# Combined Enhanced Conformational Sampling and Protein–Ligand Docking on CYP3A4 Improves *in Silico* Prediction of Drug Metabolism

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**Different CYP3A4 substrates need different active-site shapes. No single crystal structure gives you all of them.**

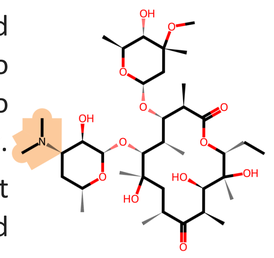
**POSTER P219**

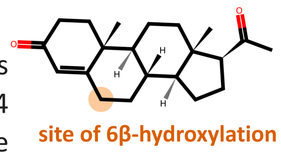
## 1 | CYP3A4: Binding isn't catalysis

CYP3A4 metabolizes more marketed drugs than any other human enzyme, so early substrate prediction is central to clearance and drug–drug-interaction risk.

Docking is the natural triage tool, but it needs a receptor conformation, and CYP3A4's active site is gated by three flexible loops that sit differently across the 121 crystal structures [1,2].

That choice matters. In erythromycin's own co-crystal structure, the site CYP3A4 demethylates sits 16.7 Å from the heme iron: bound to the protein, but much too far away to react.



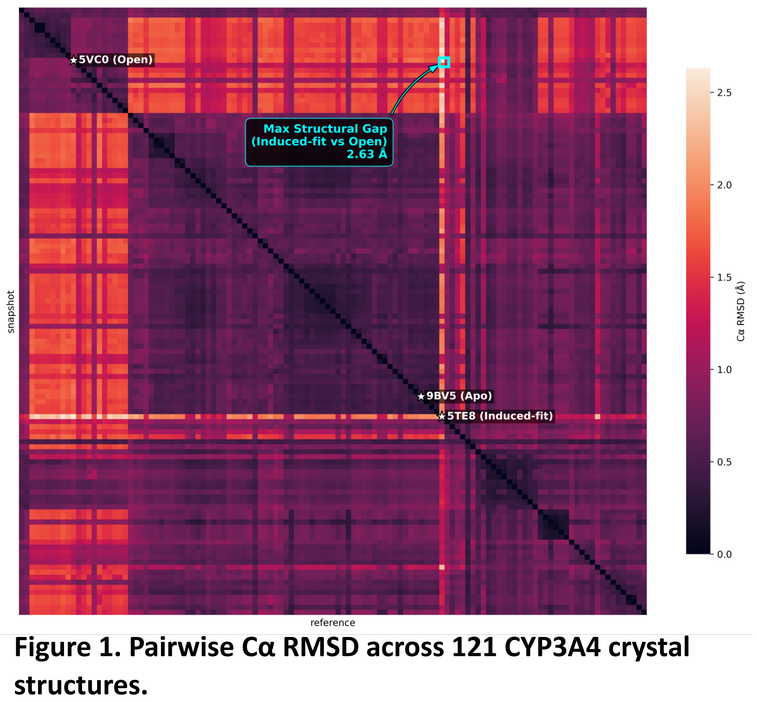




**Figure 1. Pairwise Cα RMSD across 121 CYP3A4 crystal structures.**

## Approach

To resolve this plasticity, we combined enhanced sampling with ensemble docking:

1. **3 crystallographic starts**
   apo 9BV5 · open 5VC0 · induced-fit 5TE8
2. **4.5 µs of enhanced-sampling MD**
   Gaussian accelerated MD (GaMD) [3] · 3 systems × 3 × 500 ns
3. **Cluster into distinct shapes**
   Cα RMSD, 2.25 Å cutoff
4. **13 representative conformations**
   Closed-Compact → Open-Wide
5. **Dock the compound library**
   1,343 substrates + 1,622 non-substrates [4] · AutoDock Vina [5]

## 2 | So we sampled the ensemble

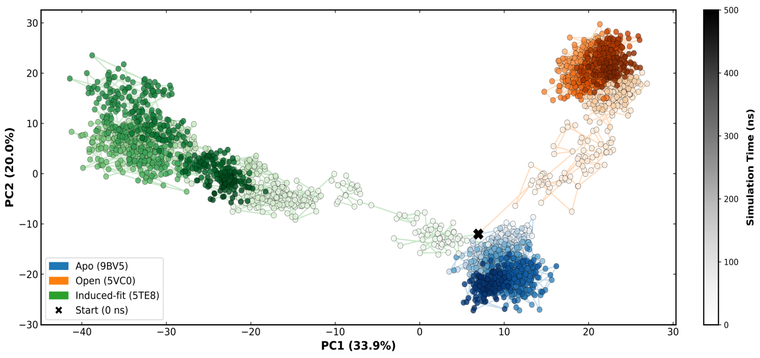



**Figure 2. Conformational displacement from the shared starting structure (✕), colored by system and shaded by simulation time. The induced-fit system travels furthest.**

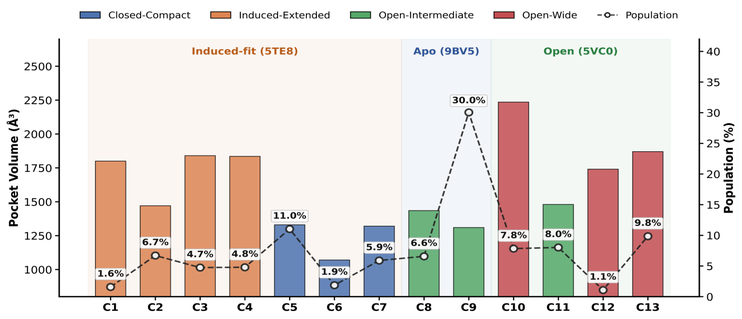



**Figure 3. Binding-pocket volume of each representative (bars) and its population in the ensemble (line). Volumes span roughly 1,050 to 2,250 Å³.**

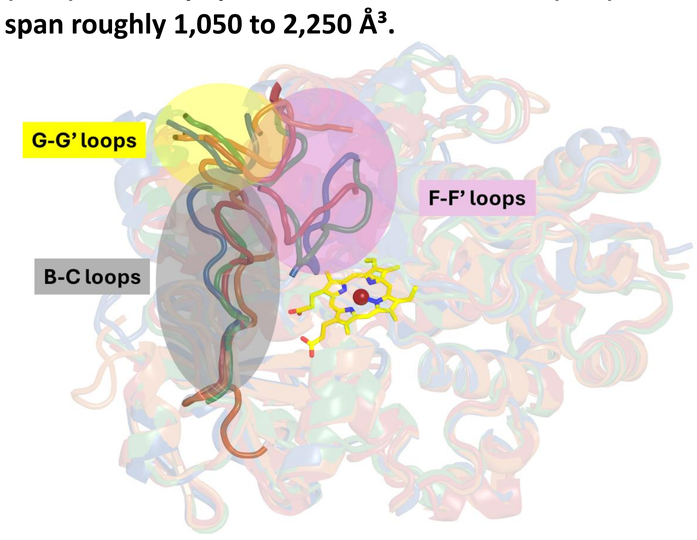



**Figure 4. Representative conformations of the four structural states: Closed-Compact, Intermediate-Extended, Open-Intermediate, Open-Wide.**

Sampling reproduced every conformational family seen experimentally and reached active-site geometries that no crystal structure shows.

The 13 representatives span four structural states, differing mainly in the F–F′, G–G′ and B–C gating loops that control access to the heme.

**The ensemble reaches open states no CYP3A4 crystal structure has captured, and those are the states bulky substrates need.**

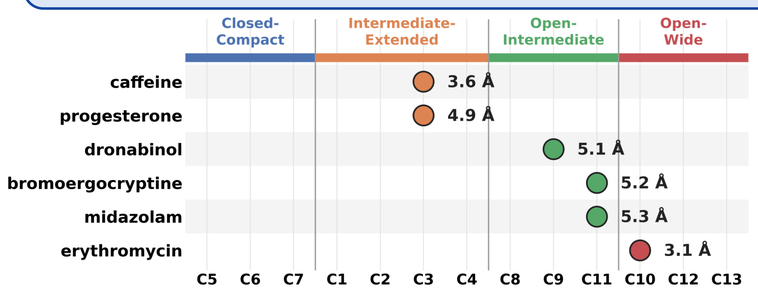



**Figure 5. The conformation giving each substrate its best productive pose (Fe–SOM ≤ 6 Å). No single conformation is productive for all six.**

## 3 | Sampling beats picking one structure

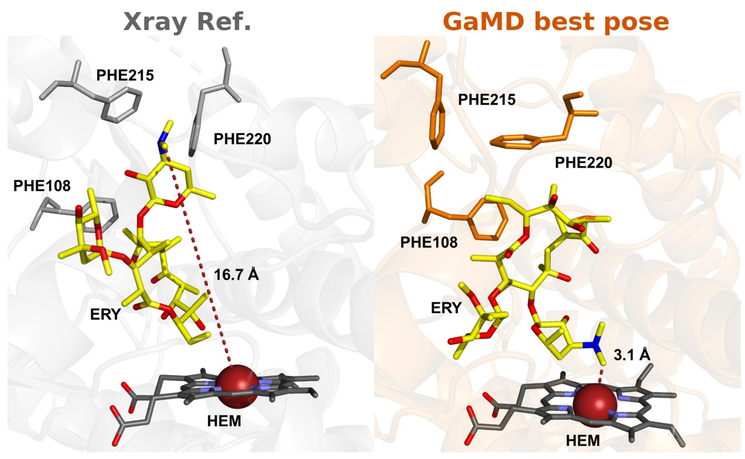



**Figure 6. Erythromycin in its co-crystal structure (left) and docked into the best GaMD conformation (right).**

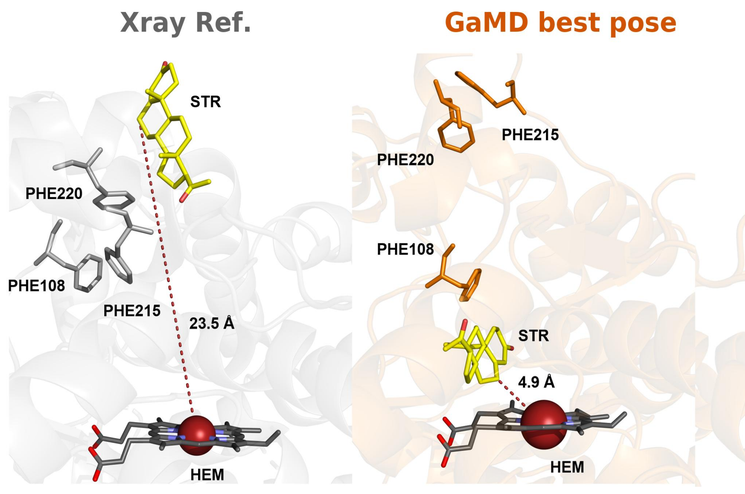



**Figure 7. Progesterone in its co-crystal structure (left) and docked into the best GaMD conformation (right).**

### Is this just molecular weight?

**Substrates are larger than non-substrates, so atom count alone yields an AUC of 0.70. Normalizing docking scores to ligand efficiency removes this bias. Under this control, single-structure performance drops to 0.65, whereas the ensemble maintains an AUC of 0.72, nearly doubling the performance advantage ($p = 1.5 \times 10^{-12}$).**

### Classification.

1,343 substrates and 1,622 non-substrates were docked against the full ensemble; classification used the 2,617 ligands scored in all 13. On docking affinities alone, the ensemble reached ROC-AUC 0.735 (95% CI 0.717–0.754) against 0.697 for the apo reference (ΔAUC +0.038, DeLong $p = 6.7 \times 10^{-8}$): it ranks a substrate above a non-substrate in 73.5% of pairs rather than 69.7%.

It also beats the best single conformation, which you could not have picked in advance (C8, 0.731; ΔAUC +0.013, $p = 0.0015$), and it no longer depends on which conformations you use.

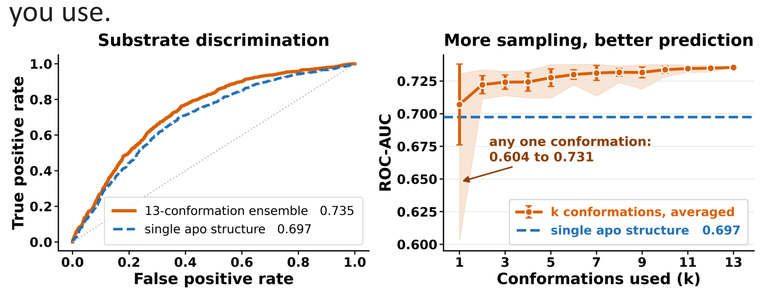



**Figure 8. Substrate discrimination and the effect of ensemble size.**

## Conclusions

1. CYP3A4 substrate prediction is limited by receptor conformation, not by scoring function.
2. Productive geometries for different substrates live in different conformations; no single structure serves all.
3. Ensemble docking removes the structure-selection gamble: it reaches those geometries without knowing in advance which conformation holds them.

Validation and controls. Five-fold stratified cross-validation, out-of-fold predictions pooled into a single ROC; logistic regression at default scikit-learn settings, no tuning. Y-randomisation over 1,000 label permutations: null AUC 0.499 ± 0.015, maximum 0.542. Size control: Vina affinity correlates with heavy-atom count ($r = -0.86$); in ligand-efficiency space the ensemble reaches 0.717 against 0.650 for the apo reference (ΔAUC +0.068, $p = 1.5 \times 10^{-12}$).

**References**

[1] Ekroos, M.; Sjögren, T. Proc. Natl. Acad. Sci. U.S.A. 2006, 103, 13682. [2] Sevrioukova, I. F.; Poulos, T. L. J. Biol. Chem. 2012, 287, 3510. [3] Miao, Y.; et al. J. Chem. Theory Comput. 2015, 11, 3584. [4] Ni, Y.-H.; et al. Sci. Data 2025, 12, 1427. [5] Eberhardt, J.; et al. J. Chem. Inf. Model. 2021, 61, 3891.

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