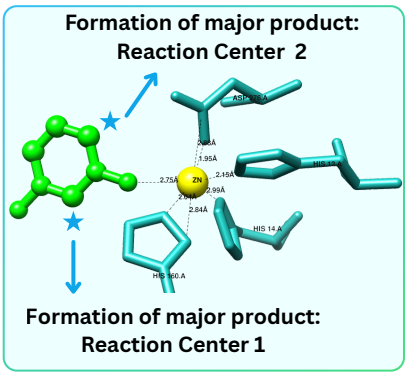
Accelerating Regioselectivity-Guided Enzyme Engineering Using AI and Molecular Simulations

Enzyme Class: Metal-dependent Decarboxylases

Application Area: Biocatalysis | Green Chemistry | Enzyme Optimization

Overview

Medvolt's AI-driven discovery pipeline integrates advance physics-based scoring, AI-assisted rescoring, and conservation/MSA-informed selection to address the challenge of engineering enzyme variants capable of performing regioselective transformations on small-molecule substrates via metal-ion coordination. Incorporating AI-based protein modelling and proprietary physics-based simulations, the platform enables accurate variant generation, ranking and robust prediction of regioselectivity

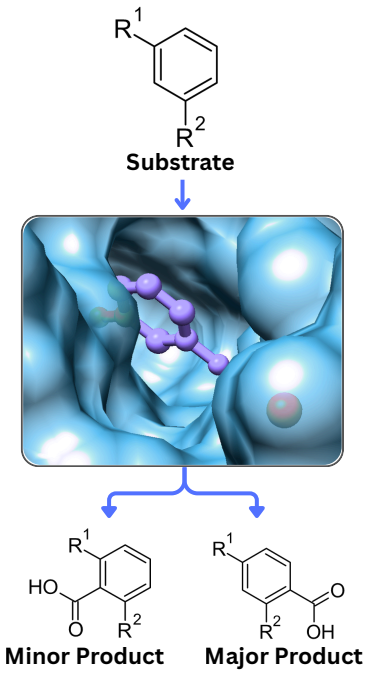


Scientific Challenge

The objective was to evaluate multiple AI-generated enzyme variants for their capacity to catalyze regioselective transformations on a common substrate. Given that reaction outcomes are strongly influenced by substrate orientation within the active site, conventional binding analyses proved inadequate. Therefore, a more comprehensive, structure-informed approach was adopted to determine whether each variant promoted the desired transformation pathway

Computational and Structural Challenges

- Predict reliable enzyme–ligand–metal ion complex structures for AI-generated variants
- Differentiate variants based on regioselective potential using spatial orientation and active-site interactions
- Strengthen confidence in predictions through multi-platform validation



Workflow

"AI-driven sequence-to-insight pipeline"

Data Collection
Sequence–Structure Data Integration

AI Modeling
Structure & AI-driven methods

Validation
Multi-Platform Pose Evaluation

Results
Predicting Regioselectivity

Our Approach

Dual-Platform Structural Modeling

AI-Driven Variant Generation
Generative AI for *de novo* mutants optimized for stability and efficiency

AI+Physics based Folding and Co-folding
Full-length enzyme structures with metal ions using AI as well as proprietary physics modelling

Validation of Pose Quality

Superimposition Analysis
Assessment against co-crystallized reference structure

Interaction Mapping
Key catalytic residue–ligand interactions and metal ion effect

Regioselectivity Assessment

Carbon Atom Selection
Reactive center selection for product formation

Distance Analysis
Differential distance (ΔD) mapping from the potential reaction sites to the catalytic metal ion

Conservation Analysis Using MSA

Sequence Alignment
60+ sequences aligned with multiple methods

Residue Mapping
Critical catalytic residues - conservation analysis

Cross-Platform Comparison

Prediction Comparison
Multi-model AI vs Medvolt pipeline comparison

High-Confidence Selection
Top-ranked common sequences from both platforms

Key Results

Quantifiable outcomes that demonstrate the effectiveness of our approach

23
Sequences identified favoring regioselectivity using AI-driven folding and co-folding pipelines

12
Sequences shortlisted using Medvolt's proprietary physics-based protocol

8
Overlapping results highlighting strong conviction between 2 pipelines

- **Key catalytic residues** were highly conserved, indicating **structural integrity**
- Ligands maintained favourable, consistent orientations across both platforms, reinforcing **prediction reliability**
- Pose alignment matched known catalytic geometries, supporting observed **regioselectivity trends**

Adaptable Use Cases →

- Enzyme Repurposing
- Selective Biocatalyst Design
- Rational Mutagenesis Planning
- Substrate Scope Expansion
- AI-guided Product Selectivity Profiling