# Structure-and-mechanism-based design and discovery of therapeutics for cocaine overdose and addiction

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(−)-Cocaine is a widely abused drug and there is currently no available anti-cocaine therapeutic. Promising agents, such as anti-cocaine catalytic antibodies and high-activity mutants of human butyrylcholinesterase (BChE), for therapeutic treatment of cocaine overdose have been developed through structure-and-mechanism-based design and discovery. In particular, a unique computational design strategy based on the modeling and simulation of the rate-determining transition state has been developed and used to design and discover desirable high-activity mutants of BChE. One of the discovered high-activity mutants of BChE has a ~456-fold improved catalytic efficiency against (−)-cocaine. The encouraging outcome of the structure-and-mechanism-based design and discovery effort demonstrates that the unique computational design approach based on transition state modeling and simulation is promising for rational enzyme redesign and drug discovery. The general approach of the structure-and-mechanism-based design and discovery may be used to design high-activity mutants of any enzyme or catalytic antibody.

## 1 Introduction

Cocaine overdose and addiction is a major medical and public health problem that continues to defy treatment.[1–4] Cocaine reinforces self-administration in relation to the peak serum concentration of the drug, the rate of rise to the peak and the degree of change of the serum level. Potent central nervous system (CNS) stimulation is followed by depression. With overdose of the drug, respiratory depression, cardiac arrhythmia and acute hypertension are common effects. The disastrous medical and social consequences of cocaine addiction, such as violent crime, loss in individual productivity, illness, and death, have made the development of an effective pharmacological treatment a high priority.[5,6] Most of the previously employed anti-addiction strategies use the classical pharmacodynamic approach, *i.e.* developing small molecules that interact with one or more neuronal binding sites, with the goal of blocking or counteracting a drug's neuropharmacological actions. However, despite decades of effort, existing pharmacodynamic approaches to cocaine abuse treatment have not yet proven successful.[5–8]

The inherent difficulties in antagonizing a blocker like cocaine have led to the development of the pharmacokinetic approach that aims at acting directly on the drug itself to alter its distribution or accelerate its clearance.[7–14] Pharmacokinetic antagonism of cocaine could be implemented by administration of a molecule, such as an anti-cocaine antibody, which binds tightly to cocaine so as to prevent cocaine from crossing the blood–brain barrier.[8,15–20] An alternative pharmacokinetic agent would be an enzyme or a catalytic antibody (regarded as an artificial enzyme) that not only binds but also accelerates cocaine metabolism and thereby

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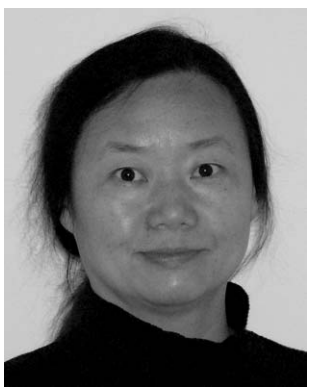

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freeing itself for further binding.[8,16–25] Usually, a pharmacokinetic agent would not be expected to cross the blood–brain barrier and thus would itself have no direct pharmacodynamic action, such as abuse liability.[5] An ideal pharmacokinetic agent for this purpose should be a potent enzyme or catalytic antibody which transforms cocaine into biologically inactive metabolites.

The primary cocaine-metabolizing pathway in primates is hydrolysis at the benzoyl ester or methyl ester group.[5,6] Benzoyl ester hydrolysis generates ecgonine methyl ester (EME), whereas methyl ester hydrolysis yields benzoylecgonine (BE). The major cocaine-metabolizing enzymes in humans are butyrylcholinesterase (BChE) which catalyzes cocaine hydrolysis at the benzoyl ester and two liver carboxylesterases, denoted by hCE-1 and hCE-2 that catalyze hydrolysis at the methyl ester and the benzoyl ester, respectively. Among the three, BChE is the principal cocaine hydrolase in human serum. Hydrolysis accounts for about 95% of cocaine metabolism in humans. The remaining 5% is deactivated through oxidation by the liver microsomal cytochrome P450 system, producing norcocaine. EME appears the least pharmacologically active of the cocaine metabolites and may even cause vasodilation, whereas both BE and norcocaine appear to cause vasoconstriction and lower the seizure threshold, similar to cocaine itself. Norcocaine is hepatotoxic and a local anesthetic. Clearly, the metabolic pathway through hydrolysis at the cocaine benzoyl ester by BChE is most suitable for amplification. However, the catalytic activity of this plasma enzyme is about a thousand-fold lower against the naturally occurring (−)-cocaine than that against the biologically inactive (+)-cocaine enantiomer.[26–29] (+)-Cocaine can be cleared from plasma in seconds and prior to partitioning into the CNS, whereas (−)-cocaine has a plasma half-life of ~45–90 min, long enough for manifestation of the CNS effects which peak in minutes.[5] Hence, a BChE mutant with a higher catalytic activity against (−)-cocaine, or a catalytic antibody with a sufficiently high catalytic activity for (−)-cocaine hydrolysis at the benzoyl ester, is highly desirable for use as an exogenous enzyme/catalytic antibody in humans.

Based on the above background, it is interesting for developing cocaine overdose and addiction therapeutics to rationally design and discover engineered enzymes and/or catalytic antibodies that have the desirable catalytic activities. To perform truly rational design and discovery of an engineered enzyme or a catalytic antibody, one first needs to understand the fundamental reaction mechanism concerning how cocaine can be metabolized by the enzyme/catalytic antibody. As discussed below, encouraging progress has been made in understanding the detailed reaction pathways and free energy profiles for hydrolysis of (−)-cocaine and (+)-cocaine in water and in BChE through state-of-the-art computational studies. New anti-cocaine catalytic antibodies and high-activity mutants of human BChE have been designed and discovered based on the detailed molecular structures and mechanisms.

## 2 Mechanism for non-enzymatic hydrolysis of cocaine in aqueous solution and design of anti-cocaine catalytic antibodies

Anti-cocaine catalytic antibodies are a novel class of artificial enzymes with unique potential as therapeutic agents for cocaine overdose and addiction.[21,22] This novel class of artificial enzymes, elicited by immunization with transition state analogs of cocaine benzoyl ester hydrolysis, have the unique potential as therapeutic artificial enzymes due to their biocompatibility and extended plasma half-life. The design of a transition state analog eliciting a catalytic antibody[30] is based on the mechanism of the corresponding non-enzymatic reaction in water, specifically the transition state structure for the rate-determining step. Hence, a more complete understanding of the mechanism for cocaine hydrolysis in aqueous solution could provide valuable insights into the rational design of more effective transition state analogs. This is why computational studies[31] for development of anti-cocaine catalytic antibodies have been focused on the reaction coordinate calculations on the detailed mechanisms for non-enzymatic hydrolysis of cocaine in water.

### 2.1 Hydrolysis of chair cocaine

The most stable conformation of cocaine in aqueous solution is the chair form (Fig. 1 and 2). As one can see in Fig. 1 and 2, a cocaine molecule has two carboxylic acid ester groups: a benzoyl ester and a methyl ester. Hence, the fundamental reaction pathway for non-enzymatic hydrolysis of cocaine at both benzoyl ester and methyl ester groups is expected to be similar to that for the usual non-enzymatic hydrolysis of a carboxylic acid ester. The hydrolysis of the majority of common alkyl esters, RCOOR′, in neutral solution occurs by the attack of hydroxide ion at the carbonyl carbon.[32–34] This mode of hydrolysis has been designated as $B_{AC}2$ (base-catalyzed, acyl–oxygen cleavage, bimolecular), and is believed to

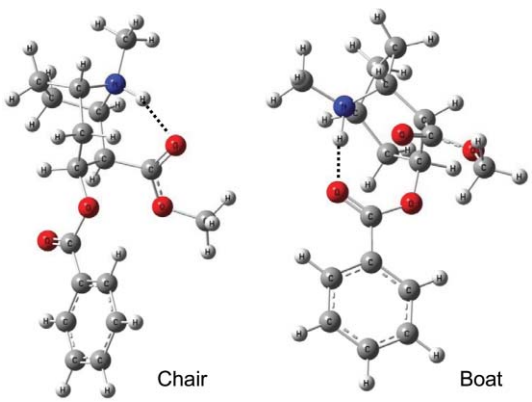


**Fig. 1** Geometries of the chair and boat conformations of (−)-cocaine optimized at the B3LYP/6-31+G* level.

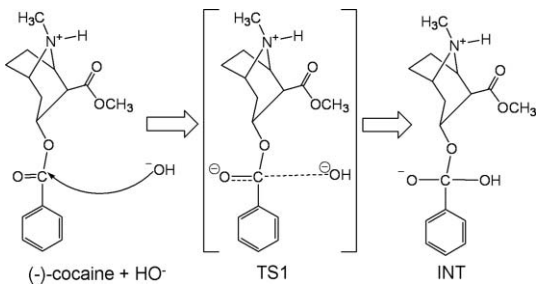


**Fig. 2** The first reaction step of (−)-cocaine hydrolysis at the benzoyl ester group in water.

occur by a two-step mechanism although a concerted pathway can arise in the case of esters containing very good leaving groups (corresponding to a low p$K_a$ value for R′OH). The generally accepted two-step mechanism consists of the formation of a tetrahedral intermediate (first step), followed by decomposition of the tetrahedral intermediate to yield products $RCOO^-$ + R′OH (second step). Degradation of cocaine may take place through the $B_{AC}2$ route of hydrolysis of either the benzoyl ester or the methyl ester group. First-principles electronic structure calculations accounting for solvent effects have been carried out to study the detailed competing reaction pathways of the non-enzymatic hydrolysis of cocaine and the corresponding free energy barriers. The first-principles computational studies[31,35] have confirmed the co-existence of the competing reaction pathways. Below, we will only discuss the hydrolysis of cocaine at the benzoyl ester group (Fig. 2), as this is the pathway relevant to the design of the desirable catalytic antibody.

Based on the first-principles reaction coordinate calculations,[31,35] the rate-determining step of the cocaine hydrolysis is the first step, *i.e.* the attack of hydroxide oxygen at the carbonyl carbon of cocaine (Fig. 2). The optimized geometry of the transition state, denoted by TS1-chair, for the dominant reaction pathway of the first step of the cocaine benzoyl ester hydrolysis is depicted in Fig. 3. This mechanistic insight suggests that rational design of a transition state analog (TSA) should be based on the transition state for the first step of the cocaine hydrolysis. Indeed, previous TSA design pioneered by Landry *et al.* was based on the first reaction step, *i.e.* design of the stable analogs of the transition state (TS1) for the first reaction step. Depicted in Fig. 4 are TSA structures that were used to successfully elicit anti-cocaine catalytic antibodies. The first anti-cocaine catalytic antibody[22] was elicited by using TSA-1 in Fig. 4. The structural change from TSA-1 to TSA-2 (by increasing the size of the TSA structure) has led to development of a new antibody[25] with a significantly improved catalytic activity for (−)-cocaine hydrolysis. The most active anti-cocaine catalytic antibody discovered so far is monoclonal antibody (mAb) 15A10 (elicited by TSA-2) with $K_M$ = 220 μM and $k_{cat}$ = 2.3 $min^{-1}$. The $k_{cat}$ value of mAb 15A10 is larger than the first-order rate constant of the non-enzymatic hydrolysis of cocaine by ~23 000-fold.[25]

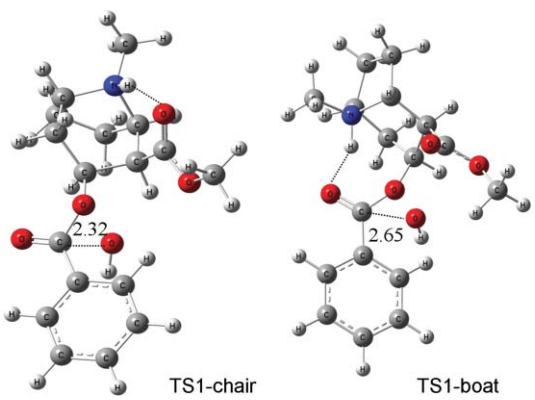


**Fig. 3** Geometries of the transition states optimized at the B3LYP/6-31+G* level for the first step of (−)-cocaine hydrolysis at the benzoyl ester group of (−)-cocaine in the chair and boat conformations.

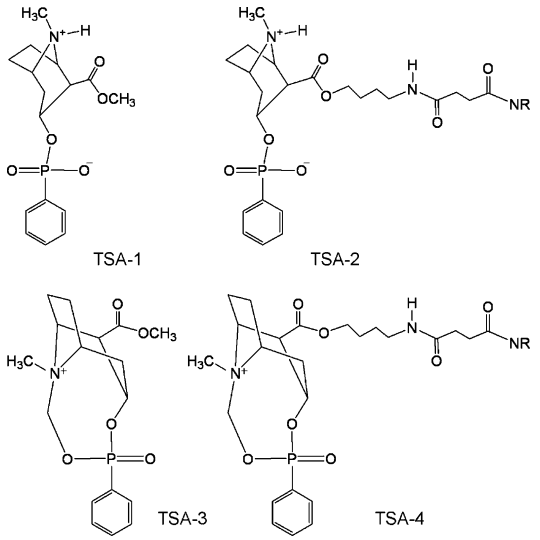


**Fig. 4** Transition state analogs that can be used to elicit anti-cocaine catalytic antibodies.

### 2.2 Hydrolysis of boat cocaine

Although the free energy of the boat conformation of cocaine (Fig. 1) is higher than that of the chair conformation, it is still interesting for TSA design to understand the reaction mechanism for boat cocaine hydrolysis. This is because an elicited antibody might be able to recruit cocaine from the chair conformation to the less stable boat form and bring the *syn*-protonated amine and benzoyl ester into proximity (Fig. 1). Based on the first-principles reaction coordinate calculations,[31] the optimized transition state geometry is shown in Fig. 3 as TS1-boat. Based on the transition state structure (TS1-boat), a new TSA structure, *i.e.* TSA-3 in Fig. 4, has been designed, synthesized, and used to successfully elicit anti-cocaine catalytic antibodies.[36] The new catalytic antibodies elicited by using TSA-3 so far are not more active than the previously discovered catalytic antibodies against cocaine. However, it might be interesting to test another possible TSA structure, *i.e.* TSA-4 depicted in Fig. 4, in the future in light of the effects of the structural change from TSA-1 to TSA-2.

## 3 Catalytic mechanism for BChE-catalyzed hydrolysis of cocaine

Reaction coordinate calculations for an enzymatic reaction begin with a concept of the enzyme–substrate binding in the prereactive enzyme–substrate complex. Different starting structures for the enzyme–substrate complex can lead to completely different reactions. The molecular docking and MD simulations[37] demonstrate that (−)/(+)-cocaine first slides down the substrate-binding gorge to bind to W82 and stands vertically in the gorge between D70 and W82 (non-prereactive complex) and then rotates to a position in the catalytic site within a favorable distance for the nucleophilic attack and hydrolysis by S198 $O^{\gamma}$ (prereactive complex). In the prereactive complex, cocaine lies horizontally at the bottom of the gorge. The main structural difference between the BChE–(−)-cocaine complexes and the corresponding BChE–(+)-cocaine complexes exists in the relative position of the cocaine methyl ester

 

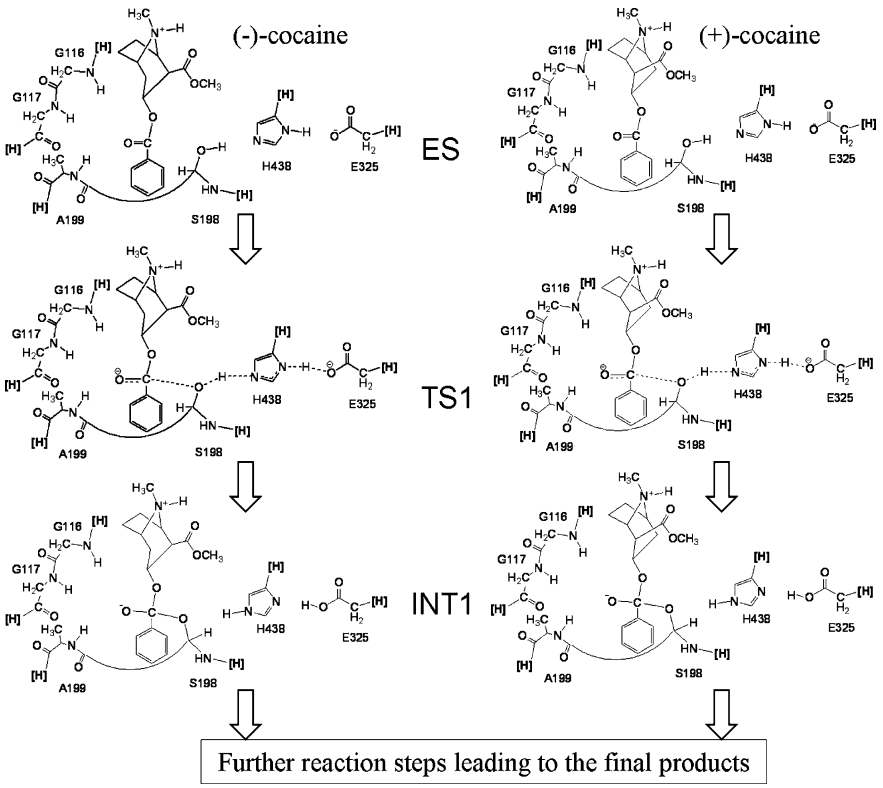


**Fig. 5** Schematic representation of the first step of the chemical reaction process for BChE-catalyzed hydrolyses of (−)- and (+)-cocaine. Notation [H] refers to a non-hydrogen atom used in a QM/MM calculation to saturate a cut covalent bond.

group.[38] The molecular structures of (−)- and (+)-cocaine can be seen in the E–S complexes depicted in Fig. 5.

As the simulated prereactive BChE–(−)-cocaine and BChE–(+)-cocaine complexes are essentially the same as the prereactive BChE–butyrylcholine (BCh) complex,[37] one can reasonably expect that BChE-catalyzed hydrolyses of (−)-cocaine and (+)-cocaine follow a reaction pathway similar to that for BChE-catalyzed hydrolysis of BCh. A remarkable difference between (−)-cocaine and (+)-cocaine is associated with the relative position of the C-2 methyl ester group. The C-2 methyl ester group of (−)-cocaine remains on the same side of the carbonyl of the benzoyl ester as the attacking hydroxyl oxygen (S198 $O^{\gamma}$), whereas the C-2 methyl ester of (+)-cocaine remains on the opposite side. This difference could cause a difference in hydrogen bonding, electrostatic, and van der Waals interactions during the catalytic reaction process, and result in a significant difference in free energy barriers (activation free energies). Nevertheless, the basic BChE mechanism for both enantiomers may resemble the common catalytic mechanism for ester hydrolysis in other serine hydrolases,[37,39] including the thoroughly investigated AChE.[40–44] This mechanistic hypothesis has been supported by reported reaction coordinate calculations using the first-principles quantum mechanics (QM) and hybrid quantum mechanics and molecular mechanics (QM/MM) methods.[37,45]

Based on the QM and QM/MM reaction coordinate calculations, the first step of the chemical reaction process is initialized by S198 $O^{\gamma}$ attack at the carbonyl carbon of the cocaine benzoyl ester to form the first tetrahedral intermediate (INT1) through the first transition state (TS1). Fig. 5 is a schematic representation of the first step of BChE-catalyzed hydrolyses of (−)-cocaine and (+)-cocaine, showing only the substrate and important groups from the catalytic triad (S198, E325, and H438) and the three-pronged oxyanion hole (G116, G117, and A199). During the formation of INT1, the C–O bond between the carbonyl carbon of the substrate and S198 $O^{\gamma}$ gradually forms, while the proton at S198 $O^{\gamma}$ gradually transfers to the imidazole N atom of H438 which acts as a general base. The QM/MM-optimized TS1 geometry for the (−)-cocaine hydrolysis is shown in Fig. 6.

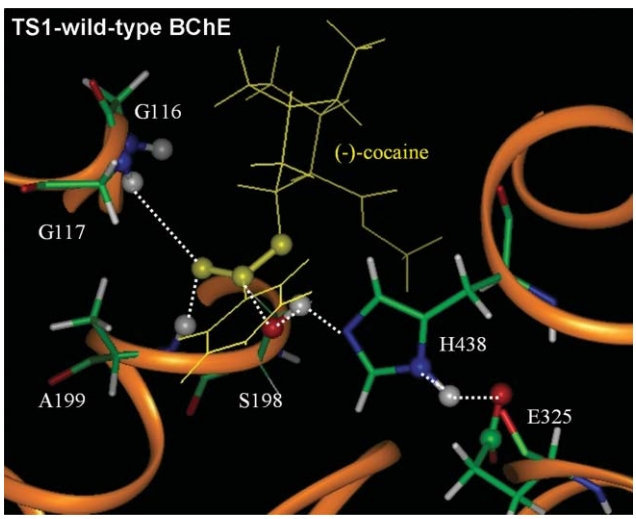


**Fig. 6** MD-simulated TS1 geometry for (−)-cocaine hydrolysis catalyzed by wild-type BChE.

All of the computational results,[37,38] in comparison with available experimental data, demonstrate that the rate-determining step of the BChE-catalyzed hydrolysis of (+)-cocaine is the chemical reaction process, whereas for (−)-cocaine the change from the non-prereactive complex to the prereactive complex is rate determining and has a free energy barrier higher than that of the chemical reaction process by ~4 kcal $mol^{-1}$.

## 4 Structure-and-mechanism-based design of high-activity mutants of BChE

### 4.1 Mutant design based on modeling and simulation of the enzyme–substrate binding

Generally speaking, for rational design of a mutant enzyme with an improved catalytic activity for a given substrate, one needs to design possible mutation(s) that can accelerate the rate-determining step of the entire catalytic reaction process while the other steps are not slowed down by the mutation(s). Now that (−)-cocaine rotation from the non-prereactive complex to the prereactive complex is rate determining for (−)-cocaine hydrolysis catalyzed by wild-type BChE, the initial design of possible high-activity mutants of BChE can be focused on the improvement of the (−)-cocaine rotation in BChE, with the aim to accelerate the change from the non-prereactive BChE–(−)-cocaine complex to the prereactive BChE–(−)-cocaine complex. A detailed analysis[37,38] of the MD-simulated structures of wild-type BChE binding with (−)-cocaine and (+)-cocaine revealed that Y332 is a key residue, hindering the structural change from the non-prereactive BChE–(−)-cocaine complex to the prereactive BChE–(−)-cocaine complex.[37,38] A number of possible mutants of BChE were proposed for *in vitro* experimental tests.[37,38,46–50] The earliest design of BChE mutants was only based on the modeled or simulated structure of the non-prereactive BChE–(−)-cocaine complex with wild-type BChE; the possible dynamics of the proposed BChE mutants were not examined. Some of the proposed mutants indeed have a significantly improved catalytic efficiency against (−)-cocaine,[37,38,46–50] *e.g.* the A328W/Y332A mutant has a ~9.4-fold improved catalytic efficiency compared to the wild-type against (−)-cocaine.

In order to more reliably predict the BChE mutants with a possibly higher catalytic efficiency against (−)-cocaine, MD simulations were also performed on the structures of (−)-cocaine binding with a number of hypothetical BChE mutants in their non-prereactive and prereactive complexes.[38] The MD simulations on the E–S structures in water led to more reliable predictions. For example, the MD-simulated E–S structures[38] suggest that both A328W/Y332A and A328W/Y332G mutants of BChE should have a higher catalytic efficiency than wild-type BChE for (−)-cocaine hydrolysis. Further, the MD simulations also suggest that the energy barrier for the (−)-cocaine rotation in A328W/Y332G BChE should be slightly lower than that in A328W/Y332A BChE and, therefore, the catalytic efficiency of A328W/Y332G BChE for the (−)-cocaine hydrolysis should be slightly higher than that of A328W/Y332A BChE.[38] In addition, the MD simulations predict that A328W/Y332A/Y419S BChE should be inactive, or have a considerably lower catalytic efficiency than the wild-type, for (−)-cocaine hydrolysis because (−)-cocaine binds with the mutant BChE in a way that is not suitable for the catalysis.[38] Following the computational predictions, *in vitro* experimental studies (including site-directed mutagenesis, protein expression, and enzyme activity assays against (−)-cocaine) were carried out.[38] The experimental kinetic data qualitatively confirm the theoretical predictions based on the MD simulations. In particular, the catalytic efficiency of A328W/Y332G BChE is indeed slightly higher than that of A328W/Y332A BChE against (−)-cocaine,[38,51] and A328W/Y332A/Y419S BChE is indeed inactive against (−)-cocaine.[38]

### 4.2 Mutant design based on modeling and simulation of rate-determining transition state

For further mutant design starting from the A328W/Y332A and A328W/Y332G mutants, a crucial question is whether the rate-determining step of the (−)-cocaine hydrolysis catalyzed by these BChE mutants is still the same as that catalyzed by the wild-type. Further computational studies and analysis of the experimental data suggest that the rate-determining reaction step for (−)-cocaine hydrolysis catalyzed by the A328W/Y332A and A328W/Y332G mutants becomes the first step of the chemical reaction process, as the hindering of the (−)-cocaine rotation from the non-prereactive BChE-(−)-cocaine complex to the prereactive BChE-(−)-cocaine complex has been removed by the Y332A or Y332G mutation.[38,52,53] Therefore, starting from the A328W/Y332A or A328W/Y332G mutant, the rational design of further mutation(s) to improve the catalytic efficiency of BChE against (−)-cocaine can aim to decrease the energy barrier for the first reaction step without significantly affecting the E–S formation and other chemical reaction steps.[52]

In principle, the free energy barrier for (−)-cocaine hydrolysis catalyzed by each hypothetical mutant of BChE can be predicted by performing QM/MM reaction coordinate calculations and the corresponding free energy calculations. Unfortunately, it would be very time-consuming to practically carry out the QM/MM reaction coordinate calculations on a lot of hypothetic mutants for the purpose of the mutant design. A unique computational strategy[52] has been developed to virtually screen various possible BChE mutants based on MD simulations of the rate-determining transition state (*i.e.* TS1). The unique computational strategy[52] makes possible the MD simulation using a classical force field on a transition state structure. In the design of a high-activity mutant of BChE against (−)-cocaine, one would like to predict some possible mutations that can lower the energy of the transition state for the first chemical reaction step (TS1) and, therefore, lower the energy barrier for this critical reaction step. Apparently, a mutant associated with the stronger hydrogen bonding between the carbonyl oxygen of (−)-cocaine benzoyl ester and the oxyanion hole of the BChE mutant in the TS1 structure may potentially have a more stable TS1 structure and, therefore, a higher catalytic efficiency for (−)-cocaine hydrolysis. Hence, the hydrogen bonding with the oxyanion hole in the TS1 structure is a crucial factor affecting the transition state stabilization and the catalytic activity. The possible effects of some mutations on the hydrogen bonding were examined by performing MD simulations on the TS1 structures for (−)-cocaine hydrolysis catalyzed by wild-type BChE and its various mutants.[52,53]

Based on extensive MD simulations[52,53] on various TS1 structures associated with wild-type BChE and its mutants, some

 

mutants were predicted to have stronger overall hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the protein environment. For example, the carbonyl oxygen of (−)-cocaine in the simulated TS1 structure associated with the wild-type has two N–H · · · O hydrogen bonds with the peptidic NH of G117 and A199 residues (Fig. 6). In the simulated TS1 structure associated with the A199S/S287G/A328W/Y332G mutant, three hydrogen bonds have been identified. As seen in Fig. 7, when residue #199 becomes a serine (*i.e.* S199), the hydroxyl group on the side chain of S199 can also hydrogen bond to the carbonyl oxygen of (−)-cocaine to form an O–H · · · O hydrogen bond, in addition to the two N–H · · · O hydrogen bonds with the peptidic NH of G117 and S199.

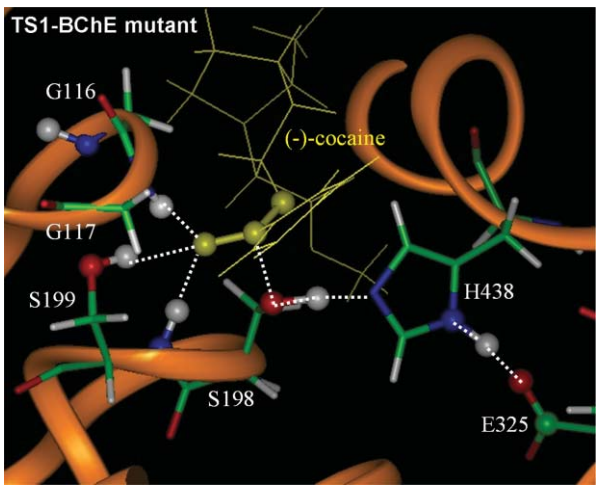


**Fig. 7** MD-simulated TS1 geometry for (−)-cocaine hydrolysis catalyzed by the A199S/S287G/A328W/Y332G mutant of BChE.

The overall hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the protein environment can be represented by the total hydrogen bonding energy (HBE) estimated by using the simulated H · · · O distances in the hydrogen bonds.[52,53] The total hydrogen bonding energies in the TS1 structures associated with the wild-type, A328W/Y332A, A328W/Y332G, A199S/F227A/A328W/Y332G, and A199S/S287G/A328W/Y332G BChEs were estimated to be −5.5, −6.2, −6.4, −9.8, and −14.0 kcal mol$^{-1}$, respectively. The estimated HBE values suggest that the transition states for the first chemical reaction step (TS1) of (−)-cocaine hydrolysis catalyzed by the A199S/F227A/A328W/Y332G and A199S/S287G/A328W/Y332G mutants should be significantly more stable than those catalyzed by the A328W/Y332A or A328W/Y332G mutants, due to the significant increase of the overall hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the oxyanion hole of the enzyme in the TS1 structure. In addition, the TS1 structure associated with the A199S/S287G/A328W/Y332G mutant should be more stable than that associated with the A199S/F227A/A328W/Y332G mutant. As the first chemical reaction step associated with TS1 should be the rate-determining step of (−)-cocaine hydrolysis catalyzed by a BChE mutant including Y332A or Y332G mutation, one may reasonably expect a clear correlation between the TS1 stabilization and the catalytic efficiency of A328W/Y332A, A328W/Y332G, A199S/F227A/A328W/Y332G, and A199S/S287G/A328W/Y332G BChEs for (−)-cocaine hydrolysis: the more stable the TS1 structure, the lower the energy barrier, and the higher the catalytic efficiency. Thus, the MD simulations and the HBE calculations predict that both A199S/F227A/A328W/Y332G and A199S/S287G/A328W/Y332G BChEs should have a higher catalytic efficiency than A328W/Y332A or A328W/Y332G BChEs for (−)-cocaine hydrolysis. Further, the A199S/S287G/A328W/Y332G mutant is expected to be more active than the A199S/F227A/A328W/Y332G mutant.

The predictions based on the transition state simulations and HBE calculations were followed by *in vitro* experiments,[52,53] including site-directed mutagenesis, protein expression, and enzyme activity assays against (−)-cocaine. The *in vitro* experiments revealed that A199S/S287G/A328W/Y332G BChE has a ∼456-fold improved catalytic efficiency against (−)-cocaine compared to the wild-type, or A199S/S287G/A328W/Y332G BChE has a $k_{cat}/K_M$ value of $\sim 4.15 \times 10^8$ M min$^{-1}$ for (−)-cocaine hydrolysis.[52] It was also determined that A199S/F227A/A328W/Y332G BChE has a ∼151-fold improved catalytic efficiency against (−)-cocaine compared to the wild-type, or A199S/F227A/A328W/Y332G BChE has a $k_{cat}/K_M$ value of $\sim 1.37 \times 10^8$ M min$^{-1}$ against (−)-cocaine.[53] By using the designed A199S/S287G/A328W/Y332G BChE as an exogenous enzyme in humans, when the concentration of this mutant is kept the same as that of the wild-type BChE in plasma, the half-life time of (−)-cocaine in plasma should be reduced from ∼45–90 min to only ∼6–12 seconds, considerably shorter than the time required for cocaine crossing the blood–brain barrier to reach the CNS.[52] Hence, the encouraging outcome of the rational design and discovery study could eventually result in valuable, efficient therapeutics for treatment of cocaine overdose and addiction.

## 5 Concluding remarks

Promising agents for therapeutic treatment of cocaine overdose and addiction have been designed and discovered based on the molecular structures and the detailed reaction mechanisms for non-enzymatic and enzymatic hydrolysis of cocaine. The stable analogs of the rate-determining transition state for non-enzymatic hydrolysis of cocaine have been designed and used to successfully elicit anti-cocaine catalytic antibodies.

The computational design of high-activity mutants of BChE has been based on not only the structure of the enzyme–substrate binding, but also the detailed catalytic mechanisms for BChE-catalyzed hydrolysis of (−)-cocaine and (+)-cocaine. Computational studies of the detailed catalytic mechanisms and the structure-and-mechanism-based computational design have been carried out through the combined use of a variety of state-of-the-art techniques of molecular modeling. The state-of-the-art computational studies have led to detailed mechanistic insights into the reaction pathways for BChE-catalyzed hydrolysis of (−)-cocaine and (+)-cocaine. These detailed mechanistic insights provide a solid basis for rational design of novel anti-cocaine medication using the high-activity mutants of human BChE against (−)-cocaine.

By using the computational insights into the catalytic mechanisms for BChE-catalyzed hydrolysis of (−)-cocaine and (+)-cocaine, it is clear that when the rate-determining step becomes the chemical reaction process, the truly rational design of the BChE mutants cannot be limited to modeling of the enzyme–substrate binding. A unique computational design strategy based

on modeling and simulation of the rate-determining transition state has been developed to design high-activity mutants of BChE for hydrolysis of (−)-cocaine, leading to the exciting discovery of BChE mutants with a considerably improved catalytic efficiency against (−)-cocaine. One of the discovered BChE mutants (*i.e.* A199S/S287G/A328W/Y332G) has a ~456-fold improved catalytic efficiency against (−)-cocaine. The encouraging outcome of the structure-and-mechanism-based design and discovery effort demonstrates that the unique computational design approach based on the transition state modeling and simulation is promising for rational enzyme redesign and drug discovery.

The general computational design strategy, particularly the structure-and-mechanism-based design of high-activity mutants of BChE, in combination with appropriate experiments including site-directed mutagenesis and enzyme activity assays may be used to design and discover high-activity mutants of any other interesting enzyme or catalytic antibody. In particular, future development of anti-cocaine catalytic antibodies should not be limited to the immunization with new transition state analogs. It should also be interesting to design and discover high-activity mutants of the currently known anti-cocaine catalytic antibodies by using a similar computational–experimental approach used for design and discovery of high-activity mutants of BChE. It is also desirable to develop high-activity mutants of other metabolic enzymes for future therapeutic treatments of metabolic diseases. A metabolic disease is a disorder caused by the accumulation of chemicals produced naturally in the body.[54,55] The metabolic diseases are usually serious, some even life threatening. Using exogenous enzymes to metabolize the chemicals is clearly an ideal therapeutic strategy, particularly when the metabolic disease is caused by a genetic defect or absence of certain metabolic enzymes. For each of the metabolic enzymes, to design the high-activity mutants against a specific compound (substrate), one first needs to uncover the fundamental catalytic mechanism and perform structure-and-mechanism-based design of mutations that can potentially stabilize the rate-determining transition state and lower the free energy barrier.

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