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**Figure 1: Structural prediction of 1,2-dihydrovomilenine 19,20-reductase using AlphaFold 3.**

1. Panel A shows the superimposition of the five predicted AlphaFold models of 1,2-dihydrovomilenine 19,20-reductase. The predicted structure is colored according to the predicted Local Distance Difference Test (pLDDT) score, which indicates the confidence of AlphaFold's model at each residue position (dark blue (>90) indicating highly reliable regions, light blue (70–90) representing confident but slightly flexible areas, yellow (50–70) corresponding to low-confidence flexible or disordered regions, and orange (<50) highlighting highly uncertain or disordered segments). Zinc ions (Zn²⁺) are represented as spheres. The overall structural alignment indicates strong conservation of the core fold, with minor deviations in loop regions.
2. Panel B shows the Predicted Aligned Error (PAE) matrix for the AlphaFold model, where darker green represents lower expected positional error (higher confidence). The low PAE values along the diagonal indicate a well-predicted protein backbone, while higher PAE values in specific off-diagonal regions suggest greater uncertainty in inter-domain orientations. Together, these results indicate that the AlphaFold model is well-resolved for most of the protein, particularly the catalytic core, but some peripheral regions may have lower reliability.

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**Figure 2. Structural prediction of 1,2-dihydrovomilenine 19,20-reductase using Chai-1.**

1. Panel A shows the superimposition of the five predicted Chai-1 models, shown in cartoon representation and colored by pLDDT scores.
2. Panel B shows the superimposition of the top-ranked AlphaFold and Chai-1 models, with the AlphaFold structure in white and the Chai-1 structure colored by pLDDT scores. The AlphaFold and Chai-1 models show strong agreement in core structural elements but differ in some flexible loops and peripheral domains. AlphaFold assigns high confidence (pLDDT > 90) to most structured regions, while Chai-1 shows greater uncertainty in binding interfaces and flexible regions. Additionally, Chai-1 predicts slightly higher ipTM (0.81) and pTM (0.87) values than AlphaFold (0.75 and 0.79), suggesting a more stable oligomeric interface in its model. However, it is important to note that Chai-1 is known to overestimate confidence scores.
3. Panel C shows a zoomed-in view of the active site from the five superimposed Chai-1 models, showing the predicted binding of the substrate (1,2-dihydrovomilenine, top ligand) and the co-factor (NADPH, bottom ligand). The overlay reveals some variation in substrate positioning across different predictions, with the ligand adopting slightly different orientations relative to key catalytic residues. The NADPH binding site appears more conserved, but subtle shifts in its placement suggest potential flexibility in co-factor binding. These differences indicate that while the general active site is well-defined, ligand positioning may be influenced by dynamic factors or uncertainty in side chain conformations, requiring further refinement or experimental validation.
4. Panel D shows the superimposition of the top Chai-1 predicted structure (white) with two experimentally solved homologs:

*3TWO: CAD from Helicobacter pylori complexed with NADP(H)*

* Shown in cyan.
* This structure includes NADPH.

*8B1V: Dihydroprecondylocarpine acetate synthase 2 from Tabernanthe iboga)*

* Shown in magenta.
* This structure includes precondylocarpine acetate, a substrate that functions similarly to 1,2-dihydrovomilenine.

This comparison evaluates the placement of the co-factor NADPH and the substrate 1,2-dihydrovomilenine by Chai-1. The NADPH orientation in Chai-1 closely matches the experimental structure of 3TWO, suggesting conserved co-factor binding. However, some variability in the placement of the substrate is observed, with the Chai-1 model differing slightly from the bound conformation in 8B1V. Additionally, the nicotinamide ring of NADPH in Chai-1 is positioned near the catalytic site but may require slight adjustments to achieve optimal van der Waals contact for hydride transfer. These differences indicate that while the Chai-1 model captures the active site architecture, refinements may be needed to precisely position the substrate for catalysis.

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**Figure 3. Active site comparison of 1,2-dihydrovomilenine 19,20-reductase and its homologs.**

1. Panel A shows the predicted active site of 1,2-dihydrovomilenine 19,20-reductase, modeled by Chai-1, shown in cartoon representation with a transparent surface to illustrate the substrate-binding pocket. The substrate (1,2-dihydrovomilenine) and co-factor (NADPH) are shown as sticks, with interacting residues also displayed as sticks.
2. Panel B shows superimposition of the homologous structures 3TWO (cyan) and 8B1V (magenta), showing their respective active sites and ligand-binding orientations. NADPH from 3TWO and the substrate analog (precondylocarpine acetate) from 8B1V are shown in stick representation. The overlay highlights a conserved co-factor binding mode but reveals differences in substrate positioning between the experimental structure and the Chai-1 prediction. While the NADPH nicotinamide ring is closely aligned with catalytic residues, the substrate in the Chai-1 model exhibits slight deviations from the experimentally determined binding pose, suggesting that refinements or alternative modeling approaches may be necessary to fully capture the productive ternary complex.