

Engineering of Halohydrin Dehalogenase IcHheG via Semi-Rational Design for Enantioselective Synthesis of (R)-5-Phenoxymethyl-2-Oxazolidinone

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Abstract

Enantiomerically pure oxazolidinones are widely used as important chiral auxiliaries in organic synthesis; however, efficient methods for their preparation remain scarce. In this study, we report the engineering of halohydrin dehalogenase *lcHheG* from *Ilumatobacter coccineus* through semi-rational design to catalyze the enantioselective ring-opening of phenyl glycidyl ether (PGE) with cyanate, as well as the direct synthesis from the precursor 1-chloro-3-phenoxy-2-propanol, enabling the efficient synthesis of (*R*)-5-phenoxyethyl-2-oxazolidinone. Through systematic single-point and combinatorial mutagenesis, we identified the optimal double mutant, *lcHheG*-L103H/N196W, which exhibited markedly improved enantioselectivity, affording the product in 95% ee. Sequential kinetic resolution of 1-chloro-3-phenoxy-2-propanol using *lcHheG*-L103H/N196W yielded (*R*)-5-phenoxyethyl-2-oxazolidinone in 99% ee (49.5% yield) and (*S*)-1a in > 98% ee. Molecular docking analysis of wild-type *lcHheG* and the mutant L103H/N196W with PGE and 1-chloro-3-phenoxy-2-propanol revealed that alterations in the substrate-binding pocket increased the distances from (*S*)-PGE and (*R*)-1-chloro-3-phenoxy-2-propanol to the catalytic residues, likely accounting for the enhanced enantioselectivity. Collectively, this mutant holds great potential as a biocatalyst for the enantioselective synthesis of chiral (*R*)-5-phenoxyethyl-2-oxazolidinone.

1 Introduction

Oxazolidinones represent a significant class of oxygen- and nitrogen-containing five-membered heterocyclic compounds that have attracted considerable attention in medicinal chemistry due to their prominent biological activities (Yuan et al. 2023). Since their biological and pharmacological activities were first reported in 1987, oxazolidinones have become valuable synthetic intermediates in organic chemistry and the pharmaceutical industry (Renslo et al. 2006; Bozdogan et al. 2004; Slee et al. 1987). Linezolid, the first oxazolidinone antibiotic approved for human use, has demonstrated remarkable efficacy against infections caused by multidrug-resistant Gram-positive bacteria (Ford et al. 2001). Enantiomerically pure 2-oxazolidinones play a pivotal role in modern organic synthesis, frequently serving as "Evans" chiral auxiliaries for various asymmetric transformations, including asymmetric C-C bond formation (Okuno et al. 2022; Nakamura et al. 1997). In recent years, an increasing number of 4- or 5-substituted 2-oxazolidinones have been identified to possess antibacterial, anti-asthmatic, and immunosuppressive activities, thus attracting widespread interest (Diekema et al. 2001; Barbachyn et al. 2003; Mukhtar et al. 2005; Renslo et al. 2006; Michalska et al. 2013; Paladino et al. 2002; Swaney et al. 1998). For instance, optically active 4-substituted 2-oxazolidinones serve as key intermediates in the synthesis of the cholesterol drug ezetimibe, the K-opioid receptor agonist CJ-15161, and the anti-migraine drug zolmitriptan (Lanoix et al. 2013; Kaul et al. 2018; Lemaire et al. 2009; Wan et al. 2019; Ishizuka et al. 1992; Vo et al. 2003). Given their importance in organic synthesis and pharmaceuticals, developing effective methods for preparing enantiomerically pure 2-oxazolidinones remains a research focus in organic synthesis (Okuno et al. 2022; Zanda et al. 2022; Li et al. 2018).

Traditional methods for preparing 2-oxazolidinones and their derivatives generally rely on enantiomerically pure β -amino alcohols via carbonylation with electrophilic C = O reagents. However, these approaches often involve the use of highly toxic and hazardous reagents such as phosgene, its derivatives, or carbon monoxide, and require harsh reaction conditions. Moreover, the preparation of chiral β -amino alcohols themselves suffers from poor selectivity, the need for excess donors, and complex synthetic routes (Li et al. 2018). In comparison, asymmetric hydrogenation of unsaturated heterocyclic compounds, while efficient, typically requires expensive and environmentally polluting metal catalysts (Xie et al. 2011; Yu et al. 2012; Zhang et al. 2016; Liu et al. 2020). The coupling of epoxides with isocyanates currently represents the most effective and convenient method for synthesizing enantiomerically pure 2-oxazolidinones (Sun et al. 2021), yet this approach suffers from poor regio- and stereoselectivity, high cost, stringent reaction temperature requirements, and long reaction times.

Biocatalysts, such as recombinant cells and purified enzymes, offer an important route for the green synthesis of oxazolidinones by providing optically pure compounds with high yield owing to their excellent regio- and enantioselectivity (Li et al. 2018). Halohydrin dehalogenases (HHDHs), as versatile biocatalysts, not only catalyze the dehalogenation of vicinal haloalcohols but also mediate the ring-opening of epoxides in the presence of nucleophiles such as N_3^- , CN^- , NO_2^- , SCN^- , OCN^- , and HCOO^- , yielding β -substituted alcohols or their derivatives (Schallmeyer et al. 2016; Hasnaoui-Dijoux et al. 2008; Haak et al. 2008; Ma et al. 2010). The use of HHDHs to catalyze epoxide ring-opening for oxazolidinone synthesis represents a promising green synthetic method. In 2008, Janssen et al. first reported the synthesis of 5-substituted 2-oxazolidinones using halohydrin dehalogenase HheC from *Agrobacterium radiobacter* via epoxide reaction with cyanate (Elenkov et al. 2008). In 2019, Wan et al. first reported the use of HheG as a biocatalyst for the synthesis of 4-aryl oxazolidinones; subsequently, they employed HheC to catalyze styrene oxide ring-opening for the synthesis of (*R*)-5-aryl-2-oxazolidinones, although the enzyme exhibited low regio- and stereoselectivity (Wan et al. 2020). In 2024, Song et al. engineered *l*cHheG using site-saturation mutagenesis and iterative combinatorial mutagenesis techniques to generate variants with enhanced regio- and stereoselectivity, with the best variant used to synthesize (*S*)-4-aryl-2-oxazolidinones and (*S*)-styrene oxide, achieving ee values up to 99% (Song et al. 2024). In 2025, Ding et al. realized the enantioselective synthesis of (*S*)-5-phenoxyethyl-2-oxazolidinone using S/HHDH as a biocatalyst (Ding et al. 2025) (Fig. 1).

Inspired by previous work, the present study aimed to further engineer halohydrin dehalogenase *l*cHheG through protein engineering to enhance its enantioselectivity in cyanate-mediated epoxide ring-opening for the synthesis of (*R*)-5-phenoxyethyl-2-oxazolidinone. In this study, key mutation sites affecting the substrate selectivity of *l*cHheG were identified through a combination of molecular docking, alanine scanning, tunnel analysis, and sequence alignment. The optimal mutant L103H/N196W with significantly enhanced regio- and stereoselectivity was obtained through site-directed and combinatorial mutagenesis. Finally, molecular docking analysis was performed to understand the mechanism underlying the enhanced enantioselectivity toward PGE.

2 Materials and Methods

2.1 Strains and Reagents

E. coli BL21(DE3) strain and pET-28a(+) expression vector were purchased from Nanjing GenScript Biotechnology Co., Ltd. Glycerol, potassium chloride, and phenyl glycidyl ether were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Yeast extract and tryptone were purchased from OXOID (Shanghai, China). Agar powder and agarose were purchased from BioFroxx (Guangzhou, China). Glucose, petroleum ether, n-hexane, isopropanol, ethyl acetate, and sodium cyanate were purchased from Tianjin Kaitong Chemical Reagent Co., Ltd. (Tianjin, China). Potassium acetate, calcium chloride, and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Kanamycin and isopropyl- β -D-thiogalactopyranoside (IPTG) were purchased from Beijing Solarbio Technology Co., Ltd. (Beijing, China). 1-Chloro-3-phenoxy-2-propanol was purchased from Jiangsu Aikon Biomedical Research and Development Co., Ltd. (Nanjing, China). Plasmid extraction kit was purchased from Nanjing Vazyme Biotechnology Co., Ltd. (Nanjing, China). Manganese chloride tetrahydrate was purchased from Tianjin Guangfu Research Institute (Tianjin, China). Ninhydrin was obtained from a commercial source. All other chemicals and reagents were of analytical grade or biochemical reagent grade and were obtained from commercial suppliers.

2.2 Expression of *lcHheG* and Its Mutants

The target gene, carried in the pET-28a(+) plasmid, was expressed in *E. coli* BL21(DE3). Recombinant *E. coli* cells were cultured in LB medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) supplemented with 50 mg/L kanamycin at 37°C. When the OD₆₀₀ reached approximately 0.6–0.8, IPTG was added to a final concentration of 0.1 mM, and the cultures were further incubated at 25°C for 16–18 h. Subsequently, cells were harvested by centrifugation at 5,000 rpm for 15 minutes at 4°C, washed with 50 mM Tris-SO₄ buffer (pH 7.5). Approximately 50 mg of induced wet cells were resuspended in 1 mL Tris-SO₄ buffer by vortexing and sonicated in an ice-water bath. Then, the cells were harvested by centrifugation at 4 °C and 13000 rpm for 1 min, washed with 50 mM TrisSO₄ buffer (pH 7.5), and used for further experiments.

2.3 Construction of *lcHheG* Mutant Library

Site-directed mutagenesis was performed by PCR using specifically designed primers and Super Pfx DNA polymerase. The first-round PCR conditions were: initial denaturation at 98°C for 2 min; 29 cycles of 98°C for 10 s, 58°C for 30 s, 72°C for 30 s; and final extension at 72°C for 10 min. The second-round PCR conditions were: initial denaturation at 98°C for 2 min; 29 cycles of 98°C for 10 s, 58°C for 30 s, 72°C for 4 min 30 s; and final extension at 72°C for 4 min. Template was digested with restriction endonuclease *Dpn* I at 37°C for 2 hours, and PCR products were used to transform *E. coli* BL21(DE3). Recombinant cells were spread on LB agar plates containing 50 mg/L kanamycin and incubated overnight at 37°C. Single colonies were picked for sequencing to identify correct transformants. Each

target amino acid residue was mutated to the other 19 amino acids, with the corresponding *E. coli*-preferred codons selected for substitution.

2.4 Molecular Modeling and Docking

The tertiary structure of *l*cHheG was retrieved from the PDB database (<https://www.rcsb.org/>) (PDB ID: 5O30). The three-dimensional homology model of *l*cHheG-L103H/N196W was generated using Modeller 10.3 software. (*R*)-PGE, (*S*)-PGE, (*R*)-1-chloro-3-phenoxy-2-propanol and (*S*)-1-chloro-3-phenoxy-2-propanol were docked into WT-*l*cHheG and the L103H/N196W mutant using AutoDock 4.0 software. The docking grid box was set to 40 × 40 × 40 Å with a spacing of 0.375 Å; other parameters followed program defaults, and the box center was positioned at the active site. PyMOL 3.1 (<https://www.pymol.org>) was used to visualize docking results and tunnels and to identify all amino acid residues involved in ligand binding. Candidate mutation sites were determined by integrating literature-reported sites with well-characterized functions, alanine scanning, substrate tunnel analysis, and sequence alignment results.

2.5 Preparative Reactions

The preparative reaction system consisted of 1–10 mL Tris-SO₄ buffer (50 mM, pH 7.5), 50 g/L *E. coli* wet cell paste (*l*cHheG WT/mutant), 10–50 mM substrate, NaOCN (1.5 eq) and 1–2.5% DMSO as co-solvent. The bacteria were re-suspended, mixed, and reacted at 200 rpm and 30 °C for 3 h. The reaction was terminated by adding an equal volume of ethyl acetate. After vortexing for 10 min, 10 µL of the upper organic phase was taken for TLC analysis with a developing solvent ratio of petroleum ether: ethyl acetate = 1:2, followed by ninhydrin staining and oven drying. Mutants showing clear product spots were marked. For enzyme samples with product formation, 500 µL of the organic supernatant was air-dried in a fume hood. The dried samples were dissolved in 1000 µL of HPLC-grade n-hexane: isopropanol (1:1), filtered through a 0.22 µm membrane filter, and analyzed by HPLC.

2.6 Analytical Methods

Product ee values were determined by high-performance liquid chromatography (HPLC) using Agilent-1220 system with a Chiralcel OD-H column (Daicel Co., Japan; 4.6 × 250 mm L, 5 µm). The mobile phase was n-hexane: isopropanol = 88:12 (v/v); flow rate 0.8 mL/min; column temperature 30°C; UV detection wavelength 220 nm. Substrate ee was calculated as: $ee_1 = [(R_1 - S_1)/(R_1 + S_1)] \times 100\%$ or $ee_1 = [(S_1 - R_1)/(S_1 + R_1)] \times 100\%$. Product ee was calculated as: $ee = [(R_2 - S_2)/(R_2 + S_2)] \times 100\%$. Enantiomeric ratio (*E* value) was calculated as: $E = \ln[(1 - ee_1)/(1 + ee_1/ee_2)] / \ln[(1 + ee_1)/(1 + ee_1/ee_2)]$.

3 Results and Discussion

3.1 Identification of Key Sites in *l*cHheG

*l*cHheG catalyzes the dehalogenation and subsequent ring-closure of (*S*)-1-chloro-3-phenoxy-2-propanol to generate the intermediate (*R*)-phenyl glycidyl ether (PGE), which is then converted to (*R*)-5-

phenoxymethyl-2-oxazolidinone from (*R*)-PGE in the presence of cyanate (van Hylckama Vlieg et al. 2001; You et al. 2012). Using AutoDock 4.0 software, (*S*)-1-chloro-3-phenoxy-2-propanol and (*R*)-PGE were individually docked into the *l*cHheG protein structure (Fig. 2), and 15 amino acid residues within 5 Å of the substrates were identified. Excluding the known catalytic triad (S152, Y165, and R169) and A153, which is situated distally from the substrate, alanine-scanning mutagenesis was performed on the remaining 11 residues: M14, Y18, V100, T101, L103, I104, T151, T195, N196, F200, and F203. Most of the resulting mutants, including Y18A, V100A, T101A, I104A, T151A, N196A, F200A, F203A, lost detectable activity. In contrast, the M14A and T195A variants retained partial activity, albeit at markedly reduced levels compared with the wild type (26% and 22%, respectively). Notably, the L103A mutant maintained approximately 99% of wild-type activity, suggesting that residue L103 represents a promising target for further mutagenesis.

Crystal structure analysis revealed that residues L103 and N196 are located near the substrate tunnel. In their study on *l*cHheG-catalyzed synthesis of (*S*)-4-aryl-2-oxazolidinones, Song et al. found through literature review and sequence alignment that *l*cHheG residues I104 and N196 correspond to I90 and N182 in the *Ab*HheG structure, respectively (Song et al. 2024) (Fig. S1). Mutations at these corresponding sites had been previously demonstrated to significantly affect the regio- and stereoselectivity of *Ab*HheG, with the optimal mutant *l*cHheG-I104F/N196W exhibiting good regioselectivity. Combining the alanine scanning results, L103, I104, and N196 were ultimately selected as candidate sites for single-point mutagenesis.

3.2 Construction and Screening of *l*cHheG Mutant Library

Site-directed mutagenesis was performed at residues L103, I104, and N196, generating 19 mutants. Their stereoselectivity toward phenyl glycidyl ether was examined by HPLC. A selection of results is presented in Table 1. Among the single-point mutants at L103, most mutants retained good enzymatic activity, but enantioselectivity was generally low. For I104 single-point mutants, although active mutants were fewer and exhibited lower activity than wild type, the I104C and I104F mutants showed enantioselectivities of 72% ee (*R*) and 85% ee (*R*), respectively, representing significant improvements over wild type. Among N196 single-point mutants, N196M and N196C showed slightly higher activity than wild type but poor enantioselectivity. N196F, N196H, and N196L exhibited much lower activity than wild type and favored the synthesis of (*S*)-configuration products. Overall, although the N196W mutant showed slightly lower activity (40%) than wild type, it exhibited good stereoselectivity (67% ee (*R*)), representing the best balance between activity and enantioselectivity among all single-point mutants. Therefore, N196W was selected as the template for subsequent iterative mutagenesis.

When selecting the double mutant, we prioritized avoiding spatially adjacent residues to minimize potential epistasis that could unpredictably impact the combined activity (Fuxreiter et al. 1997; Lipsh-Sokolik et al. 2024). Using the N196W mutant as a template, iterative mutagenesis was performed at V100, L103, and I104. Results are presented in Table 2. Mutants at Y18 were essentially inactive. Several mutants at V100 (e.g., V100A/N196W, V100L/N196W, V100Q/N196W) achieved 100% ee (*R*) but

exhibited extremely low activity (4% to 17%). In contrast, mutants at L103 showed relatively higher activity (25% to 69%). Among these, the L103H/N196W mutant displayed 37% activity with a product ee of 95%. Therefore, L103H/N196W was selected as the optimal double mutant for subsequent experiments.

3.3 Sequential Kinetic Resolution Catalyzed by Engineered IcHheG

Encouraged by these results and by previous studies showing that sequential kinetic resolution can further improve stereoselectivity (Majerić Elenkov et al. 2006), we next employed this mutant to directly catalyze the conversion of 1-chloro-3-phenoxy-2-propanol into the corresponding oxazolidinone, in an effort to further enhance the selectivity of the transformation. Related analyses were based on HPLC results (Fig. S4)

We first re-examined its performance in the PGE-based reaction. When PGE was employed as the substrate, the wild-type enzyme afforded a substrate ee of 53% and a product ee of 18% (*S*), with an *E* value below 10. In contrast, the L103H/N196W mutant delivered the product in 32% yield with 95% ee (*R*) and a substrate ee of 56%, corresponding to an *E* value of 68.6, consistent with the screening results and indicating markedly improved enantioselectivity.

When the substrate was changed to 1-chloro-3-phenoxy-2-propanol, the wild-type enzyme exhibited both substrate and product ee below 20%, with *E* < 10. Remarkably, the mutant L103H/N196W afforded the product in 49.5% yield, with the remaining substrate (*R*)-1b in >98% ee and the product in >99% ee, giving an *E* value exceeding 200. These results demonstrate that directly employing 1-chloro-3-phenoxy-2-propanol as the substrate not only dramatically enhances the product ee and enantioselectivity relative to the wild-type enzyme, but also outperforms the PGE-based reaction in terms of ee, *E* value, and product yield, thus confirming our hypothesis and establishing this as a superior synthetic strategy.

This transformation constitutes a sequential kinetic resolution, which elegantly circumvents catalyst compatibility issues and simplifies the catalytic system. In the halohydrin dehalogenase-catalyzed conversion of vicinal haloalcohols to epoxides, the introduction of a nucleophile suppresses the reverse reaction between the epoxide and the halide ion while maintaining a low steady-state concentration of the epoxide, thereby reducing spontaneous hydrolysis and enhancing the reaction rate and selectivity. Within this system, the L103H/N196W mutant preferentially converts (*S*)-1-chloro-3-phenoxy-2-propanol in the first step, generating (*R*)-PGE; the second step then preferentially transforms (*R*)-PGE. This double stereoselectivity, combined with the beneficial effects of the sequential kinetic resolution and the presence of the nucleophile, further enhances the enantiomeric excess while preventing the accumulation of intermediates. (Fig. 3).

3.4 Mechanistic Analysis of Enhanced Selectivity in Mutant L103H/N196W

To elucidate the mechanism of enhanced stereoselectivity in the mutant, a three-dimensional homology model of mutant L103H/N196W was constructed using Modeller 10.3 and structurally evaluated. The

Ramachandran plot (Fig. 4) indicated high model reliability. Literature reports indicate that enzymes can improve enantioselectivity by altering the shape and steric hindrance of substrate tunnels (Liskova et al. 2017; Wu et al. 2020; Wu et al. 2022). Static structure analysis showed that wild-type *l*cHheG has three tunnels (T1, T2, and T3) (Fig. 5a), while the mutant has only two tunnels (T1 and T2) (Fig. 5b), with the disappearance of T3 possibly related to the mutation at site 103. The position of the T1 tunnel remained essentially unchanged in the mutant, but the direction of the T2 tunnel changed, which may be associated with the mutation at site 196. Thus, tunnel alterations may be one of the reasons for the enhanced stereoselectivity of the L103H/N196W mutant.

Wild-type *l*cHheG and mutant L103H/N196W were docked with (*R*)-phenyl glycidyl ether, (*S*)-phenyl glycidyl ether, (*R*)-1-chloro-3-phenoxy-2-propanol, and (*S*)-1-chloro-3-phenoxy-2-propanol, respectively. (Fig. S2, S3).

For phenyl glycidyl ether, wild-type *l*cHheG formed strong hydrogen bonds with both (*R*)-1a and (*S*)-1a (Fig. 6a, b), with minimal differences in hydrogen bond distances, resulting in low enantioselectivity. In contrast, mutant L103H/N196W showed substantial differences in its interactions with the two substrate configurations (Fig. 6c, d). (*R*)-1a formed strong hydrogen bonds ($d = 2.5 \text{ \AA}$ and $2.6 \text{ \AA}$), while (*S*)-1a formed weaker hydrogen bonds ($d = 4.0 \text{ \AA}$ and $3.2 \text{ \AA}$), with a large difference in hydrogen bond distances. This indicates a stronger preference of the mutant for (*R*)-1a, thereby enhancing ee. This may be due to the W196 residue forming π - π interactions with (*S*)-1a during docking, which fixed the (*S*)-configured substrate, causing the oxygen atom of the epoxy ring of (*S*)-1a to be farther from the docking center hydrogen bond, affecting the reaction.

For 1-chloro-3-phenoxy-2-propanol, wild-type *l*cHheG formed strong hydrogen bonds with both (*R*)-1b and (*S*)-1b (Fig. 7a, b), with small differences in hydrogen bond distances, resulting in low ee values. However, mutant L103H/N196W formed weaker hydrogen bonds with (*R*)-1b ($d = 2.9 \text{ \AA}$ and $4.5 \text{ \AA}$) and stronger hydrogen bonds with (*S*)-1b ($d = 2.7 \text{ \AA}$ and $3.0 \text{ \AA}$), with a large difference in hydrogen bond distances (Fig. 7c, d). This indicates that the mutant has a stronger preference for substrate (*S*)-1b, thereby enhancing ee. Similarly, during docking with (*R*)-1b, π - π interactions between the benzene rings were formed in the mutant, with W196 pulling (*R*)-1b away from the active center, resulting in longer hydrogen bond distances and reduced ee for (*R*)-1b.

4 Conclusions

In this study, key amino acid sites affecting selectivity in halohydrin dehalogenase *l*cHheG were identified through molecular docking and alanine scanning. Through single-point and iterative combinatorial mutagenesis, the optimal double mutant L103H/N196W with significantly enhanced stereoselectivity was successfully obtained. This mutant effectively catalyzes cyanate-mediated ring-opening of phenyl glycidyl ether to produce the corresponding (*R*)-5-phenoxyethyl-2-oxazolidinone, achieving a product ee of 95%, which represents a substantial improvement over wild-type *l*cHheG. Experiments showed that when the substrate was switched to 1-chloro-3-phenoxy-2-propanol, the product ee could reach 99% due

to the advantage of the sequential kinetic resolution, accompanied by an increase in yield. Computational modeling and docking analysis revealed the critical roles of sites 103 and 196 in the substrate-binding pocket and substrate entry tunnel structure. The enhanced stereoselectivity of the mutant is attributed to differences in attack distances between (*S*) and (*R*) enantiomers and alterations in the substrate tunnels. This study provides a theoretical basis for the molecular engineering of halohydrin dehalogenases and offers an effective method for the enantioselective synthesis of chiral oxazolidinone compounds

Declarations

Author Contribution

Jiacen Bi: Writing-original draft, Methodology, Investigation. Ji Ding: Investigation, Validation, Formal analysis. Feng Xue: Resources, Supervision, Writing - review & editing, Project administration.

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Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures

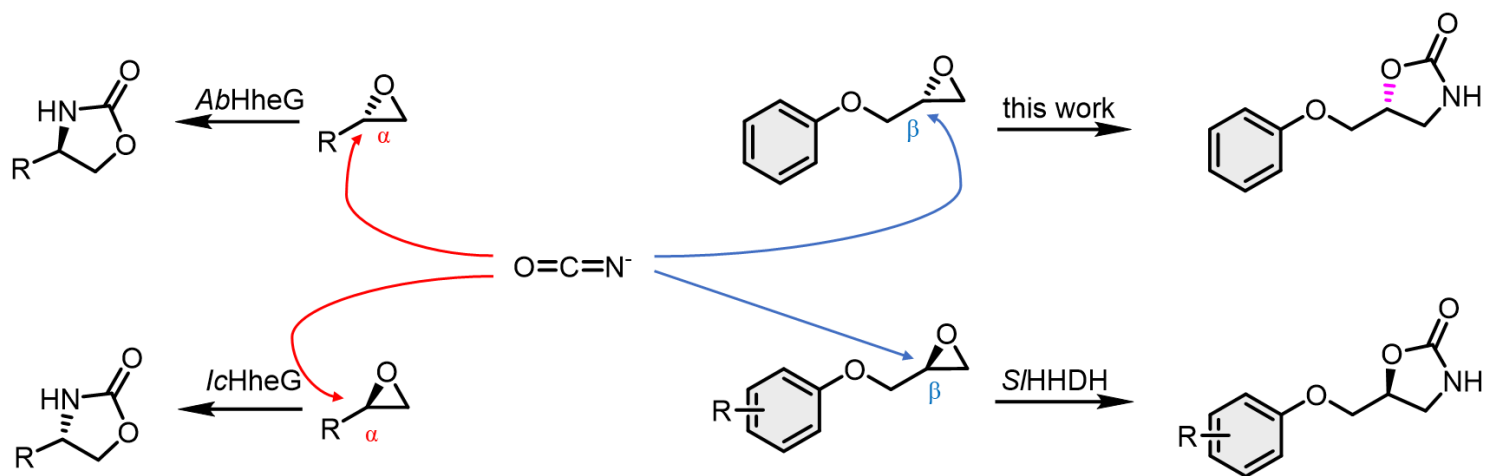


Figure 1

Possible ring-opening reactions of HHDH-catalyzed substrates with cyanate.

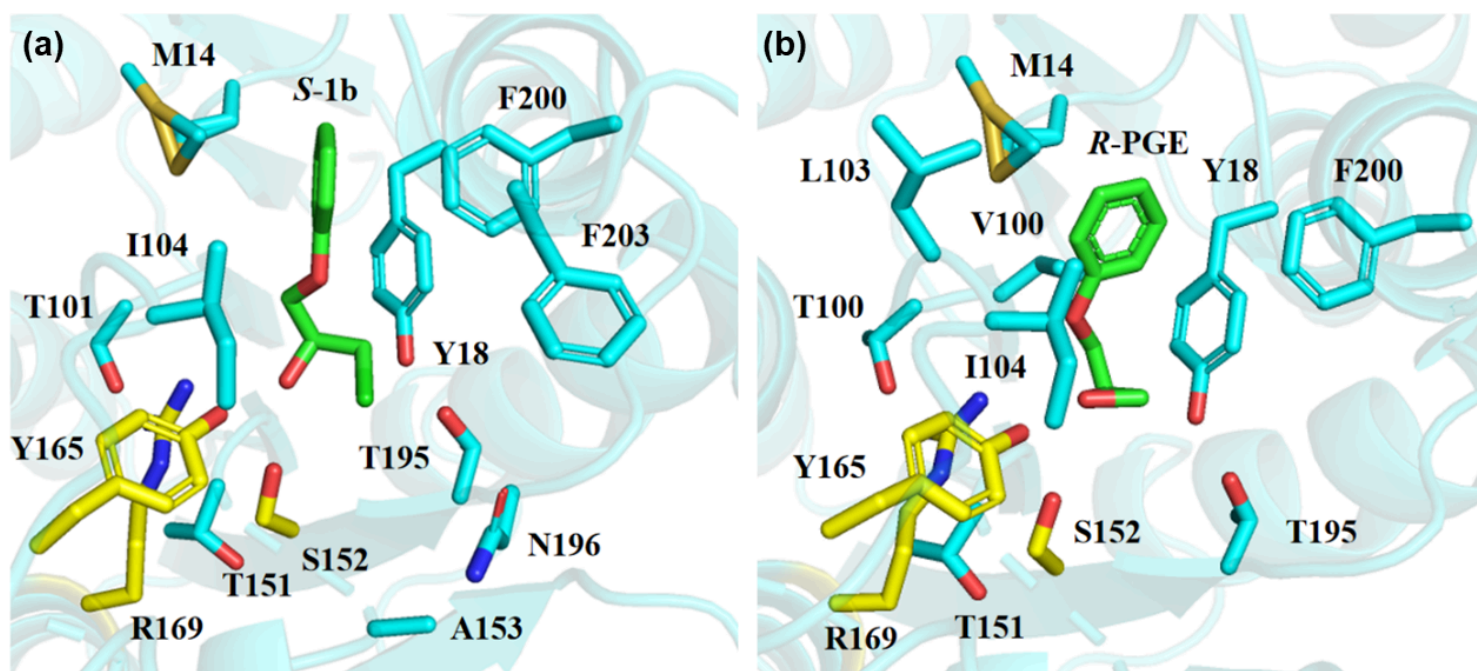


Figure 2

Docking of *IcHheG* with substrates (S)-1-chloro-3-phenoxy-2-propanol (a) and (R)-PGE (b)

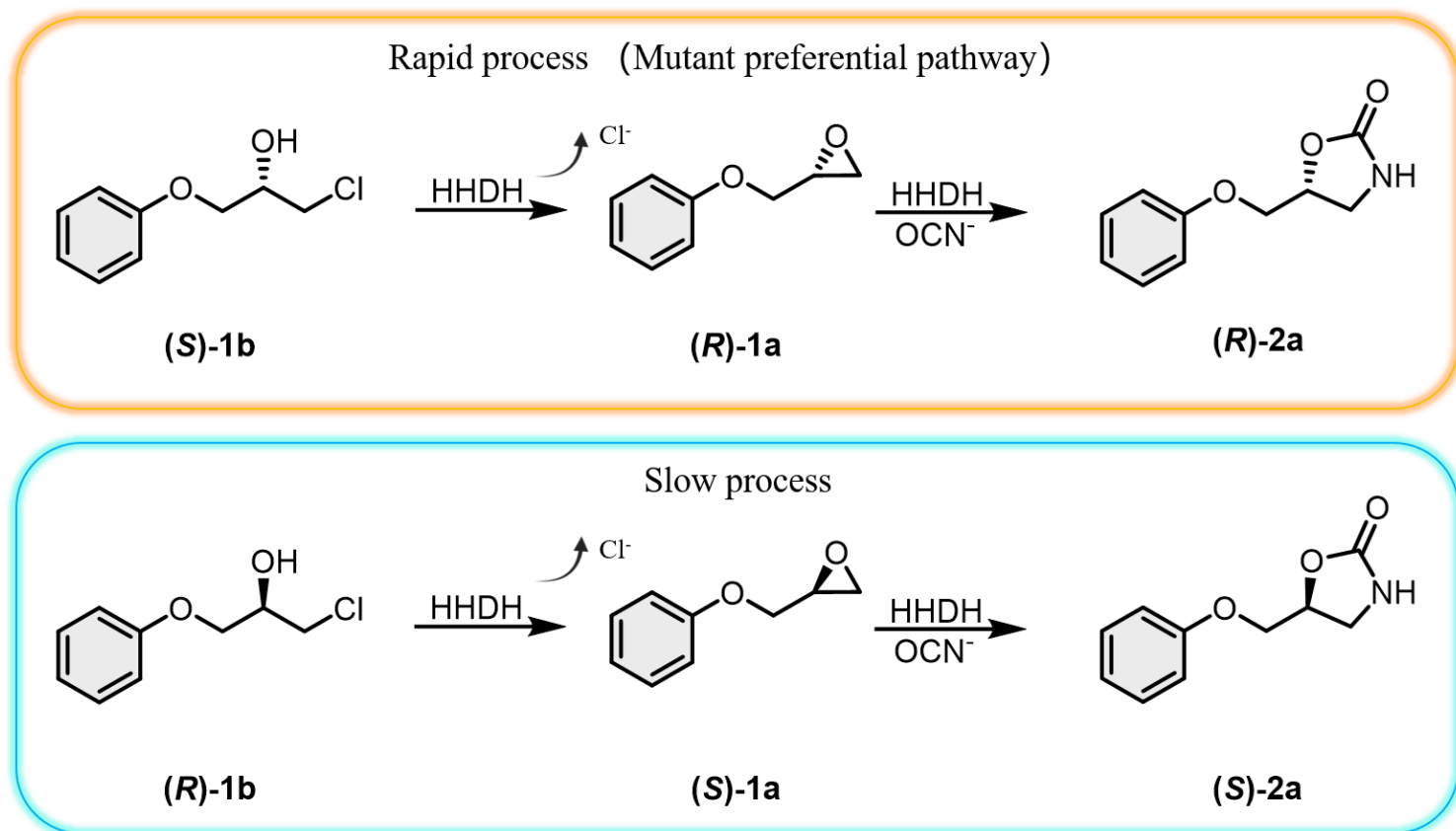


Figure 3

Sequential kinetic resolution of 1-chloro-3-phenoxy-2-propanol

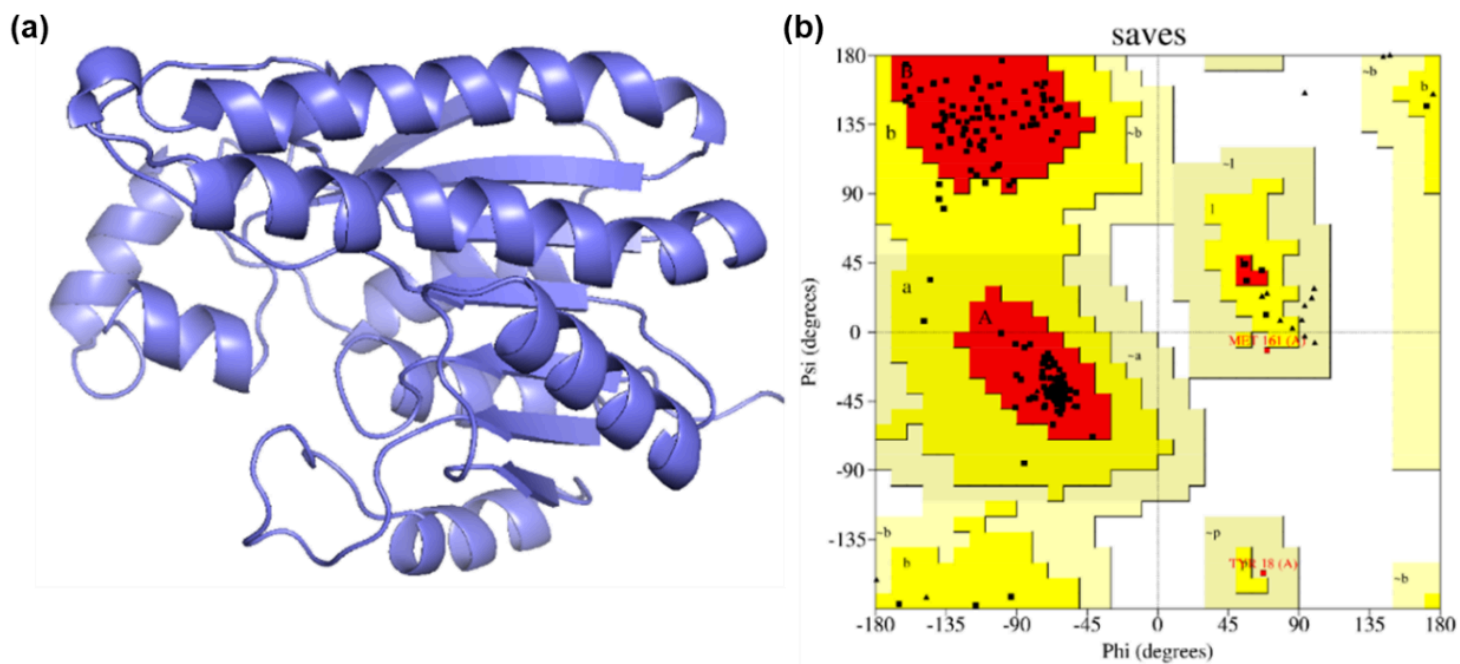


Figure 4

Access tunnels of WT-*lcHheG* (a) and mutant L103H /N196W (b). T1 is shown in blue, T2 in green, and T3 in red.

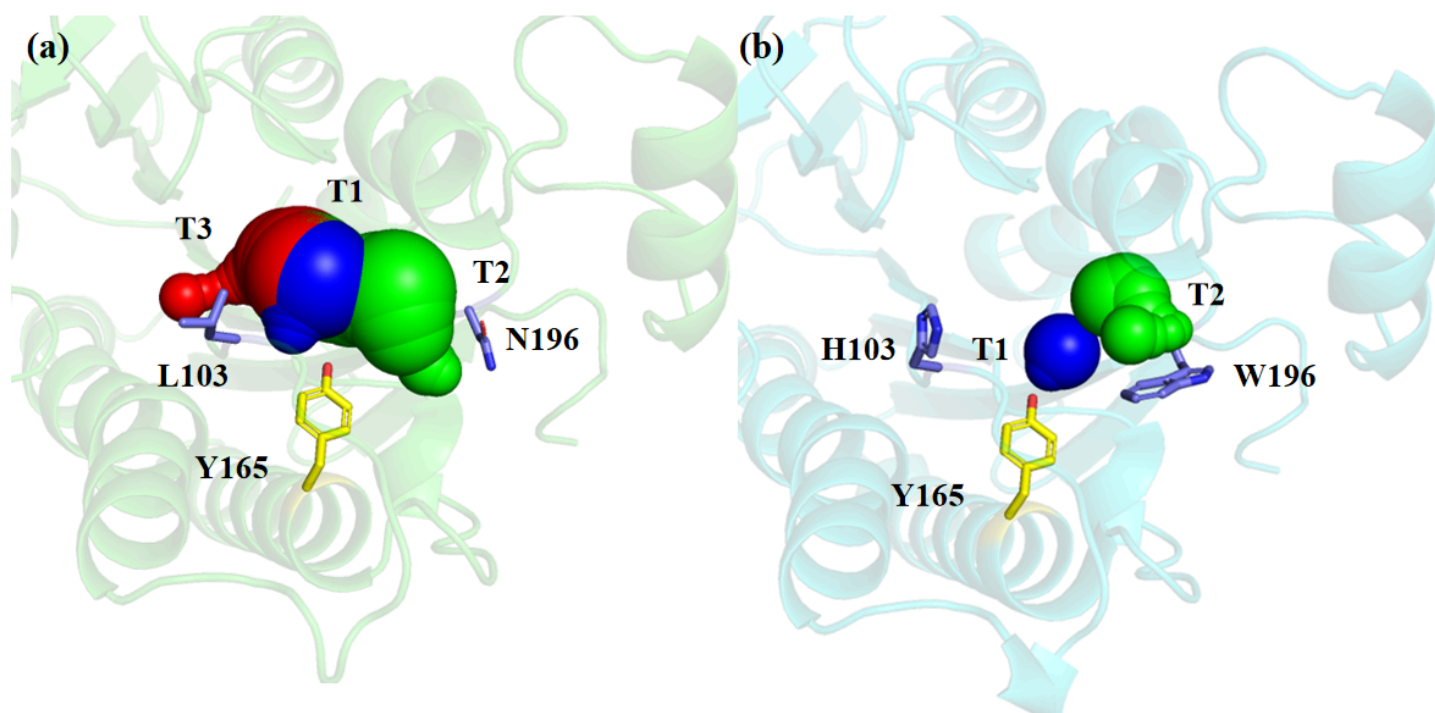


Figure 5

Structure of mutant L103H/N196W (a) and its Ramachandran plot (b)

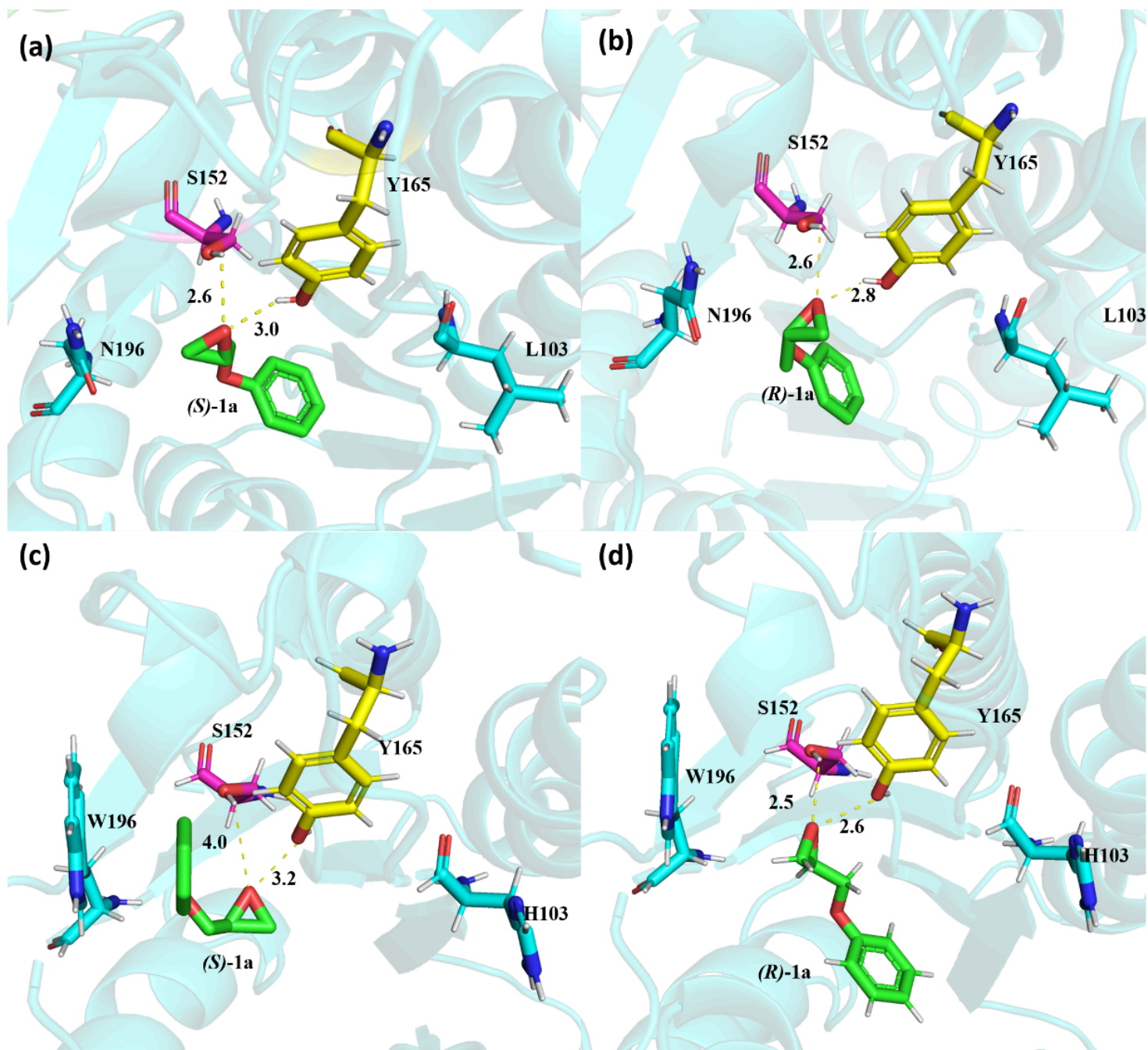


Figure 6

Molecular docking diagrams of IcHheG (a, b) and L103H/N196W (c, d) for (S, R)-PGE

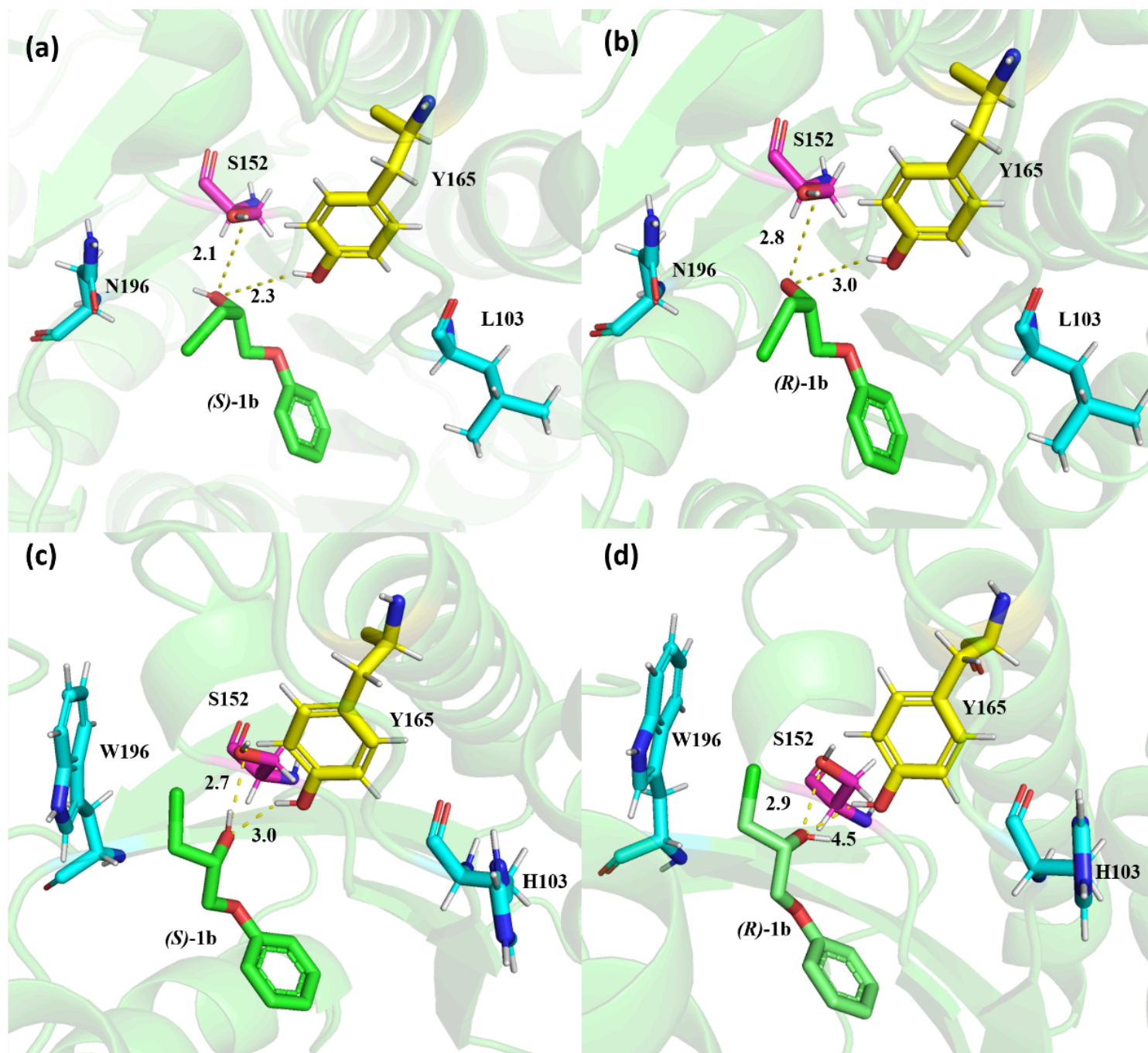


Figure 7

Molecular docking diagrams of *IChHeG* (a, b) and *L103H/N196W* (c, d) for (*S*, *R*)-1-chloro-3-phenoxy-2-propanol selectivity

Supplementary Files

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