and crystallographic ligands, 37 compounds were retained for molecular docking, comprising 19 promiscuous, 16 H1-selective, and two M2-selective ligands. All 37 compounds were successfully docked against both receptors. Docking scores differed by pharmacological class and receptor. At M2, promiscuous compounds exhibited more favorable predicted binding affinities than H1-selective compounds, with median Vina scores of -9.48 and -8.47 kcal/mol, respectively. Conversely, at H1, H1-selective compounds showed more favorable median scores than promiscuous compounds (-8.69 versus -7.80 kcal/mol). We retained two compounds with unusually unfavorable H1 docking scores after inspecting their ligand preparation and predicted binding geometries. Further examination of these outliers revealed that the relationship between docking affinity and canonical aminergic interactions was not always consistent. The top-ranked H1 pose of cyproheptadine occupied the orthosteric binding pocket but positioned its amine nitrogen 7.56 Å from Asp107. An alternative saved pose oriented toward the conserved aspartate placed the N-methyl group 1.45 Å from OD1 but received an unfavorable docking score of $+2.40$ kcal/mol. In contrast, cyproheptadine exhibited a favorable docking score at M2 (-9.78 kcal/mol), despite an amine-to-Asp103 distance of 10.2 Å. The second H1 outlier maintained an interaction with Asp107 at 3.16 Å but received a docking score of only -1.63 kcal/mol. These observations show that favorable docking scores and recovery of the canonical aminergic anchoring interaction were not consistently associated in the predicted poses.

The frequency of the conserved Asp3x32 salt bridge differed between the two compound classes and receptors (**Figure 3A; Table 2**). Among H1-selective compounds, the salt bridge was observed in 15/16 H1 poses (93.8%), compared with only 3/16 M2 poses (18.8%). For promiscuous compounds, the corresponding frequencies were 12/19 (63.2%) at H1 and 5/19 (26.3%) at M2. These results indicate that H1 selectivity was associated with retention of the Asp3x32 interaction at H1 and its frequent absence at M2. However, experimentally observed promiscuity was not consistently accompanied by formation of the canonical Asp3x32 interaction at both receptors.

Differences between the compound classes were also observed in shell and extended-pocket interactions (**Figure 3B**). H1-selective compounds more frequently contacted H1 His7x34 (6/16 versus 1/19) and Trp3x28 (4/16 versus 0/19). Conversely, promiscuous compounds more frequently contacted M2 Phe5x47 (13/19 versus 6/16) and Ala5x461 (19/19 versus 12/16). Notably, H1 Phe6x44, a receptor-specific contact identified in the crystal structure, was more frequently contacted by promiscuous compounds (15/19) than by H1-selective compounds (9/16). This observation suggests that the presence of a receptor-specific contact alone is insufficient to distinguish selective from promiscuous binding.

To further examine the relationship between docking scores and conserved anchoring interactions, three compounds were selected for detailed structural analysis: risperidone (H1-selective), clozapine (promiscuous), and cyproheptadine (promiscuous outlier) (**Figure 3C**). Both risperidone and clozapine retained the interaction with H1 Asp107 but lacked the corresponding interaction with M2 Asp103. Cyproheptadine and one H1-selective compound (CHEMBL3217984) exhibited unusually unfavorable H1 docking scores. Both cases were examined for potential ligand-preparation artifacts and retained without redocking. The top-ranked H1 pose of cyproheptadine occupied the orthosteric pocket, but its amine was located 7.56 Å from Asp107. An alternative saved pose oriented toward Asp107 placed the N-methyl group 1.45 Å from OD1 but received an unfavorable docking score of +2.40 kcal/mol. At M2, cyproheptadine scored -9.78 kcal/mol despite an amine-Asp103 distance of 10.2 Å. The second H1 outlier retained a contact with Asp107 (3.16 Å) but received a docking score of only -1.63 kcal/mol. Together, these observations indicate that favorable docking scores and recovery of the conserved Asp3x32 interaction were not consistently correlated, highlighting the limitations of interpreting docking scores as direct measures of receptor-specific ligand recognition.

Together, these data indicate that docking-derived interaction fingerprints capture differences in ligand-contact patterns between pharmacological classes. However, no individual receptor-specific residue uniquely distinguished promiscuous from selective binding.

Table 2. Key pocket differences and contact frequencies for promiscuous vs H1-selective poses.

Feature	5-HT2B	H1	M2	Promiscuous	H1-selective	Interpretation
Asp3.32 salt bridge on H1 poses	Asp	Asp	Asp	12/19	15/16	H1-selective poses keep the H1 anchor
Asp3.32 salt bridge on M2 poses	Asp	Asp	Asp	5/19	3/16	H1-selective poses usually lose the M2 anchor
3x33	Val	Tyr	Tyr	—	—	H1 and M2 share Tyr; 5-HT2B is Val
4x57	Ala; ergotamine does not contact it	Trp; contacted	Trp; contacted	—	—	H1 and M2 contact a tryptophan that 5-HT2B does not have
M2 Phe5x47	Phe	Phe	Phe	13/19	6/16	Promiscuous poses occupy this M2 shell position more often
M2 Ala5x461	Ala	Asn	Ala	19/19	12/16	Every promiscuous M2 pose contacts this shell position
H1 Phe6x44	Phe; not an ergotamine contact	Phe; doxepin contacts it	Phe	15/19	9/16	An H1 crystal-specific shell contact that is more common among promiscuous poses
H1 His7x34	Leu	His	Trp	1/19	6/16	Extended aromatic contact enriched in H1-selective poses
H1 Trp3x28	Trp	Trp	Trp	0/19	4/16	Extended aromatic contact seen in H1-selective poses and in none of the promiscuous poses

Incremental predictive contribution of docking-derived interaction features

To determine whether receptor-specific docking interactions improved the classification of binding promiscuity beyond ligand chemistry, an ablation analysis was performed using 35 compounds comprising 19 promiscuous and 16 H1-selective ligands. The dataset represented 26 distinct Bemis–Murcko molecular scaffolds. Because five-fold scaffold-grouped partitioning could not represent both classes in every test fold, we used four-fold cross-validation.

The ligand-only model, based on Morgan molecular fingerprints, achieved an out-of-fold ROC-AUC of 0.661 and an average precision (AP) of 0.629, compared with chance reference values of 0.500 and 0.543, respectively (**Figure 4**).

Incorporation of the H1 and M2 Asp3x32 salt-bridge features resulted in a modest numerical improvement, increasing ROC-AUC to 0.681 and AP to 0.645. The corresponding change in ROC-AUC relative to the ligand-only model was +0.020 (95% bootstrap interval: −0.029 to +0.088). In contrast, inclusion of shell and extended binding-pocket interaction features reduced predictive performance, yielding a ROC-AUC of 0.599 and an AP of 0.577 (Δ AUROC = −0.062; 95% interval: −0.154 to −0.010). The model incorporating the complete interaction fingerprint achieved a ROC-

AUC of 0.632 and an AP of 0.620, corresponding to a change in ROC-AUC of -0.029 (95% interval: -0.118 to +0.036) (Table3).

Table 3. Predictive performance of ligand-based and interaction-augmented classification models under scaffold-grouped cross-validation.

Model	ROC-AUC	Average precision	Δ AUROC (95% bootstrap interval)
Ligand fingerprints only	0.661	0.629	Reference
Ligand + Asp3x32 anchor	0.681	0.645	+0.020 (-0.029 to +0.088)
Ligand + shell/extended contacts	0.599	0.577	-0.062 (-0.154 to -0.010)
Ligand + complete interaction fingerprint	0.632	0.620	-0.029 (-0.118 to +0.036)

Bootstrap analysis additionally characterized the uncertainty associated with these comparisons. For the anchor-augmented model, 810 of 1,000 bootstrap resamples yielded a positive Δ AUROC, whereas 145 yielded a negative value. The confidence interval nevertheless included zero, indicating that the observed numerical improvement was not sufficiently supported to establish an additional predictive benefit. In contrast, the shell/extended interaction model produced negative Δ AUROC values in 990 bootstrap resamples and positive values in only five, with its estimated interval staying below zero. The complete interaction fingerprint model also showed no consistent improvement, with 208 positive and 754 negative bootstrap differences.

We derived these uncertainty estimates from paired scaffold-level resampling of existing out-of-fold predictions without refitting the model. They therefore describe variability within the internal evaluation and do not constitute independent external validation. Overall, docking-derived interaction features did not consistently improve on ligand molecular fingerprints under the predefined scaffold-grouped evaluation.

Discussion

The present study evaluated the contribution of receptor-specific docking interactions to predicting aminergic GPCR selectivity by distinguishing four aspects of docking performance: native-pose recovery, virtual-screening enrichment, structural interpretation, and predictive utility. Although these aspects are related, success in one does not necessarily establish dependability in the others. Native-ligand redocking successfully reproduced the experimental binding poses for 5-HT2B, H1, and M2, whereas D2 and α 1A failed the predefined rank-1 validation criteria despite sampling near-native conformations. Importantly, successful pose recovery did not consistently translate into virtual-screening performance. The 5-HT2B receptor showed near-random discrimination between active compounds and decoys despite sub-angstrom redocking accuracy. In contrast, H1 exhibited stronger early enrichment, while M2 demonstrated moderate overall discrimination but limited early enrichment. These findings highlight the importance of evaluating screening performance independently of structural pose recovery.

Comparison of the validated receptor structures revealed that only four ligand-contacting residues were conserved across all three receptors, with greater variation in the surrounding binding-pocket and extracellular regions. The extensive extracellular interaction pattern detected for 5-HT2B persisted in the complex with the smaller non-ergoline antagonist LY266097, indicating that this feature was not entirely attributable to ergotamine. Analysis of docked compounds further revealed differences in binding patterns between pharmacologically promiscuous and H1-selective ligands. H1-selective compounds more frequently maintained the conserved Asp3x32 interaction at H1, whereas promiscuous compounds did not consistently reproduce this interaction at both receptors. However, receptor-specific contacts did not uniquely distinguish the two pharmacological classes.

Furthermore, favorable docking scores were not consistently associated with recovery of canonical aminergic interactions, indicating the limitations of interpreting docking affinity independently of binding geometry. Despite these structurally interpretable differences, incorporating docking-derived interaction features did not consistently improve classification beyond ligand molecular fingerprints. Adding Asp3x32 anchoring features produced a modest increase in ROC-AUC (+0.020), although the bootstrap interval (−0.029 to +0.088) included zero. In contrast, shell and extended interaction features reduced predictive performance (Δ AUROC = −0.062; 95% interval: −0.154 to −0.010), while the complete interaction fingerprint provided no clear improvement. These results suggest that interaction features derived from predicted docking poses may not necessarily contribute independent predictive information beyond ligand chemistry, even when the underlying contacts are structurally interpretable. However, we obtained the confidence intervals by resampling existing out-of-fold predictions without refitting the model, so they do not capture the full uncertainty from model training and data partitioning.

Several limitations of the current study warrant consideration. The predictive analysis included only 35 compounds representing 26 molecular scaffolds, with too few M2-selective compounds to form a separate classification group. In addition, we evaluated enrichment using a restricted set of cationic ligands, docked a single rigid receptor conformation per target, and limited predictive modeling to a predefined regularized logistic regression framework. These factors may restrict the generalizability of the findings, and the observed performance differences should not be interpreted as evidence that receptor-specific interactions lack predictive value more broadly.

Expanding the pharmacological dataset represents an important direction for future investigation. Although 496 compounds had usable K_i measurements at both H1 and M2, an additional 3,465 compounds had measurements for only one receptor. Among these, 2,644 already satisfied either the potent- or weak-binding threshold at the measured receptor, making them potential candidates for classification if suitable measurements at the second receptor become available. Adding binding data from resources such as BindingDB (Liu et al. 2025) or updated ChEMBL (Zdrzil et al. 2024) releases, along with expanded receptor conformational sampling and independent validation, could help determine whether docking-derived interaction features provide complementary information in larger, chemically broad datasets.

Overall, these findings emphasize that structural interpretability and predictive generalization are independent properties. Receptor-specific docking interactions can provide valuable insights into ligand recognition and selectivity-related contact patterns. However, appropriately controlled comparisons against ligand-based models are necessary to establish their incremental predictive value.

Conclusion

This study assessed whether receptor-specific docking interactions provide predictive information beyond ligand molecular fingerprints for distinguishing promiscuous from H1-selective compounds. While docking-derived interaction fingerprints revealed distinct receptor–ligand contact patterns, their integration into ligand-based models did not consistently enhance predictive performance under scaffold-grouped cross-validation. Features representing the conserved Asp3x32 anchoring interaction yielded a modest but uncertain improvement, whereas inclusion of shell and extended binding-pocket contacts reduced predictive performance. These results indicate that structurally interpretable molecular interactions do not necessarily lead to improved predictive accuracy. Given the limited dataset size and chemical diversity, further research should employ larger pharmacological datasets, additional receptor systems, and independent external validation to determine the broader predictive utility of docking-derived interaction features.

Declarations

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Author Contributions

V.K.: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Software, Visualization, Writing – original draft, Writing – review & editing.

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Figures

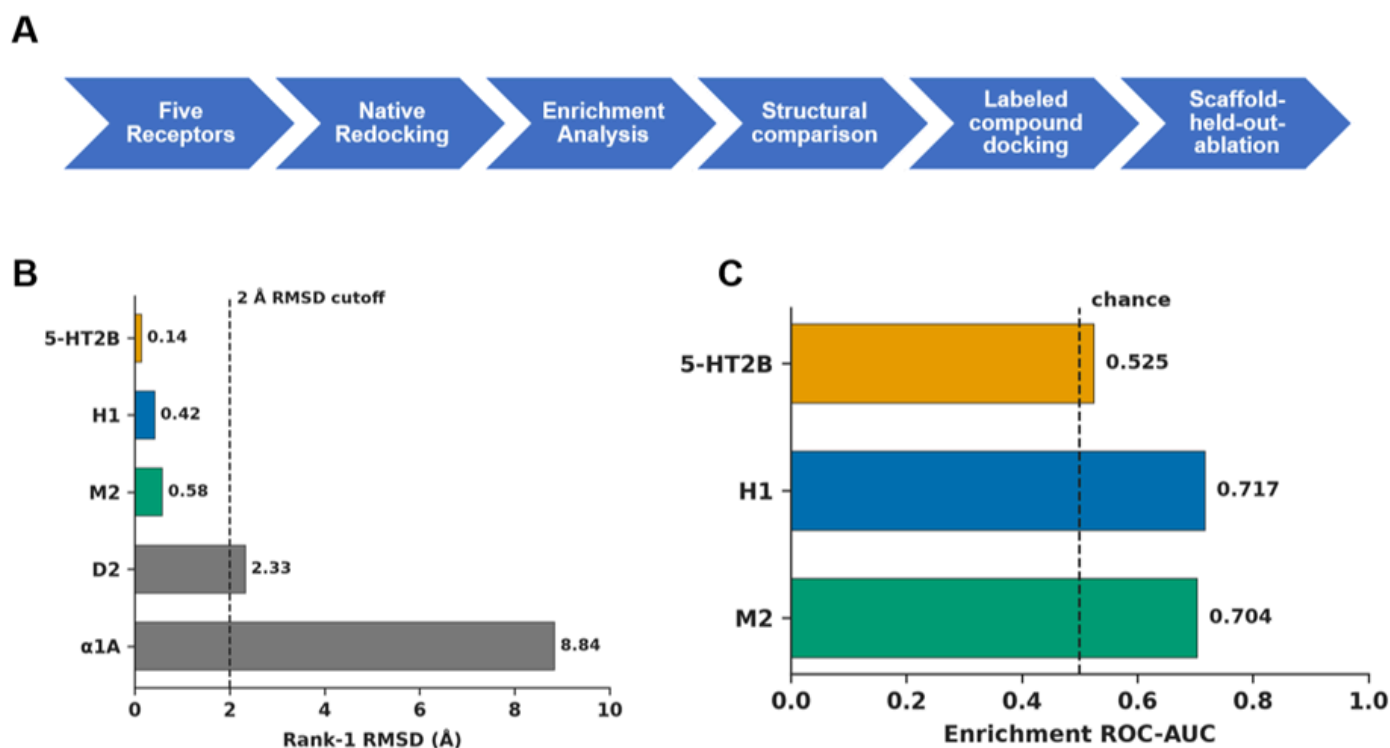


Figure 1

Study design and receptor validation. (A) Workflow from redocking through scaffold-held-out ablation. (B) Rank-1 RMSD for five receptors; dashed line = 2 Å cutoff. (C) Enrichment ROC-AUC for the three receptors that were tested.

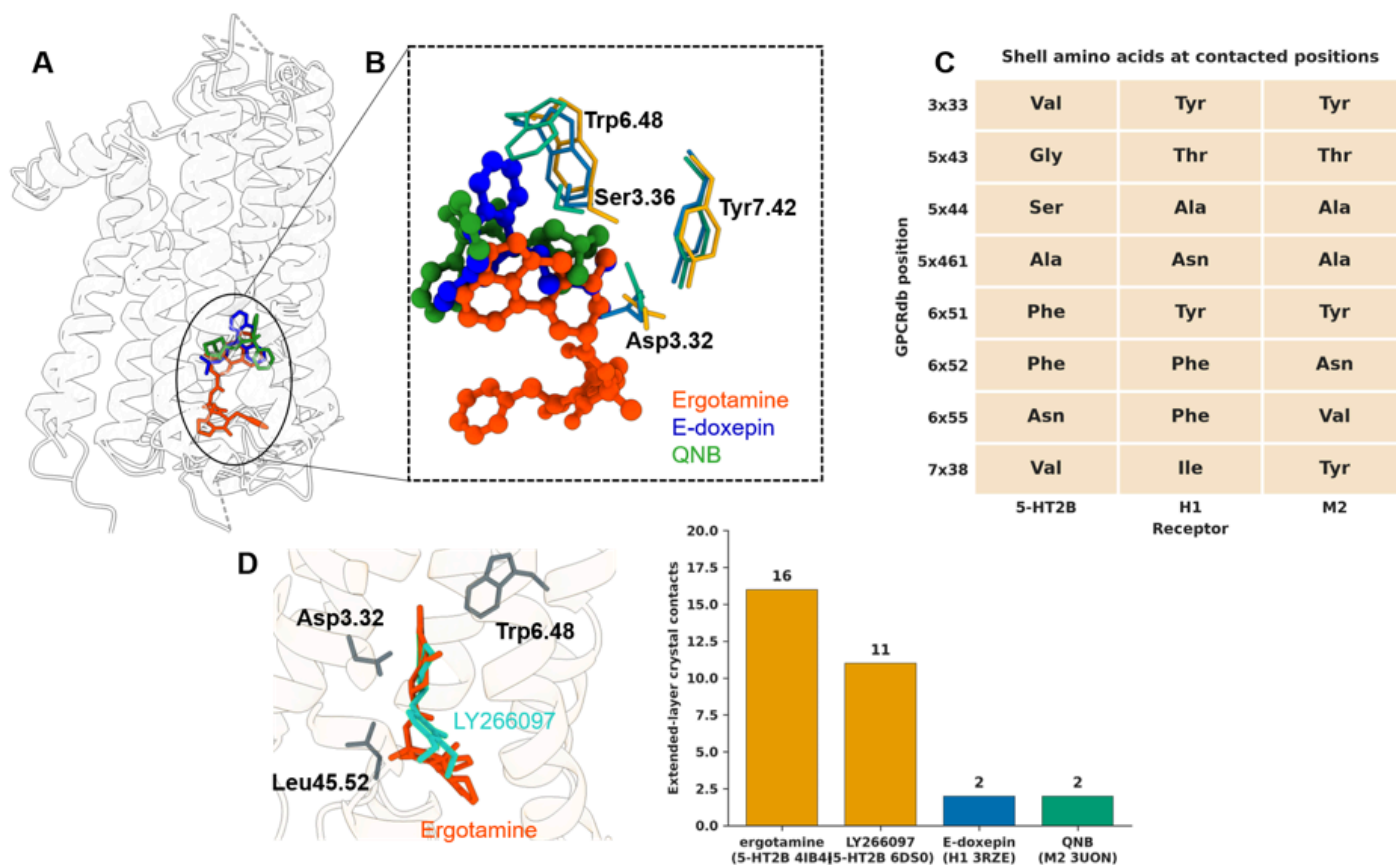


Figure 2

Structural comparison of the aminergic pockets. (A) Aligned 5-HT2B, H1, and M2 with crystal ligands (orange / blue/green). (B) Shared core residues: Asp3.32, Ser3.36, Trp6.48, Tyr7.42. (C) Divergent shell amino acids. (D) Extended-contact counts; 3D figure shows ergotamine (orange) vs LY266097 (blue).

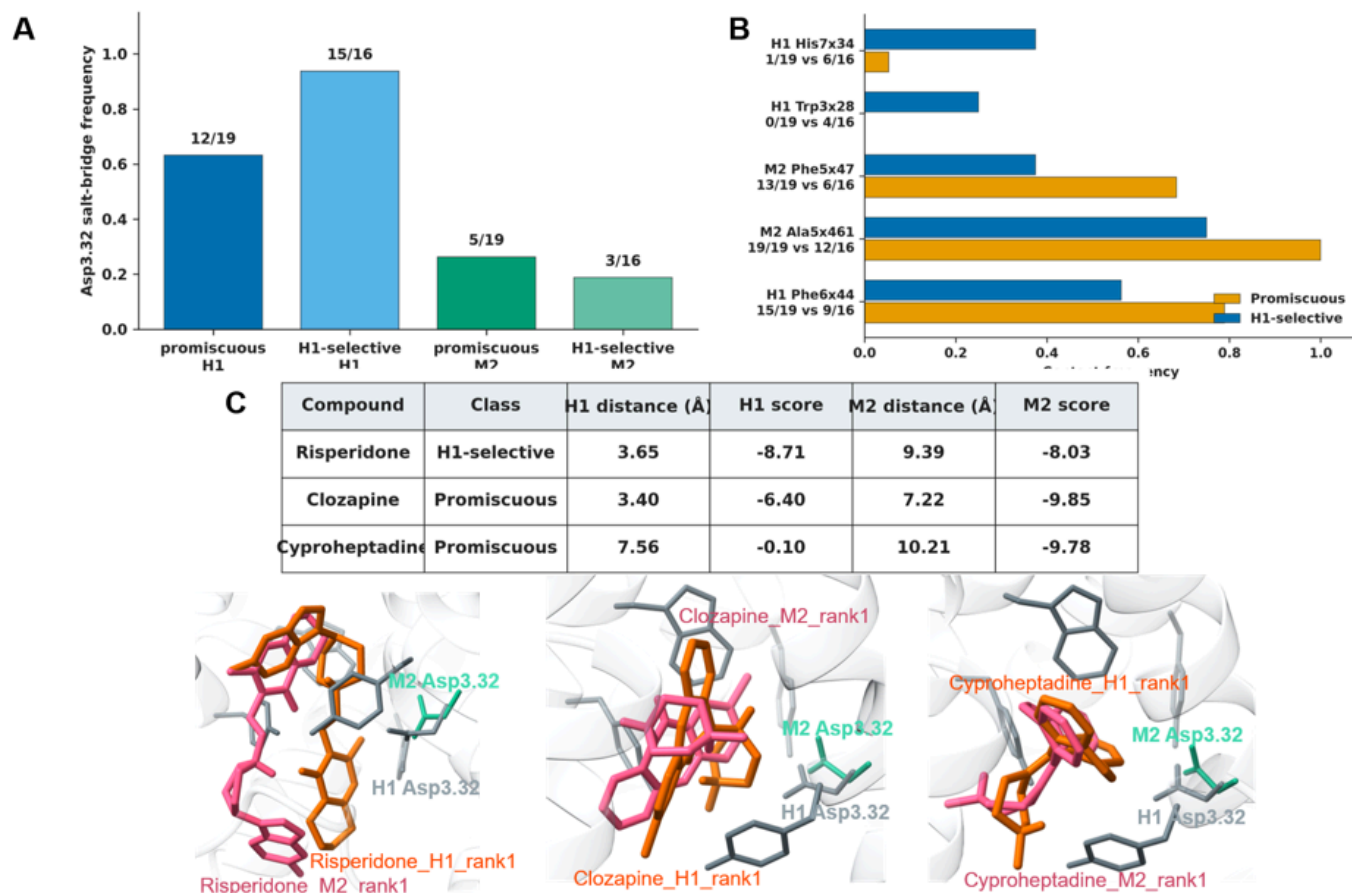


Figure 3

Contact patterns for promiscuous vs H1-selective compounds. (A) Asp3.32 salt-bridge frequency at H1 and M2. (B) Largest class differences at selected shell/extended positions. (C) Asp distance and docking score for risperidone, clozapine, and cyproheptadine, with matching 3D poses (orange = H1, pink = M2).

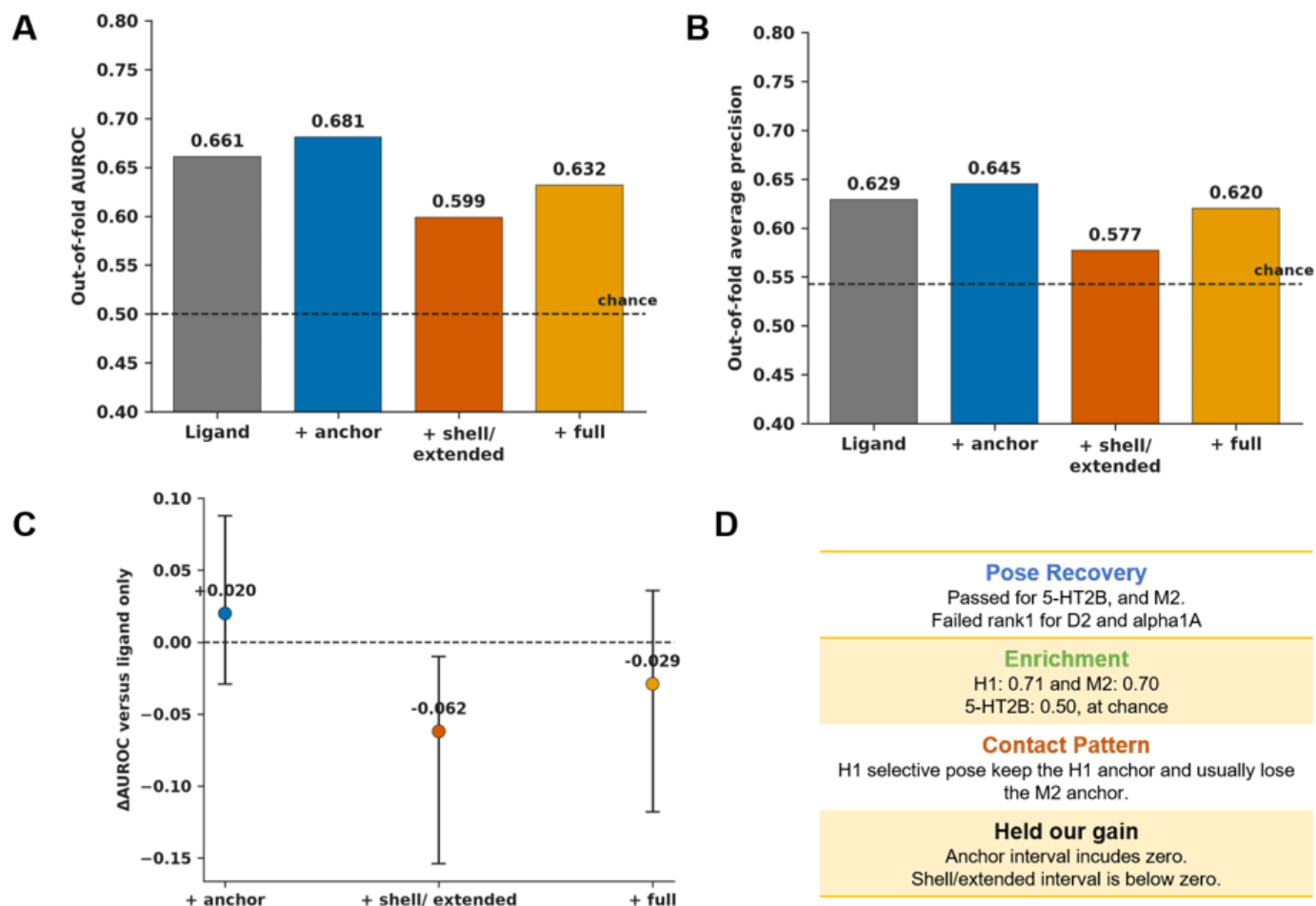


Figure 4

Does docking add predictive value beyond the ligand? (A) Out-of-fold AUROC. (B) Average precision. (C) Δ AUROC vs ligand only (95% intervals). (D) Pose recovery, enrichment, contacts, and held-out gain as separate results.