FUNCTIONAL CHARACTERIZATION OF

5-Beta Reductase Activity

A Computational Characterization Pipeline

Sequence → Motif Analysis → Structure Prediction → Docking → Functional Validation

Hypothesis & Analytical Strategy

Hypothesis: The query sequence encodes a flavoenzyme with 5-beta reductase activity, capable of reducing steroids using NADPH as an electron donor via a flavin cofactor.

1 Sequence Search

BLAST + InterPro + PROSITE

2 Motif Analysis

NADPH / FAD binding domains

3 Structure Prediction

AlphaFold3 → PDB model

4 Structural Comparison

TM-align + DALI server

5 Molecular Docking

NADPH + Steroid binding

6 Validation

Conservation + Active site

Tools Used — Phase 1: Sequence Analysis

NCBI BLASTp

blast.ncbi.nlm.nih.gov

Compares protein sequence against entire NCBI database to find homologs and infer function from known proteins.

InterPro

ebi.ac.uk/interpro

Integrates multiple protein family databases (Pfam, PRINTS, PANTHER) to classify protein families and predict domains.

PROSITE

prosite.expasy.org

Scans sequence for biologically meaningful patterns, motifs, and profiles — especially enzyme active site signatures.

Tools Used — Phase 2: Structure Prediction & Comparison

AlphaFold3 Server

alphafoldserver.com

Predicts 3D protein structure from amino acid sequence using deep learning. Outputs 5 ranked models in mmCIF format with pLDDT confidence scores.

TM-align

zhanggroup.org/TM-align

Aligns two protein structures and calculates TM-score (0–1). Score >0.5 indicates same fold family regardless of sequence identity.

DALI Server

ekhidna2.biocenter.helsinki.fi/dali

Searches entire PDB database for structurally similar proteins. Z-score >20 indicates significant structural similarity; ranks all matches.

Tools Used — Phase 3: Molecular Docking

CB-Dock2 (Auto Blind Docking)

cadd.labshare.cn/cb-dock2

Cavity-detection guided blind docking tool. Automatically detects binding pockets on protein surface and docks ligand into each, ranking by Vina score (more negative = stronger binding).

Clustal Omega (MSA)

ebi.ac.uk/Tools/msa/clustalo

Multiple sequence alignment tool. Aligns your protein with homologs (1OYA, 6QKR) to identify conserved residues — particularly catalytic residues at the active site.

PubChem (Ligand Source)

pubchem.ncbi.nlm.nih.gov

Retrieved 3D conformers of NADPH (CID: 5884) and Progesterone (CID: 5994) for use as docking ligands in CB-Dock2.

Overall Conclusion

Computational evidence supporting 5-beta reductase activity

Analysis	Tool	Key Result	Verdict
BLASTp	NCBI BLAST	Flavoenzyme hit — steroid reduction in description	✓ Positive
Domain Analysis	InterPro IPR051793	NADPH + FAD binding + bile acid degradation	✓ Strong
Motif Scan	PROSITE PS50079	FAD/NAD(P)-binding domain confirmed at pos. 611–627	✓ Positive
Structural Fold	TM-align vs 1OYA	TM-score = 0.796 — same fold as Old Yellow Enzyme	✓ Very Strong
PDB Comparison	DALI Server	NCR steroid reductase — Z-score = 52 (top hit)	✓ Very Strong
NADPH Docking	CB-Dock2	Vina = −10.6 kcal/mol; TYR167 contact confirmed	✓ Excellent
Steroid Docking	CB-Dock2	Vina = −9.2 kcal/mol; TYR167 contact confirmed	✓ Excellent
Conservation	Clustal Omega MSA	DAIHE motif, TYR167, ARG215/293 conserved	✓ Confirmed

CONCLUSION: All eight independent lines of evidence converge to support 5-beta reductase activity. The protein is a flavoenzyme that uses NADPH→FAD electron transfer to reduce steroid substrates, with TYR167 as the key catalytic residue.