Supporting Information

Rational Design, Preparation and Characterization of a Therapeutic Enzyme Mutant with Improved Stability and Function for Cocaine Detoxification

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X-ray crystallography data collection and refinement statistics (Table S1). Backbone superposition between the X-ray crystal structures of E172-173 and E196-301 (Figure S1). Time-dependence of important H···O distances (relevant to hydrogen bonds) from the MD-simulated E172-173 and E196-301 structures (Figure S2). Intermonomer disulfide bonds in the CocE mutant (E196-301) dimer refined in space group $P6_5$ (Figure S3).

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Table S1. Data collection and refinement statistics.

Wavelength (Å)	1.0
Resolution range (Å)	50-2.34 (2.42-2.34)[a]
Space group	P $6_5$22
Unit cell	a=b=106.6 Å c=220.532 Å α=β=90° c=120°
Total reflections	561710
Unique reflections	31943
Multiplicity	17.5 (9.9)
Completeness (%)	99.73 (97.41)
Mean I/sigma(I)	17.71 (2.70)
Wilson B-factor	28.84
R-sym	0.161 (0.956)
R-factor	0.1769 (0.2488)
R-free	0.2208 (0.3348)
Number of atoms	4793
macromolecules	4364
water	429
Protein residues	571
RMS(bonds)	0.005
RMS(angles)	0.89
Ramachandran favored (%)	95
Ramachandran outliers (%)	0
Clashscore	5.37
Average B-factor	27.80
macromolecules	27.40
solvent	31.80

[a]Statistics for the highest-resolution shell are shown in parentheses.

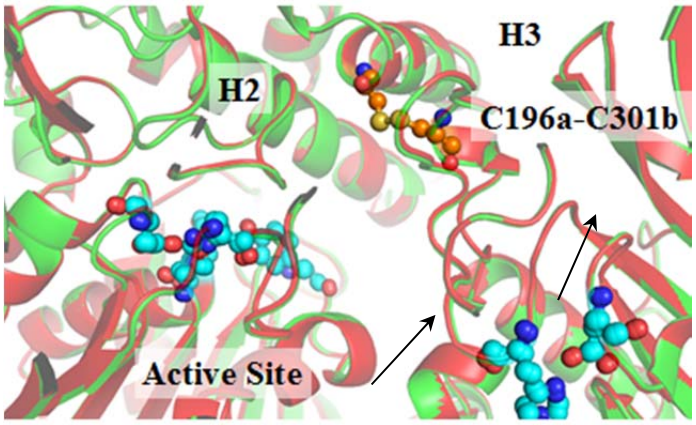

Figure S1. Backbone superposition between the X-ray crystal structures of E172-173 and E196-301. E172-173 is represented in green ribbons, and E196-301 is represented in red ribbons. The black arrow indicates the shift direction. Here, E172-173 represents T172R/G173Q CocE, and E196-301 refers to T172R/G173Q/L196C/I301C CocE.

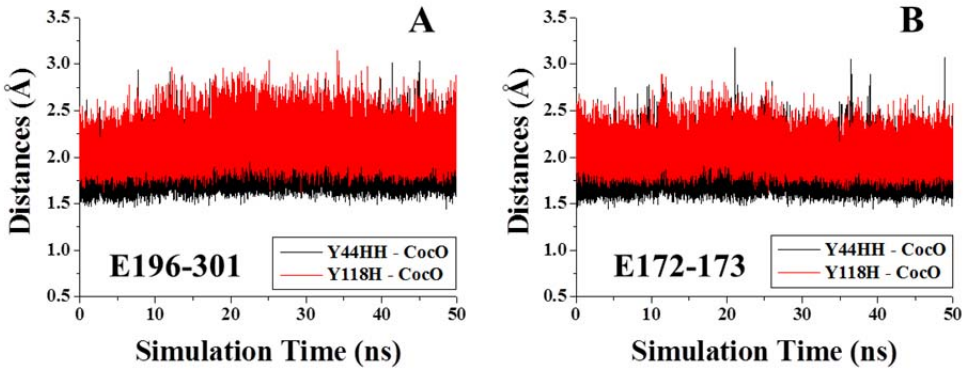

Figure S2. Time-dependence of important H···O distances (relevant to hydrogen bonds) from the MD-simulated E172-173 and E196-301 structures. Y44HH–CocO represents the distance between the hydroxyl hydrogen (denoted as HH) of the Y44 side chain and the carbonyl oxygen (denoted as CocO) of (–)-cocaine benzoyl ester. Y118H–CocO refers to the distance between hydrogen (H) of the Y118 backbone and the carbonyl oxygen (CocO) of (–)-cocaine benzoyl ester. E172-173 refers to T172R/G173Q CocE, and E196-301 refers to T172R/G173Q/L196C/I301C CocE.

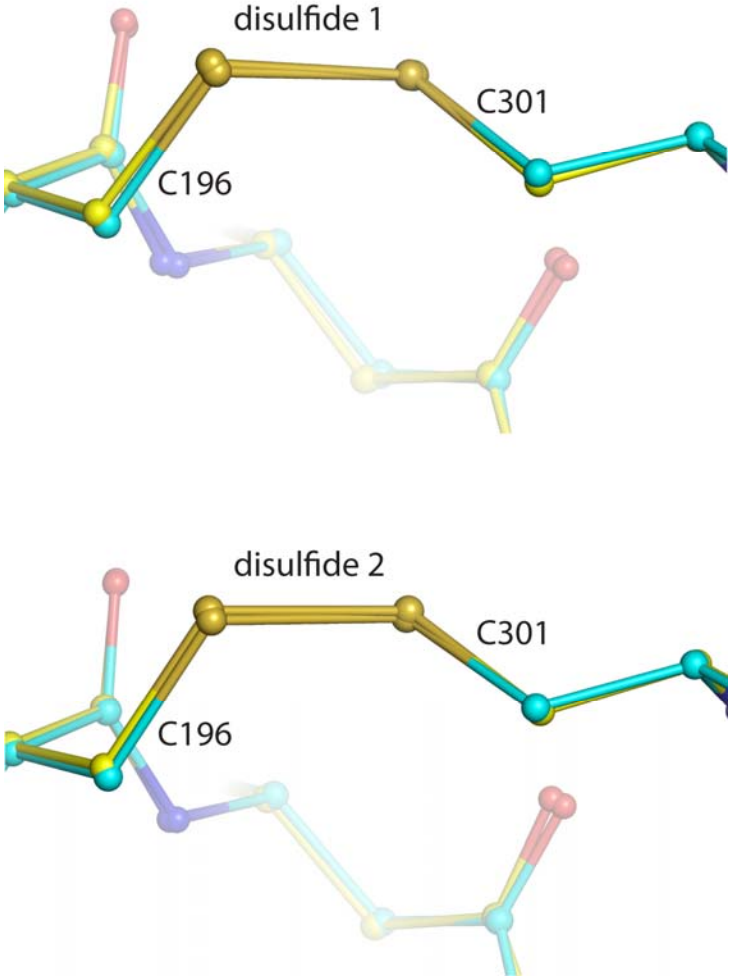

Figure S3. Intermonomer disulfide bonds in the CocE mutant (E196-301) dimer refined in space group $P6_5$. The complete process of structure determination was carried out in a space group with lower symmetry than the true space group ($P6_522$) in order to assess the effects of the dimer being located on a crystallographic two-fold axis. Noncrystallographic symmetry restraints were not used during refinement. The panels show the two, now not strictly identical, disulfide bonds for the final model (R_{work} – 0.17, R_{free} – 0.21; yellow carbons) superimposed on the identical disulfide bonds in the model refined on the crystallographic two-fold axis (cyan carbons).